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Prepared by: Packer, D.T.[1]
Geesink, G.H.[1]
Polkinghorne, R.[2]
Thompson, J.M.[1]
Ball, A.J.[3]
[1] University of New England, Armidale, NSW, 2350
[2] Birkenwood Pty Ltd, Murrurundi, NSW 2338
[3] Herefords Australia, Armidale, NSW, 2350

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The impact of oestradiol only hormone growth promotants (HGPs) on eating quality of *pasture* finished steers

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Abstract

A total of 200 *Bos indicus/Bos taurus* previously un-implanted steers were randomly allocated to two treatment groups; control (CON) or a 400 day oestradiol only (OES) HGP implant, and finished on pasture for 389 days to assess the impact of the OES implant on sensory and objective measurements of the *mm. longissimus thoracis et lumborum* (LTL) and *gluteus medius* (GM). The HGP treatment had a significant impact on live weight, carcass weight and ossification ($P<0.05$). There was a trend to decrease marbling scores, and increase ribfat, P8 fat depth and hump height. The HGP treatment interacted with days aged for all sensory scores in the LTL, apart from juiciness ($P<0.01$) whereby OES sensory scores for the LTL and GM were significantly lower than the CON group at 5 days, though the magnitude halved in the LTL after 35 days of aging. There was a trend for a days aged X HGP interaction in the GM for shear force ($P=0.057$), but not in the LTL ($P>0.05$). Shear force scores were significantly higher for the OES treatment in the LTL at 5 days ($P<0.05$), though reduced through aging. The HGP treatment significantly increased cooking loss in the LTL ($P<0.05$) but only a trend was present in the GM. The OES treatment significantly reduced L*, a* and b* colour dimensions for the LTL ($P<0.05$) but not the GM ($P>0.05$). Calpastatin activity was significantly greater for the OES treatment, which may aid in explaining part of the large impact on sensory and shear force scores at 5 days' post mortem.

Executive summary

Oestradiol only (OES) long acting Hormonal Growth Promotants (HGPs) reduced sensory scores and increased shear force when *Bos indicus/Bos taurus* composite steers were finished on pasture for ca. 400 days. The magnitude of this impact is comparable to other Australian HGP research whereby a combination trenbolone acetate and oestradiol (TBA+OES) implant was used at least once during the trial period. The negative impact of the OES implant was reduced through aging. This has implications for the northern Australian beef industry as long acting OES HGPs are commonly used in animals with a high percentage *Bos indicus* to increase productivity on pasture, whereby the extensive geography restricts the ability to re-muster for repeated HGP implantation.

HGP implants have been used in Australian extensive beef pasture systems and feedlots to improve productivity and profitability for over 30 years. Average daily gain and feed conversion improvements up to 30% and 15%, respectively, have been reported. Davies (2008) reported that HGPs added an extra $210M to the Australian beef industry in 2006-7 through heavier animals and earlier turnoff-times.

The Meat Standards Australia (MSA) beef grading model predicts the eating quality outcome of different cuts by cooking method from on-farm, carcass and processing inputs. The original MSA model did not have an adjustment for the HGP impact on eating quality, though this was later introduced after Australian research demonstrated a negative impact on sensory scores and shear force. There are minimal studies which report on the eating quality impacts of OES implants when cattle are finished on pasture, particularly long acting OES implants. From the available research, the results are contradictory, and hence some stakeholders have argued that OES implants may have less impact on eating quality than the MSA model adjustment.

This research is subsequent to the feedlot component research whereby the same line of steers were finished in a feedlot for 73 days (Project code P SHP 0688). A total of 200 composite steers were transported from their place of birth in the Northern Territory, to a central Queensland property and finished on pasture for 389 days. Animals averaging a weight of 255kg were randomly allocated to two treatment groups: CON (no implant) or OES (Compudose 400). Pastures were a mixture of tropical and native grasses, and animals were allowed access to Leucaena when protein and energy from pastures was considered limiting. Once animals reached slaughter weights (approx. 475-550 kg), they were transported to a commercial processor located at Beenleigh, QLD and humanely slaughtered. At boning, the rump (as rost biff from both sides) 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 thoracis et lumborum* (LTL – 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 impact 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 and Minolta colour dimensions (L*, a* and b*). Calpastatin activity was measured, as this is the inhibitor of Calpain-1, an important enzyme for the improvement in tenderness during aging whereby its activity may be affected by the use of HGPs.

The OES treatment group had significantly heavier liveweights and carcass weights than the untreated group. At day 371, the OES treated steers were 34kg heavier than the control group which equated to 19kg heavier carcasses. The HGP treatment significantly increased ossification scores along with a trend to decrease marbling scores, increase ribfat and P8 fat measurements. The decrease in marbling scores has been thought to be via a dilution effect as implants direct available energy towards protein accretion rather than fat synthesis. Interestingly, there was a trend to increase hump height, which in past research has been shown to be affected by TBA+OES implants and thought to be a secondary sex characteristic due to the androgen component i.e. TBA. There were no differences in pHu or AUSMEAT meat colour scores between the treatments.

The HGP treatment resulted in significantly lower sensory scores ($P<0.05$) for all measurements in both muscles at 5 and 35 days, apart from like flavour, and tenderness at 35 days for the GM. The greater impact was on the LTL which agrees with the literature whereby the greatest impact is on cuts with the greatest aging potential. This impact was reduced with aging which is important for the processing and food service sector. In agreement with the sensory scores, the OES treatment resulted in a significantly higher shear force scores in the LTL, and a numerical increase in the GM at 5 days. Again, aging for 35 days reduced the HGP impact in the LTL.

The Calpains and the inhibitor calpastatin, form a group of enzymes which partly control the tenderisation process of meat post mortem. Factors such as *Bos indicus* content have been shown to increase calpastatin activity, which in turn leads to less proteolysis when aging and therefore tougher meat. HGPs increase protein deposition by increasing protein synthesis and slowing protein degradation partly by an increase in calpastatin activity. The OES treatment significantly increased calpastatin activity, which explained 76% of the variation in shear force and around 25% of the variation for sensory scores at 5 days' post mortem. This further supports the mode of action of HGP implants is partly by increasing calpastatin activity and therefore slowing down protein degradation, leading to tougher meat.

The impact of the long acting OES treatment is comparable to the effects of TBA+OES implants reported in Australian studies, used to determine the HGP adjustment in the MSA model. Therefore, it is unlikely any modifications are required to the MSA model to differentiate alternate HGPs. The results of this experiment requires analyses of the residual MQ4 scores (actual minus predicted MQ4) to confirm the accuracy of both the HGP impact and the improvement of cuts with aging within the MSA model, and viewed collectively with the feedlot data. HGP implants are an important tool, particularly in Northern Australia, which aid in increasing liveweights and reducing turn-off times. Producers look to gain a further understanding of the effects of different HGPs on carcass traits and use this information to aid their understanding in how this may influence their MSA index score. Producers will need to continue to assess their own business in regards to HGP use in a balance between returns from productivity and premiums associated with eating quality to obtain optimal returns.

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1 Introduction

Hormonal Growth Promotants (HGPs) have been used to increase growth performance of cattle in Australia for over 30 years. The steroid based HGP implants usually consist of an oestrogen or an oestrogen combined with an androgen or progesterone, and are commonly used in Australia, Canada and America for pasture and feedlot beef production. HGP implants aid in cattle reaching target weights earlier or produce heavier animals over a set time frame. The performance outputs of HGPs in a variety of production systems are well accepted in the local and international beef industry, with reported increases in average daily gain of up to 30% and in feed conversion efficiency of as much as 15% (Preston 1999).

The Australian beef industry has transformed over the past 20 years, predominately driven by the increased focus on beef eating quality which consequently led to the introduction of the Meat Standards Australia (MSA) beef grading system (Polkinghorne *et al.* 2008). The model consists of cook by cut combinations which predict eating quality from carcass, on-farm, animal and processing data (Polkinghorne *et al.* 2008). The original MSA model did not include an adjustment for HGPs, although this was introduced in 2008 based on local and international sensory and objective beef eating quality data, which demonstrated a negative impact of HGP on meat eating quality (Watson 2008). The adjustment ranged from 3-6 meat eating quality points (MQ4) depending on cut when adjusted for ossification and marbling scores (Watson *et al.* 2008c).

There is a dearth of research comparing the eating quality impacts of OES only implants with different HGPs within the same experiment, particularly long acting OES implants when cattle were finished on pasture. Of the HGP studies that report on eating quality impacts of an OES implant or a similar implant that contains oestradiol in combination with progesterone or testosterone, the results are contradictory. Burnham *et al.* (1997) and Foutz *et al.* (1997) both reported tougher shear force scores for the OES implant when compared to controls. Conversely, Basson *et al.* (1985) reported minimal effects of various oestradiol treatments when compared to a control agreeing with shear force scores reported by Hunter (2000) and Hunter *et al.* (2000). Thompson *et al.* (2008b) demonstrated that when *Bos indicus* steers were re-implanted with short acting OES implants every 100 days, the HGP implant interacted with *Bos indicus* content whereby the HGP impact on eating quality increased as *Bos indicus* content increased.

Packer *et al.* (2017a) reported eating quality differences between OES and TBA+OES implants when used in steers finished in a feedlot for 73 days, whereby the TBA+OES implant had lower sensory scores and higher shear force than the OES treatment. The residual MQ4 scores (predicted MQ4 minus actual MQ4) from this data set demonstrated that the MSA model slightly over-penalised the OES treatment, and under-penalised the TBA+OES treatment (Packer *et al.* 2017b).

There is evidence that suggests that the negative impact on eating quality from HGP implants can be reduced by aging (Schneider *et al.* 2007; Thompson *et al.* 2008a; Packer *et al.* 2017a). This interaction has important implications for the beef industry as it allows for the use of HGPs as a production efficiency tool, with the negative eating quality effect reduced by post mortem aging. Currently there is an adjustment in the MSA model which accounts for improvement of HGP cuts via aging (Polkinghorne, R. pers. comm.) Furthermore, the HGP eating quality impact has been shown to be greater in cuts which have the greatest aging rate (Ouali *et al.* 1988; Thompson *et al.* 2008a).

Research has demonstrated that HGP implants increased muscle deposition to varying degrees partly by slowing protein degradation (VanderWal *et al.* 1975; Kerth *et al.* 2003). In the live animal protein degradation is largely influenced by calpastatin activity which has been shown to impact on eating quality in HGP treated carcasses (Gerken *et al.* 1995; Packer *et al.* 2017a).

This following paper reports on subsequent research to Packer *et al.* (2017a) aiming to compare the impact of longer acting OES implants (ca. 400 days) when cattle were finished on pasture, to control animals. Cuts with divergent aging characteristics were chosen to assess any interactions on sensory scores between the OES implant and post-mortem ageing of cuts.

2 Materials and methods

2.1 Live cattle

A total of 200 steers from a composite breed (3/8 *Bos indicus*, 1/2 *Bos taurus,* 1/8 *Bos indicus-taurus hybrid*) from the same year of birth (2013) were transported from a remote Northern Territory cattle station, to a central Queensland cattle property for finishing on pasture. The previously un-implanted steers were allowed approximately one month to acclimatise and reach weights suitable for induction into the trial (255kg). Steers were randomly allocated to an un-implanted control (CON) group, and an oestradiol only implant which has an active ingredient payout period of 400 days (OES - Compudose 400, Elanco Animal Health, Indianapolis, IN, USA; 43.9 mg oestradiol-17ß).

Animals were grazed on pastures which comprised of mixtures of tropical and native pastures, and also allowed access to Leucaena when pastures were considered limiting in protein and energy. Implant ear audits, to determine if the OES implant was present and without inhibition, along with live weights were collected at days 57, 99, 202, 299 and 371 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

After 389 days on pasture, animals were transported 864km to a commercial processing facility and slaughtered the following morning. Carcass sides were electrically stimulated and hot carcass weight plus P8 fat depth recorded, before carcasses were spray chilled. Approximately 19 hours pm carcasses were quartered at the $12^{th}/13^{th}$ rib prior to MSA grading which measured ultimate pH (pHu), hump height, eye muscle area, rib fat along with subjective scores for ossification, marbling, and meat colour scores (AUSMEAT 2005). At grading a 3mm slice from the quartered *m. longissimus thoracis et lumborum* (LTL) muscle was collected and diced. A 5 g sample placed into a sample tube and 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 carcasses. These primals were vacuum packed and chilled prior to transporting to the meat laboratory at the University of New England.

2.3 Sample preparation

The striploin and rump primals (HAM 2110, ROSTBIFF) were separated into the LTL and *m. gluteus medius* (GM) muscles and trimmed of all fat and epimysium. The LTL was cut into four portions and the GM into two portions. Within muscle, samples were rotated on position for five and 35 day aging periods, for both sensory and objective samples. Sensory samples were prepared as described by Watson *et al.* (2008a) whereby each sample portion is prepared into five 25mm thick steaks cut perpendicular to the fibre direction. Objective samples were prepared into approximately 250g blocks. All samples were aged in vacuum bags at 4°C for either 5 days or 35 days, before being frozen at -20°C.

2.4 Sensory analysis

Sensory analysis was described by Watson *et al.* (2008a). Briefly, each consumer was served seven steaks, whereby the first was a mid-range starter steak followed by six sample steaks. Sample steaks were balanced for ageing period, treatment group and cut to encompass a range of eating quality.

The five steaks from within one sample were spread across one tasting session. Product serving order was balanced in a 6x6 Latin square to ensure a balanced serving order within a tasting session. Steaks were cooked using a Silex^{TM} grill to a medium doneness prior to being rested, halved and served. The five steaks from one sample were served to ten consumers. Consumers rated each steak for tenderness, juiciness, like flavour and overall acceptability by placing a mark on a 100mm line scale anchored by the words not tender/very tender, not juicy/very juicy, and dislike extremely/like extremely for both like flavour and overall satisfaction respectively. Scores for tenderness, juiciness, like flavour and overall acceptability scores were multiplied by 0.3, 0.1, 0.3 and 0.3 respectively and summed to calculate a MQ4 score. The highest and lowest two scores from the total of ten scores per sample, were 'clipped' to reduce the standard error of the mean sensory score.

2.5 Objective measurements

Objective shear force blocks were prepared as per Perry *et al.* (2001) with slight modifications as per Packer *et al.* (2017a). Briefly, a thawed 60-80g block was prepared from each 250g sample block. Three colour readings were recorded using a Minolta Colour Meter (D65) from a cut face, following blooming for at least 20 minutes at 4°C. The mean CIE, L*, a* and b* dimensions were used in the data analysis. Blocks were cooked in unsealed vacuum bags at 70°C for 30 minutes, followed by cooling under running water for at least 20 minutes. Cooking loss was calculated as the percentage of loss from pre-cook weight, to post-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 Koohmaraie (1990) with slight modifications as outlined in Packer *et al.* (2017a). Briefly, 4g of the sample was homogenised in 20ml of extraction buffer, then spun for 15 min at 4000 x g. 12ml of supernatant was then transferred into a 15ml tube, and heated in a water bath at 95°C for 15 min. 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. Calpain-2 homogenate was prepared as per Koohmaraie (1990). Calpastatin samples were assayed against the purified Calpain-2 in a casein solution in batches of 24 to 30 samples, which contained both CON and OES samples. The activity of Calpain-2 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 the Calpain enzymes was the inverse, expressed as the amount that inhibits 1.0 unit of Calpain-2 activity, expressed in units per gram of muscle.

2.6 Statistical analysis

2.6.1 Ear pathology

At each weighing period, the ears of the OES treatment group steers were palpated to detect for implant presence and any sign of infection or related scarring. Ten animals lost their implants over the trial period. One animal lost its implant in the first 57 days and another showed signs of infection and as result had no implant at day 99. It was thought that the remaining eight animals lost their implants through scratching on shrubs or lick troughs due to the position of the implant. Often during implanting, the presence of a management ear tag did not allow the implant to be placed in the middle third of the ear. When this occurred, an alternate implant site in the front middle third of the ear, anterior to the cartilage fold, was used. The eight animals who lost their implants most likely had the implant placed in this position and therefore lost their implant. All ten animals that had lost implants by the end of the experiment were removed from the data set.

2.6.2 Liveweight

Liveweights recorded at 57, 99, 202, 299 and 371 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 not significant (P>0.05) and was not included in the final model.

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 GLM model (SAS 2002), 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) these traits 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 induction weight was not significant (P>0.05).

2.6.4 Sensory scores

Sensory scores from the LTL and GM samples 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 significant (P<0.05) for all sensory scores in the LTL, except for juiciness. This interaction was not significant (P>0.05) for the GM sample. Due to the importance of this question in the overall experimental design, this interaction was retained in both the LTL and GM statistical models.

2.6.5 Objective measurements

Within LTL and GM samples, shear force and cooking loss % were analysed using MIXED models (SAS, version 9.0) which contained terms for HGP treatment, aging and position within muscle. An interaction between HGP treatment X days aged was not significant (P>0.05) for objective measurements for either the LTL or the GM. However, given the significance of this interaction for the sensory scores it was decided to include this interaction for shear force and cooking loss % analyses for both LTL and GM analyses. Models contained a random term for animal nested within HGP treatment.

The L*, a* and b* colour dimensions were highly correlated and so a repeated measurements analysis (REM, SAS, version 9.0) was used, which contained terms for HGP treatment, days aged and position within muscle. This 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.

2.6.6 Calpastatin activity

Calpastatin activity in LTL samples collected at 20 hours pm was measured in seven batches. Each batch contained samples from each of the two 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

Live weights at each weigh day were adjusted to a mean induction weight of 255 kg. The repeated measures analysis showed a significant HGP treatment X days on pasture (DOP) interaction (P<0.001, Table 1), whereby the difference between the CON and OES groups continued to increase the longer the HGP implant was implanted. Animals in the OES group were 6 kg heavier than animals in the CON group at day 57, which increased to 34kg when cattle were finally weighed at day 371 (Figure 1). Induction weight interacted with time (P<0.001, Table 1), whereby heavier animals at induction gained more weight over the 371 trial period.

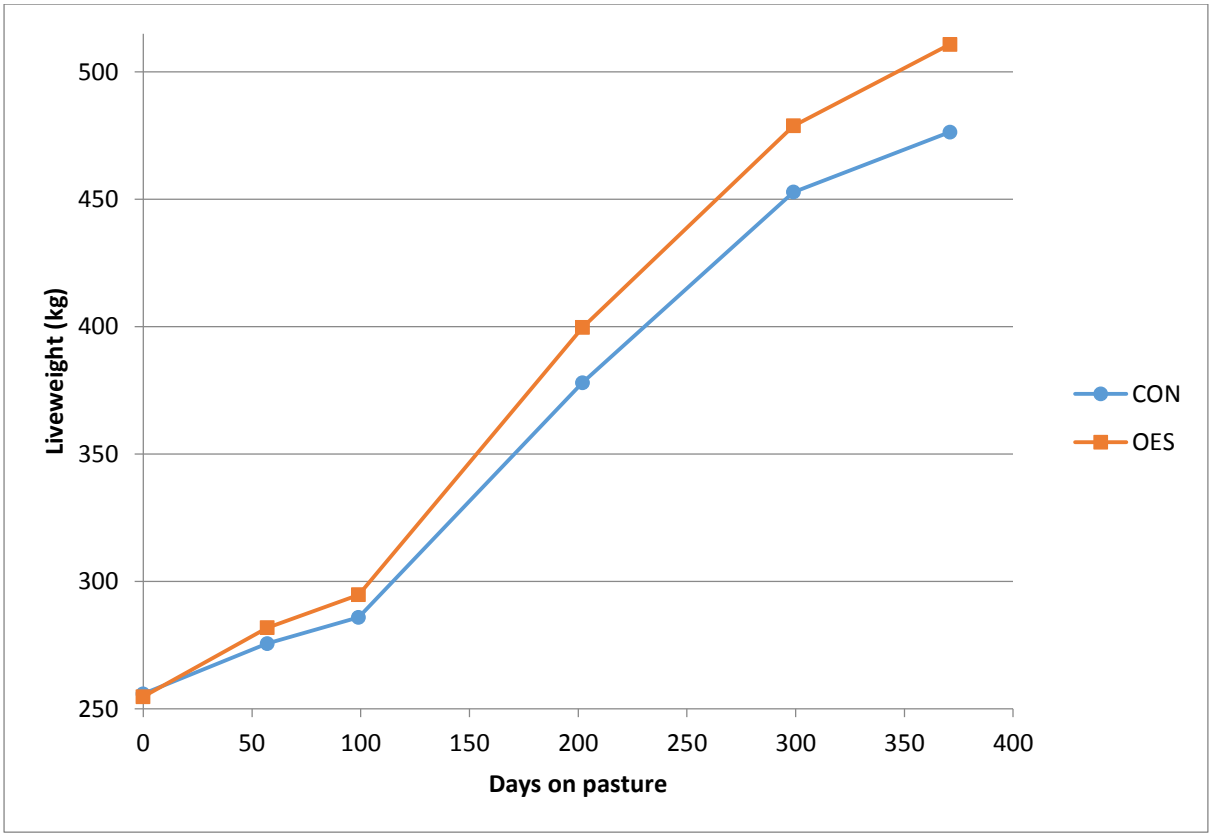

Figure 1. Predicted liveweights at 57, 99, 202, 299 and 371 days' post pasture trial induction for steers from the CON and OES groups. All liveweights were adjusted to the same induction weight of 255kg.

Table 1. F ratios for the repeated measurements analysis of OES treatment and induction weight for both mean effects and interactions with days on pasture (liveweights at days 57, 99, 202, 299 and 371 after induction)

Independent Variables	NDF,DDF	F ratio
Days on pasture (DOP)	4,744	16.63***
HGP Treatment (HGP)		
Mean HGP treatment	1,186	90.50***
DOP x HGP	4,744	47.42***
Induction weight		
Mean induction weight	1,186	75.35***
DOP x induction weight	1,744	0.73***

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

***, P<0.001

3.2 Treatment effects on carcass traits

There was a moderate range in carcass traits with marbling and ribfat, ranging from 130 to 470 MSA marbling units, and 1mm to 10mm respectively. Hot standard carcass weights (HSCW) ranged from 202 to 279kg (Table 2.). The OES treatment had a significant effect on hot carcass weight and ossification score (P<0.001, Table 3). Predicted means for the OES carcasses were 19 kg heavier than the CON group, and had a 47 unit higher ossification score than the CON carcasses at the same HSCW. There were no significant (P>0.05) HGP treatment effects on other carcass traits, although was a trend for the OES group to have a lower marbling score and higher rib fat, P8 fat and hump height measurements, when compared to the CON group.

Table 2. Means, variance and range for carcass traits for the CON and OES treatments

	Mean	**s.d.**	**Min**	**Max**
Carcass traits				
HSCW (kg)	240	15.8	202	279
Hump Height (mm)	84	9.4	60	110
Ossification score	164	35.3	100	350
Marbling score	280	60.8	130	470
Ribfat (mm)	4.1	1.57	1	10
P8 (mm)	8	2.3	3	15
Meat colour score	3.2	0.85	2	6
Eye muscle area (cm2)	62	8.4	41	87

Table 3. Predicted means for the CON and OES treatments for carcass traits including the F ratios for the HGP treatment effect and the average standard error of both treatments.

	HGP Treatment			HGP Treatment	
Carcass trait	CON	OES	s.e.	NDF, DDF	**F ratio**
HSCW (kg)	231[a]	250[b]	1.4	2,188	99.26***
Hump height (mm) #	83.5	85.4	1	2,187	1.38
Ossification score #	141[a]	188[b]	3	2,187	90.46***
Marble score #	284	276	7.1	2,187	0.49
Ribfat (mm) #	4	4.2	0.19	2,187	0.93
P8 (mm) #	7.9	8.2	0.27	2,187	0.74
pHu#	5.63	5.63	0.012	2,187	0.01
Meat colour score #	3.2	3.2	0.1	2,180	0.06
Eye muscle area (cm^2) #	61.8	62	0.97	2,187	0.00

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

Adjusted for hot carcass weight

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

3.3 Sensory scores

There was a large range in sensory and shear force scores, with up to 81 MQ4 points and 2.2 kg, and as low as 16 MQ4 points and 10.6 kg for the LTL respectively (Table 4.).

Table 4. Means, variance and range for sensory scores and objective meat quality measurements for the CON and OES treatments for the *mm. longissimus thoracis et lumborum* and *gluteus medius.*

	m. longissimus thoracis et lumborum				***m. gluteus medius***			
Trait	**Mean**	**s.d.**	**Min**	**Max**	**Mean**	**s.d.**	**Min**	**Max**
Sensory scores								
Number of samples	*366*				*378*			
Tenderness	49.2	16.98	7	84	49.2	15.15	14	82
Juiciness	53.6	13.34	23	82	57.7	11.64	25	85
Like Flavour	54.7	11.84	16	81	56.2	10.75	24	81
Overall liking	52.5	14.15	13	81	53.7	12.52	21	83
MQ4	52.1	13.5	16	81	53.3	11.92	23	79
Objective								
Number of samples	*36*				*377*			
Shear force	4.6	1.72	2.2	10.6	4.9	1.77	2.5	10.6
Cooking Loss %	21.7	2.6	12.2	29.8	22	2.53	15.5	29.3
Meat colour								
L*	36.1	2.56	30.1	46.3	35.4	2.28	29.5	41.7
a*	19.4	1.98	13.6	24.9	21.3	2.15	13.6	24.9
b*	8.3	1.35	5.1	12.8	9.6	2.77	6.2	57.1

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A HGP treatment X days aged interaction was present for the LTL samples for all sensory scores, apart from juiciness (Table 5). This interaction showed that the difference between CON and OES sensory scores at 5 days pm more than halved by 35 days for the LTL (Table 6). There was a trend for this interaction for juiciness, but failed to reach significance (P>0.05). No interactions between HGP treatment effect and days aged were present for the GM (P>0.05, Table 5).

The OES treatment resulted in significantly lower sensory scores at both 5 days and 35 days for both the LTL and GM (P<0.05), apart from like flavour at 5 and 35 days, and tenderness at 35 days, both for the GM (Table 6). There was a position effect present for the LTL whereby the anterior samples had higher sensory scores than the posterior samples (Table 5).

Table 5. F ratios for the effect HGP treatment, days aged, HGP treatment x days aged, position, and days aged x position on sensory scores for the *mm. longissimus thoracis et lumborum* and *gluteus medius.*

Trait	***m. longissimus thoracis et lumborum***						***m. gluteus medius***					
	NDF, DDF	Tenderness	Juiciness	Flavour	Overall liking	MQ4	NDF, DDF	Tenderness	Juiciness	Flavour	Overall liking	MQ4
HGP Treatment (HGP)	1,181	27.58***	22.20***	22.00***	25.00***	26.18***	1,186	6.88**	11.71***	5.41*	6.17*	7.31**
Days Aged (DA)	1,180	214.74***	119.48***	141.28***	205.06***	208.85***	1,187	268.76***	110.71***	127.23***	192.37***	216.13***
HGP*DA	1,180	20.46***	1.55	4.60*	13.53***	12.26***	1,187	1.22	0.67	0.00	0.01	0.08
Position	1,180	23.01***	36.10***	12.14**	27.94***	25.17***	1,187	0.24	0.04	0.11	0.14	0.12

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

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Table 6. Predicted means for sensory scores (tenderness, juiciness, like flavour, overall liking and MQ4 scores) for the CON and OES treatments for the *mm. longissimus thoracis et lumborum* and *gluteus medius* and the average standard error of both treatments. The model included fixed effects for HGP treatment, days aged, the interaction between these variables and position within muscle. A random term for animal number nested within treatment was also included in the model.

Sensory scores	Treatment					
	5 days aged			35 days aged		
	CON	OES	s.e.	CON	OES	s.e.
m. longissimus thoracis et lumborum						
Tenderness	48.4[a]	34.1[b]	1.53	58.9[a]	54.1[b]	1.53
Juiciness	52.0[a]	44.2[b]	1.24	61.4[a]	56.1[b]	1.24
Like Flavour	53.5[a]	45.7[b]	1.11	61.6[a]	57.4[b]	1.11
Overall liking	51.4[a]	40.6[b]	1.28	60.7[a]	56.3[b]	1.28
MQ4	51.0[a]	40.7[b]	1.22	60.2[a]	55.6[b]	1.22
m. gluteus medius						
Tenderness	44.0[a]	38.7[b]	1.37	58.4[a]	55.2[a]	1.37
Juiciness	55.5[a]	50.5[b]	1.12	63.9[a]	60.2[b]	1.12
Like Flavour	52.7[a]	50.1[a]	1.02	62.1[a]	59.4[a]	1.02
Overall liking	49.4[a]	46.0[b]	1.16	61.2[a]	57.9[b]	1.16
MQ4	49.4[a]	45.7[b]	1.09	60.7[a]	57.5[b]	1.09

Within row and days aged, means with differing superscripts indicate a significant difference ($P<0.05$).

In subsequent analyses HGP treatment effects for LTL and GM were adjusted for carcass traits in the MSA model (hump height, carcass weight, ossification and marbling scores, ribfat and ultimate pH, unpublished data, D.T. Packer). As these adjustments had little effect on the significance and magnitude of the treatment effects, these means were not presented here.

3.4 Shear force

HGP treatment significantly impacted shear force for the LTL, but not the GM samples (P<0.01, Table 7). For the LTL, at 5 days pm the OES treatment had 0.6 kg higher shear force (P<0.05, Table 8). After 35 days of aging, the OES treatment was reduced to 0.4kg and was not significant (P>0.05). Despite the change in shear force values for the LTL, the HGP treatment X days aged interaction was not significant (P>0.05). There was no difference between the two HGP treatments for the GM at 5 and 35 days, Days aged and position had a highly significant impact on shear force scores (P<0.001, Table 7.).

Table 7. F ratios for the effect HGP treatment, days aged, HGP treatment x days aged, position, and days aged x position on shear force (kg) and cooking loss percentage for the *mm. longissimus thoracis et lumborum* and *gluteus medius.*

Trait	**Shear force**				**Cooking loss %**			
	m. longissimus thoracis et lumborum		***m. gluteus medius***		***m. longissimus thoracis et lumborum***		***m. gluteus medius***	
	NND,DDF	F ratio	NNF,DDF	F ratio	NND,DDF	F ratio	NNF,DDF	F ratio
HGP Treatment (HGP)	1,181	7.43**	1,187	1.97	1,181	5.14*	1,187	3.11
Days aged (DA)	1,178	244.01***	1,185	155.69***	1,178	127.36***	1,183	93.73***
HGP*DA	1,178	1.69	1,185	3.66$^{\#}$	1,178	0.00	1,183	2.05
Position	1, 178	14.66***	1,185	40.15***	1,178	28.81***	1,183	63.57***

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

*, P<0.05; **, P<0.01; ***, P<0.001; P=0.057; #

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Table 8. Predicted means for the CON and OES shear force and cooking loss for the *mm. longissimus thoracis et lumborum* and *gluteus medius* including the average standard error of both treatments. The model included terms for HGP treatment, days aged and position.

Trait		HGP Treatment		
	Days Aged	CON	OES	s.e.
m. longissimus thoracis et lumborum				
Shear Force	**5**	5.2[a]	5.8[b]	0.15
	35	3.5[a]	3.9[a]	0.15
Cooking loss	**5**	22.6[a]	23.1[a]	0.23
	35	20.2[a]	20.8[a]	0.24
m. gluteus medius				
Shear Force	**5**	5.7[a]	5.7[a]	0.15
	35	4.4[a]	3.9[a]	0.16
Cooking loss	**5**	23.0[a]	22.9[a]	0.23
	35	21.2[a]	20.5[b]	0.23

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

3.5 Cooking loss

HGP treatment had a significant effect on cooking loss for the LTL, but not the GM (P<0.05, Table 7). The cooking loss % for the OES treatment was higher for CON samples at 5 and 35 days for the LTL, though this difference failed to reach significance (P<0.05, Table 8). The OES treatment had a lower cooking loss % than the CON for the GM, and values reached significance at 35 days. Days aged and position had a highly significant impact on cooking loss %. Cooking loss % all cuts and treatments decreased from 5 days to 35 days.

3.6 Colour

The mean HGP treatment was significant for the LTL but not the GM (P<0.01, Table 9). For the LTL samples, the OES treatment had significantly lower L*, a* and b* values when compared to the CON treatment (P<0.05, Table 10.), whereas there were only minor differences for colour dimensions in the GM. For both LTL and GM samples there were differences between the colour dimensions for samples aged for 5 or 35 days. Similarly, there were differences between positions for colour dimensions for both the LTL and GM samples (P<0.001, Table 9). As the HGP treatment effects did not interact with days aged or position these effects were not documented in this paper.

Table 9. F ratios for the repeated measurements analysis of HGP treatment, days aged and position on CIE colour dimension (average of L*, a*, b*) for the *mm. longissimus thoracis et lumborum* and *gluteus medius.*

	m. longissimus thoracis et lumborum		***m. gluteus medius***	
Independent Variables	NDF,DDF	F ratio	NDF,DDF	F ratio
Colour dimension	2,720	48501.9***	2,746	14917.4***
HGP Treatment (HGP)				
Mean treatment effect	1,360	14.01***	1,373	2.28
Colour dimension x HGP	2,720	3.55	2,746	0.32
Days aged (DA)				
Mean days aged effect	1,360	117.56***	1,373	79.94***
Colour dimension x DA	2,720	26.78***	2,746	8.96***
Position				
Mean position effect	3,360	1.32	1,373	143.81***
Colour dimension x position effect	2,720	5.32***	2,746	10.06***

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

***, P<0.001

Table 10. Predicted means for HGP treatment effect on L*, a*, b* colour dimensions after adjustment for days aged and position within the *mm. longissimus thoracis et lumborum* and *gluteus medius,* and the average standard error of both treatments.

Trait	**HGP Treatment**		
	CON	OES	s.e.
m. longissimus thoracis et lumborum			
L*	36.5[a]	35.7[b]	0.17
a*	19.7[a]	19.1[b]	0.13
b*	8.5[a]	8.1[b]	0.09
m. gluteus medius			
L*	35.6[a]	35.5[a]	0.15
a*	21.4[a]	21.2[a]	0.12
b*	9.9[a]	9.5[a]	0.20

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

3.7 Calpastatin activity

The effect of HGP treatment and Calpastatin batch had a highly significant effect on calpastatin activity for samples collected ca.19 hours pm (P<0.001, Table 11). Calpastatin activity was significantly higher for the OES treatment when compared to the CON samples (P<0.05).

Table 11. The effect of HGP treatment and batch on calpastatin activity from *m. longissimus thoracis et lumborum* sampled at 19 hours pm along with predicted means for the CON and OES treatments and the average standard error of both treatments.

Trait	NDF,DDF	F ratio	Predicted means (units of activity/g of muscle)		
			CON	OES	s.e.
HGP Treatment	1,174	14.56***	3.75[a]	3.93[b]	0.033
Calpastatin batch	6,174	229.90***			

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

***, P<0.001

Data on calpastatin activity of LTL samples at ca. 19 hours pm, provided an opportunity to assess the impact of calpastatin activity on sensory scores and shear force at 5 days for the LTL. This was established by analysing sensory scores and shear force at 5 days in a model with terms for HGP treatment, position and calpastatin batch. For shear force when calpastatin activity was then included, the F ratio decreased from 5.3 to 1.3, indicating that that calpastatin activity accounted for approximately 76% of the variance associated with HGP treatment on shear force. Similar analyses were conducted for each of the sensory scores at 5 days for the LTL. Calpastatin activity accounted for 28%, 26%, 19%, 24% and 25% of the HGP treatment effect for tenderness, juiciness, like flavour, overall liking and MQ4 respectively.

4 Discussion

Oestradiol only HGP implants decreased sensory scores and increased shear force of both the LTL and GM muscles of steer carcasses, when finished on pasture for ca. 400 days. These results agreed with Burnham *et al.* (1997) and Foutz *et al.* (1997) where oestradiol only or oestradiol benzoate plus progesterone implants decreased sensory or increased shear force measurements. Hunter *et al.* (2000) reported that the 400 day OES implant, and repeated 100 day implants, increased shear force values 0.4 kg and 0.5 kg higher respectively. Hunter (2000) reported minimal difference for shear force scores for either the OES or TBA+OES HGP treatments when compared to the un-implanted steers. Consumers though, detected a negative impact on eating quality for the repeated 100 day HGP implant treatment. Barham *et al.* (2003) reported two successive oestradiol implants had no impact on sensory scores when samples were aged for either 7 or 14 days.

Much of the research to establish the impact of HGP implants on sensory scores to further develop the MSA model, utilised combination TBA+OES implants at least once in the animals growing phase (Thompson *et al.* 2008a; Watson 2008; Watson *et al.* 2008b). Our results showed the OES implant had a similar negative impact on eating quality to other studies where combination implants or multiple implants were used. Reviews by Morgan (1997) and Tatum (2009) reported that implants that did not contain trenbolone acetate had less effect on shear force scores than TBA+OES implants. As there was no TBA+OES treatment in this study, we are unable to establish what effect a

TBA+OES implant may have had in this pasture finishing environment. The magnitude of the OES treatment in our study was much greater than reported by Packer *et al.* (2017a) when the same line of steers were implanted with OES implants and finished in a feedlot for 73 days. The reduction in MQ4 scores was only 1-2 units at 5 days pm when compared to un-implanted steers, though interestingly this effect doubled after 35 days of aging.

The steers used in this study were from the same line as reported by Packer *et al.* (2017a), therefore factors that differed between the studies may explain the larger effect observed. These include nutrition, i.e. animals had lower and variable nutrition on pasture, and the time the OES implant was active i.e. 73 days in the feedlot as opposed to 389 days on pasture. It may be that the lesser nutrition from the pasture interacted with the HGP treatment resulting in a much larger impact on sensory scores in comparison to steers finished in a feedlot. Eating quality can decrease with an increasing number of implants administered over an animal's finishing period (Samber *et al.* 1996; Scheffler *et al.* 2003; Schneider *et al.* 2007). This may be considered similar to a longer acting implant, though at the beginning of an implant payout period there is a higher release of the active ingredient which may enhance the negative impact on eating quality when successive implants are administered (Brandt 1997). The long acting OES treatment may have interacted with time, similar to continuous re-implant programs, hence having a larger impact on eating quality. Long acting implants are commonly used in extensive pasture systems of Northern Australia due to the geographical difficultly to re-muster for re-implantation. Because of this, further work may be justified to further explore both of these potential interactions.

The improvement of the eating quality in LTL of HGP treated carcasses with aging agreed with Tatum (2009) and Schneider *et al.* (2007). The reported interaction supports Packer *et al.* (2017a), though the interaction was for meat from cattle treated with a TBA+OES implant, not the OES implant. Thompson *et al.* (2008a) reported this interaction for shear force, but not sensory scores, though the HGP effect for sensory scores halved between 5 and 21 days for the LTL. This provides the processing and retail sector an opportunity to reduce the negative impact of HGP implants.

It was hypothesised by Kerth *et al.* (2003) and Thompson *et al.* (2008a) that the slowing down of protein degradation in a live animal via the use of a HGP, could be part of the mechanism which increases protein accretion, and would therefore lead to tougher meat through a decreased aging response. The Calpain system is a group of enzymes and inhibitors which are primarily responsible for myofibrillar protein degradation, both pre- and post mortem. Of these, Calpain-1 is predominantly responsible for pm proteolysis (Geesink *et al.* 2006). Calpastatin, being the inhibitor of the Calpains, has been shown to increase activity through the use of HGPs containing both androgens and oestrogens (Gerken *et al.* 1995; Packer *et al.* 2017a). This supports our findings whereby the OES treatment resulted in significantly higher calpastatin activity than the CON treatment. Calpastatin activity at ca. 19 hours pm explained 76% of the HGP treatment effect on shear force at 5 days for the LTL but less for sensory scores. These results support that OES implants may increase protein accretion in a live animal by increasing calpastatin activity and therefore slowing protein degradation, which would lead to a decrease in proteolysis post mortem and result in tougher beef.

The HGP impact was greater on the LTL when compared to GM which agreed with Watson *et al.* (2008b) and Ouali *et al.* (1988), whereby as muscles aging rate increased, so too does the negative HGP impact on eating quality. The OES treatment resulted in a negative 10 and 4 MQ4 point difference in the LTL and GM at 5 days respectively, which halved in the LTL after 35 days aging. Different muscles have different aging rates predominantly via divergent calpain to calpastatin ratio (Ouali and Talmant 1990). It may be that HGP treatment increases calpastatin synthesis in all muscles, but has the greatest impact on muscles with a high Calpain/calpastatin ratio, i.e. the LTL.

The OES treatment resulted in an additional 34kg of liveweight after 389 days when compared to the CON treatment. This translated into 19kg heavier carcasses supporting the plethora of HGP research which demonstrate an increase in average daily gain and productivity (Duckett *et al.* 1997; Hunter 2010), and with specific studies whereby performance effects from long acting oestradiol only implants were measured (Hunter 2000; Hunter *et al.* 2000; Thompson *et al.* 2008b).

The HGP treatment had an impact on other carcass traits, but most notably ossification, supporting much research demonstrating the increase in skeletal maturity due to HGPs (Apple *et al.* 1991; Thompson *et al.* 2008a; Thompson *et al.* 2008b; Watson *et al.* 2008b). Milton *et al.* (1996) reported that two oestradiol implants, which could be similar to one longer acting OES implant, increased skeletal maturity significantly. Paisley *et al.* (1999) reported that single estrogenic implants had a larger impact on skeletal maturity than a mild TBA+OES implant.

The trend for the OES treatment to reduce marbling agrees with Thompson *et al.* (2008b) when repeated 100 day oestradiol only implants were administered. It is thought the reduction in marbling may be via a dilution effect as available energy is diverted to protein accretion, rather than fat synthesis when a HGP implant is administered (Duckett *et al.* 1999). This hypothesis could explain a lower marbling score, though you would expect a slightly reduced P8 and ribfat measurements. As ribfat and P8 fat depth were higher for the OES treatment, other factors may be influencing fat and protein accretion when OES implants are used. Burnham *et al.* (1997) reported a higher rib fat depth for steers treated with oestradiol only implants, though less kidney and pelvic fat.

Interestingly, there was a trend for increased hump height for the OES treatment. Combination TBA+OES implants have shown to increase masculinity scores which has thought to be a secondary sex characteristic caused by the androgenic component of the combination implant (Apple *et al.* 1991; Sillence 2004). Packer *et al.* (2017a) found an increased hump height caused by the TBA+OES treatment, whereas the OES treatment had only a minor effect over a 73 day period in a feedlot. Herschler *et al.* (1995) reported that both combination and oestradiol implants increased masculinity scores.

The OES treatment did not alter the pHu which aligns with Thompson *et al.* (2008b) and Watson *et al.* (2008b). Converse with our findings, Scanga *et al.* (1998) reported that HGP implants, particularly aggressive repeated TBA+OES implant programs, did increase the incidence of dark cutting beef.

Cooking loss % was slightly increased by the OES treatment in the LTL, though reduced in the GM. Thompson *et al.* (2008a) reported a slightly higher cooking loss % for LTL samples from HGP treated heifers and steers.

Whilst the OES treatment resulted in lower in L* (lightness), a* (redness) and b* (yellowness) colour dimensions for the LTL, this was not visually observed in colour scores by MSA graders. Scheffler *et al.* (2003) reported only minor numerical differences in colour dimensions caused by HGP implants. Reiling and Johnson (2003) reported no effect of HGP treatments on L*, though lower a* and b* colour dimensions. Impacts on colour dimension by HGP treatments is therefore of little importance, particularly as visual grading could not detect differences.

5 Conclusion

Long acting OES HGP implants have a negative impact on eating quality when cattle are finished on pasture for extended periods. This impact is similar to the effects of TBA+OES implants and multiple implant programs reported in the literature, though this impact may be reduced through aging. In an Australian context, where carcass trait data is used to predict eating quality using the MSA beef grading model, the negative impact of the OES HGP on ossification score, marbling score and hump height should be noted.

These results have important implications for the beef industry as long acting oestradiol only implants are most commonly used in northern Australia pasture finishing productions, in animals with high *Bos indicus* content. With the increased focus on eating quality, and the MSA model forming the basis of the Australian beef industry, it is important to understand the implications of these management practices.

6 Impact on Meat and Livestock Industry

This study has benefited the industry by furthering the understanding of the impacts of long acting implants on eating quality when cattle are finished on pasture in northern Australia. The MQ4 scores for the OES group were significantly lower than the control group and comparable to the HGP impact reported in Australian studies whereby TBA+OES implants were used, on the basis of which the MSA model HGP adjustment was calculated. This most likely means that any alteration to the MSA model to differentiate HGPs is unlikely. Even so, the residual MQ4 scores assessed for model accuracy (actual minus predicted MQ4), viewed collectively with the feedlot trial, could be further examined to determine if one collective HGP adjustment continues to be justified.

Regardless of the above, both producers and the processing/retail sector can benefit from this research. Many of the processor payment grids are now underpinned by the MSA grading model, regardless of end market. This has influenced the northern Australian beef sector to increase the focus on the factors affecting beef eating quality. As long acting oestradiol only implants are predominately used on pasture in these northern systems, this research will aid as an education tool in the understanding of benefits and impacts of long acting HGPs. Producers will further understand the HGP OES effect on carcass traits, particularly ossification, hump height and marbling. As always, each individual production system needs to balance the productivity benefits of HGPs with effects on eating quality, along with factoring genetics, time until slaughter and nutrition, for an optimal return.

Whilst there is a current HGP X aging interaction in the MSA model, this trial may offer further data for refinement. The use of extended aging to reduce the negative HGP eating quality effect may benefit industry through increased MSA grading percentages, whilst retaining productivity benefits.

7 Recommendations

As mentioned previously, due to the large eating quality impact of a long acting OES implant on pasture finished cattle, it is unlikely that any model adjustments to differentiate HGP types will be justified. This will need to be confirmed via the residual MQ4 sensory score analysis. The OES implant when used in steers finished in a feedlot for ca. 70 days had far less impact on MQ4 scores when compared to the control, indicating that other factors influenced the larger HGP impact on pasture; most likely nutrition and time the implant was active. The MSA model does not differentiate between finishing types, and therefore assessment of the residual scores needs to be evaluated collectively for both the feedlot and pasture finishing studies, and presented to the MSA pathways committee to evaluate the use of a continued single HGP adjustment.

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