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The impact of two different hormonal growth promotants (HGP) on the eating quality of feedlot finished steer carcasses

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Abstract

The Meat Standards Australia (MSA) beef grading model applies a variable penalty for different cuts for Hormonal Growth Promotant (HGP) treated carcasses but does not differentiate between different HGP types. Using 300 non implanted *Bos indicus/Bos taurus* composite steers an experiment was conducted to compare the effects on eating quality in the *mm. longissimus dorsi* (LD) and *gluteus medius* (GM) muscles of an oestradiol only (OES) and a combination trenbolone acetate with oestradiol (TBA+OES) implant with non-implanted animals (CON) fed a concentrate ration for 73 days prior to slaughter. Sensory and objective LD and GM samples were aged for either 5 or 35 days before freezing at -20°C. Carcass weights from each group were significantly different ($P<0.05$). Corrected for carcass weight, both HGP treatments had a significant effect on hump height, ossification score, marble score, P8 fat depth and eye muscle area. The TBA+OES treatment resulted in significantly tougher meat than the OES and CON treatments as assessed by shear force ($P<0.05$), although this difference was reduced with aging. Sensory scores (tenderness, juiciness, like flavour, overall liking and a composite MQ4 score) confirmed a negative HGP treatment effect, whereby TBA+OES was significantly lower than the CON and OES groups after 5 days of aging, and these differences were reduced through aging. TBA+OES had a greater impact on sensory scores in the LD when compared to the GM. Both HGP treatments increased calpastatin activity, and the TBA+OES group was significantly different to the CON and OES groups ($P<0.05$). It was concluded that OES and TBA+OES implants have different impacts on meat eating quality measurements, which could have important implications for the Australian and international beef industry.

Executive summary

This experiment demonstrated that oestradiol only (OES) Hormonal Growth Promotants (HGPs) have less impact on eating quality than combination oestradiol and trenbolone acetate (TBA+OES) implants when used in feedlot finished steers fed for 73 days. Furthermore, aging of meat from animals treated with TBA+OES combination HGPs reduces the negative impact on eating quality. This could mean that the current eating quality penalty in the Meat Standards Australia (MSA) model to account for the differing impact of HGP implants may require adjustment to account for these findings.

HGPs are a tool that has been used in the Australian beef industry for over thirty years. Their productivity and economic advantages have been widely accepted with reported growth rate improvements of 10-30% and feed conversion efficiency improvements of 5-15%. Davies (2008) reported that HGPs added an extra \$210M to the Australian beef industry in 2006-7 through heavier animals and earlier turnoff-times. With the increasing focus on reducing the impact on the environment caused by agricultural production, technology such as HGP implants will continue to play a role into the future.

The MSA beef grading model predicts the eating quality outcome of different cuts by cooking method from commercial inputs of on-farm, carcass traits and processing inputs. Based on a body of Australian research in the mid-2000's it was demonstrated that HGPs have undoubtedly a negative impact on beef eating quality over and above an effect on maturity (ossification) and fat content, in the order of three to six palatability points (MQ4). Subsequently, an adjustment of approximately 4 to 5 MSA Index points is applied to any HGP treated carcass that is presented for grading.

Since the introduction of the HGP adjustment, some industry stakeholders argued that the research conducted to establish the magnitude of the HGP adjustment did not allow for the hypothesised variable effects of different HGPs used in Australia. This was because, in the majority of the studies conducted, animals received multiple implants and/or were finished with a TBA+OES HGP implant. This made individual implant contribution to eating quality effects difficult to distinguish, and therefore, to protect the consumer, the MSA pathways committee decided on one collective HGP adjustment.

A total of 300 steers were inducted into a feedlot on the Darling Downs and finished on a high energy ration for 73 days. Animals were allocated randomly into one of three HGP treatment groups; Control – CON (no implant), OES (Compudose 100) or TBA+OES (Component TE-200). Following the finishing period, animals were transported to and slaughtered at a commercial processor located at Beenleigh, Qld. At boning, the rump (as rosbiff) and left striploin were collected from each carcass, vacuum packed and chilled prior to transport to the University of New England for sampling. Four samples of the *m. gluteus medius* (GM – D rump) and *m. longissimus dorsi* (LD – striploin) from each carcass were aged either 5 days or 35 days, for both objective and consumer sensory analysis. Two primals with divergent aging characteristics were chosen for this trial, as research has shown the magnitude of the HGP on eating quality is proportional to the aging potential of the cut. For example, the striploin has the greatest aging rate in the carcass, and therefore displays the largest negative impact when a HGP is used.

Untrained consumers scored each sample for tenderness, juiciness, flavour and overall liking which were prepared using the MSA consumer testing protocols and weighted by 0.3, 0.1, 0.3 and 0.3 respectively to calculate a composite palatability score (MQ4). Objective measurements included Warner-Bratzler shear force, cooking loss, Minolta colour readings (L*, a* and b*), and pH.

Calpastatin activity, an inhibitor of μ -calpain, an enzyme responsible for proteolysis during aging, was measured.

The TBA+OES treatment resulted in the largest live and carcass weights, with a 24% and 10% respectively, advantage over the CON group. The OES treatment responses were intermediate, with a live and carcass weight advantage of 11% and 5% respectively, when compared to the CON treatment. There was a wide variance in live and carcass weights from 367 to 570kg, and 185 to 298kg respectively. Both HGP treatments increased ossification scores, decreased ribfat and marbling scores and increased eye muscle area. The impacts were larger for the TBA+OES treatment than the OES treatment. These performance and carcass results were expected, as estrogens (e.g. oestradiol) and androgens (e.g. trenbolone acetate) target different hormone receptors, and therefore any impacts are additive.

Sensory scores indicate that TBA+OES treatment had a significant negative impact on eating quality when compared to the CON or OES treatments. There was a numerical, but statistically non-significant ($P>0.05$) decrease in sensory scores as a result of OES treatment in comparison with the CON treatment. For the LD, there was an interaction between the aging and the HGP treatments, whereby the magnitude of the impact of TBA+OES treatment on eating quality reduced from 5 to 35 days. This has important implications for the processing sector as it allows for improvement of high value TBA+OES treated cuts such as the striploin, through additional aging. Shear force values supported the sensory score results, whereby the TBA+OES samples were significantly tougher than the OES and CON samples after 5 days of aging, though differences between treatments was reduced by aging 35 days. The HGP treatments increased cooking loss significantly, though numerically only by 0.4% to 0.5%. Only minor numerical differences were seen for L^* , a^* , and b^* measurements between all groups. Overall there were three carcasses with an ultimate pH >5.7 and 20 animals with a meat colour score >3 , therefore being outside the specifications to be eligible for MSA grading.

Calpastatin is an inhibitor of μ -calpain, the predominant enzyme responsible for muscle protein degradation in a live animal and as a result, meat tenderisation post mortem. It has been hypothesised that HGPs increase the level of calpastatin in muscles of live animals, thus reducing protein degradation, leading to an increase in muscle deposition. In a post mortem carcass this same enzymatic system regulates the aging process, and therefore higher levels of calpastatin would lead to a decrease in tenderisation during aging. The results from this project support this hypothesis whereby calpastatin activity was significantly increased by the TBA+OES treatment when compared to the CON and OES groups. The OES treatment increased calpastatin activity numerically but not to a statistically significant level when compared to the CON treatment. These results suggest that both oestrogen and combination androgen and oestrogen implants decrease protein degradation rates by increasing calpastatin levels, partly explaining the mode of action and their effect on meat quality.

Both HGP treatments increased live weight and carcass weight, though had negative impacts on carcass measurements correlated to eating quality, along with lower sensory scores and higher shear force scores. This impact was far greater for the TBA+OES treatment which had the largest production advantage, but the greatest impact on eating quality. The economic impacts between the three treatments will vary between production systems when balancing eating quality with productivity. Based on this study, producers look to benefit knowing that the OES treatment may result in less impact on marbling and other fat measurements, ossification and hump height, than the TBA+OES treatment when used in feedlot finished steers. The net result is a higher MSA Index score and potentially greater returns through eating quality premiums available on processor grids. The MSA Index scores for CON, OES and TBA+OES were 57.2, 51.0 and 49.9 respectively. Similarly,

OES implants have a productivity benefit over untreated animals, which may outweigh the economic differences in MSA Index scores.

Further work is underway currently to evaluate the impacts of OES implants when steers are finished on pasture for approximately 400 days. Once this component of this project is completed, both studies would need to be collectively evaluated using the predicted and actual mean MQ4 sensory scores to identify if any modifications to differentiate HGP implants in the MSA model would be justified.

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1 Introduction

Hormone growth promotants (HGPs) have been utilised in the Australian beef industry for over 30 years to improve animal weight gain and feed efficiency. They are primarily used in northern Australian grassfed production systems, as well as in feedlots. In 2007, it was estimated that approximately half of all animals slaughtered in Australia were implanted with HGPs, contributing \$210 million to the industry annually (Davies, 2008; Hunter, 2009). This contribution has declined in recent years due to the growth of HGP free markets, drought and the impact of HGPs on eating quality.

The productivity advantages of HGP implants are well understood and accepted. In an earlier review of HGPs, Preston (1999) reported improvements of 10 to 30% in growth rate and 5 to 15% in feed conversion efficiency. Australian studies have reported economic advantages of up to A\$64 per steer treated with a HGP implant (Davies, 2008). Utilising a large US feedlot implant trial database (1996-2014) along with other research, Duckett and Pratt (2014) confirmed these advantages, with the combined trenbolone acetate and oestradiol implants resulting in the highest growth performance. Dikeman (2007) commented that due to the different modes of action of androgen (e.g. trenbolone acetate) and estrogen (e.g. oestradiol) compounds in HGPs, the effects were generally additive. More recently there has been increased attention on the environmental benefits of growth enhancing technologies, such as HGP implants, to negate lifetime emissions from ruminants and improve resource efficiency (Capper & Hayes, 2012; McCrabb & Hunter, 2002)

Nichols et al. (2002) reviewed the literature on the effects of HGP implants on the tenderness of beef and concluded that for many individual experiments HGPs had only a limited effect on shear force and taste panel tenderness. Contrary to this, Watson (2008) concluded that when considered in a meta-analysis, HGP implants had a clear negative impact on both shear force and sensory tenderness. Subsequently, adjustments ranging between minus 3-6 palatability (MQ4) points depending on cut, were introduced to the MSA grading model in 2008 (Watson et al., 2008c). This adjustment was applied to all HGP treated carcasses regardless of the type of implant used.

Many of the reports in the literature have only compared one type of HGP implant against a control. Where multiple implants were used, the designs often made it difficult to compare the different HGP formulations (Hunter, 2009). However in the reviews of Tatum (2009) and Morgan (1997) a comparison between implant types was possible and although the results have been variable, they suggested that the milder oestradiol implants tended to have a smaller impact on eating quality than the implants which contained a combination of trenbolone acetate and oestradiol.

Koohmaraie et al. (2002) proposed that the net rate of muscle synthesis in the body was a function of both muscle synthesis and degradation, whereby degradation was mediated largely via the calpain/calpastatin axis. They proposed that the rate of muscle synthesis was unlikely to be associated with eating quality, rather that the eating quality of muscle in the post-mortem (pm) carcass was a function of muscle degradation rates. As suggested by Thompson et al. (2008a) if some part of the growth advantage of HGP implants in the live animal was achieved via a decreased muscle degradation rates, this would result in slower aging rates for muscles and hence tougher beef.

The Meat Standards Australia (MSA) beef grading model predicts the eating quality outcome of different cuts by cooking method from commercial inputs of on-farm, carcass traits and processing inputs (Polkinghorne et al., 2008). A feature of the MSA model is that it accounts for differential aging rates across the musculature. If different HGP formulations affect eating quality differently,

this raises the question as to whether these impacts will interact with different muscles in the carcass. Thompson et al. (2008a) reported that use of a combination trenbolone acetate and oestradiol implant resulted in much greater effects on eating quality in faster aging muscles such as the *m. longissimus dorsi* (LD), when compared to other muscles in the carcass.

Currently the MSA model does not distinguish between the different types of HGP implants. This was a deliberate strategy implemented by the MSA Pathways Committee to protect the consumer (Polkinghorne, 2015) as the Committee considered that at the time there were insufficient studies which clearly quantified the differences between HGP implants. This paper reports the results from an experiment designed to compare the impact of an oestradiol only and a combination trenbolone acetate and oestradiol implant, with non-implanted controls, when finished in a feedlot for 73 days. Given the potential of HGP implants to impact on pm aging rates, meat quality was assessed at 5 and 35 days pm using two cuts with divergent aging characteristics.

2 Materials and methods

2.1 Live cattle

A total of 300 composite steers ($3/8$ *Bos indicus*, $1/2$ *Bos taurus*, $1/8$ *Bos indicus-taurus hybrid*) from the same year of birth (2013) were used in this study. The steers were weaned on a large Northern Territory property and transported to central Queensland for backgrounding until an average feedlot entry weight of 317kg (standard deviation ± 19.3). At induction, steers were weighed and randomly allocated to the three treatment groups which comprised a control (CON), where steers received no implant, an oestradiol only implant (OES - Compudose 100, Elanco Animal Health, Indianapolis, IN, USA; 21.1 mg oestradiol-17 β), or a combination trenbolone acetate and oestradiol implant (TBA+OES - Component TE-200, Elanco Animal Health, Indianapolis, IN, USA; 200mg trenbolone acetate and 20mg oestradiol). Animals were fed a high energy grain diet as per the feedlots finishing regime for 73 days. Implant ear pathology audits and live weights were collected at day 33 and 68 after induction. Animal ethics approval was granted by the University of New England Animal Ethics Committee (authority number AEC14-045).

2.2 Slaughter and primal collection

At the completion of feeding, animals were transported 192 kilometres to a commercial abattoir and slaughtered the following morning. Carcasses were electrically stimulated and hot carcass weight and P8 fat depth recorded before carcasses were spray chilled. MSA graders assessed carcasses approximately 20 hours pm after quartering at the 12/13th rib. Grading measurements included ultimate pH (pHu), ossification score, marbling score, hump height, eye muscle area, meat colour, fat colour and ribfat depth (AUSMEAT, 2005). Directly after quartering a 3mm slice from the LD was collected from each carcass, diced and a 5g sub-sample was frozen in liquid nitrogen for calpastatin analysis.

At boning, rump primals from both sides and the striploin primal from the left side were collected from all 300 carcasses. These primals were vacuum packed and chilled prior to transport to the meat science laboratory at the University of New England.

2.3 Sample preparation

Primals were trimmed of all fat, epimysium and surrounding muscles. The trimmed LD were cut into four portions, whilst the left and right *m. gluteus medius* (GM) were each cut into two portions.

Samples for sensory and objective analyses were allocated to aging treatments by rotating samples on position within muscle. Sensory samples were prepared as described by Watson et al. (2008a), whereby each sample was cut perpendicular to the muscle fibre direction into five 25mm thick steaks. Objective LD and GM portions weighing approximately 250g were prepared. Both sensory and objective samples were aged in vacuum bags for 5 or 35 days pm at 4°C before being frozen and stored at -20°C.

2.4 Objective measurements

Shear force blocks were prepared using the methodology of Perry et al. (2001) with some modifications. Samples were thawed at 4°C for 12 hours prior to analysis. After thawing, a 60-80g block was cut (approximately 50mm x 50mm x 25mm in size) from each sample. Colour readings were taken using a Minolta Chroma Meter (D65) from a cut surface which had bloomed for at least 20 minutes. The CIE, L*, a*, b* dimensions were recorded as the average of three readings. Ultimate pH was again recorded in the laboratory.

Objective blocks were placed in unsealed vacuum bags and cooked in a water bath at 70°C for 30 minutes prior to cooling under running water for 20 minutes. Pre- and post-cook weights were used to calculate cooking loss as the % of change to the pre-cook weight. Shear force was calculated as the mean maximum force for six sub-samples cut perpendicular to the fibre direction.

Calpastatin activity was measured using the methodology of Shackelford et al. (1994) and Koochmaraie (1990) with slight modifications. Briefly, 4g of the sample collected at 20 hours pm was homogenised in 20ml of extraction buffer (100mM Tris, 5mM EDTA, 10mM mercaptoethanol, pH 8.3). Samples were spun for 15 min at 4000 x g prior to transferring 12 ml of supernatant into a 15ml tube, and heating in a water bath at 95°C for 15 min whilst gently rocking. Samples were then centrifuged for 15min at 4000 x g, and the supernatant filtered through glass wool. Samples were frozen at -20°C until analysis. An m-Calpain preparation needed to assess calpastatin activity was prepared as per Koochmaraie (1990).

Calpastatin samples were assayed against semi-purified m-calpain using casein as a substrate. The activity of m-calpain was expressed as the level of enzyme activity that increased an absorbance unit by 1.0 at 278nm in 60 mins at 25°C. Calpastatin, being the inhibitor of calpains was the inverse, expressed as the amount that inhibits 1.0 unit of m-calpain activity, expressed in units per gram of muscle.

2.5 Sensory analysis

Sensory analysis was conducted using the MSA sensory protocols described by Watson et al. (2008a). Briefly, a taste panel trial utilised 60 untrained consumers, in three sessions of 20. Each panellist was served a total of seven steaks, the first being a mid-range starter steak followed by six treatment steaks, one from each of six product groups defined by cut, treatment and days aged to establish a broad eating quality range. The five steaks from each sample were allocated to a different serving order and distributed across sessions. Product presentational order was controlled by a 6 x 6 Latin square to ensure all products were served equally across serving order, session and before and after other products. Steaks were cooked for a set time to achieve a medium doneness using a Silex™ grill. After cooking, the steaks were rested, halved and served with each sample being evaluated by ten consumers. Consumers rated each steak for four sensory attributes by marking a line on a 100mm line scale anchored by the words not tender/very tender for tenderness, not juicy/very juicy for juiciness, and dislike extremely/like extremely for both like flavour and overall satisfaction. Tenderness, juiciness, like flavour and overall acceptability scores were weighted by 0.3,

0.1, 0.3 and 0.3 respectively and summed to calculate a MQ4 score. From the ten sensory scores for each sample, the highest and lowest two scores were 'clipped' to reduce the standard error of the mean sensory score.

2.6 Statistical analysis

2.6.1 Ear pathology

When animals were weighed at 33 and 68 days, the ears of the animals in the OES and TBA+OES groups were palpated for the presence of the implant or infection. Over the 68 days of feeding, two animals had lost their implants, one by day 33 and one by day 68. At the 68 day weighing, a further two animals were noted to have extensive scar tissue which encapsulated the implant, presumably inhibiting the uptake of the active ingredients. These four animals were excluded from the analysis.

At day 68, a further 30 animals, all within the TBA+OES group, were recorded as having a small amount of fluid accumulation around the implant site which may have interfered with the release of the active ingredient. To assess whether this fluid accumulation inhibited the response to the TBA+OES implant on live weight, carcass and meat quality traits, analyses were run with models that included terms for treatment and sub-group nested within treatment for these variables (GLM, SAS version 9.0). For live weights at 33 and 68 days, hot carcass weight, carcass measurements, objective laboratory measurements and sensory scores at 5 and 35 days aged, the term for the sub-group nested within treatment did not approach significance ($P>0.05$). This indicated that for all traits the response of these 30 animals did not differ from the remaining 67 animals in the TBA+OES treatment group. On this basis data from these 30 animals which showed a small degree of fluid accumulation around the implant site were retained in the TBA+OES treatment.

2.6.2 Liveweight

Liveweights recorded at 33 and 68 days were analysed using a repeated measurements analysis (REM, SAS version 9.0) where the mean HGP treatment effect and interactions with time were tested. Induction weight was included as a covariate. The interaction between HGP treatment and induction weight was tested and found to be not significant for both the mean effect and the interaction with time ($P>0.05$).

2.6.3 Carcass measurements

AUSMeat meat colour scores 1B and 1C were transformed to a numerical scale of 1.3 and 1.7, respectively. Hot carcass weight was analysed in a model (GLM, SAS, version 9.0) which contained terms for HGP treatment. As carcass weight was generally correlated with carcass traits, (which included hump height, marbling score, ossification score, ribfat depth, P8 fat depth, pHu and meat colour scores) were examined in GLM models which contained terms for HGP treatment and hot carcass weight. For all carcass traits the interaction between HGP treatment and hot carcass weight was tested and found to be not significant ($P>0.05$).

2.6.4 Objective measurements

Shear force and cooking loss % were tested in batches of 5 to 33 samples, where each batch only contained samples within aging treatments. All batches were cooked for 30 minutes as per the above method, apart from one which was inadvertently cooked for approximately 50 instead of 30 minutes. Within LD and GM samples, shear force and cooking loss % were analysed using MIXED models (SAS, version 9.0) which contained terms for HGP treatment, aging, batch(days aged) and

position within muscle. All first order interactions were tested. Models contained a random term for animal nested within HGP treatment. For cooking loss %, but not for shear force, the days aged X position interaction was significant ($P<0.05$) for both the LD and GM samples. For shear force, the interaction between HGP treatment X days aged was significant ($P<0.05$) for LD, but not for GM ($P<0.05$). Given the importance of this question in the experimental design this interaction was included in shear force analyses for both LD and GM analyses.

As expected the L^* , a^* and b^* colour dimensions were highly correlated. To account for this the colour dimensions were analysed using a repeated measurements analyses which contained terms for HGP treatment, days aged and position within muscle. The repeated measurements analysis tested both mean of the colour dimensions and interactions between the different colour dimensions. First order interactions were tested but found to be not significant ($P>0.05$) and were not included in the final model.

2.6.5 Sensory measurements

Sensory scores from the LD and GM were analysed separately using a MIXED model which contained terms for HGP treatment, days aged and position. The interaction of HGP treatment X days aged was only significant ($P<0.05$) for tenderness and MQ4 scores in the LD. Again due to the importance of this question in the overall experimental design, this interaction was retained in both the LD and GM models.

2.6.6 Calpastatin activity

Calpastatin activity in LD samples collected at 20 hours pm was measured in ten batches. Each batch contained samples from each of the three HGP treatments. Calpastatin activity was analysed in a GLM model which contained terms for HGP treatment and calpastatin batch as independent variables.

3 Results

3.1 Live weights and carcass traits

Mean live weights and carcass traits are shown in Table 1. There was a large range in live weights at induction, day 33 and day 68. Mean induction weight was slightly less than a typical feedlot induction weight for an Australian trade steer.

Table 1. Means, variance and range for liveweight and carcass traits for the three treatment groups combined

Trait	Mean	s.d.	Min	Max
Liveweight				
Induction wt (kg)	317	19.4	273	385
Weight 33 days (kg)	391	25.6	276	458
Weight 68 days (kg)	476	33.2	367	570
Carcass traits				
Hot carcass wt (kg)	249	18.8	185	298
Hump height (mm)	91	11.0	60	125
Marble score	275	51.7	150	470
Ossification score	142	16.6	100	200
Ribfat (mm)	4.1	1.17	1	10
P8 (mm)	8.8	2.9	2	20
Ultimate pH	5.52	0.092	5.3	5.77
Eye muscle area (cm ²)	66.6	8.81	41	92
Meat colour score	2.5	0.72	1.7	6.0

Live weights at days 33 and 68 were adjusted to a mean induction weight of 317kg. The repeated measures analysis showed the interaction of treatment X time was significant ($P < 0.01$, Table 2) indicating that the differences in liveweights between treatments increased with longer time on feed. Animals in the OES and TBA+OES treatment groups were 9kg and 18kg heavier respectively than the CON group at day 33, compared with 15kg and 34kg at day 68 (Figure 1).

The range of liveweights within each of the treatments translated to a large range of carcass traits including hot carcass weight, marbling score, P8 fat depth and eye muscle area. There were three animals with an ultimate pH > 5.7 and 20 animals with an AUSMeat meat colour score greater than 3.

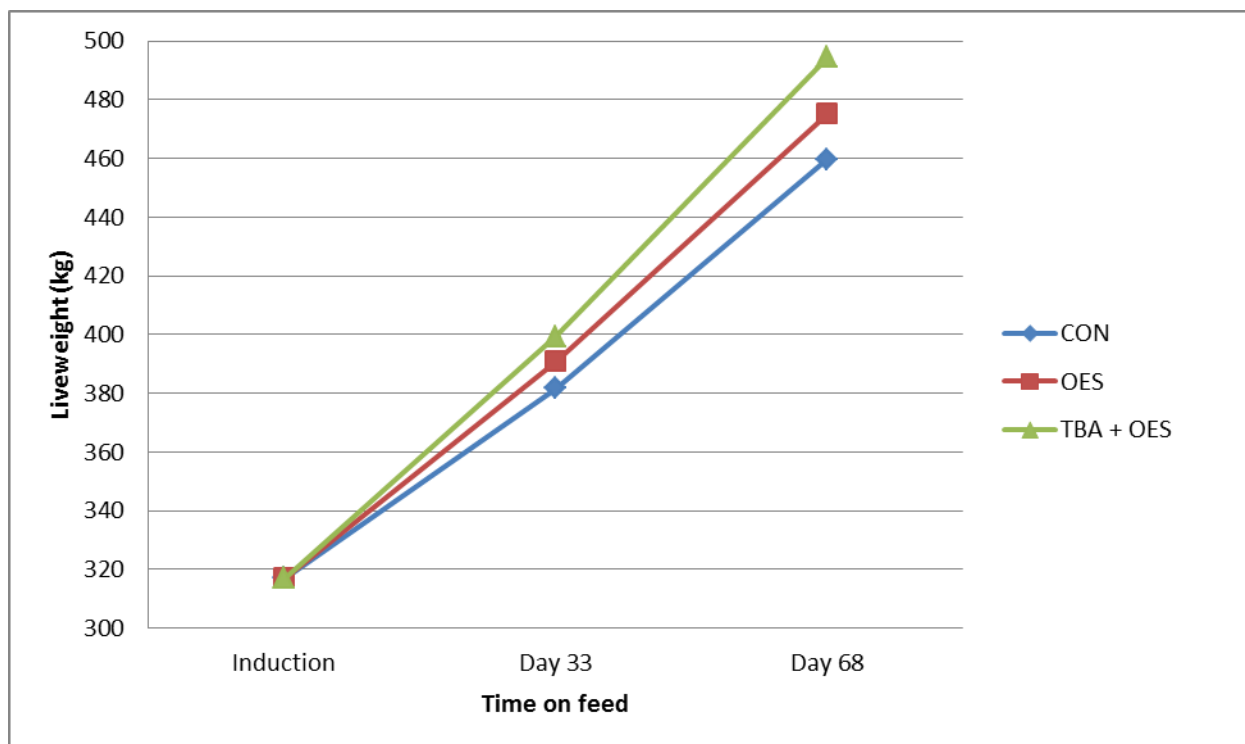


Figure 1. Predicted liveweights at 33 and 68 days after induction to the feedlot for steers from the CON, OES and TBA+OES implanted groups. All liveweights were adjusted to the same induction weight of 317kg.

Table 2. F ratios for the repeated measurements analysis of HGP treatment and induction weight for both mean effects and interactions with time on feed (liveweights at days 33 and 68)

Independent Variables	NDF,DDF	F ratio
Time on feed	1,292	8.15***
HGP Treatment		
Mean treatment	2,292	63.14***
Time x treatment	2,292	35.04***
Induction weight		
Mean induction weight	1,292	474.19***
Time x induction weight	1,292	11.67***

NDF, DDF – numerator degrees of freedom and denominator degrees of freedom

***, P<0.001

3.2 Treatment effects on carcass traits

HGP treatment had a significant effect ($P<0.05$) on hot carcass weight (Table 3). Relative to the CON group the OES treatment resulted in a 12kg increase in hot carcass weight, whilst the TBA+OES treatment resulted in a 24kg increase.

When carcass traits were adjusted to the same hot carcass weight there were significant treatment effects ($P<0.05$) on hump height, marble score, ossification score, P8 fat depth and eye muscle area. The TBA+OES treatment resulted in a 5mm increase in hump height relative to the CON group ($P<0.05$) with no significant difference in hump height between OES and CON ($P>0.05$). For ossification score, the OES treatment was 11 units higher than the CON group ($P<0.05$), whilst the TBA+OES treatment was 17 units higher than the CON group ($P<0.05$). The TBA+OES treatment resulted in a 22 unit decrease in marble score and a 0.6mm decrease in rib fat relative to the CON group ($P<0.05$). Although there were trends for lower marble score and rib fat in the OES group relative to the CON, these differences did not achieve significance ($P>0.05$). There was no significant ($P>0.05$) differences in meat colour scores between HGP treatments.

Table 3. Predicted means for the HGP treatments (CON, OES and TBA+OES), for carcass traits along with F ratios for the treatment effect and a covariate for hot carcass weight.

Carcass trait	Treatment				HGP Treatment		Covariate	
	CON	OES	TBA+OES	s.e.	NDF, DDF	F ratio	NDF, DDF	F ratio
Hot carcase weight (kg)	237 ^a	249 ^b	261 ^c	1.6	2,293	56.89***	-	-
Hump height (mm)	89 ^a	90 ^a	95 ^b	1.1	2,292	7.84**	1,292	31.54***
Marble score	287 ^a	272 ^{ab}	265 ^b	5.6	2,292	3.57*	1,292	15.21***
Ossification score	133 ^a	144 ^b	150 ^c	1.6	2,292	24.49***	1,292	8.80*
Ribfat (mm)	4.4 ^a	4.1 ^{ab}	3.8 ^b	0.13	2,292	4.21*	1,292	1.39
P8 (mm)	9	8.8	8.6	0.31	2,292	0.47	1,292	8.97**
pHu	5.52	5.53	5.52	0.010	2,292	0.23	1,292	1.46
Eye muscle area (cm ²)	64.6 ^a	66.7 ^{ab}	68.4 ^b	0.89	2,292	4.10*	1,292	24.62***
Meat colour score	2.5	2.5	2.4	0.08	2,292	0.53	1,292	0.27

NDF, DDF – numerator degrees of freedom and denominator degrees of freedom

*, $P<0.05$; **, $P<0.01$; ***, $P<0.001$.

Within rows, means with superscripts of different letters indicate a significant difference ($P<0.05$).

3.3 Objective measurements

There was a large range in both objective and sensory traits in the LD and GM cuts (Table 4). Ranges in L*, a*, b* colour dimensions were modest despite 20 animals having meat colour scores higher than 3 at MSA grading.

Table 4. Means for objective laboratory and sensory scores for the LD and GM cuts

Trait	<i>m. longissimus dorsi</i>				<i>m. gluteus medius</i>			
	Mean	s.d.	Min	Max	Mean	s.d.	Min	Max
Objective								
Shear Force	4.1	1.48	1.8	9.6	4.1	1.27	1.9	10.8
Cooking loss %	22.8	1.92	17.9	33.0	22.6	2.61	15.0	32.1
Colour								
L*	39.8	2.49	32.5	48.5	39.6	3.02	31.5	51.7
a*	20.2	1.61	15.3	26.3	22.5	2.12	16.1	29.4
b*	9.0	1.14	5.5	12.9	10.4	1.37	6.1	15.1
Sensory								
Tenderness	45.5	16.57	5	82	50.3	13.38	9	89
Juiciness	50.3	13.42	12	86	55.2	11.13	20	86
Like Flavour	51.0	11.83	19	85	55.6	9.98	17	84
Overall liking	49.4	13.96	13	85	53.8	11.32	14	86
MQ4	48.9	13.33	14	83	52.8	10.74	14	83

3.3.1 Shear force

The HGP treatment X days aged interaction was significant for shear force in the LD but not the GM ($P < 0.05$, Table 5). The interaction for the LD showed that the TBA+OES treatment had a higher shear force than the CON treatment at 5 days pm, but this was not significant ($P > 0.05$) at 35 days pm (Table 6). Position within cut had a significant ($P < 0.05$) effect for the LD, but not the GM, whereby samples at the posterior portions of the LD had higher shear force values than anterior portions.

Table 5. F ratios for the effect HGP treatment, days aged, treatment x days aged, position, and days aged x position on shear force and cooking loss % for LD and GM samples. Shear force and cooking loss % were also adjusted for cooking batch(days aged).

Trait	Shear force				Cooking loss %			
	<i>m. longissimus dorsi</i>		<i>m. gluteus medius</i>		<i>m. longissimus dorsi</i>		<i>m. gluteus medius</i>	
	NND,DDF	F ratio	NNF,DDF	F ratio	NND,DDF	F ratio	NNF,DDF	F ratio
Treatment (T)	2,289	4.94**	2,293	1.41	2,289	3.51*	2,293	3.83*
Days aged (DA)	1,254	404.54***	1,260	77.00***	1,256	1.75	1,261	17.66***
T*DA	2,254	6.30**	2,260	0.81				
Position	3,254	4.27*	1,260	0.02	3,256	30.63***	1,261	165.00***
DA*Position					3,256	3.86*	1,261	15.37***

NDF, DDF – numerator degrees of freedom and denominator degrees of freedom

*, P<0.05; **, P<0.01; ***, P<0.001

Table 6. Predicted means for shear force (kg) in the two muscles aged for 5 and 35 days along with predicted means for cooking loss percentage for samples from the two muscles. The model contained terms for HGP treatment, aging, batch(days aged) and position.

Trait	Muscle	Days Aged	Treatment			s.e.
			CON	OES	TBA+OES	
Shear Force (kg)	<i>m. longissimus dorsi</i>	5	4.4 ^a	4.6 ^{ab}	4.7 ^b	0.12
		35	3.4 ^a	3.5 ^a	3.5 ^a	0.15
	<i>m. gluteus medius</i>	5	4.4 ^a	4.5 ^a	4.7 ^b	0.11
		35	3.4 ^a	3.4 ^a	3.5 ^a	0.15
Cooking Loss %	<i>m. longissimus dorsi</i>		22.7 ^a	23.1 ^b	23.1 ^b	0.18
	<i>m. gluteus medius</i>		22.2 ^a	22.6 ^b	22.7 ^b	0.19

Within rows, means with superscripts of different letters indicate a significant difference (P<0.05).

3.3.2 Cooking loss

There was a treatment effect on cooking loss % for both the LD and GM, whereby cooking loss % increased slightly for TBA+OES and OES when compared to the CON (Table 5 & 6). The HGP treatment X days aged interaction was not significant (P>0.05) for either the LD or GM muscles, and therefore removed from the model. The position X days aged interaction was significant (P<0.05) for both muscles. Cooking loss % increased towards the anterior portion of the LD, which increased with days aged. The GM displayed a similar interaction whereby the tail portion had a higher cooking loss %, though the magnitude of the effect was reduced with days aged.

3.4 Sensory measurements

There was a significant interaction between HGP treatment X days aged for LD tenderness and MQ4 scores (P<0.05, Table 7). This interaction showed that the CON and OES treatments had significantly (P<0.05) higher sensory scores than the TBA+OES group at 5 days, although the magnitude of the effect was reduced at 35 days (Table 8). There were similar trends for LD juiciness, like flavour and overall liking, but these interactions did not achieve significance (P>0.05). The HGP treatment X days aged interaction was not significant for the GM sensory scores (Table 7).

There was a trend for OES sensory scores to be lower than CON scores, but this did not attain significance at 5 or 35 days for the LD, or 5 days for the GM (Tables 7 and 8, P>0.05). The HGP treatment effect was significant (P<0.05) for all sensory scores for the GM, whereby the TBA+OES treatment had lower sensory scores (P<0.05) than the CON group at both 5 and 35 days.

Position effects were significant for the LD whereby the anterior portions had higher sensory scores than the posterior portions. There was no significant (P>0.05) difference between the head and tail portions for the GM.

In the MSA model, HGP effects are adjusted for carcass traits (Watson et al., 2008c). As there was a significant effect of the HGP treatments on carcass traits, it was appropriate to test these after adjustment for carcass traits in the MSA model (hot carcass weight, hump height, ossification, marbling, ribfat and ultimate pH). For the four sensory and MQ4 scores and shear force scores, these adjustments for carcass traits had little effect on the significance and magnitude of the HGP treatment effects, and the means are not presented here.

Table 7. F ratios for the effect of the HGP treatments, days aged, treatments x days aged and position on sensory scores; tenderness, juiciness, like flavour, overall liking and the composite MQ4 scores for LD and GM samples.

	<i>m. longissimus dorsi</i>						<i>m. gluteus medius</i>					
	NDF, DDF	Tenderness	Juiciness	Like Flavour	Overall liking	MQ4	NDF, DDF	Tenderness	Juiciness	Like Flavour	Overall liking	MQ4
HGP Treatment (T)	2,291	14.36***	6.83**	14.19***	13.00***	13.92***	2,293	11.14***	7.75***	9.36***	9.91***	10.86***
Days Aged (DA)	1,288	709.40***	256.75***	343.29***	525.39***	574.60***	1,292	181.16***	64.65***	90.94***	115.18***	148.89***
T*DA	2,288	3.06*	1.68	2.38	2.87	3.60*	2,292	1.86	0.33	1.92	2.23	2.23
Position	3,288	17.91***	12.47***	6.61***	10.51***	13.27***	1,292	1.87	0.36	1.77	1.79	1.96

NDF, DDF – numerator degrees of freedom and denominator degrees of freedom

*, P<0.05; **, P<0.01; ***, P<0.001

Table 8. Predicted means for the effect of HGP treatment on sensory scores (tenderness, juiciness, flavour, overall liking and composite MQ4 scores). The model included terms for HGP treatment, days aged and position.

Muscle/sensory attribute	Aging							
	5 days aged				35 days aged			
	CON	OES	TBA+OES	s.e.	CON	OES	TBA+OES	s.e.
<i>m. longissimus dorsi</i>								
Tenderness	39.4 ^a	37.6 ^a	29.6 ^b	1.28	58.4 ^a	55.7 ^a	52.0 ^b	1.28
Juiciness	46.5 ^a	45.6 ^a	40.3 ^b	1.18	58.0 ^a	56.6 ^{ab}	54.5 ^b	1.18
Like Flavour	48.6 ^a	47.9 ^a	41.5 ^b	1.00	60.1 ^a	58.4 ^a	55.4 ^b	1.00
Overall liking	44.8 ^a	43.3 ^a	36.6 ^b	1.13	59.8 ^a	57.3 ^{ab}	54.4 ^b	1.13
MQ4	44.5 ^a	43.4 ^a	36.5 ^b	1.07	59.0 ^a	56.8 ^{ab}	53.9 ^b	1.07
<i>m. gluteus medius</i>								
Tenderness	48.1 ^a	45.8 ^a	41.0 ^b	1.22	59.2 ^a	54.3 ^b	53.0 ^b	1.22
Juiciness	54.2 ^a	52.8 ^a	49.7 ^b	1.08	60.7 ^a	57.9 ^{ab}	55.6 ^b	1.08
Like Flavour	54.6 ^a	53.4 ^a	49.9 ^b	0.93	61.4 ^a	57.7 ^b	56.9 ^b	0.93
Overall liking	52.4 ^a	51.1 ^a	46.6 ^b	1.06	60.6 ^a	56.7 ^b	55.6 ^b	1.06
MQ4	51.8 ^a	50.2 ^a	46.2 ^b	0.99	60.3 ^a	56.1 ^b	55.1 ^b	0.99

Within rows and aging period, HGP treatment means with superscripts of different letters indicate a significant difference ($P < 0.05$).

3.5 Colour

A significant ($P < 0.05$) interaction was present between HGP treatment X L^* , a^* , b^* colour dimension for the GM, but not the LD (Table 9). The predicted means in Table 10 showed that this interaction between treatment and colour dimensions in the GM was due to lower ($P < 0.05$) a^* and b^* dimensions and a trend for higher L^* values in the TBA+OES treatment relative to the CON and OES treatments. Overall these changes tended to cancel one another so there was no overall treatment effect on colour dimensions in the GM (Table 9). For the LD there was a trend for the OES treatment to have higher L^* , a^* and b^* dimensions compared with the CON and TBA+OES treatments, hence the mean treatment effect was significant for the LD (Table 9).

Table 9. F ratios for the repeated measurements analysis of HGP treatment (CON, OES and TBA+OES), days aged and position on CIE colour dimension (average effect of L^* , a^* , b^*).

Independent Variables	<i>m. longissimus dorsi</i>		<i>m. gluteus medius</i>	
	NDF,DDF	F ratio	NDF,DDF	F ratio
Colour dimension	2,1160	74704.8***	2,1176	72969.1***
Mean HGP Treatment	2,580	7.27***	2,588	0.70
HGP Treatment * colour dimension	4,1160	0.96	4,1176	7.33***
Mean days aged	1,580	112.74***	1,580	0.00
Days aged * colour dimension	2,1160	81.67***	2,1176	41.24***
Mean position effect	3,580	15.52***	1,588	252.88***
Position * colour dimension	6,1160	1.90	2,1176	110.95***

NDF, DDF – numerator degrees of freedom and denominator degrees of freedom

*, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$

The interaction between days aged X colour dimension was significant for both the LD and GM (Table 9). Although the predicted means were not tabulated, the LD showed an increase in L^* , a^* and b^* dimensions as samples aged from 5 to 35 days. In contrast the GM showed an increase in the L^* which was offset by a small decrease in the a^* and b^* values. Hence, whilst the mean change in colour was significant ($P < 0.05$) for the LD, it was not significant ($P > 0.05$) for the GM.

The position X colour dimension interaction was significant ($P < 0.05$) for the GM, but not the LD, whereby GM samples had lower L^* , a^* and b^* dimensions towards the tail of the GM. Position had an overall effect on colour dimensions ($P < 0.05$, Table 9) in both the LD and GM. Although not tabulated, the posterior portion of the LD had higher L^* , a^* and b^* dimensions, whereas for the GM there was an overall effect in that the tail portion had lower L^* , a^* and b^* dimensions, although the effect was greater in the L^* and a^* relative to the b^* dimension.

Table 10. Predicted means for HGP treatment effects on L*, a*, b* colour dimensions after adjustment for days aged and position within the LD and GM muscles.

Trait	Treatment			Stderr
	CON	OES	TBA+OES	
<i>m. longissimus dorsi</i>				
L*	39.7 ^a	40.0 ^a	39.7 ^a	0.16
a*	20.3 ^a	20.5 ^a	19.9 ^b	0.11
b*	9.1 ^a	9.3 ^a	8.8 ^b	0.08
<i>m. gluteus medius</i>				
L*	39.5 ^a	39.7 ^a	39.9 ^a	0.18
a*	22.7 ^a	22.6 ^a	22.1 ^b	0.13
b*	10.5 ^a	10.5 ^a	10.2 ^b	0.09

Within rows, means with superscripts of different letters indicate a significant difference (P<0.05).

3.6 Calpastatin activity

The effects of treatment and calpastatin batch were examined on calpastatin activity 20 hours pm (Table 11). HGP treatment had a significant effect (P<0.05), with the TBA+OES treatment having a higher calpastatin activity than the OES and CON treatments. As expected, calpastatin batch was also significant (P<0.05).

Table 11. The effect of HGP treatment and batch on calpastatin activity from LD sampled at 20 hours pm along with predicted means for the different HGP treatments.

Trait	NDF,DDF	F ratio	Predicted means (units of activity/g of muscle)			
			CON	OES	TBA+OES	se
HGP Treatment	2,276	9.33***	3.01 ^a	3.07 ^a	3.17 ^b	0.027
Calpastatin batch	9,276	32.81***				
Coefficient of Determination		0.532				

Within rows, means with superscripts of different letters indicate a significant difference (P<0.05).

***, P<0.001

Having calpastatin activity at 20 hours pm on LD samples, offered the prospect of quantifying the contribution of calpastatin activity to the magnitude of the HGP treatment effect for objective and sensory measurements measured at 5 day pm. This was established by analysing LD shear force at 5 days pm in a model which contained terms for HGP treatment, position and cooking batch. Calpastatin activity and calpastatin batch was then included in this model, which resulted in a decrease in the F ratio for HGP treatment from 6.37 to 3.28. This indicated that for shear force at 5 days, 49% of the variance in the HGP treatment effect was associated with calpastatin activity in the LD muscle at 20 hours pm.

Similar analyses were also performed using calpastatin activity at 20 hours pm and sensory traits for LD at 5 days pm. The initial models included HGP treatment and position as independent variables,

with tenderness, juiciness, like flavour, overall acceptability and MQ4 scores as dependent variables. When calpastatin activity and calpastatin batch were included in the models, the F ratios decreased, indicating that for tenderness, juiciness, like flavour, overall liking and MQ4 scores at 5 days pm, calpastatin activity at 20 hours pm accounted for 37, 42, 32, 37 and 36% of the HGP treatment effects, respectively.

4 Discussion

The use of an OES HGP implant had less impact on sensory scores and shear force scores compared to a combination TBA + OES implant when used in steers finished in a feedlot for 73 days. The magnitude of this TBA+OES treatment effect reflects the current adjustment of up to six MQ4 units for the striploin (Watson et al., 2008c) and reported effects of a slightly weaker (140mg trenbolone acetate + 28 mg oestradiol 17 β) combination implant (Thompson et al., 2008a), or multiple implant treatments (Watson et al., 2008b). Whilst there was a trend for the OES treatment to increase shear force and decrease sensory scores relative to the CON treatment, these effects failed to reach significance, apart from 35 days aged tenderness, like flavour, overall liking and MQ4 for the GM. The proportional increase in shear force with liveweight performance of the implant (CON<OES<TBA+OES) agreed with the reviews by Tatum (2009) and Morgan (1997). Conversely, Burnham et al. (1997) and Foutz et al. (1997) reported significant effects of oestrogen implants on shear force when compared to controls. As the correction for MSA traits resulted in minimal change for all means and did not change the levels of significance between treatment groups for shear force and sensory scores, this reconfirms an impact of HGP treatments over and above ossification and marbling as reported by Watson et al. (2008b).

The HGP treatments impacted the cut with a higher aging potential, that is the LD, more than the GM, agreeing with Thompson et al. (2008a) and Watson et al. (2008b). For the LD there was an 8.0 point difference in MQ4 scores between CON and TBA+OES treatments at 5 days pm, which decreased to 5.1 points at 35 days pm. For the GM the difference between CON and TBA+OES treatments was 5.6 points at 5 days, which only decreased to 5.1 points at 35 days pm, although a HGP treatment x cut interaction was not significant. Ouali et al. (1988) found a larger HGP impact on tenderness scores for the LD when compared to the *m. triceps brachii*, though the HGP treatment X cut interaction was not significant.

The reduction of the impact of the TBA+OES treatment on eating quality measurements through aging agreed with the results of Tatum (2009). Thompson et al. (2008a) reported a HGP treatment x aging interaction for shear force but not for sensory scores, though differences in sensory scores between HGP treated carcasses and controls halved between 5 days and 21 days aging for the LD. Schneider et al. (2007) also reported a HGP treatment X aging interaction for the LD, whereby the HGP effect significantly decreased shear force scores between 3 and 28 days of aging. The reported interaction allows the possibility for the post slaughter supply chain to minimise the magnitude of the TBA+OES effect on cuts through aging. The increase in sensory scores between 5 and 35 days pm for the OES treatment was less than the CON and TBA+OES treatments for both the LD and GM samples. However, this needs to be viewed with caution in regards to aging, as much proteolysis may have occurred prior 5 days pm.

The calpain/calpastatin system plays an important role in protein degradation in a live animal muscle, which in turn regulates the net rate of protein accretion. More importantly in respect to meat quality, this enzymatic system is involved in degradation of key myofibrillar structures pm (Goll et al., 1992). The calpain system is a group of enzymes amongst which are μ -calpain, m-calpain involved in proteolysis and the inhibitor of these enzymes, calpastatin. μ -calpain is the predominant

calpain involved in pm proteolysis, while m-calpain has been shown to have minimal impact on the degradation of myofibrillar proteins (Geesink et al., 2006; Koohmaraie & Geesink, 2006). The rate and extent of proteolysis pm is dependent on the ratio and concentration of μ -calpain and calpastatin, and can vary between muscles (Ouali & Talmant, 1990). Other factors such as genetics can influence the level of calpastatin, whereby as the percentage of *Bos indicus* increases, so too does the level of calpastatin activity (Shackelford et al., 1991).

A definitive mode of action of HGP implants is still not fully understood because of the many hormonal and metabolic pathways they influence. Nonetheless, both androgens and estrogens have direct and indirect effects that aid protein accretion, and when used together tend to have an additive effect via targeting different receptors. Protein accretion in skeletal muscle occurs by altering the ratio of protein synthesis and degradation (Goll et al., 1992; Koohmaraie et al., 2002) represented in a HGP treated animal through initially hypertrophy, followed by hyperplasia or increased satellite cell number after extended exposure (Anderson, 1991; Anderson et al., 2014; Dayton & White, 2013; Johnson et al., 1998; Johnson & Reinhardt, 2008). A decrease in nitrogen loss from HGP treated animals indicates a decrease in protein degradation in the live animal (Bouffault & Willemart, 1983).

Thompson et al. (2008a) hypothesised that HGP implants had an impact on the calpain to calpastatin ratio in a live animal, which decreased the rate of proteolysis pm. The results of this study support this theory by demonstrating that animals treated with TBA + OES implants have an increased level of calpastatin which would have resulted in less pm proteolysis and therefore tougher meat. Whilst the OES group had a numerically higher calpastatin level than the CON group, the difference was much smaller. Gerken et al. (1995) reported increased calpastatin levels in implanted clone steers. Steers treated with both estrogen implants and combination trenbolone acetate and estrogen implants had higher levels than the control group. The group implanted with trenbolone acetate (no estrogen included) alone had higher calpastatin levels than the control, but the difference did not achieve significance. These results suggest that both estrogens and androgen have an impact on calpastatin levels, and therefore impacting protein degradation rates in the live animal and subsequently aging rates and tenderness in the pm carcass.

The reported increased levels of calpastatin activity partly explain the HGP mode of action and the subsequent HGP treatment effect on sensory scores and shear force. This mode of action is supported by the authors mentioned (Dayton & White, 2013; Koohmaraie et al., 2002), as higher protein accretion is partly due to slowing down of protein degradation in the live animal. Calpastatin levels at approximately 20 hours pm accounted for approximately half of the HGP treatment effect for shear force at 5 days pm, but less so for sensory scores.

Our results align with the abundance of literature regarding the impacts of HGP implants on liveweight and carcass performance, and the flow on effects on carcass traits which influence eating quality (Duckett & Pratt, 2014; Hunter, 2009; Preston, 1999; Reinhardt & Wagner, 2014). The additive effect of an androgen with estrogen in the TBA+OES treatment seemed to translate into approximately twice the impact on liveweight, carcass weight, along with increases in hump height and ossification score and a decrease in marbling score when compared to the OES treatment.

The decrease in marbling and fat measurements for the HGP treatments, agrees with others (Duckett et al., 1997; Morgan, 1997; Thompson et al., 2008b; Watson et al., 2008b). Duckett et al. (1999) concluded that the reduction in marbling in HGP treated animals is most likely due to a dilution effect, rather than a significant reduction in the intramuscular fat levels, by diverting available nutrient pool towards protein deposition rather than adipose tissue. The HGP treatment effect on ribfat was no longer significant once corrected for carcass weight, indicating that animals

had a similar volume of fat around the rib area, just differing in overall muscle and carcass weight, again supporting an overall dilution effect. Whilst the impacts on marbling and ribfat reached significance for TBA+OES when compared to CON, the decreases were numerically only minor.

The HGP impact on ossification was greater for the TBA+OES treatment than OES treatment. These results are in accordance with the well-documented effect of HGP implants on ossification which can further increase with the addition of trenbolone acetate to oestradiol, and more so with multiple implants (Apple et al., 1991; Morgan, 1997; Platter et al., 2003; Thompson et al., 2008b). Watson et al. (2008b) and Thompson et al. (2008a) both reported a higher HGP treatment effect on ossification scores in steers than in heifers.

Interestingly, the TBA+OES treatment increased hump height. Entire *Bos Indicus* bulls have been shown to have a greater hump height than steers, a trait that can partly be considered a secondary sex characteristic (Fitzpatrick et al., 2013). Therefore, it appears likely that the androgenic effect of trenbolone acetate increased hump height. This finding was in agreement with Apple et al. (1991), who reported higher masculinity scores in animals treated with combination trenbolone acetate and oestradiol implants when compared to estrogen implants and controls. The MSA model within Australia uses hump height as an indicator of *Bos Indicus* content. Our results demonstrate that this could be somewhat confounded by the use of TBA+OES implants.

The net effect of both HGP treatments showed an increase in liveweight and carcass weight, and had negative impacts on carcass traits associated with eating quality, along with higher shear force scores and lower sensory scores. The magnitude of these effects were different for OES and TBA+OES whereby the latter had the larger impact on eating quality and carcass measurements, such as marbling, ossification, and an increase in hump height. The economic returns from the three treatment groups studied will vary between individual production systems, whereby liveweight and carcass weight performance would need to be balanced with eating quality. The MSA model underpins many of payment systems by processors in Australia, and has one HGP adjustment regardless of type of implant. The results of the study indicate producers who use OES treatments will result in higher MQ4 scores than TBA+OES treatments regardless of the HGP adjustment, as they had less impact on fat measurements, ossification and hump height. Further analysis of the actual and predicted MQ4 scores would need to be conducted to identify if OES and TBA+OES require different HGP adjustments in the MSA model.

This study examined the effect of two HGP formulations on growth carcass and eating quality traits in steers finished in a feedlot. Other questions need to be addressed including the period of implant payout and concentration, the effect of multiple oestradiol only implants, finishing environment, and *Bos indicus* content. Some of these points have been addressed by other researchers. Thompson et al. (2008b) demonstrated that the number of oestradiol only implants from one to eight over an animal's life did not impact sensory scores, when a new 100 day implant was inserted every 100 days. These results need to be viewed with caution as old implants were removed prior to the administration of a new implant. Hunter et al. (2000) reported no significant difference between shear force scores of steers treated with one 400 day oestradiol implant as opposed to four 100 day implants. The important *Bos indicus* X HGP interaction reported by Thompson et al. (2008b) needs further work to assess the repeatability of this finding. The reported synergy between *Bos indicus* content and oestradiol only implants is very important as oestradiol implants are commonly used in cattle in northern Australia which have a higher percentage of *Bos indicus* content.

Finishing environment may exacerbate the impacts of oestradiol only implants. Thompson et al. (2008b) reported an increase of up to eight MQ4 points in cattle finished on grain rather than on pasture, though did not report on any finishing regime X HGP treatment interaction. Further work is

underway to establish the effects of an oestradiol only long acting implant in cattle finished under pasture conditions.

Beyond these studies, there are other registered formulations of HGP implants which also do not contain trenbolone acetate, used in both pasture and feedlot environments. These implants contain the active ingredients oestradiol benzoate plus progesterone (for steers) or oestradiol benzoate plus testosterone (for heifers). Further work is required to establish the impact these implants have on eating quality for both steers and heifers.

5 Conclusion

This study clearly showed oestradiol only implants have less impact on meat eating quality measurements than a combination trenbolone acetate and oestradiol implant when used in short fed feedlot finished steers. The HGP impact was more pronounced in cuts which had the greatest aging potential via an increase in calpastatin concentration which in turn decreased proteolysis pm. The negative HGP impact of TBA+OES implants on eating quality was reduced through aging, which has important implications for the processing sector.

6 Impact on Meat and Livestock Industry

As discussed, further work is currently underway to establish the impact of oestradiol only implants when steers are finished on pasture for approximately 400 days. Upon completion of this project, all results can be evaluated collectively. As there is no differentiation in the MSA model between grain or grass finished animals, any modification to the HGP adjustment would have to account for both finishing systems. Furthermore, the residuals of the predicted MQ4 scores and the actual MQ4 scores from these projects will be assessed to quantify the accuracy of the current model. The full economic advantage of this overall project will not be able to be established until a decision is made by the relevant review committees as to whether, if any, MSA model adjustments are required.

Despite this, producers and the wider industry stand to benefit immediately from this project through the knowledge that oestradiol only implants when used in feedlot finishing conditions have less impact on MSA carcass measurements such as ribfat, marbling and ossification, when compared to combination trenbolone acetate and oestradiol implants, and therefore resulting in a higher MSA Index. Similarly, the use of oestradiol only implants may have an economic advantage over un-implanted animals through earlier turn-off times and/or increased carcass weight. Each production system differs in factors such as cattle type and rations, therefore each scenario would have to be analysed for economic return on an individual market basis.

Once residual analyses are conducted for predicted and actual MQ4 scores, these too can be presented as justification for possible modifications to the MSA model regarding the aging potential of TBA+OES treated cuts. Based on the data from this project, TBA+OES treated cuts improve significantly through aging. If a change was justified, there are significant benefits to the processing sector in reduced chilling times to achieve a higher eating quality of cuts from a TBA+OES treated carcasses.

7 Recommendations

Without the pasture project data, and the residual analyses for predicted and actual MQ4 scores, definitive recommendations for any MSA model modifications cannot yet be established. Despite this, the data from this project has demonstrated that there are differences between implants and therefore there may be justification in differentiating HGP implants in the MSA model.

If there were a change to the MSA model to differentiate implants, identification, auditing and producer declaration could be through:

- Ear palpation – oestradiol only implants have a silicone rubber core which remains after the payout period. Combination trenbolone acetate and oestradiol implants are compressed powder pellets which dissolve when they come into contact with blood flow. These combination implants have a leading ball bearing which remains after the pellets dissolve for detection and differentiation from oestradiol only implants.
- Trenbolone metabolites – As trenbolone acetate is a synthetic molecule, the metabolites can be detected in liver samples. Some processors are familiar with and already carrying out this testing.
- MSA Statutory declaration - an additional question could be added to the MSA statutory declaration following the HGP question to differentiate the type of implant; “oestradiol only” or “other”

Furthermore, the improvement in eating quality of TBA+OES treated cuts through aging based on the data from this project could also justify a modification in the MSA model. Again, this would have to be viewed collectively with the data from the pasture project and residuals of predicted and actual MQ4 scores.

8 Bibliography

- Anderson, P.T. 1991. Trenbolone Acetate as a Growth Promotant. *Compendium on Continuing Education for the Practicing Veterinarian* **13**(7), 1179-1190.
- Anderson, P.T., Johnson, B.J., Dikeman, M. 2014. Metabolic Modifiers. in: *Encyclopedia of Meat Sciences*, Vol. 2, Elsevier, pp. 62-69.
- Apple, J.K., Dikeman, M.E., Simms, D.D., Kuhl, G. 1991. Effects of synthetic hormone implants, singularly or in combinations, on performance, carcass traits, and longissimus muscle palatability of Holstein steers. *J Anim Sci*, **69**(11), 4437-48.
- AUSMEAT. 2005. *Handbook of Australian Meat - 7th Edition*. AUSMEAT Limited.
- Bouffault, J.C., Willemart, J.P. 1983. Anabolic activity of trenbolone acetate alone or in association with estrogens. in: *Anabolics in Animal Production - Public health aspects, analytical methods and regulation*. Office International des Epizooties, Paris, France, pp. 156-179.
- Burnham, D.L., Morris, S.T., Purchas, R.W., McCutcheon, S.N. 1997. Effects of Compudose® and Rumensin®, alone or in combination, on the growth, and carcass and meat quality of steers finished on pasture. *New Zealand Journal of Agricultural Research*, **40**(2), 231-238.
- Capper, J.L., Hayes, D.J. 2012. The environmental and economic impact of removing growth-enhancing technologies from U.S. beef production. *Journal of Animal Science*, **90**(10), 3527-37.
- Davies, B.L. 2008. Economic evaluation of hormonal growth promotants (HGPs) Meat and Livestock Australia.
- Dayton, W.R., White, M.E. 2013. Mechanisms of Anabolic Steroid Action in Bovine Skeletal Muscle. in: *Evaluating Veterinary Pharmaceutical Behavior in the Environment*, Vol. 1126, American Chemical Society, pp. 1-12.
- Dikeman, M.E. 2007. Effects of metabolic modifiers on carcass traits and meat quality. *Meat Science*, **77**(1), 121-135.
- Duckett, S.K., Owens, F.N., Andrae, J.G. 1997. Effects of implants on performance and carcass traits of feedlot steers and heifers. in: *1997 Oklahoma State University Implant Symposium*. Oklahoma, USA, pp. 63-82.
- Duckett, S.K., Pratt, S.L. 2014. Meat Science and Muscle biology symposium - Anabolic Implants and meat quality¹. *Journal of Animal Science*, **92**, 3-9.
- Duckett, S.K., Wagner, D.G., Owens, F.N., Dolezal, H.G., Gill, D.R. 1999. Effect of anabolic implants on beef intramuscular lipid content. *Journal of Animal Science*, **77**(5), 1100-4.
- Fitzpatrick, L.A., Parker, A.J., Zerby, H.N. 2013. Meat quality of grain finished entire male Bos indicus cattle. *Northern Beef Research Update Conference*, Cairns. pp. 35-42.
- Foutz, C.P., Dolezal, H.G., Gardner, T.L., Gill, D.R., Hensley, J.L., Morgan, J.B. 1997. Anabolic implant effects on steer performance, carcass traits, subprimal yields, and longissimus muscle properties. *Journal of Animal Science*, **75**(5), 1256-1265.
- Geesink, G.H., Kuchay, S., Chishti, A.H., Koohmaraie, M. 2006. μ -Calpain is essential for postmortem proteolysis of muscle proteins. *Journal of Animal Science*, **84**(10), 2834-40.
- Gerken, C.L., Tatum, J.D., Morgan, J.B., Smith, G.C. 1995. Use of genetically identical (clone) steers to determine the effects of estrogenic and androgenic implants on beef quality and palatability characteristics. *Journal of Animal Science*, **73**(11), 3317-24.
- Goll, D.E., Thompson, V.F., Taylor, R.G., Christiansen, J.A. 1992. Role of the calpain system in muscle growth. *Biochimie*, **74**(3), 225-237.
- Hunter, R.A. 2009. Hormonal Growth Promotant (HGP) use in the Australian Beef Industry. Meat and Livestock Australia. 9781741913415.
- Hunter, R.A., Magner, T., Allingham, P.G. 2000. Sustained growth promotion, carcass characteristics, and meat quality of steers treated with oestradiol-17b. *Australian Journal of Agricultural Research*, **51**, 133-138.

- Johnson, B.J., Halstead, N., White, M.E., Hathaway, M.R., DiCostanzo, A., Dayton, W.R. 1998. Activation state of muscle satellite cells isolated from steers implanted with a combined trenbolone acetate and estradiol implant. *Journal of Animal Science*, **76**(11), 2779-86.
- Johnson, B.J., Reinhardt, C.D. 2008. Growth Promotants for Beef Production: Anabolic Steroids: Performance Responses and Mode of Action. Fifth Edition ed. in: *Food Animal Practice* (Eds.) D.E. Anderson, M. Rings, Elsevier, pp. 643-651.
- Koohmaraie, M. 1990. Quantification of Ca²⁺-dependent protease activities by hydrophobic and ion-exchange chromatography. *Journal of Animal Science*, **68**(3), 659-65.
- Koohmaraie, M., Geesink, G.H. 2006. Contribution of postmortem muscle biochemistry to the delivery of consistent meat quality with particular focus on the calpain system. *Meat Science*, **74**(1), 34-43.
- Koohmaraie, M., Kent, M.P., Shackelford, S.D., Veiseth, E., Wheeler, T.L. 2002. Meat tenderness and muscle growth: is there any relationship? *Meat Science*, **62**, 345-352.
- McCraib, G.J., Hunter, R.A. 2002. Lifetime methane production is reduced when beef cattle are repeatedly treated with an hormonal growth promotant. *Proceedings of Australian Society of Animal Production*. pp. 327.
- Morgan, B. 1997. Implant program effects on USDA beef carcass quality grade traits and meat tenderness. in: *1997 Oklahoma State University Implant Symposium* Oklahoma, USA.
- Nichols, W.T., Gaylean, M.L., Thomson, D.U., Hutcheson, J.P. 2002. Effects of Steroid Implants on the Tenderness of Beef. *The Professional Animal Scientist*, **18** 202-210.
- Ouali, A., Talmant, A. 1990. Calpains and calpastatin distribution in bovine, porcine and ovine skeletal muscles. *Meat Science*, **28**(4), 331-348.
- Ouali, A., Zabari, M., Renou, J.P., Touraille, C., Kopp, J., Bonnet, M., Valin, C. 1988. Anabolic agents in beef production: Effects on muscle traits and meat quality. *Meat Science*, **24**(3), 151-161.
- Perry, D., Shorthose, W.R., Ferguson, D.M., Thompson, J.M. 2001. Methods used in the CRC program for the determination of carcass yield and beef quality. *Australian Journal of Experimental Agriculture*, **41**(7), 953-957.
- Platter, W.J., Tatum, J.D., Belk, K.E., Scanga, J.A., Smith, G.C. 2003. Effects of repetitive use of hormonal implants on beef carcass quality, tenderness, and consumer ratings of beef palatability. *Journal of Animal Science*, **81**, 984-996.
- Polkinghorne, R. 2015. Meat Standards Australia - consumer samples assessed ed, (Ed.) D.T. Packer.
- Polkinghorne, R., Thompson, J.M., Watson, R., Gee, A., Porter, M. 2008. Evolution of the Meat Standards Australia (MSA) beef grading system. *Australian Journal of Experimental Agriculture*, **48**(11), 1351-1359.
- Preston, R.L. 1999. Hormone containing growth promoting implants in farmed livestock. *Advanced Drug Delivery Reviews*, **38**, 123-138.
- Reinhardt, C.D., Wagner, J.J. 2014. High-dose anabolic implants are not all the same for growth and carcass traits of feedlot steers: A meta-analysis. *Journal of Animal Science*, **92**(10), 4711-8.
- Schneider, B.A., Tatum, J.D., Engle, T.E., Bryant, T.C. 2007. Effects of heifer finishing implants on beef carcass traits and longissimus tenderness. *Journal of Animal Science*, **85**(8), 2019-2030.
- Shackelford, S.D., Koohmaraie, M., Cundiff, L.V., Gregory, K.E., Rohrer, G.A., Savell, J.W. 1994. Heritabilities and phenotypic and genetic correlations for bovine postrigor calpastatin activity, intramuscular fat content, Warner-Bratzler shear force, retail product yield, and growth rate. *Journal of Animal Science*, **72**(4), 857-863.
- Shackelford, S.D., Koohmaraie, M., Miller, M.F., Crouse, J.D., Reagan, J.O. 1991. An evaluation of tenderness of the longissimus muscle of Angus by Hereford versus Brahman crossbred heifers. *Journal of Animal Science*, **69**(1), 171-177.
- Tatum, J.D. 2009. Growth Technologies: Performance benefits and quality considerations. *Meat Science and Muscle Biology Symposium: Balancing Live Cattle Performance and Beef Quality, Joint ADSA-CSAS-ASAS Annual Meeting*, Montreal, Quebec, Canada. pp. 1-30.

- Thompson, J.M., McIntyre, B.M., Tudor, G.D., Pethick, D.W., Polkinghorne, R., Watson, R. 2008a. 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 Journal of Experimental Agriculture*, **48**(11), 1405-1414.
- Thompson, J.M., Polkinghorne, R., Porter, M., Burrow, H.M., Hunter, R.A., McCrabb, G.J., Watson, R. 2008b. Effect of repeated implants of oestradiol-17 β on beef palatability in Brahman and Brahm cross steers finished to different market end points. *Australian Journal of Experimental Agriculture*, **48**(11), 1434-1441.
- Watson, R. 2008. Meta-analysis of the published effects of HGP use on beef palatability in steers as measured by objective and sensory testing. *Australian Journal of Experimental Agriculture*, **48**(11), 1425-1433.
- Watson, R., Gee, A., Polkinghorne, R., Porter, M. 2008a. Consumer assessment of eating quality – development of protocols for Meat Standards Australia (MSA) testing, Accesory Publication - MSA sensory testing protocols. *Australian Journal of Experimental Agriculture*, **48**(11), 1360-1367 (online version).
- Watson, R., Polkinghorne, R., Gee, A., Porter, M., Thompson, J.M., Ferguson, D., Pethick, D., McIntyre, B. 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 Journal of Experimental Agriculture*, **48**(11), 1415-1424.
- Watson, R., Polkinghorne, R., Thompson, J.M. 2008c. Development of the Meat Standards Australia (MSA) prediction model for beef palatability. *Australian Journal of Experimental Agriculture*, **48**(11), 1368-1379.