

Production and processing studies on calpain-system gene markers for beef tenderness: Consumer assessments of eating quality¹

D. L. Robinson,*†² L. M. Cafe,*† B. L. McIntyre,*‡ G. H. Geesink,*§ W. Barendse,*# D. W. Pethick,* || J. M. Thompson,*§ R. Polkinghorne,¶ and P. L. Greenwood*†

*Australian Cooperative Research Centre for Beef Genetic Technologies, University of New England, Armidale, NSW 2351, Australia; †New South Wales Department of Primary Industries, Beef Industry Centre, University of New England, Armidale, New South Wales 2351, Australia; ‡Department of Agriculture and Food, Western Australia, South Perth, WA 6151, Australia; §Department of Meat Science, University of New England, Armidale, NSW 2351, Australia; # Commonwealth Scientific and Industrial Research Organisation Livestock Industries, Queensland Bioscience Precinct, St Lucia, Qld 4067, Australia; || School of Veterinary and Biomedical Science, Murdoch University, Murdoch, WA 6150, Australia; and ¶Marrinya Agricultural Enterprises, Wuk Wuk, Vic. 3875, Australia

ABSTRACT: We investigated the effects of calpain-system genetic markers on consumer beef quality ratings, including interactions of marker effects with hormonal growth promotant (HGP) use and tenderstretch hanging. Brahman cattle in New South Wales (NSW; n = 164) and Western Australia (WA; n = 141) were selected at weaning from commercial and research herds to achieve balance and divergence in calpastatin (*CAST*) and calpain 3 (*CAPN3*) gene marker status. Genotypes for μ -calpain (*CAPN1-4751* and *CAPN1-316*) were also determined. Angus cattle (49 in NSW, 17 in WA) with favorable *CAST* and *CAPN3* alleles, balanced for *CAPN1-316* status, were also studied. Half the cattle at each site had HGP (Revalor-H, containing 200 mg trenbolone acetate and 20 mg 17 β -estradiol) implants during grain finishing. One side of each carcass was suspended from the Achilles tendon (AT) and the other from the pelvis [tenderstretch (TS)]. Meat Standards Australia consumer panels scored 7-d aged striploin steaks from both AT and TS sides, and 7-d aged rump and oyster blade steaks from the AT side of each carcass. Two favorable *CAST* alleles increased tenderness ratings of AT-striploin, TS-striploin, rump, and oyster blade steaks by, respectively, 6.1, 4.2, 4.2, and 3.1 units, and

overall liking by 4.7, 2.8, 2.9, 3.7 (all $P < 0.04$). Two favorable *CAPN1-4751* alleles increased tenderness of AT-striploin, TS-striploin, and rump steaks by 6.5, 4.3, and 3.9 units, and overall liking by 5.6, 3.1, and 4.1 units. Two favorable *CAPN3* alleles improved rump steaks by 3.7, 3.3, 3.7, and 3.5 units, for tenderness, juiciness, liking the flavor, and overall liking. There were no significant *CAPN1-316* effects. The effect of HGP was greatest for the AT-striploin (reducing tenderness and overall liking by 8.2 units, $P < 0.001$), then TS-striploin (-5.6 for tenderness, -5.0 for overall liking, $P < 0.001$), and then rump (-4.4 for tenderness, -3.3 for overall liking, $P < 0.007$). Processing conditions differed considerably between NSW and WA. Rump steaks from NSW scored about 10 units greater than those from WA, but Angus and Brahman steaks from the same location with the same marker alleles had similar scores. In contrast, NSW Angus striploin steaks scored about 15 units greater for tenderness and overall liking ($P < 0.001$) than cattle with the same marker alleles at the other 3 location \times breed combinations, which had generally similar scores. Therefore, calpain-system gene markers have beneficial effects on eating quality, consistent with our previous findings for objective meat quality.

Key words: beef quality, calpain, calpastatin, genetic marker, hormonal growth promotant, tenderstretch

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Harvey, WA; Cosign Pty Ltd, and Pfizer Animal Health Australia, in particular, Dr. Gerard Davis. The financial contribution of Meat & Livestock Australia to this work is also gratefully acknowledged. Two previous publications (Cafe et al. 2010a,b) examined the effects of genetic markers on growth, efficiency, and objective meat quality of Brahman cattle.

²Corresponding author: Dorothy.Robinson@dpi.nsw.gov.au
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INTRODUCTION

Consumers consistently rate tenderness as the most important contributor to beef palatability in Australia (Egan et al., 2001) and the United States (Koochmariaie et al., 2002). Favorable genetic markers have been shown to improve shear and compression force measurements of tenderness (Cafe et al., 2010b). It is important to verify that these improvements in objective meat quality lead to enhanced eating quality and also to quantify the benefits. Unless producers are rewarded for improvements to meat quality and increased consumer satisfaction (e.g., by including gene marker effects in beef grading systems), commercial use of marker technology may be limited.

The Meat Standards Australia (MSA) grading system was developed to predict the palatability of individual beef cuts (cooked by various methods) from commercial information, including *Bos indicus* content, hormonal growth promotant (HGP) status, tenderstretch, ultimate pH, ageing, marbling, and ossification scores, which can vary in their impact on individual cuts (Thompson, 2002; Polkinghorne et al., 2008a). The scheme is well suited to include tenderness gene markers as an additional trait.

Cafe et al. (2010a,b) examined the effect of 4 calpain-calpastatin gene markers on production, carcass, and objective meat quality traits. Cattle with favorable alleles had improved objective meat quality, reduced shear and compression forces. Although the effects varied with muscle, there were no interactions with HGP (Revalor-H) use, even though HGP operate in part via altered calpain-calpastatin activities (Gerken et al., 1995). As an initial step to incorporating the markers into MSA, we used consumer panels to quantify their effects on palatability of primal cuts from the experiments of Cafe et al. (2010a,b). The striploin, rump, and oyster blade were chosen because they vary widely in ageing rates, allowing interactions between calpain-system genes and ageing rates to be investigated.

MATERIALS AND METHODS

Use of animals and the procedures performed in this study were approved by the New South Wales (NSW) Department of Primary Industries (DPI) Orange Agricultural Institute Animal Ethics Committee (Approval Numbers ORA 06/001 and ORA 06/004), Commonwealth Scientific and Industrial Research Organisation (CSIRO) Rockhampton Animal Experimentation Ethics Committee (approval number RH216-06), and the Department of Agriculture and Food, Western Australia (WA) Animal Ethics Committee (approval number 2-06-11).

Experimental Designs

Two concurrent experiments were conducted, in NSW at the NSW DPI Agricultural Research and Advisory Station, Glen Innes (29°44'S, 151°42'E, altitude 1057m), and in WA at the WA Department of Agriculture and Food Vasse Research Station, near Busselton (33°45'S, 115°21'E, altitude 25 m). The experiments were designed to assess the effects of calpain-system gene markers for tenderness on production, carcass, and beef quality characteristics of Brahman and Angus cattle, and interactions of the calpain-system gene markers with production and processing factors (Cafe et al., 2010a,b, 2011a,b).

To establish the experimental herds, Brahman (*Bos indicus*, n = 1,664) and Angus (*Bos taurus*, n = 402) cattle in commercial and research herds (12 Brahman and 3 Angus herds) were blood sampled and genotyped at weaning for calpain-system gene markers. The calpain-calpastatin system regulates protein degradation in animals pre-mortem and post-mortem (Koochmariaie et al., 2002). The 4 markers were SNP within genes controlling the calpain-proteolytic system, specifically calpastatin (*CAST*; Barendse, 2002), calpain 3 (*CAPN3*; Barendse et al., 2008), and μ -calpain 1 (*CAPN1-4751* and *CAPN1-316*; White et al., 2005 and Page et al., 2002, respectively). The frequency of alleles in the sampled Brahman herds was reported by Cafe et al. (2010a).

Information on marker status was used to select Brahman cattle to achieve divergence in the number of favorable alleles for the *CAST* and *CAPN3* markers, with the groups being as balanced as possible for the *CAPN1-4751* marker. A small group of Angus cattle with favorable alleles for the *CAST* and *CAPN3* markers, as balanced as possible for the *CAPN1-316* marker, were also selected as positive controls for biological studies on the calpain system. Cattle were re-bled during backgrounding to confirm the status of the 4 tenderness markers.

The experiment for Brahman cattle in NSW was designed to compare the effect of favorable *CAST* alleles (0 vs. 2) \times favorable alleles for *CAPN3* (0 vs. 2) \times gender (heifer or castrate male) \times HGP treatment, with or without HGP containing 200 mg trenbolone acetate and 20 mg 17 β -estradiol (Revalor-H, registered for both steers and heifers, Virbac, Milperra, NSW, Australia), implanted in the ear 2 wk after arrival at the feedlot. The design for the Angus controls in NSW was chosen to maximize the accuracy of estimating the *CAPN1-316* marker and HGP implant effects (Robinson et al., 2007).

The WA experiment was designed to compare favorable alleles for *CAST* (0, 1, or 2) \times favorable alleles for *CAPN3* (0, 1, or 2) \times HGP treatment (with or without Revalor-H during feedlotting) in the Brahman cattle. The design for Angus cattle was chosen to maximize the

accuracy of estimating *CAPN1-316* marker and HGP implant effects. Due to the low frequency of some of the SNP in the herds tested, some combinations of markers were difficult to source. Because of this, and identification errors or inaccuracies in the original genotyping of selected animals, numbers of animals were not perfectly balanced for gene marker status. Methods used to allocate individual animals to treatment and management groups, and further details of design considerations, are described and discussed by Robinson et al. (2007) and Robinson (2009).

Experimental Procedures

The experimental procedures, including sources of the cattle, backgrounding management, feedlot management, slaughter procedures, measurements, carcass processing conditions, chiller assessments, and objective measures of beef quality, are described in detail by Cafe et al (2011a,b). Temperature and pH of the LM were measured adjacent to the 12th rib of the left side of each carcass, using TPS probes (TPS, Springwood, Qld, Australia) and temperature at pH 6 of the LM (**LTpH6**) calculated, as described by Cafe et al. (2010b). The calving period for each breed × location combination was 2 to 4 mo, but information on the birth dates of individual animals was not available. Approximate average age at slaughter was 17 mo for NSW Brahmans, 21 mo for NSW Angus, 21 mo for WA Brahmans, and 24 mo for WA Angus.

To assess the effect of tenderstretching, the left side of each carcass was suspended from the Achilles tendon (**AT**), right from the pelvis [tenderstretch (**TS**)]. Both sides were placed together in the chiller.

Preparation of Steaks

During boneout (average 28 h postmortem in NSW and 20 h postmortem in WA), the rump and oyster blade primal cuts from the Achilles-hung side plus the striploin from both the Achilles-hung and tenderstretched sides were removed, vacuum packed, and chilled to 2°C for transport to Coffs Harbour, NSW, where samples were prepared for consumer panel assessments. A breakdown on the slaughter chain at 1 slaughter in WA meant that samples for sensory testing could not be collected from 33 carcasses. Table 1 provides numbers of animals with sensory data by breed, location, marker, HGP status, and gender.

The rump primal was first seamed into component muscles to extract the gluteus medius, which was further divided along the seam of connective tissue into 2 portions, with sensory samples prepared from the larger “D” head. The LM was excised from the striploin and the supraspinatus (**SS**) from the oyster blade. Muscles

were trimmed of all fat and epimysium before slicing into 25-mm steak samples. Striploin steaks were cut from the anterior portion of the LM and oyster blade steaks from a center block of SS.

After cutting, steaks were individually wrapped and vacuum packaged in sets of 5 steaks per excised muscle sample, then stored at 2°C. Seven days after slaughter, the samples were frozen at –20°C for storage until consumer testing. This ageing period, considered appropriate for the Australian domestic market, allows direct comparison of consumer panel results with the objective meat quality measurements on 7-d aged meat from the same animals.

Consumer Assessment of Beef Quality

The development of the MSA consumer testing protocol is described by Watson et al. (2008a). Although the responses of trained panelists have less variability, their scores are not necessarily an accurate representation of the preferences of the average beef consumer. In contrast, untrained panels provide an unbiased estimate of mean consumer preferences but are likely to be more variable.

The experimental designs for consumer sensory testing were laid out using MSA protocols and software (Anon, 2008). Information on carcass suspension, HGP, breed, gene marker, and muscle were used to stratify the

Table 1. Number of cattle with 0, 1, or 2 copies of favorable alleles for each genetic marker, HGP status (0 = none, 2 = implanted), gender (0 = heifer, 2 = steer) by breed and site¹

	No. of copies			No. of copies		
	0	1	2	0	1	2
	NSW Brahmans ²			WA Brahmans ³		
CAST	66	10 ⁴	88	41	50	50
CAPN3	88	5 ⁴	71	35	59	47
CAPN1-4751	89	67	8	67	61	13
CAPN1-316	149	15	0	119	21	1
HGP	83		81	71		70
Gender	82		82			141
	NSW Angus			WA Angus		
CAST	0	0	49	0	0	17
CAPN3	0	1 ⁴	48	0	0	17
CAPN1-4751	1	18	30	0	2	15
CAPN1-316	17	21	11	3	10	4
HGP	24		25	9		8
Gender	24		25			17

¹The 4 markers were calpastatin (CAST), calpain 3 (CAPN3), and μ -calpain 1 (CAPN1-4751 and CAPN1-316). HGP = Revalor-H hormone growth promotant.

²NSW = New South Wales

³WA = Western Australia

⁴The NSW experiment was designed to compare effects of 2 vs. 0 favorable CAST or CAPN3 alleles; the small number of NSW cattle found, on retesting, to have 1 favorable allele were, however, included in the analysis.

samples into 6 groups, based on expected eating quality. The protocols then allocated the 6 groups via a 6×6 Latin square to ensure each sample was served before and after samples from all other groups. Because of concerns that serving (even randomly) a very high or low quality product in first position could bias the relativity of subsequent samples (Watson et al., 2008a), all consumers were served a mideating quality “link” product as their first sensory sample, then 1 sample from each of the 6 groups, as allocated by software routines. Each panel was allocated a mix of product, including all 4 cuts from both WA and NSW.

Consumers, recruited from clubs and societies in Sydney, NSW, were screened to be between the age of 20 and 60, eat meat at least once a fortnight, and prefer a medium degree of doneness. Each panel had 60 consumers in 3 sessions of 20 consumers at 0.75-h intervals. Previous research (Hwang et al., 2008) has shown no significant effect of demographics on MSA sensory scores, other than preferred degree of doneness.

The sensory panel test procedures are described in detail by Anon (2008). Briefly, samples were thawed at 4 °C over 24 h, transported to the venue, and cooked by grilling on a Silex S–165 clam shell grill unit (Silex Grills Australia Pty Ltd, Marrickville, NSW, Australia), set at 220 to 230°C and with 2.75 times as much heat to the top vs. bottom plates. Cooking was controlled by a timer to produce a constant medium degree of doneness. The cooked steaks were rested and halved before serving on prenumbered paper plates. Seven individual half steaks (the starter “link” sample and 6 experimental samples) were served to each consumer over a 0.75-h period. Consumers scored each sample using 100-mm line scales for tenderness, juiciness, liking the flavor, and overall liking, with 100 representing the most and 0 the least favorable scores. Results were combined into a meat quality score (MQ4), using the formula: $MQ4 = 0.4 \times \text{tenderness} + 0.1 \times \text{juiciness} + 0.2 \times \text{flavor} + 0.3 \times \text{overall liking}$ (Watson et al., 2008a). Here (and throughout the rest of the paper), references to consumer panel scores for flavor mean the scores for liking the flavor. The MSA grading system classifies meat with MQ4 scores of >46.5 as 3 star (good everyday, Watson et al., 2008a), ≥ 64 as 4 star (better than everyday), and ≥ 77 as 5 star (Polkinghorne et al., 2008a).

Statistical Analyses

Following the recommendation of Watson et al. (2008a), the 2 highest and 2 lowest of the 10 consumer scores for each sample were discarded and a “clipped” mean calculated from the remaining 6 scores. Statistical analyses were conducted on the clipped means by fitting linear mixed models for each sensory trait using the REML

methodology (Robinson, 1987) in ASREML–R software (Butler et al., 2009).

Body weight at slaughter, HCW, and LTpH6, which have the potential to influence eating quality, were also analyzed to determine the variation in these traits, including breed and location effects. The model for HCW and LTpH6 included fixed terms for breed \times location, gender, percent *Bos indicus* (BI%; average 94% for Brahman cattle in WA and 100% for Brahmans in NSW; see Cafe et al. 2010a,b), plus 3 factors for each of the 4 genetic markers (*CAST*, *CAPN3*, *CAPN1–316*, and *CAPN1–4751*), representing the number of favorable alleles (0, 1, or 2). Random effects included property of origin, kill group, and interactions of the 4 genetic markers with location (NSW or WA).

The analyses of sensory data also included HCW and LTpH6 as fixed covariates, expressed as deviations from the means for each location (262 kg and 245 kg for HCW in NSW and WA, respectively; 21.6 and 36.0°C in NSW and WA, respectively, for LTpH6) and allowing different slopes for each location. The effect of carcass weight and its interaction with location were usually significant, with heavier carcasses on average having marginally worse sensory scores, except for striploin steaks in NSW. The effects of LTpH6 were significant mainly for Achilles-hung striploin steaks in NSW ($P < 0.05$ for tenderness), with higher temperatures resulting in improved scores. Analyses omitting temperature at pH 6 produced very similar results to those from the full model when there was no significant effect of the covariate or its interaction with location. For simplicity, the results reported here are from the full model fitting separate slopes for carcass weight and LTpH6 at each location.

Interactions of *CAPN1–316* and *CAPN1–4751* with each other and with breed were also considered, as well as interactions of HGP with all 4 markers but found not to be significant ($P > 0.05$) so were not included in the final models. Because of the different processing conditions at the 2 locations, and potentially different variations within breeds, the model allowed for different residual variation for each breed at each location.

To facilitate comparisons of breeds and locations, predicted means were calculated for Brahman (adjusted to 100 BI%) and Angus steers with 2 favorable alleles for *CAST* and *CAPN3*, 1 favorable allele for *CAPN1–4751*, no favorable alleles for *CAPN1–316*, and no HGP treatment. All Angus cattle had 2 copies of *CAST*, and all except 1 had 2 copies of *CAPN3*, so additional analyses restricted to Brahman cattle were carried out to estimate and compare the effects of having 2 favorable alleles for *CAST* and *CAPN3*, plus 1 or 2 favorable alleles for *CAPN1–4751*, with the effect of having no favorable alleles for these markers. The models for these analyses used the same terms as the main analyses, omitting breed

and its interactions. Additional analyses restricted to Angus cattle were also carried out to see if any additional information could be gleaned from the small numbers of Angus cattle in this study.

RESULTS

Breed × Location Effects

The cohort of NSW Angus was ~4 mo older and about 150 kg heavier than the colocated Brahmans. Angus cattle in WA were ~3 mo older and about 75 kg heavier than WA Brahmans (Table 2).

Processing conditions, as indicated by LTpH6, differed considerably between the 2 locations. In WA, the pH of the LM declined rapidly, reaching pH 6 at an average carcass temperature of 36°C (Table 2) about 1.5 h after slaughter. In NSW, the LM reached pH 6 at average temperature of 21°C (Table 2) at an average of 6 h after slaughter. The LTpH6 was not affected by breed, HGP, or genetic marker status (Tables 2 to 7).

The different processing conditions at the 2 locations led to highly significant location × breed interactions for palatability of striploin steaks (e.g., $P = 0.001$ for tenderness of the tenderstretched striploin; interaction P -values not shown in the table). In both tenderstretched and Achilles-hung sides, average tenderness of NSW Angus striploin steaks was 16 to 17 units greater than the other 3 breed × location combinations. Scores for juiciness, liking the flavor, overall liking, and MQ4 scores were ~14 units greater (Table 2).

Tenderstretching improved tenderness of the striploin (7.7 and 5.6 units, respectively, for NSW Angus and Brahmans, 8.1 and 7.5 units for WA Angus and Brahmans, Table 2) and also reduced variability. The residual variance of tenderness scores of tenderstretched striploin was less for all breed × location combinations than the Achilles-hung striploin. The reduction was particularly noticeable for NSW Angus, where the residual variance fell from 211 to 75 units².

In contrast to breed × location effects for the striploin, the quality of rump steaks varied mainly with location. The NSW Brahman and Angus rump steaks had significantly greater sensory scores than Angus or Brahman rump steaks from WA (Table 2). However, as shown in Table 2, after adjusting for the effects of the genetic markers, the only significant difference in sensory scores for rump steaks from the same location was that Angus steaks from WA were considered less juicy than WA Brahman steaks.

Oyster blade steaks had the greatest average consumer ratings, with little variation due to breed or location. The MQ4 scores were well above the threshold of 64 for 4-star rating, with the NSW Brahman average close to the threshold of 77 for 5-star rating. The only significant breed ×

location differences ($P < 0.05$) was that WA Angus oyster blade steaks were considered less tender than NSW Angus and WA Brahmans less juicy than NSW Brahmans.

HGP

Use of HGP implants reduced tenderness, overall liking, and MQ4 scores of Achilles-hung striploin steaks by ~8 units, flavor by 7 units, and juiciness by 4.5 units ($P < 0.001$, Table 3). Sensory scores for the tenderstretched striploin were reduced by 4 to 6 units ($P < 0.001$). The effect of HGP on rump steaks was still

Table 2. Numbers of animals, approximate age of group, and predicted means¹ for BW at slaughter, carcass data, and sensory scores by breed and site

Item	New South Wales (NSW)		Western Australia (WA)		Avg. SED ²
	Angus	Brahman	Angus	Brahman	
No. of animals	49	164	17	141	
Age, mo	21	17	24	21	
BW, kg	587	433 ^a	496	421 ^a	28.4
HCW, kg	328	246 ^{ab}	266 ^a	234 ^b	15.3
LM temp. at pH 6, °C	21.6	21.1	36.9 ^a	35.7 ^a	2.4
Tenderstretched striploin sensory scores					
Tenderness	75.4	57.8 ^a	59.0 ^a	58.6 ^a	4.8
Juiciness	70.4	55.5 ^a	58.3 ^a	57.7 ^a	4.9
Like the flavor	74.8	58.9 ^a	58.4 ^a	62.1 ^a	4.1
Overall liking	75.5	59.9 ^a	61.2 ^a	62.1 ^a	4.2
MQ4 ³	74.0	58.5 ^a	59.7 ^a	60.0 ^a	4.3
Achilles-hung striploin sensory scores					
Tenderness	67.8	52.2 ^a	50.9 ^a	51.1 ^a	5.8
Juiciness	68.6	53.6 ^a	55.2 ^a	54.6 ^a	5.1
Like the flavor	68.8 ^a	56.9 ^b	61.5 ^{ab}	57.0 ^b	4.6
Overall liking	70.7	56.1 ^a	59.4 ^a	56.5 ^a	5.0
MQ4	69.2	54.8 ^a	55.9 ^a	54.5 ^a	4.9
Rump sensory scores					
Tenderness	60.7 ^a	56.4 ^a	44.5 ^b	51.5 ^b	4.6
Juiciness	64.2 ^a	58.2 ^{ab}	46.9	55.8 ^b	4.4
Like the flavor	62.5 ^a	60.7 ^a	50.2 ^b	57.4 ^{ab}	3.9
Overall liking	63.6 ^a	59.7 ^a	49.5 ^b	55.8 ^b	3.9
MQ4	61.9 ^a	58.2 ^a	46.9 ^b	53.9 ^b	3.8
Oyster blade sensory scores					
Tenderness	74.8 ^a	76.5 ^{ab}	69.4 ^b	74.4 ^{ab}	4.4
Juiciness	77.7 ^{ab}	78.2 ^a	72.8 ^{ab}	74.4 ^b	3.8
Like the flavor	72.8 ^a	75.9 ^a	69.4 ^a	72.9 ^a	4.1
Overall liking	72.5 ^a	76.3 ^a	69.3 ^a	73.2 ^a	4.2
MQ4	73.0 ^a	75.6 ^a	69.0 ^a	72.6 ^a	3.9

^{a,b}Within a row means without a common superscript differ ($P < 0.05$).

¹To facilitate comparisons of the different categories, predicted means are for averages of Brahman and Angus steers without HGP, with 2 favorable *CAST* and *CAPN3* alleles, 1 favorable allele for *CPI-475I*, and no favorable alleles for *CPI-316*. Means are predicted at carcass weights of 261.5 kg (NSW) and 245 kg (WA) and temperatures at pH 6 of 21.6 (NSW) and 36.0 (WA) °C.

²Because of the large variation in numbers of animals, the average SED provides only a rough indication of variability.

³MQ4 = meat quality score = 0.4*tenderness + 0.1*juiciness + 0.2*flavor + 0.3*overall liking. MQ4 scores <46.5 are ungraded; scores of 46.5, 64, and 77 or more are graded 3 star, 4 star, and 5 star, respectively.

significant ($P < 0.01$) but slightly smaller at 3 to 4 units. The HGP effect on oyster blade steaks was reduced to ~2 units, achieving significance only for the juiciness score. As noted in the methods section, the HGP effect did not interact with any of the tenderness markers.

CAST

The effect of 2 favorable *CAST* alleles was significant ($P < 0.05$, Table 4) for all sensory scores, except juiciness and flavor of rump and tenderstretched striploin steaks. The greatest improvement was for tenderness of Achilles-hung striploins (6 units), with improvements of 4 units for tenderness of both tenderstretched striploin and rump steaks, and 3 units for tenderness of the oyster blade (all $P < 0.025$). Only 10 NSW Brahmans had 1 favorable *CAST* allele, so information comparing the

effects of heterozygotes with homozygotes was mainly from Brahmans in WA. Standard errors of the contrast between heterozygotes and homozygotes (not shown in the Table) were greater than for comparisons of 0 vs. 2 favorable alleles, but consistent with an intermediate effect for steaks with 1 favorable allele.

CAPN3

The effect of *CAPN3* was significant only for rump steaks, where 2 favorable alleles improved tenderness, juiciness, flavor, and overall liking by 3 to 4 units (all $P < 0.025$, Table 5). There was no difference between heterozygotes (all except 1 of which were Brahmans, Table 1) and those with no favorable *CAPN3* alleles.

Table 3. Numbers of animals and predicted means¹ for BW at slaughter, carcass data, and sensory scores by hormone growth promotant (HGP) status

Item	No HGP		SED	Difference	P-value
	No HGP	HGP			
No. of animals	187	176			
BW, kg	484	521	4.0	-36.8	<0.001
HCW, kg	269	290	2.1	-21.5	<0.001
LM temp. at pH 6, °C	28.8	28.6	0.4	0.2	0.653
Tenderstretched striploin sensory scores					
Tenderness	62.7	57.2	1.3	5.6	<0.001
Juiciness	60.5	55.8	1.3	4.7	0.001
Like the flavor	63.6	59.8	1.1	3.8	0.001
Overall liking	64.7	59.6	1.2	5.0	<0.001
MQ4 ²	63.0	58.1	1.1	4.9	<0.001
Achilles-hung striploin sensory scores					
Tenderness	55.5	47.3	1.5	8.2	<0.001
Juiciness	58.0	53.5	1.4	4.5	0.002
Like the flavor	61.0	53.9	1.2	7.2	<0.001
Overall liking	60.7	52.5	1.3	8.2	<0.001
MQ4	58.6	51.0	1.3	7.6	<0.001
Rump sensory scores					
Tenderness	53.3	48.9	1.4	4.4	0.002
Juiciness	56.3	53.2	1.2	3.1	0.012
Like the flavor	57.7	54.5	1.1	3.2	0.005
Overall liking	57.2	53.9	1.2	3.3	0.007
MQ4	55.2	51.6	1.2	3.6	0.002
Oyster blade sensory scores					
Tenderness	73.8	71.7	1.2	2.1	0.080
Juiciness	75.8	73.5	1.0	2.2	0.025
Like the flavor	72.7	70.9	1.1	1.8	0.090
Overall liking	72.8	71.3	1.2	1.6	0.178
MQ4	72.5	70.8	1.0	1.8	0.089

¹ Predicted means are for averages of Brahman and Angus steers with 2 favorable *CAST* and *CAPN3* alleles, 1 favorable *CAPN1-4751* allele, and no favorable alleles for *CAPN1-316*.

²MQ4 = meat quality score = 0.4*tenderness + 0.1*juiciness + 0.2*flavor + 0.3*overall liking. MQ4 scores <46.5 are ungraded; scores of 46.5, 64, and 77 or more are graded 3 star, 4 star, and 5 star, respectively.

Table 4. Numbers of animals and predicted means¹ for BW at slaughter, carcass data, and sensory scores by number of favorable alleles for the *CAST* marker

Item	No. of favorable alleles			Avg. SED	Difference (2-0)	P-value (2-0)
	0	1	2			
No. of animals	107	60	204			
BW, kg	480	492	484	6.6	3.8	0.464
HCW, kg	265	272	269	3.5	3.4	0.229
LM temp. at pH 6, °C	29.1	29.2	28.8	0.6	-0.3	0.625
Tenderstretched striploin sensory scores						
Tenderness	58.5	60.7	62.7	2.0	4.2	0.008
Juiciness	58.9	61.5	60.5	2.0	1.6	0.315
Like the flavor	61.5	64.9	63.6	1.8	2.0	0.132
Overall liking	61.8	64.6	64.7	1.8	2.8	0.040
MQ4 ²	59.8	62.6	63.0	1.8	3.2	0.019
Achilles-hung striploin sensory scores						
Tenderness	49.4	49.6	55.5	2.3	6.1	0.001
Juiciness	53.2	55.0	58.0	2.1	4.8	0.004
Like the flavor	58.1	58.5	61.0	1.8	3.0	0.034
Overall liking	56.0	56.1	60.7	2.0	4.7	0.002
MQ4	53.5	54.2	58.6	1.9	5.1	0.001
Rump sensory scores						
Tenderness	49.1	51.2	53.3	2.1	4.2	0.010
Juiciness	53.7	54.6	56.3	1.9	2.5	0.080
Like the flavor	55.7	56.2	57.7	1.8	2.0	0.135
Overall liking	54.2	55.8	57.2	1.9	2.9	0.038
MQ4	52.1	53.7	55.2	1.8	3.1	0.022
Oyster blade sensory scores						
Tenderness	70.7	72.9	73.8	1.8	3.1	0.024
Juiciness	72.4	76.3	75.8	1.5	3.4	0.004
Like the flavor	69.6	74.1	72.7	1.6	3.2	0.012
Overall liking	69.1	73.4	72.8	1.8	3.7	0.006
MQ4	69.3	72.7	72.5	1.6	3.3	0.008

¹ Predicted means are for averages of Brahman and Angus steers without Revalor-H hormone growth promotant, with 2 favorable *CAPN3* alleles, 1 favorable *CPI-4751* allele, and no favorable alleles for *CAPN1-316*.

²MQ4 = meat quality score = 0.4*tenderness + 0.1*juiciness + 0.2*flavor + 0.3*overall liking. MQ4 scores <46.5 are ungraded; scores of 46.5, 64, and 77 or more are graded 3 star, 4 star, and 5 star, respectively.

Calpain 1 Markers

The effect of *CAPNI-4751* was highly significant, improving sensory scores for the Achilles-hung striploin by an average of 5 units (all *P* except flavor < 0.03, Table 6), with a trend for tenderstretched-striploin, where the average improvement in tenderness was 4 units (*P* = 0.058, 2-tailed). The contrasts between heterozygotes and homozygotes had larger SE, but the results suggest that steaks with 1 favorable allele are better than those with no favorable alleles but worse than those with 2. Cattle with 2 favorable alleles for *CAPNI-4751* had lighter BW at slaughter (*P* = 0.028), with a tendency (*P* = 0.096) to reduced carcass weights.

Although there was no significant interaction of *CAPNI-4751* with breed, the analysis restricted to An-

gus cattle did not show a significant effect of this marker. Consequently, it would be unwise to draw any conclusions about its effect on *Bos taurus* cattle from the small number of Angus cattle in this study.

No significant effects could be detected for *CAPNI-316* (Table 7).

Combined Effect of *CAST*, *CAPN3*, and *CAPNI-4751*

Table 8 illustrates the combined effect, for Brahman steers, of carrying 2 favorable alleles for both *CAST* and *CAPN3*, and either 1 (2_2_1) or 2 (2_2_2) favorable *CAPNI-4751* alleles, derived from the analyses of all Brahman cattle (138 had 2 favorable *CAST* alleles; 111 had 2 favorable *CAPN3* alleles; 128 and 21 had, respectively, 1 and 2 favorable *CAPNI-4751* alleles, Table 1).

Table 5. Numbers of animals and predicted means¹ for BW at slaughter, carcass data, and sensory scores by number of favorable alleles for the *CAPN3* marker

Item	No. of favorable alleles			Avg. SED	Difference (2-0)	<i>P</i> -value (2-0)
	0	1	2			
No. of animals	123	65	183			
BW, kg	489	492	484	6.7	-4.7	0.383
HCW, kg	271	272	269	3.5	-2.9	0.320
LM temp. at pH 6, °C	28.4	29.0	28.8	0.6	0.4	0.469
Tenderstretched striploin sensory scores						
Tenderness	61.1	56.5	62.7	2.0	1.6	0.306
Juiciness	59.5	57.7	60.5	2.1	1.0	0.524
Like the flavor	63.1	61.1	63.6	1.7	0.4	0.741
Overall liking	63.5	61.0	64.7	1.8	1.2	0.403
MQ4 ²	61.7	58.3	63.0	1.8	1.3	0.340
Achilles-hung striploin sensory scores						
Tenderness	53.2	52.9	55.5	2.3	2.3	0.183
Juiciness	57.8	57.4	58.0	2.2	0.2	0.885
Like the flavor	60.8	59.9	61.0	1.8	0.2	0.893
Overall liking	59.6	59.0	60.7	2.1	1.0	0.492
MQ4	57.2	56.8	58.6	1.9	1.4	0.341
Rump sensory scores						
Tenderness	49.5	50.0	53.3	2.1	3.7	0.021
Juiciness	53.0	54.6	56.3	1.9	3.3	0.023
Like the flavor	54.0	54.2	57.7	1.8	3.7	0.005
Overall liking	53.6	53.0	57.2	1.9	3.5	0.013
MQ4	51.7	51.9	55.2	1.8	3.5	0.010
Oyster blade sensory scores						
Tenderness	74.7	72.2	73.8	1.8	-0.9	0.509
Juiciness	76.3	74.8	75.8	1.7	-0.5	0.656
Like the flavor	72.4	69.3	72.7	1.8	0.3	0.813
Overall liking	73.8	70.9	72.8	1.8	-1.0	0.477
MQ4	73.5	70.8	72.5	1.6	-1.0	0.435

¹Predicted means are for averages of Brahman and Angus steers without Revalor-H hormone growth promotant, with 2 favorable *CAST* alleles, 1 favorable *CP-4751* allele, and no favorable alleles for *CPI-316*.

²MQ4 = meat quality score = 0.4*tenderness + 0.1*juiciness + 0.2*flavor + 0.3*overall liking. MQ4 scores <46.5 are ungraded; scores of 46.5, 64, and 77 or more are graded 3 star, 4 star, and 5 star, respectively.

Table 6. Numbers of animals and predicted means¹ for BW at slaughter, carcass data, and sensory scores by number of favorable alleles for the *CAPNI-4751* marker

Item	No. of favorable alleles			Avg. SED	Difference (2-0)	<i>P</i> -value (2-0)
	0	1	2			
No. of animals	157	148	66			
BW, kg	487	484	470	7.9	-17.3	0.028
HCW, kg	269	269	262	4.2	-7.0	0.096
LM temp. at pH 6, °C	28.3	28.8	29.6	0.8	1.2	0.120
Tenderstretched striploin sensory scores						
Tenderness	62.0	62.7	66.4	2.3	4.3	0.058
Juiciness	59.5	60.5	63.0	2.4	3.5	0.137
Like the flavor	62.5	63.6	65.4	2.0	2.9	0.145
Overall liking	63.7	64.7	66.8	2.0	3.1	0.131
MQ4 ²	62.1	63.0	65.6	2.0	3.5	0.083
Achilles-hung striploin sensory scores						
Tenderness	50.5	55.5	57.0	2.8	6.5	0.019
Juiciness	54.2	58.0	59.9	2.5	5.7	0.023
Like the flavor	58.5	61.0	61.5	2.1	3.0	0.170
Overall liking	56.3	60.7	61.9	2.3	5.6	0.018
MQ4	54.2	58.6	59.3	2.3	5.1	0.027
Rump sensory scores						
Tenderness	50.0	53.3	53.9	2.5	3.9	0.123
Juiciness	53.0	56.3	58.0	2.2	5.0	0.026
Like the flavor	55.6	57.7	58.9	2.1	3.3	0.108
Overall liking	54.2	57.2	58.3	2.2	4.1	0.058
MQ4	52.3	55.2	56.3	2.1	4.0	0.057
Oyster blade sensory scores						
Tenderness	72.8	73.8	73.7	2.1	0.9	0.654
Juiciness	75.1	75.8	75.5	1.8	0.4	0.830
Like the flavor	71.1	72.7	72.0	1.9	0.9	0.632
Overall liking	71.2	72.8	72.5	2.0	1.4	0.497
MQ4	71.6	72.5	72.4	1.9	0.8	0.676

¹Predicted means are for averages of Brahman and Angus steers without Revalor-H hormone growth promotant, with 2 favorable *CAST* and *CAPN3* alleles, and no favorable alleles for *CAPNI-316*.

²MQ4 = meat quality score = 0.4*tenderness + 0.1*juiciness + 0.2*flavor + 0.3*overall liking. MQ4 scores <46.5 are ungraded; scores of 46.5, 64, and 77 or more are graded 3 star, 4 star, and 5 star, respectively.

Apart from the oyster blade, which was already highly rated, the combined effect of the 3 markers made a clear and significant difference to palatability. The greatest improvement was for the Achilles-hung striploin, for which tenderness was increased from 37 (for 0_0_0) to 50 (2_2_1) and MQ4 from 42 to 53 units. The MQ4 score of rump steaks increased from 47 (for 0_0_0) to 56 (2_2_1) and the MQ4 score of tenderstretched striploin from 54 to 59.

DISCUSSION

The results presented here show that untrained consumers rated steaks from cattle with favorable calpain-system alleles as more tender and juicy, with a better-liked flavor and better liked overall, confirming that, as

Table 7. Numbers of animals and predicted means¹ for BW at slaughter, carcass data, and sensory scores by number of favorable alleles for the *CAPNI-316* marker

Item	No. of favorable alleles			Avg. SED	Difference (2-0)	P-value (2-0)
	0	1	2			
No. of animals	288	67	16			
BW, kg	484	487	480	11.6	-4.7	0.684
HCW, kg	269	268	263	6.1	-5.4	0.378
LM temp. at pH 6, °C	28.8	28.7	28.3	1.1	-0.5	0.636
Tenderstretched striploin sensory scores						
Tenderness	62.7	63.9	68.0	3.2	5.3	0.094
Juiciness	60.5	59.7	61.5	3.5	1.0	0.773
Like the flavor	63.6	63.7	66.0	2.9	2.4	0.407
Overall liking	64.7	65.2	66.2	2.9	1.6	0.598
MQ4 ²	63.0	63.6	66.3	2.9	3.2	0.262
Achilles-hung striploin sensory scores						
Tenderness	55.5	55.3	58.2	4.7	2.7	0.567
Juiciness	58.0	58.7	57.3	4.0	-0.7	0.862
Like the flavor	61.0	61.8	59.9	3.6	-1.1	0.755
Overall liking	60.7	60.9	59.4	3.9	-1.3	0.745
MQ4	58.6	58.8	59.5	3.9	0.9	0.816
Rump sensory scores						
Tenderness	53.3	52.6	52.7	3.7	-0.5	0.888
Juiciness	56.3	54.1	52.4	3.2	-3.8	0.230
Like the flavor	57.7	58.1	58.5	3.0	0.8	0.784
Overall liking	57.2	56.6	56.7	3.1	-0.4	0.885
MQ4	55.2	54.6	54.3	3.0	-0.9	0.763
Oyster blade sensory scores						
Tenderness	73.8	74.0	69.8	3.2	-4.0	0.213
Juiciness	75.8	74.1	72.0	2.6	-3.8	0.142
Like the flavor	72.7	70.8	68.6	2.8	-4.1	0.142
Overall liking	72.8	71.6	68.0	2.9	-4.8	0.103
MQ4	72.5	71.7	68.3	2.7	-4.2	0.127

¹Predicted means are for averages of Brahman and Angus steers without Revalor-H hormone growth promotant, with 2 favorable *CAST* and *CAPN3* alleles, and 1 favorable allele for *CAPNI-4751*.

²MQ4 = meat quality score = 0.4*tenderness + 0.1*juiciness + 0.2*flavor + 0.3*overall liking. MQ4 scores <46.5 are ungraded; scores of 46.5, 64, and 77 or more are graded 3 star, 4 star, and 5 star, respectively.

well as reductions in shear force and compression of the LM and semitendinosus muscles (Cafe et al., 2010b; Johnston and Graser, 2010), favorable alleles led to improved eating quality ratings for the cuts considered here. Predicted means for tenderness of Brahman steers with 2 favorable alleles for *CAST* and *CAPN3*, and 1 favorable allele for *CAPNI-4751* were 13.1 units greater for the Achilles-hung striploin steaks, 6.4 units greater for tenderstretched striploin steaks, 10.6 units greater for rump, and 2.6 units greater for oyster blade steaks. Consumers also rated the steaks from cattle with these favorable alleles more highly for juiciness, flavor, and overall liking, leading to increased MQ4 scores. Except for a possible reduction in BW at slaughter for cattle with 2 copies of *CAPNI-4751*, this improvement appears to have been achieved without any adverse effect on growth, temperament, efficiency, or carcass characteristics (Cafe et al., 2010a).

The magnitude of these improvements in eating quality can be judged in terms of the MSA grading system, which classifies meat with MQ4 scores of >46.5 as 3 star (good everyday, Watson et al., 2008a), ≥64 as 4 star (better than everyday), and ≥77 as 5 star (Polkinghorne et al., 2008a). Compared with 3-star steaks, Australian, American, and Japanese consumers say they would pay respectively 1.51, 1.64, and 1.69 times as much for 4-star product, 2.10, 2.37, and 2.86 times as much for 5-star product, but only 0.57, 0.56, and 0.48 times as much for product that does not achieve the 3-star grade (Lyford et al., 2010).

The size of the improvement varied with muscle and tenderness gene. Generally, the effects were greatest for Achilles-hung striploin steaks, somewhat less for tenderstretched striploin and rump steaks, and only just detectable in oyster blade steaks. Of the 4 markers, *CAST*, then *CAPNI-4751*, had the greatest effects, with *CAPN3* effects significant only for rump steaks. Whilst the *CAPNI-316* gene had little effect on sensory scores in this study, significant reductions in shear force of the LM and semitendinosus muscle have been noted (Johnston and Graser, 2010). The marker was not well distributed within the sample of cattle used in this experiment and so no firm conclusions can be drawn about its potential effects on sensory scores. The other important result was that there was no significant interaction among gene markers for tenderness and HGP use/nonuse; effects on sensory scores appeared to be additive.

The tenderness genes in this study act on the calpain-calpastatin system, which also causes tenderization with ageing, so it is not surprising that they interact with muscle. There are reports in the literature that muscles age at different rates, primarily because of varying calpastatin amounts (Koohmaraie and Geesink, 2006). Consequently, the favorable *CAST* allele, which de-

Table 8. Predicted means¹ from analyses of all Brahman cattle, for Brahman cattle with no favorable *CAST*, *CAPN3*, or *CAPN1-4751* (0_0_0) compared with those with 2 favorable alleles for *CAST* and *CAPN3*, and either 1 (2_2_1) or 2 (2_2_2) favorable alleles for *CAPN1-4751*

Item	Marker combination			Difference	Difference	P-value	P-value
	0_0_0	2_2_1	2_2_2	0_0_0	0_0_0	0_0_0	0_0_0
				2_2_1	2_2_2	2_2_1	2_2_2
HCW, kg	234	239	235	4.81	0.50	0.43	0.95
LM temp. at pH 6, °C	28.1	28.9	29.0	0.8	0.9	0.47	0.47
Tenderstretched striploin							
Tenderness	52.5	58.8	64.3	6.4	11.9	0.023	0.001
Juiciness	51.9	54.9	58.8	3.0	7.0	0.286	0.055
Like the flavor	56.3	59.6	64.6	3.2	8.2	0.171	0.007
Overall liking	55.6	60.4	65.1	4.8	9.5	0.047	0.002
MQ4 ²	53.9	59.3	64.1	5.4	10.2	0.024	0.001
Achilles-hung striploin							
Tenderness	36.7	49.8	51.9	13.1	15.2	<0.001	<0.001
Juiciness	44.3	52.9	56.8	8.6	12.5	0.003	0.001
Like the flavor	49.5	56.3	57.1	6.8	7.6	0.009	0.019
Overall liking	44.5	54.9	55.9	10.4	11.4	<0.001	0.001
MQ4	42.3	53.0	54.0	10.8	11.8	<0.001	<0.001
Rump							
Tenderness	44.0	54.6	55.1	10.6	11.1	<0.001	0.003
Juiciness	45.9	54.3	55.3	8.4	9.4	0.001	0.006
Like the flavor	53.2	59.9	61.2	6.7	8.1	0.009	0.015
Overall liking	49.0	57.7	58.4	8.7	9.4	0.002	0.008
MQ4	47.3	56.2	56.9	8.9	9.6	<0.001	0.004
Oyster blade							
Tenderness	72.5	75.1	76.6	2.6	4.1	0.267	0.183
Juiciness	71.4	74.3	76.0	2.9	4.6	0.151	0.081
Like the flavor	68.4	72.8	73.0	4.4	4.6	0.043	0.108
Overall liking	69.0	72.8	74.2	3.8	5.2	0.097	0.087
MQ4	70.3	72.9	74.3	2.6	4.0	0.214	0.147

¹Predicted means are for Brahman steers without Revalor-H hormone growth promotant. Analyses of meat quality data included covariates for HCW, expressed as deviations from the mean for each site: (243.7 kg and 21.4°C in New South Wales and 242.0 kg and 35.8°C in Western Australia).

²MQ4 = meat quality score = 0.4*tenderness + 0.1*juiciness + 0.2*flavor + 0.3*overall liking. MQ4 scores <46.5 are ungraded; scores of 46.5, 64, and 77 or more are graded 3 star, 4 star, and 5 star, respectively.

creases calpastatin activity (M. McDonagh, Agriculture NSW, personal communication), is expected to have a greater effect in muscles with reduced calpastatin, which age more rapidly than muscles with greater calpastatin activity. The results here show the improvement for 7-d aged steaks. Longer periods of ageing should result in even more tender meat but perhaps alter the magnitude of the difference between favorable and unfavorable alleles for the tenderness markers.

Although the HGP implant (Revalor-H) improved growth and efficiency (current experiment; Café et al., 2010a), this was accompanied by a reduction in perceived tenderness, juiciness, flavor, overall liking, and MQ4 scores for striploin and rump steaks. The reductions in tenderness ranged from 8.2 units for Achilles-hung striploin, 5.6 units for tenderstretched striploin, and 4.4 and 2.1 units for rump and oyster blade steaks, respectively. This is in agreement with the results of

Café et al. (2010b), which showed that use of this HGP increased shear and compression forces.

Our results are also consistent with a meta-analysis (Watson et al., 2008b), showing that, whilst the magnitude and significance of the HGP effect may vary in individual studies, HGP implants reduce sensory and objective meat quality of the striploin. In addition, Thompson et al. (2008) showed that the effects of HGP interact with muscle, being larger in muscles with the greatest ageing rate, such as the striploin, and also that the reductions in consumer panel scores (for steers implanted with Revalor-S and heifers implanted with Revalor-H) were greater for steaks aged for 7 d (the time at which our MSA consumer assessments are conducted) than those aged for 21 d.

In WA, steaks from Brahman cattle with 2 favorable alleles for *CAST*, *CAPN3*, and 1 favorable *CAPN1-4751* allele scored as highly as Angus cattle with the same favorable alleles. However, under the different processing

conditions in NSW, striploin steaks from NSW Angus cattle were rated more highly than the other 3 breed \times location combinations (WA Angus, NSW and WA Brahmans, which all had similar ratings). The MSA standards require the pH of the LM to fall below 6 at a temperature between 35 and 12°C (Thompson, 2002). In WA, possibly because of electrical inputs from the hide puller (B. McIntyre, personal communication), pH fell rapidly, with 88% of Angus and 65% of Brahman carcasses reaching pH 6 above 35°C, suggesting that processing conditions were not ideal. Perhaps, because of the lower variation in LTpH6 and the high proportion of carcasses reaching pH 6 above 35°C, there was no significant effect of temperature at pH 6 of the LM for the WA experiment.

Under the different processing conditions in the NSW abattoir, the decline in loin pH was generally much slower. For Brahmans, a small proportion (1.8%) of LM still reached pH 6 above 35°C, but 7.7% of Angus and 13.4% of Brahmans reached pH 6 below 12°C. Mean shear force of the Achilles-hung striploin was considerably greater in NSW (e.g., 63 N after 7 d ageing for Brahman steers, Cafe et al., 2010b) than WA (49 N for Brahman steers), but the mean sensory scores of our untrained consumer panels for NSW and WA Brahman Achilles-hung striploin steaks were similar.

The effect of LTpH6 was significant (for Achilles-hung striploin steaks), with higher temperatures resulting in greater sensory scores. The effect of LTpH6 for other cuts was smaller and not statistically significant. These results are consistent with the predictions of Thompson et al. (2006), which showed tenderness scores of Achilles-hung, but not tenderstretched, LM are related to temperature at pH 6.

The improved tenderness score of the striploin from tenderstretching (7.7 and 5.6 units, respectively, for NSW Angus and Brahmans, 8.1 and 7.5 units, respectively, for WA Angus and Brahmans, Table 2) was comparable or greater than the improvement (6.1 units) in the Achilles-hung striploin for cattle with 2 vs. 0 favorable *CAST* alleles. The larger improvements for Achilles-hung than tenderstretched striploin also suggest an interaction of marker effects with method of suspension. By preventing the striploin from shortening, tenderstretched steaks presumably have less need of improvement through protein degradation.

Variability in tenderness scores was also reduced by tenderstretching. The residual variance from the modeled tenderness scores of tenderstretched striploin was less for all breed \times location combinations than the Achilles-hung striploin. The reduction was particularly noticeable for NSW Angus, where the residual variance fell from 211 to 75 units². Such improvements in consistency with tenderstretching have been noted elsewhere (Sørheim et al.,

2001; Wolcott et al., 2009) and are generally considered of value to consumers (Polkinghorne et al., 2008b).

Implications

Seven-day aged steaks from cattle with 2 favorable alleles for *CAST* and *CAPN1-4571* were rated more highly, by the consumer panels in this trial, for tenderness, juiciness, liking the flavor, and overall liking than those with no favorable alleles. Favorable alleles for *CAPN3* improved the palatability of rump steaks. The magnitude of the difference between favorable and unfavorable alleles may vary with ageing period. Inclusion of marker information is expected to enhance prediction accuracy of the MSA model. The evidence of improved palatability for carcasses with favorable tenderness alleles could allow some currently ungraded cuts to achieve 3-star status; others (e.g., tenderstretched striploin) might achieve 4-star status. Incorporation of these markers into the MSA Grading System could result in a greater price for carcasses with favorable alleles, encouraging breeders to incorporate marker information in breeding programs and ultimately improve overall satisfaction for beef consumers.

LITERATURE CITED

- Anon. 2008. Accessory publication: MSA sensory testing protocols. *Australian J. of Experimental Agric.* 48:1360–1367.
- Barendse, W., B. E. Harrison, R. J. Bunch, and M. B. Thomas. 2008. Variation at the Calpain 3 gene is associated with meat tenderness in zebu and composite breeds of cattle. *BMC Genet.* 9:41.
- Barendse, W. J. 2002. DNA markers for meat tenderness. Inventor. The Commonwealth Scientific and Industrial Research Organization, State of Queensland through its Department of Primary Industries, University of New England, State of New South Wales through its Dep. of Agric., and Meat and Livestock Australia Limited, assignees. International Patent Publication W0 02/064820.
- Butler, D., B. Cullis, A. Gilmour, and B. Gogel. 2009. ASReml-R Reference Manual, Release 3. Queensland Dep. of Primary Industries and Fisheries, Brisbane, Australia.
- Cafe, L. M., B. L. McIntyre, D. L. Robinson, G. H. Geesink, W. Barendse, and P. L. Greenwood. 2010a. Production and processing studies on calpain-system gene markers for tenderness in Brahman cattle: 1. Growth, efficiency, temperament, and carcass characteristics. *J. Anim. Sci.* 88:3047–3058.
- Cafe, L. M., B. L. McIntyre, D. L. Robinson, G. H. Geesink, W. Barendse, D. W. Pethick et al. 2010b. Production and processing studies on calpain-system gene markers for tenderness in Brahman cattle: 2. Objective meat quality. *J. Anim. Sci.* 88:3059–3069.
- Cafe, L. M., D. L. Robinson, D. M. Ferguson, G. H. Geesink, and P. L. Greenwood. 2011a. Temperament and hypothalamic-pituitary-adrenal axis function are related and combine to affect growth, efficiency, carcass, and meat quality traits in Brahman steers. *Domestic Anim. Endocrinology* 40:230–240.

- Cafe, L. M., D. L. Robinson, D. M. Ferguson, B. L. McIntyre, G. H. Geesink, and P. L. Greenwood. 2011b. Cattle temperament: Persistence of assessments and associations with productivity, efficiency, carcass and meat quality traits. *J. Anim. Sci.* 89:1452–1465.
- Egan, A. F., D. M. Ferguson, and J. M. Thompson. 2001. Consumer sensory requirements for beef and their implications for the Australian beef industry. *Australian J. of Experimental Agric.* 41:855–859.
- Gerken, C. L., J. D. Tatum, J. B. Morgan, and G. C. Smith. 1995. Use of genetically identical (clone) steers to determine the effects of estrogenic and androgenic implants on beef quality and palatability characteristics. *J. Anim. Sci.* 73:3317–3324.
- Hwang, I. H., R. Polkinghorne, J. M. Lee, and J. M. Thompson. 2008. Demographic and design effects on beef sensory scores given by Korean and Australian consumers. *Australian J. of Experimental Agric.* 48:1387–1395.
- Johnston, D. J., and H.-U. Graser. 2010. Estimated gene frequencies of GeneSTAR markers and their size of effects on meat tenderness, marbling, and feed efficiency in temperate and tropical beef cattle breeds across a range of production systems. *J. Anim. Sci.* 88:1917–1935.
- Koohmaraie, M., and G. H. Geesink. 2006. Contribution of postmortem muscle biochemistry to the delivery of consistent meat quality with particular focus on the calpain system. *Meat Sci.* 74:34–43.
- Koohmaraie, M., M. P. Kent, S. D. Shackelford, E. Veiseth, and T. L. Wheeler. 2002. Meat tenderness and muscle growth: is there any relationship? *Meat Sci.* 62:345–352.
- Lyford, C., J. Thompson, R. Polkinghorne, M. Miller, T. Nishimura, K. Neath et al. 2010. Is willingness to pay (WTP) for beef quality grades affected by consumer demographics and meat consumption preferences? *Australasian Agribusiness Rev.* 18:1–17.
- Page, B. T., E. Casas, M. P. Heaton, N. G. Cullen, D. L. Hyndman, C. A. Morris et al. 2002. Evaluation of single-nucleotide polymorphisms in CAPN1 for association with meat tenderness in cattle. *J. Anim. Sci.* 80:3077–3085.
- Polkinghorne, R., J. Philpott, A. Gee, A. Doljanin, and J. Innes. 2008a. Development of a commercial system to apply the Meat Standards Australia grading model to optimise the return on eating quality in a beef supply chain. *Australian J. of Experimental Agric.* 48:1451–1458.
- Polkinghorne, R., R. Watson, J. M. Thompson, and D. W. Pethick. 2008b. Current usage and future development of the meat standards Australia (MSA) grading system. *Australian J. of Experimental Agric.* 48:1459–1464.
- Robinson, D. L. 1987. Estimation and use of variance components. *J. of the Royal Statistical Soc. Ser. D (The Statistician)* 36:3–14.
- Robinson, D. L. 2009. Experimental design for integrated research projects to estimate genetic and numerous treatment effects. *Livestock Sci.* 121:300–307.
- Robinson, D. L., L. M. Cafe, J. M. Thompson, and P. L. Greenwood. 2007. Designing experiments that estimate genetic marker, major gene and treatment effects. *Genetic improvement: making it happen. Proc. of the 17th Conf. of the Assoc. for the Advancement of Anim. Breeding and Gen., Armidale, New South Wales, Australia* 312–315.
- Sørheim, O., J. Idland, E. C. Halvorsen, T. Frøystein, P. Lea, and K. I. Hildrum. 2001. Influence of beef carcass stretching and chilling rate on tenderness of m. longissimus dorsi. *Meat Sci.* 57:79–85.
- Thompson, J. 2002. Managing meat tenderness. *Meat Sci.* 62: 295–308.
- Thompson, J. M., B. M. McIntyre, G. D. Tudor, D. W. Pethick, R. Polkinghorne, and R. Watson. 2008. Effects of hormonal growth promotants (HGP) on growth, carcass characteristics, the palatability of different muscles in the beef carcass and their interaction with aging. *Australian J. of Experimental Agric.* 48:1405–1414.
- Thompson, J. M., D. Perry, B. Daly, G. E. Gardner, D. J. Johnston, and D. W. Pethick. 2006. Genetic and environmental effects on the muscle structure response post-mortem. *Meat Sci.* 74:59–65.
- Watson, R., A. Gee, R. Polkinghorne, and M. Porter. 2008a. Consumer assessment of eating quality - development of protocols for Meat Standards Australia (MSA) testing. *Australian J. of Experimental Agric.* 48:1360–1367.
- Watson, R., R. Polkinghorne, A. Gee, M. Porter, J. M. Thompson, D. Ferguson et al. 2008b. Effect of hormonal growth promotants on palatability and carcass traits of various muscles from steer and heifer carcasses from a *Bos indicus*-*Bos taurus* composite cross. *Australian J. of Experimental Agric.* 48:1415–1424.
- White, S. N., E. Casas, T. L. Wheeler, S. D. Shackelford, M. Koohmaraie, D. G. Riley et al. 2005. A new single nucleotide polymorphism in CAPN1 extends the current tenderness marker test to include cattle of *Bos indicus*, *Bos taurus*, and crossbred descent. *J. Anim. Sci.* 83:2001–2008.
- Wolcott, M. L., D. J. Johnston, S. A. Barwick, C. L. Iker, J. M. Thompson, and H. M. Burrow. 2009. Genetics of meat quality and carcass traits and the impact of tenderstretching in two tropical beef genotypes. *Anim. Production Sci.* 49:383–398.