Final report

The effect of red asparagopsis oil on the eating quality of long-fed cattle

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Abstract

This project aims to evaluate the effects of feeding red asparagopsis extract in canola oil on the eating quality (EQ) of long fed Wagyu X cattle. This experiment utilised 76 long-fed (280 DOF) F1 Wagyu x Bos indicus composite cattle. The cattle in the ASP infused oil treatment group were fed the ASP oil for the whole duration of their feeding period. The cattle were fed in 2 treatment groups (control and ASP oil) in 4 weight blocks across 8 pens (n=38 per treatment). Four pens were fed a control diet, and four pens were fed the same diet with part of the oil ingredient replaced by Asp-Oil supplying 25 mg bromoform/kg feed dry matter (DM). Pens were stratified across trucks and decks during transport from Tullimba Feedlot to Stanbroke, Queensland for harvest. Identities were maintained during harvest. Two samples from the cube roll (CUB045) and rump (RMP131) were randomised for position and aged for 2 periods post-mortem – 7 days and 35 days. The cube roll plate (CUB081) was aged for 7 days post-mortem. All samples (380) were frozen prior to being allocated to their sensory sessions. Each consumer scored the samples they were delivered for tenderness, juiciness, flavour and overall liking using a 100mm line scale. Further, individual consumer sensory scores for each trait were weighted by 0.3, 0.1,0.3 and 0.3, respectively, to calculate meat quality score (MQ4) out of 100. The ASP treated oil significantly ($P<0.05$) reduced the juiciness by 4.4 points across all cuts and ageing periods, whereas other eating quality attributes (MQ4, tenderness, flavour, overall liking and satisfaction) were not significantly changed when treated with ASP oil compared to the control group. The ASP treated cuts were always numerically lower, however due to the small sample size (n=38 per treatment) and large variation in quality between the cattle, the experimental sensitivity was not high enough to show the differences were significant. Ageing of different cuts up to 35 days significantly increased all sensory attributes but there was no significant difference between treatment groups with ageing. Marbling significantly improved all eating quality attributes by 0.03 to 0.04 for every 10 point increase in marbling. The interaction between treatment and cut was not significant for any of the EQ traits. It was concluded that the administration of ASP treated oil in the long-fed cattle diet could be possible without significantly affecting the eating quality traits except juiciness, however, EQ could be improved with ageing up to 35 days.

Executive summary

Background

The livestock industry contributes 14.5% to 19% of global greenhouse gas (GHG) emissions (Gerber et al., 2013; Johnson & Johnson, 1995; Steinfeld et al., 2006). The majority of GHG emissions from livestock production is in the form of methane (CH4) from the rumen, a result of anaerobic fermentation of carbohydrates by rumen microorganisms, which are primarily caused by methanogens utilizing carbon dioxide and hydrogen in a reduction pathway (Morgavi et al., 2010). The Wagyu sector is an increasingly important high value sector to the Australian beef industry and ways to stabilise and decrease emissions of the sector will be important in the coming years.

An extract of the bioactive component named bromoform, derived from the seaweed *Asparagopsis armata* in a canola carrier oil has been demonstrated to achieve reductions in methane (CH_4) yield of 54.5 – 95.0 % when included in short-fed finisher rations at 17 – 35 mg bromoform ($CHBr_3$)/kg feed dry matter (DM) over an 81-day feeding period and potentially increase production efficiency (Cowley et al., 2023; Roque et al., 2021). Bromoform is hydrophobic so it associates well with oil giving greater stability to bromoform than what was achieved with the freeze-dried ASP powder. The ASP infused oil is shelf stable for up to 12 weeks so it looks like the more applicable/commercialisable option for ASP. However, it is not known how the roughage content of finisher rations, and duration on feed or cattle type interact with bromoform inclusion rates to mitigate methane production and overall animal performance.

The business case for adoption of Asparagopsis-derived products will not just rely on methane mitigation but also effects on cattle performance, carcase value and cost of gain. The ruminant diet can directly affect the characteristics of their carcases. Improving the quality and uniformity of beef carcases can have beneficial economic effects and, ultimately, influence consumer demand. Several studies have explored how various seaweeds with differing nutrient compositions impact the quality and storage life of meat in pork (Sardi et al., 2006; Vossen et al., 2017), lamb (Urrutia et al., 2016) and beef (Hwang et al., 2014; Stokes et al., 2016), but there is no scientific consensus among them.

For adoption of the seaweed by industry, meat quality must be maintained or improved. To collect meaningful information on cattle performance, carcase characteristics and meat quality attributes commercially applicable feeding trials are required in Australia. Research to date has consistently reported that bromoform does not transfer to meat or reduce meat quality when fed to recommended levels (Kinley, 2018), but it is necessary to determine whether this holds under Australian commercial conditions for the long-term feeding of an *Asparagopsis* derived product.

Therefore, the objective of this project is to evaluate the impact of feeding Red Asparagopsis infused oil on the meat-eating quality as well as the post-mortem ageing potential of meat from long-fed beef (280 days).

Objectives

The project objectives are:

1. Determine the impact of feeding Red Asparagopsis infused oil on the meat-eating quality of long-fed beef (280 days).
2. Determine the impact of feeding Red Asparagopsis infused oil on the post-mortem ageing potential of meat from long-fed beef (280 days).
3. Make recommendation of feeding Red Asparagopsis infused oil to the impact on meat eating quality of long-fed beef (280 days).

Methodology

Eighty F1 Wagyu steers were fed Wagyu Grower and Finisher diets in 8 pens of 10 head for a 280-day feeding period. Four pens were fed a control diet, and four pens were fed the same diet with part of the oil ingredient replaced by Asp-Oil supplying 25 mg bromoform/kg feed dry matter (DM). Five steak samples were processed from each muscle x ageing period (7 and 35 days) which were tested by untrained Australian consumers utilising MSA protocols for the grill cook method described by (Watson et al., 2008). In total from this project, 380 samples were tested by 633 consumers. Individual consumer sensory scores for each trait (tenderness, juiciness, flavour and overall liking) were weighted by 0.3, 0.1, 0.3 and 0.3, respectively, to calculate meat quality score (MQ4) out of 100. Data was analysed in a mixed effects linear model with treatment, cut, and days of ageing as fixed effects, carcase traits as covariates and animals nested within pen as random effects. The only significant carcase trait was MSA marbling. Carcase weight remained in the model (even though it wasn't significant) due to the positive correlation between HSCW and marbling. The interaction of treatment and cut on eating quality attributes was tested in this model but was not significant. All statistical tests of least-squares mean (LSM) were conducted for a significance level of $P<0.05$.

The impact of feeding red asparagopsis treated oil to the long-fed beef cattle has been analysed. Only juiciness score appears to be reduced due to ASP treatment and all other eating quality attributes remained unchanged. Eating quality was also improved with ageing up to 35 days for all of the selected cuts.

Key findings

- Feeding an Asparagopsis extract in canola oil (Asp-Oil) at 25 mg/kg DM to F1 Wagyu steers for 280 days did not significantly affect sensory panel attributes except the juiciness by 4.41 points.
- MQ4 was increased by 0.03 to 0.04 points for every 10 point increase in marbling
- Eating quality was also improved with ageing up to 35 days for the rump and cube roll independent of treatment group.

Benefits to industry

This research represents one of the commercial trials of Asparagopsis products conducted in conditions relevant to the Australian feedlot industry, and it is also one of the longest feeding experiments involving Asparagopsis products. Consequently, it offers crucial data to support the adoption of ASP in extended feeding programs in feedlots, particularly concerning meat eating quality.

The findings of this study indicate that the ASP in canola oil treatment doesn't affect most eating quality attributes except for a 4.4 points decrease in juiciness. Improvement in tenderness and MQ4 scores was notable with increased marbling, although statistical models detect these differences as significant due to the large dataset.

Future research and recommendations

Currently, there is no established supply chain for Asparagopsis sp. for livestock feed, and the feasibility and costs associated with scaling up production of these species remain undetermined. For livestock producers, it is crucial to assess the economic advantages of any potential seaweed product. Even if regulations require the use of seaweed or other products to mitigate methane emissions, farmers may face increased financial burdens if animal performance does not concurrently improve (e.g., through enhanced productivity, efficiency, health, or product quality). Therefore, the value gained from such improvements must justify the cost of the product, or else additional incentive programs will be necessary to encourage widespread adoption. Regardless, these results from this project give confidence that there is a minimal effect on meat quality across the cuts and ageing timepoints measured.

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Executive summary

1. Background

The livestock industry contributes 14.5% to 19% of global greenhouse gas (GHG) emissions (Gerber et al., 2013; Johnson & Johnson, 1995; Steinfeld et al., 2006). According to Meat and Livestock Australia (2023), emissions from the red meat sector accounted for 10.3% of the total national greenhouse gas emissions in 2020. The majority of GHG emissions from livestock production is in the form of methane (CH4) from the rumen, a result of anaerobic fermentation of carbohydrates by rumen microorganisms, which are primarily caused by methanogens utilizing carbon dioxide and hydrogen in a reduction pathway (Morgavi et al., 2010). Enteric methane emissions not only add to the overall greenhouse gas emissions from agriculture but also result in an energy loss equivalent to as much as 11% of the energy consumed in the diet (Moraes et al., 2014). Therefore, lowering methene (CH_4) emissions would benefit the environment and possibly the efficiency of livestock production by preserving feed energy.

Using methane inhibitors may be an effective strategy for reducing emissions from enteric fermentation, while diet manipulation is considered the most straightforward and efficient approach for mitigating methane (CH_4) emissions in ruminant production systems (Beauchemin et al., 2008). Various methods offer the potential for reducing enteric methane emissions, emphasizing the utilization of feed additives, adjustments in diet composition, and enhancements in forage quality (Hristov et al., 2013).

Seaweeds have been a traditional part of livestock diet and have been used since the recording of agricultural practices began (Evans & Critchley, 2014). There have been several studies on seaweeds to characterize their effects as livestock feeds and their potential to manipulate rumen fermentation and methane production. The effectiveness of the seaweeds has been shown to have a relationship with the level of inclusion in the diet (Kinley et al., 2016; Li et al., 2016; Machado et al., 2016) and only Asparagopsis (ASP) has been demonstrated to remain effective and antimethanogenic without negative impacts on rumen function and at low inclusion levels in animal diets (Kinley et al., 2016; Li et al., 2016).

An extract of the bioactive component named bromoform, derived from the seaweed *Asparagopsis armata* in a canola carrier oil has been demonstrated to achieve reductions in methane (CH_4) yield of 54.5 – 95.0 % when included in short-fed finisher rations at 17 – 35 mg bromoform ($CHBr_3$)/kg feed dry matter (DM) over an 81-day feeding period and potentially increase production efficiency (Cowley et al., 2023; Roque et al., 2021). Bromoform is hydrophobic so it associates well with oil giving greater stability to bromoform than what was achieved with the freeze-dried ASP powder. The ASP infused oil is shelf stable for up to 12 weeks so it looks like the more applicable/commercialisable option for ASP. However, it is not known how the roughage content of finisher rations, and duration on feed or cattle type interact with bromoform inclusion rates to mitigate methane production and overall animal performance.

The business case for adoption of Asparagopsis-derived products will not just rely on methane mitigation but also effects on cattle performance, carcase value and cost of gain. The ruminant diet can directly affect the characteristics of their carcases. Improving the quality and uniformity of beef carcases can have beneficial economic effects and, ultimately, influence consumer demand. Several studies have explored how various seaweeds with differing nutrient compositions impact the quality and storage life of meat in pork (Sardi et al., 2006; Vossen et al., 2017), lamb (Urrutia et al., 2016) and beef (Hwang et al., 2014; Stokes et al., 2016), but there is no scientific consensus among them.

Further, it is not clear whether a decrease in CH_4 production leads to consistent improvement of animal performance, information that will be necessary for adoption by producers. Most published research on feeding Asparagopsis to beef cattle has been conducted with small numbers of cattle (~20 total) in individual pens, and the longest feeding period tested to date has been in North America over 147 days with rations based on lucerne, wheaten hays with dry distillers' grain and rolled maize (Roque et al., 2021). The experiment comprised four treatment groups, including control groups along with low, medium, and high levels of ASP oil inclusion, each consisting of five animals. Although the meat quality of these 20 animals was assessed, the findings were inconclusive due to the limited sample size within each treatment group and substantial variation observed within the groups, attributed to the differing quality of animals.

For adoption of the seaweed by industry, meat quality must be maintained or improved. To collect meaningful information on cattle performance, carcase characteristics and meat quality attributes commercially applicable feeding trials are required in Australia. Research to date has consistently reported that bromoform does not transfer to meat or reduce meat quality when fed to recommended levels (Kinley, 2018), but it is necessary to determine whether this holds under Australian commercial conditions for the long-term feeding of an *Asparagopsis* derived product. Increased understanding of the seaweed supplementation related to rumen fermentation and its effect on animal performance and CH_4 emissions in ruminants may lead to novel strategies aimed at reducing greenhouse gas emissions while improving animal productivity.

Therefore, the objective of this project is to evaluate the impact of feeding Red Asparagopsis infused oil on the meat-eating quality as well as the post-mortem ageing potential of meat from long-fed beef (300 days).

2. Project objectives

The project objectives are:

4. Determine the impact of feeding Red Asparagopsis infused oil on the meat-eating quality of long-fed beef (300 days).
5. Determine the impact of feeding Red Asparagopsis infused oil on the post-mortem ageing potential of meat from long-fed beef (300 days).
6. Make recommendation of feeding Red Asparagopsis infused oil to the impact on meat eating quality of long-fed beef (300 days).

3. Methodology

3.1 Animals and experimental design

The effect of Asp-Oil on Wagyu cattle emissions and feedlot performance was tested in a randomised block design with 2 dietary treatments (Asp-Oil or Control). All procedures were approved by the University of New England Animal Ethics Committee (Approval number ARA22-014).

The cattle were randomly allocated and fed in 4 initial cattle liveweight blocks across 8 pens (location x weight), running North to South as shown in Table 1 (n = 10 head per pen but few cattle have been removed over the feeding period due to health issues) with 2 treatments represented (n=38 per treatment).

This experiment utilised 76 long-fed (280 DOF) F1 Wagyu x Bos indicus composite cattle. The cattle in the ASP-infused oil treatment group were fed the ASP oil for the whole duration of their feeding period. The treatment diets were a modification of the AACo 300-day Wagyu feeding program without (Control) and with Asparagopsis oil (ASP) replacing part of the canola oil component of the total mixed ration (TMR).

Table 1: Allocation of animals per block and pen

Feedlot Pen	Weight Block	Head	Treatment
1	A	10	ASP
2		10	Control
3	B	10	ASP
4		10	Control
5	C	10	ASP
6		10	Control
7	D	10	ASP
8		10	Control

3.2 Animals and husbandry

Five hundred Wagyu steers (sourced from 3 AACo breeding properties) were backgrounded at Goonoo Feedlot (Qld). All cattle were inducted into the feedlot into low density pens for 43 days and onto a silage-based ration. Only animals that were eating the ration in these pens were considered for the trial. Eighty steers were selected from the group of 500. On day 7 before experimental adaptation commenced, the 80 head were transported to the University of New England's Tullimba Research Feedlot, near Armidale NSW, and placed into two large pens in the feedlot. At induction, the cattle were stratified into 4 weight blocks, each of 20 steers. Then, the 20 cattle in each block were randomly assigned to one of the two experimental diets: Control or ASP supplemented (n = 10 animals per treatment). From day 3 to 18 days on feed, treatments were housed adjacently in two 40-head pens (one per treatment) and fed in a single bunk per pen, until commencing the grower ration. This was to facilitate feeding of a high roughage transition diets that would fail to flow through auto-feeders in the experimental pens. On day 18, the cattle completed the adaptation to the treatment diets, were re-drafted into their experimental group and pens (10-head per pen) and commenced the experimental period. The 4 blocks of paired Control and Asp-Oil treatments were randomly located within a contiguous group of 8 pens in the feedlot, and the location of each treatment within a contiguous blocked pair of treatments was also randomised, generating a 'home pen' for each animal. The pens were orientated with a slope 3˚ West to East (from front of pen to back), along the row of pens. Each pen provided 50 m2/head pen space and was fitted with a reticulated water trough of 150 cm length. All animals were checked for health daily. The details of dietary treatment, measurement of cattle performance, slaughter and carcase measurements procedure had been reported by Fran Cowley (2023).

3.3 Design of truck loading, and kill order and boning

Animals were transported on the 15th January of 2023 from Tullimba Feedlot to Stanbroke abattoir in two trucks. All animals stayed within their designated pen treatment groups throughout transportation and while being held in a lairage, with no mixing of animals from different pens (Table 2). The loading and kill order were stratified to balance the treatment groups and weight blocks. At the kill on 16th January 2023, ear tags were collected, NLIS buttons were scanned and ID against body number was maintained. A liver and muscle sample were taken for residue testing by AACo.

Table 2: Design of truck loading, and kill

Feedlot Pen #	Weight Block	Head	Treatment	Truck	Deck	Front/Back of deck	Loading order	Kill order
7	4	10	Control	1	Top	Front	1	1
2	1	8	Control	1	Top	Back	2	2
4	2	10	Control	2	Bottom	Front	7	5
5	3	10	Control	2	Bottom	Back	8	6
8	4	10	Asp	1	Bottom	Front	3	3
1	1	9	Asp	1	Bottom	Back	4	4
3	2	9	Asp	2	Top	Front	5	7
6	3	10	Asp	2	Top	Back	6	8

3.4 Primal processing and sample collection

At boning on the 17th of January 2023, the cube roll and rostbif were collected from all 76 head (Table 3). The cube roll was cut 60:40, with UNE collecting all the larger portions from the cranial end for consumer testing. The caudal end was sent to UQ for trained panel testing. The rostbif was further split by slicing into the Rump 131 and Rump 231. The Rump 131 was utilised by UNE for untrained consumer testing and the Rump 231 was sent to UQ for trained panel testing. The experiment was powered to pick up a 4 point difference in eating quality between treatments. With a standard deviation of 10 between animals, 38 samples per treatment group are needed at 95% significance level to give an experimental power of 53%.

Table 3: Number of different cuts per treatment group

Cut	ASP	Control
CUB045	76	74
CUB081	38	37
RMP131	76	74

The primals allocated to UNE were further processed at the UNE Meat Science lab. Two samples from the cube roll (CUB045) and rump (RMP131) were randomised for position and aged for 2 periods post-mortem – 7 days and 35 days. The cube roll plate (CUB081) was consumer tested with 7 days post-mortem ageing.

3.5 Sensory and cooking protocol

Five steak samples were processed from each muscle x ageing period which were tested by untrained Australian consumers utilising MSA protocols for the grill cook method described by (Watson et al., 2008). In total from this project, 380 samples were consumer tested. Briefly, all samples were thawed in a fridge (4^0 C for 24 hours) prior to each sensory session before being lifted onto trays 30 mins before cooking to allow them to reach room temperature. A Silex clamshell grill was set at 195°C on the top plate and 210°C on the bottom plate and allowed to heat up 45 minutes prior to cooking the first steaks. Ten starter steaks were cooked and discarded to ensure a stable cooking temperature was achieved. All samples were grilled for 5 minutes and 15 seconds to a medium degree of doneness following the sensory grill protocol. Steaks were placed on the grill in the order they appeared on the sheets to ensure EQSRef codes could be tracked to the consumer.

A common first sample known as a link (LNK) was served to each group of 10 consumers to allow a base for statistical analysis. A 6x6 Latin Square design was employed to ensure each muscle was eaten by the same number of consumers in all serving positions (2-7) effectively balancing the trial for order effects. Each of the 5 samples from each muscle were cut in half into two equal sized rectangular pieces after cooking to allow for consumer testing by 10 consumers. Each consumer scored the samples they were delivered for Tenderness, Juiciness, Flavour and overall liking using a 100 mm line scale (0 being not very tender and 100 being very tender; 0 being not very juicy and 100 being very juicy; 0 being not very flavourful and 100 being very flavourful; 0 being not overall liked and 100 being very overall liked). In addition, consumers were then asked to mark the sample as unsatisfactory, good everyday, better than everyday or premium quality. Every sample was tested 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. Further, individual consumer sensory scores for each trait were weighted by 0.3, 0.1, 0.3 and 0.3, respectively, to calculate meat quality score (MQ4) out of 100.

MQ4 = (Tenderness × 0.3) + (Juicyness × 0.1) + (Overall liking × 0.3) + (Flavour × 0.3)

The 3 cuts chosen were cube roll which has a moderate ageing rate and moderate/high quality, cube roll plate which has a very fast ageing rate and high quality and the rump centre which has a lesser ageing rate and lower quality. This gives the variation in the experiment needed to determine if the effect of ASP on eating quality is via proteolysis or the concentration of connective tissue accumulation.

3.6 Statistical analysis

All data was analysed in R (Team, 2021), and several models were tested for each response. Data merging and manipulation, data visualisations and summary data were conducted using the ‘dplyr’ (Hadley et al., 2019), ‘ggplot2’ (Wickham et al., 2019) and ‘table1’ (Rich, 2018) packages respectively. Preliminary data have been conducted using linear mixed effects model from the ‘lme4’ package (Bates, 2016) and estimated marginal means were generated using the ‘emmeans’ package. The model of best fit is chosen by the lowest Akaike Information Criterion (AIC) value. Data from a single observation (meat quality attributes) were analysed in a mixed effects linear model with treatment, cut, and days of ageing as fixed effects, carcase traits as covariates and animals nested within pen as random effects. The only significant carcase trait was MSA marbling. Carcase weight remained in the model (even though it wasn’t significant) due to the positive correlation between HSCW and marbling. The interaction of treatment and cut on eating quality attributes was tested in this model but was not significant. All statistical tests of least-squares mean (LSM) were conducted for a significance level of $P<0.05$.

Mixed effect model for meat quality traits:

(Tenderness, Juiciness, Flavour, Satisfaction, MQ4) = lmer (treatment + cut + treatment * cut + ageing + marbling) + (1 | animal ID/pen)

4. Results

4.1 Carcase traits

Summary statistics with mean, standard deviation, minimum and maximum values are presented in Table 4. The average hot standard carcase weight (HSCW), ossification and eye muscle area (EMA) were higher in the control group compared to the ASP-treated group and rib fat and hump height were slightly higher in ASP treated group than the control group, however, none of them were statistically significant. Only significant ($p<0.05$) differences were found for MSA marbling and intramuscular fat percentage (IMF%).

Table 4: Summary statistics (mean, standard deviation, minimum and maximum) for different carcase traits

Variables	Treatment	Mean	SD	Min	Max
HSCW (kg)	ASP	389.7	27.6	336	446
	Control	409.4	34.7	327	511
MSA Marbling (100 – 1190)	ASP	522.1	111.5	320	740
	Control	520.5	108.9	330	780
Ossification score (100 – 590)	ASP	165.0	20.2	140	230
	Control	167.0	17.1	140	200
IMF (%)	ASP	10.4	2.9	5.1	17.2
	Control	10.0	2.9	5.0	16.9
Rib fat (mm)	ASP	11.6	4.7	4	27
	Control	11.4	3.3	5	20
Hump height (mm)	ASP	108.1	18.5	75	185
	Control	105.5	12.8	70	130
EMA (cm^2)	ASP	97.2	8.0	75	111
	Control	97.7	8.1	84	121

HSCW= Hot standard carcase weight; IMF% = Intramuscular fat percentage, EMA=Eye Muscle Area.

4.2 Effect of treatment on meat eating quality

4.2.1 Effect of treatment and ageing on tenderness

In general, there was no significant difference in tenderness due to ASP inclusion in the diet, although the ASP treatment reduced the tenderness by 1.32 points (Table 5, Fig. 1). Although the interaction between treatment and cut did slightly reduce the score, the difference was not statistically significant. The tenderness score for the cut CUB081 was notably higher by 20.10 points, whereas for RMP131, it was lower by 16.98 points compared to CUB045. Furthermore, tenderness significantly improved with aging, increasing by 8.8 points for both cuts, with variations ranging from 6.94 to 10.77 points. Additionally, marbling significantly impacted tenderness, with a 0.04 point increase for every 10 point rise in marbling.

Table 5: Effect of treatment on tenderness

Predictors	**Estimates**	**CI**	**p**
Intercept	41.11	19.22 – 62.99	**<0.001**
TreatASP	-1.32	-5.29 – 2.65	0.513
CutCUB081	20.10	16.63 – 23.58	**<0.001**
CutRMP131	-16.98	-19.71 – -14.26	**<0.001**
Days of ageing35	8.86	6.94 – 10.77	**<0.001**
Marbling	0.04	0.02 – 0.05	**<0.001**
HSCW	0.01	-0.04 – 0.07	0.583
TreatASP: CutCUB081	-0.37	-5.06 – 4.32	0.877
TreatASP: CutRMP131	-2.33	-6.16 – 1.49	0.231

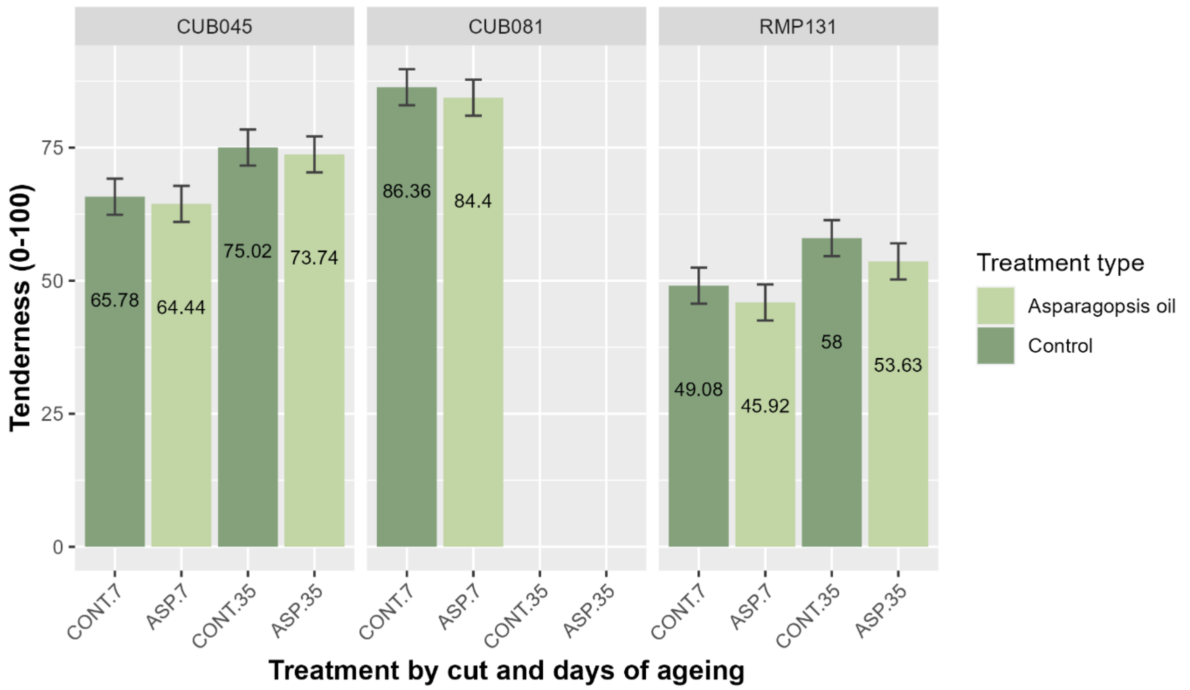

Figure 1: Effect of treatment on tenderness for different cuts and ageing

4.2.2 Effect of treatment and ageing on Juiciness

Juiciness was significantly (P<0.05) decreased by 4.41 points when ASP was included in the diet (Table 6, Fig. 2). Compared to CUB045, the cut CUB081 demonstrated an improvement in juiciness by 12.96 points, while RMP131 showed a decrease by 19.33 points. Moreover, juiciness significantly improved with aging, with an increase of 5.85 points for both cuts, varying between 3.98 to 7.72 points. There was no significant interaction between treatment and cut regarding juiciness. Additionally, marbling had a notable impact on juiciness, with a 0.04 point increase for every 10 point increase in marbling.

Table 6: Effect of treatment on Juiciness

Predictors	Estimates	CI	p
Intercept	52.53	32.52 – 72.53	**<0.001**
TreatASP	-4.41	-8.11 – -0.72	**0.019**
CutCUB081	12.96	9.57 – 16.35	**<0.001**
CutRMP131	-19.33	-21.99 – -16.67	**<0.001**
Days of ageing35	5.85	3.98 – 7.72	**<0.001**
Marbling	0.04	0.02 – 0.05	**<0.001**
HSCW	-0.00	-0.05 – 0.04	0.907
TreatASP: CutCUB081	3.33	-1.25 – 7.90	0.154
TreatASP: CutRMP131	0.86	-2.87 – 4.60	0.650

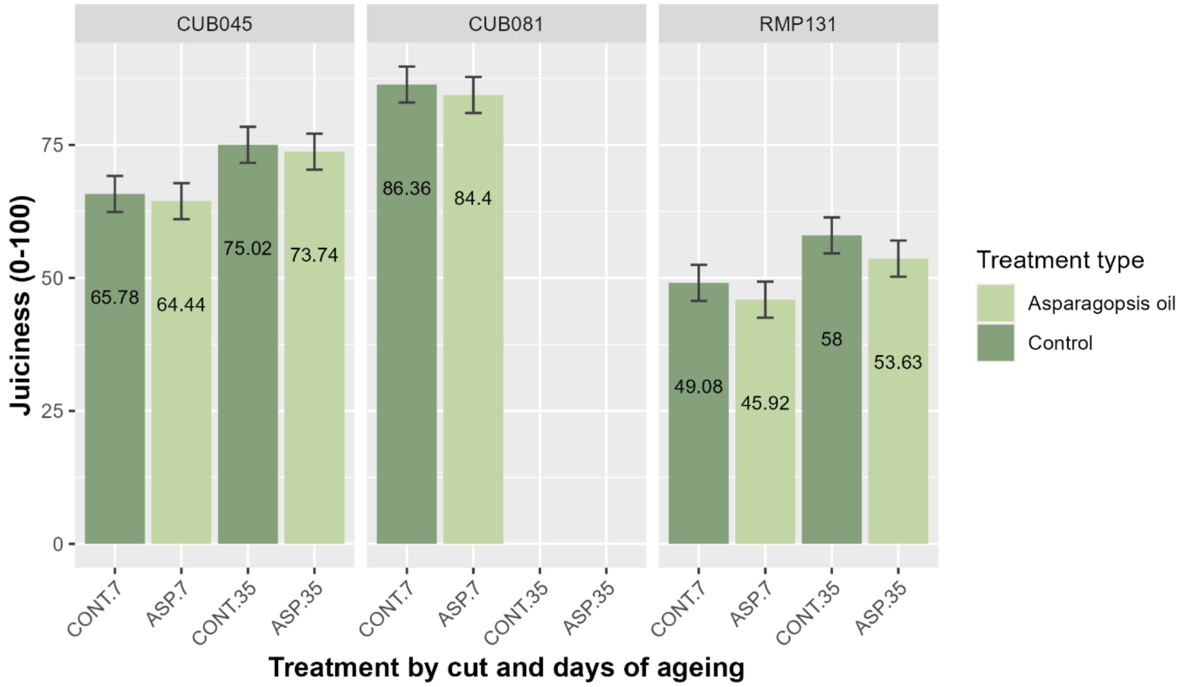

Figure 2: Effect of treatment on juiciness for different cuts and ageing

4.2.3 Effect of treatment and ageing on flavour

There were no statistically significant differences (P>0.05) observed for flavor between the treatment and control groups, as indicated in Table 7 and Figure 3. Compared to CUB045, the cut CUB081 exhibited an improvement in juiciness by 14.36 points, while RMP131 showed a decrease by 13.22 points. Additionally, juiciness significantly improved with aging, with an increase of 4.25 points for both cuts, ranging from 2.72 to 5.77 points. There was no significant interaction between treatment and cut regarding juiciness. Furthermore, marbling had a notable impact on juiciness, with a 0.03 point increase for every 10 point increase in marbling.

Table 7: Effect of treatment on flavour

Predictors	Estimates	CI	p
Intercept	52.77	36.48 – 69.06	**<0.001**
TreatASP	-1.39	-4.36 – 1.67	0.381
CutCUB081	14.36	11.60 – 17.13	**<0.001**
CutRMP131	-13.22	-15.39 – -11.05	**<0.001**
Days of ageing35	4.25	2.72 – 5.77	**<0.001**
Marbling	0.03	0.02 – 0.04	**<0.001**
HSCW	0.01	-0.03 – 0.05	0.733
TreatASP: CutCUB081	-0.92	-4.65 – 2.82	0.629
TreatASP: CutRMP131	-1.64	-4.69 – 1.41	0.292

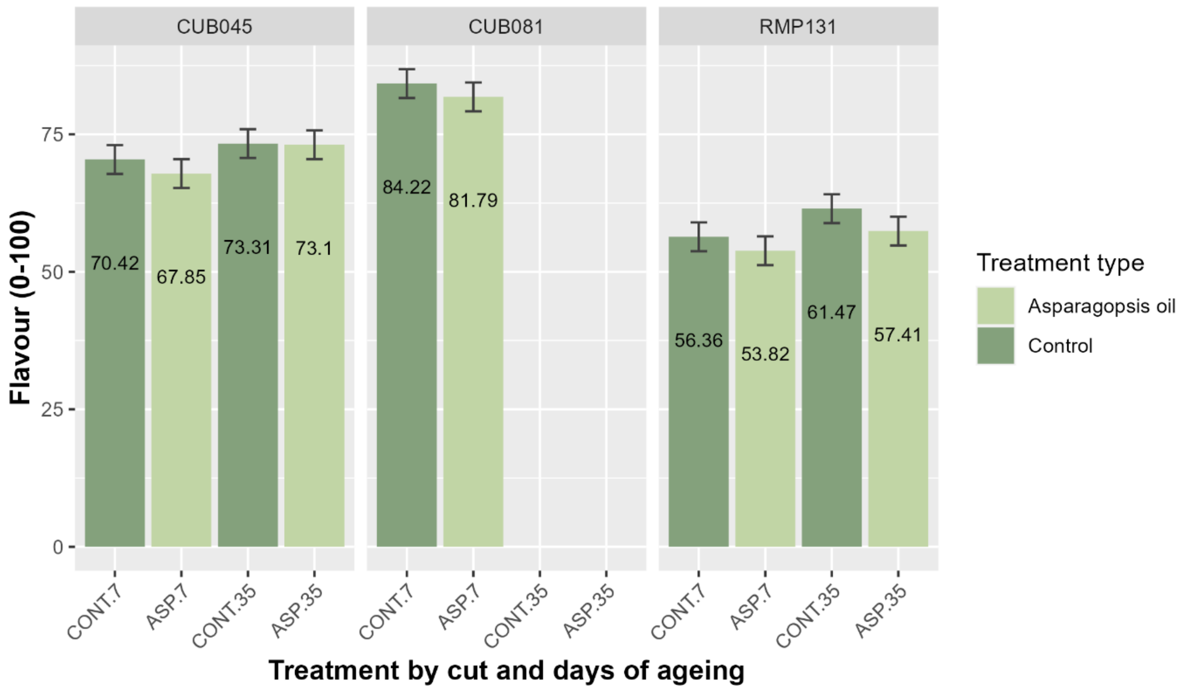

Figure 3: Effect of treatment on flavour for different cuts and ageing

4.2.4 Effect of treatment and ageing on satisfaction

There were no statistically significant differences (P>0.05) observed for satisfaction between the treatment and control groups (Table 8 and Figure 4). In comparison to CUB045, CUB081 demonstrated an improvement in satisfaction by 0.84 points, whereas RMP131 showed a decrease by 0.62 points. Moreover, satisfaction significantly increased with aging, with a rise of 0.25 points for both cuts, ranging from 0.18 to 0.32 points. There was no significant interaction between treatment and cut regarding satisfaction.

Table 8: Effect of treatment on satisfaction

Predictors	Estimates	CI	p
Intercept	3.13	2.35 – 3.92	**<0.001**
TreatASP	-0.08	-0.22 – 0.07	0.294
CutCUB081	0.84	0.70 -0.97	**<0.001**
CutRMP131	-0.62	-0.72 - -0.51	**<0.001**
Days of ageing35	0.25	0.18 - 0.32	**<0.001**
Marbling	0.00	0.00 – 0.00	**<0.001**
HSCW	-0.00	-0.00 – 0.00	0.743
TreatASP: CutCUB081	-0.11	-0.29 – 0.07	0.247
TreatASP: CutRMP131	-0.04	-0.19 – 0.11	0.587

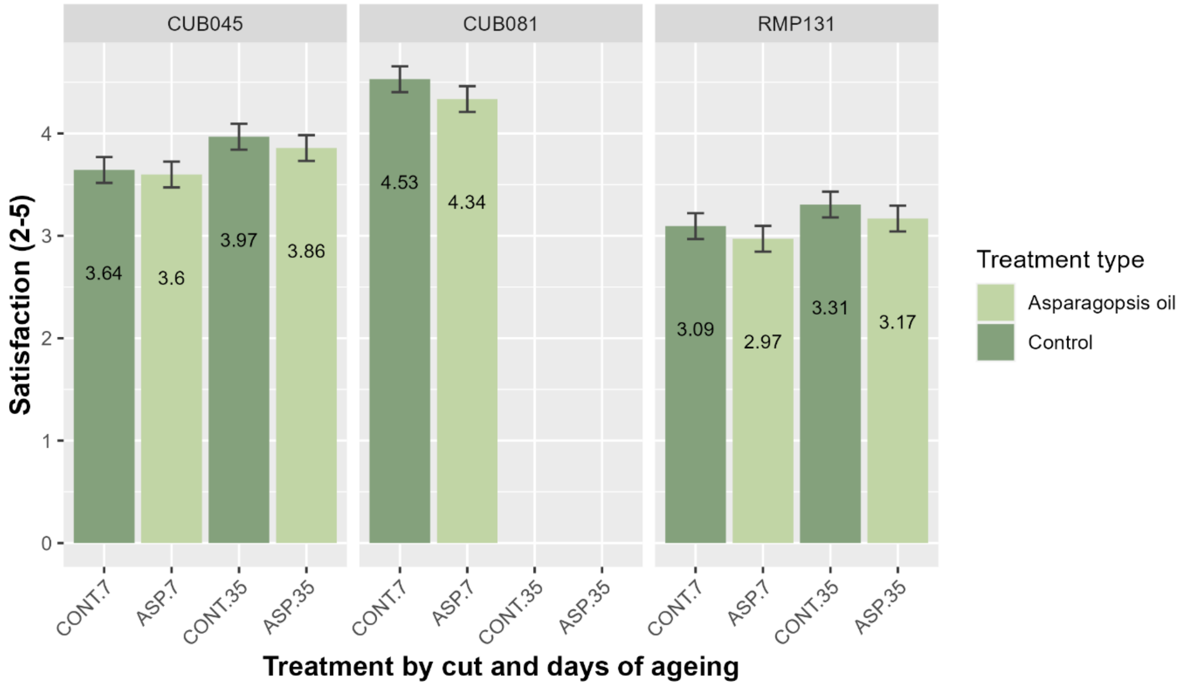

Figure 4: Effect of treatment on satisfaction for different cuts

4.2.5 Effect of treatment on overall liking

There were no statistically significant differences (P>0.05) observed for overall liking between the treatment and control groups, as indicated in Table 9. Compared to CUB045, CUB081 showed an improvement in satisfaction by 16.14 points, while RMP131 demonstrated a decrease by 15.35 points. Additionally, satisfaction significantly increased with aging up to 35 days, with a rise of 6.02 points for both cuts, ranging from 4.38 to 7.66 points (Figure 4). There was no significant interaction between treatment and cut regarding satisfaction. Additionally, marbling had a notable impact on overall liking, with a 0.03 point increase for every 10 point increase in marbling.

Table 9: Effect of treatment on overall liking

Predictors	Estimates	CI	p
Intercept	48.25	28.86 – 67.64	**<0.001**
TreatASP	-1.34	-4.82 – 2.15	0.450
CutCUB081	16.14	13.16 – 19.11	**<0.001**
CutRMP131	-15.35	-17.68 – -13.01	**<0.001**
Days of ageing35	6.02	4.38 – 7.66	**<0.001**
Marbling	0.03	0.02 – 0.05	**<0.001**
HSCW	0.01	-0.04 – 0.05	0.733
TreatASP: CutCUB081	-0.89	-4.91 – 3.12	0.663
TreatASP: CutRMP131	-1.97	-5.25 – 1.30	0.237

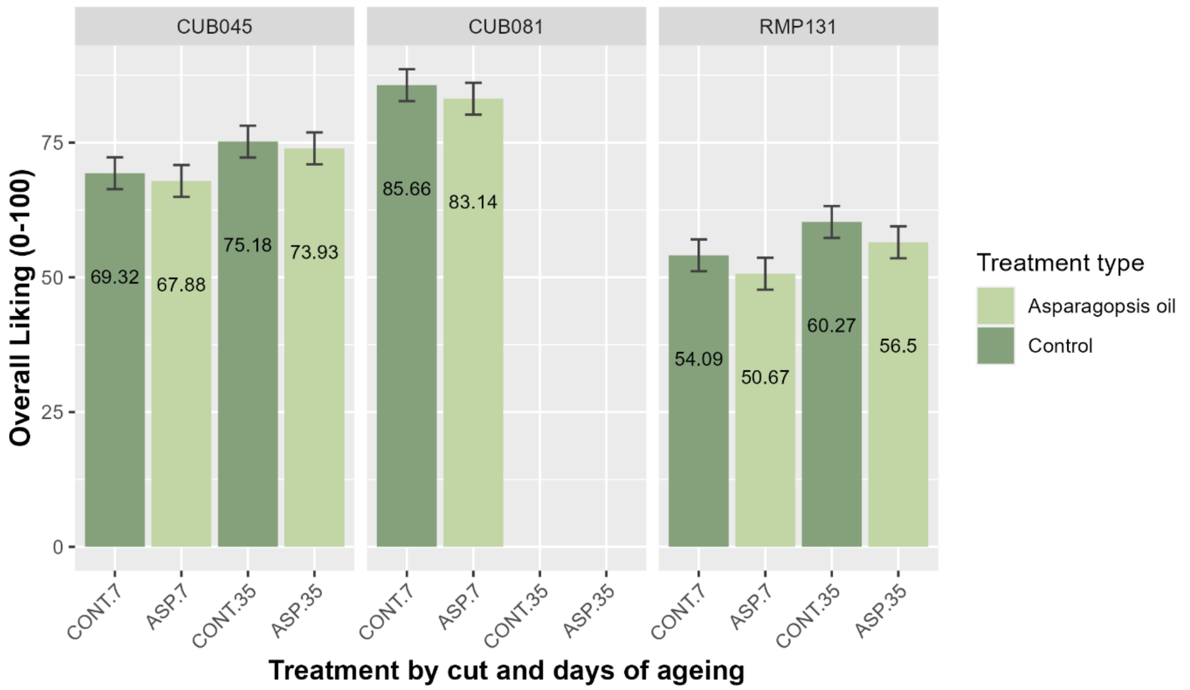

Figure 5: Effect of treatment on overall liking for different cuts

4.2.6 Effect of treatment on MQ4

Likewise all other meat quality attributes, there were no statistically significant differences (P>0.05) observed for MQ4 between the treatment and control groups (Table 10). In comparison to CUB045, CUB081 exhibited an improvement in satisfaction by 16.50 points, while RMP131 showed a decrease by 15.45 points. Additionally, MQ4 significantly increased with aging up to 35 days, with a rise of 6.09 points for both cuts, ranging from 4.51 to 7.66 points (Figure 6). There was no significant interaction between treatment and cut regarding MQ4. Additionally, marbling had a notable impact on MQ4, with a 0.03 point increase for every 10 point increase in marbling.

Table 10: Effect of treatment on MQ4

Predictors	Estimates	CI	p
Intercept	47.80	29.06 – 66.55	**<0.001**
TreatASP	-1.52	-4.88 – 1.84	0.374
CutCUB081	16.50	13.64 – 19.36	**<0.001**
CutRMP131	-15.45	-17.70 – -13.21	**<0.001**
Days of ageing35	6.09	4.51 – 7.66	**<0.001**
Marbling	0.03	0.02 – 0.04	**<0.001**
HSCW	0.01	-0.03 – 0.05	0.671
TreatASP: CutCUB081	-0.54	-4.40 – 3.32	0.784
TreatASP: CutRMP131	-1.46	-4.62 – 1.69	0.363

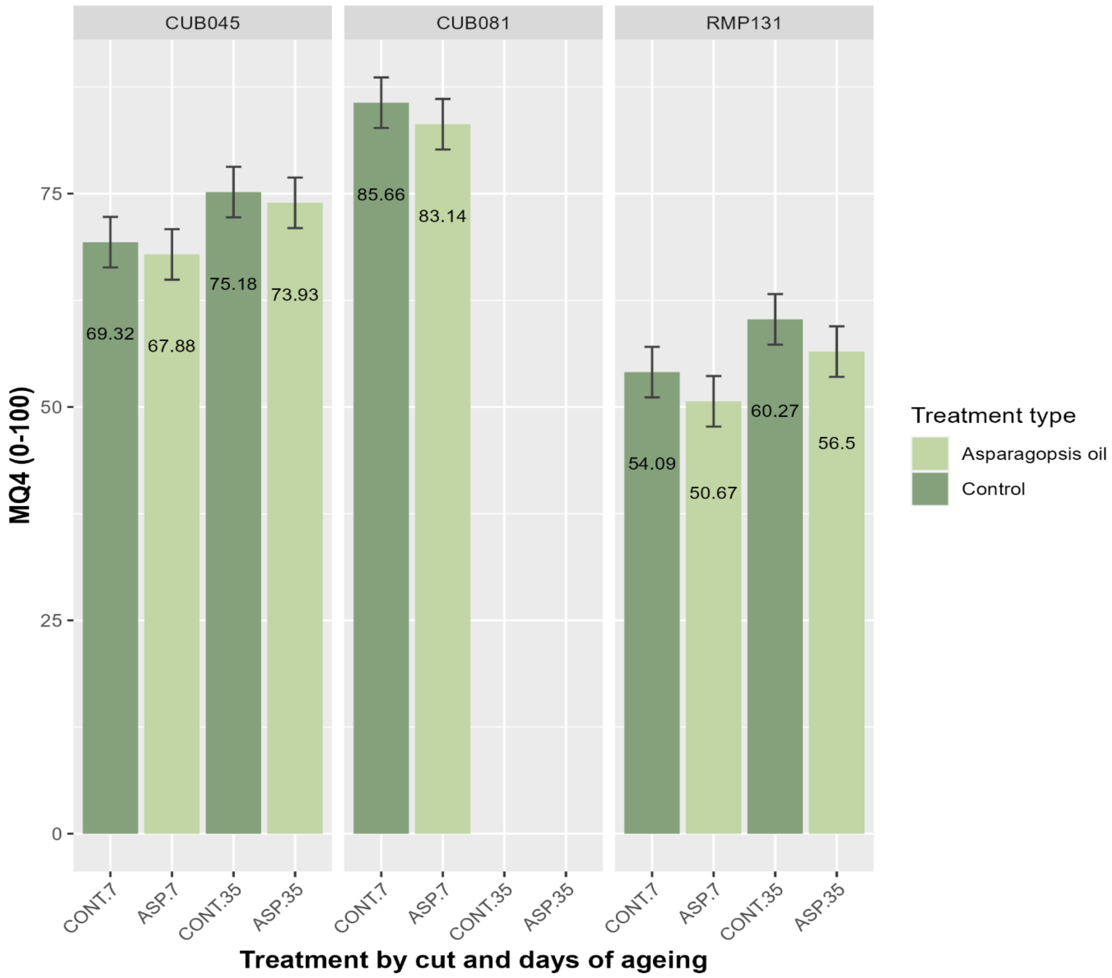

Figure 6: Effect of treatment on MQ4 for different cuts

5. Discussion

Effects of *Asparagopsis* supplementation on marbling have not been previously reported. In the current study, all animals were of similar age and breed, with only a slight, but statistically significant, difference observed in average marbling scores. Urrutia et al. (2016) suggested that marine microalgae increased lipid oxidation in lamb. In beef, previous research showed that the algae supplementation improved fatty acid composition while saturated fatty acids, unsaturated fatty acids, polyunsaturated fatty acids (PUFA), and monosaturated fatty acids (MUFA) were not affected (Bolkenov et al., 2021; Hwang et al., 2014; Stokes et al., 2016). Conversely, in pork, marine algae did not change the meat colour and lipid oxidation rate (Sardi et al., 2006; Vossen et al., 2017). The lipid oxidation in the current study could affect the slight improvement in marbling. However, the previous studies used different types of seaweed with different nutrient compositions must be taken into account.

High importance is placed on tenderness in the marketplace, with studies indicating that consumers are willing to pay more for more tender beef (Lamare et al., 2002; Miller et al., 2001). Various factors, including the age at which animals are slaughtered, their breed, the amount of fat and collagen (connective tissue) contained in a particular cut and diet, can significantly influence meat tenderness and other sensory properties (Blank et al., 2017; Corbin et al., 2014; Warner et al., 2010). The lack of significant differences in meat quality factors except juiciness (reduced by 4.4 points) in the current study suggests that the supplementation of Asparagopsis did not affect the meat quality attributes. This finding aligns with previous research on meat taste assessment, which also found no differences between beef cattle supplemented with Asparagopsis and those that were not (Hwang et al., 2014; Kinley et al., 2020; Roque et al., 2021). On the contrary, Urrutia et al. (2016) reported, algae had adverse effects on meat quality, with greater lipid oxidation and reduced ratings for odour and flavour. Bolkenov et al. (2021) investigated the shelf life of steaks from the low (0.25%) and high dose (0.5%) groups of ASP compared to the control group and found that the shelf life for the low dose group remained the same as the control group but the steaks from the high dose had a darker colour with higher microbial counts that causes a shortened shelf life due to undesirable appearance and faster microbial spoilage. Overall, both the current study and previous research indicate that supplementing cattle with Asparagopsis does not have a significant impact on overall meat quality or alter the sensory properties of beef.

Further, all the sensory properties for the selected cuts improved with ageing up to 35 days for both control and treatment groups. The improvement of sensory qualities of all cuts with ageing is attributed to proteolysis and connective tissue changes, fundamental processes in beef that lead to improved tenderness and flavour, making aged beef highly desirable for its enhanced eating qualities. Tenderness is primarily influenced by connective tissue and the muscle contractile apparatus, with collagen being a key component (Greaser, 1986). As beef ages, collagen transforms into gelatin through proteolysis, particularly involving the calpain system. The μ-calpain system plays a crucial role in early post-mortem aging, while m-calpain becomes more prominent in long-term aging (Parr et al., 2007). Moreover, the influence of tenderness varies across cuts and is affected by factors like fat content. Weight-bearing muscles and those used frequently contain more collagen, while cuts with higher fat content tend to exhibit greater tenderness due to reduced muscle use. While there were variations in sensory properties across different cuts, both the treated and control groups showed improvement with aging. This indicates that the presence of ASP does not impact the aging process for these cuts.

Conclusions/Recommendations

The impact of feeding red asparagopsis treated oil to the long-fed beef cattle has been analysed. Only juiciness score appears to be reduced due to ASP treatment and all other eating quality attributes remained unchanged. Eating quality was also improved with ageing up to 35 days for all of the selected cuts.

5.1 Key findings

- Feeding an Asparagopsis extract in canola oil (Asp-Oil) at 25 mg/kg DM to F1 Wagyu steers for 280 days did not significantly affect sensory panel attributes except the juiciness by 4.41 points.
- Marbling was slightly increased by 0.03 to 0.04 points due to the inclusion of ASP in the diet.
- Eating quality was also improved with ageing up to 35 days for all of the selected cuts.

5.2 Benefits to industry

This research represents one of the commercial trials of Asparagopsis products conducted in conditions relevant to the Australian feedlot industry, and it's also one of the longest feeding experiments involving Asparagopsis products. Consequently, it offers crucial data to support the adoption of Asp-Oil in extended feeding programs in feedlots, particularly concerning meat eating quality.

The findings of this study indicate that the ASP treatment doesn't affect most eating quality attributes except for a decrease in juiciness. Improvement in tenderness and MQ4 scores was notable with increased marbling, although statistical models detect these differences as significant due to the large dataset.

5.3 Future research and recommendations

Currently, there is no established supply chain for Asparagopsis sp. for livestock feed, and the feasibility and costs associated with scaling up production of these species remain undetermined. For livestock producers, it's crucial to assess the economic advantages of any potential seaweed product. Even if regulations require the use of seaweed or other products to mitigate methane emissions, farmers may face increased financial burdens if animal performance does not concurrently improve (e.g., through enhanced productivity, efficiency, health, or product quality). Therefore, the value gained from such improvements must justify the cost of the product, or else additional incentive programs will be necessary to encourage widespread adoption.

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