Australian Good Meat Education resources are developed by MLA Meat & Livestock Australia

TEACHER GUIDE

Senior Agriculture Resource

Elective 2: Climate Challenge

Analyse a Research Study

Analyse a research study of the development and/or implementation of climate variability or management strategies related to climate variability in terms of:

- Design of the study
- Methodology of the study
- Collection of data for the study
- Presentation of data
- Analysis of the data
- Conclusions and recommendations

Australian Good Meat Education resources are developed by MLA MEAT & LIVESTOCK AUSTRALIA

TEACHER GUIDE

Research Study: ***Asparagopsis feedlot feeding trial***

https://www.mla.com.au/contentassets/120dea2a6b504401baeedfd303794361/b.flt.0394_final_report.pdf

Prepared by: Rob Kinley
Commonwealth Scientific and Industrial Research Organisation

Date published: 31 May 2018

Scan QR code to access research study

OUTCOMES

A student:

- **H3.4** evaluates the management of the processes in agricultural systems
- **H4.1** justifies and applies appropriate experimental techniques, technologies, research by methods and data presentation and analysis in relation to agricultural problems and situations
- **H5.1** evaluates the impact of innovation, ethics and current issues on Australian agricultural systems.

Aligned to NSW Stage 6 Agriculture, HSC course, Electives.

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ACTIVITY 1 – *Asparagopsis* and Ruminant Methane Emissions

1. *Asparagopsis* feedlot feeding trial
2. Carbon neutral 2030 R&D | Meat & Livestock Australia
3. Digital devices and headphones
4. About FutureFeed (3:04)

ACTIVITY 2 – Analyse a Research Study

1. *Asparagopsis* feedlot feeding trial
2. Digital devices
3. Highlighters and pens
4. Student Learning Resource

ACTIVITY 3 – Rolling Revision

1. Scissors, glue, or sticky tape
2. Printed copy of Rolling Revision

Lesson guide

ACTIVITY 1 – *Asparagopsis* and Ruminant Methane Emissions

Students watch a video and answer questions as an introduction to the research study analysis.

1. Introduce the chosen research study on the benefits of *Asparagopsis* as a feed additive in feedlot beef cattle to analyse for this elective.
2. Distribute or digitally share a copy of *Asparagopsis* and Ruminant Methane Emissions.
3. Divide students into pairs and allow them time to watch the video About FutureFeed (3:04) and record their answers to the questions provided.
4. As a class, discuss the responses to the questions on the worksheet. (Answers page 8)

ACTIVITY 2 – Analyse a Research Study

Students use the Student Learning Resource (with teacher guidance) to analyse the research study investigating the benefits of *Asparagopsis* as a feed additive in feedlot beef cattle. Students are supported through an in-depth analysis of the research report.

Note: The student booklet for this task is extensive. Teachers have the choice of either providing students with printed or digital access (featuring fillable answer spaces) to the Student Learning Resource. This is dependent on their student's needs, access to technology, and choice of lesson methodology.

During the analysis, students are required to highlight and annotate various aspects of the Student Learning Resource, so consideration should be given to the most suitable method of presentation and delivery.

1. Provide students with a digital or printed copy of the research paper *Asparagopsis* feedlot feeding trial.
2. Provide students with digital or printed copy of the Student Learning Resource.

***Lesson guide** continued next page*

Lesson guide *continued*

3. Project a copy of the Student Learning Resource in a central area and progress through the various sections of analysing the research paper.

   Note: Suggested answers are provided throughout the Teacher Guide to use as a basis for discussion. These responses are provided as a guide only and should be viewed as a foundation for fostering meaningful discussions and critical thinking within the classroom.

ACTIVITY 3 – Rolling Revision

Students challenge peers using a revision game to address a section or components of the research report in an engaging, hands-on way to summarise the research study.

1. Divide students into pairs or groups of three.
2. Provide students with a printed copy of Rolling Revision, scissors, and glue or sticky tape.
3. Students cut out and construct the dice.
4. One student rolls the dice and reads the section of the research study that is facing up. The other students recall a key aspect of that section or component of the research. Students continue to take turns rolling the dice and revising topics. If a topic is rolled twice (or more), students must not repeat a 'fact' that has been previously stated.
5. This activity may be extended in many ways. As an example, students and teachers may develop a list of questions to be asked when each section is rolled. E.g. When 'Design of study' is rolled, questions asked could include, "Explain what a variable and a control are, and how were variables controlled in this study?" or "What was the aim of this study?" etc.

Contents

Asparagopsis and Ruminant Methane Emissions

Asparagopsis is a red seaweed that is being investigated as a potential feed additive for livestock due to its ability to reduce methane emissions produced during digestion in ruminant animals. Methane is a potent greenhouse gas that is produced during enteric fermentation in the rumen of cattle and other ruminants, and reducing these emissions can have a significant impact on the environment, as well as increasing the productivity of these livestock.

1. Use the QR code or access the link below, to learn how an innovative livestock feed supplement initiative developed by the CSIRO, Meat & Livestock Australia, and James Cook University utilises a specific type of seaweed which can increase productivity and reduce methane emissions. Answer the questions on the following page.

About FutureFeed (3:04)

Activity 1 continued next page

2. Explain how ruminants such as cattle, sheep, and goats produce methane. How is it then released into the atmosphere?

   As cattle and sheep digest their feed, microorganisms in their digestive system (the rumen), called methanogens, produce methane as the feed is fermented.

   From the research paper 'Enteric CH_4 is a by-product from anaerobic fermentation of feed organic matter (OM) by a microbial consortium in the rumen. This enteric fermentation produces carbon dioxide (CO_2) and hydrogen (H_2), which is then instrumental in the formation of CH_4 in a reduction pathway by microbial methanogenic archaea'.

   Methane is released into the atmosphere by cows belching (burping).

3. Identify the chemical formula for methane.

   CH_4

4. Describe why methane production by ruminants is a significant focus for stakeholders in the meat and livestock industry.
   Carbon neutral 2030 R&D | Meat & Livestock Australia

   Methane is a potent greenhouse gas. Methane from livestock producers accounts for about 15% of global greenhouse gas emissions.

   The Australian red meat industry has set a target to be carbon neutral by 2030 (CN30). This means that by 2030, Australian beef, lamb, and goat production, including lot feeding and meat processing, aims to make no net release of greenhouse gas (GHG) emissions into the atmosphere.

   Belched methane represents energy lost from the production system that might otherwise be converted to the meat, milk, or fibre that generates income. So, reducing methane loss leads to productivity gains by the ruminants.

5. Describe how the benefits of feeding cattle seaweed were first discovered.

   It was observed that cattle by the sea that ate seaweed were more productive, grew faster, and were healthier and easier to manage than other cattle. Scientist, Dr Rob Kinley, discovered that methane emissions were much lower in these seaweed-eating cattle when studying why this occurred.

6. Identify the seaweed with the most 'methane busting potential'.

   Asparagopsis

Analyse a Research Study

Topic one: Design of the Study

1. **Title of the research study** *(Final Report page 1 of 42)*

   a. Highlight the name of the study and copy the name in the space provided.

CSIRO mla MEAT & LIVESTOCK AUSTRALIA

final report

Project code:	B.FLT.0394
Prepared by:	Rob Kinley Commonwealth Scientific and Industrial Research Organisation
Date published:	31 May 2018

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Asparagopsis feedlot feeding trial

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Asparagopsis feedlot feeding trial

Topic 1 continued next page

Topic 1 continued

Activity 2

2. Authors and organisations *(Final Report page 1 of 42)*

a. Identify the author and the organisation where this researcher was based.

> Robert D. Kinley
> Commonwealth Scientific and Industrial Research Organisation (CSIRO)

3. Date published *(Final Report page 1 of 42)*

a. Identify the published date of the final report.

> 31 May 2018

4. Design and format of the research report *(Final Report pages 1–42)*

a. List the order of contents of the research report.

> Abstract
> Executive summary
> Background
> Project objectives
> Methodology
> Results
> Discussion
> Conclusions/recommendations
> Key messages
> Bibliography
> Appendix I

Topic 1 continued next page

Activity 2

5. **Abstract** *(Final Report page 2 of 42)*

An abstract is a short summary at the beginning of the research that outlines all components of the report.

The significance of the abstract is to provide readers with an overview of the relevance of the paper including the:

- Reason/need for the research
- Problem being investigated
- Overview of the methodology
- Results
- Implications for future research or developments.

a. Read the abstract and highlight the main points summarising the research report. Attempt to find at least one point focused on each of:

- The importance and need for the research
- An aspect of the methodology
- The significance of the findings
- A future direction for associated research.

Abstract

It's a global initiative to decrease methane emissions and increase productivity of cattle to benefit the environment, food production, and profitability of beef production. The seaweed *Asparagopsis taxiformis* was evaluated for its capability to reduce methane emissions and improve productivity in Brangus steers. It was included in the feedlot ration at 0.05%, 0.10%, and 0.20% of organic matter. Emissions were monitored in respiration chambers fortnightly during 90 days treatment. Daily feed intake was measured and steers weighed weekly. Steers receiving *Asparagopsis* demonstrated reduced methane up to 98%. There was no change in feed intake, however with inclusion of 0.10%, and 0.20% there was indication of weight gain improvement of 53% and 42%, respectively, which requires to be demonstrated and confirmed in a commercial environment. Hot carcass weight and dressing percentage were not different. *Asparagopsis* had no effect on meat eating quality. The bioactive bromoform was not detectable in tissues of treated steers, given a two day withdrawal period. Response is expected to change with feed base, thus investigation is required to identify appropriate inclusion levels in variable feed formulations. If the feeding of *Asparagopsis* ultimately proves feasible, the beef industry will benefit with improved image, environmental footprint, and profitability.

Topic 1 continued next page

Activity 2

6. **Executive summary** *(Final Report pages 3–5 of 42)*

This research study provides an Executive summary. This is a more detailed summary of the research, results, and the significance of the work. This section is not commonly found in journal articles. It is included here to provide a specific summary to industry members within the meat and livestock industry.

a. Read the **Executive summary** and highlight the main points summarising the research report. Attempt to find at least one point focused on each of:

- The importance and need for the research
- An aspect of the methodology
- The significance of the findings
- The future direction for associated research.

Executive summary

Methane (CH_4) in the atmosphere is a highly a potent greenhouse gas (GHG) with a global warming potential 28 times greater than carbon dioxide (CO_2, IPCC 2014). Agriculture is a major contributor to the global GHG inventory and ruminant enteric fermentation is the largest agricultural source and is responsible for 60% of agriculture's contributions and primarily as methane. The antimethanogenic properties of using many types of seaweeds (macroalgae) as feed additives has now been confirmed by many researchers.

This project B.FLT.0394 was a beef feedlot simulation that followed the sheep supplementation study B.CCH.2095 both designed to confirm in ruminant animals the findings of the in vitro project B.CCH.6420 based in the Meat & Livestock Australia (MLA) guided National Livestock Methane Program (NLMP). The in vitro work screened 20 algae species and identified the red seaweed (macroalgae) *Asparagopsis taxiformis* as the best and subsequent primary candidate for progression to demonstration in animal experiments. The in vitro work demonstrated the lack of negative effects on rumen fermentation while elucidating inclusion level effects for feed formulation of this highly potent antimethanogenic agent. In the outcomes of the NLMP program, *Asparagopsis* was identified as a high priority for research investment by MLA based on its capacity to reduce methane (CH_4) emissions and potential for productivity gains. The experiment was conducted at a simulated feedlot at CSIRO Lansdown Research Station, Townsville, using 28 Brangus breed steers (Brahman-Angus cross). The content of Brahman in the individuals was unspecified and varied using the proxy assessment of hump height with average hump of 106 mm. Steers were allocated across four groups to determine the CH_4 abatement, changes in hydrogen (H_2) emissions and productivity in response to graded levels of freeze dried *Asparagopsis taxiformis*.

Topic 1 continued next page

Activity 2

The experimental treatment levels were 0.00%, 0.05%, 0.10%, and 0.20% of dietary OM representing the Control, Low, Mid, and High levels, respectively. Steers were maintained in individual pens for accurate intake monitoring and were cycled through five sessions in respiration chambers for emissions monitoring and weekly monitoring of body weight. After the chamber sessions steers were sampled for rumen metabolites and at termination of the feedlot finishing period their carcasses were assessed by Meat Standards Australia (MSA) followed by meat eating quality sensory evaluations. Determination of the effects of *Asparagopsis* on rumen fermentation parameters of volatile fatty acids (VFA) and ammonia (NH_3-N) was completed via rumen samples collected by stomach tubing. These sample sets were collected three hours post-feeding, on the day ending their respective respiration chamber sessions where measurements for CH_4 and H_2 emissions were collected. Liveweight (LW) was determined prior to morning feeding for consistency between measurements using a certified Gallagher Smart TSi walkover scale system (Hamilton, NZL) equipped with True Test HD1010 weigh bars (Brisbane, AUS). Weight measurements coincided with movement of animals into and out of the animal house and chambers resulting in weight measurements every week.

At the completion of the 90 d feedlot period, the inclusion of *Asparagopsis* in the diets was ceased and the steers received the control TMR and Rhodes grass ad libitum for two days. All animals were transported at the same time to JBS Australia in Townsville, Qld (45 km), an export accredited abattoir, and slaughtered the following day using commercial best practice. MSA was on site to grade carcasses and collected samples for meat eating quality sensory evaluation (MQ4). Carcass characteristics (carcass weight, rump fat depth [P8], ribeye muscle area [EMA], rib fat depth) and MSA scores (including meat and fat colour, marbling and ossification, EMA) were recorded for each animal. Meat samples were collected in line with MSA sampling and sampling of depot fat (brisket) and whole kidneys for bromoform residue analysis was completed at the JBS processing line.

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Activity 2

The primary effects of *Asparagopsis* were to dramatically reduce CH_4 and increase H_2 emissions in a both linear and quadratic response. Methane measured as g/kg DMI was reduced 98% (11.0 down to 0.26 g/kg DMI) by adding *Asparagopsis* at the High level of 0.20% of the TMR (OM bases) while H_2 was increased 17 fold (0.10 g/kg up to 1.80 g/kg DMI). There was also a linear increase in average daily weight gain (ADWG) of 53% and 42% during the period of final inclusion levels of *Asparagopsis* at the Mid level of 0.10% and High level of 0.20%, respectively. The statistical significance observed with ADWG improvements were muted by high variability from the feed conversion ratio (FCR) which marginally failed to be a trend (P=0.124) of improvement with *Asparagopsis* inclusion. The TVFA remained without significant change and there was no significant difference in any of the VFA species however both acetate and propionate only marginally failed test for significance with P-values of 0.054 and 0.051, respectively. Therefore both represent a strong trend with proportional reduction in acetate and increase in propionate with increasing *Asparagopsis*. Then the acetate:propionate ratio (A:P) did have a significant linear decrease indicating strong shift from acetate to propionate species with increasing *Asparagopsis* inclusion. All other aspects and variables remained without significant change with inclusion of *Asparagopsis* within the range included in the rations of lot fed Brangus cattle in this study.

Asparagopsis taxiformis was demonstrated to be an even more potent antimethanogenic agent for cattle when included in a grain based feedlot diet as compared to sheep on a legume-grain mix diet and compared to in vitro assessments using a grass based substrate. The amount of *Asparagopsis* required to achieve near elimination of CH_4 was surprisingly low and represents the single most important finding of this study. This was the first study using a high grain feedlot ration and with additives such as monensin in the TMR. Even with 1/10 of expected required levels of inclusion of the seaweed, CH_4 production was reduced by 9%, 38% and 98% at levels of 0.05%, 0.10%, and 0.20% of dietary OM, respectively. This has the potential to make significant impact for the red meat industry toward the achievement of MLA's commitment to carbon neutrality by 2030 (CN2030).

Although H_2 was demonstrated to significantly increase it did not increase at previously observed levels relative to CH_4 reductions in a study using chloroform as the antimethanogenic agent. The excess H_2 had no negative effect on the steers DMI and productivity but H_2 emissions represent feed energy loss and so methods of conserving the H_2 would further the benefits of *Asparagopsis* and it is recommended that such methods be explored.

Topic 1 continued next page

Activity 2

Importantly, in achieving extensive CH_4 reductions, the DMI of the cattle in this study was not negatively affected as feed consumption remained consistent for all treatment groups. The response in ADWG to *Asparagopsis* was also better than expected by demonstrating ADWG improvements of 53% and 42% for those cattle receiving 0.10% and 0.20% of dietary OM, respectively. However this outcome should only be regarded as an indicator due to the small number of cattle in each treatment group and results are susceptible to variation in individual animal performance. It is possible that the *Asparagopsis* supplemented cattle were inherently the most productive individuals in the feeding scenario of this study.

It is recommended that the efficacy of *Asparagopsis* to reduce CH_4 emissions be investigated as an inclusion in diets of variable formulation. Diets containing variable levels of roughage of variable sources, and also with inclusions such as monensin, have potential to alter the efficacy of *Asparagopsis*. This is expected to change the inclusion level required for large reduction in CH_4 emissions. However, if increasing the inclusion level of *Asparagopsis* is necessary for effective reduction of CH_4 in any feeding system it will be necessary to carefully monitor potential toxicity and concomitant detrimental effects on animal health. Seaweeds are known to concentrate potentially toxic entities such as heavy metals and minerals, and *Asparagopsis* tends to accumulate iodine. Techniques of cultivation and processing should be developed that will reduce the level of seaweed required for effective CH_4 reduction and to reduce exposure to entities such as iodine. To widely utilise *Asparagopsis* as a livestock feed additive knowledge of the interaction and efficacy in variable feed formulations would be intrinsic in the variable cattle feeding systems in Australia and globally. The ADWG improvements suggested by this study should be demonstrated and refined in a large scale study in a commercial feedlot environment. This is required for confirmation that *Asparagopsis* can increase ADWG under typical feedlot conditions, provide confidence, and promote commercial acceptance and subsequent adoption.

Topic 1 continued next page

Activity 2

There was no significant difference in initial LW prior to *Asparagopsis* treatment, and after completion of the treatment period the final LW was marginally higher for the treated steers (P=0.034). However, the Mid inclusion level treatment group was the only group with significantly greater final LW compared to the Control. Using ADWG as a proxy for change in LW there was a significant increase in LW during the treatment period for treated steers compared to steers without *Asparagopsis*. Although mean initial LW's were not significantly different the LW was variable within the groups, and there was enough difference between the groups to significantly favor ADWG of the treated steers. Significant increase was demonstrated for both the Mid and High treatment groups resulting from LW increases of 137 and 130 kg, respectively, compared to 113 kg for the Control. Although there was marginal significant difference in the final LW but with high variability it was not enough to impact carcass weight. The carcass weight means were consistent between all treatment groups and Control and overall mean carcass weight was 322 kg. Carcass weights were not significantly different, however only marginally failed to achieve a trend (P=0.133). Therefore these irregularities lead to the conclusion for the need for caution on interpretation of the results as evidence or confirmation of enhanced ADWG induced by *Asparagopsis*. Thus there it is essential to reproduce the apparent enhanced ADWG in a large scale project in a commercial feeding environment.

The only measured noteworthy changes in rumen fluid due to *Asparagopsis* as a feed additive was a trend toward concomitant decreased acetate and increased propionate without effect on TVFA. This has now been demonstrated as a consistent concomitant outcome with reduced CH_4 emissions induced by *Asparagopsis* as a feed additive at low levels. Carcass characteristics and meat eating quality were not changed as confirmed by MSA consumer testing protocols and all the treatment groups were MSA graded. No bromoform could be detected in any meat, kidney, or fat collected from *Asparagopsis* treated steers. However, due diligence recommends continued monitoring and further necroscopy exploration as the feed inclusion periods get longer and/or the inclusion and intake levels are substantially increased which may be the case in dairy systems. Overall, it was demonstrated that *Asparagopsis* is the most promising antimethanogenic agent for feedlot production systems currently in the development pipeline. It is recommended that further study be completed to address changing feed base, confirmation of productivity enhancement using large numbers of test animals, and commercial scale systems and environment applications.

b. Identify the management strategy related to climate variability being investigated in the research study and summarise aspects of the **executive summary** and **abstract** on the following page.

Topic 1 continued next page

Suggested points to include:

Management strategy related to climate variability

- The seaweed *Asparagopsis taxiformis* was used as a feed supplement/ingredient in Brangus (Brahman-Angus cross) steers to see if this reduced the production of methane and improved productivity.

The importance and need for the research

- There is a global initiative to decrease methane emissions and increase productivity of cattle to benefit the environment, food production, and profitability of beef production.

An aspect of the methodology

- *Asparagopsis* was included in the feed of Brangus cross steers at 0.00%, 0.05%, 0.10%, and 0.20% of feed organic matter.
- Emissions were monitored in respiration chambers fortnightly over 90 days of treatment.
- Feed intake was monitored daily.
- Steers were weighed weekly prior to feeding.
- After the chamber sessions steers were sampled for rumen metabolites and at termination of the feedlot finishing period their carcasses were assessed by Meat Standards Australia (MSA) followed by meat eating quality sensory evaluations.

The significance of the findings

- Steers receiving *Asparagopsis* demonstrated reduced methane by up to 98%.
- The inclusion (at low rates) of *Asparagopsis* in ruminant diets has the potential to significantly contribute to the red meat industry's goal of achieving carbon neutrality by 2030 (CN2030).
- If the feeding of *Asparagopsis* ultimately proves feasible, the beef industry will benefit from improved image, reduced environmental footprint, and increased profitability.

Future direction for associated research

- It is recommended that further study be completed to address changing feed base, confirmation of productivity enhancement using large numbers of test animals, and commercial scale systems and environment applications.
- Studies into methods of conserving H_2 loss would further the benefits of feeding *Asparagopsis*, as increased H_2 emissions represent feed energy loss.
- Techniques of cultivation and processing of *Asparagopsis* that will reduce the level of seaweed required for effective CH_4 reduction and reduce exposure to entities such as iodine should be developed.

Topic 1 continued next page

Activity 2

7. **Introduction/Background** *(Final Report pages 8–9 of 42)*

The introduction is presented at the start of a research study. It is the information that the reader needs to know about the topic that the research is focused on before they engage in reading how the study was performed and what results were obtained.

The significance of the introduction/background is that it provides the reader with significant findings from previous research, relevant information on the topic, and clarifies the importance of the study. It also provides details about the specific research question/problem being investigated.

a. Read the background information below and highlight the main points.

1. Background

Methane (CH_4) in the atmosphere is a highly a potent greenhouse gas (GHG) with a global warming potential 28 times greater than carbon dioxide (CO_2, IPCC 2014). Agriculture is a major contributor to the global GHG inventory and ruminant enteric fermentation is the largest agricultural source and is responsible for 60% of agriculture's contributions (Olivier et al. 2005). Enteric CH_4 is a consequence of fermentation of feed organic matter (OM) by a microbial consortium that produces CO_2 and H_2 utilised in the formation of CH_4 in a reduction pathway used by microbial methanogenic archaea (Morgavi et al. 2010). Much research has been directed in the search for feed additives to disrupt this pathway or otherwise reduce methanogen populations. Patra (2012) reviewed ruminant feed additive options and reported that an antimethanogenic feed additive may be concomitant with detriment to livestock productivity often as a result of reduced feed intake. Therefore, it is critical to develop additives that do not impair productivity or otherwise improves productivity.

The antimethanogenic properties of using many types of seaweeds (macroalgae) as feed additives has now been confirmed by many researchers (Wang et al. 2008; Dubois et al. 2013; Kinley and Fredeen 2014; Maia et al. 2016, Li et al. 2018, among others). The antimethanogenic potency and impacts of macroalgae on the rumen ecosystem is highly genus and species specific (Kinley et al. 2016b). However, one marine species of red macroalgae characterized by secondary metabolites with antibacterial properties (Paul et al. 2006) demonstrates a potent antimethanogenic effect without detriment *in vitro* (Kinley et al. 2016a), and *in vivo* (Li et al. 2018).

Topic 1 continued next page

In the *in vitro* work described by Kinley et al. (2016a) *Asparagopsis* consistently eliminated CH_4 from rumen fermentations at inclusion rates as low as 1% (OM basis) of the Rhodes grass feed base and in the fermentations did so without detriment to other parameters of rumen digestion. Supplementing *Asparagopsis* to sheep confirmed the antimethanogenic capability of this macroalgae (Li et al. 2016). Methane was reduced by 80% when 3% of the organic matter intake was supplemented as seaweed. The feeding regime with the sheep was restricted to help ensure higher level of intake of the seaweed which confounded demonstration of productivity enhancement using average daily weight gain (ADWG) as the metric. Here it is important to clarify that the sheep in the higher additive levels in the Li et al. (2016) study did not eat all the *Asparagopsis* offered to them. The diet was a pelleted formulation of the pulse/legume lupins with oats-barley-wheat, and the seaweed was added on top of the pellets causing separation due to specific gravity and selection by the sheep. Also, the *Asparagopsis* used was of similar quality based on the bromoform content compared to the *in vitro* work, and of particular importance, older and of much lower quality compared to the present beef supplementation study. Bromoform is the bioactive ingredient responsible for antimethanogenesis and its content in the *Asparagopsis* used in each study was 1723, 1750, and 6550 mg/kg of dry matter (DM) for the in vitro, sheep, and beef feedlot studies, respectively. The in vitro studies used Rhodes grass feed and the sheep study used a legume and grain feed base, however in contrast the beef feedlot study used a high grain diet based on steam rolled barley representative of commercial feedlots. It has been considered throughout these associated studies that feed base composition relative to roughage content and addition of entities such as monensin could have as yet unidentified effect on the antimethanogenic efficacy of *Asparagopsis* as a feed additive.

The effect of seaweeds such as *Asparagopsis* as feed additives on carcass and meat eating quality may have a direct impact on the consumer perspective of product value and hence the viability for producer adoption of the additive. Studies have been conducted on carcass and meat eating quality to determine changes relative to modifying the diet using energetic by-products of food processing (Duynisveld and Charmley 2016) showing major diet changes without detriment is possible using appropriate substrate. Adding *Ascophyllum nodosum* (Tasco), a brown seaweed, at levels of 2% of dietary intake to the feed formulation of Brangus steers has proven to induce improvements in carcass, shelf life, and meat eating qualities with little change and no detrimental effects observed (Braden et al. 2007). The environmental benefits of *Asparagopsis* to rumen fermentations observed to date has been compelling and the successful use of other types of seaweed provides further promise. Therefore it is practical to develop knowledge of *Asparagopsis* effect on carcass and meat eating quality, and explore possibility of bromoform residue in food products when this seaweed is included in beef feed formulations.

Topic 1 continued next page

Activity 2

The hypotheses investigated was that the addition of *Asparagopsis* to a feedlot diet formulation fed to Brangus steers would result in CH_4 reduction similar to those demonstrated *in vitro* where a grass based diet was used, and in vivo in sheep where a legume and grain mix diet was used, and do so using the same successful inclusion levels as used in the in vitro and sheep projects. It was also hypothesised that the energy otherwise lost as CH_4 gas would be conserved in the animal and be converted to some extent into productivity as enhanced growth parameters with improvements to product quality.

Specifically we sought to determine the effect of *Asparagopsis* supplementation on enteric CH_4 production, animal productivity, parameters of fermentation, and meat product quality relevant to intensive ruminant production systems, namely Australian beef feedlots, by:

a) Establishing the effect of *Asparagopsis* inclusion in feedlot diets on animal productivity and individual intakes;

b) Defining the inclusion level response relationship for *Asparagopsis* across four inclusion levels and reductions in enteric CH_4 for individual animals;

c) Establishing the effects of algae feeding on carcass characteristics and Meat Standards Australia (MSA) grading outcomes;

d) Conducting the meat and eating quality assessments to ascertain consumer acceptance of algae supplemented beef product; and

e) Establishing whether the feeding of algae results in potential chemical residues in the meat of fed cattle.

Topic 1 continued next page

Topic 1 continued

Activity 1

b. Summarise the highlighted sections from the background information.

Suggested points to include:

Background summary

- Methane (CH_4) in the atmosphere is a highly a potent greenhouse gas (GHG) with a global warming potential 28 times greater than carbon dioxide (CO_2).
- Enteric CH_4 is a consequence of fermentation of feed organic matter (OM) by a microbes (methanogenic archaea) that produce CO_2 and H_2 which form CH_4 (in a reduction pathway).
- The antimethanogenic potency and impacts of macroalgae on the rumen ecosystem is highly genus and species specific. Many types of seaweeds (macroalgae) as feed additives have been studied.
- *In vitro* work in 2016 showed that *Asparagopsis* consistently eliminated CH_4 from rumen fermentations at inclusion rates as low as 1% (OM basis) of the Rhodes grass feed base and without detriment to other parameters of rumen digestion.
- Supplementing *Asparagopsis* to sheep confirmed the antimethanogenic capability of this macroalgae. Methane was reduced by 80% when 3% of the organic matter intake was supplemented as seaweed.
- The effect of seaweeds such as *Asparagopsis* as feed additives on carcass and meat eating quality may have a direct impact on the consumer perspective of product value and hence the viability for producer adoption of the additive.
- Studies have been conducted on carcass and meat eating quality to determine changes relative to modifying the diet using energetic by-products of food processing showing major diet changes without detriment is possible.
- Adding *Ascophyllum nodosum* (Tasco), a brown seaweed, at levels of 2% of dietary intake to the feed formulation of Brangus steers has proven to induce improvements in carcass, shelf life, and meat eating qualities with little change and no detrimental effects observed.
- The environmental benefits of *Asparagopsis* to rumen fermentations observed to date has been compelling and the successful use of other types of seaweed provides further promise.
- It is practical to develop knowledge of *Asparagopsis* effect on carcass and meat eating quality, and explore possibility of bromoform residue in food products when this seaweed is included in beef feed formulations.

Topic 1 continued next page

8. Aim/Objectives *(Final Report pages 9–10 of 42)*

The ***Aim/Objective*** of a report is the stated area of investigation that researchers are interested in and are focused on in the research study.

a. Read and highlight the main points in each objective.

The objectives of this project were to:

2.1 B.FLT.0394 Project Objectives

Determination of the effect of *Asparagopsis* supplementation on enteric CH_4 production and animal productivity relevant to intensive ruminant production systems, namely Australian beef feedlots, by:

2.1.1 Establishing the effect of Asparagopsis

Establishing the effect of *Asparagopsis* inclusion in feedlot diets on animal's productivity (ADWG) and individual intakes.

2.1.2 Defining the inclusion level response

Defining the inclusion level response relationship for *Asparagopsis* across groups of steers receiving four inclusion levels and reductions in enteric CH_4 for individual animals.

2.1.3 Effects on carcass characteristics

Establishing the effects of algae feeding on carcass characteristics and MSA grading outcomes.

2.1.4 Meat and eating quality

Conducting the meat and eating quality assessments to ascertain consumer acceptance of algae supplemented beef product.

2.1.5 Chemical residues

Establishing whether the feeding of algae results in potential chemical residues in the meat of fed cattle.

Topic two: Methodology of the Study

1. **Methodology** *(Final Report page 10 of 42)*

   The methodology of a study aims to provide the reader with a set of instructions about how the research was performed. Specific information should be provided detailing the materials and strategies used to complete the research.

   The significance of the methodology section is to provide the reader with enough insight into how the research was performed so that they could carry out the task themselves.

   There are many components that are addressed within a methodology including:

   - Control
   - Independent and dependent variables
   - Standardisation
   - Replication
   - Randomisation

   These elements are important to enhance the reliability, validity and accuracy of a research study.

   - Reliable (how consistently something is measured during the trial/s or how consistent the information is between sources)
   - Valid (how accurately something is measured or the appropriateness of the information – is the research/er qualified, current, biased?)
   - Accurate test (being both reliable and valid).

Topic 2 continued next page

Activity 2

2. The components of methodology

a. As a group, discuss each of these components of the methodology in a research report. Collaborate to:

- Define the term
- Provide reason/s why each component is significant to an experiment or trial
- Give an example of each component in an experiment

Control:

Suggested information to include:

Definition: A control is an experimental condition (a variable) that is designed to remain constant and unchanged and is used as a reference point to compare against the other experimental conditions.

Significance: The purpose of including a control is to ensure that any changes or effects observed in the experimental group are due to the specific variable being tested and not due to other factors. Controlled variables enhance the reliability and validity of an experiment and help establish a correlational or causal relationship between the variables being tested.

Example: Researchers want to test the effect of a new fertiliser on the growth of tomato plants. Researchers would design the experiment with a control group and an experimental group. The control group would receive the same amount of water, sunlight, humidity, soil type, and other conditions as the experimental group, but without any of the new fertiliser being tested. By comparing the growth of the experimental group to the control group, researchers could determine if any changes in growth are due to the fertiliser being tested or if they are just variations that occur without any fertiliser treatment.

Topic 2 continued next page

Activity 2

Independent and dependent variables:

Suggested information to include:

Definition: Variables are factors or characteristics that can be measured in scientific research. eg. height, temperature, mass, age etc.

The **independent variables** are the factors that researchers manipulate or control in an experiment. (An easy way to remember this is "**i**" for independent variable means "**I** control the independent variable")

The independent variable is the 'cause' in a cause-and-effect relationship.

The **dependent variable** is the 'effect' in a cause-and-effect relationship. Its value depends on changes of the independent variable and cannot be controlled by researchers.

Significance: Variables are used to test hypotheses and to determine the relationships between different factors or conditions. It is important to be able to identify variables to accurately measure the effects of a specific factor on an outcome and draw valid and reliable conclusions.

Example: Researchers want to study the effects of caffeine on heart rate in humans. Caffeine would be the independent variable (the cause). Its value is independent of the other variables in the research study. The heart rate would be the dependent variable (the effect).

Topic 2 continued next page

Standardisation:

Suggested information to include:

Definition: Standardisation is a process used to control for potential sources of variation and ensure that all groups, samples, or participants are treated equally.

Significance: It is important to use the same procedures across all groups, samples, or participants in an experiment. The groups should only differ in the manipulation of the independent variable so that researchers can measure its effect on the dependent variable. By standardising procedures, measurements, and conditions, researchers can minimise the effects of extraneous variables (variables not being investigated, but may affect the results if not controlled for) and increase the validity of their study. Standardisation also helps to ensure that the results of a study can be compared and replicated by other researchers.

Example: Researchers want to investigate the effect of a new feed supplement on the weight gain of cattle. To ensure standardisation in this experiment, you would need to ensure that both the treatment group and control group were housed in identical conditions (amount of shade, access to water, etc) and received the same type and amount of feed, (except that the treatment group would receive the new feed supplement in addition to the regular feed). The weight of both groups of cattle would also need to be measured using exactly the same methods to be confident that any differences in weight gain between the treatment and control groups were due to the feed supplement rather than other factors such as the breed, age, range, location etc.

Topic 2 continued next page

Replication:

Suggested information to include:

Definition: Replication is the process of repeating an experiment or entire study to test the validity and reliability of the results. Replication can also occur within a research study if a large, statistically significant number of repeats are used.

Significance: Replication is an essential aspect of scientific research, as it helps to improve accuracy and reliability.

Example: Researchers want to investigate how temperature affects the growth of a particular plant species. They set up an experiment where they grow 100 plants (of the same species) under controlled conditions in a greenhouse, with each group of plants exposed to a different temperature. They measure the height and weight of each plant after two weeks and record their findings.

To improve the validity and reliability of their results, researchers replicate the experiment by repeating it, with new plants of the same species, using the same methods. By comparing the results of the original experiment with the results of the replicated experiment, the researchers can determine whether their findings are consistent and reliable, or whether they were specific to the particular set of plants or conditions studied.

Topic 2 continued next page

Randomisation:

Suggested information to include:

Definition: Randomisation is a process used to assign treatments or conditions to different groups or participants in an unpredictable or random way. Random assignment in experimental design with multiple groups helps balance the characteristics of groups so that there are no systematic differences between them.

Significance: Randomisation is used to help prevent potential biases that may arise from a non-random assignment. It helps to minimise the effects of unknown or unmeasured variables that could otherwise confound or skew the results of the study, making it easier to determine the true effects of the variable being studied.

Example: Researchers want to test a new antiparasitic drug for sheep. Researchers should randomly assign each sheep to either the treatment group (receiving the new drug) or the control group (receiving the placebo, or another parasitic drug). Randomisation could be achieved using computer-generated random number software, ensuring each sheep has an equal chance of being assigned to either group. Once the sheep are assigned to their respective groups, the researchers can compare the results of the treatment group to the control group to determine the effects of the new drug. Because the sheep were randomly assigned to the groups, the researchers can be more confident that any differences in results were due to the new drug being tested and not to other factors, such as age, sex, previous health etc., that may have differed between the groups if non-random assignment was used.

Topic 2 continued next page

b. Read the methodology and highlight the main strategies used to conduct the experiment. Annotate the words 'control', 'randomisation', 'replication', 'variables/standardisation', and 'data', where they are referred to.

c.

3 Methodology

3.1 Asparagopsis taxiformis and feedbase

The red seaweed *Asparagopsis taxiformis* in the filamentous gametophyte phase was collected from a site near Humpy Island, Keppel Bay, Qld (23o13'01"S, 150o54'01"E) by MACRO (Center for Macroalgal Resources and Biotechnology) of James Cook University (JCU) in Townsville, QLD. The collected biomass was frozen and stored at -15°C then shipped to Forager Food Co. (Red Hills, TAS), where it was freeze dried to approximately 95% dry matter as the best available method to retain volatile bioactive compounds (Vucko et al. 2016). The dried *Asparagopsis* biomass consisting of 50% OM was milled (2-3 mm) to ensure a uniform product and prior to feeding was incorporated into a high grain total mixed ration (TMR) similar to typical feedlot TMR in Australia. The TMR was manually prepared by CSIRO from pre-formulated ingredients in a steam rolled barley base (Riverina Pty Ltd, Oakey, Qld, AUS). Based on preliminary in vitro testing the originally intended final inclusion levels were designed to deliver the equivalent of 1.0%, 1.5% and 2.0% *Asparagopsis* (OM basis). The mixing system was a custom built horizontal paddle mixer calibrated to mix each batch containing all ingredients for 4 minutes which was confirmed to consistently provide homogenous mixtures (coefficient of variation <8%).

Topic 2 continued next page

The steers were fed *ad libitum* on the steam rolled barley based formulation using the TMR's defined in Table 1 for the four individual *Asparagopsis* inclusion levels (Control, Low, Mid, High). Individual nutrients for the basal TMR as determined by analysis of four compiled composite samples are presented in Table 2. Composites consisted of samples collected weekly, stored at -20^{0}C, subsampled at the end of the project and thoroughly mixed, and analysed by Feed Central Laboratories (Toowoomba, QLD). Individual nutrient, feed additive, and bioactive contents of the respective TMR's used for TMR formulation at the beginning of the treatment period are presented in Table 3. Steers were adapted to the control diet (no *Asparagopsis*) over 45 days by ramping the grain level using starter-split-intermediate-split-final diet steps with slow ramping between stages. At the end of the project all steers had received the final TMR exclusive of *Asparagopsis* for 100 days. Steers were then adapted and adjusted to their final respective inclusion levels of *Asparagopsis* in an exploratory research process over 30 days to characterise the appropriate inclusion range for the experiment. The details of this characterisation process were reported in the Discussion section (5.1.1) because this represents the most significant outcome for discussion. The additive requirements of *Asparagopsis* were confirmed to be dramatically lower than indicated by the preliminary assessments and previous *in vitro* studies using *Asparagopsis*. The final inclusion levels were determined by reversing the seaweed adaptation process and proceeding by placing animals in one of four open circuit respiration chambers of internal volume 23 m^3 for two hour monitoring periods to identify at what inclusion CH_4 was disappearing and from that information created an updated inclusion range of 0.00, 0.05, 0.10, and 0.20% of the TMR's OM content. *Ad libitum* feeding was maintained by daily monitoring of individual as-fed intakes and adding or removing the TMR in increments of 500 g daily as necessary.

Topic 2 continued next page

Activity 2

Prior to beginning inclusion of *Asparagopsis* the steers were weighed for allocation to treatment groups followed by a baseline 24 h respiration chamber session for all steers and immediately following each session the groups of four began adaptation to the seaweed. When the final *Asparagopsis* inclusion levels were achieved the steers remained for 60 days on their respective final levels leading to slaughter. Due to the staggered baseline measurements the steers received *Asparagopsis* for a total of 86-94 days which is reflective of six groups of steers in the queue for 24 h respiration chamber sessions in four chambers. Due to logistics, and health and safety regulations, it was not possible to have steers in chambers overnight on weekends. The project adhered as closely as possible to a 90 day finishing period that began with adaptation to inclusion of *Asparagopsis*, however given logistics, chamber session scheduling, and JBS Townsville requirements of delivery on a Monday (16th October) there was the variation from the first to last group of eight days on *Asparagopsis* but only varied by two days on the final inclusion levels. However each of the six measurement groups consisted of a representative from each treatment group therefore for each treatment the average days since beginning of adaptation to the seaweed was c. 90 days. All steers finished their dietary treatment with *Asparagopsis* on the day following the final chamber session to ensure they went to slaughter with an equivalent withholding period (2 days).

Table 1: Ingredient composition of the total mixed ration for each *Asparagopsis* inclusion level

Ingredient As Fed-Basis, %	Control	0.05% OM (Low)	0.10% OM (Mid)	0.20% OM (High)
Ground *Asparagopsis*	nil	0.09	0.18	0.36
Rhodes Grass Hay	8.00	8.00	8.00	8.00
Steam rolled barley	70.8	70.7	70.6	70.4
Limestone ($CaCO_3$)	1.00	1.00	1.00	1.00
Vegetable Oil	3.20	3.20	3.20	3.20
Whole Cottonseed	9.00	9.00	9.00	9.00
Molasses Vit./mineral Blend	8.00	8.00	8.00	8.00

Topic 2 continued next page

Activity 2

Table 2: Nutrient composition of the basal total mixed ration as analysed on four composite samples compiled from series samples collected weekly throughout the project.

	Avg
Rhodes grass hay (% DM)	80.6
Rhodes grass hay NDF (% DM)	72.3
Rhodes grass hay ADF (% DM)	46.9
TMR Dry Matter (% DM as fed)	88.5
Ash (% DM)	8.57
TDN (% DM)	71.9
Organic Matter (% DM)	92.3
ME (Mcal/kg DM)	2.96
NEm (Mcal/kg DM)	1.74
NEg (Mcal/kg DM)	1.12
Starch (% DM)	23.2
Fat (% DM)	8.33
NDF (% DM)	30.6
CP (% DM)	15.5
DIP (% DM)	7.89
Ca (% DM)	0.60
P (% DM)	0.35
Mg % DM)	0.23
K (% DM)	0.68

Note table 3 omitted.

3.2 Experimental design

The Brahman-Angus cross (Brangus) steers were maintained at the CSIRO Lansdown Research Station in Townsville according to current guidelines of the Australian code for the care and use of animals for scientific purposes (NHMRC 2013) and approved by the local animal ethics committee on permit A10/2015. After adaptation to the TMR and the day before the first baseline respiration chamber sessions 28 Brangus steers (24 experimental, 4 spares) were weighed and allocated to four blocks of seven individuals based on ascending live weight (LW). From the LW blocks individuals were randomly allocated to the four treatment groups of six steers each and a spare group of four in a randomised incomplete block design. The initial LW average was 477 kg and the initial LW's and variation of treatment groups are described in Table 4. The baseline chamber sessions were completed as experiment schedule Day 0 (Fig. 1) which was immediately prior to beginning the *Asparagopsis* treatment period on Day 1.

Topic 2 continued next page

Although the project was largely completed according to the above design with 6 steers per group the final data analysis was confined to 5 animals per treatment group due to a few individuals that had to be removed from the project or excluded from data analysis. There was some incidences injury, illness, and repeated poor feed intake during the measurement sessions and residence in respiration chambers. Although most problematic steers were members of the Control and Low *Asparagopsis* inclusion groups the steers per group was reduced by one after allocation of applicable spares and all treatment groups were balanced at n=5.

Original planned treatment groups:
1) Control; high grain TMR (without *Asparagopsis*);
2) Low - High grain TMR with 1.0% *Asparagopsis* (OM basis);
3) Mid - High grain TMR with 1.5% *Asparagopsis* (OM basis);
4) High - High grain TMR with 2.0% *Asparagopsis* (OM basis).

The original planned treatment groups were designed based on in vitro demonstrated response with a Rhodes grass substrate (Kinley et al. 2016a). This treatment set was initiated during the adaptation period, however the response in vivo when included in the feedlot ration was demonstrated to be greater than the *in vitro* studies and thus a replacement treatment set was formulated based on observations during adaptation to *Asparagopsis* inclusion as follows:

Final treatment groups:
1) Control; high grain TMR (without *Asparagopsis*);
2) Low - High grain TMR with 0.05% *Asparagopsis* (OM basis);
3) Mid - High grain TMR with 0.10% *Asparagopsis* (OM basis);
4) High - High grain TMR with 0.20% *Asparagopsis* (OM basis).

On arrival to Lansdown RS on 16-06-2015 the Brangus steers at average weight 186 kg were segregated in a paddock with access to Rhodes grass hay and clean water. They had already been vaccinated with Zoetis Ultravac® 5 in 1 for defense against clostridial diseases. They remained together in a separate paddock and on 27-02-2017 commenced periodic training for residence in pens and chambers. The steers were treated with pour-on Elanco Demize™ for defense against buffalo fly on 20-06-2017 and pour-on Zoetis CattleGuard® an endectocide for defense against parasites on 18- 07-2017. Their National Livestock Identification System (NLIS) devices were recorded and they were ear tagged with CSIRO identification devices and weighed. The steers were not implanted with hormone growth promotants (HGP). This was avoided primarily to eliminate possibility of infection at the implant site because there was limited trained cattle in the correct weight range therefore the risk and implications of losing cattle to infection was too high. Also, without HGP there was increased ability to attribute any differences in growth rate to *Asparagopsis* treatment and remove possibility of HGP as a confounding factor. The project required significant animal handling and movement between pens and respiration chambers, therefore occasionally during their paddock residence, and intensively approaching adaptation to the diet, they received training for handling and confinement in individual pens and respiration chambers.

Topic 2 continued next page

This training continued throughout the project to limit stress and maximise feed intake while residing in the pens and respiration chambers. All feed formulations of control and those containing *Asparagopsis* were fed *ad lib*, in one daily feeding. Throughout the feed adaptation and treatment periods steers were maintained in sheltered and shaded individual pens. The pens were located adjacent to the animal house where the respiration chambers were located. The pens had dimensions of 2.14 m × 4.25 m (9.1 m^2). Throughout the 90 day treatment period all animals from each treatment group were rotated at approximately 14 d intervals through individual pens and open circuit respiration chambers (1 d) to determine daily individual feed dry matter intakes (DMI) and CH_4 and H_2 emissions. Animals were grouped in fours with one from each *Asparagopsis* treatment group of similar weight allocated to one of the four open circuit respiration chambers. In this way all treatment groups were represented every session, and all steers were in every chamber at least once in the treatment period. This routine provided five independent measures of CH_4 production for each animal and allowed determination of efficacy of each of the three inclusion levels of *Asparagopsis* over time.

The timeline in Fig. 1 is an illustrative description of how the steers were progressed through adaptation to the feedlot diet and *Asparagopsis*, and then monitored for CH_4 and H_2 emissions throughout the finishing period. Full adaptation with ramping grain content in the diet had the duration of 45 days when the final TMR was achieved. This was straightaway followed by the baseline respiration chamber session. Following the baseline sessions (0 d) the steers began to be ramped in receiving their adaptation to *Asparagopsis* which as described in detail in the discussion section, and briefly above, required modification due to the unexpected heightened efficacy of *Asparagopsis*. On this discovery the steers were rolled back in inclusion and an exploratory research process of determination of effective level of *Asparagopsis* as a feed additive in the feedlot TMR was initiated. Thus each animal was placed in a respiration chamber for 2 hours on a rotating bases until the inclusion correction process was close to refinement, then a 24 h chamber session was completed at 25 days following which final treatment levels were set for initiation at 30 days of *Asparagopsis* adaptation. In this way it was determined that CH_4 was eliminated at 0.20% of OM and the levels of 0.05 and 0.10% were also assigned to provide for construct of an inclusion level response curve. Although the final inclusion range was provided for 55 days leading up to their final chamber session the steers were receiving *Asparagopsis* for a total of 86-94 days due to the staggered baseline chamber sessions. The steers began their final regime after 30 days adaptation and thereafter entered 24 hour respiration chamber sessions fortnightly. Thus each steer had a total of five 24 hour chamber sessions preceding slaughter, 55 days on treatment to their final chamber session, and 60 days receiving their respective final inclusion levels of *Asparagopsis* prior to slaughter. Note that no rumen samples were collected after the chamber session at 25 days of *Asparagopsis* adaptation.

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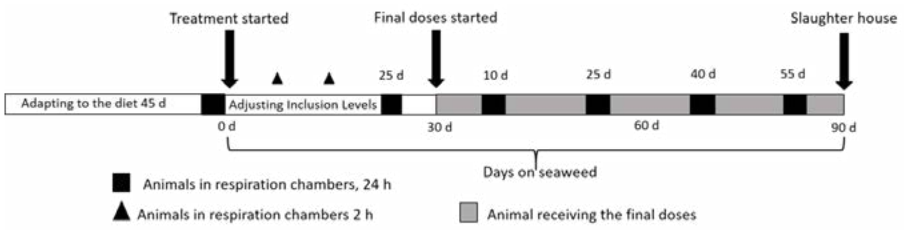

Fig. 1: Experimental timeline including adaption, reformulation (adjusting) of inclusion levels, feeding and treatment periods, respiration chamber sessions, and total days on *Asparagopsis* (seaweed) preceding slaughter.

3.3 Sampling and analysis

3.3.1 Feed analysis

Dry matter content of the TMR was determined on each feed batch by achievement of constant weight at 105°C. Bromoform content of the *Asparagopsis* was determined by the team at MACRO using extraction in MeOH with naphthalene as internal standard and the extract analysed using gas chromatography and mass spectrometry (GC-MS) as described by Vucko et al. (2016) and Paul *et al*. (2006). All other feed analyses were completed on composite samples as described in Section 3.1 through commercial feed analyses services at Feed Central (Toowoomba, QLD). Analysis of *Asparagopsis* was completed on two representative composite samples consisting of sixty subsamples each by Symbio Laboratories (Brisbane, QLD).

3.3.2 Methane and hydrogen emissions

Methane and H_2 emissions were measured for all treatment groups using the four respiration chambers at Lansdown Research Station near Townsville, Qld, AUS. Five chamber session periods were performed consisting of seven cycles using the four chambers (total 28 steers) with one steer from each of the four treatment groups in each of the cycles (including the spare steers). Prior to commencement of the measurement phases of the project all participating steers were trained in the chambers over two months by gradually adapting them to increasing periods of residence in the chambers so that all steers had several experiences in the chambers for the 24 h duration prior to commencement of the *Asparagopsis* dietary inclusions and feedlot finishing period. Animal gas production values have accounted for ambient air contribution and chamber air exchange rates.

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Martinez-Fernandez et al. (2016) and Charmley et al. (2016) described in detail the technical parameters of the Lansdown RS respiration chambers used for emissions measurements from the steers in this project. Their pertinent descriptions are interpreted here to be specific to the experimental design and protocols of this project. Four open-circuit respiration chambers were used with CH_4 and H_2 emissions collected over 24 h. Dimensions of the chambers was 4.0 m × 2.4 m × 2.4 m for 23 m³ internal volume and was constructed of a galvanised steel frame with 4.5 mm clear polycarbonate attached and sealed providing full visibility, for and between, each animal. Each chamber was equipped with a water trough and feed bin containing the daily ration. A modified squeeze crush within each chamber defined a confinement area that accommodated cattle of different sizes. Each chamber was fitted with a door (1050 X 2100 mm) at either end for entry and exit of the animal, and to prevent suffocation the doors would open automatically in the event of power failure. Animals were fed at *ad libitum* levels established before each chamber session. Measurements were taken over 24 h for CH_4 and H_2 production. Fantech TD800/200N fans (Melbourne, Vic, AUS) provided air from external to the animal house building, and chamber exhaust was vented through the roof line. The inline fans were fitted with variable speed controllers which maintained the flow rate of 3000 L/min through a 250 mm duct and a slight negative pressure was maintained within each chamber. Relative humidity and temperature (HMT 330, Vaisala, Melbourne, Vic, AUS) and pressure (QBM75-1U/C, Siemens, Zurich, CHE) sensors installed in each chamber permitted air flow to be corrected to standard temperature and pressure. Exact flow rates were corrected to measured conditions for temperature and pressure for each individual chamber and were used in calculations for CH_4 and H_2 production (Takahashi et al. 1999; Williams et al. 2007). Air flow was measured on the exhaust with thermal flow sensors (SS20.500 SCHMIDT® Flow sensor, St Georgen, DEU). The atmosphere inside the chambers was maintained at 2°C below ambient temperature, atmospheric pressure of approximately -10 Pa, and relative humidity in the range of 50 to 75% throughout the 24 h residence sessions. Air sampling for gas analysis was drawn from a point in the exhaust duct through polyurethane tubing at 4.5 L/min, using a micro diaphragm pump (SW & WS Burrage, Ashford, Kent, GBR) located between a multiport gas-switching unit and membrane drier (Perma Pure LLC, Toms River, NJ, USA). Following particulate filtering and dehumidifying using a four pot refrigerated drier (AF30-02, SMC Pneumatics Australia, Sydney, NSW, AUS), air samples entered the multiport gas switching unit that sampled each chamber and two outside air ports. Air samples then passed through the membrane drier and through independent rotameters before analysis for CH_4 (Servomex 4100, Servomex Group Ltd, Egham, Surrey, GBR) and H_2 (Servomex Chroma). Data for flow rate, temperature and chamber pressure, and CH_4 and H_2 content of the exhaust air for the final 315 s of each sampling event was used to calculate CH_4 and H_2 flux. Sampling events, internal monitoring of chamber conditions and data management were handled by Innotech® processors (Genesis II, Innotech®, Brisbane, Qld, AUS) using digital I/O at 4-20 mA. All data were compiled in a dedicated computer by using a structured query language database. System recoveries were assessed by releasing CH_4 (99.9% purity) at known rates (g/min) and regressed against chamber readings between each experimental period."

Topic 2 continued next page

3.3.3 Feed intake

All animals (including spares) were maintained in individual pens throughout the study from beginning of adaptation to the high grain feed and through to the final sessions in respiration chambers at the conclusion of the c. 90 day *Asparagopsis* treatment period. Each day prior to feeding the intake was determined by measurement of difference between offered feed and feed remaining after 24 h. From this value the feed offered each day at 09:30 for the upcoming 24 h was adjusted based on consistency over several days and either offering more or reducing in 500 g increments (as fed). The intakes were recorded throughout the study to determine the individual and treatment group DMI's during the measurement and supplementation period as impacted by *Asparagopsis* inclusion in the diet. These values were used to express variables on a per kg DMI basis.

3.3.4 Body weight, average daily weight gain and feed conversion ratio

Following along with the measurement of individual and treatment group intakes the respective weight gains were monitored and recorded every week. Animal weights were consistently measured every seven days (Friday) at c. 08:30 prior to the daily feeding. This provided for tracking and demonstration of effect of *Asparagopsis* levels in our inclusion range on ADWG between individuals and treatment groups during the supplementation period. The feed conversion ratio (FCR) was calculated as follows: feed intake:weight gain, therefore a lower FCR indicates greater kg weight gain per kg intake.

3.3.5 Volatile fatty acids production

Volatile fatty acids were measured in rumen fluid collected by stomach tubing the steers on the day of exit from the respiration chambers and the VFA's were quantified for acetate, propionate, nbutyrate, iso-butyrate, iso-valerate and n-valerate. Protocols were as described by Kinley et al. (2016a) and Gagen et al. (2014). The preparation of rumen fluid for VFA analysis was at a ratio of 4 mL of rumen fluid to 1.0 mL of fresh 20% metaphosphoric acid and stored at -20°C. When thawed for analysis the mixture was vortexed and a 1.5-mL subsample was centrifuged for 15 min at 13,500 g and 4°C (Labnet Prism R; Edison, NJ, USA). Then 0.50 mL of clear supernatant was extracted by pipette, spiked with 0.05 mL of 11 mM 4-methylvaleric acid (Sigma-Aldrich; Castle Hill, NSW, Australia) as internal standard then analysed using a Shimadzu GC-2010 equipped with a Restek Stabilwax (30 m × 0.25 mm × 0.25 mm) fused silica column and flame ionisation detector. The column was ramped from 90°C to 155°C at 3°C/min and held for 8.3 min. The temperature was 220°C in the injector and 250°C in the FID. Ultra high purity N_2 was the carrier gas at 1.5 mL/min and the injection was 1.0 mL. Total VFA's (TVFA) were calculated as the cumulative mM of the above listed VFA species and the individual species were reported based on their contribution (%) to the TVFA.

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3.3.6 Ammonia production

Ammonia concentration was measured in rumen fluid that was collected by stomach tubing each steer on the day of exit from the respiration chambers. The rumen fluid for NH_3-N analysis was prepared at a ratio of 4 mL of rumen fluid to 1.0 mL of fresh 25% metaphosphoric acid, snap frozen with dry ice and stored at -20 °C. The NH_3-N concentration was determined by the colorimetric method described by Chaney and Marbach (1962). The rumen fluid was thawed overnight at 4°C, vortexed and a 2 mL portion was centrifuged for 20 min at 12,000 g and 4°C. Then 0.04 mL of supernatant was extracted from the centrifuge vial and added to 2.5 mL of phenol reagent dispensed into 5 mL Eppendorf tubes. Then 2.0 mL of alkaline hypochlorite reagent was added to each tube and vortexed. The mixtures were then incubated in a 37°C water bath for 10 mins for reaction and color development. After incubation 0.30 mL from each tube was then dispensed into wells of a microtiter plate (Thermo Scientific Nunclon Sterile 96 well plates with lids). Once completed the plate was transferred into a plate reader and analysis performed using a SpectraMax Plus 384 Spectrophotometer (Molecular Devices LLC, San Jose, CA, USA). The analysis data was recorded and managed with Soft Max Pro software.

3.3.7 Carcass characteristics

All the steers from all treatment groups were slaughtered using commercial best practice at JBS Australia an export-accredited abattoir in Townsville, Qld. After slaughter carcasses were hung and prepared as per JBS standards and chilled and automatically maintained at less than 7°C for 24 h by a thermostat controller with a probe inserted in a carcass. Observing slaughter and carcass preparation were MLA-MSA professional graders onsite for assessment and grading of the hot and chilled carcasses. The Carcass characteristics of hot carcass weight (kg), fat thickness at the rump (P8; mm), fat thickness at the ribs (mm), ribeye muscle area size (EMA; cm^2), pH, and MSA scores (including meat and fat colour, marbling and ossification) were recorded for each animal. Grading was completed as described in the MLA Cattle Assessment Manual (MLA 2017a).

3.3.8 Meat eating quality

From every carcass, one LD (*M. Longissimus dorsi*) was boned-out after 24 h chilling at less than 7°C and separated into cranial and caudal portions. Under management of MSA officials caudal portions were vacuum packed and chilled to –0.5°C over a 5 h period. All samples were aged for 7 days prior to freezing and stored at –20°C until sensory evaluation was completed. Caudal portions of the LD was used for consumer sensory evaluation of flavour, tenderness, juiciness and overall acceptance to generate clipped MQ4 scores (clipped = 2 highs and 2 lows removed). Objective measures of beef quality; color, peak force (PF) and initial yield (IY), of cooked samples were conducted on the cranial portions of each LD.

Topic 2 continued next page

The subjective testing was completed according to the MSA Grill protocol with the *Asparagopsis* project samples nested within other variable quality meat samples from different animals and cuts to ensure each consumer received a range. The MSA protocols were based on previous work in development of MSA standard protocols of consumer assessment as described by Thompson et al. (2005) and Watson et al. (2008). Every sample was tasted by ten consumers and then the two highest and two lowest responses were removed and the mean of the remaining six consumer scores produced the clipped scores which eliminates any outlier effect. The MQ4 score is considered the best overall assessment and is formulated as:

$$MQ4 = (\text{Tenderness} \times 0.3) + (\text{flav} \times 0.3) + (\text{Overall} \times 0.3) + (\text{Juicyness} \times 0.1)$$

All consumers were served a mid-quality starter steak in first position followed by 6 test samples with the test meat samples (including the *Asparagopsis* Control, Low, Mid and High) allocated via a 6 × 6 Latin square to ensure each was served equally before and after each other product and equally in each order. The five steaks within each sample (steer) were served in five different positions with each allocated to a sub set of twelve consumers across the sixty samples.

3.3.9 Residues of bromoform

Samples of depot fat (brisket) and kidney were collected at the JBS processing line immediately after the kill room as the hot carcasses came through and entrails were being sorted. After 24 h of chilling at less than 7°C the meat samples (*M. Longissimus dorsi*) were collected from all carcasses in each treatment group. Samples were placed on dry ice and transferred to -80°C storage until residue analysis for bromoform by the National Measurement Institute (NMI), Melbourne, VIC. Analysis was in accordance with the Australian Pesticides and Veterinary Medicines Authority (APVMA) guidelines. Samples of fat, kidney, and meat from two randomly selected steers representing the treatment groups were sent on dry ice to NMI for analysis using APVMA approved NMI method VL 234 consisting of purge and trap gas chromatography/mass spectrometry (GC/MS). In this method whole samples were homogenised and then sub-samples of 5.0 g (+/- 0.10 g) were analysed. Samples were incubated at 100 degrees for 15 minutes in headspace sample vials with an internal standard added into each sample. The generated vapour was then analysed by GC/MS according to specifics of the instrumentation operated by NMI (operational parameters not released by NMI). This method had a limit of detection of 0.05 mg/kg of bromoform in the respective samples.

Topic 2 continued next page

Activity 2

c. Mark the respective column in the table below if it's possible to identify the component of methodology in the study. Complete the summary table based on collaborative discussion.

Component	Identified
Control	✓
Replication	✓
Standardisation	✓
Randomisation	✓

d. As a class, discuss why the methodology of the study was appropriate.

e. All research must be designed and conducted responsibly and ethically. Research involving animals must include evidence in the form of a statement identifying the institutional and/or licensing committee approving the experiments. Locate the statement referring to animal ethics and fill in the missing words.

The Brangus steers in this project were maintained at the [CSIRO] [Lansdown] Research Station in Townsville according to current guidelines of the [Australian] code for the [care] and [use] of [animals] for scientific . purposes (NHMRC 2013) and approved by the local [animal] [ethics] committee with permit number [A10/2015].

Topic 2 continued next page

Activity 2

f. As a group, discuss why animal ethics and welfare is crucial for all research studies involving animals, including this one. Note some key points.

Suggested points to include:

- Animals used in research studies deserve to be treated with respect and care. Having a legislated requirement for animal ethics in research ensures that animals are treated ethically and that their welfare is a priority.
- Animal welfare is a significant concern for many people, and public perception can affect the funding and acceptance of research studies. Ethical treatment of animals in research can help to maintain public support for research.
- Studies can be more valid if animals are treated in a humane way. If animals are mistreated or subjected to unnecessary suffering, it can compromise the scientific validity of the study.
- Ethical considerations in animal research can also promote the development and use of alternative methods, such as computer simulations and modelling which can reduce the need for using animals while still producing valid scientific results.

(National Health and Medical Research Council, 2018)

Topic 2 continued next page

Activity 2

3. The Control

a. Outline how this component of methodology is addressed in the research report and use examples/quotes to support the answer.

Control
Examples
• The rations fed to the steers were controlled by collecting the *Asparagopsis* from the same area, storing and preparing it in the same way and then incorporating it into a high grain total mixed ration which was then fed *ad libitum* to the steers. 'The red seaweed *Asparagopsis taxiformis* in the filamentous gametophyte phase was collected from the same site, frozen and stored at -15°C then shipped to Forager Food Co. (Red Hills, TAS), where it was freeze dried to approximately 95% dry matter' • One group of five Brangus steers was designated as a control group and fed a high grain total mixed ration (TMR) consisting of no *Asparagopsis* for 90 days. • The other three groups of five steers had differing levels of *Asparagopsis* added to their TMR (as a percentage of organic matter), 0.05%, 0.10%, and 0.20% *Asparagopsis* was added to their TMR. • The three treatment groups consuming *Asparagopsis* were compared to the control group to determine their weight gain, daily individual feed dry matter intake, CH_4 and H_2 emissions. 'Throughout the 90 day treatment period, all animals from each treatment group were rotated at approximately 14 d intervals through individual pens and open circuit respiration chambers (1 d) to determine daily individual feed dry matter intakes (DMI) and CH_4 and H_2 emissions.' • All steers finished their dietary treatment with *Asparagopsis* on the day following the final chamber session to ensure they went to slaughter with an equivalent withholding period (2 days). • The three treatment groups were also compared to the control group to determine meat eating quality as well as bromoform residues once slaughtered.

Topic 2 continued next page

4. Standardisation

a. Outline how this component of methodology is addressed in the research report and use examples/quotes to support the answer.

Standardisation
Examples
• The *Asparagopsis* was collected from the same area, stored and prepared in the same way and then incorporated into a high grain total mixed ration which was then fed *ad libitum* to the steers. • Steers were maintained at the CSIRO Lansdown Research Station in Townsville according to current guidelines of the Australian code for the care and use of animals for scientific purposes. • Final treatment groups were set as *Asparagopsis* (as a percentage of organic matter) 0.00, 0.05, 0.10, and 0.20% of the high grain TMR. • All participating steers were trained in the chambers over two months by gradually adapting them to increasing periods of residence in the chambers so that all steers had several experiences in the chambers for the 24 h duration prior to commencement of the *Asparagopsis* dietary inclusions and feedlot finishing period. • All steers from each treatment group were managed on 14 d cycles to allow comparable periods in individual pens and open circuit respiration chambers (24 h) to determine daily individual feed dry matter intake (DMI) and CH_4 and H_2 emissions. • All treatment groups were represented every sesson, and all steers passed through each respiration chamber. • Rumen fluid was collected by stomach tubing the steers on the day of exit from the respiration chambers. • Each steer had a total of five 24 hour chamber sessions preceding slaughter, 55 days on treatment to their final chamber session, and 60 days receiving their respective final inclusion levels of *Asparagopsis* prior to slaughter. • All steers finished their dietary treatment with *Asparagopsis* on the same day following the final chamber session of the last group to ensure they were slaughtered with an equivalent withholding period (2 d). • All the steers from all treatment groups were slaughtered using commercial best practice at JBS Australia an export-accredited abattoir in Townsville, Qld. Carcasses were hung and prepared as per JBS standards. • Meat samples (M. Longissimus dorsi) were collected from all carcases in each treatment group. • Every cooked meat sample was tasted by ten consumers who ranked the eating experience for tenderness, juiciness, flavour and overall acceptance.

Topic 2 continued next page

Activity 2

The study satisfied the element of standardisation (conditions/variables that do not change) in the trial.

- Twenty Brahman-Angus cross (Brangus) steers were involved in the study.
- Steers were randomly allocated into four groups of five.
- All groups were fed the same total mixed ration (TMR) for 90 days. The only difference between the groups was the amount of *Asparagopsis* in the ration. One group was designated a control group and fed a TMR consisting of no *Asparagopsis*. The other groups had differing levels of *Asparagopsis* added to their TMR (as a percentage of organic matter). Low (0.05%), Mid (0.10%), and High (0.20%) *Asparagopsis* was added to their TMR.
- All groups were fed the total mixed ration ad libitum, meaning that the steers had access to as much feed as desired.
- All groups were housed in the same conditions (pen size).
- All groups were managed on 14 day cycles to allow comparable periods in individual pens and open circuit respiration chambers (24 h) to determine daily individual feed dry matter intake (DMI) and CH_4 and H_2 emissions.
- All groups finished their dietary treatment with *Asparagopsis* on the same day following the final chamber session of the last group to ensure they were slaughtered within an equivalent withholding period (2 d).
- Meat samples (M. Longissimus dorsi) were collected from all carcases in each treatment group.

Topic 2 continued next page

5. Replication

a. Outline how this component of methodology is addressed in the research report and use examples/quotes to support the answer.

Replication
Examples
Twenty Brahman-Angus cross (Brangus) steers were maintained at the Commonwealth Industrial and Scientific Research Organisation (CSIRO), Lansdown Research Station in Townsville, QLD, Australia. • All treatment groups were accommodated in each measurement period. • This routine provided a pre-treatment baseline and five independent measures of enteric CH_4 and H_2 production for each steer. • Twenty Brahman-Angus cross (Brangus) steers were involved in the study. • Steers were allocated into four groups of six (only 5 were used for final analysis). • Each steer, prior to the introduction of *Asparagopsis* into their TMR had their enteric CH_4 and H_2 production measured. This provided a pre-treatment baseline and five independent measures of enteric CH_4 and H_2 production for each steer.

Topic 2 continued next page

6. **Randomisation**

   a. Outline how this component of methodology is addressed in the research report and use examples/quotes to support the answer.

Randomisation
Examples
'After adaptation to the TMR and the day before the initial baseline respiration chamber sessions, 28 Brangus steers (24 experimental, 4 spares) were weighed and were randomly allocated to the four treatment groups of six steers each and a spare group of four in a randomised incomplete block design. The study used a randomised incomplete block design (RIBD) to evaluate the effectiveness of *Asparagopsis* supplementation. Cattle were divided into blocks (or groups of six) based on their weight and then randomly assigned to one of four treatments: A control group that received a standard total mixed ration and no *Asparagopsis* supplementation (0.00% (OM), a group that received a Low dose of *Asparagopsis* supplementation (0.05% OM), a group that received a Mid dose of *Asparagopsis* supplementation (0.10% OM), and a group that received a High dose of *Asparagopsis* supplementation (0.20% OM). Randomisation is used to help prevent bias in a study of two or more research groups. The use of a RIBD in this study allowed for the control of potential confounding factors, such as weight and age of the cattle, that could affect the results. This helps to increase the validity of the research study. Randomisation was also used when assessing meat eating quality and testing for residues of bromoform. 'The five steaks within each sample (steer) were served in five different positions with each allocated to a sub set of twelve consumers across the sixty samples.' 'Samples of fat, kidney, and meat from two randomly selected steers representing the treatment groups were sent on dry ice to NMI for analysis'

Topic three: Collection of data for the study

1. **The collection of data for the study** *(Final Report pages 13–19 of 42)*

Researchers need to decide on the type of data recorded and how and when it is recorded. Both qualitative and quantitative data can be collected during a research study.

Collecting quantitative data allows for meaningful analysis and researchers can use this to determine the **statistical significance** of their findings, i.e. the study results didn't happen by chance; they occurred due to the factor/variable of interest.

In this study, a large amount of data was collected for analysis.

a. *Table 1: Examples of data collected* summarises some of the data collected by researchers during the trial. Study the table and answer the questions associated with the collection of data for the study.

Table 1: Examples of data collected.

Data collected	Description of the data	Reason for collection of data
Methane and hydrogen emissions	The average methane and hydrogen emissions (g/kg DMI) were collected for each of the four treatment groups.	To determine whether the H_2 and CH_4 that the cattle emitted differed according to whether the cattle had consumed no (0%), Low (0.05%), Mid (0.10%), or High (0.20%) levels of *Asparagopsis*.
Productivity and feed efficiency	The live weights (LW) were taken for each individual in each of the four treatment groups. Average daily weight gain (ADWG) in kg/day was calculated (n = 5 per treatment group). The mass of the feed consumed was measured for each treatment group and along with the data on weight gain, feed conversion ratios (FCR) were calculated for each treatment group. (Feed intake /weight gain).	Allows for studying how the different inclusion levels of *Asparagopsis taxiformis* at 0.00% (Control), 0.05% (Low), 0.10% (Mid), and 0.20% (High) of organic matter intake affect the weight gain of the Brangus steers. This can be used to determine the productivity and feed efficiency which would affect a producer's profitability.

Topic 3 continued next page

Activity 2

Data collected	Description of the data	Reason for collection of data
Feed intake	The dry matter intake was calculated as the amount of feed consumed per day on a dry (moisture-free) basis. Measured in kg/day.	To determine if the steers consuming differing levels of *Asparagopsis* were eating more or less than the control group.
Volatile fatty acid concentration	The concentration of volatile fatty acids found in the rumen fluid (acetate, propionate, and butyrate) were measured (in mM) and added together to give a total volatile fatty acid concentration (tVFA).	The most important end products of carbohydrate breakdown in the rumen of cattle are volatile fatty acids (VFAs). These acids are the major source of energy (70%) (Moran, 2005). Researchers wished to investigate whether the tVFAs would change in the steer's rumens with differing inclusion levels of *Asparagopsis*.
Ammonia concentration	The concentration of ammonia found in the rumen fluid was measured (in mM).	Monitoring ammonia levels in the rumen of cattle when introducing a new diet is important as excessive ammonia can elevate rumen pH, disrupting the microbial balance and impeding digestion.
Carcass characteristics	Carcasses were graded by Meat Standards Australia (MSA) inspectors.	To determine if the *Asparagopsis* fed to the steers at differing levels made any difference, either positive or negative, to the quality of their meat.
Meat eating quality	Subjective testing was completed according to the MSA Grill protocol to calculate a MQ4 score.	To determine if the *Asparagopsis* fed to the steers at differing levels made any difference, either positive or negative, to the eating quality of their meat.
Residues of bromoform	Meat, kidney, fat, (and faeces) were investigated for any residues of bromoform.	Bromoform was measured to see if it was found within the meat, kidney, or fat collected from steers in this study. It is important to see if this compound is stored in any of the animals' muscles or organs which would be consumed, as it has harmful effects on humans.

b. Recall the data that was collected during the research study.

Students use information from the table to select three examples of data collected using columns one and two.

Topic 3 continued next page

Activity 2

c. Explain the importance of data collection during a research study.

Collecting quantitative data allows for meaningful analysis and can allow researchers to determine the statistical significance of their findings.

d. Name the type of data that was collected during a research study and explain why this information was important to the study.

Students use information from the table to select approximately three examples of data collected.

e. In pairs or as a class, collaborate to rank the data in ***Table 3a: Examples of data collected***, from the most meaningful data to the least important data to address the aim/objectives.

Responses will depend on individual classes.

f. Discuss the following questions.

- What were the reasons for ranking the information in the way you did?
- Was it possible to agree on the most important and least important data?
- Is data only meaningful if it is collected in many ways?
- Why do you think the researchers of this study collected the variety of data they did?

g. Summarise the main points from the above discussion questions.

Responses will depend on individual classes.

Topic four: Presentation of data

1. **The results** *(Final Report pages 19–27 of 42)*

   The results section of a research study is the section that summarises what data was collected and presents the key findings of the study.

   The results section summarises the large amount of data collected during the study and displays this information in a summarised format. Data can be presented using text, tables, graphs, pictures, photos, or spatial data/images.

   The results are presented logically to allow the reader to understand the data collected and the trends and importance of the findings. By using different methods to present data, the reader can better understand information that might be difficult to represent in only a textual format.

2. **Presentation of results**

   The importance of textual presentation is to present a clear and concise description of the data obtained in the study and communicate them to others. The textual presentation also gives the reader context for the results obtained.

   The advantage of using tables to present results is to allow a large amount of data to be displayed clearly in a relatively small space. Tables can enhance data visualisation by presenting data in a clear and concise format. They also allow the reader to compare and contrast the data as multiple datasets can be displayed side by side.

   Graphical representations of data, such as graphs and charts, provide a visual representation of (sometimes complex) information that is easier to understand than tables of data. Graphs can highlight patterns and trends in data and relationships between variables which can help researchers communicate their findings more effectively.

   a. In the research study, the data is presented using text and graphs. Complete the following table, *Table 2: Data presentation* to fill in the missing information or cross out the incorrect words. Discuss whether the presentation of the results is effective in conveying information with a peer and record a Yes or No in the last column.

Topic 4 continued next page

Activity 2

Table 2: Data presentation

Method of data presentation	Final Report Page	Task	Effective way to present results?
Text 4.1.1 Methane and hydrogen emissions	20	As the seaweed inclusion increased the production of CH_4 decreased as g/day and g/kg DMI in linear and quadratic fashion. Compared to the Control group which received no seaweed the reduction in CH_4 emissions as g/kg DMI was apparent for the Low treatment of 0.05% of dietary OM and significant (P=0.008) for Mid treatment of 0.10% and High treatment of 0.20% with reductions of 9 %, 38 %, and 98 % for each of the incremental inclusion levels, respectively. As the seaweed inclusion increased, there was significant increase in H_2 emissions but on a lesser scale where the steers without *Asparagopsis* feed additive emitted almost zero H_2 but which increased both linear and quadratic with increasing *Asparagopsis* feed inclusion. However, the increased H_2 emissions at Low and Mid treatment were not significant and the result was a 17 fold rise which equates to an increase of 1.7 g/kg DMI for the High treatment steers.	YES ✓ NO ☐
Graph Fig 2. 4.1.1 Methane and hydrogen emissions	20	As *Asparagopsis taxiformis* inclusion in Brangus steers consuming a a feedlot (high grain) total mixed ration increases, enteric methane (CH_4) emissions from the Brangus steers decreases significantly. As *Asparagopsis taxiformis* inclusion in Brangus steers consuming a high grain total mixed ration increases, hydrogen (H_2) emissions from the Brangus steers increases.	YES ✓ NO ☐

Topic 4 continued next page

Topic 4 continued

Activity 2

Method of data presentation	Final Report Page	Task	Effective way to present results?
Graph Fig 3. 4.1.2 Feed intake	21–22	Brangus steers consuming different levels of *Asparagopsis* had little effect on their [dry] [matter] [intake] – (DMI, kg/d) during the study. The steers receiving the mid treatment demonstrated [significantly] [greater] DMI than the low treatment steers.	YES ☑ NO ☐
Text 4.1.3 Average daily weight gain	21–22	During the feed additive treatment period with *Asparagopsis* and where seaweed inclusion induced significant reduction in CH_4 (Fig. 2) there was concomitant significant increase in [Average] [Daily] [Weight] [Gain]. Compared to the Control group which received no seaweed there was [no] [difference] in ADWG for the inclusion of 0.05% of feed OM. However, a [significant] [increase] was demonstrated for both the [Mid] treatment (P=0.009) and the [High] treatment (P=0.024) resulting from LW increases of 137 and 130 kg, respectively, compared to 113 kg for the Control. After the full [90 day] treatment period with *Asparagopsis* the increase in ADWG compared to the Control was [26] % and [22] % for the Mid and High inclusion levels, respectively. During the 60 d period of receiving the final inclusion levels there was LW increases of 80 and 75 kg, for the Mid and High groups, respectively, compared to 53 kg for the Control. The [increase] in ADWG compared to the Control during that period was [53] % and [42] % for the Mid and High treatments, respectively.	YES ☑ NO ☐

Topic 4 continued next page

Activity 2

Method of data presentation	Final Report Page	Task	Effective way to present results?
Graph Fig 4. 4.1.3 Average daily weight gain	22	Note: Columns identified with different letters were significantly different at P < 0.05. ADWG is represented by columns and FCR is represented by line points. Average Daily Weight Gain (ADWG) [increased] across all treatment groups. When compared with the Control, the increase in ADWG at Low inclusion level was ~~significantly different~~ / not significantly different. When compared with the Control, the increase in ADWG at Mid inclusion level was significantly different / ~~not significantly different~~. When compared with the Control, the increase in ADWG at High inclusion level was significantly different / ~~not significantly different~~. The FCR [decreased] [slightly] as inclusion levels of *Asparagopsis* increased.	YES ☑ NO ☐
Text 4.1.4 Volatile fatty acids concentration	22–23	There was [no] significant change in rumen fluid tVFA due to inclusion of *Asparagopsis* in the rations of Brangus lofted steers. Acetate tended to be [reduced] compared to the Control group and propionate tended to [increase] . The acetate:propionate ratio (A:P) was [significantly] reduced with increasing *Asparagopsis* inclusion in the ration of the steers. Even with tendency for changes in acetate, propionate, and A:P, the butyrate VFA species remained consistent [without] effect from *Asparagopsis*.	YES ☑ NO ☐

Topic 4 continued next page

Activity 2

Method of data presentation	Final Report Page	Task	Effective way to present results?
Graph Fig 5 4.1.4 Volatile fatty acids concentration	22–23	Total volatile fatty acid (tVFA) concentration during the concluding 60 d of the study showed that the tVFA (when compared with the Control), decreased for all inclusion levels of *Asparagopsis*. The decrease in tVFA at all inclusion levels was ~~significantly different~~ / not significantly different.	YES ✓ NO
Text 4.1.6 Carcass characteristics	25	All carcases from this study were graded by Meat Standards Australia (MSA) inspectors. Average grades were of MSA 3-Star so the steers were of excellent quality and not significantly different as determined by professional MSA graders. Compared to steers that did not receive *Asparagopsis* there was no significant difference in the carcasses induced by inclusion of *Asparagopsis* in the feedlot rations of the Brangus steers in this study.	YES ✓ NO
Text 4.1.7 Meat eating quality	26	The presence of *Asparagopsis* in the diet at the treatment levels described in Table 1 did not impact negatively or positively on the meat eating quality.	YES ✓ NO
Text Table 6 4.1.8 Residues of bromoform	26	Residues of bromoform were not detected in any meat, kidney, or fat collected from steers in this study.	YES ✓ NO

Topic five: Analysis of data

1. **Data analysis** *(Final Report pages 19–27 of 42)*

   The results of a research study detail what researchers have found during the investigation. Data can then be analysed in many ways. Researchers seek to find the significance of their results by using different mathematical and statistical tests that are appropriate to their study type. They vary depending on the number of variables, degree of replication available during the study etc. An F test analyses the difference between two data sets, whereas an ANOVA test looks for significance between more than two treatment groups. These are just two of the many tests that can be used for data analysis.

   The results for this study are extensive and many aspects of data collected were analysed. One of the statistical tests that were used was a ***least significant difference (LSD)*** procedure, a two-step testing procedure for pairwise comparisons of several treatment groups (Meier, 2006). The LSD method is used in ANOVA to create confidence intervals for all pairwise differences between means. In this study, the test was run to evaluate the relationships between the four different treatment groups receiving differing levels of *Asparagopsis* in their TMR.

   The main purpose of this section is to show the reader the data collected from the trial in an understandable format. Data analysis allows researchers to determine the statistical significance of their findings, i.e. the study results didn't happen by chance; they occurred due to the factor/variable of interest. The reader should be able to understand the data collected during the study and, after it has been analysed and presented, also understand whether the research question/problem was addressed and answered.

2. **Results**

   a. Divide into groups and allocate the six results sections from the **Results** section of the report to different members/groups.

   b. Scan the assigned section for the most important data that has been analysed and record it on a sticky note (also note how the results have been presented: text, table or graph).

   c. Share your result (data) and record each main point from the other members in the space provided.

Topic 5 continued next page

Results – Member 1 *(Final Report pages 20–21 of 42)*

4.1.1 Methane and hydrogen emissions

The performance of *Asparagopsis* as an inclusion in the feedlot ration at the inclusion levels described in Table 1 and intended for reduction in rumen CH_4 production was exceptional. As the seaweed inclusion increased the production of CH_4 decreased as g/day and g/kg DMI in linear and quadratic fashion. Compared to the Control group which received no seaweed the reduction in CH_4 emissions as g/kg DMI was apparent for the Low treatment of 0.05% of dietary OM and significant (P=0.008) for Mid treatment of 0.10% and High treatment of 0.20% with reductions of 9, 38, and 98% for each of the incremental inclusion levels, respectively (Fig. 2). Conversely, there was significant increase in H_2 emissions but on a lesser scale where the steers without *Asparagopsis* feed additive emitted almost zero H_2 but which increased both linear and quadratic with increasing *Asparagopsis* feed inclusion. However, the increased H_2 emissions at Low and Mid treatment were not significant and the result was a 17 fold rise which equates to an increase of 1.7 g/kg DMI for the High treatment steers.

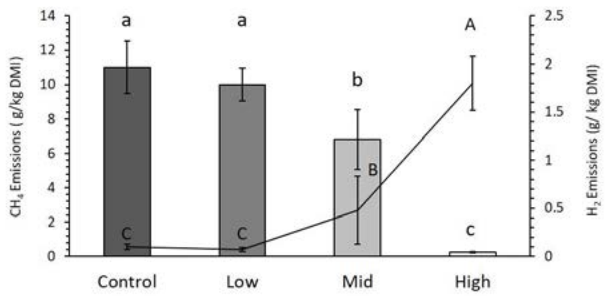

Fig. 2: Methane (CH_4) and hydrogen (H_2) emissions as produced by Brangus steers consuming a feedlot total mixed ration with increasing *Asparagopsis taxiformis* inclusion at the four inclusion levels of 0.00, 0.05, 0.10, and 0.20% of organic matter intake (*n*=5 per treatment group). Columns and line points identified with different letters were significantly different at P<0.05.

The efficacy of *Asparagopsis* may be reduced in scenarios of long term feed inclusion particularly at low levels. This may be a result of variable levels of adaptation to bromoform and is a response that is expected to be more pronounced as the level is reduced. A 90 d treatment period was a moderate duration of exposure and the steers received higher inclusions in the early stage of the exploratory research while defining optimum inclusion levels, and the final levels were maintained for 55 d. The time series response to *Asparagopsis* on CH_4 emissions is presented in Appendix I in Table AI-V as g CH_4/d and in Table AI-VI as g CH_4/kg DMI. Reduction in CH_4 inhibition efficacy during the treatment period was evident for the Low (P=0.002), less so for the Mid (P=0.015) and not evident for the High inclusion level (P=0.159) treated steers.

Topic 5 continued next page

Activity 2

Results – Member 2 *(Final Report pages 20–21 of 42)*

4.1.2 Feed intake

The inclusion of *Asparagopsis* in the feedlot ration of Brangus steers at the levels described in Table 1 had little effect on DMI overall during the treatment period (Fig. 3). However, the steers receiving the mid treatment demonstrated significantly greater DMI than the low treatment steers. Compared to the Control group which received no seaweed the DMI was only marginally lower (10.8%) in the low group receiving *Asparagopsis* at 0.05% of OM, and marginally higher (7.5%) in the Mid level group receiving 0.10%, and was similar to the DMI of the steers receiving the highest inclusion level of 0.20%. There was no significant difference in DMI of steers not receiving *Asparagopsis*, compared to those in the treatment groups and within groups DMI was fairly consistent with relatively small variability as indicated by the SE bars.

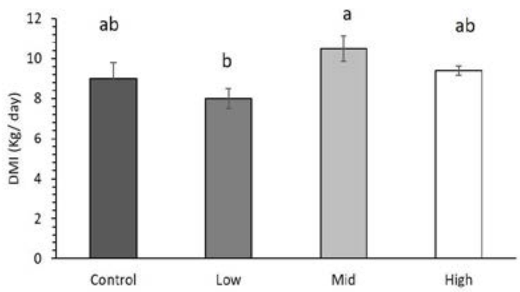

Fig. 3: Dry matter intake (DMI) while in individual pens as measured in Brangus steers consuming a feedlot total mixed ration with increasing *Asparagopsis taxiformis* inclusion at 4 inclusion levels of 0.00, 0.05, 0.10, and 0.20% of organic matter intake (*n*=5 per treatment group). Columns identified with different letters were significantly different at P<0.05.

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4.1.3 Average daily weight gain

The performance of *Asparagopsis* as an inclusion in the feedlot ration at the treatment levels described in Table 1 and hypothesised to improve productivity in the form of ADWG was good (Fig. 4). During the feed additive treatment period with *Asparagopsis* and where seaweed inclusion induced significant reduction in CH_4 (Fig. 2) there was concomitant significant increase in ADWG (Table 4; Fig. 4). Compared to the Control group which received no seaweed there was no difference in ADWG for the inclusion of 0.05% of feed OM. However, a significant increase was demonstrated for both the Mid treatment (P=0.009) and the High treatment (P=0.024) resulting from LW increases of 137 and 130 kg, respectively, compared to 113 kg for the Control. Table 4 shows that after the full 90 d treatment period with *Asparagopsis* the increase in ADWG compared to the Control was 26% and 22% for the Mid and High inclusion levels, respectively. During the 60 d period of receiving the final inclusion levels there was LW increases of 80 and 75 kg, for the Mid and High groups, respectively, compared to 53 kg for the Control. The increase in ADWG compared to the Control during that period was 53% and 42% for the Mid and High treatments, respectively. When examined relative to FCR, the CH_4 reductions depicted in Fig. 2 indicates a significant conservation of feed energy otherwise lost as CH_4, however due to variability the FCR data fails significance and trend (Fig. 4; Table 4).

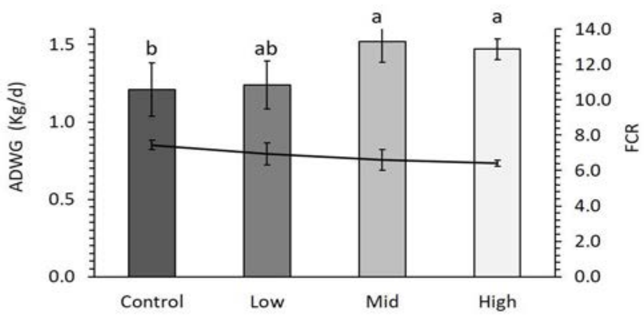

Fig. 4: Changes induced in average daily weight gain (ADWG) and feed conversion ratio (FCR) in Brangus steers consuming a feedlot total mixed ration with increasing *Asparagopsis taxiformis* inclusion at 4 treatment levels of 0.00, 0.05, 0.10, and 0.20% of organic matter intake (*n*=5 per treatment group). Columns identified with different letters were significantly different at P<0.05.

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4.1.4 Volatile fatty acids concentration

At the inclusion levels applied in the present study there was no significant change in rumen fluid TVFA due to inclusion of *Asparagopsis* in the rations of Brangus lotfed steers (Fig. 5). The Low group was equivalent to the Control however a not significant dip in TVFA occurred in the mean concentrations for the Mid steers but this was not observed with the High treatment group.

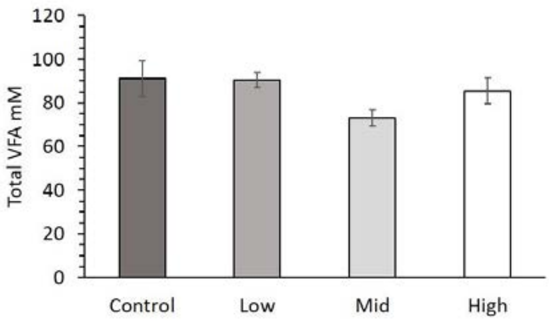

Fig 5: Changes induced in total volatile fatty acid (TVFA) concentration in rumen fluid extracted from Brangus steers consuming a feedlot total mixed ration with *Asparagopsis taxiformis* included at 4 treatment levels of 0.00, 0.05, 0.10, and 0.20% of organic matter intake (*n*=5 per treatment group). Columns are not identified with different letters because they were not significantly different at P<0.05.

The major VFA's in Fig. 6 accounted for 95% of TVFA and tended to be different as induced by with increasing inclusion level. Acetate only marginally failed test for significance (P=0.054) and there was a trend toward reduction in the acetate proportion of the TVFA with decrease compared to the Control group of 4%, 14%, and 20% for the Low, Mid, and High treatment groups, respectively (Fig. 6-A). However, the linear and quadratic contrasts were not significant. Conversely, the propionate proportion tended to increase (Fig. 6-B) as acetate decreased and with P=0.051 (Table 4) it was very close to a significant increase. Nonetheless, the acetate:propionate ratio was significantly reduced with increasing inclusion (Fig. 6-D) indicating a significant linear shift to propionate in TVFA with increasing *Asparagopsis* in the ration of the steers. Even with tendency for changes in acetate, propionate, and A:P, the butyrate VFA species remained consistent without effect from *Asparagopsis* (Fig. 6-C).

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The minor VFA's in Fig. 7 accounted for 5% of TVFA and did not demonstrate alterations induced by *Asparagopsis* with increasing inclusion level. There was no significant changes in isobutyrate (Fig. 7-A), valerate (Fig. 7-B) or isovalerate (Fig. 7-C) even though the graphic representation appears to indicate differences. The valeric species with small concentrations and proportions of TVFA were susceptible to high levels of variability which muted or created illusion of effect induced by *Asparagopsis*. However, isobutyrate remained more consistent in a similar way as its sister species butyrate.

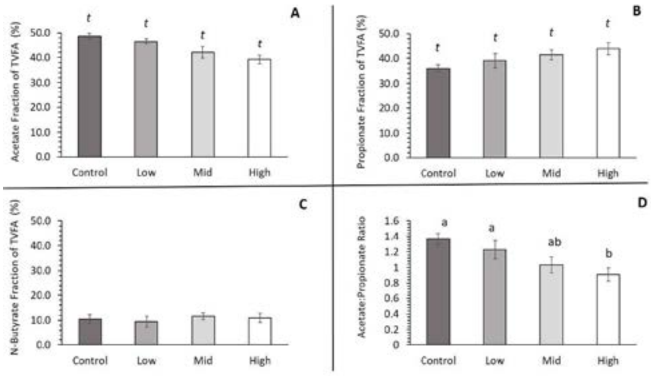

Fig 6: Changes induced in acetate (**A**), propionate (**B**), and butyrate (**C**) the three major volatile fatty acids fractions of the total volatile fatty acids (TVFA) and the acetate to propionate ratio (**D**) in rumen fluid extracted from Brangus steers consuming a feedlot total mixed ration with increasing *Asparagopsis taxiformis* inclusion at 4 treatment levels of 0.00, 0.05, 0.10, and 0.20% of organic matter intake (*n*=5 per treatment group). Columns identified with different letters were significantly different at P<0.05 and those with identified with a '*t*' were different as a trend at P≥0.05 and P<0.10.

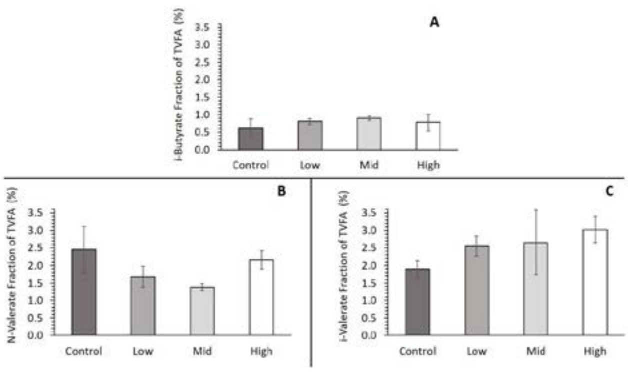

Fig 7: Changes induced in isobutyrate (**A**), valerate (**B**), and isovalerate (**C**) three of the minor volatile fatty acids fractions of the total volatile fatty acids (TVFA) in rumen fluid extracted from Brangus steers consuming a feedlot total mixed ration with increasing *Asparagopsis taxiformis* inclusion at 4 treatment levels of 0.00, 0.05, 0.10, and 0.20% of organic matter intake (*n*=5 per treatment group). Columns are not identified with different letters because they were not significantly different at P<0.05.

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4.1.5 Ammonia concentration

The inclusion of *Asparagopsis* in the feedlot ration of Brangus steers at the levels described in Table 1 had no significant effect on NH_3-N concentration in their rumen fluid (Table 4). In the graphical representation of the response to *Asparagopsis* at increasing inclusion levels there appears to be a significant drop in NH_3-N at the Mid treatment. However, high variability between steers within the treatment groups muted the possibility of significance between groups even though compared to the Control there was a numerical increase of 23% and reduction of 53% for the Low and Mid treatment groups, respectively. The Control and the High group had equivalent NH_3-N concentrations in rumen fluid. It is worthy of note that NH_3-N had greatest variability of the variables monitored in this study.

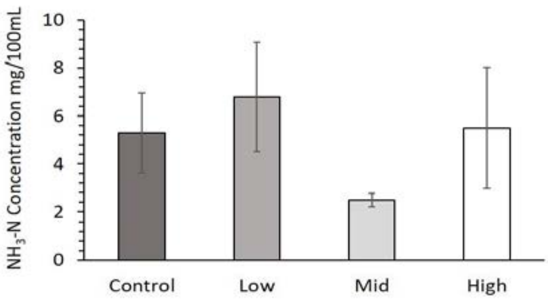

Fig 8: Changes induced in ammonia nitrogen (NH_3-N) concentrations in rumen fluid extracted from Brangus steers consuming a feedlot total mixed ration with increasing *Asparagopsis taxiformis* inclusion at 4 treatment levels of 0.00, 0.05, 0.10, and 0.20% of organic matter intake (*n*=5 per treatment group). Columns are not identified with different letters because they were not significantly different at P<0.05.

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4.1.6 Carcass characteristics

The *Asparagopsis* treatment and Control groups qualified and graded MSA with average grades of MSA 3-Star so the steers were of excellent quality and not significantly different as determined by professional MSA graders. Table 5 shows that compared to steers that did not receive *Asparagopsis* there was no significant difference in the carcasses induced by inclusion of *Asparagopsis* in the feedlot rations of the Brangus steers in this study. The carcass weight means of the treatment groups were consistent between all treatment groups and Control and overall mean carcass weight was 322 kg.

The initial and final LW are shown in Table 4. After completion of the treatment period the LW between treatment groups was higher for the Mid inclusion level steers. There was no significant difference in the final LW between the Control, Low, and High treatment groups, however overall there was a marginally linear higher LW for the treated steers (P=0.034). Using ADWG as a proxy for change in LW during the treatment period, it was evident that the change was significantly more for the Mid and High *Asparagopsis* treated steers (P=0.010). With only the Mid group having higher final LW the carcass weights for treated steers were not significantly different from the control group. The Mid group had the apparent heaviest carcass weights but was not significantly higher, however only marginally failed to achieve a trend (P=0.133). These irregularities lead to the conclusion of the need for caution on interpretation of the results as evidence or confirmation of enhanced ADWG induced by *Asparagopsis*.

There was no difference in average dressing percentage with variation less than 0.5% between treatment groups. With deposition of rump fat (P8) there was more variation between group means but the High treatment group was analogous to the Control group and differed by only 1.5%. As well, there was consistency in the size of the rib-eye muscle area (EMA) at an overall average size of 72.5 cm^2. Numerically the High group developed the largest mean EMA of 75.8 cm^2 which was not significant but 6.5% larger than the Control group. Rib fat depth appears on first glance to be higher for steers receiving *Asparagopsis* but the difference of 53% was muted by internal variability of the treatment groups and Control and thus was not significant but was approaching a trend (P=0.128).

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Table 5: Treatment (Control, Low, Mid and High) effects on carcass weight, P8, EMA and rib fat of Brangus steers treated for 55 days at their maximum respective inclusion of *Asparagopsis taxiformis*.

						P-value	
	Control	Low	Mid	High	SE	Treatment	Contrast
Number of steers (*n*)	5	5	5	5			
Carcass weight (kg)	320.5	321.3	324.2	320.2	3.168	0.133	*n/s*
Dressing %	53.7	53.8	53.6	53.2	0.143	0.315	*n/s*
P8 (mm)	13.6	15.6	16.2	13.4	0.922	0.258	*n/s*
EMA (cm^2)	71.2	71.0	72.0	75.8	1.973	0.611	*n/s*
Rib fat (mm)	6.4	9.8	9.0	9.8	0.611	0.128	*n/s*

SE: Standard error.

[a–c]Within a row treatment means without a common superscript differ, P < 0.05.

Contrast: Significant (P <0.05) linear (*L*) or quadratic (*Q*) effects of the response to incremental inclusion of seaweed estimated by polynomial contrast or *n/s* for not significant.

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Results – Member 6 *(Final Report pages 26–27 of 42)*

The *Asparagopsis* treatment and Control groups qualified and graded MSA with average grades of MSA 3-Star so all treatment groups were of excellent quality and not different as determined by professional MSA graders and MSA Grill Protocols for consumer sensory evaluations. However, in each of the treatment groups there was two steers that graded premium MSA 4-Star and two steers that marginally failed to MSA grade. Therefore, the presence of *Asparagopsis* in the diet at the treatment levels described in Table 1 did not impact negatively or positively on the meat eating quality. Table 6 shows that compared to steers that did not receive *Asparagopsis* there was no significant difference in meat eating quality and sensory judgement by consumers induced by inclusion of *Asparagopsis* in the feedlot rations of the Brangus steers in this study.

We observed a slight deviation in the scores for meat from steers consuming the Mid level of *Asparagopsis*. Although not significant there was generally lower scores for meat from the Mid inclusion treated steers and particularly for liking of flavor which was less preferred compared to meat from Control group and the Low and High treatment groups. The lowest scores in all categories were for the Mid group steers, however the MQ4 score still graded MSA 3-Star and the noise associated with variability in consumer testing scores and small animal number muted the possibility of contrast significance between treatment groups for any test scores associated with meat eating quality.

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Table 6: Treatment (Control, Low, Mid and High) effects on consumer sensory perception of tenderness, juicyness, flavour, overall liking, satisfaction , and MQ4 scores of meat samples from Brangus steers treated for 55 days at their maximum respective inclusion levelss of *Asparagopsis taxiformis*.

	Control	Low	Mid	High	SE	*P*-value Treatment	*P*-value Contrast
Number of steers (*n*)	5	5	5	5			
Tenderness	59.7	55.3	43.2	56.9	1.662	0.514	*n/s*
Juicyness	65.9	59.2	53.8	61.4	1.951	0.432	*n/s*
Liking of flavour	63.1	59.9	48.1	60.6	1.454	0.423	*n/s*
Overall Liking	60.2	59.6	48.8	57.1	1.723	0.685	*n/s*
Satisfaction	3.10	3.20	3.00	3.13	0.044	0.993	*n/s*
MQ4	61.3	58.3	47.5	59.1	1.457	0.562	*n/s*

SE: Standard error;

[a–c]Within a row treatment means without a common superscript differ, P < 0.05.

Contrast: Significant (P <0.05) linear (*L*) or quadratic (*Q*) effects of the response to incremental inclusion of seaweed estimated by polynomial contrast or *n/s* for not significant.

4.1.8 Residues of bromoform

None of the samples of meat, kidney, or fat collected after slaughter from the Brangus steers in this study were found to contain detectable levels of residual bromoform. The treated steers had received TMR with inclusion of *Asparagopsis* at the levels defined in Table 1 during a 90 day feedlot finishing period. Samples were cryovac packed and snap frozen with dry ice at JBS Australia and stored at -80°C until analysis by NMI. All treatment groups were found to be free of bromoform at the NMI detection level of <0.05 mg/kg.

Table 6: Deposition of bromoform in the meat, fat and kidneys of Brangus steers consuming total mixed rations with inclusion of increasing inclusion levels (Control, Low, Mid and High) of the seaweed *Asparagopsis taxiformis*.

Tissue Type	Bromoform Residue (mg/kg) Control	Low	Mid	High
Number of steers (*n*)	2	2	2	2
Meat (striploin; Longissimus dorsi))	<0.05	<0.05	<0.05	<0.05
Kidney (whole organ)	<0.05	<0.05	<0.05	<0.05
Fat (brisket)	<0.05	<0.05	<0.05	<0.05

<0.05 mg/kg: limit of detection of the NMI analysis methodology

Activity 2

Topic six: Discussion, conclusions and recommendations

1. **Discussion** *(Final Report pages 27–34 of 42)*

   The discussion of a study aims to offer an analysis of the results, providing an understanding of their broader implications. It situates the findings within existing literature, elaborates on their significance, acknowledges potential limitations, and suggests areas for future exploration. This section doesn't just present data but interprets it, connecting the study's outcomes to the wider field of research.

   The significance of the discussion section is to provide a complete picture of the research's relevance. It aids readers in grasping not only the direct results but also their wider implications, ensuring the study's findings are understood in context. Additionally, critiquing and synthesising results showcases the depth of the researcher's understanding and sets the stage for subsequent studies.

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5 Discussion

5.1 Methane and hydrogen emissions

The supplementation of *Asparagosis* in the diets Brangus steers in our simulated feedlot scenario had a profound effect of reducing CH_4 emissions, little effect on feed intake, and significant effect of increasing ADWG during the period of final inclusion levels of *Asparagopsis* supplementation. Clearly the capability of the seaweed is confirmed based on progressing demonstration through successive studies beginning at the in vitro scale (Kinley et al. 2016a) then supplemented to sheep on a legume and grain mixed diet (Li et al. 2018). The efficacy and treatment effect in diets with variable digestibility, or grass based diets including graded roughage and grain contents remains to be demonstrated and represents a knowledge gap in the *Asparagopsis* product development. The project was confounded (in a good way) during the adaptation of the steers to the seaweed. Based on the previous studies (Kinley et al. 2016a; Li et al. 2016) a planned inclusion level range for the seaweed was set to be representative of previous knowledge. Steers were adapted to the base TMR (Control) and then began adaptation to *Asparagopsis* in their respective treatment groups. The timeline of discovery and recovery in inclusion level adaptation is displayed in Fig. 1. Animals started the adaptation to the initial proposed treatments (1.0%, 1.5% and 2.0% of TMR OM) and increased progressively with inclusion into the TMR. After 10 days of treatment when the steers had reached the inclusion level of 0.8% they were placed in respiration chambers for a snapshot look at CH_4 production during 2 hour sessions. Surprisingly no CH_4 was detected in any of the steers receiving *Asparagopsis* (data not shown). However, steers not receiving *Asparagopsis* were emitting CH_4 as expected, therefore as a precaution steers were moved into different chambers and with no change to the observation thus eliminating a chamber malfunction. Based on these results inclusion levels were adjusted by reduction to 0.01%, 0.2% and 0.3%. On day 16, animals were again placed in respiration chambers for 2 h and again there was no detection of CH_4 emitted by any steers receiving *Asparagopsis*. A further reduction was designed to identify the required inclusion range and set at 0.005%, 0.02% and 0.04%. On day 25 of *Asparagopsis* adaptation animals were placed in

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respiration chambers for 24 h and samples collected and no effect on any of the gas and rumen fermentation parameters could be detected (Table AI-I; Appendix I). Based on this lack of response we set the final treatment levels to 0.05%, 0.1% and 0.2%. In spite of the issues with determining appropriate inclusion levels it was demonstrated that levels as low as 1/100 of the highest intended inclusion (2%) had potency to reduce CH_4 below detection in the short term. However, the rumen microbiology began to adapt to these extremely low inclusion and CH_4 emissions began to recover. Remarkably, it was demonstrated that CH_4 can be reduced for a period of time at dietary *Asparagopsis* inclusion lower than 0.02% of OM intake. After a series of inclusion adjustments we confirmed efficacy while reducing levels to c. 1/10 of the original treatment plan representing the single most important outcome of this project. Even with powerful mitigation ability at low inclusion it was observed that very low levels of 0.10% and lower are likely to suffer decreasing efficacy over time (Appendix I: Table AI-V and AI-VI). This was not observed at 0.20% inclusion within the duration and conditions of the present study, however longer term studies are necessary to characterise the consistency of antimethogenic efficacy in extended feed inclusion scenarios of some feedlot systems and typical of dairy feeding systems. It was observed that CH_4 emissions returned to normal very quickly after *Asparagopsis* was removed from the feed and conversely was reduced to zero again very quickly when the seaweed was returned to the feed. The final treatment levels were set at 0.05%, 0.10%, and 0.20% of OM intake and the steers were static at those levels for 60 days to finish the treatment period. Table 4 presents the preslaughter outcomes after the final measurements were collected at the end of the project. Appendix I and Tables AI-I, II, III, IV presents data recorded during the exploratory process (AI-I) and progressive respiration chamber sessions during the final treatment levels period. Each steer returned to the respiration chambers for four sessions (c. fortnightly) while achieving these final inclusion levels. The expectations of the efficacy of *Asparagopsis* in a feedlot diet was dramatically exceeded relative to the response in reduction of CH_4 emissions. This will be reflected in associated dramatic improvements in the cost of the supplement and closing of gaps in the supply chain. There is strong potential that the feed energy otherwise lost as CH_4 could be redirected to more beneficial metabolism and improve the ruminant animal's productivity. The potential for adoption of the technology will likewise be improved. When included in a feedlot diet formulation at 0.20% of OM intake *Asparagopsis* can virtually eliminate CH_4 emissions and at half that inclusion there is still close to 40% reduction (Fig. 2). This has far reaching implications in the search for methods to alleviate the contribution of agriculture, and more specifically livestock, to the global greenhouse gas (GHG) inventory and subsequently climate change. *Asparagopsis* has capability for the red meat industry in providing significant impact toward achieving the MLA goal of carbon neutrality by 2030 (CN2030). An example of other products currently of interest for antimethanogenic potential is one of chemical origin namely 3-nitrooxypropanol (NOP). This product has received large investment in both development and marketing. The study of Vyas et al. (2016) applied NOP in rations of 84 yearling steers over 238 days including backgrounding (138 d) and finishing (105 d) periods with no apparent detriment to the meat

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quality. On DMI bases, and at their highest dose (200 mg/kg DMI), during backgrounding the efficiency of NOP was at 29% reduction in CH_4, and most notably during finishing the reported response was 81%. However, during the finishing phase CH_4 reductions came at the cost of 16% and 10% reductions in DMI and ADWG, respectively. Also, there was no apparent benefit of NOP at their low dose (100 mg/kg DMI) which confounds development of a dose response model. As a natural product and seaweed, *Asparagopsis* stands to be more popular and effective as an agent for environmental benefit. There is a potential chain of value in cultivation and use of the seaweed product. It could create new economies in impoverished regions using low skilled labor while remediating hyper nutrient enriched water and consuming dissolved CO_2 thus fighting ocean acidity. When fed to cattle it would dramatically reduce the GHG contribution from agriculture and there is early indication of potential for increase in livestock productivity. The cause or factor behind the 10 fold reduction in requirement of *Asparagopsis* is unclear and requires exploration and characterisation. There are several possibilities that create differences from the previous studies compared to the present feedlot study. Firstly this was a high grain diet and low in forage content and is thus digested more efficiently than the grass and legumes used in the previous studies where more *Asparagopsis* was required for mitigation of CH_4. Secondly, a molasses based blend containing vitamins, minerals, and more importantly monensin, was included. To elucidate the cause it is necessary to further investigate dietary formulation in a study that incrementally increases the forage component, and reduces the monensin and other components while adjusting the *Asparagopsis* inclusion to maintain CH_4 inhibition. If increasing inclusion of *Asparagopsisis* necessary for effective reduction of CH_4 in any feeding system it will be necessary to carefully monitor potential toxicity and concomitant detrimental effects on animal health. Seaweeds are known to concentrate potentially toxic entities such as heavy metals and minerals. Dry processed *Asparagopsis* used in the present study contained 7945 mg/kg iodine. When combined with the 0.86 mg/kg in the TMR this resulted in delivery of 8.77, 16.69, and 32.51 mg/kg DM of iodine in the TMR for the Low, Mid, and High treatment groups, respectively (Table 3). The maximum tolerable limit (MTL) for cattle and sheep has been reported as 50.0 mg/kg DM (NRC 2005), therefore increasing the High inclusion level by a further 55% to achieve 0.31% of TMR OM will equal the MTL. The manifest symptoms of prolonged exposure to toxic levels of iodine will vary with dose level, exposure duration, animal species and individuals. Symptoms include as examples but not limited to: decreased DMI; coughing; nasal discharge; scaly skin, and in severe cases, pneumonia (Paulikova et al. 2002). Also, iodine can be transferred into milk which has implications for use in dairy cattle and where dietary iodine is very high it may impact calves. This study was not designed to study health effects of iodine and no associated examinations were part of this work, however there was no observed negative health effects from any source during 90 d of dietary inclusion and the steers without *Asparagopsis* appeared to be more problematic than their seaweed treated counter parts. This observation is further elucidated in the Feed Intake discussion. It is beneficial to limit iodine in the TMR and maintain iodine intake at the low end of the safe concentration range (Newton et al. 1974). In the

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development of a commercial supply and subsequent product improvements, the level of iodine in the seaweed may be managed through various production techniques such as: i) limiting iodine uptake through reduced concentration in cultivation media, particularly in tank production; ii) genetic selection and breeding of strains with low iodine accumulation; iii) increasing OM content by removal of excess salt through iodine free rinsing; and iv) genetic selection and breeding of strains with high bromoform accumulation thus further reducing the inclusion level. Also, iodine and other nutrients inherent in the *Asparagopsis* product may no longer be required in the feed formulation when the seaweed is added. The other aspect of emissions monitoring is the increase in H_2 emissions with increasing *Asparagopsis* treatment level (Fig. 2). This means that as CH_4 was reduced the H_2 was increased which explains partially where CH_4 is being diverted. Emissions of H_2 represents a loss of energy and if greatly increased, H_2 pressure in the rumen has potential to reduce intake and impair rumen function thus negatively impacting productivity (Martinez-Fernandez et al. 2017). In a study to track the flow of H_2 when CH_4 emissions are reduced from Brahman steers using chloroform, Martinez-Fernandez et al. (2016) showed that high concentrate diets resulted in higher H_2 emissions compared to diets with more grass-roughage relative to the reduction in CH_4. They demonstrated that a reduction of 58% CH_4 emissions resulted in H_2 emissions of 3.16 g/kg DMI. We demonstrated in this study using *Asparagopsis* that the level of H_2 emissions from steers with 98% CH4 reduction was 1.8 g/kg of DMI which is effectively half the H_2 emissions of Martinez-Fernandez et al. (2016) but with double the CH_4 reduction. Alternatively, Vyas et al. (2016) demonstrated H_2 emissions induced by NOP of approximately 2.0 g/kg DMI which was roughly 11% higher than our High treatment using *Asparagopsis* and 317% higher than our Mid inclusion which reduced CH_4 by 40%. Comparatively, no effect on CH_4 was demonstrated using NOP at 100 mg/kg DMI but H_2 emitted was similar to *Asparagopsis* at the same inclusion (Mid). It is worthy to note that *Asparagopsis* by weight is mostly minerals and other nutrients where NOP is a pure chemical. The bioactive bromoform was 6550 mg/kg of *Asparagopsis* DM. Although the per DMI emissions of H2 relative to CH4 reduction was demonstrated to be much lower using *Asparagopsis* as the antimethanogen compared to raw chemical chloroform this still represents a loss of feed energy. Research has shown that the rumen microbial consortium can be tweaked to favour growth of H_2 utilising bacteria resulting in H_2 diversion to alternative sinks through nutritional stimulation of specific microbial groups (Martinez-Fernandez et al. 2017). This exciting development suggests that the energy lost as H_2 with reduction in CH_4 may also be conserved to some extent and that the negative impact of H_2 accumulation in the rumen under suppression of methanogenesis can be ameliorated by the provision of novel compounds that have nutritional value for the animal when degraded by reductive processes. More research is necessary to explore methods to harness rumen H_2 through use of specific proteins in the feed formulation and enhancement of hydrogenotrophic bacteria in the rumen.

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5.2 Feed intake

In spite of the dramatic reduction in CH_4 emissions there was no detriment to feed intake induced by *Asparagopsis* feed inclusion at the levels utilised in this study. The observed differences were marginal increases in DMI and were for steers demonstrating significant reductions in CH_4 and increases in H_2 emissions. Increased H_2 pressure in the rumen had no apparent detrimental effect which is a concern with CH_4 abatement technologies because it has been demonstrated that concomitant increase in H_2 pressure has the effect of reducing DMI (Martinez-Fernandez et al. 2016). In fact, the most restless treatment group was the Control and these steers were most difficult to handle and had least close to normal DMI in chambers compared to pens (Table 4). This compliments the report that seaweed assists in resistance to stress in livestock (Evans and Critchley 2014) which has been a long standing anecdotal claim by producers adding seaweeds to ruminant diets. The significant difference reported in Table 4 was a result of the Mid treatment steers having a significantly higher DMI than the Low treatment steers. However, the High treatment demonstrated little effect on DMI, and relative to steers that did not receive the seaweed, DMI was marginally but not significantly increased. The treatment groups were fairly well balanced and there was representation from individuals with inherently low and high intakes. This resulted in a clear demonstration that *Asparagopsis* at this inclusion level does not negatively impact DMI (Fig. 3). This study monitored the concomitant H_2 production and there was lower H_2 produced relative to the level of CH_4 reduction described in the Martinez-Fernandez et al. (2016) study. The lower H_2 emissions increase relative to CH_4 decrease suggests redirection of part of the feed energy otherwise lost as CH_4 into more beneficial metabolic use for growth of the steers. This would partially explain the demonstrated ADWG improvements and the shift from acetate to propionate in the steers receiving. In general, intake relative to ADWG is crucial to productivity benefits because productivity improvements and hence profitability can be realised as: (i) increased ADWG without changing DMI; (ii) no change in ADWG with lower DMI; (iii) and optimally, increased ADWG with lower DMI. This has to occur without detriment to meat quality or animal health, and at a price point and systems compatibility that will allow for increased profit.

5.3 Average daily weight gain and feed conversion ratio

The differences in ADWG and FCR observed between steers receiving, and those not receiving, a dietary inclusion of *Asparagopsis* supports the theory of redistribution of the elements and energy conserved with extensive CH_4 inhibition. The rumen management of H_2 would play a role in its capture for beneficial metabolism in the rumen microbiome (Martinez-Fernandez et al. 2016). This offerssome reasoning why steers receiving *Asparagopsis* at 0.10% and 0.20% of dietary OM demonstrated high levels of reduction in CH_4 production (Fig. 2) with concomitant increases in ADWG (Fig. 4; Table 4). In the study of NOP inclusion in a high grain finishing feed formulation it was

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Activity 2

demonstrated that compared to steers with no NOP both DMI and ADWG tended to be reduced (Vyas et al. 2016) in contrast to the increases noted in the present study. However, the Vyas et al. (2016) NOP study utilised group pen feeding and group averaged DMI and ADWG. This group housing likely reduced stress, more closely mimicked a feedlot atmosphere, and likely impacted the DMI and ADWG in a positive way between individuals in each group. This may partially explain why ADWG was higher overall compared to the present study utilising individual pens and DMI measurement. The average ADWG during the treatment period in the present study was 1.36 kg/d compared to 1.47 kg/d in the NOP study. Our steers were also handled more frequently and had more frequent chamber sessions which contributes to stress and a moderately lower average ADWG. It is worthy of note that the Mid and High *Asparagopsis* treated steers had ADWG of 1.50 kg/d thus were marginally higher than the NOP steers which also fits with the weight gain range reported for commercial feedlot steers of 1.1-1.7 kg/d (DAF 2018). Notably this marginally exceeds the 1.4 kg/d ADWG reported by DAF (2018) and did so with lower DMI compared to the typical DMI of 3% of LW reported for Australian lot-fed steers. The level of increase in ADWG and FCR demonstrated in the *Asparagopsis* treated steers is appealing however this requires elucidation. Even though ADWG was demonstrated to be significant the FCR was not significant even with 35% and 37% improvement for the Mid and High does groups, respectively. Although the increases seems very large there is also an underlying large variability between the individuals in each treatment group. The groups were well balanced with representatives across the DMI range but there was much variation in ADWG thus reducing relative significance. This reflects on the prospect of steers achieving this ADWG enhancement or demonstrating it consistently. For example, we started with 24 steers contributing to treatment group data and after data collection throughout the study we included five steers per group in the final statistical analysis due to issues with a few individuals including injury, illness, and repeated poor DMI during respiration chamber sessions. Also, with only five or six steers per group the possibility exists for inadvertent allocation of the best steers to the *Asparagopsis* treatments. In a small study one or two exceptional performers can impact the apparent improvement of the group. For that reason it is imperative that a large commercial scale study be completed to confirm the improvements in ADWG indicated in this study. The lower the number of steers, the lower is the reliability of the ADWG. However this is not the case with CH4 reduction where near elimination of emissions occurs in all steers in the treatment group. Also, benefits of ADWG improvements relative to DMI as described above in the DMI discussion will increase interest in using *Asparagopsis* as a feed additive. When our demonstrated gains in ADWG are confirmed in a commercial environment the potential for adoption of the product will increase substantially.

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5.4 Volatile fatty acids production

The energetic molecules of VFA's have an important role in the productivity of ruminants. A decrease in TVFA is undesirable due the potential for a concomitant impairment of productivity. However, it has been demonstrated with *Asparagopsis* inclusion that VFA are not detrimentally impacted. Kinley et al. (2016a) demonstrated that at in vitro inclusion up to 5% there was little change in TVFA, however in an in vivo study with sheep at inclusion up to 3% the TVFA were seen to reduce somewhat with inclusion level but not relative to time on treatment (Li et al. 2018). In the present study TVFA were quite stable (Fig. 5) within the 10 fold lower inclusion levels compared to the sheep study. It is a universal phenomenon in *Asparagopsis* inclusion studies that as CH_4 emissions are reduced there is concomitant reduction in acetate and increase in propionate as *Asparagopsis* level increases (Machado et al. 2016; Kinley et al. 2016a; Li et al. 2018). Martinez-Fernandez et al. (2016; 2017) also report this phenomenon using chloroform which suggests that this is common trait with CH_4 mitigation in the rumen. Propionate acts an alternative sink for both carbon (C) and H_2 partially explaining CH_4's C and H_2 redistribution. There has been little change in butyrate proportions in rumen fluid in any of the aforementioned studies which leave little to discuss except an increase in butyrate would offer another alternative sink for C and H_2 otherwise lost as CH_4 emissions. Impact on butyrate requires to be further explored in terms of rumen microbial modification as *Asparagopsis* technology progresses. On the other hand, in vitro and in vivo studies using halogenated compounds such as bromochloromethane (BCM) or NOP consistently demonstrate an increase of branched-chain fatty acids when methane was decreased 30% to 50%, which suggests that deamination of amino acids was not negatively affected (Denman et al., 2007; Mitsumori et al., 2012). This effect was not significant in the present study with isobutyrate and isovalerate. However isobutyrate was numerically increased compared to the Control by 29%, 45%, and 26%, as well isovalerate demonstrated numerical increase of 32%, 42%, and 58% for the Low, Mid, and High treatment groups, respectively, but with high variability within treatment groups. Feed is hydrolysed to peptides and amino acids in the rumen with some amino acids degraded into VFA, NH_3 and CO_2. The deamination of some amino acids are in part converted to VFA such as valine which is degraded to isobutyric acid. From this we know that branched-chain acids found in rumen fluid originate from amino acids (McDonald et al. 2011). However, all of the above remains collective to the maximum 90 day *Asparagopsis* feedlot ration inclusion period and further understanding of long term exposure and changes in dietary composition remains to be elucidated.

5.5 Ammonia production

Free ammonia in rumen fluid, typically expressed as NH_3-N, is an important precursor for synthesis of microbial protein and an intermediate molecule in protein degradation of feed in the rumen. Typical concentrations are in the range of 5.0 mg/100 mL of rumen fluid (McDonald 2011) which agrees with

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concentrations measured in the present study. McDonald et al. (2011) explain that the main organisms responsible for branched chain VFA due to amino acid degradation are Prevotella ruminicola, Peptostreptococci species and the various protozoa. The ammonia produced, together with some small peptides and free amino acids, are utilised in the rumen microbial consortium to synthesise microbial proteins which make up the bulk of protein utilised by the ruminant. Some of the microbial protein is degraded during rumen fermentation and the nitrogen is recycled in the system. When the organisms are carried through to the abomasum and small intestine, their cell proteins are digested and absorbed. An important feature of the formation of microbial protein is that bacteria are capable of synthesising indispensable as well as dispensable amino acids, therefore the ruminant can be independent of dietary supply but dietary protein aids in high level productivity. Unfortunately, the measure of NH_3-N in rumen fluid of the steers in the present study does not provide a clear picture of the effect of *Asparagopsis* in the rumen NH_3-N cycle. The analysis of samples indicates some intriguing and unlikely results (outliers) that require, if necessary, confirmation through analysis of secondary (backup) samples collected. The odd results appear a result of analysis or sample disruption as opposed to dietary differences since the oddities are not confined to *Asparagosis* treatment groups. Most of the oddities in NH_3-N values occurred in the samples collected mid-project (Appendix 1) and the final measurements at the end of the treatment period are somewhat more as expected. Overall, the NH_3-N concentrations in rumen fluid appear normal with occasional outliers, however this does not impact the main message of this study which was near elimination of CH_4 and strong potential of good productivity gains.

5.6 Carcass characteristics

The carcass characteristics of the Brangus steers that had received *Asparagopsis* as a feed additive were not significantly different between treatment groups and control and there was little numerical variation with exception of rib fat which was somewhat thicker for the steers receiving the seaweed. Compared to those not receiving *Asparagopsis* the rib fat on steers from both the Low and High groups was 53% thicker and 41% thicker in the Mid group, however variation between individuals in the groups was too high for significant difference. This is a feature that should be observed in a larger group of steers in a commercial scale study. Respective of the traditional measures of carcass characteristics it would be valuable to include data and observations on points of evaluation as can be obtained with comprehensive necroscopy. Li et al. (2018) in a 72 day *Asparagopsis* feed inclusion regime with sheep reported little change in haematological parameters and all animals remained within normal clinical range and there was no impairment to liver function. There was some discoloration, nodular proliferation, and blunting of papillae of the rumen wall in some sheep receiving *Asparagopsis* but no significant lesions were noted. The changes could not be confirmed as a response to the seaweed and it was suspected to be a response to parasitism that would have occurred before the sheep were inducted into the study. As inclusion of *Asparagopsis* in feed of

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livestock progress and duration of exposure becomes extended it will be important to monitor for even unlikely health issues. Long term studies will need to be cognisant of the presence of bromoform even though there has been no evidence of health issues and considering the very low inclusion of *Asparagopsis* that will be included in the rations. Thus more work and observation on carcass, organs, and other features of necroscopy are warranted as long term feeding studies are performed going forward.

5.7 Meat eating quality

Consumers are acutely affected by unreliable meat eating quality and this can cause a shift in consumer purchase trends to alternative products because meat quality affects desirability of meat even more than price. Achieving an MSA grade is reflective of higher quality and hence value at retail for meat products that bear MSA grades which aids producers to achieve consistency and benefit from improvement in consumer meat purchase habits (MLA 2017b). The Brangus steers receiving *Asparagopsis* inclusion in their feed formulations did not demonstrate any detriment to quality compared to those not receiving the seaweed. Also, in all treatment groups some of the steers graded MSA 4-Star for premium quality indicating that at the inclusion levels utilised in this study there was no negative effect on meat quality. Statistical analysis indicates that the Mid treatment steers did not have significantly different meat eating quality however clearly this group was at least slightly different. What could have caused that is not clear. The High treatment steers had the numerical best quality (MQ4) of the treatment groups and the Control group had the highest MQ4 score overall. With the exception of the overall liking score the High group was most similar to the Control group. Overall, there was no significant difference in meat eating quality with inclusion of *Asparagopsis* included in the feedlot feed formulation. There was indication that *Asparagopsis* increased growth rate, therefore as development of a commercial product of *Asparagopsis* continues, the effect on meat quality will be of interest. There exists potential to further improve meat quality particularly in development of next generation products of *Asparagopsis* that may consist of mixtures with other entities that have capability to improve meat eating quality such as other seaweeds. Going forward it is critical that feed additives do not impair meat eating quality in any way and optimally would create a greater consumer acceptance of the meat products. For example, this could be realised from a number of perspectives along with improving MQ4 such as “green” labelling indicating certified production using an environmentally beneficial technology, using a “natural” feed additive as opposed to chemicals or pharmaceuticals.

5.8 Residues of bromoform

The NMI uses an analysis protocol accepted by the APVMA for bromoform residues. At NMI’s limit of dection of <0.05 mg/kg presence of bromoform residues could not be detected in any of the meat,

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kidney, or fat collected from the Brangus steers in this study (receiving or not receiving *Asparagopsis*). This was also the case with meat, kidney, and fat from sheep receiving *Asparagopsis* as a feed additive (Li et al. 2018). The analytical detection limit offered by NMI is half the recommended interim drinking water guidelines of 0.10 mg/L (Cotruvo et al. 1982). In progressing research it should be continued to monitor for bromoform residues routinely and will be an even more important feature if *Asparagopsis* is included in the diets of dairy cattle because they eat more and hence consume more *Asparagopsis* per kg LW and milk is more likely to contain novel compounds of feed origin, the same is true of iodine which can be concentrated in seaweeds (Table 3). There is limited literature that reports on the effects of bromoform in animals and those are based on studies in mice and rats. Condi et al. (1983) gavaged (forced intake with a huge stress component) mice with pure bromoform at doses of 72-289 mg/kg LW, and Chu et al. (1982) added pure bromoform to drinking water of rats at up to 2500 ppm. The Brangus steers in the current study received *Asparagopsis* resulting in a seaweed bromoform exposure of 0.36 mg/kg.d LW. Condi's and Chu's exposure is exponentially higher at their low end than the highest treatment group in our feedlot cattle, representing multiples of 200 and 566 times more, respectively. Condi et al. (1983) reported degenerative effects (hyperplasia, hypertrophy) only at their highest dose which was a multiple of 803 times higher than the High level used in the present study, and as expected, at the highest level of gavaging, a couple of the mice demonstrated some degenerative health issues. However among the THM's tested bromoform was ranked the least toxic with chloroform being the highest. Chu et al. (1982) reported that their highest dose, which was a multiple of 2022 times higher per kg LW/d than the Brangus steers received, only created minor histological changes in rats and those disappeared after cessation of intake of bromoform.

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2. **Conclusions and recommendations** *(Final Report pages 34–36 of 42)*

The conclusions and recommendations of a study aim to provide the reader with the answer or significance of the outcomes of the project. Researchers provide:

- Recommendations for further research
- Developments/modifications to the technology being trialled
- Suggest whether the results and outcomes are beneficial for future trials, research, and investigation.

The significance of the conclusions and recommendations section is to provide the reader with an understanding of the viability of future developments and research in the focus area/s and the implications of the findings for the future of the industry. Possible courses of action for future developments may be included.

a. Read the conclusions/recommendations and key messages sections of the report and highlight the main points in each section.

6. Conclusions/ recommendations

Asparagopsis taxiformis was demonstrated to be an even more potent antimethanogenic agent for cattle when included in a grain based feedlot diet as compared to sheep on a legume-grain mix diet and compared to in vitro assessments using a grass based substrate. The amount of *Asparagopsis* required to achieve near elimination of CH_4 was surprisingly low and represents the single most important finding of this study. However, this was the first study using a high grain feedlot ration and with additives such as monensin in the TMR. Even with 1/10 of expected required levels of inclusion of the seaweed CH_4 production was reduced by 9%, 38% and 98% at treatment levels of 0.05%, 0.10%, and 0.20% of dietary OM, respectively. This has potential to make significant impact for the red meat industry toward the achievement of MLA's commitment to carbon neutrality by 2030 (CN2030).

Although H_2 was demonstrated to significantly increase it did not increase at previously observed levels relative to CH_4 reductions in a study using chloroform as the antimethanogenic agent. The excess H_2 had no negative effect on the steers DMI and productivity but H_2 emissions represent feed energy loss and so methods of conserving the H_2 would further the benefits of *Asparagopsis* and it is recommended that such methods be explored.

Importantly, in achieving extensive CH_4 reductions the DMI of the cattle in this study was not negatively affected as feed consumption remained consistent for all treatment groups. The response

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in ADWG to *Asparagopsis* was also better than expected by demonstrating ADWG improvements of 53% and 42% for those cattle receiving 0.10% and 0.20% of dietary OM, respectively. However this outcome should only be regarded as an indicator due to the small number of cattle in each treatment group and results are susceptible to individual animal performance. It is possible that the *Asparagopsis* supplemented cattle were inherently the most productive individuals or the Control biased by less stress tolerant individuals.

The ADWG improvements indicated by this study should be demonstrated and refined in a large scale study in a commercial feedlot environment. This would confirm that *Asparagopsis* can increase ADWG under typical feedlot conditions, provide confidence, and promote commercial acceptance and subsequent adoption.

It is recommended that the efficacy of *Asparagopsis* to reduce CH_4 emissions be investigated as an inclusion in diets of variable formulation. Diets containing variable levels of roughage of variable sources, and also with inclusions such as monensin have potential to alter the efficacy of *Asparagopsis*. This is expected to change the inclusion level required for large reduction in CH_4 emissions. To widely utilise *Asparagopsis* in livestock feed formulations this knowledge would be intrinsic in the variable cattle feeding systems in Australia and globally. Increasing the inclusion of *Asparagopsis* requires attention to potentially toxic entities that tend to accumulate in seaweeds. *Asparagopsis* may accumulate iodine at levels that may exceed the MTL at higher feed inclusion and research is required in the seaweed cultivation industry to increase quality and OM content and reduce the amount iodine and the amount of the seaweed product required for methane reduction.

The only noteworthy changes in rumen fluid due to *Asparagopsis* as a feed additive was a trend toward concomitant decreased acetate and increased propionate without effect on TVFA. This has now been demonstrated as a consistent outcome with reduced CH_4 emissions induced by *Asparagopsis* as a feed additive at low inclusion. Carcass characteristics and meat eating quality were not significantly changed and all the treatment groups were MSA graded. There is no evidence to support the use of *Asparagopsis* as an agent for improving meat eating quality, however at the increasing inclusion used in this study *Asparagopsis* did not reduce meat eating quality as confirmed by MSA consumer testing protocols. No bromoform could be detected in any meat, kidney, or fat collected from *Asparagopsis* treated steers. However, due diligence recommends continued monitoring and further necroscopy exploration as the feed inclusion periods get longer and/or the inclusion and intake levels are substantially increased which may be the case in dairy systems. Overall, it was demonstrated that *Asparagopsis* is the most promising antimethanogenic agent for feedlot production systems currently in the development pipeline. It is recommended that further study be completed addressing changing feed base, confirmation of productivity enhancement using large numbers of test animals, and commercial scale environment applications.

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Activity 2

b. Using the highlighted points, complete the cloze passage focused on this section of the research study.

Conclusions

The potential of *Asparagopsis* *taxiformis* as a tool to minimise methane/CH_4 emissions was investigated in feedlot beef cattle using Brangus steers.

The amount of *Asparagopsis* required to achieve near elimination of CH_4 was surprisingly low and represents the single most important finding of this study.

Asparagopsis was included in the high grain total mixed ration at 0.0% (control), 0.05% (Low), 0.10% (Mid), and 0.20% (High) of diet organic matter.

- CH_4 production (g/kg DMI) was reduced by 9 % at the Low inclusion level of 0.05%,
- CH_4 production (g/kg DMI) was reduced by 38 % at the Mid inclusion level of 0.10%,
- CH_4 production (g/kg DMI) was reduced by 98 % at the High inclusion level of 0.20%.

Although H_2 was demonstrated to significantly increase it did not increase at previously observed levels relative to CH_4 reductions.

The DMI of the cattle in this study was not negatively affected as feed consumption remained consistent for all treatment groups.

The ADWG was also better than expected, showing improvements of 53 % and 42 % for those cattle receiving 0.10% and 0.20% of dietary OM, respectively.

The only noteworthy changes in rumen fluid due to *Asparagopsis* as a feed additive was a trend toward concomitant decreased acetate and increased propionate without effect on TVFA. This has now been demonstrated as a consistent outcome with reduced CH_4 emissions induced by *Asparagopsis* as a feed additive at low inclusion.

Carcass characteristics and meat eating quality were not significantly changed and all the treatment groups were MSA graded. There is no evidence to support the use of *Asparagopsis* as an agent for improving meat eating quality , however at the increasing inclusion used in this study *Asparagopsis* did not reduce meat eating quality as confirmed by MSA consumer testing protocols.

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Activity 2

No [bromoform] could be detected in any meat, kidney, or fat collected from *Asparagopsis* treated steers.

Asparagopsis is the most promising [antimethanogenic] agent for feedlot production systems currently in the development pipeline.

3. Benefits to the industry

a. Using the highlighted points, complete the cloze passage focused on the benefits to the industry.

The implication of this study is that enteric [Methane (CH_4)] could be virtually eliminated while using *Asparagopsis* as a feed ingredient in the high grain TMR of [feedlot] beef cattle.

Reducing [methane] emissions in the agricultural sector benefits the environment by reducing the effects of [global] [warming]. Emission reduction is also of benefit to cattle producers. Feeding *Asparagopsis* to cattle has been shown to increase productivity by increasing the daily weight gain of cattle.

Opportunities will also emerge for new [industries] in the production and processing of [*Asparagopsis*] as a feed ingredient for the livestock sector.

Asparagopsis has capability for the red meat industry in providing significant impact toward achieving the MLA commitment of carbon [neutrality] by 2030 (CN2030).

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4. Future research and recommendations

a. Using the highlighted points, identify any recommendations for future development that are identified in the research study.

Suggested points to include:

Future research and recommendations

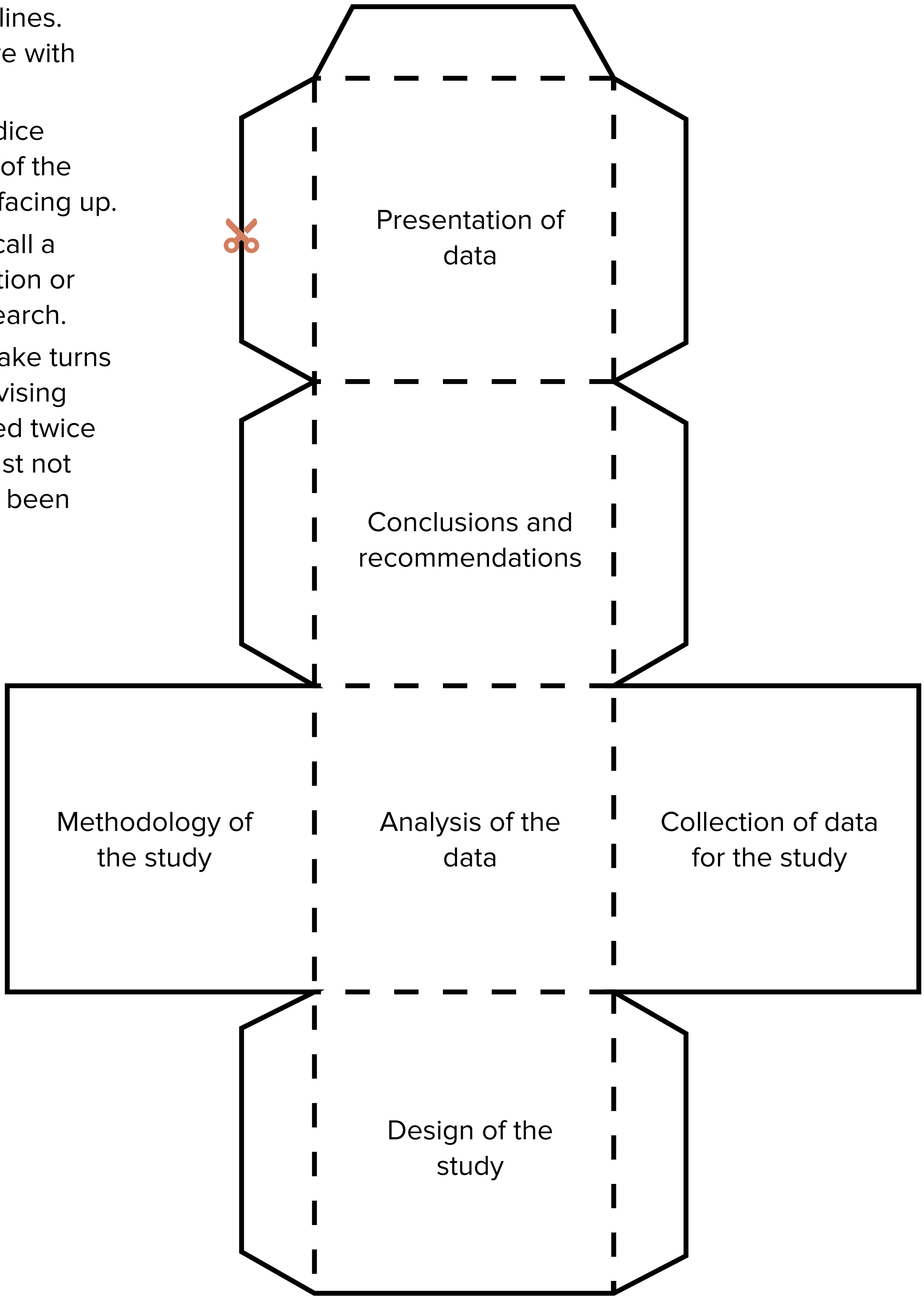
- Further studies to address changing feed base, confirm productivity enhancement, and application in commercial feedlots is required with larger numbers of animals/ larger sample sizes.
- Due diligence with respect to bromoform is required, particularly with longer term feed inclusion and higher level of intake as compared with duration and inclusion levels of this study.

PLEASE NOTE: This activity requires single-sided printing

Activity 3

Rolling Revision

1. Cut along the outside lines. Fold in the tabs. Secure with sticky tape or glue.
2. One student rolls the dice and reads the section of the research study that is facing up.
3. The other students recall a key aspect of that section or component of the research.
4. Students continue to take turns rolling the dice and revising topics. If a topic is rolled twice (or more), students must not repeat a 'fact' that has been previously stated.



References

FutureFeed. (2021, October 21). About *FutureFeed*. Vimeo. https://vimeo.com/637673333?embedded=true&source=vimeo_logo&owner=147791771

Kinley, R. (2018). *Asparagopsis* feedlot feeding trial. https://www.mla.com.au/contentassets/120dea2a6b504401baeedfd303794361/b.flt.0394_final_report.pdf

Meat & Livestock Australia. (n.d.). *Carbon neutral 2030 R&D | Meat & Livestock Australia*. MLA Corporate. https://www.mla.com.au/research-and-development/Environment-sustainability/carbon-neutral-2030-rd

Meier, U. (2006). A note on the power of Fisher's least significant difference procedure. *Pharmaceutical Statistics*, 5(4), 253–263. https://doi.org/10.1002/pst.210

Moran, J. (2005). *How the rumen works*. CSIRO Publishing; Landlinks Press. https://www.publish.csiro.au/ebook/chapter/SA0501041

National Health and Medical Research Council. (2018). *Animal ethics | NHMRC*. Nhmrc.gov.au. https://www.nhmrc.gov.au/research-policy/ethics/animal-ethics