Final report

Wagyu Beef Eating Quality and MENA Sensory Testing

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Abstract

This study aimed to evaluate the potential effect of Wagyu breed content on eating quality, when carcases were selected in a case-control fashion based on marbling. Three cohorts of 36 carcases were selected from Angus, Wagyu Angus F1 cross, high content crossbred Wagyu (F3, 87%+ Wagyu), Wagyu *Bos indicus* F1 cross and purebred Wagyu (unregistered), all of which had been long fed (≥200 DOF – cohort 1, ≥300 DOF – cohorts 2 and 3). The MSA marbling scores averaged 474 to 705 across the three cohorts and ranged from 320 to 1150. The chuck roll, bolar blade, striploin, D-rump, and outside flat were consumer tested using the grill, shabu shabu and yakiniku cook methods. Sensory scores for tenderness, juiciness, liking of flavour, overall liking and MQ4 were analysed within cohorts against breed, cut and carcase traits for Australian and Middle Eastern consumers. The results showed that cut and carcase characteristics were better predictors of eating quality than Wagyu breed content. Wagyu breed content did affect some intrinsic eating quality traits, but the effect was not consistent (sometimes negative, sometimes positive) across cook methods or cuts making it difficult to implement changes to the MSA prediction model. The grill and yakiniku cook methods were scored higher by consumers than the shabu shabu cook method. Middle Eastern consumers scored the rump and striploin cuts higher than Australian consumers, the rump cap lower while there was no difference in the outside flat and blade scores.

Executive summary

The Australian Wagyu cattle breed has dominated premium international markets over recent decades. However, within the Meat Standards Australia (MSA) eating quality grading system there is currently no adjustment for Wagyu breed as differences in eating quality are explained through variables existing in the model. There is a perceived assumption of a benefit of Wagyu cattle over and above other cattle breeds and which is not explained by the current MSA model predictions. Within the existing version of the MSA model, Wagyu influenced cattle represent around 2% of the data underpinning the predictions which include animals with MSA marbling scores up to the maximum 1190. The objectives of the project were to 1) determine if there is a 'Wagyu effect' on eating quality that is over and above the current MSA model prediction utilising long fed (≥200 DOF – cohort 1, ≥300 DOF – cohorts 2 and 3) high content crossbred Wagyu, purebred Wagyu (unregistered) and Wagyu and Angus F1 animals; 2) In the event of the identification of an effect, to quantify this based on tenderness, juiciness, flavour and overall liking and 3) conduct a subset of consumer sensory testing in the Middle East to expand knowledge of international consumers for high value Australian product.

Methodology

Three (3) cohorts of 36 carcases were selected for consumer testing from much larger kill groups;

- cohort 1 compared non-Wagyu to Wagyu content animals using 18 Angus and 18 Wagyu Angus F1 cross, fed for ≥200 days;
- cohort 2 compared two different levels of Wagyu content using 18 high content crossbred Wagyu (F3 and higher >87.5% Wagyu) and 18 Wagyu *Bos indicus* F1 cross, fed for ≥300 days; and
- cohort 3 compared two different levels of Wagyu content using 18 purebred Wagyu (non-registered) and 18 Wagyu Angus F1 cross, fed for ≥300 days .

The chuck roll, bolar blade, striploin, D-rump, and outside flat were collected from cohorts 1 and 2 for fabrication into grill, shabu shabu and yakiniku sensory samples and aged for 7 days. The anterior end of the striploin was collected from cohort 3 for grill samples. Untrained Australian consumers evaluated the samples for tenderness, juiciness, liking of flavour and overall liking, in accordance with MSA sensory testing protocols, and the overall eating quality score (MQ4) was calculated. Sensory scores for tenderness, juiciness, liking of flavour, overall liking and MQ4 were analysed within cohorts against breed, cut and carcase traits.

Samples from cohort 1 were also consumer tested in the Middle East. All samples for Middle East testing were grill samples all aged for 7 days post-mortem and then shipped frozen other than the striploin 045 which had 2 samples aged for 7 and 35 days. The other samples were the Blade 096, Outside 005, Rump 131, Rump 231, and Rump 005.

Results/key findings

Cut and carcase characteristics were better predictors of eating quality in the majority of cases. Grilled samples in cohort 1 showed that Wagyu Angus F1 samples were more tender than Angus, however there was no breed effect for juiciness, flavour, overall liking or MQ4. There was no effect of Wagyu content level for cohort two. In cohort 3 the Wagyu Angus F1 steers were significantly higher in tenderness, juiciness, flavour, overall liking and raw MQ4 score when compared to purebred Wagyu (unregistered) steers ($P < 0.05$). Interestingly, there was a negative impact of hump height on the Wagyu steers that was not evident for Wagyu Angus F1 steers, though this requires further investigation due to low numbers. There was no breed effect for shabu shabu in any muscle in cohorts 1 or 2 for tenderness, juiciness, liking of flavour, overall liking and MQ4 ($P > 0.05$).

Consumers rated Wagyu Angus F1 yakiniku samples higher than Angus in cohort 1 for flavour and tended to rate them higher for juiciness, however there was no effect of Wagyu content on tenderness, overall liking and MQ4. In cohort two, high content crossbred Wagyu yakiniku samples were more tender and overall liking tended to be higher, however juiciness, flavour and MQ4 scores were not influenced by breed.

The consumers from Middle East scored the tenderness, juiciness, flavour, overall liking and MQ4 score of the RMP005 lower than Australian consumers, but scored the RMP131, RMP231 and STR045 higher than Australian consumers for tenderness, juiciness, overall liking and MQ4 score ($P<0.05$). However, there was no difference in consumer sensory scores between the 2 countries for the MQ4 score of BLD096 or OUT005 ($P>0.05$).

Benefits to industry

These results suggest that there are inconsistent effects of increasing the Wagyu breed content on different intrinsic eating quality parameters, which change with cook type and across cuts. The differences in eating quality are explained to a greater extent by cut and carcase characteristics already in the MSA model. Further work is required to substantiate if there is any consistent breed effect. However, given the results of this project showing both positive and negative outcomes for cohorts of animals with varying Wagyu content, and following review from the MSA Pathways committee, it has been recommended that no change is made to the MSA eating quality prediction model. It was noted that the cohorts in this project were of limited size and did not cover the breadth of the Wagyu breed allowing for further quantification of the Wagyu effect in coming years. However, premium brands utilising high marbling carcases could benefit from MSA grading underpinning branding ensuring consistency of product to consumers, as marbling alone does not explain all variation in eating quality.

Overall, Middle East consumers are very similar to Australian consumers and identify similar differences between breeds within a cut for all sensory traits. The rump cap is not as appreciated in the Middle East and somewhat re-ranks, however many cuts score slightly higher for Middle Eastern consumers than Australian consumers indicating that premium Australian beef is highly appreciated in the Middle East.

Future research and recommendations

Future research could concentrate on understanding the effect of marbling fineness and distribution, the impact of Bos indicus content and hump height in Wagyu and Wagyu cross cattle plus gain a greater understanding of the effect of fatty acid profiles on eating quality. It is paramount that more consumer data is collected utilising Southern multibreed Angus and Wagyu steers which have been born, raised and harvested together. Further data underpinning MSA model predictions for high ossification (>200) and high MSA marbling (>800) would refine predictions for the MSA grading model further.

It is recommended from this research project that no adjustments to the current MSA model are necessary for Wagyu or Wagyu content and that the data generated by this project be utilised to improve the accuracy of prediction for many cut x cook combination from highly marbled carcases. This data will assist in refining predictions for high marble score carcases, for certain cuts, such as the rump cap.

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1. Background

The Wagyu breed was established in Australia in the 1990's with the importation of embryos, semen and live animals from Japan via the United States of America (Zhang et al., 2015). Since then the largest population of Wagyu cattle outside of Japan has developed in Australia (Australian Wagyu Association, 2016). The Wagyu breed typically exhibit high degrees of intramuscular fat, which results in ninety percent of Australian Wagyu products being exported to premium international markets each year (Zhang et al., 2015; Australian Wagyu Association, 2019).

The ability of the Wagyu breed to produce a premium product is not currently recognised as a breed effect, but rather an intramuscular fat effect in the current Meat Standards Australia (MSA) model of consumer eating quality outcomes (Polkinghorne et al., 2008). This has led to doubts about the suitability of the MSA model to predict the eating quality of Wagyu beef due to its high degree of intramuscular fat and the resulting disruption of connective tissue which would mean it could be vastly different to the carcases used to develop the MSA model (Australian Wagyu Association, 2019), even though the current MSA model is based upon meat eaten from carcases across the range of MSA marble scores. However, it is unknown from literature if the disruption of connective tissue due to high marbling is equivalent across all breeds.

Throughout literature a difference in fatty acid profiles, fibre type, connective tissue disruption and degree of marbling has been observed between Wagyu and other *Bos taurus* breeds (Iwamoto et al., 1991; May et al., 1993; Nishimura et al., 1999; Duarte et al., 2013). The Wagyu breed have been shown to produce more monounsaturated fatty acids then other cattle breeds, which decreases the melting point of the fat, potentially altering the eating experience (Sturdivant et al., 1992; May et al., 1993). Iwamoto et al. (1991), found that red muscle fibres in Wagyu cattle were of a higher frequency and larger diameter than in other breeds which may affect the tenderness and texture of the meat. Nishimura et al. (1999) also found that the development of intramuscular fat appears to disorganise the intramuscular connective tissue sturctures in Wagyu cattle which may contribute to a more tender eating experience even as the animals age and ossification score increases. These factors all have the potential to impact the traits of meat eating quality.

Additionally, it is also not well known or understood how Middle Eastern consumers appreciate high quality premium beef and if their perception differs to that of domestic Australian consumers. At present the Middle Eastern consumer has not been characterised.

The aim of this project is to identify the potential effects of Wagyu breed content on juiciness, tenderness, flavour, overall liking and the resulting meat quality score (MQ4) for various cuts when they are cooked using grill, shabu shabu and yakiniku cook methods. Plus, additionally characterise the perception of Middle Eastern consumers for premium quality Australian beef.

2. Objectives

1. To determine if there is a Wagyu breed effect on eating quality that is over and above the current MSA model prediction utilising long fed (≥200 DOF) high content crossbred Wagyu, purebred and F1 animals.

2. In the event of the identification of a breed effect, to quantify this based on tenderness, juiciness, flavour and overall liking.

3. Conduct sensory testing in the Middle East (MENA) to expand knowledge of international consumers.

3. Methodology

3.1 Carcase selection

Carcases were selected from commercial slaughter animals from multiple sources. The selection criteria was such that cattle were fed together in the same feedlot pen and include at least two breeds, one of which was Wagyu or Wagyu cross. Table 1 outlines the breeds collected across three cohorts.

Table 1. Cattle breeds and number of carcases selected for cohorts 1, 2 and 3, including number of days on a high energy feedlot ration (DOF)

Breed	Cohort 1 (200 DOF)	Cohort 2 (300 DOF)	Cohort 3 (300 DOF)
Angus	18	-	-
Wagyu x Angus	18	-	18
Wagyu x *Bos indicus*	-	18	-
High content crossbred Wagyu	-	18	-
Wagyu Purebred	-	-	18

3.1.1 Cohort 1

The 36 animals for this kill were sourced from a single feedlot and had been fed together in the same pen for 200 days, prior to processing at a commercial abattoir on the same day. The cattle consisted of Angus (AAAA) and F1 Angus Wagyu cross cattle (WYAA). In total, 18 hd of Angus and 18 hd of F1 Angus Wagyu were selected to be part of the cohort out of a larger kill group. Animals were selected as case and control pairs with Angus and F1 Angus Wagyu carcases matched as close on marbling and ossification as possible. From these 36 head, the striploin, bolar blade, outside flat, D-rump and chuck roll were collected and cut into grill, shabu shabu and yakiniku samples.

3.1.2 Cohort 2

The 36 animals for this kill were sourced from a single feedlot and all cattle had been fed together in the same pen for 300+ days prior to processing at a commercial abattoir on the same day. The cattle consisted of F1 Wagyu *Bos indicus* cross cattle (WYXX) and high content crossbred Wagyu (WYWY) cattle (F3 or higher). In total 18 head of high content crossbred Wagyu and 18 head of F1 Wagyu were selected to be part of the cohort out of a much larger kill group. Animals were selected as case and control pairs with high content crossbred Wagyu and F1 Wagyu carcases matched as close on marbling, ossification and hump height as possible. The high content crossbred Wagyus were identified by genotyping all cattle in the pen prior to slaughter and were classified as anything that was F3 or higher (≥87.5% Wagyu). From these 36 head, the striploin, bolar blade, outside flat, D-rump and chuck roll were collected and cut into grill, shabu shabu and yakiniku samples.

3.1.3 Cohort 3

The 36 animals for this kill were sourced from a single feedlot and all cattle had been fed in three different pens for 300+ days prior to processing a commercial abattoir on the same day. The cattle consisted of F1 Wagyu Angus cattle (WYAA) and purebred Wagyu cattle (WYWY). In total 18 head of purebred cattle and 18 head of F1 Wagyu were selected to be part of the cohort. Six F1s and 6 purebreds were selected from each of the 3 pens. Animals were selected as case and control pairs with pure bred and F1 Wagyu carcases matched as close on marbling and ossification as possible. From these 36 head, the striploin was collected and cut into grill, shabu shabu and yakiniku samples.

3.2 Slaughter Procedure, Carcase Grading and Primal Collection

Each cohort group was slaughtered separately at commercial abattoirs in Queensland, Australia. A temperature and pH rate of decline was recorded for each carcase at hourly intervals, from the time of entry into the chiller until the muscle pH fell below 6. This was done to identify potential risk of heat toughening (i.e. carcase reached pH 6 while temperature was above 35 °C) or cold shortening. This ensured only carcases which conformed to MSA pH and temperature decline requirements were selected for further use in the study. Only carcases which dropped below pH 6 while the temperature ranged between 15°C and 35 °C were selected for these cohorts.

The carcases were graded against the Aus-Meat Chiller Assessment Standards and the Meat Standards Australia Grading Standards (AUS-MEAT Limited, 2005) by a single grader at 20 hours post slaughter. The measurements taken included hot standard carcase weight (HSCW), ossification, hump height (mm), eye muscle area (EMA; cm^2), subcutaneous rib fat depth (mm), AUS-MEAT marble score, MSA marble score, meat colour and fat colour, and ultimate pH (pH_u).

The Bolar Blade (HAM #2302), Chuck Roll (HAM #2275), Striploin (HAM #2140), D-Rump (HAM #2100) and Outside Flat (HAM #2050) were collected for cohorts 1 and 2, whilst just the Striploin was collected from cohort 3 at boning. These primals were then packed into vacuum sealed bags and chilled for 24hrs prior to collection from the abattoir. For further processing these primals were transported at 1 °C to the processing site at the University of New England.

3.3 Sample preparation

On the 6th day from primal collection, the primals were trimmed of external fat and epimysium. Primals were broken down into individual muscles; Bolar Blade into BLD096 (*M. triceps brachii caput longum*) and BLD097 (*M. triceps brachii caput mediale*); Chuck Roll into CHK074 (*M.semispinalis capitis*), CHK078 (*M. serratus ventralis*) and CHK081 (*M. spinalis dorsi*); Striploin into STR045 (*M. longissimus lumborum*); D-Rump into RMP005 (*M. biceps femoris*), RMP131 (eye rump centre; *M. gluteus medius*), and RMP231 (eye rump side; *M. gluteus medius*); OUT005 (*M. biceps femoris*). Table 2 outlines the cuts utilised for each cook method.

Sensory samples were prepared according to MSA protocols, as reported by (Watson et al., 2008b). The grill (GRL) samples were prepared from a 75 x 25 x 150 mm block. Each sample was individually wrapped in freezer film then vacuum packed. Shabu shabu (SSB) samples of 2 mm slices were cut from a 100 x 50 x 20 mm block, perpendicular to the fibre. Yakiniku (YAK) samples were prepared into a 4 mm slice, from 90 x 20 x 75 mm blocks. Both SSB and YAK blocks were prepared and frozen, with slices taken from the par-frozen sample prior to cooking. All samples were aged 7 d, frozen and stored at ~-20 °C until sensory testing. If more than 1 sample was coming from a muscle, the samples were balanced for muscle position across the treatment groups.

Table 2. Muscles tested by cook method

Muscle	Grill	Shabu Shabu	Yakiniku
BLD096 (*M. triceps brachii caput longum*)	Y		
BLD097 (*M. triceps brachii caput mediale*)			Y
CHK074 (*M.semispinalis capitis*)		Y	Y
CHK078 (*M. serratus ventralis*)	Y	Y	Y
CHK081 (*M. spinalis dorsi*)	Y		
OUT005 (*M. biceps femoris*)	Y	Y	Y
RMP005 (*M. biceps femoris*)	Y		
RMP131 (*M. gluteus medius*)	Y		
RMP231 (*M. gluteus medius*)	Y		
STR045 (*M. longissimus lumborum*)	Y	Y	Y

3.3.1 Sample fabrication for the Middle East

Beef from cohort 1 for this project were utilised for testing in the Middle East. From the 36 head, the striploin, bolar blade, outside flat, D-rump and chuck roll were collected and cut into sensory samples. Grill samples from the Blade 096, Outside 005, Rump 131, Rump 231, and Rump 005 for Middle East testing were aged for 7 days post-mortem and then frozen. Two samples were taken from each Striploin 045 which were aged for 7 and 35 days. The 4 cartons of meat (252 samples) were exported to the United Arab Emirates (UAE) from an export licensed processor in Australia. As per the grill samples consumer tested in Australia, all meat collected was fabricated into grills samples utilising the MSA protocols for the fabrication of sensory samples (Watson et al., 2008).

3.4 Consumer sensory testing

The consumer sensory testing procedures were conducted in line with the MSA protocols, as reported by (Watson et al., 2008b).

Briefly, groups (picks) of 60 untrained consumers were recruited. Each consumer tasted 7 samples with the first sample being a standard sample among the consumers. The following 6 samples were controlled in a 6 x 6 Latin Square design which ensured each piece was eaten equally before and after each other piece.

Twenty (20) GRL, 8 SSB and 9 YAK picks were collected for sensory testing in Australia, totalling 2,220 consumers. An additional 6 picks were collected for sensory testing in the Middle East.

The consumers scored each sample on a 0 to 100 scale line for tenderness, juiciness, flavour, and overall liking. From this, the meat quality score (MQ4) was calculated using a weighted average, where tenderness was rated 0.3, juiciness 0.1, flavour 0.3 and overall liking 0.3.

Equation 1. Calculation of Meat Quality Score (MQ4)

$$Meat\ Quality\ (MQ4) = 0.3 \times tenderness + 0.1 \times juiciness + 0.3 \times\ flavour + 0.3 \times\ overall\ liking$$

The consumers also marked each sample whether it was unsatisfactory, good every day, better than every day or premium.

3.4.1 Grill (GRL)

Samples were grilled on a Silex Clamshell Grill (Silex, Hamburg, Germany) set at 195°C for the top cast iron plate and 210°C for the bottom cast iron plate to produce a medium steak (Watson et al., 2008c). The grill was turned on and left to reach the desired temperature 45 minutes prior to cooking. A set of 10 “starter” steaks were placed on the grill to create a stable temperature before the cooking cycle commenced for the samples used in the study. Steaks were placed on the grill in accordance with their order on the sheets; link steaks were cooked in the first round, followed by the 6 sample steaks specific to the study.

The grill cooking procedure followed a strict time schedule to ensure that the time spent cooking was uniform to achieve medium doneness and in the correct sequence. In each round of cooking, 10 steak samples were loaded onto the bottom plate within 45 seconds before closing the lid of the Silex grill. Once the cooking time interval of 5 minutes and 15 seconds was completed samples were placed on a cutting board and left to rest for 3 minutes. In this time the next round of samples were placed on the bottom grill to start the cooking process. Once the rest period was completed the samples were cut through the middle to yield two equal sized portions that were placed onto paper plates. These plates contained a corresponding sample reference code and consumer number and were used to serve the samples to consumers. The sample reference code and consumer number were also on the top of the consumer surveys so the plate could be cross checked by staff when samples were placed before the consumer.

3.4.2 Shabu Shabu (SSB)

The Shabu Shabu broth was created by boiling 20 L of water, 35 g of fine salt, 300 g of fresh celery, 1 kg of frozen sliced carrots and 1 kg of frozen sliced onions for 45 mins. After boiling, the broth was strained through a fine sieve and returned to rolling boil. Boiling broth (approx. 100 °C; 300 mL) is poured into a plastic container and the Shabu Shabu samples were cooked singly by moving the sample around within the broth until the sample has turned pinkish grey (approximately 30 sec). Once the broth is used for the one sample it is discarded and a new container is used for the next sample to prevent carry over flavour. The samples were served immediately post cooking. All samples were concealed from consumers to reduce visual biases before and during cooking.

3.4.3 Yakiniku (YAK)

Yakiniku samples were placed on a preheated hotplate at 250-260 °C. Samples were turned when moisture pooled on the surface of the meat. When moisture pooled on the second side of the meat it was served to the consumer, ensuring the meat was cooked to a medium degree of doneness.

3.4.4 Middle East consumer sensory testing

Consumer sensory testing was carried out in Dubai in the UAE by Polkinghorne’s Pty Ltd. MLA in Dubai and a recruiting company supported the sensory testing using 420 consumers of Middle Eastern heritage to eat the 252 samples of grilled beef. Each consumer ate 7 samples (a link plus 6 experimental samples) and scored them for Tenderness, Juiciness, Flavour and Overall Liking. Ten consumers tested each sample.

3.5 Statistical analysis

All statistical analysis was conducted in R (R Core Team, 20121). Data cleaning, visualization and summary were found using the "tidyverse" (Wickham et al., 2019), and "emmeans" (Length, 2021) respectively. Australian consumer sensory scores were analysed using a linear model, with tenderness, juiciness, flavour, overall liking and MQ4 score as the dependant variables, and cut, breed type, ossification, MSA marbling, rib fat, hump height, hot standard carcase weight, pHu, as well as higher order polynomials plus relevant interactions as response variables. Non-significant terms were removed in a step-wise manner for each trait.

Statistical analysis for Middle East data was conducted in R using a linear mixed effects model where country (Middle East or Australia, Cut and Breed (Angus or F1 Wagyu) were included as fixed effects and animal ID was included as the random term.

4. Results

4.1 Carcase traits

4.1.1 Cohort 1 – Angus and F1 Angus x Wagyu

The hot standard carcase weight (HSCW) was significantly higher in the Angus steers by 12.7 ± 4.97 kg compared to the F1 Angus cross Wagyu steers (P<0.05, Table 3). The ultimate pH (pHu) was significantly higher in the F1 Angus cross Wagyu steers by 0.03 ± 0.01 compared to the Angus steers (P<0.05, Table 3). There was no significant difference in hump height, eye muscle area (EMA), rib fat depth, ossification score or MSA marble score between the Angus (AAAA) and F1 Angus cross Wagyu (WYAA) steers (Table 3).

Table 3. Mean (± SEM), minimum and maximum for the carcase characteristics of hot standard carcase weight (HSCW), hump height, eye muscle area (EMA), rib fat depth, ossification, MSA marbling score and ultimate pH (pHu) for the cohort 1 Angus (AAAA) and F1 Angus x Wagyu (WYAA) steers.

	Breed	
Carcase Trait	WYAA	AAAA
Total Carcases	18	18
HSCW (kg)*	386.2 ± 2.8 (334, 454)	398.5 ± 4.1 (348, 472)
Hump (mm)	78.6 ± 0.9 (65, 95)	80.6 ± 1 (65, 100)
EMA (cm^2)	94.8 ± 1.1 (72, 109)	95.5 ± 1 (75, 111)
Rib fat depth (mm)	13.1 ± 0.5 (9, 28)	13.2 ± 0.5 (8, 22)
Ossification (100 – 590)	155.1 ± 1.6 (130, 180)	155.6 ± 2.1 (140, 200)
MSA Marbling (100 – 1190)	501.4 ± 17.3 (320, 850)	473.9 ± 11 (340, 660)
pHu (0-14) *	5.5 ± 0.007 (5.39, 5.62)	5.47 ± 0.008 (5.38, 5.62)

* Row is significantly different (P<0.05)

4.1.2 Cohort 2 – High content crossbred Wagyu and F1 Wagyu x *Bos indicus* composites

The hot standard carcase weight, hump height, rib fat depth and pHu was higher in the F1 Wagyu cross *Bos indicus* steers by 32.9 ± 4.27 kg, 16.9 ± 2.09 mm, 2.83 ± 0.81 mm and 0.09 ± 0.008, respectively compared to the high content crossbred Wagyu steers (p<0.05, Table 4). The ossification score was higher in the high content crossbred Wagyu steers by 15.9 ± 5.79 compared to the F1 Wagyu cross *Bos indicus* steers (P<0.05, Table 4). There was no difference in EMA or MSA marble score between the F1 Wagyu cross *Bos indicus* and the high content crossbred Wagyu steers (Table 4).

Table 4. Mean (± SEM), minimum and maximum for the carcase characteristics of hot standard carcase weight (HSCW), hump height, eye muscle area (EMA), rib fat depth, ossification, MSA marbling and ultimate pH (pHu) for the cohort 2 F1 Wagyu cross Brahman (WYXX) or F3 to F5 Wagyu cross *Bos indicus* (WYWY) steers.

	Breed	
Carcase Trait	WYWY	WYXX
Total Carcases	18	18
HSCW (kg)*	353.6 ± 2.3 (322, 386)	386.5 ± 3.7 (342, 460)
Hump (mm)*	78.4 ± 0.8 (65, 90)	95.3 ± 2 (70, 120)
EMA (cm^2)	99.6 ± 0.9 (85, 112)	98.8 ± 1 (85, 110)
Rib fat depth (mm)*	11.3 ± 0.4 (6, 19)	14.2 ± 0.7 (7, 25)
Ossification (100 – 590) *	215.3 ± 3.4 (150, 250)	199.4 ± 4.8 (140, 250)
MSA Marbling (100 – 1190)	705.4 ± 22.9 (400, 1150)	659.4 ± 20 (410, 1080)
pHu (0-14) *	5.46 ± 0.006 (5.36, 5.59)	5.55 ± 0.005 (5.46, 5.62)

* Row is significantly different (P<0.05)

4.1.3 Cohort 3 – Purebred Wagyu and F1 Angus x Wagyu

The ossification score was higher in the purebred Wagyu steers by 26.7 ± 5.71 points compared to the F1 Angus cross Wagyu steers (P<0.05, Table 5). There was no difference in hot standard carcase weight, hump height, EMA, pHu, rib fat depth or MSA marble between the purebred Wagyu and F1 Angus cross Wagyu steers (Table 5).

Table 5. Mean (± SEM), minimum and maximum for the carcase characteristics of hot standard carcase weight (HSCW), hump height, eye muscle area (EMA), rib fat depth, ossification, MSA marbling score and ultimate pH (pHu) for the cohort 3 purebred Wagyu (WYWY) and F1 Angus x Wagyu (WYAA) steers.

	Breed	
Carcase Trait	WYAA	WYWY
Total Carcases	18	18
HSCW (kg)	434.4 ± 9.5 (355.5, 518.5)	421.4 ± 8.5 (363, 485.5)
Hump (mm)	92.8 ± 2.6 (75, 115)	97.5 ± 2.8 (75, 120)
EMA (cm^2)	103.2 ± 2 (88, 121)	104.7 ± 2.2 (87, 118)
Rib fat depth (mm)	13.3 ± 0.7 (6, 22)	13.5 ± 1 (7, 23)
Ossification (100 – 590) *	157.2 ± 3.1 (140, 190)	183.9 ± 4.8 (140, 230)
MSA Marbling (100 – 1190)	680 ± 15.3 (570, 790)	699.4 ± 22.4 (560, 880)
pHu (0-14)	5.44 ± 0.014 (5.34, 5.56)	5.44 ± 0.008 (5.38, 5.51)

* Row is significantly different (P<0.05)

4.2 Sensory

The following section outlines the sensory outcomes (GRL, SSB and YAK) for each cohort.

4.2.1 Grill

4.2.1.1 Cohort 1 Grill – Angus and F1 Angus x Wagyu

All fixed effects used to model tenderness, juiciness, flavour, overall liking and MQ4 scores in cohort 1 are presented in Table 6 below. The fixed effects used to model GRL samples for cohort 1 were good predictors of tenderness (R2 = 0.638). The main driver of tenderness for GRL samples in cohort 1 was cut ($P < 0.001$). There was a breed effect for tenderness ($P = 0.043$), with AAAA scoring 59.5 ± 1.02 compared to WYAA at 62.3 ± 1.00. Ossification ($P = 0.013$) and pH_u ($P = 0.012$) also had an impact. The curvilinear response of MSA marble score showed a tendency for effect ($P = 0.082$), as did the hump height by HSCW interaction ($P = 0.057$). Figure 1 (below) appears to show a difference between the breeds for the CHK078, however there was no interaction between breed type and cut ($P = 0.343$).

Figure 1. Tenderness ± SE of cohort 1 Angus (AAAA) and Wagyu x Angus F1 (WYAA) carcases within cuts

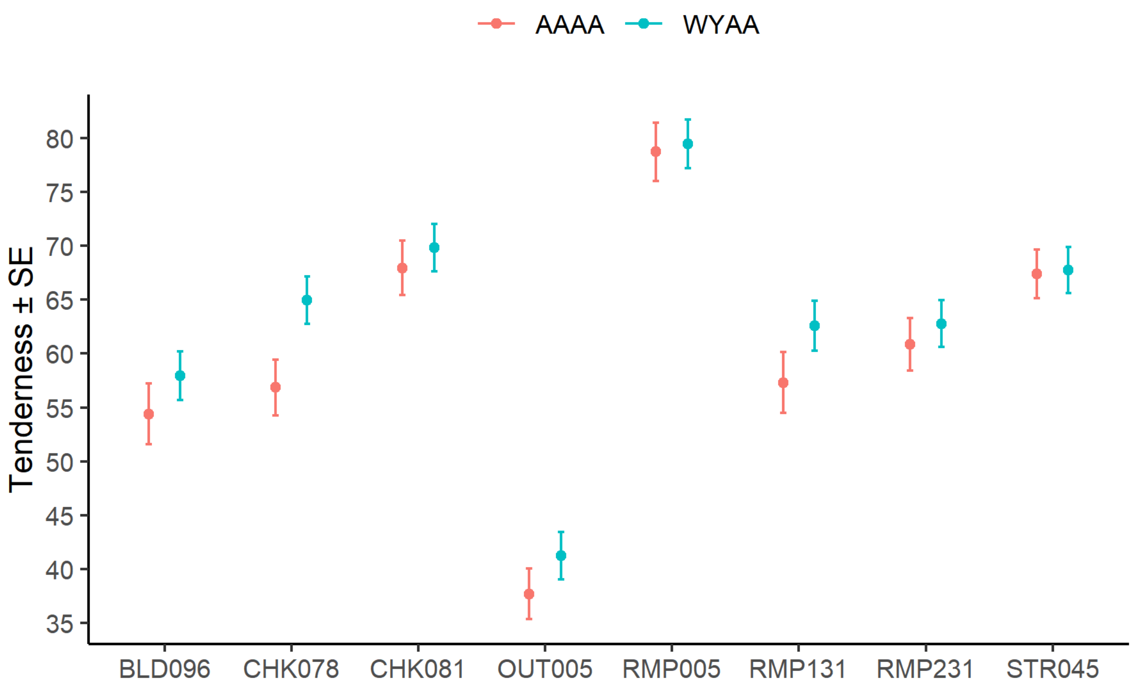


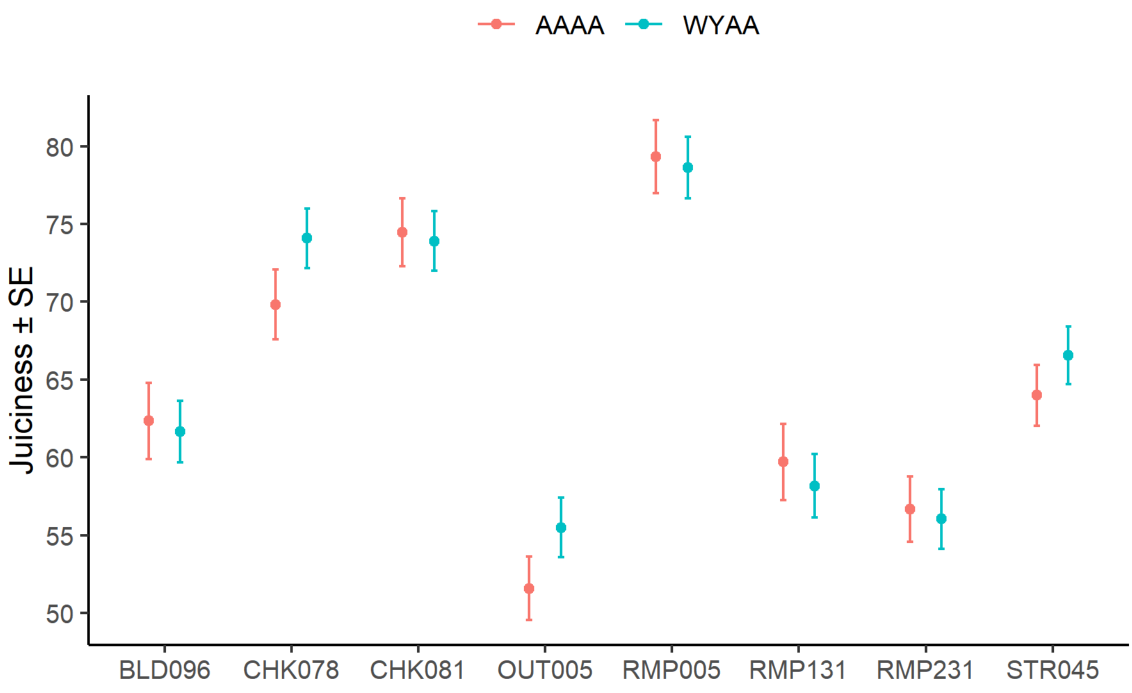
Table 6. The numerator degree of freedom (NDF), denominator degree of freedom (DDF), F-value and the p-value for each of the factors which compose the models to predict tenderness, juiciness, flavour, overall liking and MQ4 for cohort 1 Grill samples.

Trait	Tenderness		Juiciness		Flavour		Overall Liking		MQ4	
	NDF,DDF	F-value	NDF,DDF	F-value	NDF,DDF	F-value	NDF,DDF	F-value	NDF,DDF	F-value
Breed	1, 227	5.73**	1, 228	0.81	1, 227	1.31	1, 228	3.22*	1, 227	3.54*
Cut	7, 227	48.29***	7, 228	39.34***	7, 227	34.63***	7, 228	43.48***	7, 227	48.30***
MSA MB	1, 227	6.87***	1, 228	14.49***	1, 227	34.72***	1, 228	19.80***	1, 227	21.47***
Ossification	1, 227	5.37**	1, 228	0.08	1, 227	0.53	1, 228	2.16	1, 227	3.01*
Hump (mm)	1, 227	0.41	1, 228	1.07	1, 227	0.06	1, 228	0.10	1, 227	0.00
Ultimate pH	1, 227	4.58**	1, 228	2.33	1, 227	2.01	1, 228	3.12*	1, 227	5.07**
Rib Fat (mm)	1, 227	0.32	1, 228	0.03	1, 227	1.67	1, 228	0.06	1, 227	1.25
HSCW (kg)	1, 227	3.89**	1, 228	0.65	1, 227	1.86	1, 228	3.10*	1, 227	3.18*
Breed : Cut	7, 227	0.58	7, 228	0.64	7, 227	1.32	7, 228	1.04	7, 227	0.84
Hump : HSCW	1, 227	3.75*	228, 228							
Ossification : HSCW					1, 227	3.58*				
MSA MB : HSCW									1, 227	3.95**

*P<0.1, ** P<0.05, *** P<0.01

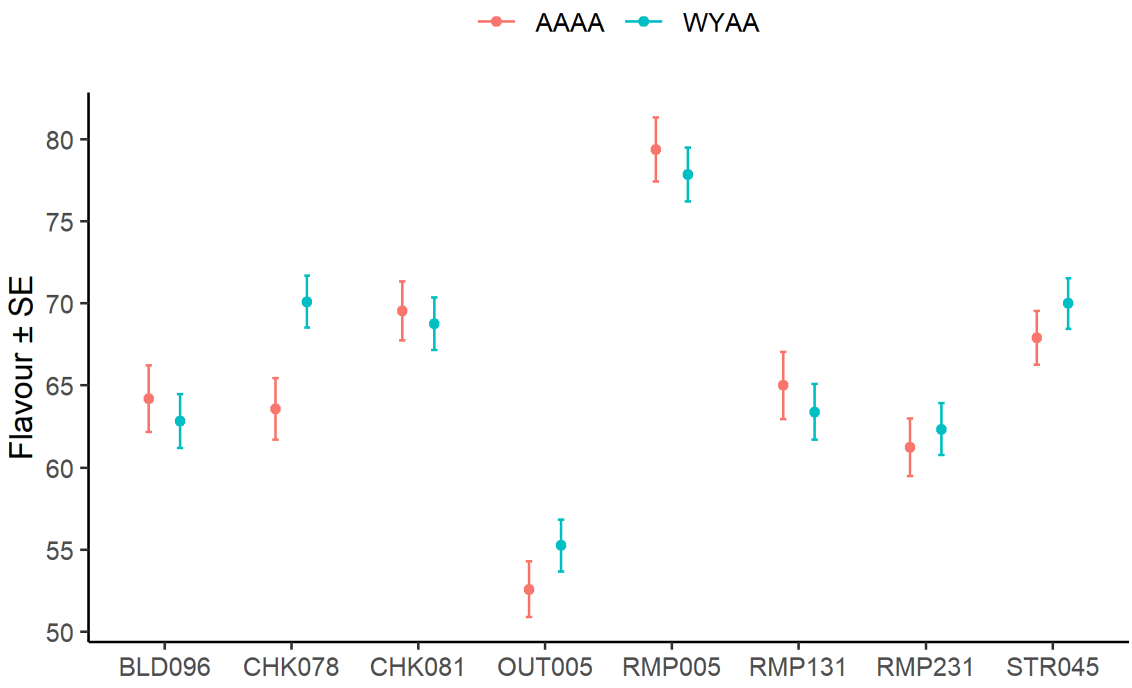
Juiciness scores of GRL samples appeared to be determined by a combination of cut and MSA marble score ($P < 0.001$; $R^2 = 0.575$). All other carcase traits were not reliable indicators of juiciness, and breed had no impact on juiciness scores ($P = 0.37$; Fig. 2).

Figure 2. Juiciness ± SE of cohort 1 Angus (AAAA) and Wagyu x Angus F1 (WYAA) carcases within cuts



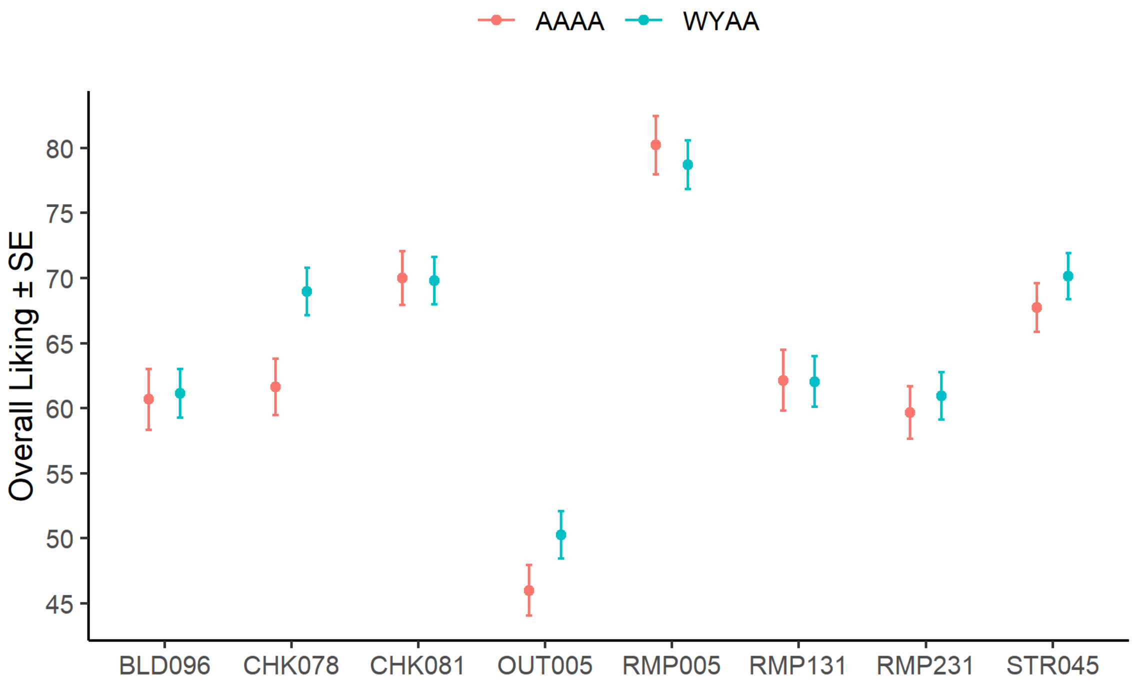
Consumer liking of flavour was also influenced by cut and MSA marble score ($P < 0.001$; $R^2 = 0.587$), however there appeared to be an interaction between ossification and hump height ($P = 0.06$). Breed had no impact on flavour ($P = 0.254$; Fig. 3).

Figure 3. Flavour ± SE of cohort 1 Angus (AAAA) and Wagyu x Angus F1 (WYAA) carcases within cuts



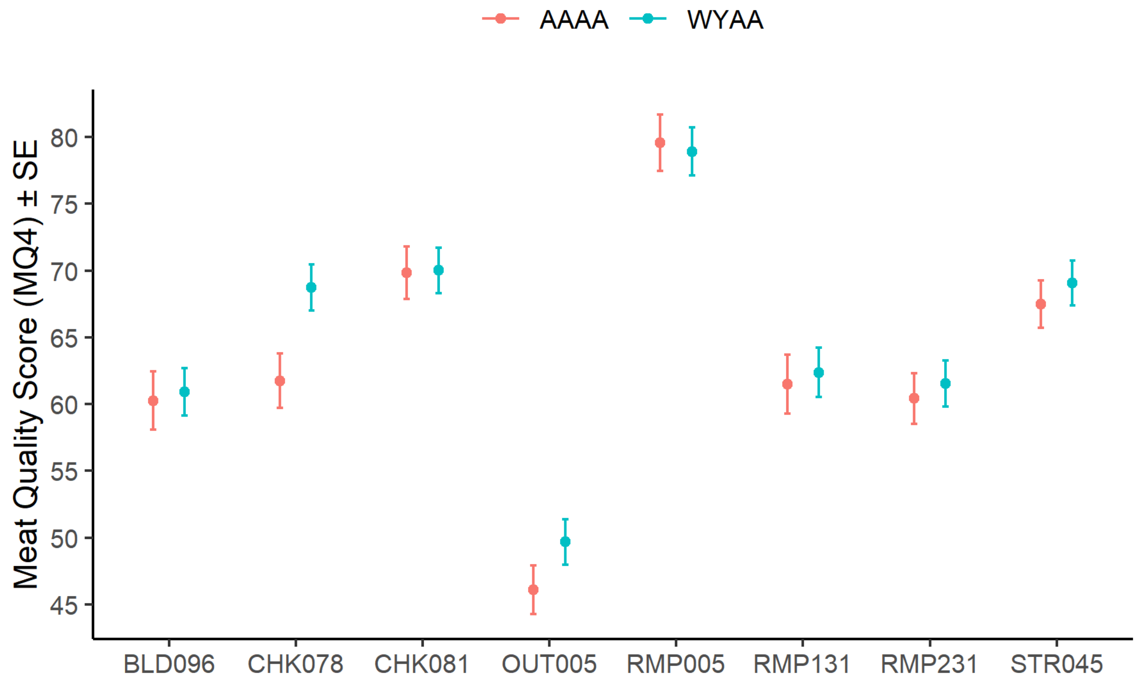
There was a tendency for breed to impact consumer sensory perception of overall liking (P = 0.074), however this was again associated with cut and MSA marble score (P < 0.001). Interestingly, there was also a tendency for increasing HSCW to decrease overall liking (P = 0.079).

Figure 4. Overall liking ± SE of cohort 1 Angus (AAAA) and Wagyu x Angus F1 (WYAA) carcases within cuts



The MQ4 scores provided by consumers appeared to be mostly driven by cut (Fig. 5) and MSA marbles score (P < 0.001) as well as pHu (P = 0.025) and a MSA marble score and HSCW interaction (P = 0.048). However, there were tendencies for ossification (P = 0.084) and HSCW (P = 0.076) to have an impact on the MQ4 outcomes. Overall, the MQ4 model accounted for a reasonable amount of variation observed in MQ4 score (R^2 = 0.64).

Figure 5. Meat quality score ± SE of cohort 1 Angus (AAAA) and Wagyu x Angus F1 (WYAA) carcases within cuts



4.2.1.2 Cohort 2 Grill – High content crossbred Wagyu (F3 or higher ≥ 87.5% Wagyu) and F1 Wagyu x *Bos indicus* composites

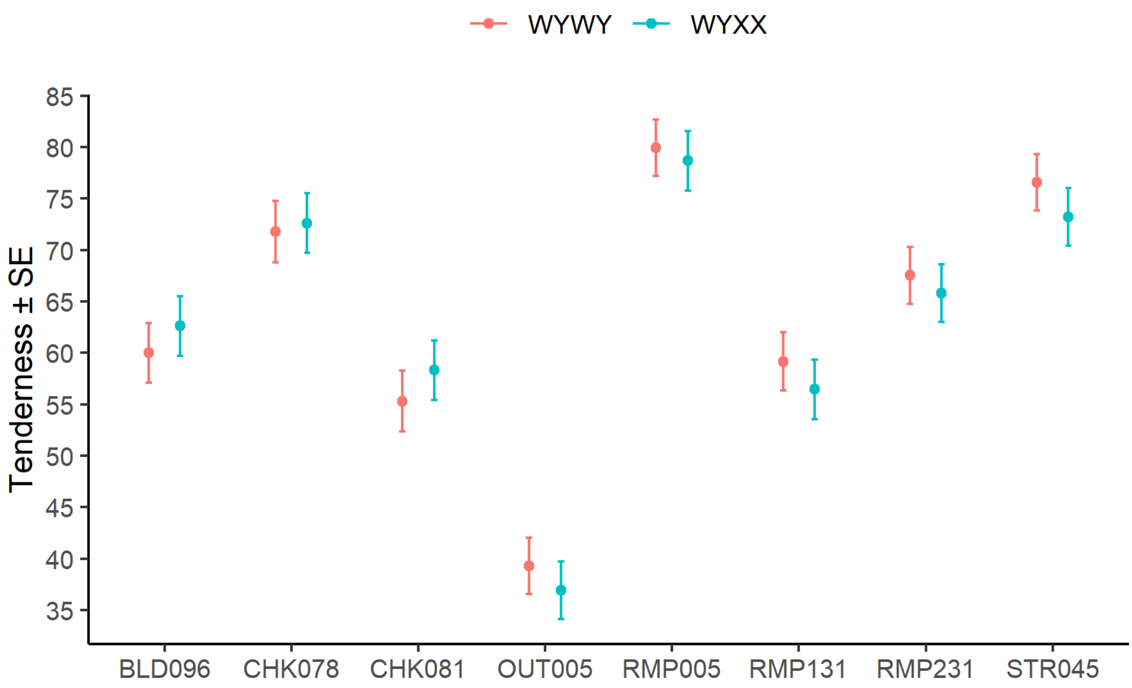
All fixed effects used to model tenderness, juiciness, flavour, overall liking and MQ4 scores in cohort 2 are presented in Table 7 below. Cohort 2 tenderness scores were influenced by cut ($P < 0.001$), ossification ($P = 0.012$), rib fat ($P = 0.007$) and the curvilinear response to rib fat ($P = 0.023$). The breed by cut interaction did not appear to be a driver of tenderness in cohort 2 (Fig. 6).

Table 7. The numerator degree of freedom (NDF), denominator degree of freedom (DDF), F-value and the p-value for each of the factors which compose the models to predict tenderness, juiciness, flavour, overall liking and MQ4 for cohort 2 Grill samples.

Trait	Tenderness		Juiciness		Flavour		Overall Liking		MQ4	
	NDF,DDF	F-value	NDF,DDF	F-value	NDF,DDF	F-value	NDF,DDF	F-value	NDF,DDF	F-value
Breed	1, 243	0.09	1, 244	1.62	1, 243	0.16	1, 243	0.06	1, 243	0.80
Cut	7, 243	52.53***	7, 244	44.22***	7, 243	27.65***	7, 243	38.90***	7, 243	46.32***
MSA MB	1, 243	1.51	1, 244	0.57	1, 243	3.00*	1, 243	2.18	1, 243	0.50
Ossification	1, 243	6.45**	1, 244	1.92	1, 243	0.74	1, 243	3.59*	1, 243	3.80*
Hump (mm)	1, 243	0.03	1, 244	0.00	1, 243	0.02	1, 243	0.01	1, 243	0.24
Ultimate pH	1, 243	1.65	1, 244	0.18	1, 243	4.94**	1, 243	5.06**	1, 243	7.03***
Rib Fat (mm)	1, 243	7.36***	1, 244	4.19**	1, 243	5.79**	1, 243	5.39**	1, 243	10.94***
HSCW (kg)	1, 243	1.15	1, 244	3.24*	1, 243	0.56	1, 243	0.74	1, 243	0.94
MSA MB^2									1, 243	4.78**
Rib Fat (mm)^2	1, 243	5.23**			1, 243	0.81	1, 243	3.96**	1, 243	9.03***
Breed x Cut	7, 243	0.44	7, 244	1.04	7, 243	4.90**	7, 243	0.67	7, 243	0.59
Ossification x HSCW									1, 243	3.95**
MSA MB x HSCW									1, 243	8.37***

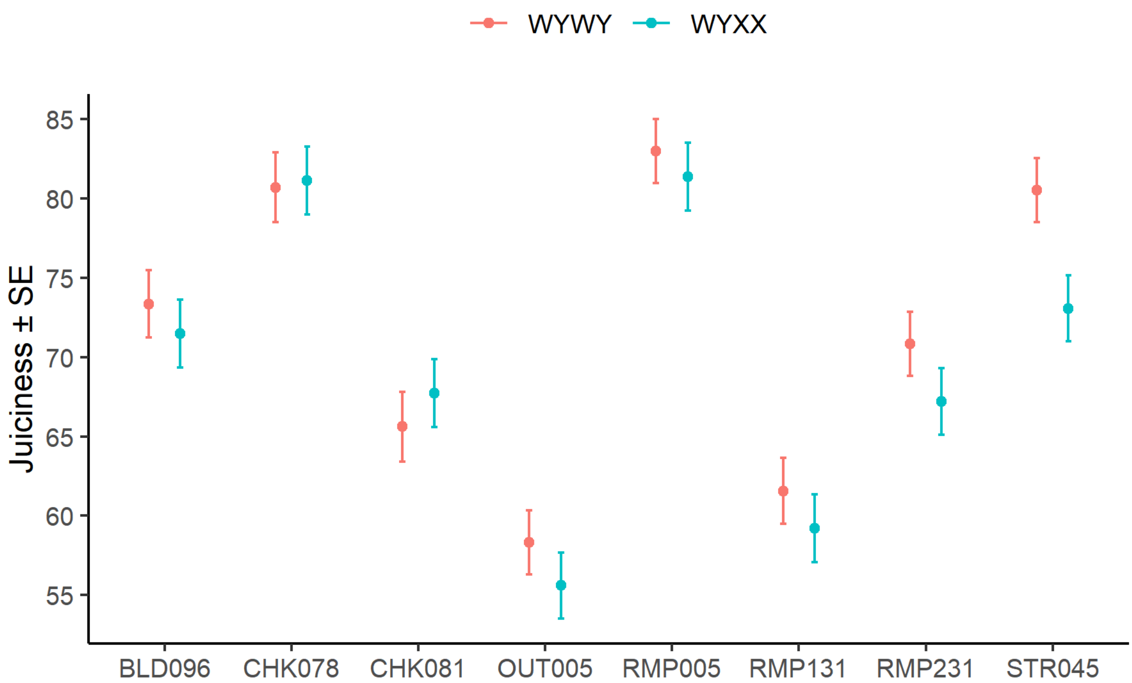
*P<0.1, ** P<0.05, *** P<0.01

Figure 6. Tenderness ± SE of grill samples for cohort 2 high content crossbred Wagyu (WYWY, F3 or higher ≥ 87.5% Wagyu) and Wagyu *x Bos indicus* F1 (WYXX) carcases within cuts



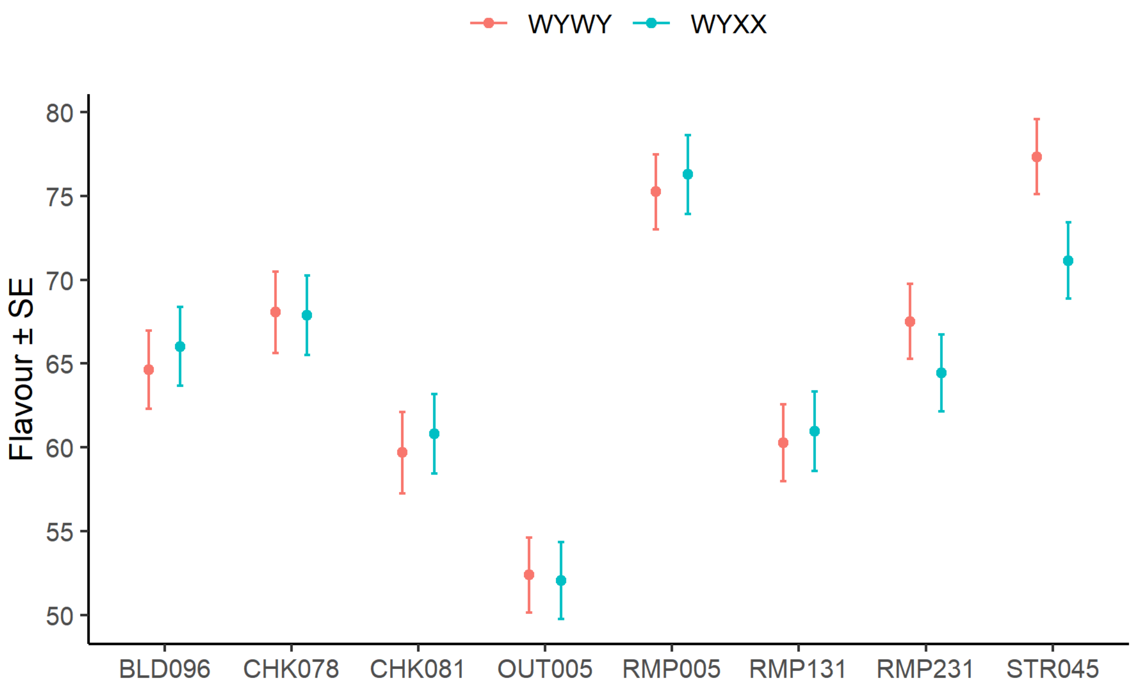
Juiciness was associated with cut (P < 0.001), rib fat (P = 0.004) and the curvilinear response to rib fat (P = 0.011), HSCW (P = 0.039), and an interaction between HSCW and MSA marble score (P = 0.023, Table 7). There was no breed effect or interaction between breed and cut (P > 0.05; Fig. 7).

Figure 7. Juiciness ± SE of grill samples for cohort 2 high content crossbred Wagyu (WYWY, F3 or higher ≥ 87.5% Wagyu) and Wagyu x *Bos indicus* F1 (WYXX) carcases within cuts



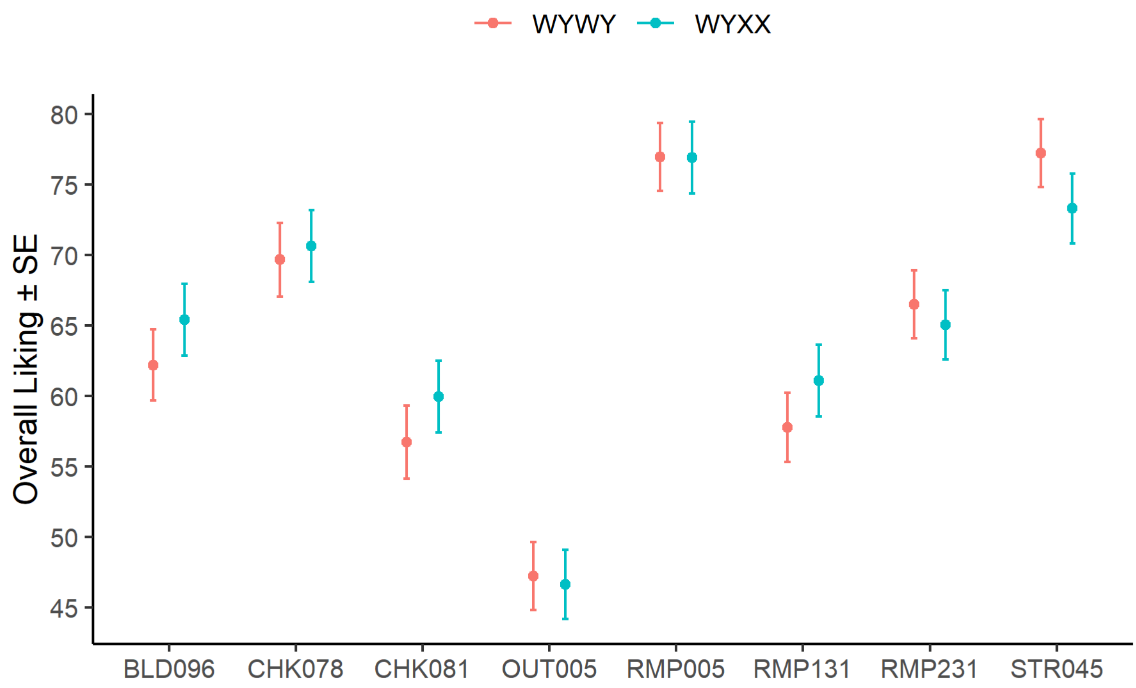
Flavour appeared to be driven by similar fixed effects as juiciness, with cut ($P < 0.001$), pHu ($P = 0.005$), rib fat ($P = 0.002$) and its curvilinear response ($P = 0.004$), and the MSA marble score and HSCW interaction ($P = 0.007$). Breed had no impact on flavour ($P = 0.512$), with a predicted flavour of 65.1 ± 1.26 for the high content crossbred Wagyu and 66.5 ± 1.55 for the Wagyu composite F1 carcases. The breed by cut interaction shows this outcome (Fig. 8).

Figure 8. Flavour ± SE of grill samples for cohort 2 high content crossbred Wagyu (WYWY, F3 or higher ≥ 87.5% Wagyu) and Wagyu x *Bos indicus* F1 (WYXX) carcases within cuts



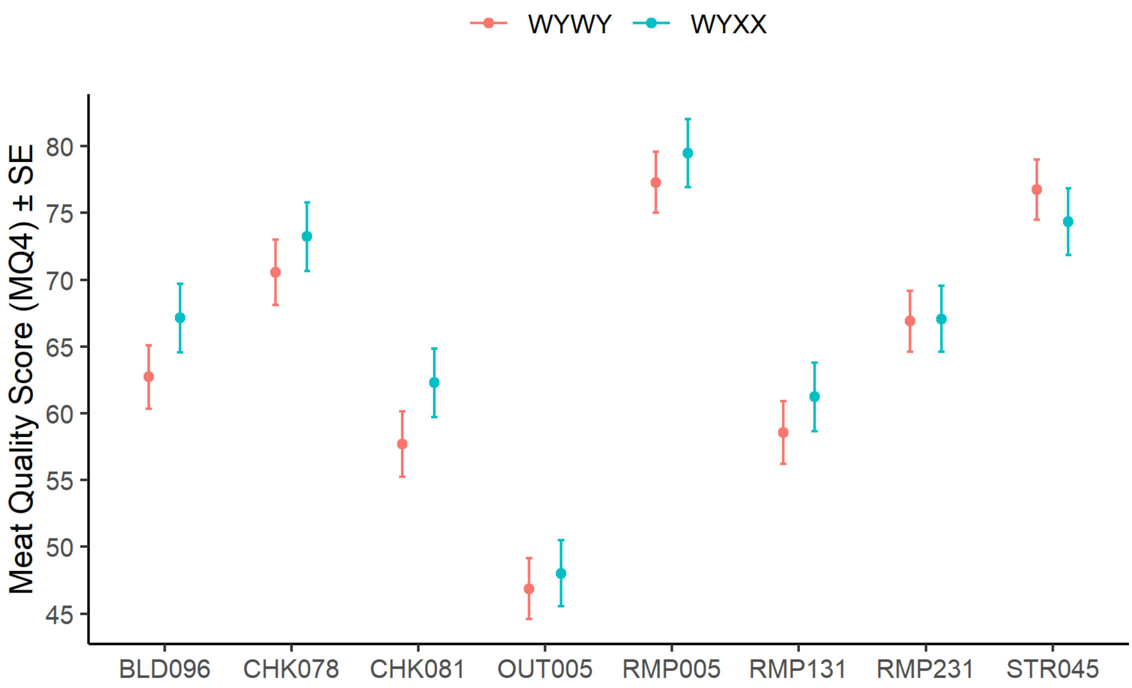
The overall liking scores from consumers were explained by cut ($P < 0.001$), pHu ($P = 0.004$), rib fat ($P = 0.003$) and its curvilinear response ($P = 0.008$), the curvilinear response to MSA marble score ($P = 0.037$) and the interaction between HSCW and MSA marble score ($P = 0.006$). There was a tendency for ossification ($P = 0.058$) and the ossification and HSCW interaction ($P = 0.075$). Breed had no impact ($P = 0.198$), nor did the breed by cut interaction ($P = 0.692$; Fig. 9).

Figure 9. Overall liking ± SE of grill samples for cohort 2 high content crossbred Wagyu (WYWY, F3 or higher ≥ 87.5% Wagyu) and Wagyu x *Bos indicus* F1 (WYXX) carcases within cuts



Meat quality score was similarly explained by cut (P < 0.001), pHu (P = 0.009), rib fat (P = 0.001) and its curvilinear response (P = 0.003), the curvilinear response to MSA marble score (P = 0.030) and the interaction between HSCW and MSA marble score (P = 0.004) and the ossification and HSCW interaction (P = 0.048). There was a tendency for ossification (P = 0.052). Breed had no impact (P = 0.371), nor did the breed by cut interaction (P = 0.761; Fig. 10).

Figure 10. Meat quality score ± SE of grill samples for cohort 2 high content crossbred Wagyu (WYWY, F3 or higher ≥ 87.5% Wagyu) and Wagyu x *Bos Indicus F1* (WYXX) carcases within cuts



4.2.1.3 Cohort 3 Grill – Purebred Wagyu (unregistered) and F1 Angus Wagyu

For cohort 3, breed type, rib fat, ossification and an interaction between breed type and hump height had a significant effect on the meat-eating quality score (MQ4) and tenderness, as well as an interaction between hump height and rib fat depth for overall liking (Table 8, P<0.05). Breed, rib fat depth and ossification had a significant effect on the flavour scores (Table 8, P<0.05) while breed type, hump and an interaction between breed type and hump had a significant effect on the juiciness score (Table 8, P<0.05).

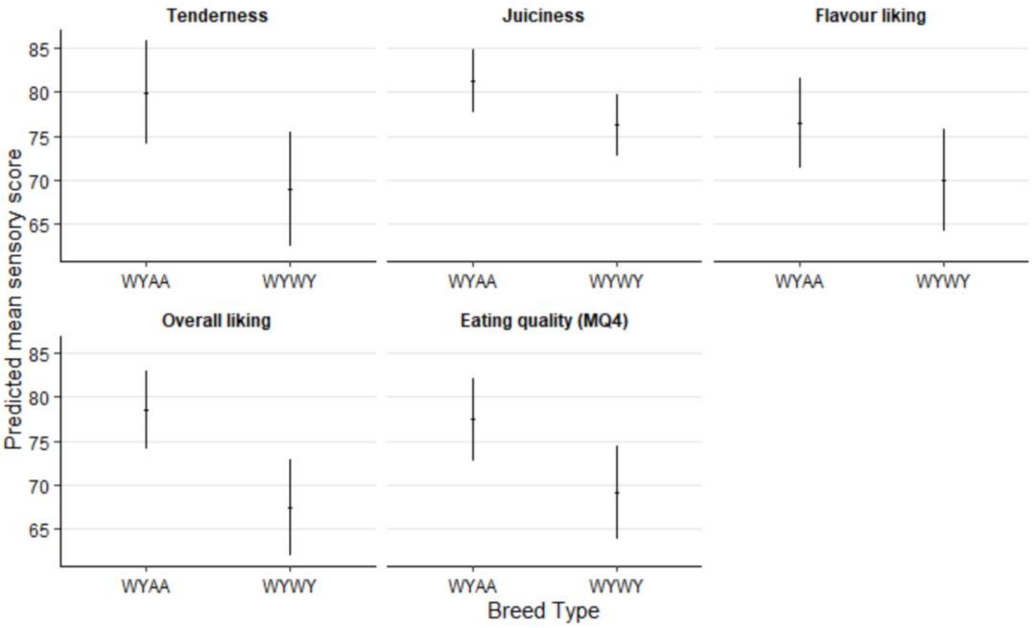
Table 8. The numerator degree of freedom (NDF), denominator degree of freedom (DDF), F-value and the p-value for each of the factors which compose the models to predict tenderness, juiciness, flavour, overall liking and MQ4 for cohort 3 Grill samples.

Term	Tenderness		Juiciness		Flavour		Overall Liking		MQ4	
	NDF,DDF	F-Value	NDF,DDF	F-Value	NDF,DDF	F-Value	NDF,DDF	F-Value	NDF,DDF	F-Value
(Intercept)	1,28	2828.83***	1,32	4275.76***	1,28	3467.48***	1,27	4971.91***	1,28	4141.77***
Breed Type	1,28	7.56**	1,32	6.29**	1,28	2.53	1,27	7.21**	1,28	6.04**
Hump	1,28	2.77	1,32	4.39**	1,28	1.68	1,27	5.07**	1,28	3.87*
Rib Fat	1,28	1.23			1,28	1.30	1,27	2.54	1,28	1.84
Rib fat^2	1,28	6.72**			1,28	6.13**	1,27	7.86***	1,28	7.98***
Ossification	1,28	1.40			1,28	0.88	1,27	1.06	1,28	1.47
Ossification^2	1,28	5.59**			1,28	5.36**	1,27	9.41***	1,28	7.30***
Breed Type: Hump	1,28	6.92**	1,32	8.67***	1,28	3.79*	1,27	4.21**	1,28	5.23**
Hump: Rib Fat							1,27	4.17*		

*P<0.1, ** P<0.05, *** P<0.01

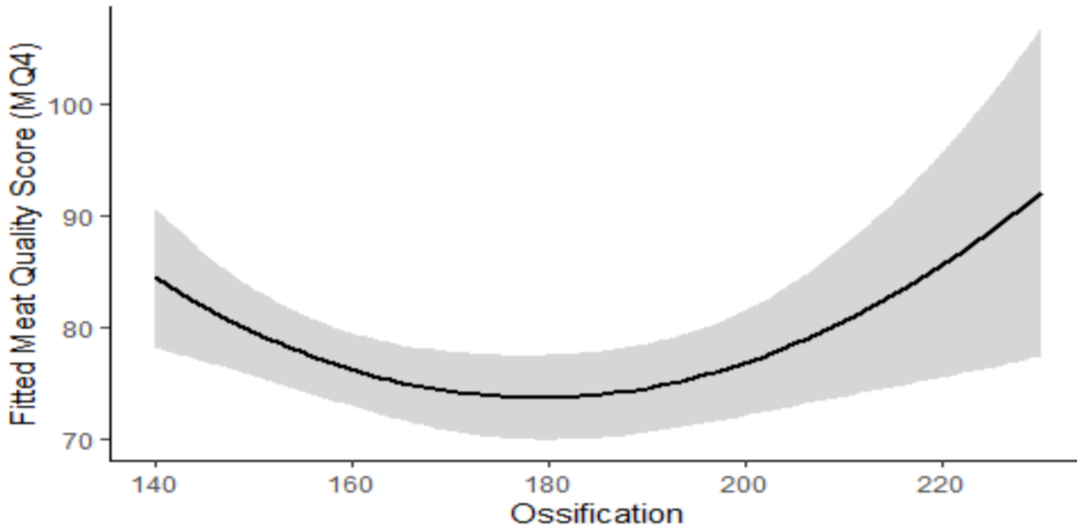
The consumer tenderness and juiciness scores for the F1 Angus x Wagyu steers grilled STR045 was 11.00 ± 3.98 and 5.03 ± 2.44 points higher than those from the purebred Wagyu steers (P<0.05, Fig. 11). The consumer flavour liking scores for the F1 Angus x Wagyu steers grilled STR045 was 6.51 ± 3.52 points higher than those from the purebred Wagyu steers (P=0.075, Fig. 11). The consumer overall liking scores were 11.2 ± 3.41 points higher (P = 0.003, Fig. 11) and the MQ4 scores were 8.37 ± 3.23 points higher (P = 0.015, Fig. 11) for the F1 Angus x Wagyu steers grilled STR045 than those from the purebred Wagyu steers.

Figure 11. Estimated marginal means with 95% confidence intervals of consumer sensory scores for the tenderness, juiciness, flavour liking, overall liking and eating quality (MQ4) scores of the STR045 (*M. longissimus lumborum*) from unregistered purebred Wagyu (WYWY) and F1 Wagyu cross Angus (WYAA) steers consumed using the grill cook method.



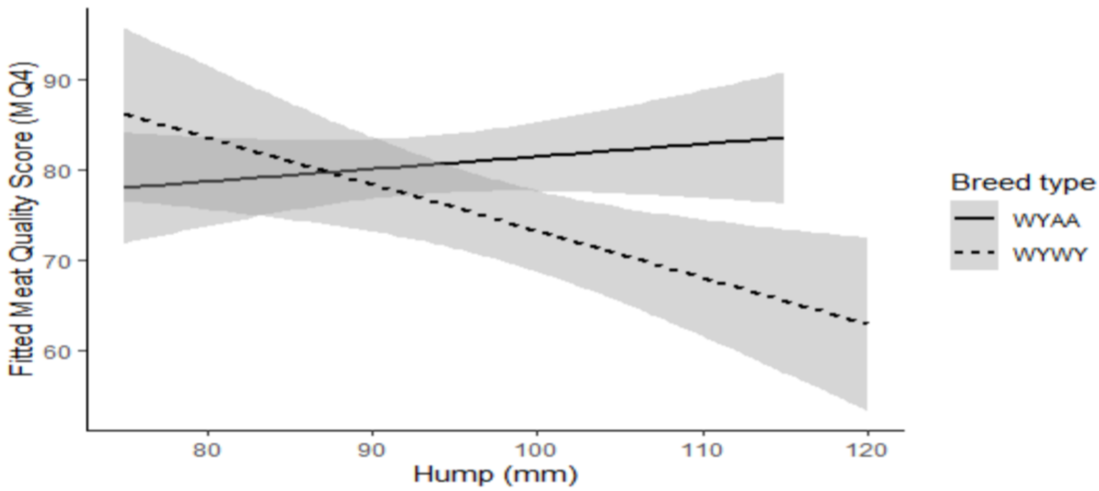
In cohort 3 there was a curvilinear relationship between ossification score and the fitted MQ4 score (Fig. 12). The MQ4 score decreased as ossification increased from 140 to 180 then increased from 180 to 230. Ossification also had a curvilinear relationship on tenderness, liking of flavour and overall liking (P<0.05, Table 8).

Figure 12. The effect of ossification on the fitted MQ4 score for the STR045 cooked using grill cook method for cohort 3.



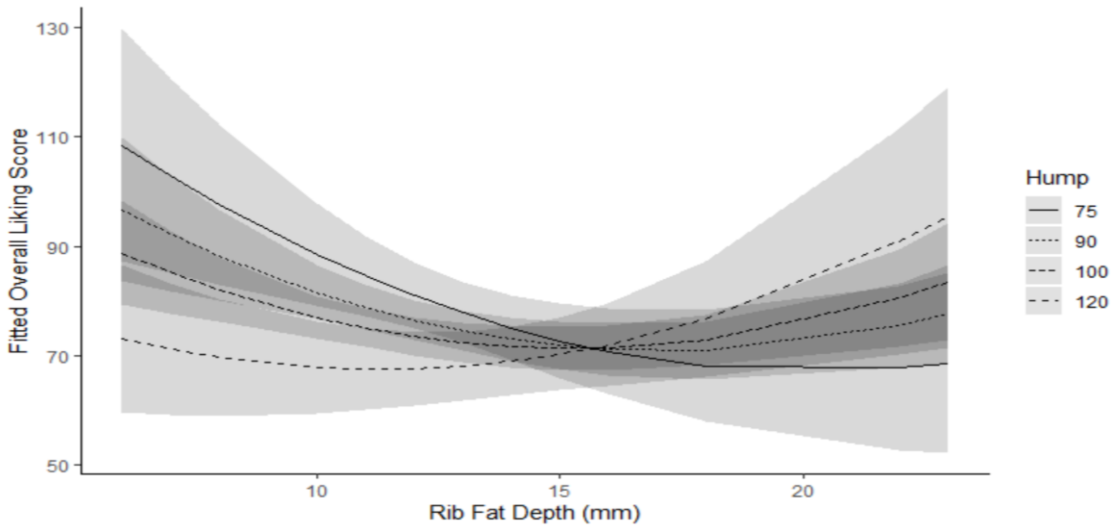
In cohort 3, there was a negative relationship between hump height and the fitted MQ4 score for the purebred Wagyu between 75mm and 120mm, whilst the fitted MQ4 score for the F1 Wagyu had a slight positive relationship with hump height between 75mm and 115mm (Fig. 13). The tenderness, juiciness, and overall liking scores for the purebred Wagyu decreased as the hump height increased from 75mm to 120mm.

Figure 13. The effect of hump on the fitted MQ4 score for the STR045 cooked using grill cook method for cohort 3.



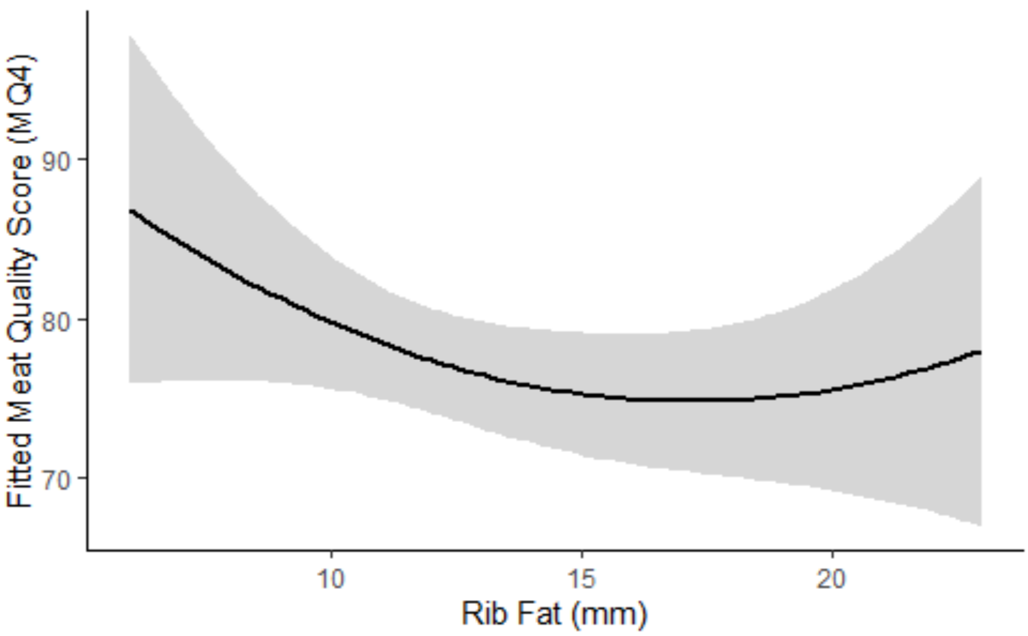
In cohort 3 there was a curvilinear relationship between the fitted overall liking score and the rib fat depth for hump height (and the fitted MQ4 score (Fig. 14, P<0.05, Table 8)). When hump height is low the fitted overall liking score decreases dramatically between the rib fat depth of 6 to 15mm, then plateaus from 15 to 23mm. Whereas the higher hump height starts at a lower fitted overall liking value between 6 and 15mm then increases from 15 to 23mm (Fig. 14).

Figure 14. The effect of rib fat depth and hump height on the fitted overall liking score for the STR045 cooked using grill cook method for cohort 3.



In cohort 3 there was a curvilinear relationship between rib fat depth and the fitted MQ4 score (Fig. 15). The MQ4 score decreased as rib fat increased from 6 to 15mm then had no change between 15 to 23mm. Rib fat depth also had a curvilinear relationship on tenderness, liking of flavour and overall liking (P<0.05, Table 8).

Figure 15. The effect of rib fat depth on the fitted MQ4 score for the STR045 cooked using grill cook method for cohort 3.



4.2.2 Shabu Shabu

4.2.2.1 Cohort 1 Shabu Shabu – Angus and Wagyu X Angus F1's

Cut, rib fat depth and marbling had a significant effect on the meat eating quality score (MQ4) and overall liking score of SSB samples (Table 9, P<0.05). Cut, ossification and marbling had a significant effect on the tenderness and juiciness scores (Table 9, P<0.05) while cut, rib fat and marbling had a significant effect on the liking of flavour score (Table 9, P<0.05).

There was no significant effect of breed type on the tenderness, juiciness, liking of flavour, overall liking or MQ4 score of the CHK074, CHK078, OUT005 and STR045 from AAAA steers and WYAA steers consumed using the Shabu Shabu cook method (Fig. 16). There was no significant difference in MQ4, tenderness, juiciness, flavour and overall liking scores between the CHK074, CHK078 and STR045 (Fig. 16). The OUT005 had the lowest tenderness scores and was significantly lower than the CHK074, CHK078 and STR045 by 12.69 ± 2.55, 12.21 ± 2.52, and 15.79 ± 2.51 points respectively (P<0.05, Fig. 16). The OUT005 had the lowest juiciness scores and was significantly lower than the CHK074, CHK078 and STR045 by 6.36 ± 2.02, 8.08 ± 2.01, and 7.97 ± 1.99 points respectively (P<0.05, Fig. 16). The OUT005 had the lowest flavour scores and was significantly lower than the CHK074 and STR045 by 5.95 ± 1.82, and 8.36 ± 1.79 points respectively (P<0.05, Fig. 16). There was no significant difference between the flavour scores for the OUT005 and the CHK078 (P= 0.0593, Fig. 16). The OUT005 also had the lowest overall liking scores and was significantly lower than the CHK074, CHK078 and STR045 by 8.38 ± 1.87, 7.49 ± 1.85, and 10.81 ± 1.84 points respectively (P<0.05, Fig. 16). The OUT005 also had the lowest eating quality (MQ4) scores and was significantly lower than the CHK074, CHK078 and STR045 by 8.82 ± 1.85, 8.33 ± 1.83, and 11.65 ± 1.82 points respectively (P<0.05, Fig. 16).

Figure 16. Estimated marginal means with 95% confidence intervals of consumer sensory scores for the tenderness, juiciness, flavour liking, overall liking and eating quality (MQ4) scores of the CHK074 (*M.semispinalis capitis*), CHK078 (*M. serratus ventralis*), OUT005 (*M. biceps femoris*) and STR045 (*M. longissimus lumborum*) from Angus (AAAA) and F1 Angus x Wagyu (WYAA) steers consumed using Shabu Shabu cook method.

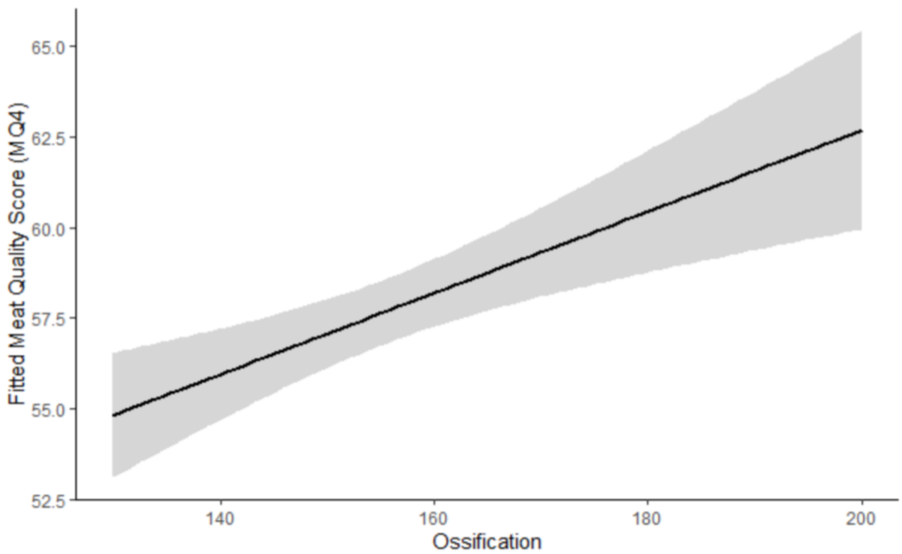
Table 9. The numerator degree of freedom (NDF), denominator degree of freedom (DDF), F-value and the p-value for each of the factors which compose the models to predict tenderness, juiciness, flavour, overall liking and MQ4 for cohort 1 Shabu Shabu samples.

*P<0.1, ** P<0.05, *** P<0.01

Term	Tenderness		Juiciness		Flavour		Overall Liking		MQ4	
	NDF,DDF	F-Value	NDF,DDF	F-Value	NDF,DDF	F-Value	NDF,DDF	F-Value	NDF,DDF	F-Value
(Intercept)	1,101	2724.28***	1,101	5433.12***	1,101	8290.96***	1,101	6880.80***	1,101	6780.04***
Cut	3,101	15.60***	3,101	7.43***	3,101	7.79***	3,101	13.06***	3,101	15.42***
Breed Type	1,101	1.38	1,33	7.32	1,101	2.09	1,101	3.46*	1,101	2.22
Ossification	1,33	6.29**	1,101	0.79**			1,31	5.29**	1,31	6.02**
Rib fat					1,32	0.27	1,31	0.04	1,31	0.90
Rib fat^2					1,32	4.44**	1,31	5.12**	1,31	4.91**
MSA Marbling	1,33	9.33***	1,33	8.384***	1,32	8.47***	1,31	11.06***	1,31	9.39***

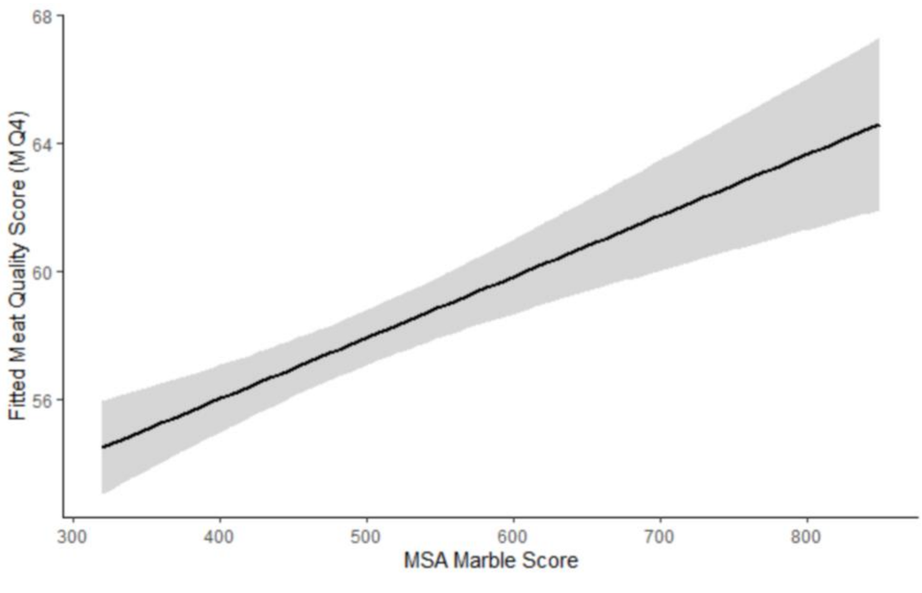
In cohort 1 there was a positive relationship between 130 to 200 ossification score and the fitted MQ4 score (Fig. 17; P<0.05, Table 9). Ossification also had a positive relationship on tenderness, juiciness and overall liking between the range of 130 to 200 in cohort 1 (P<0.05, Table 9). This is possibly due to consumers enjoying meat with more connective tissue when cooked using Shabu Shabu.

Figure 17. The effect of ossification score on fitted MQ4 score for all cuts eaten using the Shabu Shabu cook method in cohort 1.



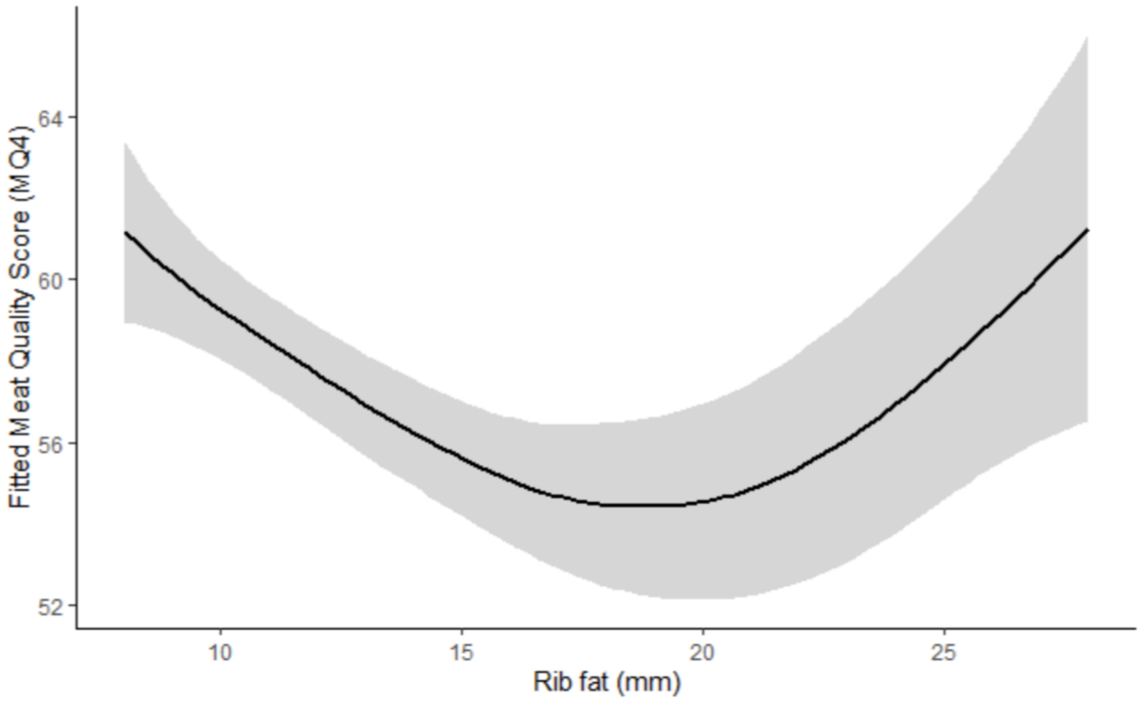
In cohort 1 there was a positive relationship between 320 to 850 MSA marble score and the fitted MQ4 score (Fig. 18; P<0.05, Table 9). Marbling also had a positive relationship on tenderness, juiciness and overall liking between the range of 320 to 850 in cohort 1 (P<0.05, Table 9).

Figure 18. The effect of MSA marble score on fitted MQ4 score for all cuts eaten using the Shabu Shabu cook method in cohort 1.



In cohort 1, there was a curvilinear relationship between rib fat depth and MQ4 score (Fig. 19; P<0.05, Table 9). MQ4 score decreased as rib fat depth increased from 8 to 20, but then as rib fat increased from 20 to 28 the MQ4 score also increased (Fig. 19). Rib fat depth also had a curvilinear relationship with liking of flavour and overall liking (P<0.05, Table 9).

Figure 19. The effect of rib fat depth on fitted MQ4 score for all cuts eaten using the Shabu Shabu cook method in cohort 1.



4.2.2.2 Cohort 2 Shabu Shabu – High content crossbred Wagyu (F3 or higher ≥ 87.5% Wagyu) and F1 Wagyu x Bos indicus composites

For cohort 2, cut, rib fat depth, an interaction between cut and ossification as well as an interaction between breed type and rib fat depth all had a significant effect on the meat-eating quality score (MQ4), flavour and overall liking (Table 10, P<0.05). Cut, rib fat depth and MSA marbling had a significant effect on the juiciness scores (Table 10, P<0.05) while cut and an interaction between cut and ossification had a significant effect on the tenderness score (Table 10, P<0.05).

Table 10. The numerator degree of freedom (NDF), denominator degree of freedom (DDF), F-value and the p-value for each of the factors which compose the models to predict tenderness, juiciness, flavour, overall liking and MQ4 for cohort 2 Shabu Shabu samples.

Term	Tenderness		Juiciness		Flavour		Overall Liking		MQ4	
	NDF,DDF	F-Value	NDF,DDF	F-Value	NDF,DDF	F-Value	NDF,DDF	F-Value	NDF,DDF	F-Value
(Intercept)	1,103	3039.64***	1,106	5717.25***	1,103	5017.16***	1,103	5963.65***	1,103	5157.93***
Cut	3,103	34.48***	3,106	19.92***	3,103	18.20***	3,103	24.06***	3,103	29.27***
Breed Type	1,29	1.7	1,30	3.45*	1,29	2.43	1,29	2.14*	1,29	2.8
Ossification	1,29	1.91			1,29	3.03*	1,29	4.07	1,29	3.227*
Rib fat	1,29	2.1	1,30	11.69***	1,29	4.62**	1,29	6.86**	1,29	4.67**
MSA Marbling	1,29	3.05*	1,30	7.67***	1,29	2.51	1,29	3.56*	1,29	3.57*
Cut: Ossification	3,103	3.63**			3,103	4.85***	3,103	6.55***	3,103	5.27***
Breed Type: Rib fat	1,29	3.14*	1,30	3.81*	1,29	5.10**	1,29	6.88**	1,29	5.57**

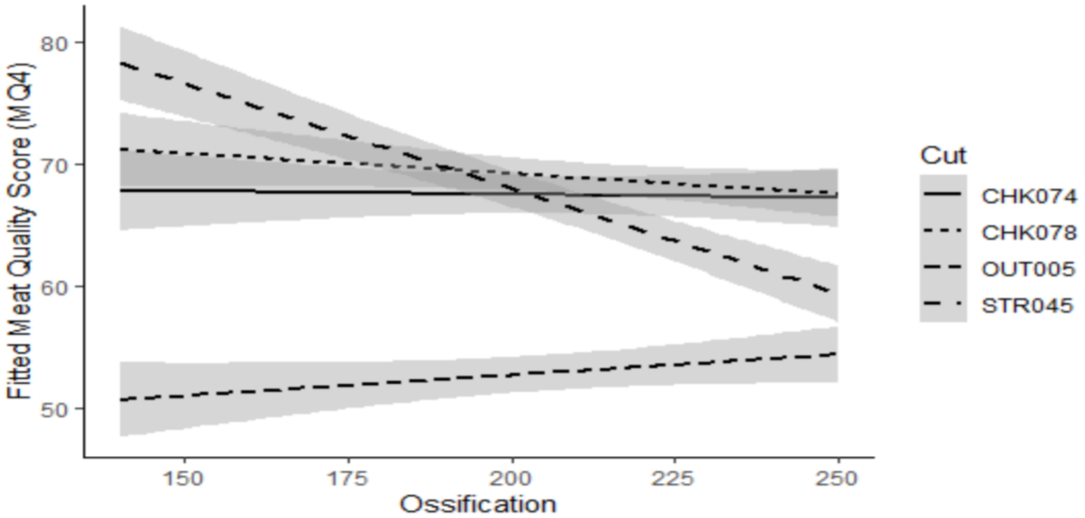
*P<0.1, ** P<0.05, *** P<0.001

There was no significant effect of breed type on the tenderness, juiciness, liking of flavour, overall liking or MQ4 score of the CHK074, CHK078, OUT005 and STR045 from WYWY steers and WYXX steers consumed using Shabu Shabu cook method (Fig. 20). There was no significant difference in tenderness, juiciness, flavour liking, overall liking and MQ4 scores between the CHK074, CHK078 and STR045 (Fig. 20). The OUT005 had the lowest tenderness scores and was significantly lower than the CHK074, CHK078 and STR045 by 20.26 ± 2.60, 22.59 ± 2.49, and 18.25 ± 2.56 points respectively ($P<0.05$, Fig. 20). The OUT005 had the lowest juiciness scores and was significantly lower than the CHK074, CHK078 and STR045 by 10.71 ± 2.13, 15.04 ± 2.03, and 11.51 ± 2.10 points respectively ($P<0.05$, Fig. 20). The OUT005 had the lowest flavour liking scores and was significantly lower than the CHK074, CHK078 and STR045 by 11.59 ± 2.00, 11.41 ± 1.91, and 11.299 ± 1.97 points respectively ($P<0.05$, Fig. 20). The OUT005 also had the lowest overall liking scores and was significantly lower than the CHK074, CHK078 and STR045 by 12.87 ± 2.04, 14.11 ± 1.95, and 12.886 ± 2.01 points respectively ($P<0.05$, Fig. 20). The OUT005 had the lowest eating quality (MQ4) scores and was significantly lower than the CHK074, CHK078 and STR045 by 14.36 ± 2.01, 15.52 ± 1.92, and 13.72 ± 1.98 points respectively ($P<0.05$, Fig. 20).

Figure 20. Estimated marginal means with 95% confidence intervals of consumer sensory scores for the tenderness, juiciness, flavour liking, overall liking and eating quality (MQ4) scores of the CHK074 (*M.semispinalis capitis*), CHK078 (*M. serratus ventralis*), OUT005 (*M. biceps femoris*) and STR045 (*M. longissimus lumborum*) from high content crossbred Wagyu (WYWY, F3 or higher ≥ 87.5% Wagyu) and F1 Wagyu cross *Bos indicus* (WYXX) steers consumed using Shabu Shabu cook method.

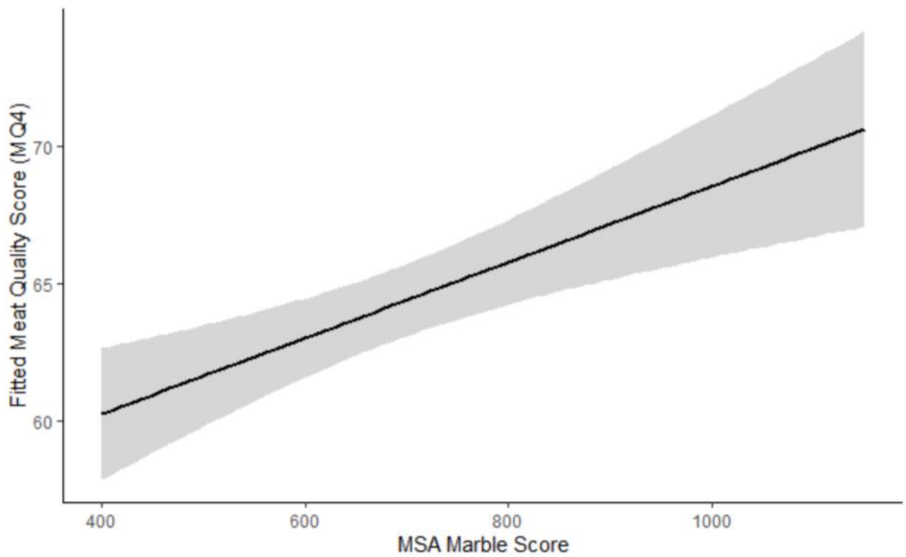
In cohort 2 there was a strong negative linear relationship between ossification score and the fitted MQ4 score for the STR045 but a positive linear relationship for the OUT005, whereas no relationship for CHK074 and the CHK078 as ossification increased from 140 to 250 (Fig. 21, $P<0.05$, Table 10). Ossification also had a positive relationship on tenderness, liking of flavour and overall liking for the OUT005 which increased as ossification increased from 140 to 250 ($P<0.05$, Table 10). Although ossification also had a negative relationship on tenderness, liking of flavour and overall liking for the STR045 and no relationship for the CHK078 and CHK074 which decreased as ossification increased from 140 to 250 (P<0.05, Table 10).

Figure 21. The effect of ossification score on the fitted MQ4 score for each cut cooked using Shabu Shabu cook method for cohort 2



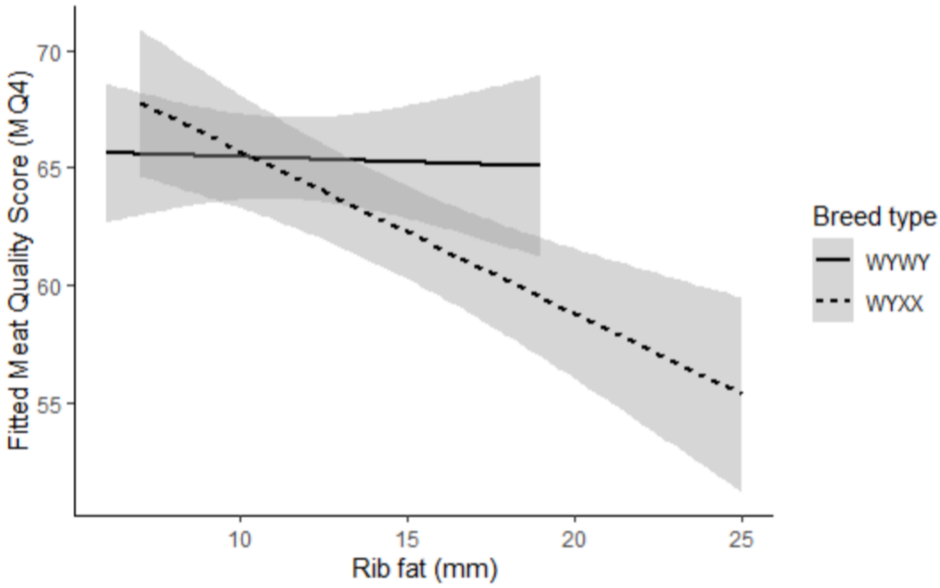
In cohort 2 there was a positive relationship between marbling score and the fitted MQ4 score (Fig. 22, P<0.05, Table 10). Averaged across all muscles (due to no cut by marbling interaction), MQ4 score increased as MSA marbling increased from 400 to 1180 (P<0.05). Marbling also had a positive relationship on tenderness, juiciness, liking of flavour and overall liking as marbling increased from 400 to 1150 (P<0.05, Table 10).

Figure 22. The effect of MSA marble score on the fitted MQ4 score for all cuts cooked using Shabu Shabu cook method for cohort 2



In cohort 2 there was a negative relationship between the fitted MQ4 score and the F1 Wagyu x *Bos indicus* rib fat measurement between 7 to 25 mm, whilst there was no relationship between the fitted MQ4 score and the WYWY (F3 to F5 Wagyu) rib fat measurement between 6 to 19 mm (Fig. 23, P<0.05, Table 10)**.** The liking of flavour and overall liking scores for the F1 Wagyu decreased as rib fat increased from 7 to 25 mm and for the F2 to F5 Wagyu there was no relationship for the rib fat measurement between 6 to 19 mm (P<0.05, Table 10).

Figure 23. The effect of rib fat depth on the fitted MQ4 score for each breed type cooked using Shabu Shabu cook method for cohort 2



4.2.3 Yakiniku

4.2.3.1 Cohort 1 Yakiniku – Angus and F1 Angus x Wagyu

All fixed effects used to model tenderness, juiciness, flavour, overall liking and MQ4 scores in cohort 1 are presented in Table 11 below. There was no relationship between breed type and the tenderness of YAK samples (P = 0.806; R^2 = 0.412). However, both cut and MSA marble score appeared to be the main drivers of the tenderness of YAK samples (P < 0.001). Ultimate pH (positive; P = 0.025), rib fat (negative; P = 0.049) and the curvilinear response to rib fat (positive; P = 0.022) were also related to tenderness scores. There was a negative relationship between tenderness and the interaction of MSA marble score and HSCW (P= 0.023), and a tendency for the interaction between breed type and cut (Fig. 24).

Table 11. The numerator degree of freedom (NDF), denominator degree of freedom (DDF), F-value and the p-value for each of the factors which compose the models to predict tenderness, juiciness, flavour, overall liking and MQ4 for cohort 1 Yakiniku samples.

Trait	Tenderness		Juiciness		Flavour		Overall Liking		MQ4	
	NDF,DDF	F-value	NDF,DDF	F-value	NDF,DDF	F-value	NDF,DDF	F-value	NDF,DDF	F-value
Breed	1, 118	0.06	1, 118	3.42*	1, 115	7.20***	1, 116	2.68	1, 118	1.03
Cut	3, 118	14.88***	3, 118	8.44***	3, 115	6.48***	3, 116	9.89***	3, 118	10.81***
MSA Marbling	1, 118	14.91***	1, 118	6.31**	1, 115	0.07	1, 116	0.78	1, 118	11.76***
Ossification	1, 118	0.59	1, 118	1.96	1, 115	3.81*	1, 116	2.21	1, 118	2.19
Hump (mm)	1, 118	0.07	1, 118	3.27*	1, 115	4.44**	1, 116	3.79*	1, 118	1.78
Ultimate pH	1, 118	5.13**	1, 118	0.75	1, 115	2.50	1, 116	2.43	1, 118	3.10*
Rib Fat (mm)	1, 118	3.97**	1, 118	0.12	1, 115	0.81	1, 116	1.21	1, 118	1.61
HSCW (kg)	1, 118	0.04	1, 118	0.02	1, 115	2.63	1, 116	0.00	1, 118	0.12
MSA MB^2					1, 115	11.19***	1, 116	4.70**		
Rib Fat (mm)^2	1, 118	5.42**								
HSCW (kg)^2					1, 115	4.65**				
Breed x Cut	3, 118	2.42*	3, 118	0.51	3, 115	0.82	3, 116	1.25	3, 118	1.51
Ossification x HSCW			1, 118	3.99**	1, 115	12.03***	1, 116	8.12***		
MSA MB x HSCW	1, 118	5.34**	1, 118	13.44***	1, 115	16.70***	1, 116	10.62***	1, 118	3.99**
MSA MB x Rib Fat					1, 115	15.30***	1, 116	11.77***	1, 118	7.29***

Figure 24. Tenderness ± SE of cohort 1 Angus (AAAA) and Wagyu x Angus F1 (WYAA) carcases within cuts

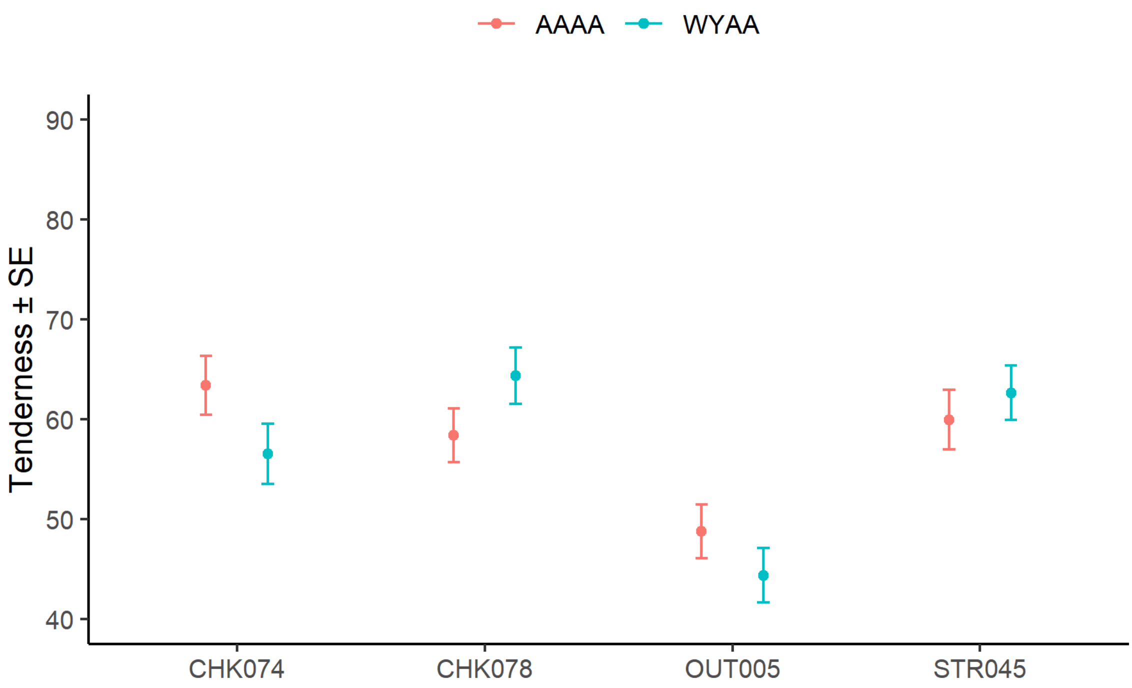
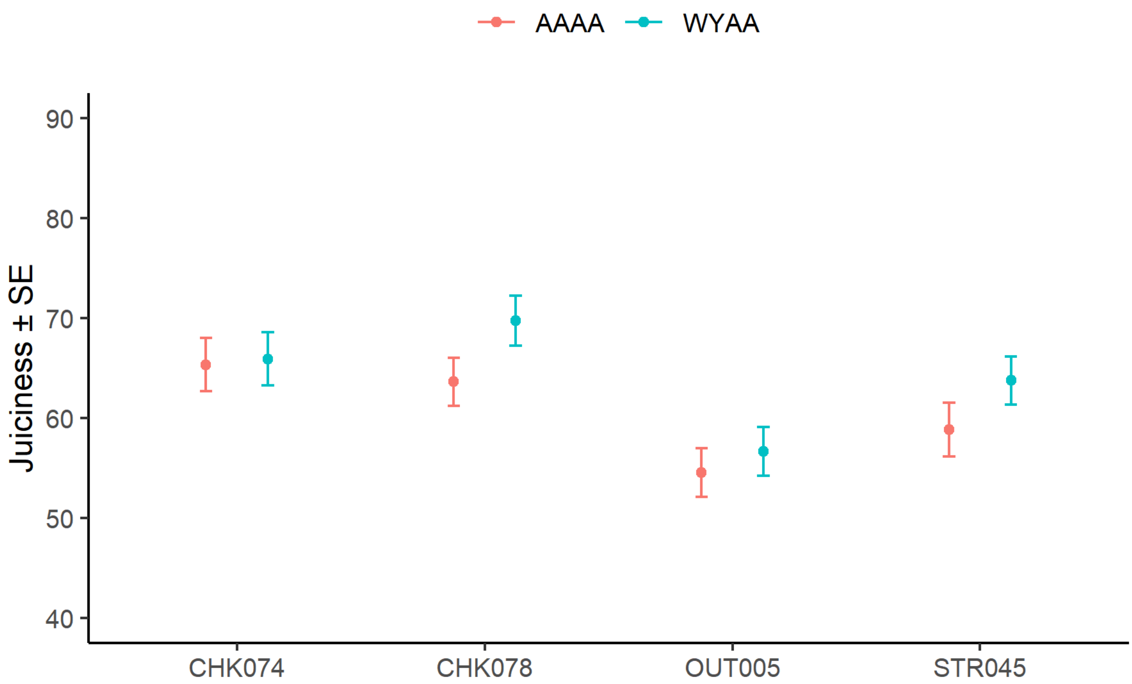


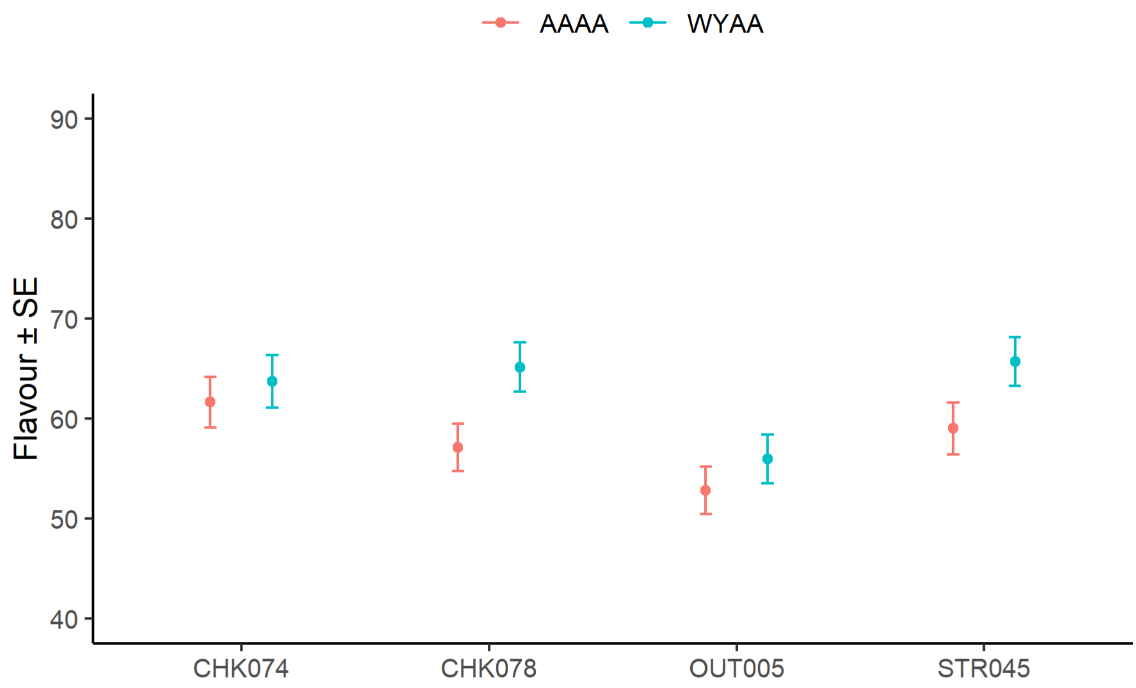
Figure 25. Juiciness ± SE of cohort 1 Angus (AAAA) and Wagyu x Angus F1 (WYAA) carcases within cuts



There was a tendency for breed type (AAAA = 60.6 ± 1.31 vs WYAA = 64.0 ± 1.29; P = 0.067) and hump height (P = 0.073) to impact on the juiciness of YAK samples. The fixed effects that had an impact on juiciness included cut ($P < 0.001$), MSA marble score ($P = 0.013$), the MSA marble score and HSCW interaction ($P <0.001$) and ossification and HSCW interaction ($P = 0.048$). There was no interaction between breed type and cut ($P = 0.676$; Fig. 25). Overall, the model was a reasonable estimator of juiciness with a residual standard error (R^2) of 0.3315.

Flavour scores for YAK samples were reasonably predicted by the fixed effects ($R^2 = 0.388$). Breed type (AAAA = 57.7 ± 1.54 vs WYAA = 62.6 ± 1.62; $P = 0.008$), cut ($P < 0.001$), hump height ($P = 0.037$), the curvilinear effects of MSA marble score ($P = 0.001$) and the curvilinear effects of HSCW ($P = 0.033$) had an effect on juiciness. Furthermore, the interactions between ossification and HSCW ($P = 0.001$), MSA marble score and HSCW ($P < 0.001$), and MSA marble score and rib fat ($P < 0.001$) all had a relationship with the juiciness score of the YAK samples. The breed type by cut interaction did not have a relationship with juiciness ($P = 0.485$; Fig. 26).

Figure 26. Flavour ± SE of cohort 1 Angus (AAAA) and Wagyu x Angus F1 (WYAA) carcases within cuts

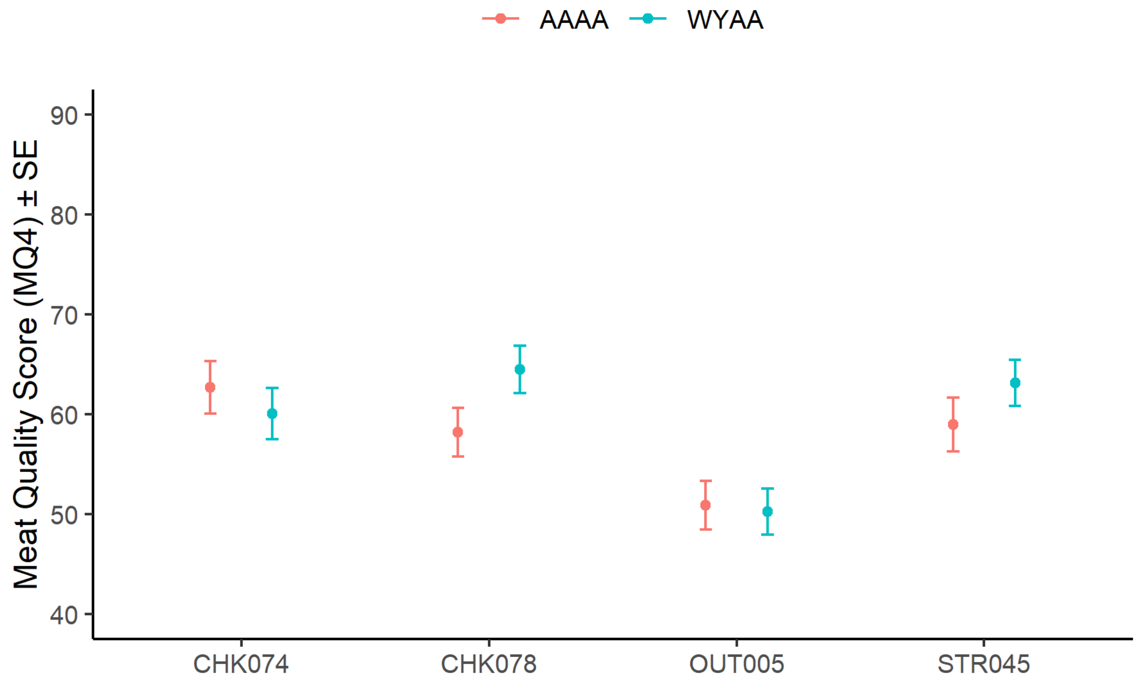


Overall liking was driven by cut ($P < 0.001$), the curvilinear relationship of MSA marble score ($P = 0.032$), and the interactions between ossification and HSCW ($P = 0.005$), MSA marble score and HSCW ($P = 0.001$), and MSA marble score and rib fat ($P = 0.001$). There was a tendency for hump height to impact on overall liking ($P = 0.054$). Breed type did not affect overall liking ($P = 0.104$), nor did the breed type by cut interaction ($P = 0.296$; Fig. 27). Overall, the model was a reasonable estimator of overall liking with a R^2 of 0.409.

The model for the prediction of MQ4 score of cohort 1 ($R^2 = 0.377$) identified cut ($P < 0.001$), MSA marble score ($P = 0.001$), the MSA marble score and HSCW interaction ($P = 0.048$), and the MSA marble score and rib fat interaction ($P = 0.008$) drivers of eating quality. While there was a tendency for pHu to have an effect ($P = 0.081$), both breed type ($P = 0.312$) and the breed type by cut interaction ($P = 0.216$) had no impact on the final outcome (Fig. 28).

Figure 27. Overall liking ± SE of cohort 1 Angus (AAAA) and Wagyu x Angus F1 (WYAA) carcases within cuts

Figure 28. Meat quality score ± SE of cohort 1 Angus (AAAA) and Wagyu x Angus F1 (WYAA) carcases within cuts



4.2.3.2 Cohort 2 Yakiniku – High content crossbred Wagyu (F3 or higher ≥ 87.5% Wagyu) and F1 Wagyu x *Bos indicus* composites

All fixed effects used to model tenderness, juiciness, flavour, overall liking and MQ4 scores in cohort 1 are presented in Table 12 below. Cohort 2 tenderness scores were linked ($R^2 = 0.478$) to the breed type (WYWY = 68.5 ± 1.64 vs WYXX = 61.7 ± 2.06; $P = 0.037$), cut ($P < 0.001$), ossification ($P = 0.013$), rib fat ($P = 0.004$) and the curvilinear response to HSCW ($P = 0.001$). There was also an interaction between hump height and HSCW ($P = 0.001$), however the breed type by cut interaction was not a good predictor of tenderness ($P = 0.145$; Fig. 29). There was a tendency hump height ($P = 0.072$) and pHu ($P = 0.095$) to impact on the outcome of tenderness in YAK samples.

Figure 29. Tenderness ± SE of yakiniku samples for cohort 2 high content crossbred Wagyu (WYWY) and Wagyu F1 x *Bos indicus* (WYXX) composite carcases within cuts

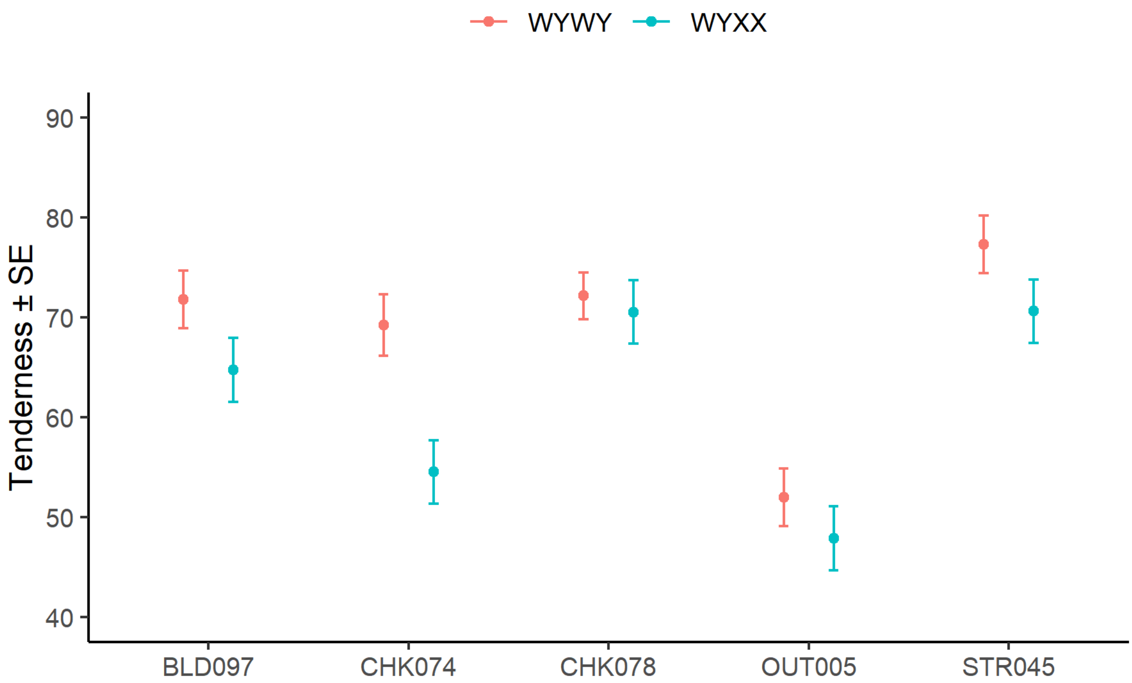
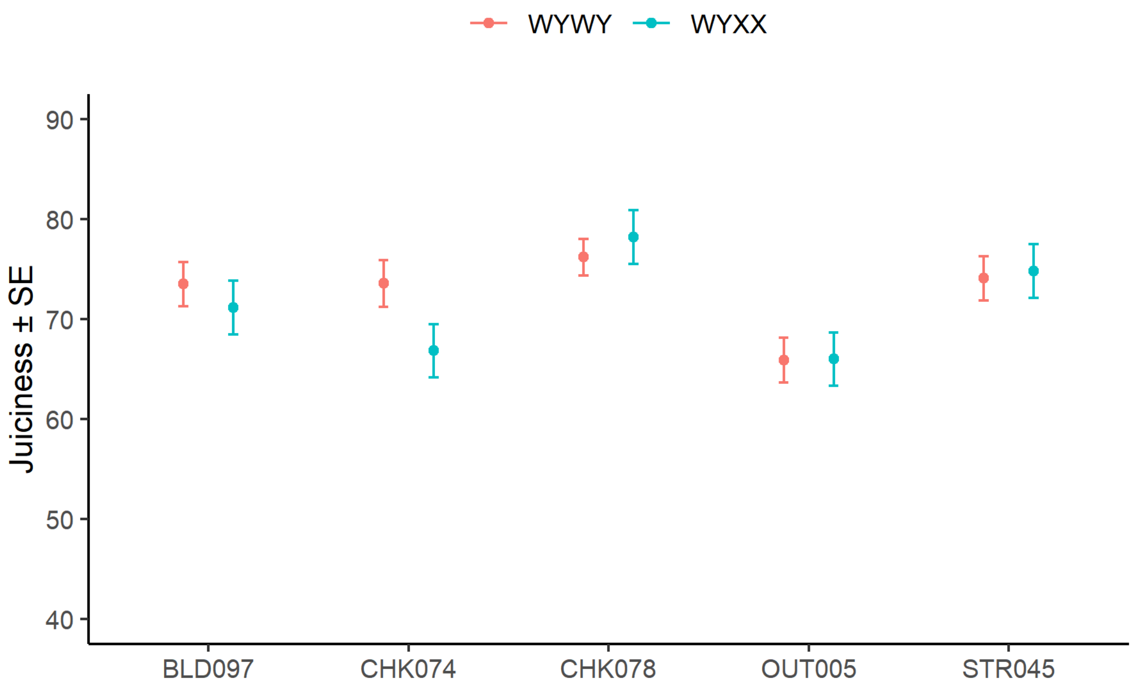


Table 12. The numerator degree of freedom (NDF), denominator degree of freedom (DDF), F-value and the p-value for each of the factors which compose the models to predict tenderness, juiciness, flavour, overall liking and MQ4 for cohort 2 Yakiniku samples.

Trait	Tenderness		Juiciness		Flavour		Overall Liking		MQ4	
	NDF,DDF	F-value	NDF,DDF	F-value	NDF,DDF	F-value	NDF,DDF	F-value	NDF,DDF	F-value
Breed	1, 169	4.41**	1, 168	0.16	1, 168	2.32	1, 168	2.76*	1, 168	1.76
Cut	4, 169	25.64***	4, 168	9.33***	4, 168	10.19***	4, 168	15.15***	4, 168	18.12***
MSA Marbling	1, 169	2.12	1, 168	10.40***	1, 168	8.94***	1, 168	8.34***	1, 168	9.36***
Ossification	1, 169	6.28**	1, 168	3.01*	1, 168	3.96**	1, 168	3.85*	1, 168	3.79*
Hump (mm)	1, 169	3.27*	1, 168	1.40	1, 168	1.40	1, 168	1.32	1, 168	1.38
Ultimate pH	1, 169	2.83*	1, 168	0.00	1, 168	1.86	1, 168	1.05	1, 168	0.68
Rib Fat (mm)	1, 169	8.42***	1, 168	17.78***	1, 168	16.31***	1, 168	16.57***	1, 168	17.69***
HSCW (kg)	1, 169	0.79	1, 168	0.05	1, 168	0.56	1, 168	0.46	1, 168	0.36
Ossification^2			1, 168	8.33***	1, 168	9.32***	1, 168	9.86***	1, 168	9.40***
HSCW (kg)^2	1, 169	11.03***	1, 168	10.47***	1, 168	12.11***	1, 168	13.20***	1, 168	11.90***
Breed x Cut	4, 169	1.73	4, 168	1.48	4, 168	0.80	4, 168	1.99*	4, 168	1.57
Ossification x HSCW			1, 168	18.53***	1, 168	17.10***	1, 168	20.24***	1, 168	18.43***
Hump x HSCW	1, 169	11.03***								

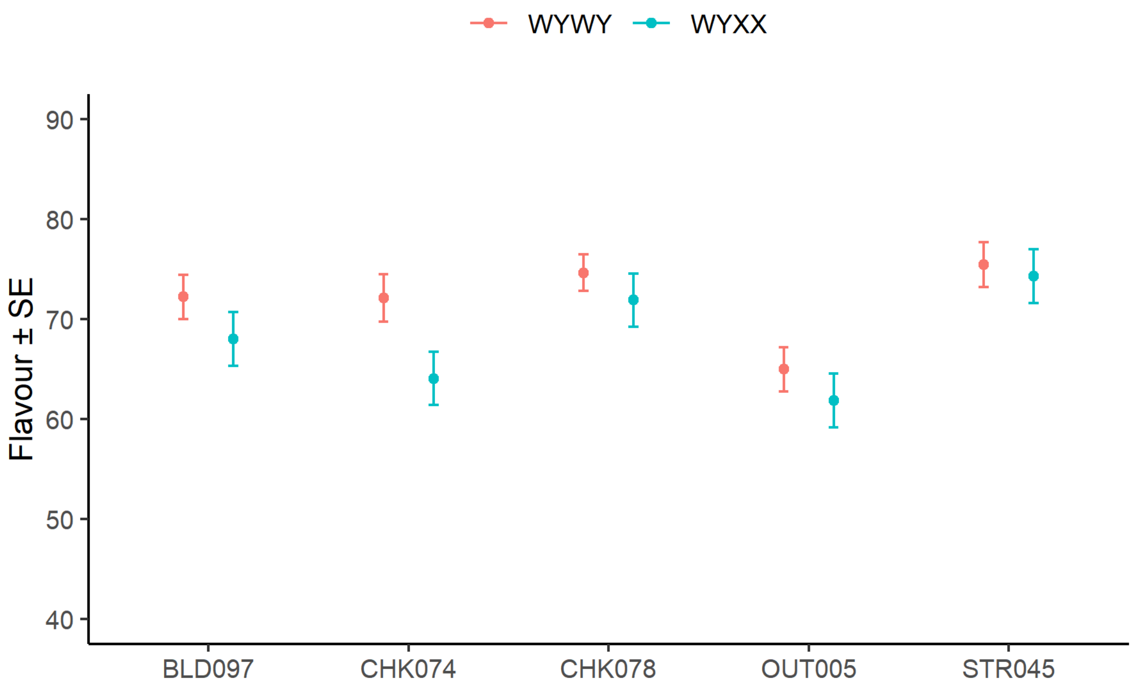
Modelling the impact of the fixed effects on juiciness (R^2 = 0.39) found that cut (P < 0.001), rib fat (P < 0.001) and the interaction between ossification and HSCW (P < 0.001) had the largest effects on juiciness of YAK samples. The curvilinear relationships of ossification (P = 0.004) and HSCW (P = 0.001) also showed a relationship with juiciness. The interaction between breed type and cut had no effect on juiciness (P = 0.211; Fig. 30).

Figure 30. Juiciness ± SE of yakiniku samples for cohort 2 high content crossbred Wagyu (WYWY, F3 or higher ≥ 87.5% Wagyu) and Wagyu x *Bos indicus* F1 (WYXX) carcases within cuts



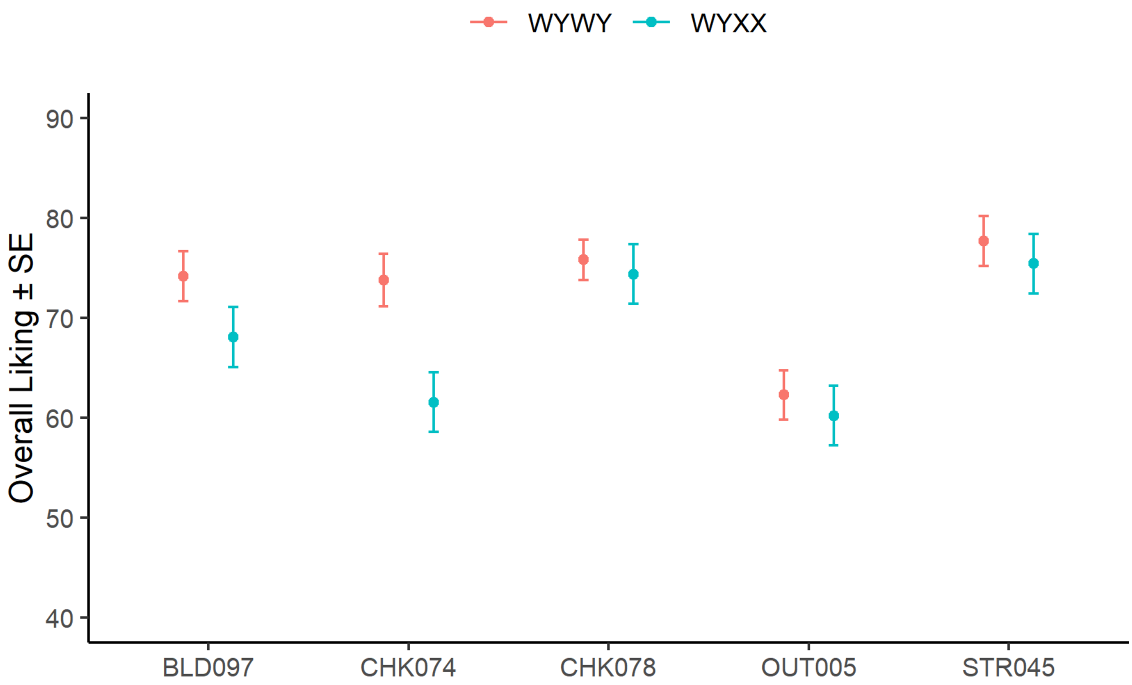
Flavour of YAK samples was driven by cut ($P < 0.001$), rib fat ($P < 0.001$), MSA marble score ($P = 0.003$), ossification ($P = 0.048$) and the curvilinear effect of ossification ($P = 0.003$), the curvilinear effect of HSCW ($P = 0.001$), and the interaction between ossification and HSCW ($P < 0.001$) in a moderately strong model ($R^2 = 0.41$). The breed type by cut interaction did not appear to have an impact on flavour ($P = 0.528$; Fig. 31).

Figure 31. Flavour ± SE of yakiniku samples for cohort 2 high content crossbred Wagyu (WYWY, F3 or higher ≥ 87.5% Wagyu) and Wagyu x *Bos indicus* F1 (WYXX) carcases within cuts



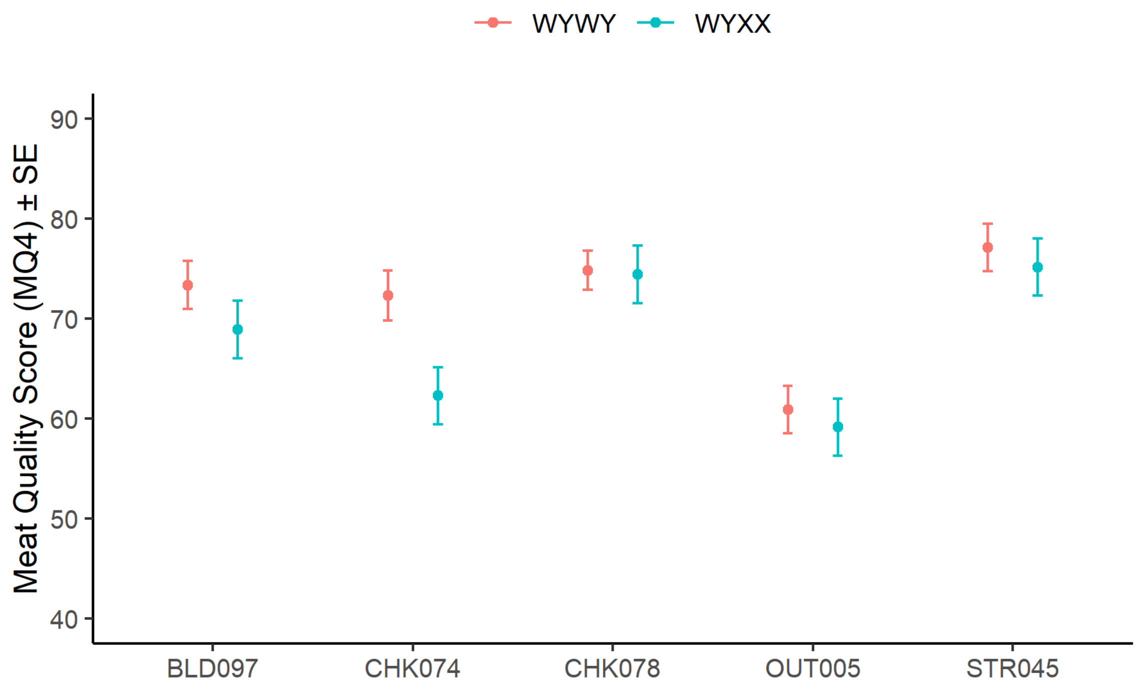
The overall liking scores of YAK samples showed a relationship with cut ($P < 0.001$), rib fat ($P < 0.001$), MSA marble score ($P = 0.004$), the curvilinear effect of both ossification ($P = 0.002$) and HSCW ($P < 0.001$), and the interaction between ossification and HSCW ($P < 0.001$, Table 12). Breed type ($P = 0.099$) and the breed type by cut interaction ($P = 0.098$) both had a tendency to impact overall liking (Fig. 32). The WYWY carcases averaged an overall liking score of 72.7 ± 1.48, as compared to the WYXX carcases with 67.9 ± 2.18 points. The strength of the model was moderate ($R^2 = 0.46$).

Figure 32. Overall liking ± SE of yakiniku samples for cohort 2 high content crossbred Wagyu (WYWY, F3 or higher ≥ 87.5% Wagyu) and Wagyu F1 x *Bos indicus* (WYXX) composite carcases within cuts



The breed type had no impact on the MQ4 score of the YAK samples (P = 0.187), however cut (P < 0.001), MSA marble score (P = 0.003), rib fat (P < 0.001), the curvilinear effect of both ossification (p = 0.003) and HSCW (P = 0.001), as well as the interaction between HSCW and ossification (P < 0.001) all had an impact on eating quality. Ossification showed a tendency for effect (P = 0.053), however there was no interaction between breed type and cut (P = 0.184; Fig. 33). All other fixed effects did not appear to be drivers of the overall eating quality of YAK samples from cohort 2 in what was a moderately strong model (P > 0.05; R^2 = 0.471).

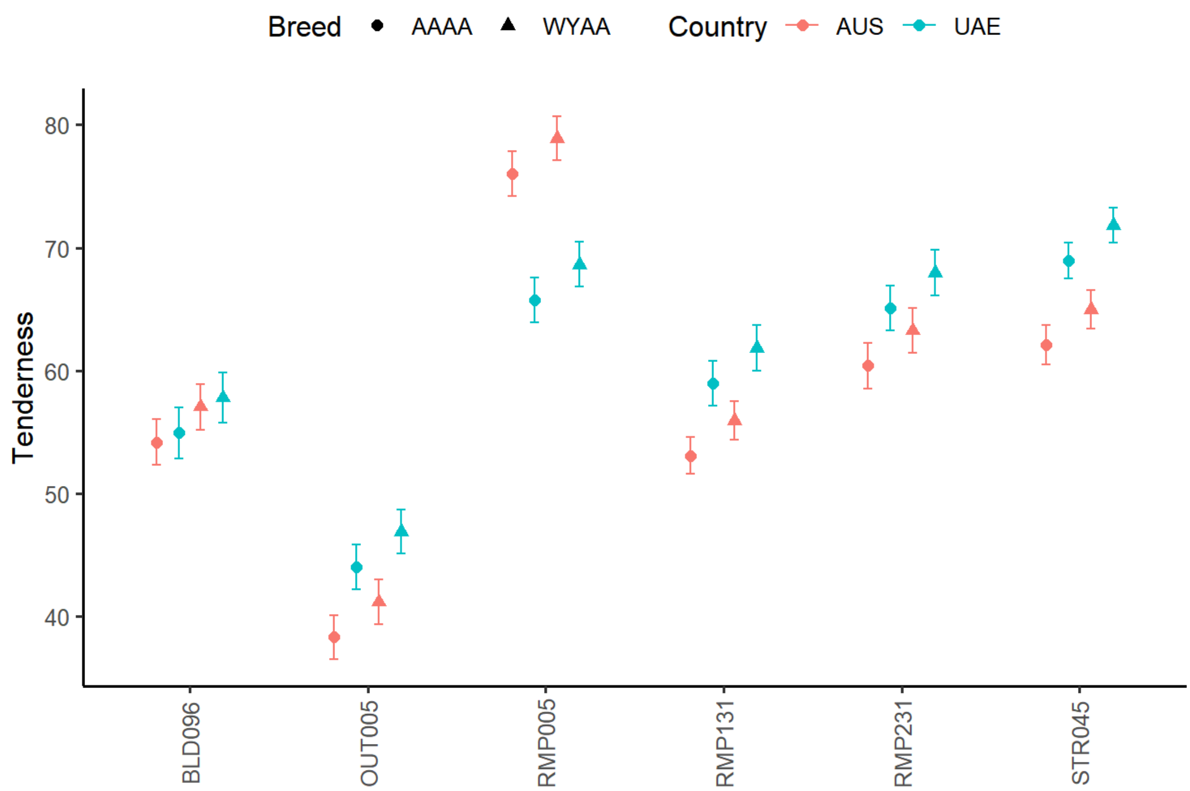
Figure 33. Meat quality score ± SE of yakiniku samples for cohort 2 high content crossbred Wagyu (WYWY, F3 or higher ≥ 87.5% Wagyu) and Wagyu F1 (WYXX) carcases within cuts



4.3 Middle East Grill consumer sensory

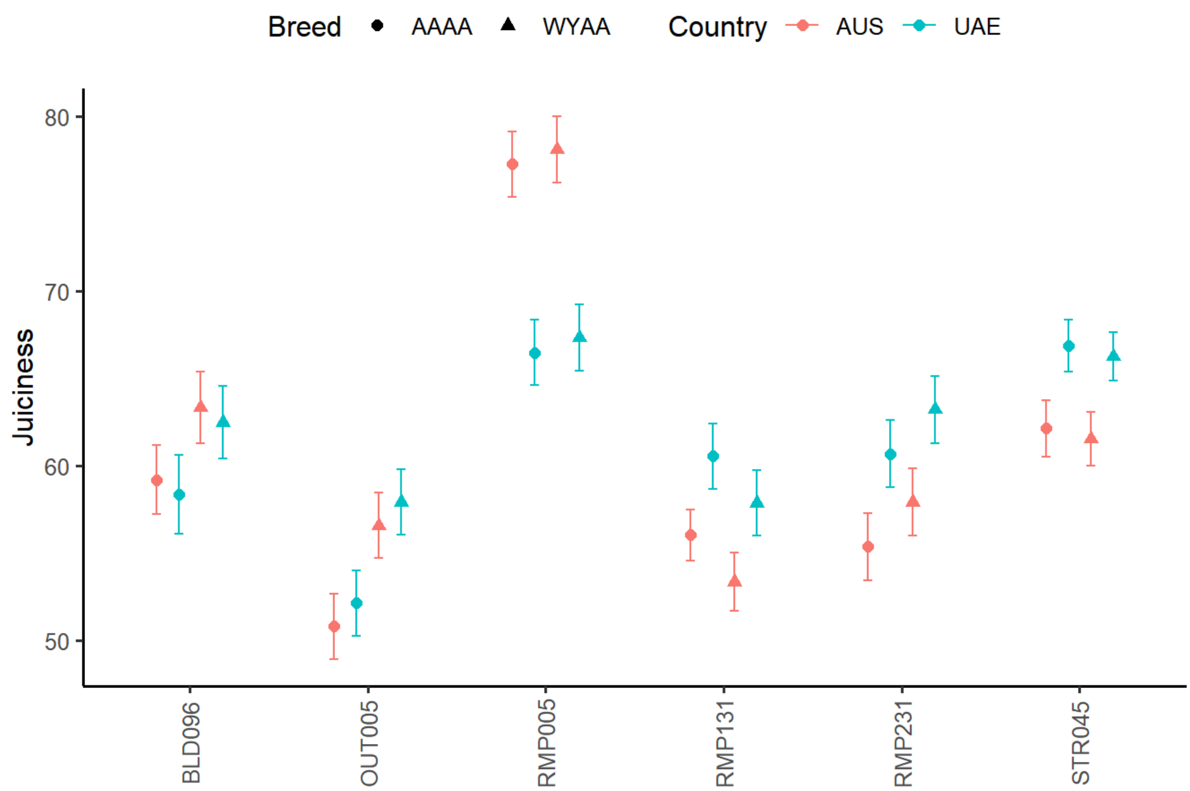
There was a significant effect of country, cut and breed type on tenderness from Angus steers and F1 Angus x Wagyu steers from cohort 1 consumed using the grill cook method (Fig. 34). The consumers from the Middle East scored the RMP005 lower than Australian consumers for tenderness, but scored the OUT005, RMP131, RMP231 and STR045 higher than Australian consumers (P<0.05, Fig. 34). However, there was no difference in consumer sensory scores for tenderness between the 2 countries for BLD096 (P>0.05, Fig. 34). The OUT005 had the lowest tenderness scores and was significantly lower than all the other cuts (P<0.05, Fig. 34).

Figure 34. Tenderness estimated marginal means with standard errors of consumer sensory scores for the BLD096 (*M. triceps brachii caput longum*), OUT005 (*M. biceps femoris*), RMP005 (*M. biceps femoris),* RMP131 (*M. gluteus medius)*, RMP231 (*M. gluteus medius*) and STR045 (*M. longissimus thoracis et lumborum*) from Angus (AAAA) and F1 Angus x Wagyu (WYAA) steers consumed using grill cook method in Australia and the Middle East (UAE).



There was a significant effect of cut and significant interaction between cut and breed plus cut and country on juiciness from Angus steers and F1 Angus x Wagyu steers consumed using the grill cook method (Fig. 35). The consumers from the Middle East scored the RMP005 lower than Australian consumers for juiciness, but scored the RMP131, RMP231 and STR045 higher than Australian consumers (P<0.05, Fig. 35). However, there was no difference in consumer sensory scores between the 2 countries for OUT005 or BLD096 (P>0.05, Fig. 35). The OUT005 had the largest breed effect on juiciness (P<0.05, Fig. 35). The relationships between the breeds for juiciness were echoed by consumers across both countries.

Figure 35. Juiciness estimated marginal means with standard errors of consumer sensory scores for the BLD096 (*M. triceps brachii caput longum*), OUT005 (*M. biceps femoris*), RMP005 (*M. biceps femoris),* RMP131 (*M. gluteus medius)*, RMP231 (*M. gluteus medius*) and STR045 (*M. longissimus thoracis et lumborum*) from Angus (AAAA) and F1 Angus x Wagyu (WYAA) steers consumed using grill cook method in Australia and the Middle East (UAE).

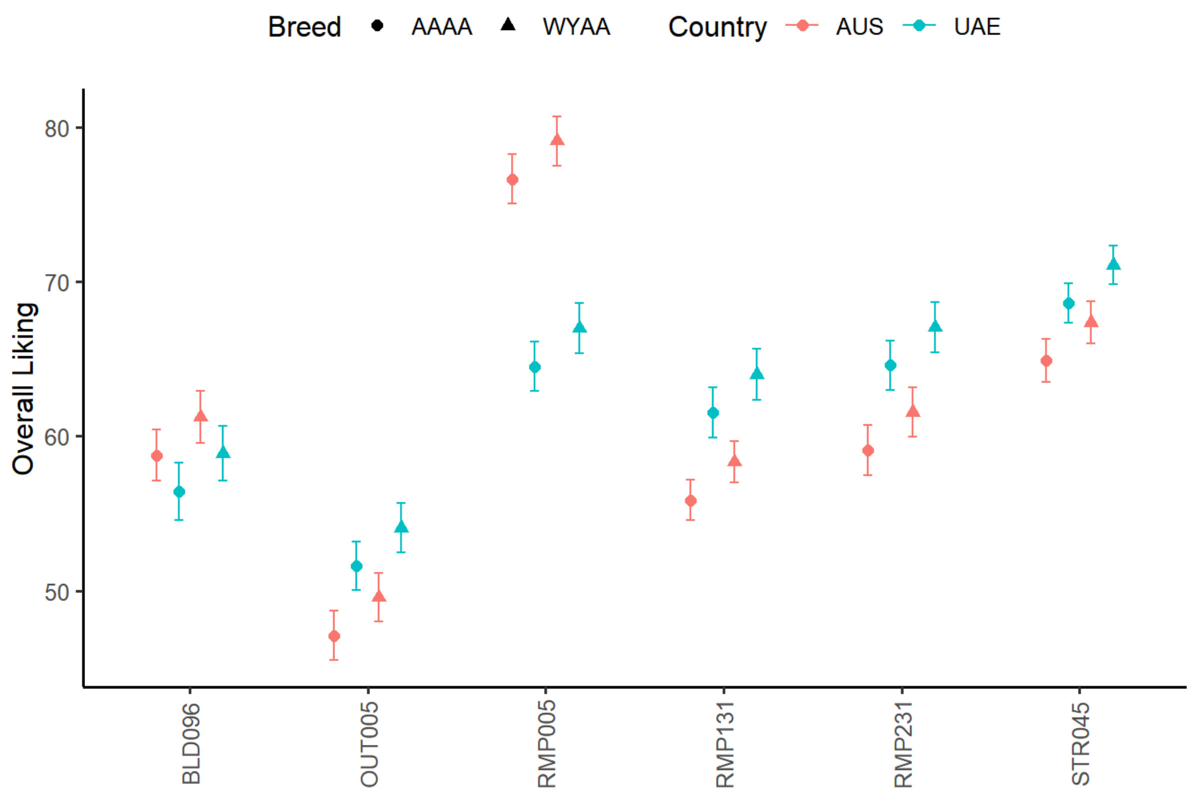


There was a significant effect of country, cut, breed type and a significant interaction between cut and country on flavour from Angus steers and F1 Angus x Wagyu steers consumed using the grill cook method (Fig. 36). The consumers from the UAE scored the flavor of the RMP005 and BLD096 much lower than Australian consumers (P<0.05, Fig. 36). However, there was no difference in consumer sensory scores between the 2 countries for OUT005, RMP131, RMP231 and STR045 (P>0.05, Fig. 36). The relationships between the breeds for flavour were echoed by consumers across both countries.

Figure 36. Flavour estimated marginal means with standard errors of consumer sensory scores for the BLD096 (*M. triceps brachii caput longum*), OUT005 (*M. biceps femoris*), RMP005 (*M. biceps femoris),* RMP131 (*M. gluteus medius)*, RMP231 (*M. gluteus medius*) and STR045 (*M. longissimus thoracis et lumborum*) from Angus (AAAA) and F1 Angus x Wagyu (WYAA) steers consumed using grill cook method in Australia and the Middle East (UAE).

There was a significant effect of cut, breed type and a significant interaction between cut and country on Overall Liking from Angus steers and F1 Angus x Wagyu steers consumed using the grill cook method (Fig. 37). The consumers from the UAE scored the overall liking of the RMP005 lower than Australian consumers, but scored the OUT005, RMP131, RMP231 and STR045 higher than Australian consumers ($P<0.05$, Fig. 37). However, there was no difference in consumer sensory scores between the 2 countries for BLD096 ($P>0.05$, Fig. 37). The OUT005 had the lowest Overall liking scores and was significantly lower than all the other cuts ($P<0.05$, Fig. 37) within each country.

Figure 37. Overall Liking estimated marginal means with standard errors of consumer sensory scores for the BLD096 (*M. triceps brachii caput longum*), OUT005 (*M. biceps femoris*), RMP005 (*M. biceps femoris),* RMP131 (*M. gluteus medius)*, RMP231 (*M. gluteus medius*) and STR045 (*M. longissimus thoracis et lumborum*) from Angus (AAAA) and F1 Angus x Wagyu (WYAA) steers consumed using grill cook method in Australia and the Middle East (UAE).



There was a significant effect of cut, breed type and a significant interaction between cut and country on MQ4 score from Angus steers and F1 Angus x Wagyu steers consumed using the grill cook method (Fig. 38). The consumers from the UAE scored the MQ4 score of the RMP005 lower than Australian consumers, but scored the RMP131, RMP231 and STR045 higher than Australian consumers (P<0.05, Fig. 38). However, there was no difference in consumer sensory scores between the 2 countries for BLD096 or OUT005 (P>0.05, Fig. 38). The OUT005 had the lowest MQ4 scores and was significantly lower than all the other cuts (P<0.05, Fig. 38).

Figure 38. MQ4 score estimated marginal means with standard errors of consumer sensory scores for the BLD096 (*M. triceps brachii caput longum*), OUT005 (*M. biceps femoris*), RMP005 (*M. biceps femoris),* RMP131 (*M. gluteus medius)*, RMP231 (*M. gluteus medius*) and STR045 (*M. longissimus thoracis et lumborum*) from Angus (AAAA) and F1 Angus x Wagyu (WYAA) steers consumed using grill cook method in Australia and the Middle East (UAE).

5. Discussion

This experiment analysed the influence of Wagyu breed content on eating quality when balanced for carcase characteristics using the yakiniku, shabu shabu and grill cooking methods. The results showed that there is no consistent eating quality benefit of having a higher proportion of Wagyu when carcase characteristics are comparable.

The differences stated in literature which elucidate that Wagyu cattle could produce superior eating quality compared to other *Bos taurus* cattle breeds (Iwamoto et al., 1991; May et al., 1993; Nishimura et al., 1999; Duarte et al., 2013), has not been able to result in any superior difference in this experiment. The carcases in this experiment were selected in a case and control fashion, where carcases with similar attributes differed in Wagyu breed content. This shows that when traits are the same or when statistical models are adjusted for carcase attributes, there is no difference between the breeds, in fact the F1 Angus Wagyu's in cohort 3 were better than the unregistered purebred Wagyu's for all eating quality traits. Alternatively, the differences may be smaller than 4 MQ4 score points which is what the experiment's statistical power was modelled on using standard deviation of 10, power of 0.7 and statistical difference of 0.05.

Wagyu cattle have been shown to dissociate connective tissue crosslinks with high degrees of marbling which gives them higher tenderness (Nishimura et al., 1999; Weston et al., 2002) even when physiological maturity or ossification increase. However, in this experiment tenderness was only significantly higher for cattle with more Wagyu breed content for cohort 1 grills and cohort 2 yakiniku. This significance was driven by the CHK078 in cohort 1 grills and the CHK074 in the cohort 2 yakiniku results, hence the effect seems to be sporadic and across different muscles and cook methods. This suggests that the relationship seen in the paper by Nishimura et al. (1999) where more intramuscular fat creates more dissociation of the connective tissue matrix could occur in all breeds of *Bos taurus* cattle not just specific to Wagyu.

Wagyu influenced cattle have been shown to produce higher monounsaturated fatty acids and less saturated fatty acids than other *Bos taurus* animals (May et al., 1993). This change in fatty acid profiles is hypothesised to effect eating quality aspects due to the lower melting points of unsaturated fatty acids, although none of the results from cohort 1, 2 or 3 show significant differences where a difference in fatty acid profiles could be an explanation. This may be due to large variation within the Wagyu breed, as seen in the paper by Sturdivant et al. (1992), which explains that the degree of effect on increased monounsaturated fatty acids is dependent on the region within Japan the animal originates. Since the Australian Wagyu have been crossed between the available family groups without outside genetic influence, the variation in eating quality between Wagyu influenced animals may be minimalised as generations progress, however this would need further investigation. There could also be a difference in the fatty acid profiles between Australian Wagyu and Japanese Wagyu due to the different diets the animals are fed during finishing (Vatansever et al., 2000). The results from this experiment suggest that nutrition of the animals may have had a bigger impact on fats and flavour profiles than genetics and breeds, evidenced through the variation in eating quality scores between the cohorts. However, comparison between cohorts was not the focus of this experiment.

The proposed higher proportion and larger size of type I muscle fibres seen in Wagyu cattle compared to other *Bos taurus* breeds (Iwamoto et al., 1991; Gotoh, 2003) also did not produce any breed effect on any eating quality traits in these experiments. The impact of changes in muscle fibre type could have been suppressed due to all cook methods, where samples were cut across the grain and in 2mm slices for shabu shabu resulting in bundles of muscle cells not thick enough to cause a difference in tenderness (Joo et al., 2017; Mashima et al., 2019). This experiment has shown when the cattle have the same carcase parameters, there is no positive additive breed effect of increasing the proportion of Wagyu breed content on the parameters of eating quality.

5.1 Cook methods and cuts

The shabu shabu cook method scored lower than grill and yakiniku for all eating quality parameters. Tenderness scores for shabu shabu could be effected by having less to chew as the size of sample and therefore muscle fibre bundles is reduced (Watanabe et al., 2019). The tenderness could also be impacted by the internal temperature of the samples, as the grill would have a lower internal temperature and therefore less connective tissue shrinking compared to shabu shabu samples.

As the shabu shabu is a method of cooking which boils the meat compared to grilling, the flavour profile can be altered due to the rendering out of some fats from the sample, the lack of Maillard reaction and removal of soluble flavour compounds from the sample (Mottram, 1998; Koutsidis et al., 2008; Ngapo et al., 2012; Watanabe et al., 2019). The juiciness and flavour of the samples may be impacted by significantly lower quantities of total fat in the sample compared to grilled samples also due to the size of the sample (Polkinghorne et al., 2011). Although there were these differences between the shabu shabu cook method and other cook methods, there was also significant differences between cuts which did not re-rank across cook methods.

In both cohort 1 and 2 the *M. biceps femoris* (OUT005) was significantly lower in eating quality to the other muscles across all cook methods. This may be due to the significantly higher total collagen content within the outside flat compared to the striploin (Jeremiah et al., 2003). Dashdorj et al. (2017) also found that type I and type III collagen content is higher in locomotive muscle than postural muscles. Collagen contributes to variation in meat tenderness by creating a matrix of connective tissue which becomes more thermally stable and less soluble with age (Weston et al., 2002). This stable connective tissue matrix makes it harder to chew through and results in lower tenderness scores (Weston et al., 2002). The high correlation between tenderness and the other sensory traits would have also have caused the OUT005 to be lower than the other muscles for all eating quality traits (Polkinghorne et al., 2011). The surprise outcome from this research was the very high scores for the rump cap (RMP005) when grilled from cohort 1 and 2. It appears that in these higher IMF carcases, the rump cap has exceptional eating quality. The Middle Eastern consumers did not agree with the Australian consumers and scored the rump cap in alignment with previous research where it had a similar score to the striploin.

5.2 Significant contributors to models

A significant breed and hump height interaction was seen in the grill results of cohort 3. This interaction showed that unregistered purebred Wagyu cattle had a decreasing score for tenderness, juiciness, overall liking and MQ4 when hump height increased. This indicates a chance for there to be a similar hump height effect caused by the relationship with calpastatin as seen in *Bos indicus* cattle (Casas et al., 2005). The mechanism seen in this effect is determined by reduced calpain activity due to an over expression of calpastatin causing the meat to be less tender (Casas et al., 2005; Bhat et al., 2018). However this effect must be studied to a greater extent in future work because in the current study it is plausible that this effect was driven by a couple of purebred Wagyu cattle that had high hump heights. The hump heights recorded in the purebred might have been a dominance of the small proportion of *Bos indicus* still in the purebreds which could be further quantified using a genotype test. Further quantification of hump height could also be conducted in Full blood Wagyu's from the Southern Multi Breed project.

Within cohort 1 and experiment 2, the level of marbling had a significant positive relationship in predicting tenderness, juiciness, overall liking and MQ4 score. Marbling effects eating quality by melting during the cooking process, which makes the sample more tender and can stimulate the salivary glands, which can increase the perceived juiciness (Miller, 2014). The effect of marbling on eating quality parameters may also be of a higher magnitude in the shabu shabu cook method as this method would have a reduced total fat content due to its smaller consumer sample size compared to grill samples.

Within cohort 1 for shabu shabu, the degree of ossification had a significant positive relationship in predicting each aspect of eating quality, whereas in cohort 3, ossification had a significant negative relationship with tenderness, flavour, overall liking and MQ4 score. The results from cohort 3 were more aligned with literature as ossification is used to describe maturity related collagen crosslink development, showing that when increasing ossification, the tenderness decreases (Bonny et al., 2016). Although some of these previous studies may not have had high enough IMF values to see impact of disorganised connective tissue, which is why there is an unfamiliar relationship between ossification and eating quality in cohort 1 (Nishimura et al., 1999; Bonny et al., 2016). The effect of cook method could also have produced an unfamiliar relationship between ossification and eating quality as the connective tissue found in higher ossification animals would have been disrupted by slicing the portion of meat so finely (2mm) for shabu shabu. This was found in Bonny et al. (2016), where the Korean BBQ (4mm) samples had a slight positive relationship with MQ4 score and the grill (25mm), roast (10mm) and slow cook (21mm) samples all had negative relationships with MQ4 (Watson et al., 2008a).

Although within shabu shabu results of cohort 2, a significant interaction between cut and ossification for MQ4 tenderness, flavour and overall liking highlighted that the STR045 had a negative relationship but the others had slightly positive relationships or no relationship. It seems that the STR045 follows the normal relationship with ossification stated by literature (Bonny et al., 2016). Although the OUT005 significantly increased in MQ4 score when the ossification increased, but the CHK074 and the CHK078 MQ4 scores were not significantly affected. The explanation for the OUT005 results is unknown and further research into the relationship between ossification and the eating quality of different muscles using the shabu shabu cook method is recommended. The results overall indicate that the negative effect of ossification on current eating quality predictions (Polkinghorne et al., 2008; Bonny et al., 2016) might need re-assessing for the Meat Standards Australia models depending on cook method and cut.

Within cohort 1, the amount of rib fat thickness had a significant negative relationship when predicting flavour, overall liking and MQ4. These results may have been observed due to the selection pressure for increased intramuscular fat and reduced rib fat depth within the Angus and Wagyu breeds which could result in a change of fat deposition away from the external areas and towards intramuscular areas (Gotoh et al., 2009; Australia, 2020). This could have also driven the significant interaction between breed type and rib fat depth in experiment 2 for juiciness, overall liking and MQ4 score. However, these results are opposite of the effect of rib fat in the MSA models for consumer sensory score (Polkinghorne et al., 2008), thus the eating quality predictions might need adjusting based on breed, cook method and cut.

6. Conclusion

The results of this project have demonstrated that at times, for some intrinsic eating quality traits, in some cuts for some cook methods, Wagyu and Wagyu influenced cattle have superior eating quality outcomes to comparison cattle, however, this effect is not consistent whereby the comparison cohorts had superior eating quality across different cuts and cooking methods for some cohorts. This variability makes it difficult to model any consistent advantage of the Wagyu and Wagyu influenced cattle within the MSA model. The majority of the variation in eating quality could be explained by carcase traits already measured and utilised as part of the MSA model including hump height, marbling and ossification.

The results also showed that grill and yakiniku cook methods were better than the shabu shabu cook method for all muscles. The consumer sensory scores for many cuts in this study scored very highly with the rump cap excelling with Australian consumers and appears to re-rank higher than the striploin in highly marbled animals.

Data captured as part of the MENA sensory testing shows that the MENA consumers have similar appreciation of grilled beef samples to Australian consumers, apart from the rump cap, which was scored lower by MENA consumers compared to Australian consumers.

6.1 Key findings

- MSA marble score (MSA Marbling average of cohorts 474 to 705) and cut differences were the main variables that explained eating quality of cohort 1 for tenderness, juiciness, flavour, overall liking and MQ4 score. While a breed effect (Wagyu content 50% – F1) was observed for cohort 1 tenderness, the rest of the impact of breed on eating quality was negligible for juiciness and flavour, and tended to be important for overall liking and MQ4. Furthermore, where a difference was noted, it was only small with 2-3 eating quality points difference. This would indicate that a breed effect may indeed be evident in some cases, however higher Wagyu content was not an influencing factor in cohort 2 or cohort 3, whereby the comparison cattle had no difference or consistently superior eating quality for grill. More research would need to be conducted to determine any breed effect. Furthermore, additional research would need to be targeted and indicative of all Wagyu present in the current value chain, including a range and knowledge of the genetic traits driving eating quality e.g. Marble score estimated breeding value, breed proportions, pre-feedlot entry effects and other variables which may drive variability in outcomes. The Southern Multi-breed project will provide an opportunity to compare full blood Angus and full blood Wagyu cattle which were born together, raised together and finished together.
- In cohort 2, the differences in eating quality were explained predominately by carcase traits to a far greater extent than breed.
- In cohort 3, the F1 Wagyu x Angus outperformed the unregistered purebred Wagyu's across all intrinsic eating quality traits for the striploin as a grill.
- MENA consumers have similar appreciation of grilled beef samples to Australian consumers. Rump cuts (131 and 231) and the striploin consistently scored higher for tenderness, juiciness, overall liking and MQ4 score compared to Australian consumers. The major difference was only the RMP005 (rump cap) which the MENA consumers scored lower for all intrinsic meat quality traits. There was no difference between MENA and Australian consumers for the blade or outside flat.

6.2 Benefits to industry

- The data generated for consumer sensory scores on high marbling animals will help to improve the accuracy of the MSA model for high marbling carcases across a broad range of breeds, cuts and cook types.
- The benefits to the wider industry are improved accuracy of eating quality predictions for premium long fed carcases aiding consistency within beef in premium brands, improving the consumer satisfaction with Australian highly marbled beef.
- This project has also helped the Australian beef industry understand the preferences of MENA consumers of premium quality beef. In general, 3 cuts (2 rump muscles and striploin) were scored higher by MENA consumers while the blade and outside were not different and the rump cap was scored lower by MENA consumers.
- This project also clearly demonstrates that Wagyu content or marble score are not the only important determinants of eating quality but are more important terms used in marketing. Premium brands would benefit from MSA grading and segregation based on MSA eating quality predictions.

7. Future research and recommendations

7.1 Future research

- Effect of fatty acid profiles and total IMF % on eating quality across all the cut by cook combinations tested in this project – 7 of the major fatty acids have been analysed on all cuts from all animals.
- Analyse the impact of hump height within purebred Wagyus or fullbloods Wagyus and composite F1's on eating quality. Understand the relationship between hump height, calpastatin activity and desmin reduction, and tenderness on Wagyu full bloods and F1's.
- Consumer testing in older Wagyu and Wagyu F1's with higher IMF to further build on existing high marbled (up to MSA Marbling 1190) Wagyu and Wagyu X data sets driving eating quality prediction in the MSA model. A greater understanding of the relationship between high ossification scores and high marbling scores is warranted.
- Cut collection and sensory testing from a single cohort of AAAA and WYWY cattle born together, raised together and killed together. The Southern Multibreed project provides this opportunity, and offer a range within breed in genetic traits which influence eating quality outcomes e.g. IMF %.
- Gain a greater understanding of the impact of marbling fineness and distribution on sensory outcomes using camera grading technologies.
- Understand the relationship between fat melting point and eating quality.
- Analysis of the residuals between the MSA predictions for each cut x cook and the actual scores from consumers for MQ4 scores.

7.2 Recommendations

- No adjustments to the current MSA model are necessary for Wagyu or Wagyu proportion based on the variability in eating quality outcomes from this project. These results were independently reviewed by statisticians and the MSA pathways committee, a group of independent scientists, who supported that no change to the MSA model.
- The data from trail will underpin refinements to the MSA model, for certain cuts, such as the RMP005 (*M. biceps femoris*) which had superior eating quality.
- There is a need to further understand the variability within and between all Wagyu and Wagyu influenced cattle especially those that have some *bos indicus* content.

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