

# The variation in the eating quality of beef from different sexes and breed classes cannot be completely explained by carcass measurements

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*Delivering beef of consistent quality to the consumer is vital for consumer satisfaction and will help to ensure demand and therefore profitability within the beef industry. In Australia, this is being tackled with Meat Standards Australia (MSA), which uses carcass traits and processing factors to deliver an individual eating quality guarantee to the consumer for 135 different 'cut by cooking methods' from each carcass. The carcass traits used in the MSA model, such as ossification score, carcass weight and marbling explain the majority of the differences between breeds and sexes. Therefore, it was expected that the model would predict with eating quality of bulls and dairy breeds with good accuracy. In total, 8128 muscle samples from 482 carcasses from France, Poland, Ireland and Northern Ireland were MSA graded at slaughter then evaluated for tenderness, juiciness, flavour liking and overall liking by untrained consumers, according to MSA protocols. The scores were weighted (0.3, 0.1, 0.3, 0.3) and combined to form a global eating quality (meat quality (MQ4)) score. The carcasses were grouped into one of the three breed categories: beef breeds, dairy breeds and crosses. The difference between the actual and the MSA-predicted MQ4 scores were analysed using a linear mixed effects model including fixed effects for carcass hang method, cook type, muscle type, sex, country, breed category and postmortem ageing period, and random terms for animal identification, consumer country and kill group. Bulls had lower MQ4 scores than steers and females and were predicted less accurately by the MSA model. Beef breeds had lower eating quality scores than dairy breeds and crosses for five out of the 16 muscles tested. Beef breeds were also over predicted in comparison with the cross and dairy breeds for six out of the 16 muscles tested. Therefore, even after accounting for differences in carcass traits, bulls still differ in eating quality when compared with females and steers. Breed also influenced eating quality beyond differences in carcass traits. However, in this case, it was only for certain muscles. This should be taken into account when estimating the eating quality of meat. In addition, the coefficients used by the Australian MSA model for some muscles, marbling score and ultimate pH do not exactly reflect the influence of these factors on eating quality in this data set, and if this system was to be applied to Europe then the coefficients for these muscles and covariates would need further investigation.*

**Keywords:** MSA, prediction of beef eating quality, European Union, sex, breed

## Implications

Variable eating quality is a major factor in declining beef consumption. In Australia, this is addressed with Meat Standards Australia (MSA), which uses carcass measurements to deliver an individual eating quality guarantee. In contrast to Australia, young bulls and dairy breeds are very important for European beef production. If a similar system

was to be used in Europe, it must take these types of production into account. This study found that variation in eating quality due to breed and sex is not completely explained by the current MSA model, and would therefore need separate adjustments in an equivalent European model.

## Introduction

The inability of consumers to reliably select beef of a consistent quality is seen as a major factor in the global decline

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in beef consumption (Morgan *et al.*, 1991; Polkinghorne *et al.*, 2008a). In Australia, this issue is being addressed with the MSA system. Through a unique 'cut by cooking method' eating quality prediction model, MSA uses carcass traits to deliver beef to consumers with an eating quality guarantee (Polkinghorne *et al.*, 2008a and 2008b; Watson *et al.*, 2008b). Such a system to guarantee beef eating quality would be well accepted by European beef consumers (Verbeke *et al.*, 2010), and would also enable products within such a system to command a premium price (Lyford *et al.*, 2010).

At present, only females and castrated males have been tested with MSA protocols and are eligible for grading with the MSA system (Polkinghorne *et al.*, 2008a). However, young bulls form an important part of many different production systems, particularly in Europe. In addition, a large proportion of beef production in Europe is from dairy breeds and dairy crosses as a by-product of the dairy industry (Hocquette and Chatellier, 2011). Therefore, for any eating quality prediction system to be relevant in these markets, meat from bulls and dairy breeds would also need to be considered.

There are a number of differences between bulls, heifers and steers that have been identified within the beef production industry. It is well established that bulls grow more rapidly, are more feed efficient and produce higher yielding carcasses with less fat than steers (Field, 1971). Female cattle also have more favourable genes for fat deposition and a hormonal profile that directly influences fatty acid proportion and distribution in muscles (Venkata Reddy *et al.*, 2015). Along with the effect on lean meat yield, it is likely that these differences in adiposity would have an effect on eating quality. Many studies have shown increased marbling level, or intramuscular fat (IMF) is associated with greater tenderness, juiciness, flavour liking and overall liking (Thompson, 2004; Chriki *et al.*, 2012). Therefore, the lower levels of IMF and lower marbling scores of bulls (Drayer, 2003; Choat *et al.*, 2006; Chriki *et al.*, 2013) would result in a lower eating quality. In addition to the sex effect on adiposity, the tenderness of meat from female cattle would be positively affected by the smaller fibre diameter and, in some cases, less collagen than meat from bulls (Boccard *et al.*, 1979; Seideman *et al.*, 1989; Chriki *et al.*, 2013). These differences, combined with the increased IMF effectively diluting the collagen within the muscle (Lee *et al.*, 1990), are reflected by the lower shear force values for meat from heifers (Morgan *et al.*, 1993; Chriki *et al.*, 2013) and higher tenderness scores (Dikeman *et al.*, 1986; Morgan *et al.*, 1993; Węglarz, 2010). However, these results are not consistently reported in the literature and other studies have also found no difference in shear force (Drayer, 2003) and scores for tenderness and flavour (Mandell *et al.*, 1997) between bulls, heifers and steers. Therefore assuming that the key difference between the sexes will be marbling, the current MSA model, whereas not having a separate adjustment for bulls, does account for the effect of marbling on eating quality and therefore will adequately describe the eating quality of bulls when classed as steers in the model.

As with sex, the amount, composition and distribution of adipose tissue within a carcass is one of the most distinct differences between beef and dairy breeds. Holsteins tend to deposit marbling at a younger age and have less subcutaneous fat (Garcia-de-Siles *et al.*, 1977; Lizaso *et al.*, 2011) than beef breeds. This led to higher juiciness and flavour scores for the loins of Holsteins when compared with a beef breed (Lizaso *et al.*, 2011). As adipose tissue is late maturing, the higher IMF levels may be related to the earlier age at maturity exhibited by dairy breeds (Lawrie, 1985). However, an earlier age at maturity may also be the cause of increased collagen and reduced collagen solubility seen in the loin of Holsteins when compared with beef breeds (Boccard *et al.*, 1979; Christensen *et al.*, 2011; Lizaso *et al.*, 2011). Nonetheless, these differences in collagen did not translate to any differences in shear force (Christensen *et al.*, 2011; Lizaso *et al.*, 2011). Furthermore, many studies have failed to find any difference in sensory scores or consumer acceptability between dairy and beef breeds raised under similar circumstances (Mills *et al.*, 1992; Christensen *et al.*, 2011; Lizaso *et al.*, 2011). In contrast, McKay (1970) found no difference in collagen content between breeds, despite the beef (Hereford) samples scoring higher for tenderness and overall preference than Holstein samples. Similarly, Boccard *et al.* (1979) found that beef breed samples had higher collagen solubility and tenderness scores than dairy breed samples. It is likely that the majority of the variation in the literature can be explained by differences in feeding regimes and the age of the animal at slaughter. Consequently, assuming that the difference between breeds is attributable to IMF and growth path differences, the current MSA model should have the capacity to account for these differences with an adjustment for both marbling score and growth path as described by ossification score and carcass weight.

Therefore, based on the balance of the evidence available, we hypothesise that meat from bulls would have lower consumer scores than heifers and steers, and that this will be largely driven by differences in marbling. As such the MSA model should accurately predict the eating quality of bulls when classed as steers. In addition, we hypothesise that dairy breeds will exhibit moderately increased eating quality mediated through higher levels of IMF and different growth paths to slaughter. Therefore, given that the MSA model contains adjustments for both marbling score and growth path (ossification and carcass weight), these differences would therefore also be adequately explained by the MSA model.

## Material and methods

### *Animals and muscle samples*

The data set was formed through combining the records of animals selected for a number of specific, smaller, experiments. As a result this data set provides across-section of European cattle types (Table 1). The Polish carcasses were processed at three facilities situated in the north-east of Poland. The Irish carcasses were processed at two

commercial abattoirs and one pilot-scale abattoir. The French carcasses were processed at a single facility in the west of France. The carcasses from Northern Ireland were processed at five different facilities distributed across the region. All cattle travelled <5 h to reach the abattoirs. The cattle were slaughtered commercially according to standard practice in each country. Post slaughter carcasses were either hung by the Achilles tendon or they underwent tenderstretching, indicating they were instead hung by the obturator foramen or the pelvic ligaments. Tenderstretching was only performed at a subset of the abattoirs. There was a range of 5 to 28 days *postmortem* ageing for the samples, and all samples were wet aged.

All carcasses were graded by personnel trained in MSA and United States Department of Agriculture (USDA) meat grading according to standard MSA protocols for characteristics such as ossification (an estimate of maturity), marbling and ultimate pH. Ultimate pH was recorded at 24 h post slaughter. Ossification score was measured following the guidelines from the USDA (1997). It is a visual measure of the calcification in the spinous processes in the sacral, lumbar and thoracic vertebrae and provides a scale between 100 and 590, in increments of 10 for MSA, and is an assessment of physiological age of a bovine carcass (Anonymous, 2005). Marbling score is a measure of the fat deposited between

individual fibres in the rib eye muscle ranging from 100 to 1100 in increments of 10. Marbling was assessed at the quartering site of the chilled carcass and was calculated by evaluating the amount, piece size and distribution of marbling in comparison with the MSA reference standards (Anonymous, 2005; MLA, 2006). Ultimate pH was recorded at 24 h post slaughter. All cattle were growth-promotant free as these are prohibited in the European Union. There was a wide range in the other carcass traits measured such as marbling score and carcass weight; however, due to the constraints of such an observational study not all measurements were recorded for all carcasses (Table 2). A total of 18 different muscles were collected, though not all muscles were collected from each carcass (Table 3).

There was an uneven distribution of cattle and samples amongst the effects controlled for in this study (Tables 1 and 3). This distribution within the data set reflects the differences in beef production/consumption in the different countries. Animal breed was divided into three categories: beef breeds, dairy breeds and crosses between the beef and dairy breeds. The beef breeds were made up of Angus (six), Hereford (three), Murray grey (19), Shorthorn (two), Belted Galloway (one), Belgian blue (26), Charolais (99), Blonde d'Aquitaine (11), Limousin (48), Montbeliarde (one), Romagnola (one) and Simmental (10). The dairy breeds were made up of Holstein (150), Ayrshire (one) and Normande (four). The cross-breeds were crosses between the previously mentioned beef and dairy breeds, with varying percentages of beef and dairy genetics. In total, 16 different muscles were represented in the 7542 different samples; however the number and type of muscles sampled varied between carcasses, countries and other factors in the study (Tables 1 and 3).

**Table 1** Number of carcasses from which muscle samples were taken within subgroups of the data set

	Sex			Breed		
	B	F	S	Cross	Dairy	Beef
Hang						
AT	55	155	165	142	95	138
TX	41	31	202	30	94	150
Country						
Australia	3	–	40	20	–	20
France	–	45	–	7	19	22
Ireland	–	70	16	86	–	–
NI MED	–	2	16	–	–	18
NI WD	41	37	183	–	95	166
Poland	51	17	–	29	38	1
Cook						
Grill	91	164	255	133	150	227
Roast	88	87	132	92	80	135
Slow cook	20	10	–	14	16	–
Thin slice	–	20	–	20	–	–
Days aged						
5	20	40	20	18	26	36
7	44	59	206	28	104	177
10	34	47	–	17	41	23
14	–	65	18	81	1	11
≥21	40	40	151	8	81	142

B = bull; F = female; S = steer; Cross = beef and dairy breed cross; Dairy = dairy breed; Beef = beef breed; AT = Achilles hung; TX = tenderstretch hung; NI MED = the carcasses from Northern Ireland from which meat samples were prepared to a medium doneness; NI WD = the carcasses from Northern Ireland from which meat samples were prepared to a well-done doneness; Days aged = the number of days a meat sample is aged *postmortem* before preparation.

#### Meat preparation and consumer panels

Meat preparation and consumer assessment of eating quality for the four cooking methods were performed according to protocols for MSA testing (Anonymous, 2008; Watson *et al.*, 2008a). The grill cooking method was performed in all countries and the roast cooking method was performed in all countries except for France. In Northern Ireland the roast and grill samples were prepared to either a medium or a well-done cooking

**Table 2** Number of carcasses and the raw maximum, minimum, mean and SD

	Carcasses	Mean	SD	Minimum	Maximum
Ossification score	521	190	99.5	110	590
Age (days)	480	906	731	369	6133
Ultimate pH	521	5.60	0.19	5.33	7.15
Carcass weight (kg)	521	327	53.0	188	515
Marbling score	521	331	113	100	820
Hump height (cm)	437	63.9	13.8	25.0	115
Eye muscle area (cm <sup>2</sup> )	439	72.1	19.0	30.0	140

Ultimate pH, ossification and marble score were recorded as standard Meat Standards Australia measurements by trained graders. The number of carcasses varies for each measure because not all measurements were recorded for all carcasses.

**Table 3** Different muscles tested by breed class

Muscles	Number of samples			
	Beef	Cross	Dairy	Total
<i>M. triceps brachii caput longum</i> <sup>1</sup>	20	87	25	132
<i>M. serratus ventralis cervicis</i> <sup>2</sup>	19	17	22	58
<i>M. longissimus thoracis et lumborum</i> <sup>3</sup>	0	24	14	38
<i>M. spinalis dorsi</i> <sup>4</sup>	0	13	16	29
<i>M. semitendinosus</i> <sup>5</sup>	34	83	16	133
<i>M. rectus femoris</i> <sup>6</sup>	163	118	79	360
<i>M. vastus lateralis</i> <sup>7</sup>	30	24	23	77
<i>M. biceps femoris</i> <sup>8</sup>	268	151	196	615
<i>M. infraspinatus</i> <sup>9</sup>	60	19	25	104
<i>M. tensor fasciae latae</i> <sup>10</sup>	0	12	12	24
<i>M. gluteus medius</i> <sup>11</sup>	637	188	268	1093
<i>M. gluteus medius</i> <sup>12</sup>	310	26	118	454
<i>M. longissimus thoracis et lumborum</i> <sup>13</sup>	1374	397	590	2361
<i>M. psoas major</i> <sup>14</sup>	159	115	108	382
<i>M. adductor femoris</i> <sup>15</sup>	146	7	55	208
<i>M. semimembranosus</i> <sup>16</sup>	773	381	320	1474
Total	3993	1662	1887	7542

<sup>1</sup>Blade (BLD096).<sup>2</sup>Chuck (CHK078).<sup>3</sup>Cube roll (CUB045).<sup>4</sup>Cube roll (CUB081).<sup>5</sup>Eye round (EYE075).<sup>6</sup>Knuckle (KNU066).<sup>7</sup>Knuckle (KNU099).<sup>8</sup>Silverside (OUT005).<sup>9</sup>Blade (OYS036).<sup>10</sup>Rump tail (RMP087).<sup>11</sup>Eye of rump centre (RMP131).<sup>12</sup>Eye of rump side (RMP231).<sup>13</sup>Striploin (STR045).<sup>14</sup>Tenderloin (TDR062).<sup>15</sup>Topside (TOP001).<sup>16</sup>Topside (TOP073).

doneness. All other samples were prepared to a medium cooking doneness. The slow cooking method was only used in Poland and the Korean BBQ was tested only in Ireland. As the samples were prepared in batches, each consumer only scored samples prepared by a single cooking method. For each of the four cooking methods each consumer received seven portions: the first portion (a 'link' sample) was derived from either a generic striploin or rump muscle and expected to be of average quality – the sensory scores for this portion were not part of the final statistical analysis. The remain six portions of beef tested by the consumer were from one of the experimental samples. These samples were served in accordance with a 6 x 6 Latin square, designed to present each consumer with a diverse quality range and to balance potential order or halo effects.

In total, there were 69 770 consumer responses, with each individual consumer giving six separate responses meaning ~11 300 consumers or people. The consumer demographics are explained in further detail by Bonny *et al.* (2015). Consumers scored meat from their country of origin and were sourced through both commercial consumer testing organisations and local clubs and charities. They were selected to reflect the general population with the only requirement

being that they considered meat an important part of their diet. Consumers scored samples for tenderness, juiciness, flavour liking and overall liking, by making a mark on a 100-mm line scale, with the low end of the scale representing a negative response and the high end of the scale representing a positive response. For a more detailed description of the testing procedures see Anonymous (2008).

#### Meat quality (MQ4) score

Within each country each muscle from each carcass was assessed by 10 individual untrained consumers. The tenderness, juiciness, flavour liking and overall liking values were weighted and combined to create a single MQ4 score. The weightings were calculated using a discriminant analysis, as performed by Watson *et al.* (2008a) and are 0.3 × tenderness, 0.1 × juiciness, 0.3 × flavour liking, 0.3 × overall liking. The highest and lowest two scores for each trait and MQ4 score were removed and an average was calculated for the remaining six scores. These clipped scores were aligned with the muscle, carcass and animal traits for analysis. There is a high correlation between all four sensory scores with a minimum partial correlation coefficient between any of the scores of 0.66 calculated on a subset of the data (Bonny *et al.*, 2015). The predicted MQ4 scores were calculated using the current 2009 MSA model with the bulls being classed as steers.

#### Statistical analysis

Both the actual consumer observed MQ4, and the difference between the actual and the predicted MQ4, from the current MSA model (SP2009), were analysed using a linear mixed effects model (SAS v9.1). Initially, a base model was established which included fixed effects for carcass hanging method, cooking method, muscle type, sex, country and breed. *Postmortem* ageing period in days was included as a covariate. The samples from Northern Ireland were split into two groups such as: NI MED, the samples from Northern Ireland from which were prepared to a medium doneness and NI WD, the samples from Northern Ireland from which were prepared well done. These two groups of samples were classed as separate countries in the statistical models, that is NI MED and NI WD, therefore encompassing the variation due to the different cooking doneness and negating the need for a cooking doneness term within the model. Animal identification number within carcass source country, kill group (animals slaughtered on the same day at the same abattoir) and consumer country were included as random terms. Terms in the model and their first-order interactions were removed in a step-wise fashion is non-significant.

The predicted means for the sexes and breeds were compared using the LSDs, generated using the PDIF function in SAS (SAS v9.1). The DF was determined using the Kenward and Rodger technique (SAS v9.1). For the model with the difference between the actual MQ4 and the MSA-predicted MQ4 as the dependent variable, significant effects in the model indicated that the

accuracy of the prediction differed between subgroups with numbers further away from 0, indicating lower prediction accuracies.

## Results

### Actual MQ4

The *F* values for the core model are presented in Table 4. Cooking method, muscle type and sex were significant main effects in the model and the sex effect did not vary within any of the other terms in the model. The predicted mean of the actual MQ4 of samples from bulls ( $52.1 \pm 1.40$ ) was lower ( $P < 0.05$ ) than both the females ( $54.4 \pm 1.32$ ) and steers ( $56.0 \pm 1.32$ ), which did not differ from each other. When the covariates of marbling and ossification score were included in the model, the difference between sexes did not change.

There were marked differences between breeds (breed  $\times$  cut interaction,  $P < 0.05$ ; Table 4), but this was only evident for five out of the 16 muscles tested (Table 5). Balanced comparisons for breed could only be made within subgroups of cooking method and hang method and so only the grilled samples from carcasses Achilles hung carcasses are reported. As the relationship between breed and MQ4 did not vary between cooking and carcass hang methods (Table 4), the results presented can be considered representative of all other cooking and carcass hang methods in the study. In each of the five muscles where differences were evident, beef breeds had MQ4 scores that were on average about 7 units lower than the dairy and/or cross-breeds. Alternatively, the comparison between dairy and cross-breeds varied across the muscles. In two cases, for the *m. biceps femoris* and the *m. rectus femoris*, the dairy breeds had  $\sim 6$  units lower eating quality ( $P < 0.05$ ) than the cross-breeds, whereas for the *m. gluteus medius* and the *m. longissimus thoracis et*

*lumborum* there were no differences between dairy and cross-breeds. Alternatively, for the *m. semimembranosus* the dairy breeds had 5 units higher eating quality ( $P < 0.05$ ) than the cross-breeds. None of the covariates tested had any effect on the differences between breed in the model.

### MSA prediction accuracy

The *F* values from the core model predicting the difference between the predicted and the actual MQ4 are presented in Table 6. As with the model predicting the actual MQ4, breed interacted with muscle type and cooking method, muscle type and sex were significant as main effects. The predicted mean of the actual MQ4 of samples from bulls ( $-3.82 \pm 1.45$ ) was smaller ( $P < 0.05$ ) than the females ( $-1.25 \pm 1.38$ ). Steers ( $-1.89 \pm 1.34$ ) did not differ from bulls and females. The small negative values indicate that in all cases the MQ4 was slightly over predicted by the MSA model.

When ultimate pH was included as a covariate in the model, the difference between the bulls and either the females or the steers was increased by  $\sim 1$  MQ4 point (data not shown). There was no change in the difference between the females and the steers. Similarly, when marbling score was added to the model as a covariate, the difference between the bulls and the females or the steers increased by  $\sim 1.5$  MQ4 points (data not shown) suggesting that the distribution of marbling score and pH in this data set actually masked differences between the sexes and that the coefficients for marbling score and ultimate pH in the MSA model are not adequately describing the influence of these carcass traits on the eating quality of meat from bulls. There was no change in the difference between the females and the steers. No other covariates tested had an effect on sex in the model.

Similar to the previous model predicting the actual MQ4, balanced comparisons for the prediction accuracy of the different breed categories could only be made within subgroups of cooking method and hang method. As with the previous model, only the grilled samples from carcasses Achilles hung are reported (Table 7). As the relationship between breed and MSA prediction accuracy did not vary between cooking and carcass hang methods, the results presented can be considered representative of all other cooking and carcass hang methods in the study. The degree of under or over prediction of the MSA model varied between muscles ( $P < 0.05$ ; Table 6), with positive values indicating an under prediction of the actual MQ4 score by the MSA model and negative values indicating an over prediction by the MSA model. The ability of the MSA model to predict eating quality also differed between the breeds ( $P < 0.05$ ; Table 6) for six out of the 16 muscles tested (Table 7). For the muscles with significant effects, the beef breeds generally had lower scores ( $P < 0.05$ ) than the cross and dairy breeds, by between 2.5 and 7.5 units. This is evidenced by the predicted means for the beef breeds which were either closer to 0 where the MSA system had under predicted the muscles, or more negative where the MSA system had over predicted the muscles. The contrast to this trend was for the *m. infraspinatus* where the beef breed had eating quality

**Table 4** *F* values for the core model predicting actual meat quality score (MQ4<sup>^</sup>)

Effects	Core model		
	NDF	DDF	<i>F</i> value
Hang	1	7234	59.17***
Sex	2	273	10.95***
Cook method	3	6988	9.79***
Muscle type	14	7232	32.68***
Days aged	1	7313	0.07
Breed class	2	1446	0.24
Days aged $\times$ muscle type	13	7236	6.71***
Days aged $\times$ hang	1	7065	9.7**
Cook method $\times$ muscle type	22	7124	10.7***
Hang $\times$ muscle type	11	7093	12.07***
Hang $\times$ cook method	1	7151	45.02***
Breed class $\times$ muscle type	28	7213	7.32***

NDF = numerator degrees of freedom; DDF = denominator degrees of freedom.

<sup>^</sup>MQ4 is a weighted combination (0.3, 0.1, 0.3, 0.3) of four sensory scores: tenderness, juiciness, flavour liking and overall liking as scored by untrained consumers.

\*\* $P < 0.01$ , \*\*\* $P < 0.001$ .

**Table 5** Predicted means ( $\pm$  SE) of the actual meat quality score (MQ4<sup>^</sup>) for each of the muscles within each breed for the grilled samples from Achilles hung carcasses (n)

Muscles	Beef	Cross	Dairy
<i>M. triceps brachii caput longum</i> <sup>1</sup>	58.8 $\pm$ 2.86 (20)	53.5 $\pm$ 2.44 (33)	–
<i>M. serratus ventralis cervicis</i> <sup>2</sup>	50.2 $\pm$ 2.89 (19)	–	–
<i>M. longissimus thoracis et lumborum a</i> <sup>3</sup>	–	61.7 $\pm$ 5.34 (9)	73.5 $\pm$ 72.3 (7)
<i>M. spinalis dorsi</i> <sup>4</sup>	–	76.4 $\pm$ 10.65 (6)	73.4 $\pm$ 4.16 (6)
<i>M. semitendinosus</i> <sup>5</sup>	–	49.7 $\pm$ 2.52 (41)	–
<i>M. rectus femoris</i> <sup>6</sup>	48.6 $\pm$ 1.83 (59) <sup>a</sup>	59.3 $\pm$ 2.00 (43) <sup>b</sup>	54.1 $\pm$ 1.86 (60) <sup>c</sup>
<i>M. vastus lateralis</i> <sup>7</sup>	–	51.4 $\pm$ 10.6 (4)	41.2 $\pm$ 5.00 (4)
<i>M. biceps femoris</i> <sup>8</sup>	31.5 $\pm$ 1.73 (95) <sup>a</sup>	40.6 $\pm$ 1.73 (86) <sup>b</sup>	34.2 $\pm$ 1.71 (85) <sup>c</sup>
<i>M. infraspinatus</i> <sup>9</sup>	67.5 $\pm$ 2.08 (60)	63.5 $\pm$ 2.84 (17)	62.4 $\pm$ 2.55 (25)
<i>M. tensor fasciae latae</i> <sup>10</sup>	59.3 $\pm$ 2.13 (49)	–	58.2 $\pm$ 2.14 (53)
<i>M. gluteus medius</i> <sup>11</sup>	45.8 $\pm$ 1.45 (251) <sup>a</sup>	53.6 $\pm$ 1.72 (88) <sup>b</sup>	54.8 $\pm$ 1.57 (139) <sup>b</sup>
<i>M. gluteus medius</i> <sup>12</sup>	52.9 $\pm$ 2.78 (38)	58.4 $\pm$ 5.13 (13)	56.2 $\pm$ 3.32 (11)
<i>M. longissimus thoracis et lumborum b</i> <sup>13</sup>	54.0 $\pm$ 1.40 (478) <sup>a</sup>	58.1 $\pm$ 1.55 (179) <sup>b</sup>	58.4 $\pm$ 1.49 (211) <sup>b</sup>
<i>M. psoas major</i> <sup>14</sup>	75.4 $\pm$ 1.61 (127)	78.4 $\pm$ 1.78 (71)	76.4 $\pm$ 1.71 (85)
<i>M. adductor femoris</i> <sup>15</sup>	38.8 $\pm$ 2.63 (18)	–	37.9 $\pm$ 4.43 (12)
<i>M. semimembranosus</i> <sup>16</sup>	35.7 $\pm$ 1.43 (312) <sup>a</sup>	40.1 $\pm$ 1.56 (171) <sup>b</sup>	44.8 $\pm$ 1.55 (149) <sup>c</sup>

– = cells without data.

<sup>a,b,c</sup>Values within a row with different superscripts differ significantly at  $P < 0.05$ .<sup>^</sup>MQ4 is a weighted combination (0.3, 0.1, 0.3, 0.3) of four sensory scores: tenderness, juiciness, flavour liking and overall liking as scored by untrained consumers.<sup>1</sup>Blade (BLD096).<sup>2</sup>Chuck (CHK078).<sup>3</sup>Cube roll (CUB045).<sup>4</sup>Cube roll (CUB081).<sup>5</sup>Eye round (EYE075).<sup>6</sup>Knuckle (KNU066).<sup>7</sup>Knuckle (KNU099).<sup>8</sup>Silverside (OUT005).<sup>9</sup>Blade (OYS036).<sup>10</sup>Rump tail (RMP087).<sup>11</sup>Eye of rump centre (RMP131).<sup>12</sup>Eye of rump side (RMP231).<sup>13</sup>Striploin (STR045).<sup>14</sup>Tenderloin (TDR062).<sup>15</sup>Topside (TOP001).<sup>16</sup>Topside (TOP073).**Table 6** F values and DF for the core model analysing the difference between the predicted and actual meat quality score (MQ4<sup>^</sup>)

Effects	NDF	DDF	F value
Hang	1	7234	2.02
Sex	2	375	3.36*
Cook method	3	6877	5.17**
Muscle type	14	7215	4.19***
Days aged	1	7281	0.25
Breed class	2	1556	2.25
Country	5	34.3	4.34**
Days aged $\times$ muscle type	13	7220	5.73***
Cook method $\times$ muscle type	22	7101	16.63***
Hang $\times$ muscle type	11	7070	5.92***
Hang $\times$ cook method	1	7138	49.57***
Breed class $\times$ muscle type	28	7183	8.55***

NDF = numerator DF; DDF = denominator DF.

<sup>^</sup>MQ4 is a weighted combination (0.3, 0.1, 0.3, 0.3) of four sensory scores: tenderness, juiciness, flavour liking and overall liking as scored by untrained consumers; predicted MQ4 was calculated with carcass traits using the Meat Standards Australia model.\* $P < 0.05$ , \*\* $P < 0.01$ , \*\*\* $P < 0.001$ .

scores about 8.5 units higher than the cross or dairy breeds. None of the covariates tested had any effect on the prediction of breed in the model.

## Discussion

### Sex

Aligning with our hypothesis, samples from bulls had lower eating quality scores than samples from females and steers. This effect was still present after correcting for marbling score, despite evidence that this was likely to be due to differences in IMF (Drayer, 2003; Choat *et al.*, 2006; Chriki *et al.*, 2013). Furthermore, it was not affected by correction for any of the other covariates tested in this study. This suggests that a more complex relationship exists between marbling, sex and eating quality than could be identified in this analysis, or that other factors which were not measured such as fibre diameter and/or collagen content may be driving this difference (Chriki *et al.*, 2013).

Contrary to our hypothesis, the prediction accuracy for bulls, classed as steers within the MSA prediction model, was

**Table 7** Predicted means ( $\pm$  SE) of the difference between the actual and the predicted meat quality score (MQ4<sup>^</sup>) for each of the muscles within each breed for the grilled samples from Achilles hung carcasses (n)

Muscle	Beef	Cross	Dairy
<i>M. triceps brachii caput longum</i> <sup>1</sup>	2.82 $\pm$ 2.60 (20)	0.35 $\pm$ 2.25 (33)	–
<i>M. serratus ventralis cervicis</i> <sup>2</sup>	– 1.74 $\pm$ 2.64 (19)	–	–
<i>M. longissimus thoracis et lumborum</i> <sup>3</sup>	–	– 1.34 $\pm$ 3.49 (9)	3.46 $\pm$ 3.89 (7)
<i>M. spinalis dorsi</i> <sup>4</sup>	–	6.10 $\pm$ 4.16 (6)	10.8 $\pm$ 4.16 (6)
<i>M. semitendinosus</i> <sup>5</sup>	–	3.24 $\pm$ 2.19 (41)	–
<i>M. rectus femoris</i> <sup>6</sup>	2.62 $\pm$ 1.84 (59) <sup>a</sup>	11.7 $\pm$ 2.02 (43) <sup>b</sup>	6.30 $\pm$ 1.84 (60) <sup>a</sup>
<i>M. vastus lateralis</i> <sup>7</sup>	–	3.58 $\pm$ 5.00 (4)	2.84 $\pm$ 5.00 (4)
<i>M. biceps femoris</i> <sup>8</sup>	– 10.8 $\pm$ 1.68 (95) <sup>a</sup>	– 3.66 $\pm$ 1.73 (86) <sup>b</sup>	– 9.42 $\pm$ 1.69 (85) <sup>a</sup>
<i>M. infraspinatus</i> <sup>9</sup>	1.61 $\pm$ 1.88 (60) <sup>a</sup>	– 7.06 $\pm$ 2.72(17) <sup>b</sup>	– 6.92 $\pm$ 2.36 (25) <sup>b</sup>
<i>M. tensor fasciae latae</i> <sup>10</sup>	– 0.19 $\pm$ 1.93 (649)	–	– 1.00 $\pm$ 1.89 (53)
<i>M. gluteus medius</i> <sup>11</sup>	– 4.64 $\pm$ 1.47 (251) <sup>a</sup>	0.78 $\pm$ 1.72 (88) <sup>b</sup>	4.62 $\pm$ 1.54 (139) <sup>c</sup>
<i>M. gluteus medius</i> <sup>12</sup>	– 0.14 $\pm$ 2.09 (38)	– 1.56 $\pm$ 3.00 (13)	2.77 $\pm$ 3.21 (11)
<i>M. longissimus thoracis et lumborum</i> <sup>13</sup>	– 2.57 $\pm$ 1.42 (478) <sup>a</sup>	0.90 $\pm$ 1.57 (179) <sup>b</sup>	– 1.01 $\pm$ 1.47 (211) <sup>ab</sup>
<i>M. psoas major</i> <sup>14</sup>	– 1.66 $\pm$ 1.60 (127)	– 0.24 $\pm$ 1.79 (71)	– 2.50 $\pm$ 1.69 (85)
<i>M. adductor femoris</i> <sup>15</sup>	– 0.67 $\pm$ 2.65 (18)	–	– 2.62 $\pm$ 3.09 (12)
<i>M. semimembranosus</i> <sup>16</sup>	1.38 $\pm$ 1.45 (312) <sup>a</sup>	3.97 $\pm$ 1.57 (171) <sup>a</sup>	8.90 $\pm$ 1.52 (149) <sup>b</sup>

– = cells without data.

<sup>a,b,c</sup>Values within a row with different superscripts differ significantly at  $P < 0.05$ .

<sup>^</sup>MQ4 is a weighted combination (0.3, 0.1, 0.3, 0.3) of four sensory scores: tenderness, juiciness, flavour liking and overall liking as scored by untrained consumers; predicted MQ4 was calculated with carcass traits using the Meat Standards Australia model.

<sup>1</sup>Blade (BLD096).

<sup>2</sup>Chuck (CHK078).

<sup>3</sup>Cube roll (CUB045).

<sup>4</sup>Cube roll (CUB081).

<sup>5</sup>Eye round (EYE075).

<sup>6</sup>Knuckle (KNU066).

<sup>7</sup>Knuckle (KNU099).

<sup>8</sup>Silverside (OUT005).

<sup>9</sup>Blade (OYS036).

<sup>10</sup>Rump tail (RMP087).

<sup>11</sup>Eye of rump centre (RMP131).

<sup>12</sup>Eye of rump side (RMP231).

<sup>13</sup>Striploin (STR045).

<sup>14</sup>Tenderloin (TDR062).

<sup>15</sup>Topside (TOP001).

<sup>16</sup>Topside (TOP073).

lower than for females. Our expectation that both sexes would be predicted with similar accuracy was based upon our assumption that IMF was the factor driving this difference, which would have been accounted for by the marbling score adjustment in the MSA model. Yet, contrary to this, a further correction of the MSA prediction accuracy model for either marbling score or ultimate pH actually increased the differences in the prediction accuracy between the sexes. This demonstrates that the distribution of marbling score and ultimate pH within this data set was actually masking or minimising the differences in prediction accuracy between the sexes. In the absence of differences in IMF driving the differences in eating quality, other factors such as variations in fibre diameter and collagen content could be playing a role (Boccard *et al.*, 1979; Seideman *et al.*, 1989; Chriki *et al.*, 2013). However, these findings indicate that even after accounting for differences in carcass traits, bulls still differ in eating quality when compared with females and steers and this would need to be taken into account when estimating

the eating quality of meat sourced from bull carcasses. In addition, the coefficients used by the Australian MSA model for marbling score and ultimate pH do not exactly reflect the influence of these factors on eating quality in this data set. However, as a result of the relatively small subsample of data used in this experiment, additional data are required to properly elucidate the slope of these relationships for European consumers.

Within the data there was a suggestion of a reduced capacity of the meat from bulls to improve with ageing (data not shown), which could have resulted from differences in muscular calpastatin activity and rates of protein turnover between the sexes (Morgan *et al.*, 1993; Koohmaraie *et al.*, 2002). However, due to the structure of the data set comparisons of the ageing rate within bulls compared with the other sexes was confounded, usually by country. Therefore, to explore this comparison properly, future experiments should make this comparison using samples consumed within the same taste panel session.

### Breed

Aligning with our hypothesis, dairy breeds generally had higher sensory scores than beef breeds, however this was for certain muscles only (Table 5). This agrees with the work of Lizaso *et al.* (2011), who found higher juiciness and flavour scores for the loins of Holsteins when compared with a beef breed. However, in contrast to previous work, this was not explained by marbling score, an estimate of IMF (Lizaso *et al.*, 2011), or any other of the covariates tested. Therefore, it is possible that other factors, such as collagen content or fibre type, are responsible for the difference seen in eating quality between the breeds (Boccard *et al.*, 1979; Christensen *et al.*, 2011; Lizaso *et al.*, 2011). Alternatively, this result may be due to the limitations of marbling score, which is measured on the striploin, to describe adiposity within the diverse range of muscles found over an entire carcass which differ in structure and function. This is evidence by work in beef (Brackebush, 1991), and lamb (Anderson *et al.*, 2015) which demonstrates considerable variation in IMF correlations between the loin muscle and other muscles throughout the carcass. Furthermore, differences in production methods and feeding regimes and age at slaughter present in this study would also complicate the results. Similar production and physiological differences are likely to be underpinning the eating quality differences between the dairy breeds and the cross-breeds (Table 5). However, as the percentage of beef or dairy genetics in the cross-breeds was not fixed in this study, it is likely to have led to the greater variability in the results.

Contrary to our hypothesis, the MSA model did not predict different breeds with equal accuracy and the difference in accuracy varied by muscle (Table 7). Where there were significant differences, the beef breeds had consistently lower predicted means than both the dairy and cross-breeds. Hence, the difference between breeds is not accounted for by the existing adjustments within the MSA model for factors such as marbling score, ossification, carcass weight or fatness. Furthermore, the difference in prediction accuracy between breeds was unchanged by any of the covariates tested in the model, demonstrating that the inaccuracy is not simply a case of needing to adjust the coefficients for these terms within the MSA model. It is important to note that the MSA model also varied in its prediction accuracy of individual muscles, therefore a combination of a muscle-based adjustment along with a single breed adjustment is required to raise all breeds and muscles to similar prediction accuracies.

### Conclusion

The MSA beef quality prediction system in Australia improves consumer satisfaction by delivering beef of a consistent and guaranteed quality. It is well known that part of the variation in meat can be attributed to breed and sex, and the MSA model reflects this with adjustments for *Bos indicus* content and sex (heifer or steer). Bulls and dairy breeds are an important part of the beef industry in Europe and would need to be considered for any meat quality prediction system to be relevant. However, there is little information on the ability of the MSA

model to predict bulls and dairy breeds. This study has identified that there are differences in eating quality between the sexes and breeds. Previous work has indicated that a proportion of the differences between the sexes and breeds can often be explained by factors such as marbling and maturity score, which are included in the MSA model. Eating quality differences were not able to be explained by simple relationships between breed, sex, ossification score and marbling or by the more complex eating quality prediction model in the MSA system, which encompasses a range of other carcass traits. However, the remaining differences in quality could be encompassed by further dairy breed and bull adjustments along with some optimisation of other coefficients such as marbling and ultimate pH. Therefore, with minor adjustments, a complex eating quality prediction system such as the MSA model is flexible enough to adequately describe eating quality within the European beef production system.

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### References

- Anonymous 2005. Handbook of Australian meat. AUS-MEAT Ltd, Brisbane, Australia.
- Anonymous 2008. Accessory Publication: MSA sensory testing protocols. Australian Journal of Experimental Agriculture 48, 1360–1367.
- Anderson F, Pethick DW and Gardner GE 2015. The correlation of intramuscular fat content between muscles of the lamb carcass and the use of computed tomography to predict intramuscular fat percentage in lambs. *Animal* 9, 1239–1249.
- Boccard RL, Naudé RT, Cronje DE, Smit MC, Venter HJ and Rossouw EJ 1979. The influence of age, sex and breed of cattle on their muscle characteristics. *Meat Science* 3, 261–280.
- Bonny SPF, Gardner GE, Pethick DW, Legrand I, Polkinghorne RJ and Hocquette J-F 2015. Biochemical measurements of beef are a good predictor of untrained consumer sensory scores across muscles. *Animal* 9, 179–190.



- Bonny SPF, Pethick DW, Legrand I, Wierzbicki J, Allen P, Farmer LJ, Polkinghorne RJ, Hocquette J-F and Gardner GE 2015. Ossification score is a better indicator of maturity related changes in eating quality than animal age. *Animal*, First view, 1–11.
- Brackebush SA MF, Carr TR and McLaren DG 1991. Relationship between longissimus composition and the composition of other major muscles of the beef carcass. *Journal of Animal Science* 69, 631–640.
- Choat WT, Paterson JA, Rainey BM, King MC, Smith GC, Belk KE and Lipsey RJ 2006. The effects of cattle sex on carcass characteristics and longissimus muscle palatability. *Journal of Animal Science* 84, 1820–1826.
- Chriki S, Gardner GE, Jurie C, Picard B, Micol D, Brun JP, Journaux L and Hocquette J-F 2012. Cluster analysis application identifies muscle characteristics of importance for beef tenderness. *BMC Biochemistry* 13, 29.
- Chriki S, Renand G, Picard B, Micol D, Journaux L and Hocquette J-F 2013. Meta-analysis of the relationships between beef tenderness and muscle characteristics. *Livestock Science* 155, 424–434.
- Christensen M, Erthbjerg P, Failla S, Sañudo C, Richardson RI, Nute GR, Olleta JL, Panea B, Alberti P, Juárez M, Hocquette J-F and Williams JL 2011. Relationship between collagen characteristics, lipid content and raw and cooked texture of meat from young bulls of fifteen European breeds. *Meat Science* 87, 61–65.
- Dikeman ME, Reddy GB, Arthaud VH, Tuma HJ, Koch RM, Mandigo RW and Axe JB 1986. Longissimus muscle quality, palatability and connective tissue histological characteristics of bulls and steers fed different energy levels and slaughtered at four ages. *Journal of Animal Science* 63, 92–101.
- Drayer ML 2003. Evaluation of intact males and steers for growth performance and meat quality. California State University, Fresno, CA, USA.
- Field RA 1971. Effect of castration on meat quality and quantity. *Journal of Animal Science* 32, 849–858.
- Garcia-de-Siles JL, Ziegler JH, Wilson LL and J.D. S 1977. Growth, carcass and muscle characters of Hereford and Holstein steers. *Journal of Animal Science* 44, 973–984.
- Hocquette J-F and Chatellier V 2011. Prospects for the European beef sector over the next 30 years. *Animal Frontiers* 1, 20–28.
- Koohmaraie M, Kent MP, Shackelford SD, Veiseth E and Wheeler TL 2002. Meat tenderness and muscle growth: is there any relationship? *Meat Science* 62, 345–352.
- Lawrie RA 1985. *Meat science*. Pergamon, Oxford, UK.
- Lee CY, Henricks DM, Skelley GC and Grimes LW 1990. Growth and hormonal response of intact and castrate male cattle to trenbolone acetate and estradiol. *Journal of Animal Science* 68, 2682–2689.
- Lizaso G, Beriain MJ, Horcada A, Chasco J and Purroy A 2011. Effect of intended purpose (dairy/beef production) on beef quality. *Canadian Journal of Animal Science* 91, 97–102.
- Lyford C, Thompson J, Polkinghorne R, Miller M, Nishimura T, Neath K, Allen P and Belasco E 2010. Is willingness to pay (WTP) for beef quality grades affected by consumer demographics and meat consumption preferences? *Australasian Agribusiness Review* 18, 1–17.
- Mandell IB, Gullett EA, Wilton JW, Kemp RA and Allen OB 1997. Effects of gender and breed on carcass traits, chemical composition, and palatability attributes in Hereford and Simmental bulls and steers. *Livestock Production Science* 49, 235–248.
- McKay DG 1970. Some aspects of meat quality in dairy beef. McGill University, Ann Arbor, Canada.
- Mills EW, Comerford JW, Hollender R, Harpster HW, House B and Henning WR 1992. Meat composition and palatability of Holstein and beef steers as influenced by forage type and protein source. *Journal of Animal Science* 70, 2446–2461.
- MLA 2006. Module 6: Meat Standards Australia Grading, version 5.0, pp. 1–111. Meat & Livestock Australia, Sydney.
- Morgan JB, Savell JW, Hale DS, Miller RK, Griffin DB, Cross HR and Shackelford SD 1991. National beef tenderness survey. *Journal of Animal Science* 69, 3274–3283.
- Morgan JB, Wheeler TL, Koohmaraie M, Savell JW and Crouse JD 1993. Meat tenderness and the calpain proteolytic system in longissimus muscle of young bulls and steers. *Journal of Animal Science* 71, 1471–1476.
- Polkinghorne R, Thompson JM, Watson R, Gee A and Porter M 2008a. Evolution of the Meat Standards Australia (MSA) beef grading system. *Australian Journal of Experimental Agriculture* 48, 1351–1359.
- Polkinghorne R, Watson R, Thompson JM and Pethick DW 2008b. Current usage and future development of the Meat Standards Australia (MSA) grading system. *Australian Journal of Experimental Agriculture* 48, 1459–1464.
- Seideman SC, Cross HR and Crouse JD 1989. Carcass characteristics, sensory properties and mineral content of meat from bulls and steers. *Journal of Food Quality* 11, 497–507.
- Thompson JM 2004. The effects of marbling on flavour and juiciness scores of cooked beef, after adjusting to a constant tenderness. *Australian Journal of Experimental Agriculture* 44, 645–652.
- USDA 1997. United States standards for grades of carcass beef. United States Department of Agriculture, Washington, DC.
- Venkata Reddy B, Sivakumar AS, Jeong DW, Woo Y-B, Park S-J, Lee S-Y, Byun J-Y, Kim C-H, Cho S-H and Hwang I 2015. Beef quality traits of heifer in comparison with steer, bull and cow at various feeding environments. *Animal Science Journal* 86, 1–16.
- Verbeke W, Van Wezemael L, de Barcellos MD, Kügler JO, Hocquette J-F, Ueland Ø and Grunert KG 2010. European beef consumers' interest in a beef eating-quality guarantee: insights from a qualitative study in four EU countries. *Appetite* 54, 289–296.
- Watson R, Gee A, Polkinghorne R and Porter M 2008a. Consumer assessment of eating quality – development of protocols for Meat Standards Australia (MSA) testing. *Australian Journal of Experimental Agriculture* 48, 1360–1367.
- Watson R, Polkinghorne R and Thompson JM 2008b. Development of the Meat Standards Australia (MSA) prediction model for beef palatability. *Australian Journal of Experimental Agriculture* 48, 1368–1379.
- Węglarz A 2010. Quality beef from semi-intensively fattened heifers and bulls. *Animal Science Papers and Reports* 28, 207–218.