Final report

Impact of red asparagosis oil on the eating quality of mid-fed cattle

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Abstract

The red seaweed *Asparagopsis*, and the bioactives thereof, are highly effective in reducing enteric methane when fed as feed additives to ruminants. This project aimed to evaluate the effects of feeding SEAFEED™, a bromoform-based canola oil infusion of *Asparagopsis armata (*Asp-Oil) on the eating quality (EQ) of beef from mid-fed Angus cattle. This experiment utilised 600 mid-fed (150 days on feed – DOF) Angus cattle, divided into control and Asp-Oil treatment groups across three blocks and six pens. The cattle in the treatment group were fed rations containing Asp-Oil for the final 100 days of their feeding period, which included Asp-Oil containing 25.6 mg bromoform ($CHBr_3$)/kg feed dry matter (DM). Cattle were transported and processed across three groups. All carcases were MSA graded, then 24 carcases from both the control and treated group of each group were selected for cut collection. In total, cuts were collected from 72 treated and 72 control cattle. Two samples from the anterior striploin (STR045) and eye round (EYE075) were randomised in position and aged for two different periods post-mortem – 7 days and 28 days, from each of the selected carcases, for consumer testing. The oyster blade (OYS036) was aged for 7 days post-mortem. All samples (n=720) were frozen before being allocated to their sensory sessions and each sample was tested by 10 untrained consumers. 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 the meat quality score (MQ4) out of 100. There were 1,200 Australian consumers utilised to sample the meat from this project in the North-west, North Coast, Central West and Mid North Coast regions of NSW. Live animal data and consumer sensory data were compiled and analysed. The addition of ASP oil to the diet did not have a significant impact on carcass traits. The cuts from the treated group fed Asp-Oil were always numerically lower for meat quality traits (tenderness, Juiciness, flavour, overall liking and MQ4), however, not statistically significant ($p>0.05$). Post-mortem ageing of the STR045 and EYE075 cuts from 7 days to 28 days significantly improved all sensory attributes. In conclusion, there was no adverse impact of Asp-Oil inclusion in the diet on eating quality, nor any changes in carcase attributes.

Executive summary

Background

This project was a joint initiative between the University of New England, Rangers Valley Cattle Station Pty Ltd, Meat and Livestock Australia (MLA) and Sea Forest Limited, aimed to determine the impact of feeding SEAFEED™, a bromoform-based canola oil infusion of *Asparagopsis armata* on the carcase attributes, meat eating quality and on the post-mortem ageing potential of mid-fed beef (150 days). Rangers Valley has a large suite of high-quality beef brands prominent in domestic and international markets.

Previous studies have investigated the impact of freeze-dried seaweed supplementation on enteric methane emissions and meat quality in cattle. However, the selection of carcases for consumer sampling was potentially confounded, suggesting that the animals chosen were not uniform across the control and treated groups regarding carcase characteristics. This could possibly lead to misleading results however that will always be unknown.

An extract of the bioactive derived from the seaweed *Asparagopsis,* called bromoform, 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 (Cowley et al., 2023). It is unknown 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. Further, the business case for the adoption of bromoform based products will not just rely on methane mitigation but also effects on cattle performance, carcase value and cost of gain.

Most recent published research on feeding freeze-dried *Asparagopsis* to beef cattle has been conducted with small numbers of cattle (~20 total) in individual pens in North America over 147 days with rations based on lucerne, wheaten hay with dry distillers' grain and rolled maize (Roque et al., 2021). Although the meat quality of these 20 animals was assessed through consumer sensory, 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. To collect meaningful information on cattle performance, carcase characteristics and meat quality attributes commercially applicable feeding trials are required in Australia. Cowley et al. (2023) reported conducting the longest feeding trial (275 days) of SEAFEED™ on Wagyu steers in Australia. Research to date has consistently reported that bromoform does not transfer to meat or reduce meat quality when fed to recommended levels, but it is necessary to determine whether this holds under Australian commercial conditions for the mid-term feeding of bromoform based products.

Therefore, this commercial feeding trial evaluated the inclusion of SEAFEED™ (Asp-Oil) in a total mixed ration (TMR) for Angus steers over the last 100 days across 3 independent kill groups. Mean inclusion of Asp-Oil in the TMR prepared on a daily basis was 1.1% by weight determined by Bovine Dynamics to deliver 25.6 mg bromoform per kg feed (predicted). Average pen data was used to determine feedlot performance, feed efficiency ratio and cost of inclusion. Carcase data was collected on individual animals and samples of muscle and meat eating quality attributes.

The results of this trial will help inform Sea Forest Ltd and Rangers Valley on the potential scaling up for supply and use of SEAFEED™ in finishing periods and provide (at the discretion of Rangers Valley) the Australian feedlot sector with information on the application of the technology in commercial operations.

Objectives

The objectives of this project were to determine the effect of Asp-Oil inclusion in a feedlot total mixed ration on:

- The meat eating quality of mid-fed beef (150 days, 100 days on Asp-Oil).
- The post-mortem ageing potential of meat from mid-fed beef (150 days, 100 days on Asp-Oil).
- The carcase traits of mid- fed beef (150 days, 100 days on Asp-Oil).

Methodology

This experiment utilised 600 mid-fed (150 DOF) Angus cattle. The cattle in the Asp-Oil treatment group were fed the ASP infused oil for the final ~100 days of their feeding period. The cattle were fed in 3 replicates across 6 pens (n = 100 head per pen but some cattle were removed over the feeding period). These cattle were fed in 3 consignments/kill groups (n= 200 head per consignment) with two treatments within each consignment- Control (n = 100) and Asp-Oil (n=100). At 50 days of feed, a pen of 200 was divided into 2 pens of 100 for the experiment based on vendor and stratified for induction weight. All animals remained within their designated pen groups during transport from Rangers Valley Feedlot to the abattoir, where they were held according to their pen treatment groups in the lairage area.

Immediately post slaughter and dressing, carcase characteristics such as, hot standard carcase weight (HSCW), hump height (mm) and P8 fat (mm) were determined according to AUS-MEAT carcase standards (AUS-MEAT, 2005). At >60 hours post-mortem, each carcase was evaluated between the 12/13th rib by a Meat Standards Australia (MSA) grader (Meat Standards Australia 2007) for MSA marbling score; rib fat depth (mm); ossification score; ultimate pH (pHu); eye muscle area (EMA, cm^2), meat colour (MC) and fat colour (FC).

Samples selected for meat eating quality were collected from Striploin, Oyster Blade and Eye Round. Across the three kill groups, cuts were collected from 72 treated and 72 control cattle (n = 144 total, 48 per kill group, 24 control and 24 Asp-Oil). Two samples each from the anterior striploin (STR045) and eye round (EYE075) were aged for 7 and 28 days post-mortem, while a single sample from the oyster blade (OYS036) was aged for 7 days. Consumer sensory evaluation used a 6 x 6 Latin square to ensure each sample was served equally before and after other products and equally in each order. Samples were assessed for tenderness, juiciness, flavour and overall liking using four unstructured, 100-mm lines defined by the words very tough–very tender for tenderness, very dry–very juicy for juiciness and extremely dislike–extremely like for both flavour and overall liking. In addition, consumers were asked to mark the sample as unsatisfactory (2 star), good every day (3 star), better than everyday (4 star) or premium quality (5 star).

Results/key findings

- Feeding a ration containing SEAFEED™, a bromoform-based canola oil infusion of *Asparagopsis armata* (Asp-Oil) at a dose rate of 25.6 mg/kg dry matter to Angus cattle for 100 days with Asp-Oil out of 150 days of feeding did not significantly affect meat quality attributes as determined by a untrained consumer sensory panel.
- Eating quality was improved with ageing from 7 to 28 days for all of the selected cuts, independent of any treatment effects.
- Carcase traits (HSCW, hump height, EMA, ossification, MSA marbling, AUSMarbling, MSA Index, P8 fat, subcutaneous rib fat, meat colour and fat colour) were not impacted by the Asp-Oil treatment.
- The inclusion of Asp-Oil in the diet did not impact feed intake or carcass weight of the treated cattle.

Benefits to industry

This research represents the largest commercial trials of Asp-Oil products on carcase and meat quality conducted in conditions relevant to the Australian feedlot industry. 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-Oil treatment does not affect eating quality attributes or carcase traits. This consistency is crucial for maintaining consumer satisfaction and meeting quality standards in the meat industry. These findings support the economic feasibility of incorporating Asp-Oil into extended feeding programs.

Future research and recommendations

Future research is needed to optimize feeding programs for Asp-Oil supplements, including testing different varieties, and new synthetic variations of bromoform with various formulations. However, thorough sensory testing is essential if these supplements are to be applied commercially.

Currently, there is a limited supply chain for *Asparagopsis* spp. for livestock feed, and the feasibility and costs associated with scaling up production of these species remain uncertain. 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.

The feed intakes, growth rates and HSCW of any replicate were not impacted by the inclusion of Asp-Oil into their diet. To date, many experiments have reported variation in feed intakes after inclusion of the oil. Hence these results suggest that having cattle on finisher ration or giving them a long lead time (50 days in this case) is beneficial to ensure that the inclusion of the Asp-Oil does not impact animal performance.

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1. Background

The agricultural sector is responsible for 13.5% of the total emissions in Australia, and the livestock industry contributes 14.5% to 19% of global greenhouse gas (GHG) emissions (Johnson and Johnson, 1995; Steinfeld et al., 2006; Gerber et al., 2013). However, total GHG emissions attributed to the red meat industry were 51.25 Mt CO2-e in 2020, representing a 6.4% decrease compared to 2019 and a 64.9% decrease compared to the reference year of 2005. These emissions represented 10.3% of national GHG emissions in 2020 (MLA, 2022). More than 90% of red meat industry GHG emissions were associated with grazing and land management. Feedlot production contributed 5.8% of total GHG emissions, and processing contributed another 2.1% (MLA, 2022). Australia's latest feedlot survey results reveal an increase in cattle on feed to 1,354,747 head in the first quarter (Jan-Mar, Q1) of 2024, making a record high and an increase of 60,216 head increase from the December 2023 quarter (Oct-Dec, Q4) (ALFA, 2024), reflecting a general herd rebuild associated with higher prices for feeder cattle and supply limitations.

The majority of greenhouse gases (GHG) emission from livestock production is in the form of methane (CH_4) 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, referred to as enteric emission (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 methane (CH_4) emissions would benefit the environment and possibly the efficiency of livestock production by preserving feed energy.

Technologies targeting direct abatement through the use of additives to significantly mitigate enteric emissions from beef cattle will become increasingly important as the livestock sector faces abatement targets set for 2030 and 2050. Solutions to decrease methane emissions of beef cattle are important to also align with the CN30 initiative as outlined in the Red Meat 2030 strategic plan. The amount of enteric methane produced by cattle is dependent on the level of feed intake and diet composition, including the type of carbohydrate and the impact of additives such as ionophores and oils (McAllister et al. 1996). 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).

Australian feedlots are integral in maintaining a supply of high-quality red meat to overseas and domestic markets and continue to service a growing demand for Australian beef. This industry currently contributes 0.5% of greenhouse gases, including enteric methane, to the national inventory (MLA, 2021). In general, larger cattle with high maintenance and growth requirements compared to animals not in a production feeding operation consume more feed and generate greater enteric methane emissions. The composition of the feed also influences enteric methane losses (Johnson and Johnson, 1995); over the short-term (weeks) diets consisting of high forage increase methane production (g CH_4/day), yield (g CH_4/kg dry matter intake) and loss as % of gross energy (GE) intake relative to high grain diets (Beauchemin and McGinn, 2005). Moving forward, their intensive management enables them to consider implementing dietary changes, thereby playing a significant role in stabilizing and reducing emissions within their sector. This underscores their continued importance in the years to come.

A bioactive extract derived from the seaweed *Asparagopsis* spp. in a canola carrier oil (SEAFEED™; Asp-Oil) has been demonstrated to achieve reductions in methane 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 (Cowley et al., 2023). Bromoform is hydrophobic so it associates well with oil giving greater stability to bromoform than what was achieved with the freeze-dried *Asparagopsis* (ASP) powder. 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 and bromoform based products such as Asp-Oil will not just rely on methane mitigation, but also effects on cattle performance, carcase value and cost of gain. 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. 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. Further, the cost of Asp-Oil is sufficiently high that its uptake and commercialisation will likely be seen in high-quality beef brands in the first instance. Due to the high cost per kilogram, it is important these brands do not jeopardise their intrinsic eating quality of the beef they produce. 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.

Most published research on feeding freeze-dried *Asparagopsis* to beef using a smaller titration nutrition experiment has been conducted using 20 animals 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 hay 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 *A. taxiformis* inclusion based on organic matter intake, each consisting of five animals. Although the meat quality of these 20 animals was assessed through consumer sensory, 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. This meant that the animals and meat samples selected were not uniform across the control and treated groups in terms of their carcase attributes and primal location, possibly leading to confounded results however that will always be unknown. In addition, carcases and meat samples selected for the consumer testing were not done as per standard MSA protocol.

For adoption of bromoform based products by the beef 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. 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 a bromoform based product. Recently, the longest feeding trial (275 days) of a canola oil-based product (Asp-Oil) was on 76 Wagyu cross steers in Australia reported to date by Cowley et al. (2023) followed by an additional untrained consumer analysis of meat eating quality. Increased understanding of Asp-Oil 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.

This project is a joint initiative between Rangers Valley Cattle Station Pty Ltd, Meat and Livestock Australia (MLA) and Sea Forest Limited and aimed to determine the impact of feeding SEAFEED™ (Asp-oil) infused oil on the meat eating quality and post-mortem ageing potential of mid-fed beef (150 days, 100 days on Asp-Oil). Rangers Valley has a large suite of high-quality beef brands which are prominent in the domestic and international markets. This commercial feeding trial evaluated the inclusion of Asp-Oil in a total mixed ration for Angus steers over the last 100 days across three independent kill groups. Carcase data was collected for individual animals and samples of muscle for the determination of bromoform in meat eating quality attributes.

This is important research for the Australian beef industry to allow for Asp-Oil to be commercially accepted across the industry. If Asp-Oil does impact eating quality, its use will be limited to low quality beef cattle. The results of this trial will help inform Sea Forest Ltd and Rangers Valley on the potential scaling up for supply and use of Asp-Oil in finishing periods and provide (at the discretion of Rangers Valley) the Australian feedlot sector with information on the application of the technology in commercial operations.

2. Objectives

The objectives of this project were to determine the effect of Asp-Oil inclusion in a feedlot total mixed ration on:

- The meat eating quality of mid-fed beef (150 days, 100 days on Asp-Oil).
- The post-mortem ageing potential of meat from mid-fed beef (150 days, 100 days on Asp-Oil).
- The carcase traits of mid- fed beef (150 days, 100 days on Asp-Oil).

3. Methodology

3.1 Experimental Design

The effect of Asp-Oil inclusion in a finisher total mixed ration (TMR) on animal performance, carcase characteristics and meat-eating quality of Angus steers was assessed with two dietary treatments (with Asp-Oil or without Asp-Oil [Control]). Asp-Oil was included in the TMR for the final 100 days of their finishing period. The trial was conducted at Rangers Valley Feedlot, near Glen Innes NSW. Three standard feedlot pens (1650 m^2) in the same row with a pen separating treatments were allocated to the trial. The pens were orientated with a slope 2.5% east to west (from front of pen to back), along the row of pens. Each pen provided 16.17 m^2/head pen space and was fitted with a reticulated water trough of 3.0m length. The Asp-Oil treated pens were at the lowest point in the row to prevent any contamination of feed runoff down the feed trough. The trial design had two treatments and three blocks per treatment, blocked by days on feed (DOF) and equally allocated by vendors.

3.2 Animals and feeding

This experiment utilised 600 mid-fed (150 DOF) Angus cattle. Cattle were transported to feedlots by truck and feedlot entry was managed under standard industry practice through the on-site processing shed. They were initially inducted after arrival and all cattle received new ear tags, and initial live weight was recorded. All steers were HGP free. Steers were allocated to a treatment group based on vendor and stratified for induction weight to ensure an equal distribution between treatments.

These cattle were fed in 3 consignment/kill groups (n = 200 head per consignment). They were initially fed in pens of 200 for the first 50 days to acclimate to feedlot feeding and ration composition. Following the initial 50 days the groups were allocated to treatment groups divided into 2 pens of 100 in Table 1 (n = 100 head per pen but some cattle were removed over the feeding period due to health issues) for the experiments based on vendor and stratified for induction weight.

Table 1: Kill dates, number in each pen replicate, total days on feed, days fed the Asparagopsis oil, loading order out of the feedlot and kill order at the abattoir

Feedlot Pen	Kill Date	Head	Treatment	Total Days on feed	Days on Asp-Oil	Loading order	Kill order
1	27/01/23	98	Control	150	0	1	1
2	27/01/23	99	Asp-Oil	150	136	2	2
3	10/03/23	97	Control	180	0	2	2
4	10/03/23	98	Asp-Oil	180	112	1	1
5	26/05/23	94	Control	120	0	1	1
6	26/05/23	94	Asp-Oil	120	108	2	2

The treatment diets were a standard Rangers Valley mid fed ration with Asp-Oil or without (Control). The Asp-Oil treated diet contained Asp infused oil as a partial substitute of normal vegetable oil inclusion to ensure diets were isoenergetic. Mean inclusion of Asp-Oil in the ration prepared on a daily basis was 1.1% by weight to deliver 25.6 mg/kg Bromoform per kilogram of feed. The Asp-Oil diet was a preparation of solvent-extracted canola oil as a carrier for the halogenated bioactive compounds (namely bromoform) extracted from the gametophyte of *Asparagopsis armata* grown by Sea Forest Ltd in Tasmania.

- Treatment 1 (without Asp-Oil - Control): received a normal mid-fed ration
- Treatment 2 (with Asp-Oil): received a mid-fed ration with Asp-Oil inclusion

3.3 Slaughter and carcase characteristics

All steers were RFID scanned and bulk weighed in mobs of 20 and the total weight for the 20 head was recorded over a weighbridge between 0700-0900, dispatched at 1400-1600 (consignment 1&2) or 1300-1500 (consignment 3) as per processors' trucking schedule, transported by road (345 km) from feedlot to abattoir as a pen treatment group and slaughtered following standard industry practice. Animals were penned for lairage overnight (No mixing of pens occurred). Processing commenced at 0500 in order of truck arrival. The loading and kill order of treatments was changed each kill. The kill order of treatments was changed for each kill. At the kills, ear tags were collected, NLIS buttons were scanned and ID against body number was maintained.

Immediately post slaughter and dressing, hot standard carcase weight (HSCW, kg) and P8 fat depth (mm) were determined according to AUS-MEAT carcase standards (AUS-MEAT, 2005). Each carcase was evaluated between the 12/13th rib by a Meat Standards Australia (MSA) grader (Meat Standards Australia 2007), hump height (mm); MSA marbling score; subcutaneous rib fat depth (mm); ossification score; ultimate pH (pHu) and temperature; eye muscle area (EMA, cm^2), meat colour (MC) and fat colour (FC) were recorded. MSA marbling score was evaluated in chilled carcases against MSA reference standards at the quartering site and was estimated by scoring the amount, piece size and distribution of intramuscular fat deposited between individual fibres, on a scale ranging from 100 to 1100 (Romans et al., 1985; AUS-MEAT, 2005; MLA, 2007).

Subcutaneous rib fat depth was measured using a graduated metal ruler, at the quartering site positioned between the 12th and 13th rib (AUS-MEAT, 2005). Ossification score was determined on a scale described by Romans et al. (1985). Ultimate pH and loin temperature were measured in the M. *longissimus thoracis* at the time of carcase grading. Carcase temperature and pH were measured using MSA approved temperature and pH probes (TPS WP-80M pH Meter, TPS Pty Ltd., Springwood, Brisbane, Qld, 4127, Australia). Eye muscle area was determined for each carcase by measuring the M. *longissimus thoracis* at the quartering site using a standardised grid (AUS-MEAT, 2005).

3.4 Cooking protocol and Sensory Evaluation

Samples selected for meat eating quality were derived from the Striploin, Oyster Blade and Eye Round (Table 2). Across the three kill groups, cuts were collected from 72 treated and 72 control cattle (n = 144). Every 4th carcase for the kill was originally chosen for collection across the 2 treatment groups to balance for time of kill within the treatment. Additionally, the carcase attributes of the control and treated carcases were balanced to have similar means and standard deviations for MSA marbling, ossification and hot standard carcase weight. This meant that carcase selections deemed to be outliers were altered once the averages were analysed.

Consumer sensory evaluation of the samples was conducted by the University of New England. Five steak samples were processed from each sample (muscle x ageing period) and were assessed by untrained Australian consumers utilising MSA protocols for the grill cook method as described by (Watson et al., 2008). In total from this project, 720 samples were consumer tested. Briefly, each sample was denuded of all fat and epimysium and then cut to produce five consumer samples slices which were individually wrapped in plastic sheets before being placed into labelled cryovac bags. Two samples from the anterior striploin (STR045) and eye round (EYE075) were randomised for position and aged for two periods post-mortem – 7 and 28 days from each of the carcases selected. The oyster blade (OYS036) was aged for 7 days post-mortem. All samples were frozen prior to being allocated to their sensory sessions.

All samples were thawed in a refrigerator at 2°C for 24 hours before each sensory session and were then placed on trays 30 minutes prior to cooking to allow them to come to 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.

All consumers were served a starter steak in the first position followed by 6 test samples with the test meat samples allocated via a 6 x 6 Latin square to ensure each was served equally before and after other products and equally in each order. 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 asked to mark the sample as unsatisfactory (2 star), good every day (3 star), better than everyday (4 star) or premium quality (5 star). Every sample was tested by ten individual 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 (Watson et al., 2008).

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

The 3 cuts chosen are striploin (STR045) which has a moderate ageing rate and moderate/high quality, oyster blade (OYS036) which has a very fast ageing rate and high quality and the eye round (EYE075) which has a slow ageing rate and lower quality. This provided the variation in the experiment needed to determine if any effect of ASP on eating quality is via proteolysis or the concentration of connective tissue accumulation.

Table 2. Number and weight of Striploin, Oyster Blade and Eye Round collected at slaughter for meat eating quality assessment

Cut	Kill 1	Kill 2	Kill 3	(n)
Striploin	48	48	47	143
Oyster Blade	48	48	47	143
Eye Round	48	48	47	143

3.5 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 analyses 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.

Carcase traits were analysed in a linear model with treatment, kills, average daily gain (feedlot entry weight to exit weight), entry weight and HSCW as fixed effects based on the significance level of each variable (Table 3).

Table 3: Statistical models used for the carcase traits

Traits	Model
HSCW	Treatment + kills +ADG (feedlot entry to exit weight) + entry weight Or: Treatment + kills + entry weight
Hump	Treatment + kills + HSCW
EMA	Treatment + kills + HSCW
Ossification	Treatment + kills + HSCW + ADG (feedlot original entry weight to entry weight)
AUS-Meat marbling	Treatment + kills + HSCW
MSA marbling	Treatment + kills + HSCW
MSA Index	Treatment + kills + HSCW
P8 Fat	Treatment + kills + HSCW
Rib Fat	Treatment + kills + HSCW
Meat colour	Treatment + kills + HSCW
Fat colour	Treatment + kills + HSCW
pH	Treatment + kills + HSCW

Meat quality attributes for tenderness, juiciness, flavour, overall liking, MQ4 and satisfaction were analysed in a linear mixed effects model with treatment, cut, and days of ageing as fixed effects, and animal nested within pen as the random effect. 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 experiment was powered to pick up a 4-point difference in eating quality between treatments. With a standard deviation of 10 between animals, 72 samples per treatment group are needed at a 95% significance level to give an experimental power of 80%.

Mixed effect model for meat quality traits:

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

4. Results

4.1 Effect of treatment on carcase traits

4.1.1 Hot Standard Carcase Weight

There was no significant effect of treatment on hot standard carcase weight (HSCW, kg) regardless if the statistical model was corrected for average daily gain (ADG) from the feedlot entry weight to exit weight (Fig. 1 and Table 4) or not (Fig. 2 and Table 5). The first kill group had the highest carcase weights for both control and treated groups followed by the second and third kill groups. In the first analysis, ADG was significant when included as a covariate ($p<0.001$). As ADG increased by 1kg per day, carcass weight decreased by -15.01kg indicating that cattle that grow faster were lighter in HSCW which may relate to being at a younger stage of maturity (Table 4).

In both analyses of HSCW, entry weight had a significant effect on HSCW ($p<0.001$). In the model corrected for ADG, as entry weight increased by 1kg, HSCW increased by 0.64 (Table 4). However, in the model not adjusted for ADG, as entry weight increased by 1kg, HSCW increased by 0.53 (Table 5). Hence heavier at feedlot entry gives heavier carcasses by the dressing percentage equivalence.

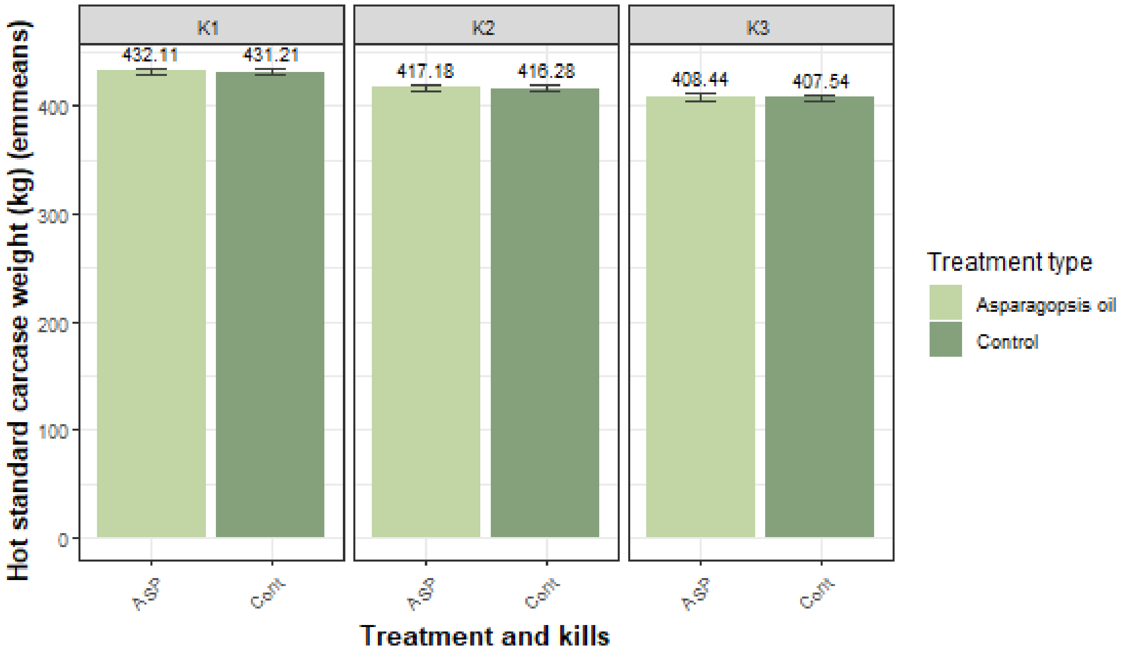

Figure 1. Effect of treatment and kill group on hot standard carcase weight (kg) having average daily gain (ADG) and error bar represent confidence intervals (CI) around the estimated marginal means

Table 4. Effect of treatment on carcase weight (Including ADG)

Predictors	Estimates	95% Confidence Interval	p
Intercept	98.97	73.37 – 124.56	**<0.001**
Treatment Asp-Oil	0.90	-2.15 – 3.94	0.562
KillsK2	-14.93	-18.67 – -11.18	**<0.001**
KillsK3	-23.67	-27.70 – -19.64	**<0.001**
ADG	-15.01	-19.30 – -10.72	**<0.001**
Entry weight	0.64	0.59 – 0.70	**<0.001**

ADG= Average daily gain (from feedlot entry day to research allocation day); Entry weight: Average weight on the research allocation day; and p= p-value

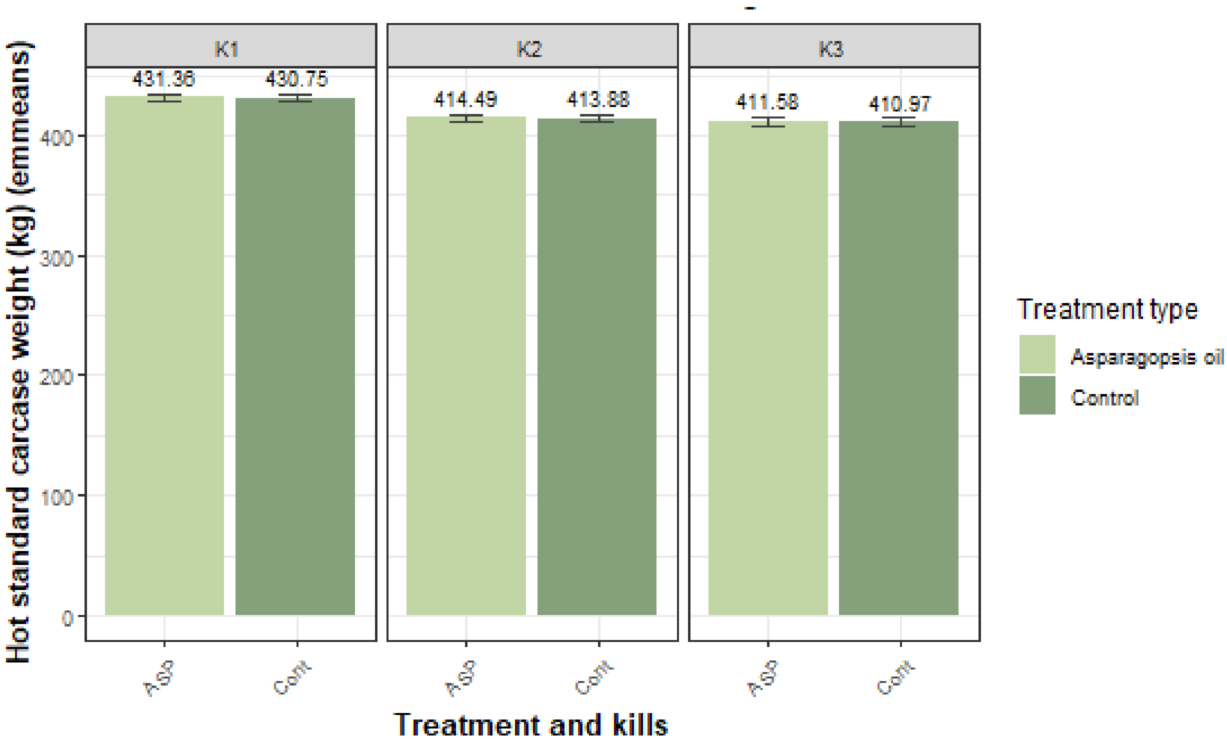

Figure 2. Effect of treatment and kill group on hot standard carcase weight (kg) without average daily gain (ADG) and error bar represent confidence intervals (CI) around the estimated marginal means

Table 5. Effect of treatment on carcase weight (Without ADG)

Predictors	Estimates	95% Confidence Interval	p
Intercept	137.59	113.59 – 161.59	**<0.001**
Treatment Asp-Oil	0.61	-2.55 – 3.78	0.704
KillsK2	-16.87	-20.72 – -13.02	**<0.001**
KillsK3	-19.78	-23.81 – -15.76	**<0.001**
Entry weight	0.53	0.49 – 0.58	**<0.001**

Entry weight: Average weight on the research allocation day; and p= p-value

4.1.2 Hump height

Hump height was measured by the MSA accredited grader and recorded in gradients of 5mm. It is primarily used to verify the tropical breed content indicated on the MSA vendor declaration. The average hump height was trending higher in the Asp-Oil treated group by 1.35mm compared to the control group (Table 6, p=0.068, Figure 3). HSCW significantly impacted hump height ($p<0.001$, Table 6) with a 1kg increase in HSCW increasing hump height by 0.12mm.

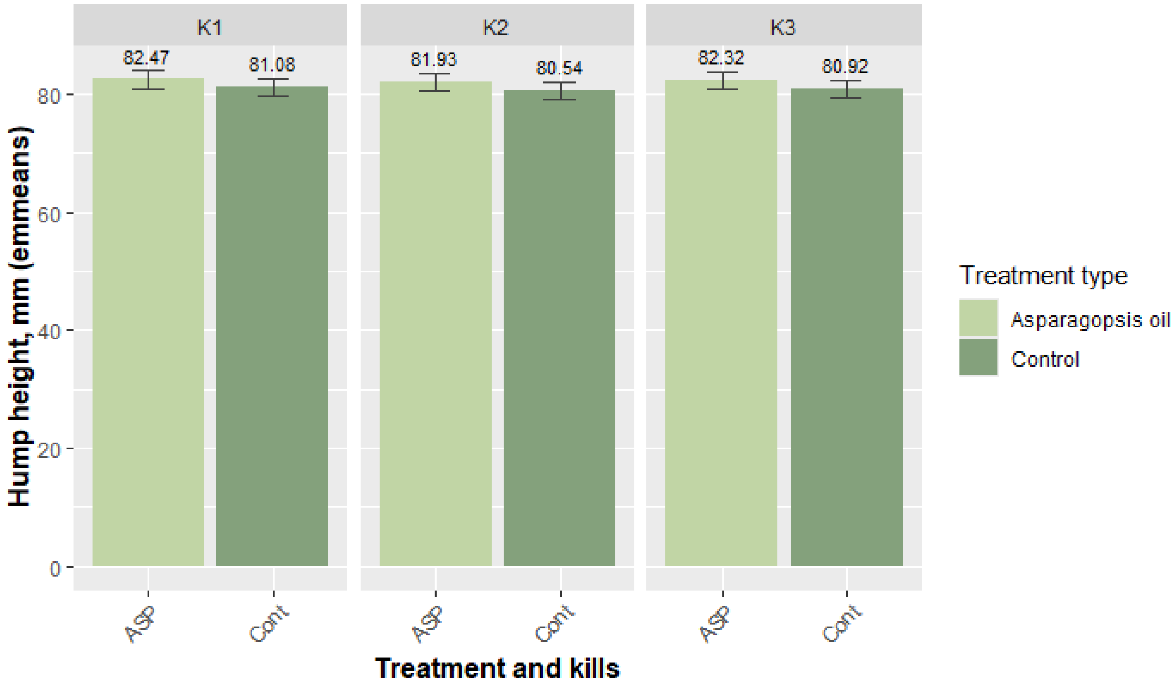

Figure 3. Effect of treatment and kill group on hump height (mm) and error bar represent confidence intervals (CI) around the estimated marginal means

Table 6. Effect of treatment on hump height (mm)

Predictors	Estimates	95% Confidence Interval	p
Intercept	31.03	19.56 – 42.49	**<0.001**
Treatment Asp-Oil	1.35	-0.10 – 2.81	0.068
KillsK2	-1.26	-3.07 – 0.55	0.174
KillsK3	-1.40	-3.20 – 0.39	0.125
HSCW	0.12	0.09 – 0.15	**<0.001**

HSCW= Hot standard carcase weight; and p= p-value

4.1.3 Eye muscle area

Eye Muscle Area (EMA) is the area of the Eye Muscle (*longissimus dorsi*) measured in square centimetres. MSA Graders used an AUSMEAT-approved grid for taking the measurement. There was no statistically significant difference (p>0.05) between the control and treatment group for the eye muscle area (Fig. 4 and Table 7). However, the EMA for the second and third kill groups were 1.69 and 5.8cm^2 larger than the first kill group (p<0.05). HSCW also had a significant effect on EMA (p<0.001, Table 7). As HSCW went up by 1 kg, EMA increased by 0.13cm^2.

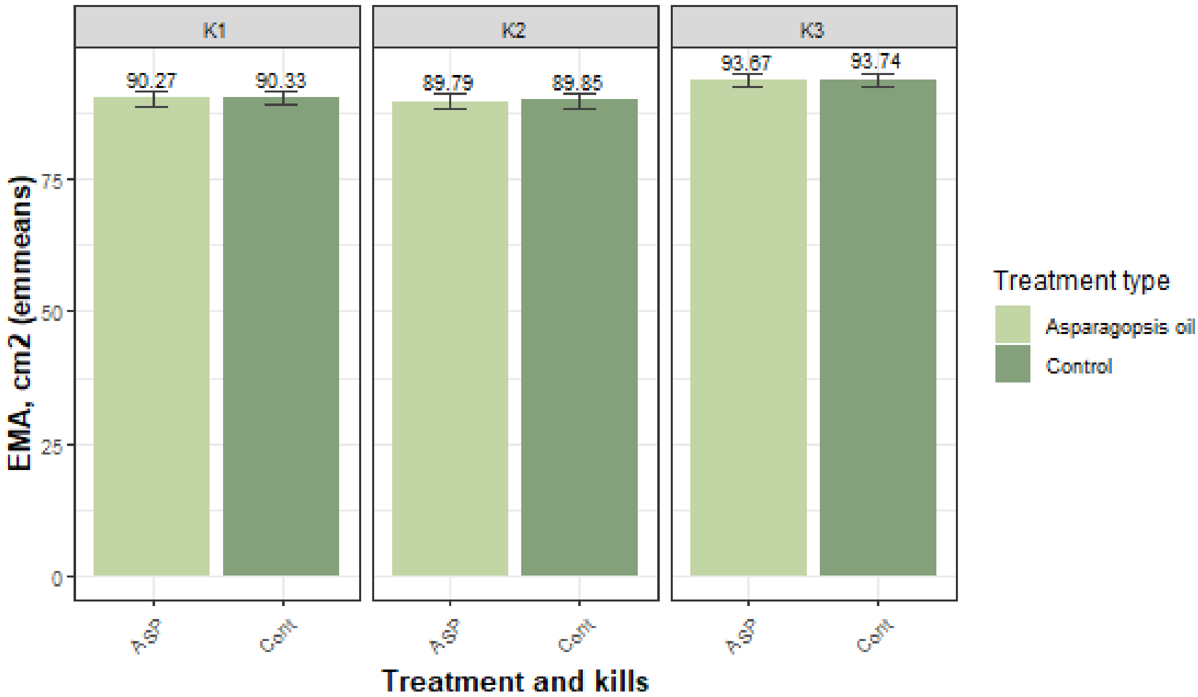

Figure 4. Effect of treatment and kill group on Eye Muscle Area and error bar represent confidence intervals (CI) around the estimated marginal means

Table 7. Effect of treatment on EMA (cm^2)

Predictors	Estimates	95% Confidence Interval	p
Intercept	34.33	24.60 – 44.07	**<0.001**
Treatment Asp-Oil	-0.16	-1.40 – 1.07	0.795
KillsK2	1.69	0.15 – 3.23	**0.031**
KillsK3	5.80	4.28 – 7.33	**<0.001**
HSCW	0.13	0.11 – 0.15	**<0.001**

HSCW= Hot standard carcase weight and p= p-value

4.1.4 Ossification score

The ossification score, alongside the hot standard carcase weight (HSCW), is utilized to develop the physiological maturity of beef carcases by assessing bone development in the vertebrae. This measurement correlates with the level of collagen fibre development, which impacts the tenderness of cooked meat. Higher levels of ossification can toughen meat when cooked, with the extent of toughening varying across different cuts based on their connective tissue content. Ossification scores range from 100 to 590 and increase in increments of 10, with lower scores indicating less physiological maturity.

There were no significant differences ($p>0.05$) between the control and treated groups (Fig. 5 and Table 8). There were also no significant differences ($p>0.05$) between the kill groups for ossification (Fig. 5 and Table 8). There was a significant impact of HSCW and ADG on ossification score ($p<0.001$). As HSCW increased by 1 kilogram, ossification also increased by 0.11 and as ADG increased by 1kg per day, ossification decreased by 4.29 points (Table 8).

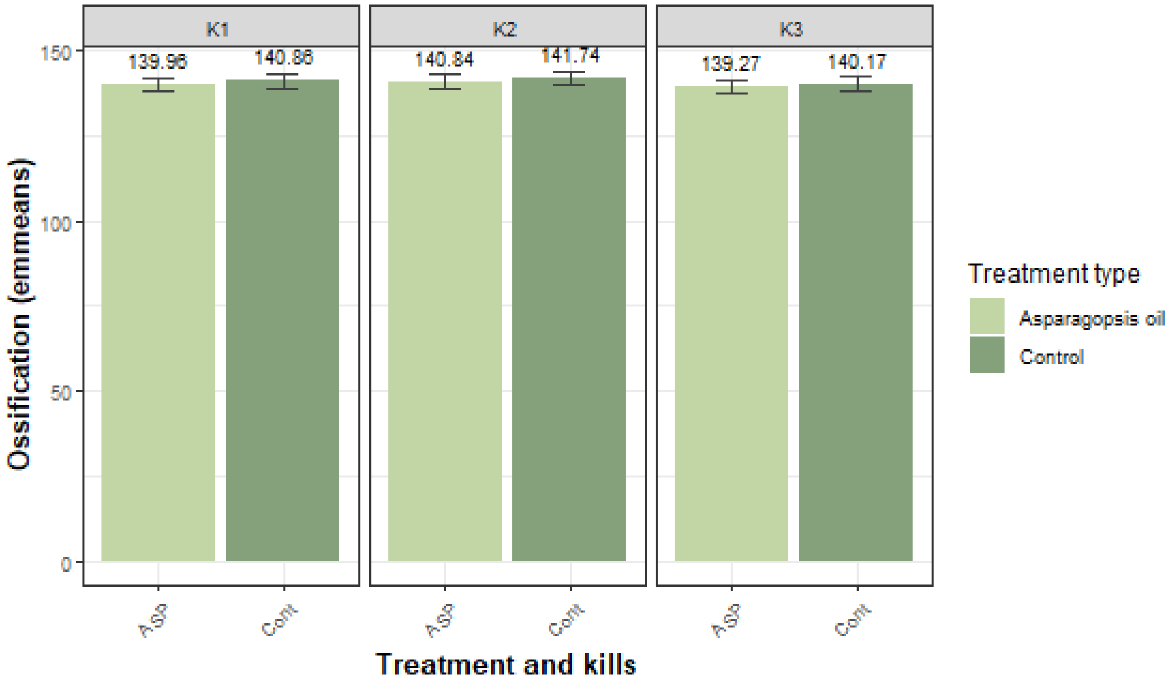

Figure 5. Effect of treatment and kill group on ossification (MSA grader) and error bar represent confidence intervals (CI) around the estimated marginal means

Table 8. Effect of treatment on ossification

Predictors	Estimates	95% Confidence Interval	p
Intercept	101.51	85.54 – 117.48	**<0.001**
Treatment Asp-Oil	-0.90	-2.92 – 1.13	0.384
KillsK2	0.88	-1.70 – 3.45	0.504
KillsK3	-0.69	-3.19 – 1.80	0.586
HSCW	0.11	0.07 – 0.15	**<0.001**
ADG	-4.29	-6.65 – -1.93	**<0.001**

HSCW= Hot standard carcase weight; ADG= Average daily gain (from feedlot entry day to research allocation day); and p= p-value

4.1.5 MSA Marbling score

The term marbling refers to the appearance of white flecks or streaks of fatty tissue between the muscle fibres in meat. Marbling evaluation typically occurs from the 5th to the 13th rib on the carcase, involving the observation of fat deposits within the muscle tissue. Meat Standards Australia (MSA) utilizes marbling standards to estimate the amount, piece size and distribution of fat within the eye muscle. Scores range from 100 to 1190 in increments of ten. Marbling is deposited unevenly throughout the body, increasing towards the head and decreasing towards the tail.

Marbling was not significantly ($p>0.05$) impacted by the ASP treatment (Figure 6 and Table 9). The marbling for the third group was significantly higher compared to the first kill group (p=0.005) and HSCW also had a significant impact on MSA marbling (Table 9, p<0.001). As HSCW increased by 1kg, MSA marbling increased by 0.66 points (Table 9).

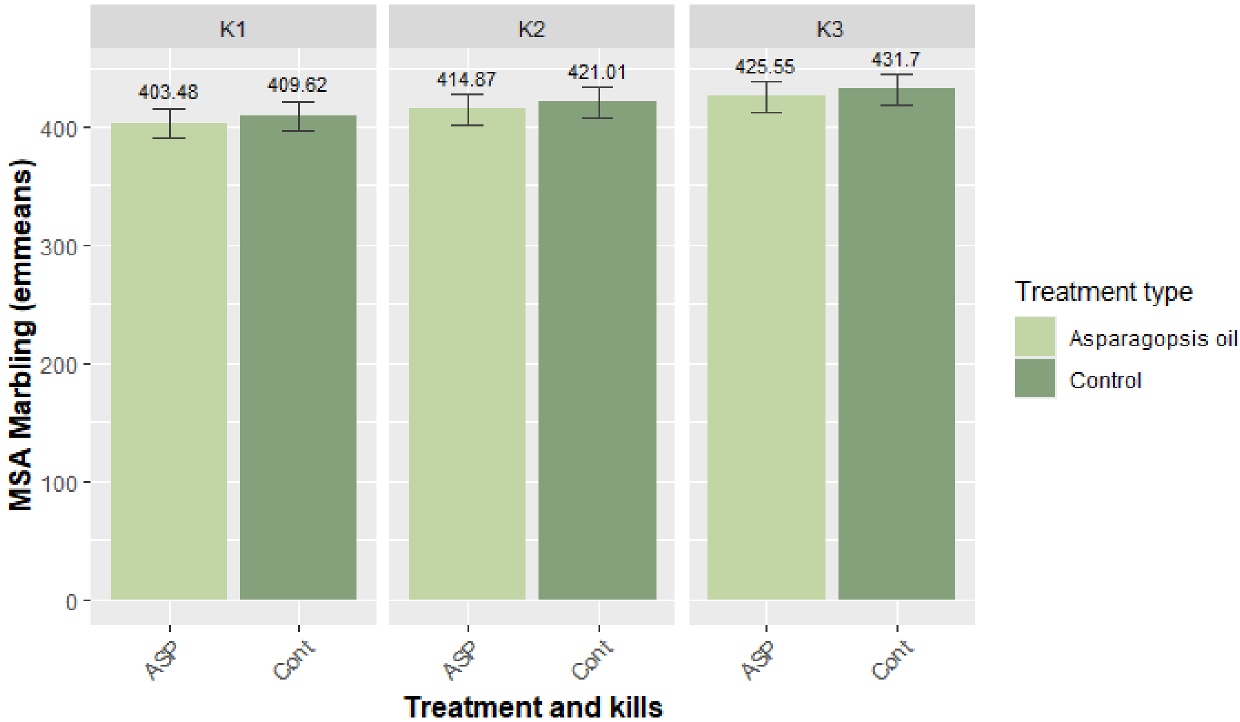

Figure 6. Effect of treatment and kill group on marbling and error bar represent confidence intervals (CI) around the estimated marginal means

Table 9. Effect of treatment on MSA marbling

Predictors	Estimates	95% Confidence Interval	p
Intercept	132.11	32.90 – 231.33	**0.009**
Treatment Asp-Oil	-6.14	-18.73 – 6.44	0.338
KillsK2	11.39	-4.29 – 27.07	0.154
KillsK3	22.08	6.56 – 37.60	**0.005**
HSCW	0.66	0.43 – 0.89	**<0.001**

HSCW= Hot standard carcase weight; CI= Confidence interval and p= p-value

4.1.6 AUS-MEAT Marbling

AUS-MEAT marbling is the intramuscular fat present on the eye muscle at the assessment site. MSA graders use the AUS-MEAT range 0 (no intramuscular fat) to 9 (extreme amounts of intramuscular fat). The Asp-Oil treatment did not affect AUS-MEAT marbling (p>0.05, Figure 7 and Table 10). The AUS-MEAT marbling was also significantly higher for the third kill group than the first kill group by 0.19 scores (p=0.023, Table 10). HSCW also had a significant impact on AUSMEAT marble score with high carcass weights increasing the AUS-MEAT marble scores (p<0.001, Table 10).

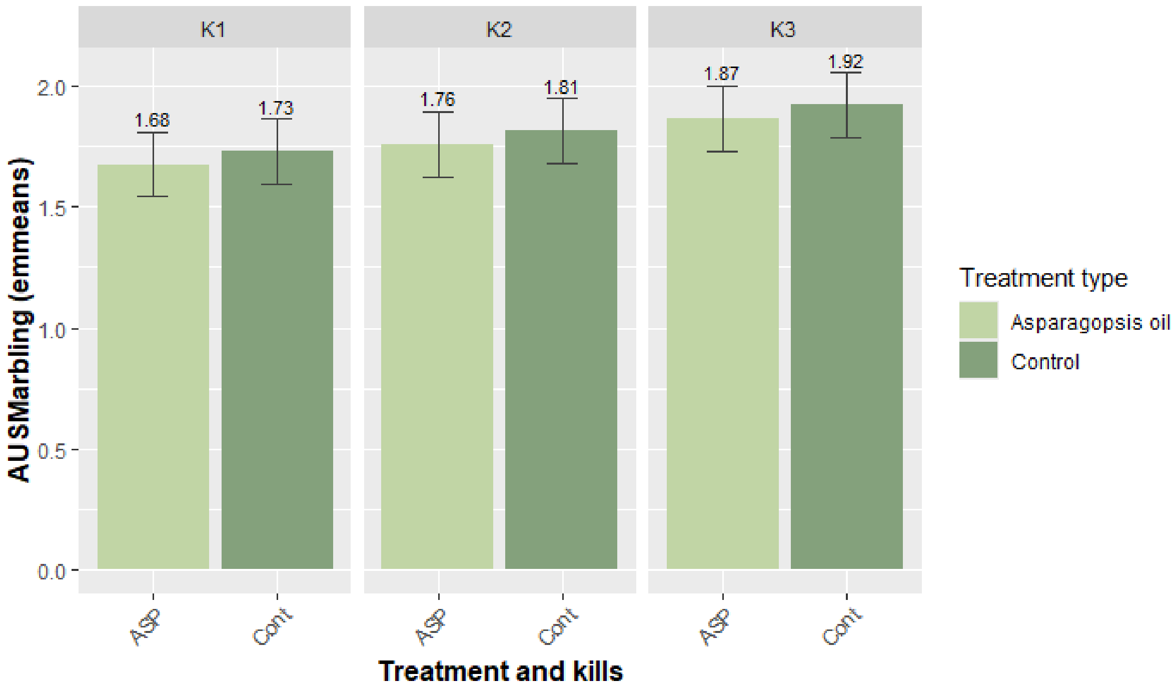

Figure 7. Effect of treatment and kill group on AUS-MEAT Marbling and error bar represent confidence intervals (CI) around the estimated marginal means

Table 10. Effect of treatment on AUS-MEAT Marbling

Predictors	Estimates	95% Confidence Interval	p
Intercept	-1.01	-2.06 – 0.05	0.062
Treatment Asp-Oil	-0.05	-0.19 – 0.08	0.440
KillsK2	0.08	-0.08 – 0.25	0.322
KillsK3	0.19	0.03 – 0.36	**0.023**
HSCW	0.01	0.00 – 0.01	**<0.001**

HSCW= Hot standard carcase weight; and p= p-value

4.1.7 MSA Index

The Meat Standards Australia (MSA) Index was developed for producer feedback systems to rank the potential eating quality of beef carcases (McGilchrist et al., 2019). The MSA index is a score between 0 -100, applied to each carcase (that meets MSA specifications) and is independent of any processing impacts. Generally, an increase in the MSA index will result in a carcase with increased MSA eating quality scores. The MSA Index was not affected by the inclusion of Asp-Oil in the mixed ration (p>0.05, Table 11 and Figure 8). Kill 3 had an MSA Index 0.42 points higher than kill 1 (Table 8, p=0.004) and HSCW had a significant positive effect on MSA index. As HSCW increased by 1kg, MSA Index increased by 0.01 (p<0.001, Table 11).

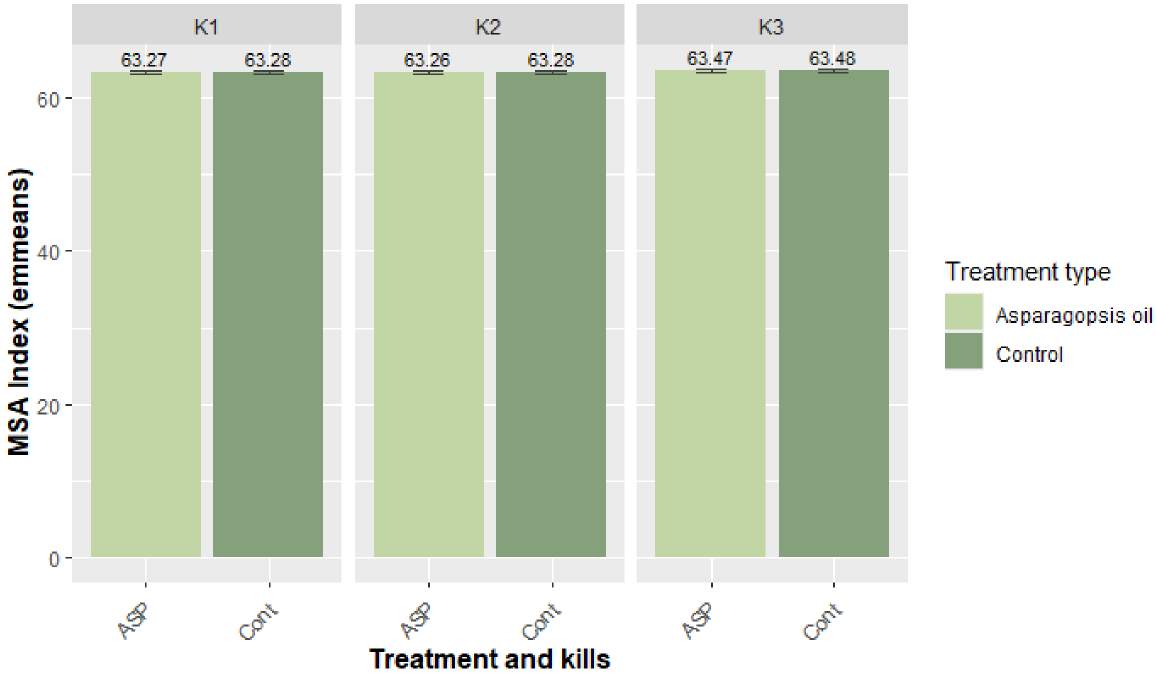

Figure 8. Effect of treatment and kill group on MSA Index and error bar represent confidence intervals (CI) around the estimated marginal means

Table 11. Effect of treatment on MSA Index

Predictors	Estimates	95% Confidence Interval	p
Intercept	58.39	56.55 – 60.24	**<0.001**
Treatment Asp-Oil	-0.01	-0.24 – 0.23	0.960
KillsK2	0.12	-0.17 – 0.41	0.420
KillsK3	0.42	0.13 – 0.71	**0.004**
HSCW	0.01	0.01 – 0.02	**<0.001**

HSCW= Hot standard carcase weight; and p= p-value

4.1.8 P8 Fat

Fatness plays a crucial role in beef carcases and the MSA accredited grader must assess the distribution of fat over primals to ensure coverage is adequate to prevent severe chilling. The P8 site is defined as the point at the junction of a line centered on the crest of the third (high) sacral vertebra and a line parallel to the backbone, centered on the pin bone. Carcases with very low-fat depths, such as 0, 1, or 2 millimetres, might be considered excessively lean, while those between 12 to 15 millimetres could be deemed optimal and those exceeding 20 millimetres overly fatty. The fat depth at the P8 site reflects the carcase fat percentage and subsequent meat yield.

The P8 fat depth remained unaffected by the ASP treatment (*p*>0.05, Table 12); however, the kill 2 group had significantly lower fat depth at the P8 site by 3.49mm compared to the kill group 1 (p<0.001, Figure 9, Table 12). HSCW had a significant positive effect on P8 fat (p=0.025) with a 1kg increase in HSCW increasing P8 Fat by 0.02mm (Table 12).

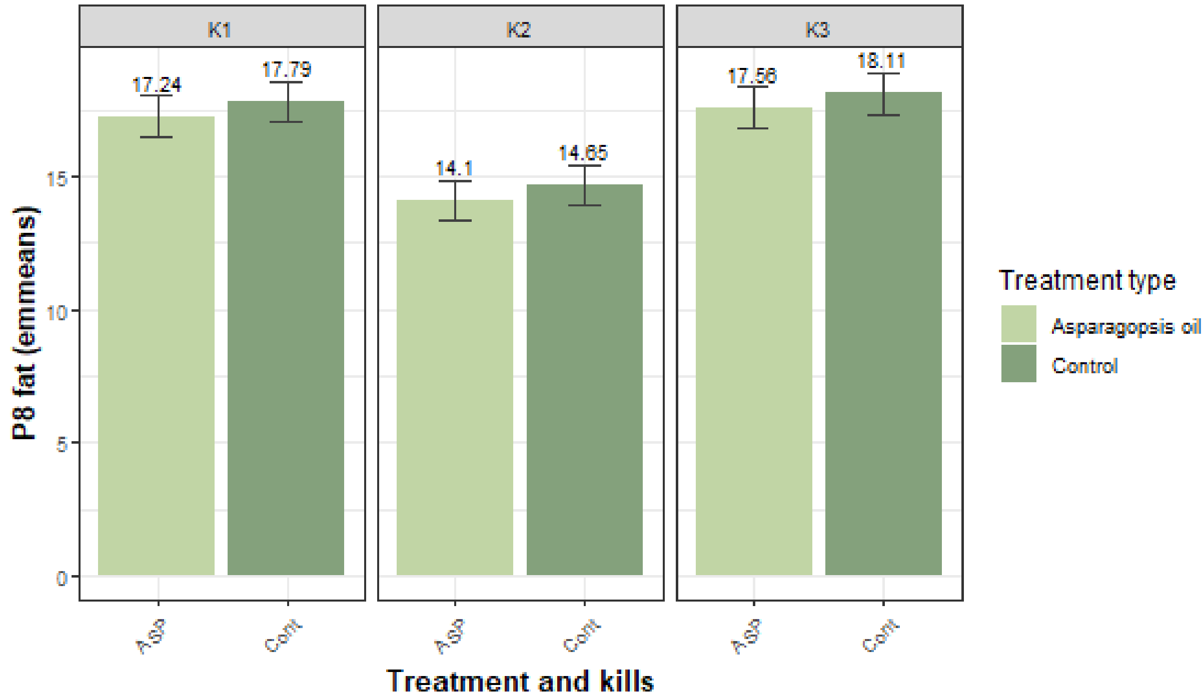

Figure 9. Effect of treatment and kill group on P8 fat and error bar represent confidence intervals (CI) around the estimated marginal means

Table 12. Effect of treatment on P8 fat

Predictors	Estimates	95% Confidence Interval	p
Intercept	11.58	5.74 – 17.43	**<0.001**
Treatment Asp-Oil	-0.57	-1.31 – 0.17	0.132
KillsK2	-3.49	-4.41 – -2.57	**<0.001**
KillsK3	-0.30	-1.21 – 0.62	0.524
HSCW	0.02	0.00 – 0.03	**0.025**

HSCW= Hot standard carcase weight; and p= p-value

4.1.9 Subcutaneous Rib fat (mm)

Subcutaneous rib fat represents the depth of subcutaneous fat over the quartered rib site, between the 5th to the 13th ribs. Rib fat thickness may vary across sites and tends to increase towards the head (5th rib). Unlike P8 fat, which is measured on the hot carcase on the slaughter floor, rib fat is measured after chilling and quartering the carcase.

ASP treatment did not affect rib fat thickness (*p*>0.05); however, the kill 3 group showed a significant increase in fat thickness by 0.83mm compared to the kill 1 group (p=0.039, Figure 10 and Table 13). HSCW had a significant positive effect on rib fat (p=0.002) with a 1kg increase in HSCW increasing rib fat by 0.02mm (Table 13).

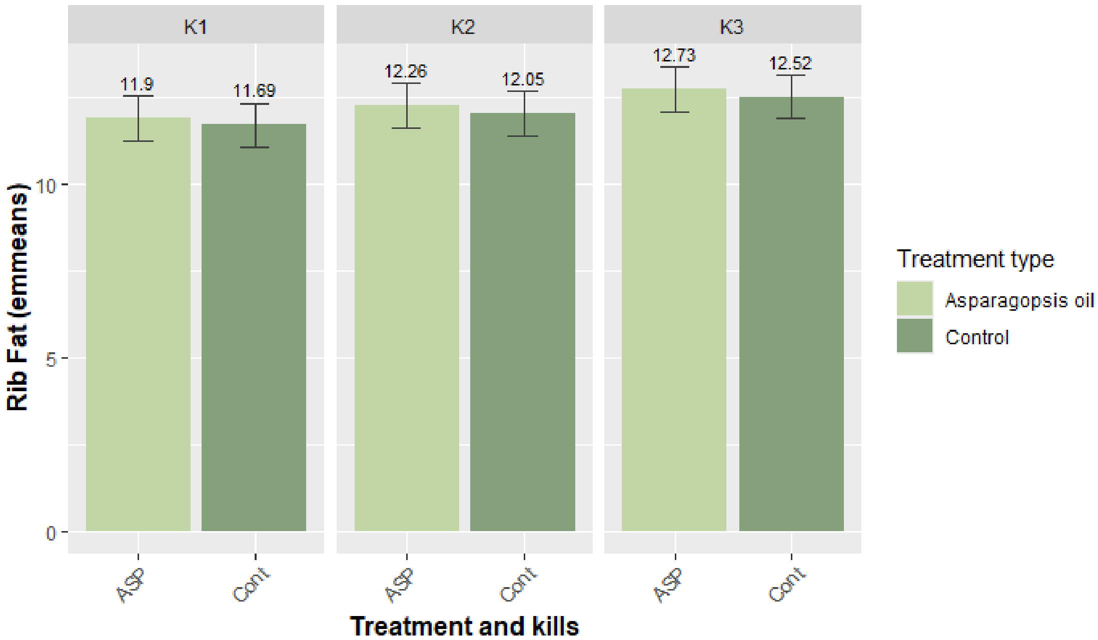

Figure 10. Effect of treatment and kill group on Rib fat (MSA grader) and error bar represent confidence intervals (CI) around the estimated marginal means

Table 13. Effect of treatment on Rib fat

Predictors	Estimates	95% Confidence Interval	p
Intercept	3.90	-1.12 – 8.91	0.127
Treatment Asp-Oil	0.21	-0.43 – 0.85	0.517
KillsK2	0.36	-0.43 – 1.15	0.373
KillsK3	0.83	0.04 – 1.61	**0.039**
HSCW	0.02	0.01 – 0.03	**0.002**

HSCW= Hot standard carcase weight; CI= Confidence interval and p= p-value

4.1.10 Meat Colour

Meat colour is evaluated at the rib eye muscle (M. *longissimus thoracis*) on the chilled carcase and scored against the AUS-MEAT Meat colour reference standards 1A (very pale) to 7 (very dark purple). In this study, the inclusion of Asp-Oil in the diet had no impact on meat colour ($p>0.05$, Table 14 and Figure 11). There was no difference between kill groups ($p>0.05$) but HSCW did significantly impact meat colour ($p=0.005$, Table 14).

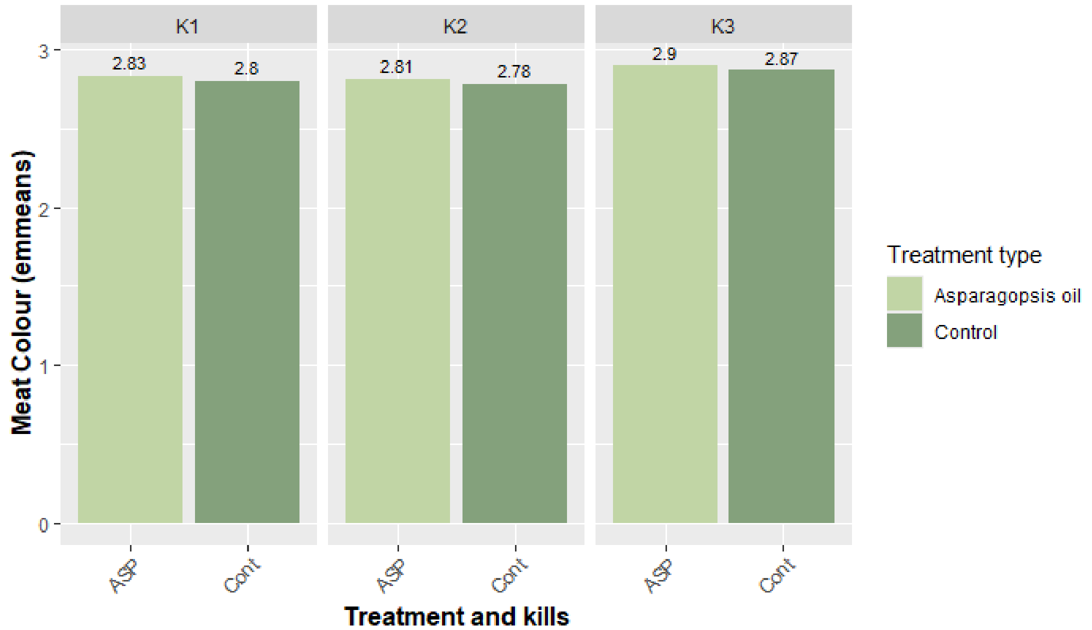

Figure 11. Effect of treatment and kill group on Meat colour and error bar represent confidence intervals (CI) around the estimated marginal means

Table 14. Effect of treatment on meat colour

Predictors	Estimates	95% Confidence Interval	p
Intercept	3.90	3.14 – 4.67	**<0.001**
Treatment Asp-Oil	0.04	-0.05 – 0.14	0.376
KillsK2	0.01	-0.12 – 0.13	0.929
KillsK3	0.05	-0.06 – 0.17	0.371
HSCW	-0.001	-0.00 – -0.00	**0.005**

HSCW= Hot standard carcase weight; CI= Confidence interval and p= p-value

4.1.11 Fat Colour

Fat colour is usually recorded using AUS-MEAT chips from 0 (white) to 9 (yellow). The Asp-Oil inclusion in the diet did not significantly ($p>0.05$) affect the fat colour; however, the second and third kill groups had significantly higher fat colours by 0.32 units compared to kill 1 group ($p<0.001$, Figure 12, and Table 15).

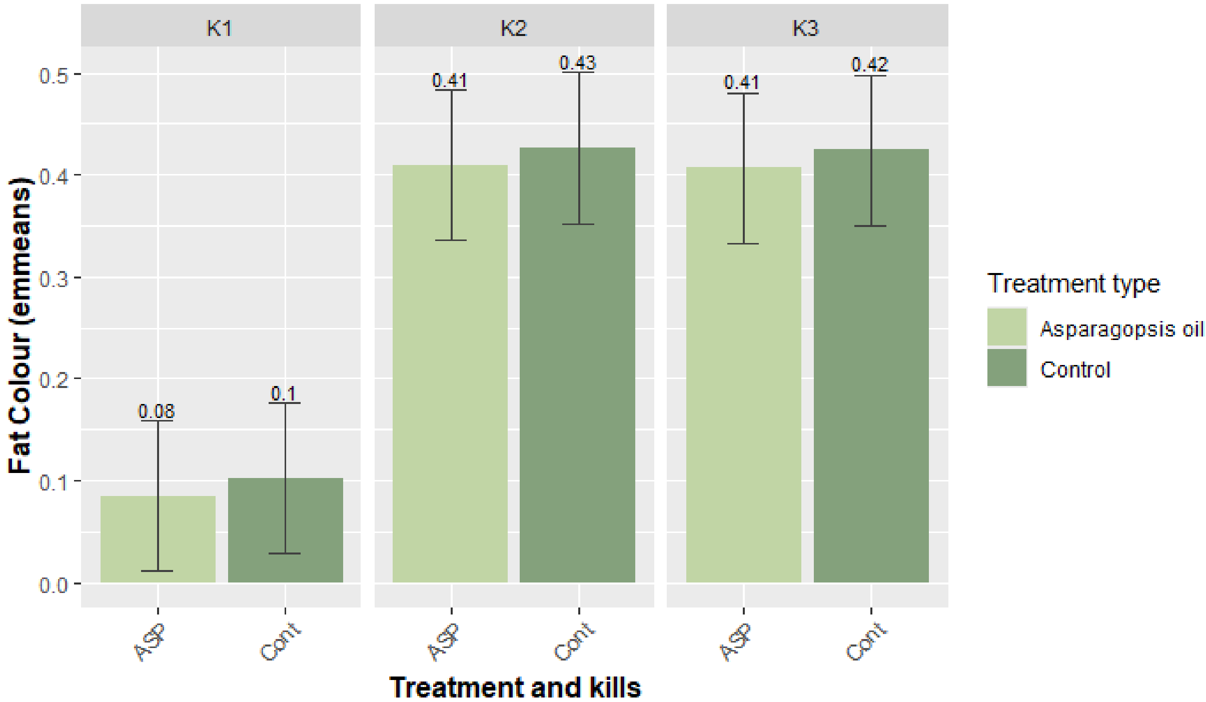

Figure 12. Effect of treatment and kill group on Fat colour and error bar represent confidence intervals (CI) around the estimated marginal means

Table 15. Effect of treatment on fat colour

Predictors	Estimates	95% Confidence Interval	p
Intercept	0.52	-0.06 – 1.10	0.076
Treatment Asp-Oil	-0.02	-0.09 – 0.06	0.645
KillsK2	0.32	0.23 – 0.42	**<0.001**
KillsK3	0.32	0.23 – 0.41	**<0.001**
HSCW	-0.00	-0.00 – 0.00	0.144

HSCW= Hot standard carcase weight; and p= p-value

4.1.12 Loin pH (Ultimate pH)

Ultimate pH is a measurement of lactic acid within the muscle. The optimum pH level of meat is 5.3 - 5.7, with levels above this being downgraded to a non-MSA product. The ultimate pH was significantly (p<0.05) reduced by the inclusion of Asp-Oil in the diet by 0.03 pH units (p<0.001, Figure 13, and Table 16). The 3 kill groups also all differed in pH_u (p<0.001, Figure 13, and Table 16) with kill group 2 having the lowest pH_u and kill group 3 having the highest. HSCW also had a significant effect on pH_u (p<0.001, Table 16) with heavier carcasses having lower pH's.

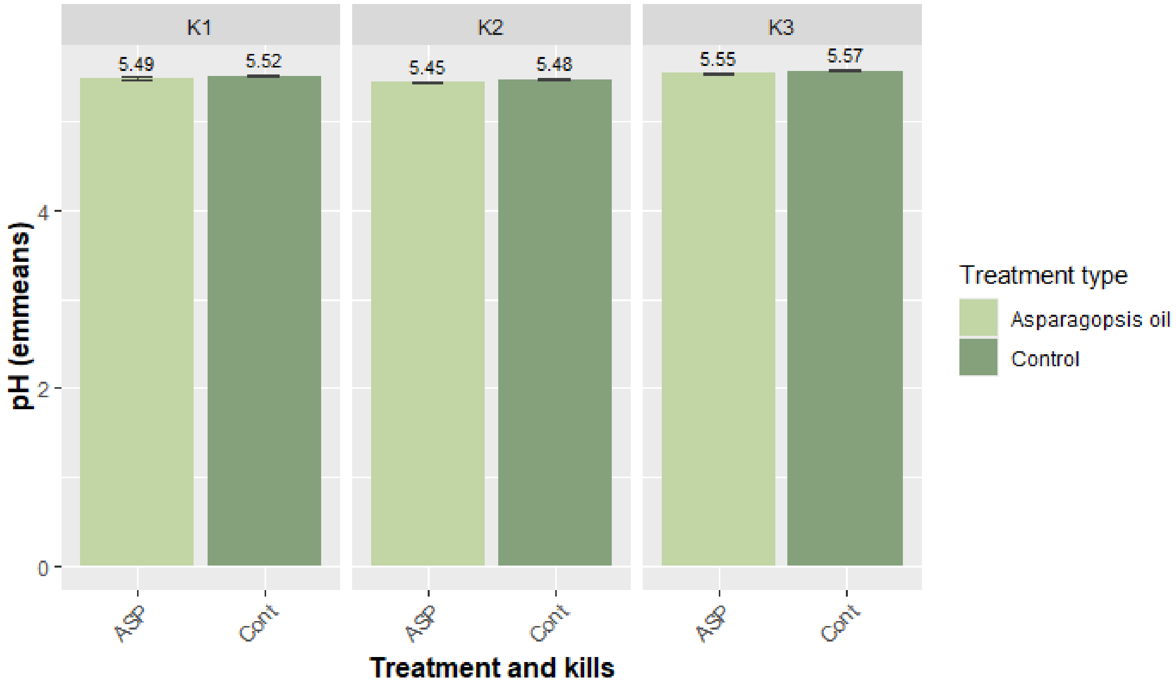

Figure 13. Effect of treatment and kill group on loin pH and error bar represent confidence intervals (CI) around the estimated marginal means

Table 16. Effect of treatment on loin pH

Predictors	Estimates	95% Confidence Interval	p
Intercept	5.69	5.60 – 5.77	**<0.001**
Treatment Asp-Oil	-0.03	-0.04 – -0.02	**<0.001**
KillsK2	-0.04	-0.06 - -0.03	**<0.001**
KillsK3	0.05	0.04 – 0.07	**<0.001**
HSCW	-0.00	-0.00 - -0.00	**<0.001**

HSCW= Hot standard carcase weight and p= p-value

4.2 Effect of treatment on meat eating quality

4.2.1 Effect of treatment and ageing on tenderness

There was no significant difference in tenderness due to Asp-Oil inclusion in the diet between the control and treatment groups, however, tenderness was reduced by 0.32 points (P>0.05, Figure 14 and Table 17) which is minimal. Notably, cuts OYS036 and STR045 exhibited significantly higher tenderness scores by 41.0 and 35.1 points, respectively, when compared to EYE075 points (*p<0.001*, Figure 14 and Table 17). These results imply the inherent properties of different muscles, for instance, muscles that are more frequently used have more connective tissue and are less tender.

Moreover, tenderness showed improvement with ageing, with an increase of 4.84 points, ranging from 3.31 to 6.47 (*p<0.001*, Figure 14 and Table 17). There was no significant interaction between cut and ageing indicating that both the EYE075 and STR045 both aged the same amount. This improvement with ageing is consistent with the general understanding that meat tenderness tends to enhance over time due to the enzymatic breakdown of muscle fibres and to a lesser extent, connective tissues. Additionally, marbling and hump height also had significant influences on tenderness (*p<0.05*, Table 17). As marbling increased by 100 units, the tenderness score increased by 2 points while an increase in hump height by 10mm increased tenderness scores by 1.7 points (Table 17).

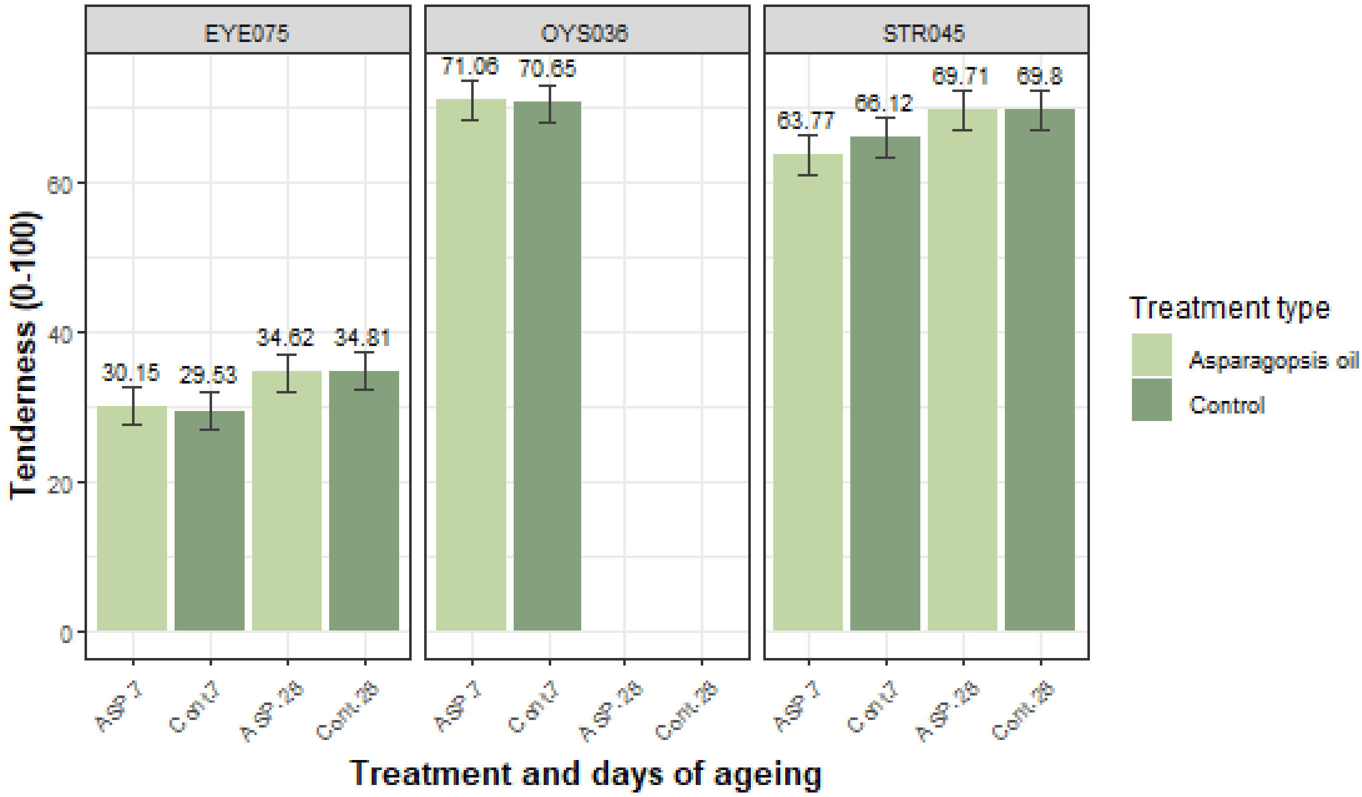

Figure 14. Effect of treatment and ageing on tenderness and error bar represent confidence intervals (CI) around the estimated marginal means

Table 17. Effect of treatment, cut, postmortem ageing, carcass weight, marbling and hump height on tenderness

Predictors	**Estimates**	**95% Confidence Interval**	**p**
Intercept	21.67	4.22 – 39.12	**0.015**
Treatment Asp-Oil	-0.32	-2.68 – 2.04	0.729
CutOYS036	41.00	38.98 – 43.02	**<0.001**
CutSTR045	35.07	33.54 – 36.60	**<0.001**
Days of ageing28	4.84	3.31 – 6.37	**<0.001**
HSCW	-0.04	-0.08 – 0.01	0.129
MSA Marbling	0.02	0.01 – 0.04	**0.010**
Hump Height	0.17	0.02 – 0.31	**0.023**

HSCW= Hot standard carcase weight; and p= p-value

4.2.2 Effect of treatment and ageing on juiciness

Inclusion of Asp-Oil did not have a significant effect on juiciness (*P*=0.148), yet it showed a reduction of 1.77 points (Figure 15 and Table 18). Compared to EYE075, the OYS036 and STR045 exhibited higher juiciness by 34.72 and 25.98 points, respectively (p<0.001, Figure 15 and Table 18). This indicates that the OYS036 and STR045 cuts are inherently juicier than EYE075, which could be because of fat content and connective tissue distribution.

Moreover, ageing postmortem up to 28 days led to a significant improvement in juiciness by 4.22 points for both the EYE075 and STR045 (p<0.001 and Table 18), suggesting the enzymatic breakdown of muscle fibres and connective tissues, which helps to improve texture and moisture retention. Additionally, MSA marbling and hump height also had significant influences on juiciness (*p<0.05*, Table 18). As marbling increased by 100 units, the juiciness score increased by 2 points while an increase in hump height by 10mm increased juiciness scores by 1.5 points (Table 18).

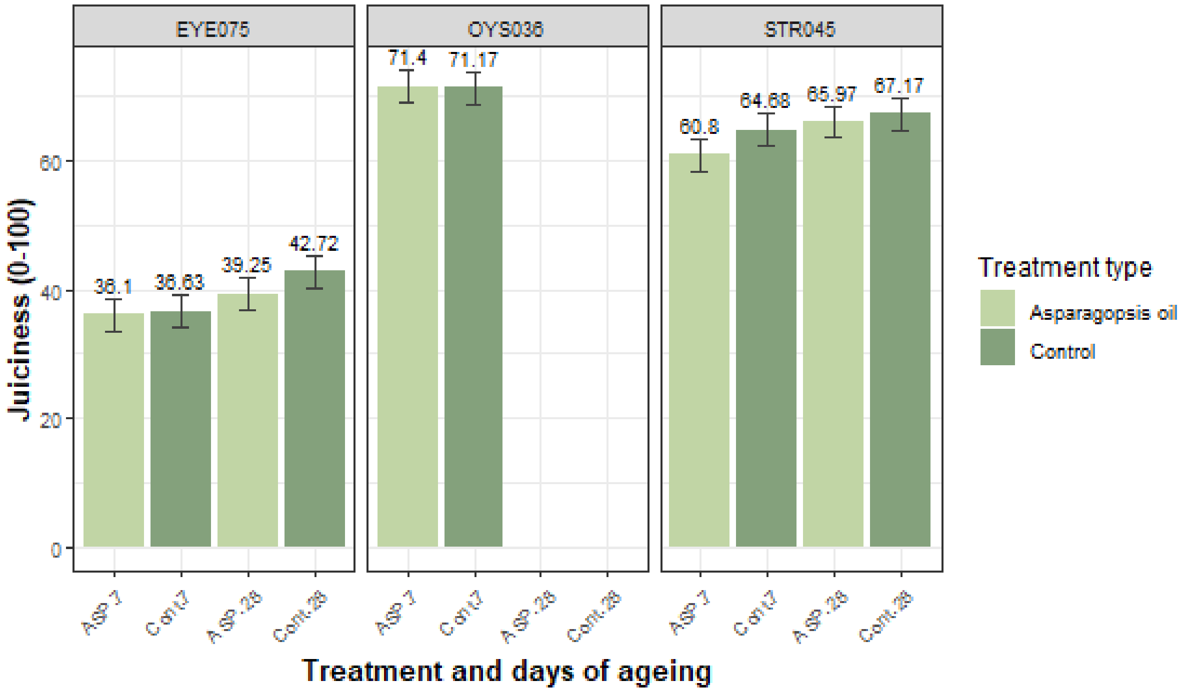

Figure 15. Effect of treatment and ageing on juiciness and error bar represent confidence intervals (CI) around the estimated marginal means

Table 18. Effect of treatment, cut, postmortem ageing, carcass weight, marbling and hump height on Juiciness

Predictors	Estimates	95% Confidence Interval	p
Intercept	26.79	9.10 – 44.48	**0.003**
Treatment Asp-Oil	-1.77	-4.16 – 0.63	0.148
CutOYS036	34.72	32.87 – 36.58	**<0.001**
CutSTR045	25.98	24.57 – 27.38	**<0.001**
Days of ageing28	4.22	2.82 – 5.63	**<0.001**
HSCW	-0.03	-0.07 – 0.02	0.238
MSA Marbling	0.02	0.01 – 0.04	**0.009**
Hump Height	0.15	0.01 – 0.30	**0.039**

HSCW= Hot standard carcase weight; CI= Confidence interval and p= p-value

4.2.3 Effect of treatment and ageing on flavour

No statistically significant differences (*P*=0.529, Figure 16, Table 19) were observed in flavour between the treatment and control groups, although there was a decrease of 0.63 points. Like tenderness and juiciness, OYS036 and STR045 showed improved flavour by 26.76 and 21.48 points respectively compared to EYE075 (*P*<0.001, Figure 16, Table 19). Flavour in the EYE075 and STR045 also experienced an improvement of 3.26 points when aged for 28 days (*P*<0.001, Figure 16, Table 19). Additionally, MSA marbling (p=0.014) and hump height (p=0.055) also had significant influences on flavour (Table 19). As marbling increased by 100 units, the flavour score increased by 2 points while an increase in hump height by 10mm increased flavour scores by 1.2 points (Table 19).

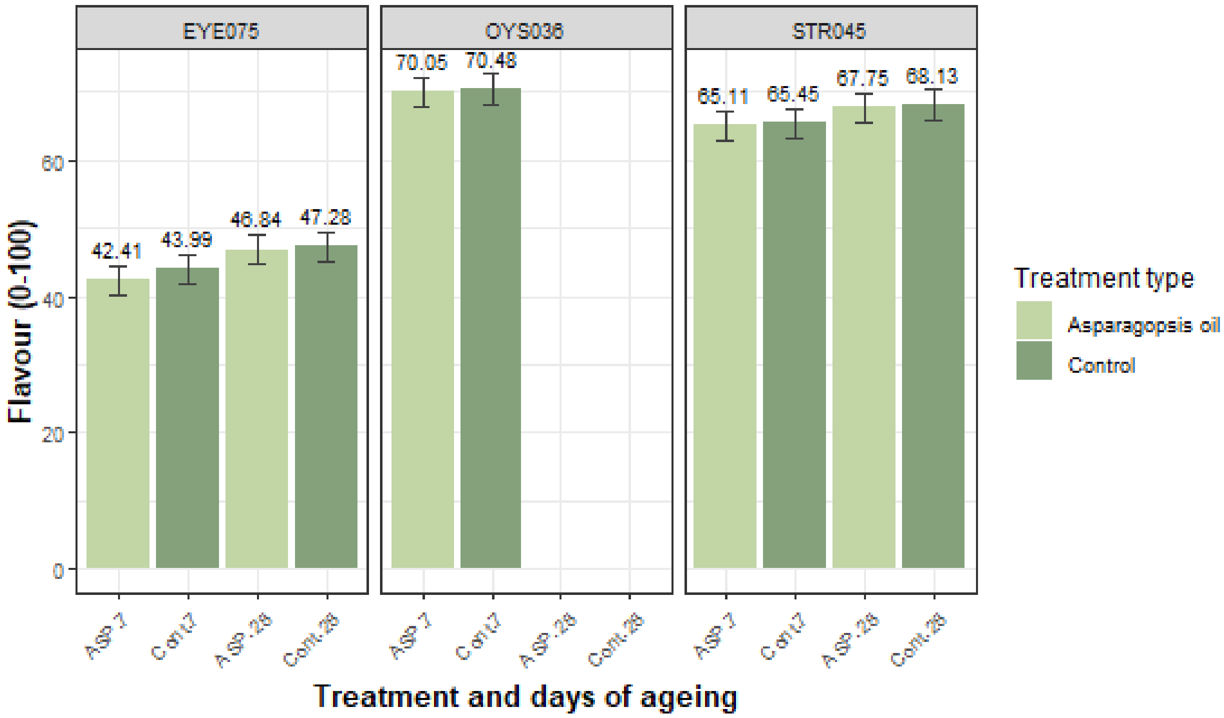

Figure 16. Effect of treatment and ageing on flavour and error bar represent confidence intervals (CI) around the estimated marginal means

Table 19. Effect of treatment, cut, postmortem ageing, carcass weight, marbling and hump height on Flavour

Predictors	Estimates	95% Confidence Interval	p
Intercept	37.51	22.89 – 52.13	**<0.001**
Treatment Asp-Oil	-0.63	-2.61 – 1.34	0.529
CutOYS036	26.76	25.04 – 28.49	**<0.001**
CutSTR045	21.48	20.18 – 22.79	**<0.001**
Days of ageing28	3.26	1.96 – 4.56	**<0.001**
HSCW	-0.03	-0.06 – 0.01	0.182
MSA Marbling	0.02	0.00 – 0.03	**0.014**
Hump Height	0.12	-0.00 – 0.24	0.055

HSCW= Hot standard carcase weight; CI= Confidence interval and p= p-value

4.2.4 Effect of treatment and ageing on overall liking

There were no statistically significant differences (*P*=0. 554) observed for overall liking between the treatment and control groups, although Asp-Oil reduced the score by 0.67 points (Table 20). This suggests that Asp-Oil treatment did not result in perceptible differences compared to the control group. The OYS036 and STR045 scored 32.43 and 26.4 points higher than the EYE075 respectively ($p<0.001$, Figure 17, Table 20). There was no significant cut by treatment interaction. The OYS036 and STR045 might have superior qualities such as better marbling, fat distribution, or tenderness compared to EYE075, resulting in higher overall liking. Cuts with more favourable attributes such as enhanced flavour, juiciness, and tenderness are generally preferred by consumers, leading to higher overall liking scores.

The postmortem ageing of 28 days also significantly improved the overall liking by 4.39 points ($p<0.001$, Table 20), but there was no significant cut by ageing interaction indicating that the overall liking of both cuts improved by the same amount. In addition, marbling and hump height both had a significant impact on overall liking ($p<0.05$, Table 20). As marbling increased by 100 units, the overall liking score increased by 2 points while an increase in hump height by 10mm increased overall liking scores by 1.5 points (Table 20).

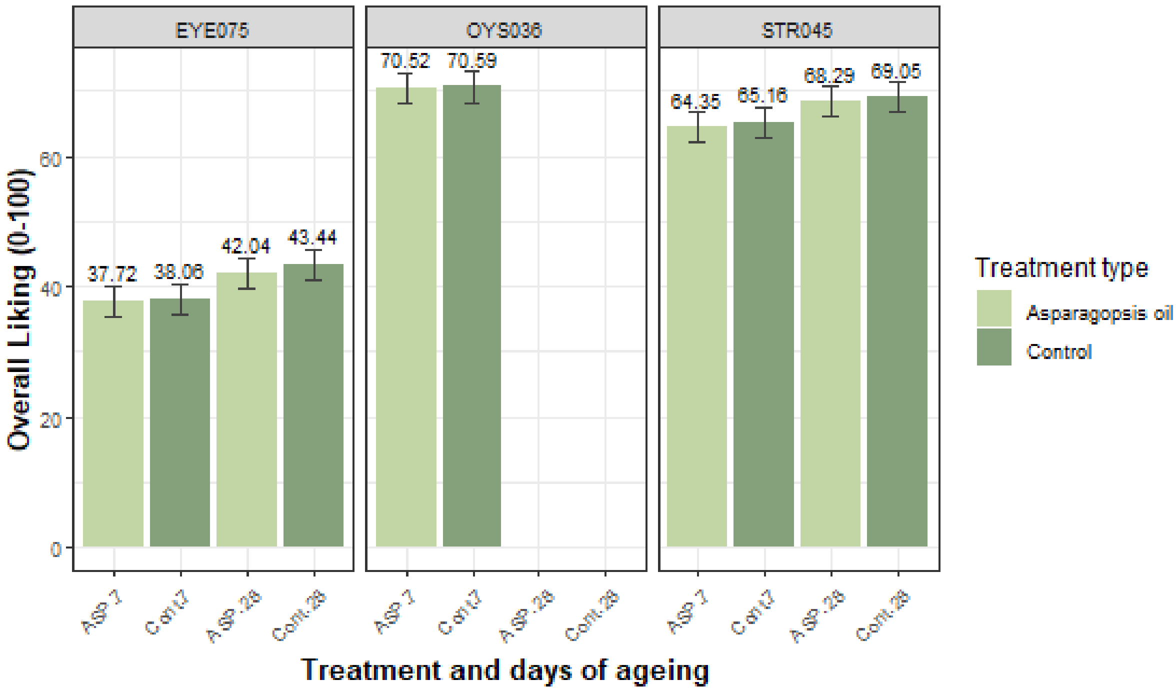

Figure 17. Effect of treatment and ageing on overall liking and error bar represent confidence intervals (CI) around the estimated marginal means

Table 20. Effect of treatment, cut, postmortem ageing, carcass weight, marbling and hump height on overall liking

Predictors	Estimates	95% Confidence Interval	p
Intercept	28.99	12.46 – 45.52	**0.001**
Treatment Asp-Oil	-0.67	-2.91 – 1.56	0.554
CutOYS036	32.43	30.65 – 34.21	**<0.001**
CutSTR045	26.40	25.05 – 27.74	**<0.001**
Days of ageing28	4.39	3.04 – 5.73	**<0.001**
HSCW	-0.03	-0.07 – 0.01	0.186
MSA Marbling	0.02	0.01 – 0.04	**0.006**
Hump Height	0.15	0.01 – 0.28	**0.034**

HSCW= Hot standard carcase weight; CI= Confidence interval and p= p-value

4.2.5 Effect of treatment and ageing on MQ4

There were no statistically significant differences observed for MQ4 between the treatment and control groups (*P*=0.603, Figure 18 and Table 21), suggesting that any observed changes might be due to random variability rather than the treatment itself. Compared to EYE075, the OYS036 and STR045 demonstrated an improvement in MQ4 score by 32.86 and 27.02 points, respectively ($p<0.001$, Figure 18 and Table 21). This is due to differences in tenderness, juiciness, flavour, or overall liking intrinsic to these cuts. Furthermore, MQ4 was significantly increased by 28 days postmortem ageing, showing a rise of 4.13 points for both EYE075 and STR045, with a range from 2.86 to 5.4 points ($p<0.001$, Figure 18 and Table 21). Additionally, both MSA marbling and hump height had a notable significant impact on MQ4 ($p<0.05$ and Table 21). As marbling increased by 100 units, the MQ4 score increased by 2 points while an increase in hump height by 10mm increased MQ4 score by 1.5 points (Table 21).

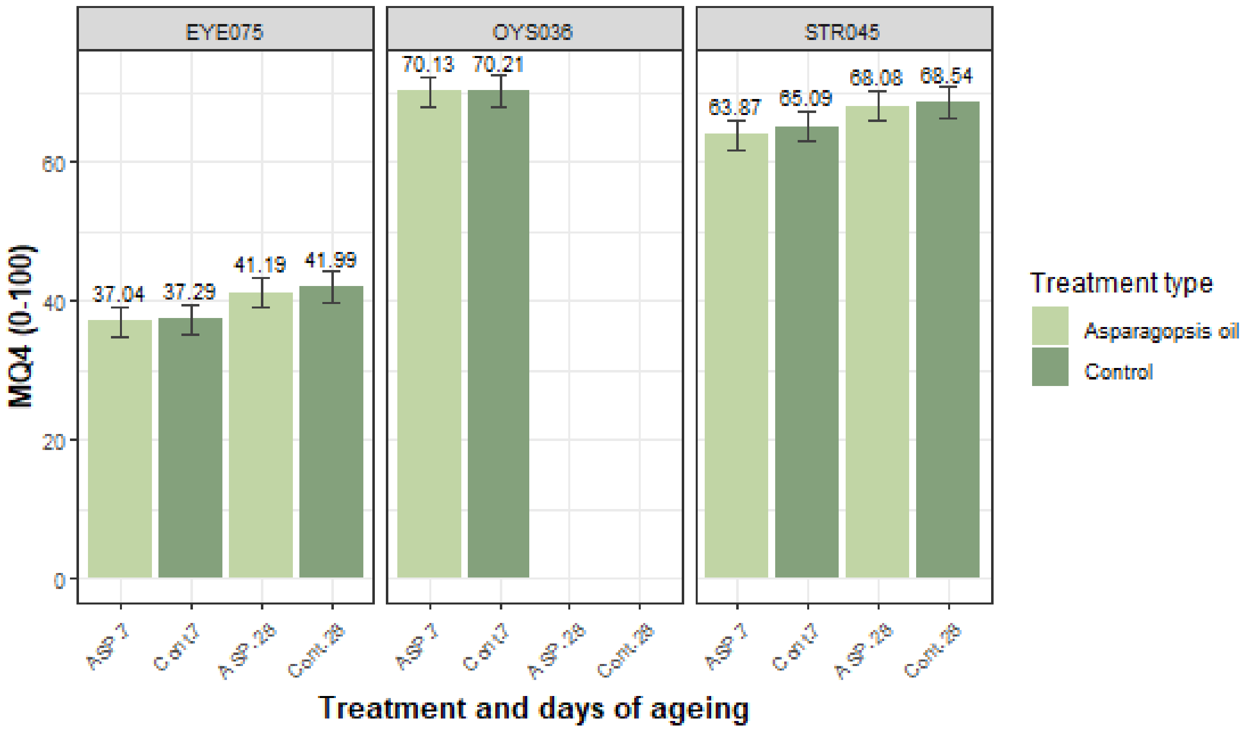

Figure 18. Effect of treatment and ageing on MQ4 and error bar represent confidence intervals (CI) around the estimated marginal means

Table 21. Effect of treatment, cut, postmortem ageing, carcass weight, marbling and hump height on MQ4

Predictors	Estimates	95% Confidence Interval	p
Intercept	29.64	13.94 – 45.34	**<0.001**
Treatment Asp-Oil	-0.56	-2.69 – 1.56	0.603
CutOYS036	32.86	31.18 – 34.54	**<0.001**
CutSTR045	27.02	25.75 – 28.29	**<0.001**
Days of ageing28	4.13	2.86 – 5.40	**<0.001**
HSCW	-0.03	-0.07 – 0.01	0.132
MSA Marbling	0.02	0.01 – 0.04	**0.007**
Hump Height	0.15	0.02 – 0.28	**0.023**

HSCW= Hot standard carcase weight; and p= p-value

4.2.6 Effect of treatment and ageing on satisfaction

Consumers were asked to mark the sample as unsatisfactory (2 star), good every day (3 star), better than everyday (4 star) or premium quality (5 star). The treatment did not significantly affect (*P*=0.474) satisfaction score (Figure 19 and Table 22), indicating that the treatment did not lead to notable changes in how consumers rated their overall satisfaction. The satisfaction scores for OYS036 and STR045 were significantly higher by 1.2 and 0.9 points, respectively, compared to EYE075, suggesting the qualities of OYS036 and STR045, highlighting their superior quality. Furthermore, ageing up to 28 days contributed to a small but statistically significant increase in satisfaction, underscoring the benefits of proper ageing in improving meat quality. MSA Marbling and hump height also had a significant effect on the satisfaction of meat (P<0.05).

Overall, the characteristics of the meat samples themselves, particularly marbling, hump height along with ageing time, played a significant role in determining consumer preferences and satisfaction levels. At the same time, the treatment did not have a major impact on any of these meat quality attributes.

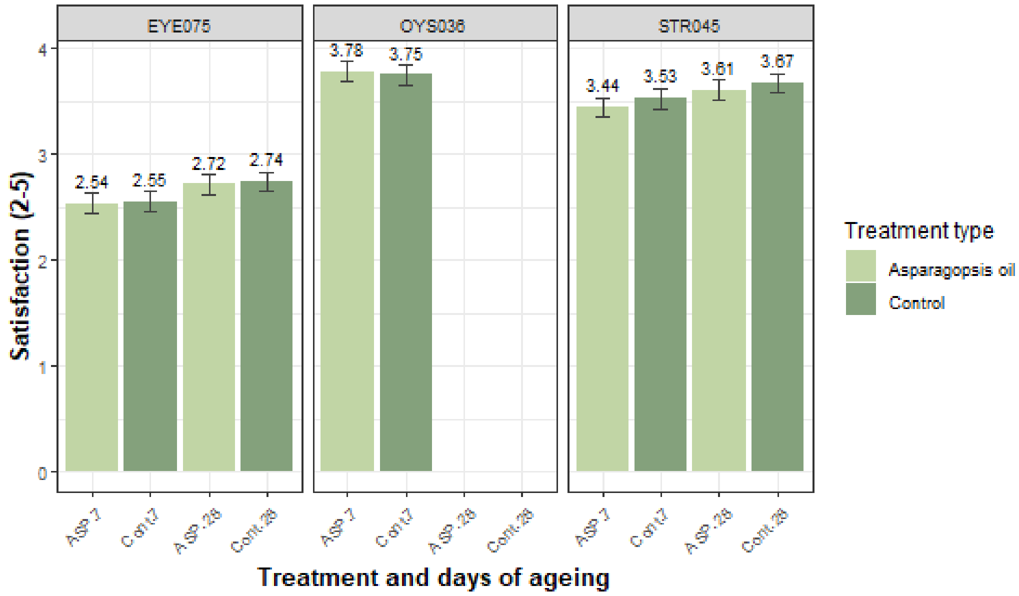

Figure 19. Effect of treatment and ageing on satisfaction and error bar represent confidence intervals (CI) around the estimated marginal means

Table 22. Effect of treatment, cut, postmortem ageing, carcass weight, marbling and hump height on Satisfaction

Predictors	Estimates	95% Confidence Interval	p
Intercept	2.27	1.65 – 2.88	**<0.001**
Treatment Asp-Oil	-0.03	-0.11 – 0.05	0.474
CutOYS036	1.22	1.14 – 1.29	**<0.001**
CutSTR045	0.93	0.87 – 0.98	**<0.001**
Days of ageing28	0.17	0.11 – 0.23	**<0.001**
HSCW	-0.00	-0.00 – 0.00	0.099
MSA Marbling	0.00	0.00 – 0.00	**0.016**
Hump Height	0.01	0.00 – 0.01	**0.009**

HSCW= Hot standard carcase weight; and p= p-value

5. Discussion

The current study indicates that supplementing feed with SEAFEED™, a bromoform canola oil-based infusion of *Asparagopsis armata* (Asp-Oil) did not significantly (*p*>0.05) impact meat quality attributes, although all the sensory attributes had a minimal numerical reduction due to the inclusion of Asp-Oil in the diet. This finding aligns with previous research on meat taste assessment, which also found no differences between beef cattle supplemented with *Asparagopsis* spp. derived products and the control group (Kinley et al., 2020; Roque et al., 2021; George et al., 2024). Kinley et al. (2020) reported that supplementation with freeze-dried *Asparagopsis* biomass did not influence meat eating quality characteristics. However, they noted variability in consumer testing scores and a limited sample size, which may have obscured potential differences between treatment groups. Similarly, Roque et al. (2021) found no differences in beef cattle supplemented with freeze-dried *Asparagopsis* and those that were not. Similarly, George et al. (2024) showed that tenderness or juiciness was not impacted by the inclusion of Asp-Oil using a trained taste panel on 160 cattle (p=0.07). 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. This highlights the potential drawbacks of higher supplementation levels on meat appearance and microbial spoilage. Overall, both this study and prior research suggest that supplementing cattle with bromoform based products does not significantly affect overall meat quality or alter the sensory characteristics of beef. Variations in study results may be due to differences in product inclusion rates, forms (such as biomass versus extract), muscle types, and methodologies used across the studies.

Further, all the sensory properties for the selected cuts improved with ageing up to 28 days for both control and treatment groups. High importance is placed on tenderness in the marketplace, with studies indicating that consumers are willing to pay more for more tender beef (Miller et al., 2001; Lamare et al., 2002). Tenderness is primarily influenced by the three factors- collagen, sarcomere length and proteolysis (Koohmaraie et al., 1988). The improvement of sensory qualities of all cuts with ageing is attributed to proteolysis and connective tissue changes, development of complex flavour compounds, and changes in fat and water distribution- fundamental processes in beef that lead to improved tenderness and other meat quality attributes, making aged beef highly desirable. This is particularly noticeable in cuts with higher connective tissue content.

As beef ages, collagen transforms into gelatine through proteolysis, particularly involving the calpain system. The µ-calpain system plays a crucial role in early post-mortem ageing, while m-calpain becomes more prominent in long-term ageing (Parr et al., 2007). 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 ageing. This indicates that the presence of Asp-Oil does not impact the ageing process for these cuts. However, *Asparagopsis* supplementation, known for its potential to reduce methane emissions in ruminants due to its high content of bioactive compounds like bromoform and dibromoacetic acid, may also interact with metabolic processes in animal's digestive system. While primarily studied for its environmental benefits, the presence of bioactive compounds in *Asparagopsis* based products could lead to variations in proteolytic breakdown rates and subsequently influence meat tenderness and other sensory attributes. Further, various factors, including the age at which animals are slaughtered, their breed, and the amount of fat contained in a particular cut, can significantly influence meat tenderness and other sensory properties (Warner et al., 2010; Corbin et al., 2014; Blank et al., 2017).

In this study, all animals were of similar age and breed, with only a significant reduction observed in loin pH due to the Asp-Oil inclusion. 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 (Hwang et al., 2014; Stokes et al., 2016; Bolkenov et al., 2021). Conversely, in pork, marine algae did not change the meat colour and lipid oxidation rate (Sardi et al., 2006; Vossen et al., 2017). The current study is also in agreement with the previous research that the inclusion of Asp-Oil did not impact carcase and eating quality. However, the previous studies used different types of seaweed with different nutrient compositions and need to be taken into consideration.

6. Conclusions/Recommendations

The analysis examined the effects of feeding Asp-Oil at a dose rate of 25.6 mg Bromoform per kilogram of feed to mid-fed beef cattle. There were no significant effects of Asp-Oil treatment found on meat quality attributes as well as carcase traits. However, eating quality improved with ageing up to 28 days for all selected cuts.

6.1 Key findings

- Feeding a ration containing SEAFEED™ (Asp-Oil) at a dose rate of 25.6 mg/kg dry matter to Angus or Angus crossbred cattle for 100 days or more days with Asp-Oil out of 150 days or more days of feeding did not significantly affect meat quality attributes as determined by a sensory panel.
- Eating quality was also improved with ageing from 7 to 28 days for all the selected cuts, independent of any treatment effects.
- Carcase traits (HSCW, hump height, EMA, ossification, MSA marbling, AUS-MEAT Marbling, MSA Index, P8 fat, subcutaneous rib fat, meat colour and fat colour) were not impacted by the Asp-Oil treatment.
- The feed intake, growth rates and HSCW of the treated cattle were not impacted by the inclusion of the Asp-Oil after 50 days on feed already.

6.2 Benefits to industry

This research represents one of the largest commercial trials of Asp-Oil on carcase and meat quality conducted in conditions relevant to the Australian feedlot industry. 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-Oil treatment doesn't affect most eating quality attributes. This consistency is crucial for maintaining consumer satisfaction and meeting quality standards in the meat industry. These findings provide evidence supporting the economic feasibility of incorporating Asp-Oil into extended feeding programs.

6.3 Future research and recommendations

Future research is needed to optimize feeding programs for Asp-Oil supplements, including testing different varieties, and new synthetic variations of bromoform with various formulations. However, thorough sensory testing is essential if these supplements are to be applied commercially.

Currently, there is no established supply chain for *Asparagopsis* spp. 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.

The feed intakes, growth rates and HSCW of any replicate were not impacted by the inclusion of Asp-Oil into their diet. To date, many experiments have reported variation in feed intakes after inclusion of the oil. Hence these results suggest that having cattle on finisher ration or giving them a long lead time (50 days in this case) is beneficial to ensure that the inclusion of the Asp-Oil does not impact animal performance.

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