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Development of a vaccine to enhance lean muscle yield in beef cattle – A feasibility study

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Abstract

Myostatin is the protein that negatively controls muscle growth, however breeding programs focused on selecting for Myostatin mutations in cattle to increase muscle mass and carcase value can be challenging. Immune-stimulation against myostatin is an alternative approach and has been successfully used in mice resulting in hyper-muscularity. This study evaluated both immunological and physiological responses to 4 different peptide-conjugated candidate vaccines in non-HGP treated feedlot heifers with known Estimating Breeding Values (EBVs). The immustimulation approach used herein did not include the application of genetic manipulation technology. They received two subcutaneous vaccinations four weeks apart post-weaning. Slaughter occurred 33 weeks later. Growth, body composition, feed efficiency and carcass measurements (including Computer Tomography (CT) scans of primal cuts) were recorded. Immune response was variable but there was a significant increase in muscle and decrease in fat percentage (1.45% and 1.42%) in the CT scanned primal from carcasses of heifers in the group which had the most consistent immune response ($P<0.05$) compared to control. There was also a significant yet negative impact of vaccination on the visual marble scores of carcasses from this group ($P=0.07$) and 5 ($P<0.01$), yet there was no difference between groups for chemically determined IMF ($P=0.61$). The results are encouraging and indicate further vaccine development is warranted.

Executive summary

Myostatin is a natural occurring protein responsible for suppressing muscle growth *in vivo* and suppressing proliferation and differentiation of cultured myogenic cells. It is mainly found in developing and adult skeletal muscle. Natural mutations in the myostatin gene, causing loss of function, have been recorded in humans and other mammals. Myostatin mutations are associated with muscle hypertrophy and hyperplasia. A number of different mutations have been documented in bovine myostatin and these different mutations are present in many breeds. Whilst myostatin mutation in cattle is linked with better feed efficiency, an increase in muscularity, a decrease in fatness and consequently an increase in carcass yield and value; it can also be associated with undesirable traits such as decreased in fertility, a higher incidence of birth difficulties and decreased fitness (mainly due to smaller size vital organs).

Terminal breeding systems which aim for the production of progeny which are heterozygous myostatin carriers can be implemented and may be profitable, however they require intense inputs and for these reason are not widely adopted. The advantages in carcass yield of cattle with a myostatin mutation cannot be achieved easily without the negative impacts. Administration of endogenous proteins and immune-stimulation applying genetic manipulation has previously been described to block or disrupt myostatin activity in laboratory animals; however these methods may attract negative public perception if used in livestock destined for human consumption. The present study for this reason evaluated the feasibility of 4 myostatin peptide-conjugated candidate vaccines. Of the chosen peptides, one was a published peptide (group 2) and 3 were novel peptides (groups 3, 4 and 5). To test the concept, a discrete design aiming to optimise carcass muscle /fat deposition in young heifers finished under feedlot conditions was chosen; as it has been reported that this production system tends to result in over-fat, out of specification carcasses. Young Breedplan registered Angus heifers (average 164 ± 12.7 days) with known Estimating Breeding Values (EBVs) were used. Fifty heifers were allocated to 5 experimental groups: 14 in the non-treated control and 9 in each of the 4 experimental vaccine treated groups. The design aimed to identify differences between the treated groups and the control, not differences between treated groups. Treatment groups were balanced for all relevant phenotypic parameters at weaning. The heifers received two subcutaneous vaccinations, 4 weeks a part post weaning, and their immune response was monitored via ELISA testing for serum antibodies. Live and carcass measurements were taken including: live weight, eye muscle area, rump fat, rib fat and intramuscular fat pre- and at two and five months post-vaccination. The heifers were also submitted to a net feed intake test. Slaughter occurred 33 weeks post-vaccination at an average age of 436 ± 12.7 days. Extensive carcass and meat quality tests were carried out, including computer tomography (CT) scans of all untrimmed primals.

Immune response was variable and in most instances did not strongly correlate with phenotypic responses. Nevertheless in treatment group 2, the group with the most consistent immune response, a significant increase in muscle % and decrease in fat % in the primal, as determined by CT scans, was observed. Muscle % in this group was 1.45% higher and fat % was 1.42% lower than the control (not treated). In addition effect of rib fat EBV on primal CT scanned fat % and muscle % was significantly different between group 2 and control. This suggests that cattle with a greater genetic potential to be fat had a greater response to myostatin blockage. Serum myostatin was then measured by ELISA however levels were not related to EBV or to immune response.

No significant effect was noticed in the live animal measurements and no significant differences were seen in any of the carcass measurements (including MSA index), except for MSA marbling score. Groups 2 ($P=0.07$) and 5 ($P<0.01$) had significantly lower marbling

score than the control group; however there was no difference in chemically measured intramuscular fat (P=0.61). The discrepancy between the two measurements could be explained by variation in visual appearance of the intramuscular fat between the groups which was recorded as different when visually appraised. There was no significant effect of treatment group on shear force or cooking loss of the *longissimus thoracis* which had been aged for 14 days (P>0.05). However there was a slight trend (P<0.1) for group 2 to be more tender than the control group.

Previous work in cattle visually selected for muscle divergence, found that high muscling heterozygous myostatin steers had 3.5% higher meat yield (estimated from commercial bone-out) than low muscling wild-type steers (no myostatin mutation). Thus a muscle difference of 1.45% in heifers with no visual muscling score divergence in an initial research study is a modest but considerable result. The variation in muscle % measured in the present study would translate into an estimated increase of approximately 3.5 kg in saleable meat from a 240kg carcass. Further improvement in the vaccine design, targeting a higher immune response, can probably be achieved through adjuvant enhancement and possibly use of multiple peptides.

Complementary information:

- Given the regulatory complexity of developing novel products for livestock destined for human consumption and the difficulty in obtaining objective information from regulatory bodies, it is thought that if further work is to be carried out a specialised consultant should be engaged from the early stages.

- The trend to positive fat EBVs in bulls can potentially result in a higher incidence of non-compliance.

- If the vaccine is further developed and the results are favourable for commercialisation, the impact on eating quality should then be extensively tested in the MSA model.

- This approach has potential to be cost effective for adoption by the beef industry.

- The presence of existing patent claims on future vaccine development will largely depend on formulation(s) to be pursued if further work is to proceed.

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1.0 Background

The myostatin protein is a growth differentiation factor belonging to the transforming growth factor beta superfamily (Elkasrawy and Hamrick, 2010; Lee, 2012). This protein potently and negatively regulates muscle growth by affecting both muscle cell size and the total number of muscle cells (Lee, 2012). It also plays a role in the deposition of adipose tissues throughout the body (Kim *et al.*, 2001). A number of different mutations have been identified in bovine myostatin and they are present in many breeds. These mutations cause the affected animal to develop hypermuscularity, with reduced adipose tissue synthesis and a potential increase in feed conversion efficiency (Fiems, 2005; Hanset *et al.*1987; Karim *et al.* 2000; O'Rourke *et al.* 2009). The severity of phenotypic expression observed in cattle depends on the type of mutation present and whether it is a homozygote or heterozygoes for that myostatin mutation (Casas *et al.* 2004, Dunner *et al.* 2003, Smith *et al.* 2000, Sellick *et al.* 2007). A common example of these mutations in cattle is that seen in the Belgium Blue breed, in which the resulting muscle hypertrophy is quite marked.

Bovine myostatin mutations can affect carcass charactristics by significantly increasing retail beef yield, decreasing fat and increasing the proprotion of 'expensive cuts' (Casas *et al.* 2004, Gill *et al.* 2008, O'Rourke *et al.* 2009, Sellick *et al.* 2007, Cafe *et al.* 2012). Research has also shown that feed efficiency may be beneficially affected in cattle with myostatin mutation (Cafe *et al.* 2012). The extreme muscularity observed in some of the myostatin mutation carriers can be associated with undesirable effects such as: significant reduction in reproductive traits, particularly in breeding females; a decrease in calf survival and decreased fitness due to reduced vital organs capacity (Arthur, 1995; Arthur, 1988; Hanset, 1979; Vissac, 1973). These issues are mainly connected with homozygous expression. Breeding strategies can be implemented by beef producers to select for heterozygous; benefiting from positive traits and minimising negatives ones. Due to the extra demands required to adequatly manage myostatin mutation herds (strict breeding documentation and management, DNA test, etc), this practice is one that should not be widely advocated (Casas *et al.* 2004, Alford *et al.* 2009).

A recent Australian study estimated that feedlot cattle under short (around 100 days on feed) and long fed systems (220 plus days on feed) have non-compliance levels with beef market specifications of 28 and 29%, respectively (Slack-Smith *et al.* 2009). The cost of non-compliance for P8 fat (fat cover measured at a standard site on the rump) specification alone can vary between $16 and $80 per carcass. Heifers have earlier maturing growth pathways than steers, and under a feedlot finishing situation this can represent a challenge for meeting market specification, as carcasses from heifers tend to have more fat and higher ossification scores than those from steers (McIntyre *et al.* 2009, McKenna *et al.* 2002) at a given live weight. The outcomes from the recently concluded MLA funded Maternal Productivity Project has indicated that selection for positive genetic fat is very important to maintain desired fertility levels in beef breeding herd, especially in tough seasons. Anecdotal evidence from Southern Australian states as well as from New Zealand suggests that over that last couple of years there has been a higher preference for positive fat EBV sires in beef bull sales. This trend to increase genetic fat in beef herds may results in higher incidences of non-compliance due to over fatness especially in feedlot finished animals. Given the effects that myostatin mutations have on muscle development and fat deposition, myostatin presents as a prime candidate for manipulation to increase the yield, reduce the proportion of over-fat progeny and lift production efficiency of beef cattle.

Research in laboratory animals using myostatin targated immunotherapies or endogenous enzymes, have shown that if myostatin is absent in the body, or its pathway is disrupted, increased muscle development is observed (Lee, 2007; Liang *et al.* 2007, Miyake *et al.* 2010; Zhang *et al.* 2011 and Fuentes *et al.* 2013). Much of the previous experimental work to

block/disrupt myostatin action involved genetic manipulation and focused on the potential application in human muscle disorders (Zhang *et al.* 2011, Lee 2007, Whittemore *et al.* 2003). A more innate immuno-therapy approach (no genetic modification involved) was tested in the current study. The feasibility of four myostatin peptide-conjugate vaccines was tested. In the present study, the vaccine was inoculated in young terminal heifers to evaluate efficacy to induce an immune response to myostatin, decrease myostatin activity and thereby promote increased muscle growth and possibly decreased fat deposition.

The generation of a successful immune and physiological response to myostatin may have vast applications and implications for the livestock industry. For the purpose of the present feasibility study, a feedlot finished heifer system was used because it offered the ideal discrete situation to test the proposed hypothesis. Angus heifers with known EBVs were used.

2.0 Projects objectives

The aims of this project were:

- Investigate the best approach for the development of a candidate vaccine against myostatin that have the highest probability to an induce immune response in cattle
- Identify and develop the best candidate vaccines
- Measure immune response in treated young cattle
- Measure muscle growth response
- Measure carcass attributes in treated animals including meat quality
- Cost benefit analysis

3.0 Methodology

3.1 Animals

3.1.1 Type of cattle

The female cattle selected for this experiment were derived from a herd of purebred Black Angus cattle from Department of Agriculture and Food of Western Australia's Vasse Research Centre. The breeding herd was part of the Beef CRC Maternal Productivity herd and consisted of dams selected for extreme genetic fatness based on Estimated Breeding Values (EBV) for rib fat (Laurence, 2010). To generate the heifers for the present study, the dams were joined over a nine week period to bulls with equivalent estimated breeding values for genetics fatness i.e. high fat EBV dams were joined with high fat EBV sires and low fat EBV dams were joined with low fat EBV sires. The resulting progeny were therefore classed as either genetically fat or genetically lean.

3.1.2 Selection of cattle

Calves born between May and August 2012 were weaned in early January 2013. The base Angus herd had 73 female progeny that would be at least 6 months old by the time the first vaccine was administered, from which 50 heifers were ultimately selected for this experiment. Upon weaning each heifer was weighed, ultrasound scanned and had their hip height measured. The ultrasound scans were carried out by an Agricultural Business Research Institute accredited scanner using a 3.5 MHz/180mm linear array animal science

probe (Esoate Pie Medical, The Netherlands). Hip height was measured using a retractable tape measure, affixed to the top of the crush. The distance from the floor to the top of the crush was recorded and individual heifer measurements form the top of the crush to their hip later subtracted from this value to produce the hip height measurement. Breedplan parental average EBVs (not including heifers own measurements) were obtained for each of the progeny for a variety of production traits including birth weight, 200 day weight (weaning weight), 400 day weight (yearling weight), carcass weight, eye muscle area (EMA), rib fat, rump fat, retail beef yield and intramuscular fat (IMF). The EMA, rib fat and IMF for each animal was measured using a hand held portable ultrasound machine placed dorsally over the intercostal space between the 12^{th} and 13^{th} rib. The rump fat was measured by placing the ultrasound over the P8 site.

Selection occurred at weaning when the heifer progeny were approximately five months of age (average 164 days). Allocation to treatment groups were based on a variety of phenotypic and genetic (based on relevant parental average EBVs) ensuring groups were balanced for the various parameters. The phenotypic measurements used for selection included birth weight, weaning weight, hip height and ultrasound scanning results for EMA, rib fat, rump fat and IMF at weaning. The EBVs for EMA, rib fat, rump fat, IMF, 400 day growth, retail beef yield and carcass weight were then assessed to ensure the groups were balanced.

As described earlier, these measurements were used along with chosen EBVs to select 50 of the original heifers and allocate them in a balanced manner to one of 5 treatment groups. They consisted of 14 heifers in the control group and 9 heifers in 4 candidate vaccine groups. The higher number of heifers in the control group was aimed at increasing the statistical power to identify physiological responses. The design therefore aimed to identify differences between the treated groups and the control, not differences between treated groups. No significant variations existed between the treatment groups for the EBVs relating to EMA, rib fat, rump fat, IMF, 400 day growth, retail beef yield or carcass weight ($P>0.05$). No significant variations existed between the treatment groups for the phenotypic measurements which included actual birth weight, weaning weight, EMA, rib fat, rump fat, IMF or hip height ($P>0.05$).

3.1.3 Myostatin mutation screening

All heifers were tested for the presence of naturally occurring myostatin mutations. The testing screened for the *Nt419(del7-ins10)*, *Q204X, E226X, Nt821(del11), E291X, C313Y, D182N* and *S105C* mutations which are known to cause the double muscling phenotype (Berry *et al.*, 2002; Cappucio, 1998; Grobet *et al.*, 1997; Grobet *et al.*, 1998; Kambadur *et al.*, 1997; Karim *et al.*, 2000; McPherron and Lee, 1997). The mutations were screened for using single nucleotide polymorphism assays on DNA obtained from hair samples collected from each animal. All animals were found to be genetically free of these mutations.

3.2 Housing

The heifers were housed at Vasse Research Centre throughout the duration of the study. Upon weaning they were housed as a group of 50 within a small paddock for 12 weeks before entering the feedlot. The lot feeding period consisted of two phases. The first 15 weeks included feeding in individual pens for residual feed intake (RFI) test (see section 3.7). This was followed by 4 weeks of feeding in groups to finish the heifers and to minimise the effects of stress from mixing prior to slaughter. The individual pens consisted of $100m^2$ (20m by 5m dimensions), each with a 1.2m long, 0.5m wide, 0.5m deep metal feed bunk on a 2.5m concrete pad which was covered with a metal roof. A plastic auto re-filling water trough (approximately 16L) was located at the back of the pen. The individual pens were centralised

around a handling area consisting of yards, races and a twin crush system allowing for easy and minimal handling during the individual lot feeding period. For the final 4 weeks before slaughter, the three group pens consisted of 803m^2 (51m by 15.75m dimensions), each with a 10m long, 0.6m wide, 0.5m deep concrete feed bunk on a 2.15m concrete pad. A metal roof covered the feed pad while the centre of the pen, where a concrete water trough (approximately 700L) was located, was protected by shade cloth. During the group feeding phase the heifers were drafted by weight into three groups so that light, medium and heavy weight animals were housed separately. To eliminate pen effect the 3 groups were rotated in the 3 pens returning to a different pen each time live weight measurement was carried out.

3.3 Feed rations and regime

Before entering the feedlot the heifers were fed ration 0 which consisted of *ad libitum* access to good quality hay. When they were placed in the individual pens they were fed hay on the first day then adaptation to feedlot ration started. Rations with increase grain inclusions were used (20% to 40% to 60%). The remainder of the ration consisted of hay and mineral supplementation. The initial protocol consisted to feed Ration 1 for the first four days followed by ration 2 for the following four days before introducing animals to the final ration (Ration 3). In reality, to avoid issues with indigestion and consequent welfare, progress to following diet did not occur until all animals' daily intake of diet reached at least 1.5% of their live weight for at least 2 consecutive days. Complete adaptation was achieved within 2 weeks. Ration 3 was then fed until the end of the individual lot feeding period. During the final month prior to slaughter when the heifers were placed in group pens, they were fed ration 2 until slaughter as many had already reached minimum market specifications for fat and weight. The composition and nutritional analysis of each ration fed during the individual feeding period is outlined in Table 1. Both ration and water were fed *ad libitum.*

Table 1: Ration composition and nutritional analysis

	Ration 0	Ration 1	Ration 2	Ration 3
Ingredients	Hay	Hay (80%), Grain (20%), Minerals	Hay (60%), Grain (40%), Minerals	Hay (40%), Grain (60%), Minerals
DM (%)	91.5	90.7	90.8	91.2
Crude protein (%)	12.5	11.5	12.0	12.9
DMD (%)	59	62.0	67.1	71.0
Ash (%)	4	5.3	5.9	5.8
Organic matter (%)	96	94.7	94.1	94.2
OMD (%)	59	62.3	67.2	71.3
DOMD (%)	57	59.0	63.2	67.2
ME (mj/kg DM)	8.4	8.8	9.6	10.3
NDF	67.4	60.1	57.3	53.3

Dry matter (DM), Dry matter digestibility (DMD), Organic matter digestibility (OMD), Dry organic matter digestibility (DOMD), Metabolisable energy (ME), Neutral detergent fibre (NDF)

3.4 Cattle treatments

3.4.1 Routine treatments

All heifers received standard immunisation (1 initial dose followed by booster dose 4 weeks later) for Clostridium disease (7 in 1) and Pestivirus (Pestigard) prior to weaning. A follow up dose of 7 in 1 was also administered prior to introduction to feedlot. During week 8 of the study, each animal received vaccinations against the *Mannheimia haemolytica* pathogen (bovine respiratory disease) and bovine viral diarrhoea virus (bovine pestivirus). A booster dose of each vaccine was given during week 14. During week 10 each animal was back-lined for routine intestinal parasite and ectoparasite control using 0.5 mg/kg of 'Cydectin' (moxidectin, triclobendazole). A timeline of the activities throughout the experiment are illustrated in Table 2.

Table 2: Activity timeline for the 50 heifers throughout the experimental period

Week	Activity	Housing
	Weeks 0 to 12	
0	LW, scan, group allocation	Paddock
1	LW, bleed, 1st vaccination	Paddock
2	LW, bleed	Paddock
4	LW, bleed, 2nd vaccination	Paddock
6	LW, bleed	Paddock
8	LW	Paddock
	Weeks 13 to 27	
13	LW, feedlot induction	Feedlot (group pens)
14	LW, scan	
15-27	LW (weekly), DFI, bleed (week 20), scan (week 27)	Feedlot (individual pens)
	Weeks 28 to 33	
28-32	Finishing	Feedlot (group pens)
33	Slaughter, bleed	Feedlot (group pens)

Live weight (LW), Daily feed intake (DFI)

3.4.2 Vaccine design

The rationale behind the vaccine design is that when animals are injected with a preparation containing proteins an immune response will generally be generated resulting in the production of antibodies (i.e. molecules of the immune system specific for the injected preparation). Myostatin is a protein that is naturally produced so it is not normal for an animal to raise an immune response against a protein that is seen as "self". To do this a small part of the myostatin protein (a peptide) was attached (conjugated) to a much larger foreign carrier protein. When the protein complex is injected along with an adjuvant the animal raises an immune response against the entire complex including the small part (peptide) of myostatin. Thus antibodies are produced by the animal which recognise and bind to parts of the myostatin, thereby inhibiting the action of myostatin.

3.5 Experimental vaccination protocol

Each animal from the original groups 2, 3, 4 and 5 received an initial dose (1ml) of the respective vaccine , administered subcutaneously on the crest of the neck. Two weeks later, the animals then received a second dose containing the same peptide as their initial vaccination. The animals in the control group did not receive any treatment. Table 3 shows the experimental design.

Table 3: Experimental design

Treatment group	Number of heifers	Intervention
1 (Control)	14	No inoculation
2	9	Inoculation of peptide 1 vaccine
3	9	Inoculation of peptide 2 vaccine
4	9	Inoculation of peptide 3 vaccine
5	9	Inoculation of peptide 4 vaccine

3.6 Immune response measurements

Multiple blood samples were taken from each animal throughout the experiment to measure the antibody titre levels generated against the target myostatin peptide. A total of 6 blood samples were taken from the jugular vein of each animal. The first sample was taken immediately prior to the administration of the initial experimental vaccination to measure a baseline for comparison of antibody titres in post-vaccination blood samples. Blood was also collected 2, 4, 6 and 20 weeks post-initial vaccination as well as at slaughter. The presence of antibodies formed against each of the target peptides were evaluated by enzyme-linked immunosorbent assay (ELISA) testing on serum samples obtained from the blood collections. The immune fold change in antibody titre levels relative to the baseline levels observed in the pre-vaccination bleed for each individual animal was used to determine whether an immune response was generated and to assess the efficacy of the secondary vaccination. An immune response was defined as a titre increase of 12.5% (i.e 1.125 fold increase) or greater above the pre-immune levels. The response was then categorised as a fixed effect in: > 1.25 High response, >1.12 Moderate response, <1.12 low response. For statistical analysis the animals deemed as non-responders or having low response (from original treatments 2, 3, 4 and 5) were allocated to a 6th treatment group.

The 6 resulting groups were again analysed for variations in phenotypic measures and EBVs to ensure that the groups were balanced prior to statistical analysis proceeded (Table 4).

Measurement of Serum Myostatin

The level of myostatin in the serum of each individual animal was quantified using the R&D Systems Quantikine® ELISA (cross reactivity was not identified in other commercially available kits) for GDF-8/myostatin as per the manufacturer's instructions. GDF-8 in each serum sample was activated and diluted as directed for human serum and plasma.

Table 4: Descriptive statistics of the phenotypic pre-treatment measures and EBVs of heifers in the 6 treatment groups generated post-immune response analysis

	Treatment Group						
	Group 1	Group 2	Group 3	Group 4	Group 5	Group 6	
	Control	Peptide 1	Peptide 2	Peptide 3	Peptide 4	Nil response	Statistical Sig.
N	14	8	4	6	8	10	
	Pre-treatment Measures						
Birth Weight (kg)	35.6 ± 0.9	35.5 ± 1.2	34.3 ± 1.7	35.7 ± 1.4	33.1± 1.3	32.5± 1.1	NS
Weaning Weight (kg)	268.3 ± 12.6	276.9± 14.6	262.3± 18.4	270.3± 16.1	252.8± 14.6	253.3± 13.8	NS
EMA (cm2)	48.5 ± 1.9	52.4± 2.5	47.3± 3.4	47.2± 2.8	49.2± 2.5	47.8± 2.2	NS
Rib Fat (mm)	5.4 ± 0.5	5.6± 0.6	5.8± .8	5.8± 0.7	4.6± 0.6	5.1± 0.5	NS
P8 fat (mm)	6.8 ± 0.6	6.4± 0.7	7.0± 1.0	7.0± 0.8	5.9± 0.7	6.1± 0.7	NS
IMF (%)	2.3 ± 0.2	2.4± 0.3	2.3± 0.4	2.6± 0.3	2.4± 0.3	2.7± 0.3	NS
Hip Height (cm)	112.2 ± 1.3	112.3± 1.6	109.3± 2.1	111.9± 1.8	109.4± 1.6	110.0± 1.5	NS
	Estimated Breeding Values (EBVs)						
EMA EBV	1.9± 0.5	1.9± 0.5	1.9± 0.5	1.6± 0.5	1.7± 0.5	2.0± 0.5	NS
Rib Fat EBV	M-0.2 ± 0.3	M-0.2 ± 0.3	M-0.4 ± 0.4	0.1± 0.3	M-0.3 ± 0.3	M-0.1 ± 0.3	NS
P8 fat EBV	M-0.2 ± 0.4	M-0.2 ± 0.4	M-0.3 ± 0.4	0.0 ± 0.4	M-0.3 ± 0.4	M-0.1 ± 0.4	NS
IMF EBV	0.5 ± 0.2	0.5 ± 0.2	0.6 ± 0.2	0.6 ± 0.2	0.5 ± 0.2	0.5 ± 0.2	NS
400 day growth EBV	60.5^{A} ± 3.7	61.1^{A} ± 4.0	59.9^{AB}± 4.6	61.7^{AB}± 4.2	56.9^{AB}± 4.0	57.7^{B}± 3.9	NS
Retail Beef Yield EBV	0.2 ± 0.2	0.2 ± 0.2	0.1 ± 0.2	0.0 ± 0.2	0.2 ± 0.2	0.2 ± 0.2	NS
Slaughter age	440± 4	434± 5	433± 6	435± 6	432± 5	432± 5	NS

^{A,B} Groups within a row with different letters are different (P<0.05)

3.7 Feed efficiency

A standard net feed intake test was performed (Exton, 2001). Feed intake was recorded for each animal during the individual pen feeding phase. The weight of feed offered each morning to individual heifers was recorded. Any uneaten feed the following morning was removed and its weight was deducted from the amount offered in order to determine the feed intake for each animal on a daily basis (daily intakes and live weight measured prior to the full adaptation to final ration were not used in the feed efficiency calculation). Live weight data was recorded on a fortnightly basis whilst the heifers were housed together and then on a weekly basis during the individual lot feeding phase. No fasting was applied prior to weighing and heifers were always weighed at the same time of the day (early in the morning) to minimise variation. Live weight was recorded for each animal by running them through a crush system equipped with electronic scales. Their weight gain whilst on individual rations was used to calculate their ADG. Performance on feed was measured in two ways – feed conversion ratio (FCR) and residual feed intake. The FCR is simply a measure of how well an individual converts a mass of feed into a mass of body weight. This ratio can be calculated by dividing the animal's average daily feed intake by its average daily gain (ADG). The RFI is also a measure of feed efficiency, which predicts the expected feed intake for an individual animal and uses this to calculate the residual portion between the expected feed intake and the actual feed intake. Slaughter measurements

3.8 Carcass measurements

All 50 heifers were slaughtered on the same day at an average of 436 ± 12 days of age. They were transported to the abattoir on the previous day and remained in their respective group (3) until time of slaughter. The management tag and time of slaughter was recorded for each animal. A blood sample was collected upon exsanguination to assess for antibody titres. Each body was then electrically stimulated using low voltage to ensure they reached the correct pH and temperature window. No fat was trimmed from the left side of the body. Kidney, knob and channel fat (KKCF) from the left side was removed and weighed, so the contribution of these deposits to total body fat could be later determined. The bodies were Achilles hang in the chiller and Meat Standards Australia (MSA) graded at a square cut made between the 12^{th} and 13^{th} rib approximately 12 hours after slaughter (AUS-MEAT, 2005; MLA, 2006). Relevant data taken for MSA grading included hot standard carcass weight (HSCW), EMA, rib fat, rump fat, ossification and MSA marbling score. For further information see McGilchrist *et al.* (2012).

The carcasses were chilled overnight and the left hand side was boned the following day. The primals were removed manually by natural fall. Table 5 outlines the boning protocol that was followed for the removal of untrimmed primals from all carcasses (AUS-MEAT, 2005). AUSmeat fat trimming was not carried out on any of the carcasses or primals and the only trimming allowed was that required for hygienic reasons. Any trim from the bones from the forequarter and hindquarter were retained and packed separately. Bones were removed from the forequarter and hindquarter and these weighed. Once removed, the primals were vacuum packed individually, weighed and boxed according to the body number they were removed from.

Table 5: Boning Protocol of the left hand side carcass

Primal	AUSmeat Primal no.
Forequarter	
Blade	2300
Chuck rib flap	2265
Chuck neck	2280
Chuck roll	2275
Chuck tender	2310
Cube roll	2240
Point end brisket	2330
Rib set flap	2223
3 rib flap	-
Intercostals	2430
Forequarter shank	2360
Forequarter trim	-
Hindquarter	
Striploin	2142
Tenderloin	2150
Topside	2000
Rump	2090
Outside	2020
Navel end brisket	2340
Thick flank	2060
Flank steak	2210
Flap meat	2206
Flap fat	-
Hindquarter shin and heel	2360
Hindquarter trim	-

3.8.1 Computed Tomography Scanning

The untrimmed primals listed in Table 5 from the left side of each carcass were weighed before being CT scanned within 96 hours of slaughter. This provided an accurate weight measurement for correlation with the CT scan data. A Siemens Somatom Emotion 16 slice CT Scanner (Seimens Ltd, Munich, Germany), was used to scan the untrimmed primals. Once all the primal cuts of each bed load were in the scanning field, spiral abdomen protocol was selected.

The settings for each scan were set as follows: field of view 500mm, current 200 mA, and voltage 110 kV. The scan slice width was 10 mm, with each slice taken serially. The pixel height and depth ranged between 0.834 and 0.977 mm depending on the size of the bed load with a mean of 0.969mm. The images produced from the CT scanner were edited using

Image J version 1.37v (National Institute of Health, USA) to partition off areas of the images that were irrelevant for determining composition.

The quantity of muscle and fat was analysed using the thresholding method. The discrimination point to identify the hounsfield barriers for associating pixels with muscle or fat were –235 to 2.3 for fat and 2.4 to 164.3 for lean. Microsoft Excel® was used to partition each image into muscle and fat based on these hounsfield thresholds. An estimate of volume using cavalieri's method (Gundersen *et al.*, 1988; Gundersen and Jensen, 1987) was calculated as follows:

$$\text{Volume}_{\text{Cav}} = d \times \sum_{g=1}^{m} \text{area}_g - t \times \text{area}_{max}$$

Where m is the number of CT scans taken and d is the distance between cross-sectional CT scans, in this case 1 cm. The value of t is the thickness of each slice (g), in this example 1 cm, and area max is the maximum area of any of the m scans.

The average of the hounsfield units of the pixels of each component was then determined and converted into density (kg/L) using a linear transformation (Mull, 1984). This was then used along with the volume of each component to determine the weight of fat and muscle, which was then expressed as a percentage of primal weight at the time of scanning.

3.8.2 Intra-muscular fat analysis

Approximately 40 g of diced muscle from the 12th and 13th rib section of the *M. longissimus thoracis* was collected in 50 ml tubes. Samples were weighed and stored at -20°C until subsequent freeze drying. Samples were freeze-dried using a Scanvac Coolsafe PRO 95-15™ (Labogene APS, Lynge, Denmark) and then re-weighed. The IMF% was determined using a near infrared procedure in a Spectro Star 2400 (Unity Scientific Inc., Model No. 2400, Brookfield, Connecticut, USA). NIR readings were validated with chemical fat determinations using solvent extraction. IMF was expressed as percentage fat on a wet matter basis.

3.8.3 Shear force measurement

Approximately 200g muscle from the 12th and 13th rib section of the *M. longissimus thoracis* was used for shear force testing. Samples were vacuum packed and frozen at -20°C after 14 days till subsequent testing. Steaks were thawed (4ºC) until an internal temperature of 2 to 5 ºC was reached. They were cooked in plastic bags in a water bath for 35 min at 71°C, and were cooled in running water for 30 min after cooking. Five cores (~3 - 4 cm long, 1 cm^2 cross sectional) from each loin sample were cut and shear force was measured using a Lloyd texture analyser (Model LRX, Lloyd Instruments, Hampshire, UK) with a Warner-Bratzler shear blade fitted. Each core was sheared once.

3.9 Statistical analysis

The higher number of heifers in the control group had the objective of increasing the statistical power to identify physiological responses. The design therefore aimed to identify differences between the treated groups and the control, not differences between the various treatment groups.

Measurements of live weight, EMA, rib fat, rump fat, IMF, at 2, 5 and 7 months post vaccination, feed efficiency measures plus carcass traits were analysed using a linear mixed effect model in SAS (SAS, 2001). The fixed effect in the model was treatment group and sire was used as the random term as there were multiple progeny from the same sire. For the

measurements taken at 2 or 5 months post vaccination or at slaughter, the corresponding 2 or 5 month live weight or carcass weights was included in the model as a covariate but for all live and carcass measures, the inclusion of weight in the model did not affect the significance of the treatment term so weight was removed from the model. The corresponding EBV to the dependent variable (i.e. the IMF EBV when analysing IMF percentage or MSA marbling) was also included in the model as a covariate and interacted with treatment but the parent mid-point EBVs did also not affect the significance of the treatment term. Terms and interactions that were non-significant (P>0.05) were removed from the model in a step-wise fashion.

CT Muscle and fat percentages were also analysed using a linear mixed effect model in SAS (SAS, 2001). The fixed effects in the model were treatment group and carcass primal plus their interaction. Sire and carcass ID were used as random terms. Rib fat EBV was also included in the model as a covariate, plus the interaction with treatment. Interactions that were non-significant (P>0.05) were removed from the model.

4.0 Results

4.1 Live animal measurements

4.1.1 Immune response

The immune response was measure via ELISA in bloods collected from live animals at four occasions. The blood collected at slaughter was deemed not to be of satisfactory quality and therefore not used. Each bleed was considered an independent measure so rather than average the antibody response over the four bleeds, the highest response over the four bleeds was used for each animal. As mentioned earlier, animals with low or nil response were allocated in the analysis to treatment group 6. Table 6 shows the immune response for the various treatment groups.

Table 6: Treatment group least squared means (± standard error) for immune maximum immune response to candidate vaccines

	Treatment Group						
	Group 1	Group 2	Group 3	Group 4	Group 5	Group 6	
	Control	Peptide 1	Peptide 2	Peptide 3	Peptide 4	Nil response	Statistical Sig.
N	14	8	4	6	8	10	
Immune response	1.0 ±0.05[a]	1.76 ±0.07[f]	1.34 ±0.10[c]	1.59 ±0.08[e]	1.45 ±0.07[d]	1.13 ±0.06[b]	P<0.05

[a,b,c,d,e,f] Groups within a row with different letters are different (P<0.05)

4.1.2 Phenotypic measurements

Treatment group did not have a significant effect on live weight at two months, five months or 7 months post treatment (Table 6), irrespective of whether the model was corrected for 400 day growth EBV. Treatment group did not have a significant effect on scanned EMA at either two months or five months post treatment (Table 7). The inclusion of live weight in the statistical model did not impact the significance of the treatment group effect on EMA. Treatment group did not have a significant effect on rib fat or rump fat at either two months or five months post treatment (Table 7). The inclusion of rib fat EBVs, rump fat EBVs and live weight in the statistical models did not impact the significance of the treatment group on rib or rump fat. Treatment group did not have a significant effect on IMF at either two months or five months post treatment (Table 7). The inclusion of IMF EBVs and live weight in the statistical model at either time points did not impact the significance of the treatment group effect on IMF.

Table 7: Phenotypic measurements taken at two, five and seven months post initial vaccination treatment

	Treatment Group						
	Group 1	Group 2	Group 3	Group 4	Group 5	Group 6	
	Control	Peptide 1	Peptide 2	Peptide 3	Peptide 4	Nil response	Statistical Sig.
N	14	8	4	6	8	10	
	Two Months Post-treatment						
Scanned EMA (cm^2)	54.8±1.74	58.0±2.28	54.9±3.20	58.7±2.63	55.3±2.28	52.9±2.05	NS
Scanned Rib fat (mm)	4.96±0.30	4.91±0.38	5.17±0.51	5.22±0.43	4.41±0.38	4.76±0.34	NS
Scanned Rump fat (mm)	6.10±0.32	5.66±0.40	6.14±0.55	6.07±0.46	5.42±0.40	5.09±0.37	NS
Scanned IMF (%)	2.83±0.18	2.98±0.24	2.76±0.33	3.22±0.27	2.62±0.24	2.84±0.21	NS
Live Weight (kg)	293±13.0	302±14.8	292±18.2	298±16.2	273±14.8	278±14.1	NS
	Five Months Post-treatment						
Scanned EMA (cm^2)	74.8±1.79	72.4±2.42	72.3±2.63	75.3±2.45	71.6±2.18	72.4±2.02	NS
Scanned Rib fat (mm)	9.19±0.60	9.86±0.69	9.32±0.84	8.98±0.75	8.83±0.69	8.64±0.65	NS
Scanned Rump fat (mm)	11.7±0.90	12.1±1.07	10.2±1.36	12.2±1.18	10.2±1.07	10.9±1.00	NS
Scanned IMF (%)	6.34±0.24	6.51±0.31	5.93±0.44	5.65±0.36	6.05±0.31	5.90±0.28	NS
Live Weight (kg)	421±16.0	433±17.9	414±21.4	423±19.3	395±17.9	397±17.1	NS
	Seven Months Post-treatment (point of slaughter)						
Final live weight (kg)	458±16.9	467±18.8	449±22.3	449±20.2	435±18.8	430±18.0	NS

4.1.3 Performance and feed efficiency measurements

Treatment group did not have a significant effect on ADG, feed conversion ratio or residual feed intake as indicated in Table 8. The inclusion of live weight or 400 day weight EBV in the statistical model did not impact the significance of the treatment group effect on ADG, feed conversion ratio or residual feed intake.

Table 8: Live animal growth performance and feed efficacy traits

	Treatment Group						
	Group 1	Group 2	Group 3	Group 4	Group 5	Group 6	
	Control	Peptide 1	Peptide 2	Peptide 3	Peptide 4	Nil response	Statistical Sig.
N	14	8	4	6	8	10	
Average daily gain (kg)	1.47±0.08	1.56±0.09	1.41±0.11	1.48±0.09	1.42±0.09	1.39±0.08	NS
Feed conversion ratio	6.63±0.24	6.33±0.29	6.71±0.38	6.69±0.33	6.45±0.29	6.66±0.27	NS
Residual feed intake	-0.02±0.12	-0.12±0.16	0.14±0.23	0.22±0.19	-0.04±0.16	-0.04±0.14	NS

4.2 Post mortem measurements

4.2.1 Carcass measurements

Treatment group did not have a significant effect on HSCW (Table 9). The inclusion of carcass weight EBVs in the statistical model did not impact the significance of the treatment group effect on HSCW.

Treatment group did not have a significant effect on rib fat, rump fat, ossification, EMA or MSA index (Table 8). The inclusion of HSCW in the statistical model did not impact the significance of the treatment group.

Treatment group did have a significant effect on visually assessed MSA marbling score ($P<0.05$) but not on chemically determined IMF percentage (Table 9). Group 5 had the lowest marbling score and was 22.8 ± 6.82 and 16.3 ± 7.42 units lower than groups 1, and 6 ($P<0.01$) respectively. Group 1 (controls) had the highest marbling score, which was 12.66 ± 6.82 units higher than group 2 ($P=0.07$). No significant differences were seen between the other treatment groups for MSA marbling scores.

There was no significant effect of treatment group on shear force or cooking loss of the *longissimus thoracis* which had been aged for 14 days ($P>0.05$). However there was a slight trend ($P<0.1$) for group 2 to be more tender than the control group.

4.2.2 Yield measurements

Treatment group did not have a significant effect on carcass dressing percentage, percentage of bone or the percentage of KKCF (Table 9).

Treatment group had a significant effect on the percentage of muscle and fat in the CT scanned primals ($P<0.05$, Table 9). Group 2 had 1.45%, 1.95%, 2.87%, 2.23% and 2.37% greater muscle yield in the primals compared to groups 1, 3, 4, 5 and 6 respectively ($P<0.05$). The CT primal muscle percentages of all other treatment groups were not different. Meanwhile group 2 similarly had 1.42%, 1.94%, 2.87%, 2.24% and 2.42% lower fat yield in the primals compared to groups 1, 3, 4, 5 and 6 respectively ($P<0.05$). The CT primal fat percentages of all other treatment groups were not different.

The interaction between rib fat EBV and treatment group had a significant effect on the CT primal percentage of muscle and fat (Figures 4 and 5, $P<0.05$). Figures 4 and 5 show that in Group 2 animals, as rib fat EBV increased, the percentage of muscle and fat in the primals as determined by CT scans did not change significantly. However in the control group 1, as rib fat EBV increased, CT primal fat percentage increased by 4.34% (Figure 5) from 36.1 ± 1.5 to 40.4 ± 1.6 percent ($P<0.05$). In the control group 1, CT primal muscle percentage decreased by 4.31% as rib fat EBV increased from -1 to 1mm (Figure 4) from 63.9 ± 1.46 to 59.6 ± 1.58 ($P<0.05$). As an attempt to further understand the interaction between EBV and physiological response in Group 2 as compared to control serum myostatin (measured by ELISA) was measured in stored samples. There was no significant correlation between EBV

nor immune response with the serum concentration of myostatin.

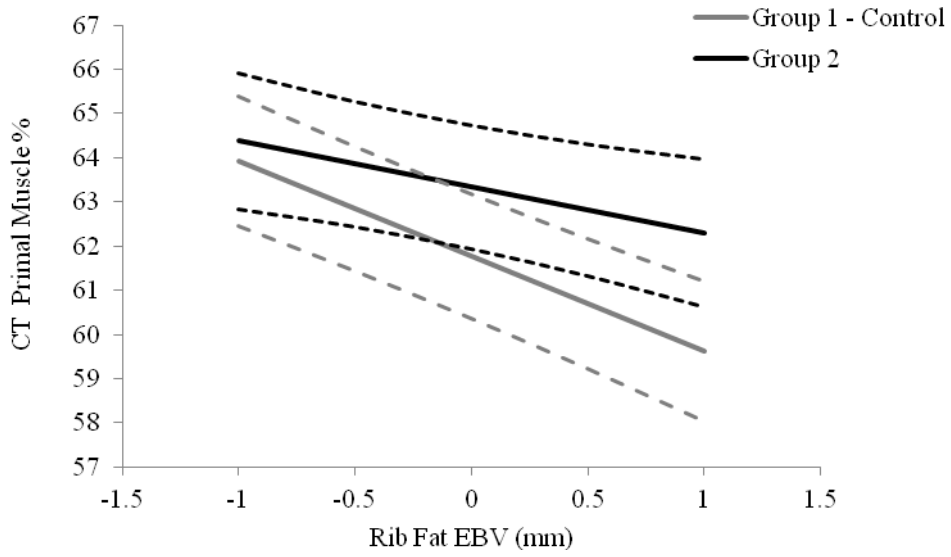

Figure 4: The effect of Rib Fat estimated breeding value (EBV) on CT primal muscle percentage. Full lines represent averages and dashed lines standard errors.

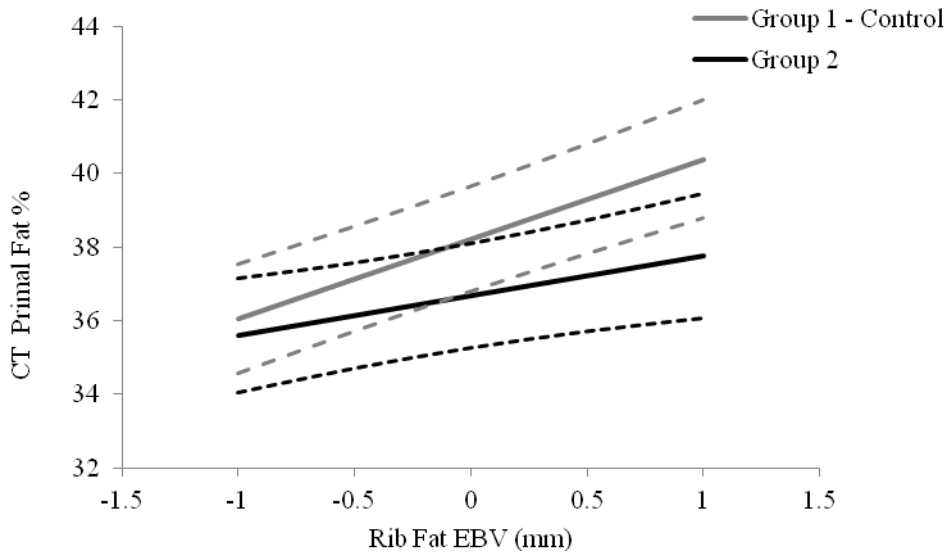

Figure 5: The effect of Rib Fat estimated breeding value (EBV) on CT primal fat percentage. Full lines represent averages and dashed lines standard errors.

Primal cut had a significant effect on the percentage of muscle and fat as determined by CT scanning ($P<0.001$). Figure 6 shows that the Topside had the highest percentage of muscle and the lowest percentage of fat, while the flank fat had the highest percentage of fat and lowest percentage of muscle. The interaction of primal with treatment group was not significant.

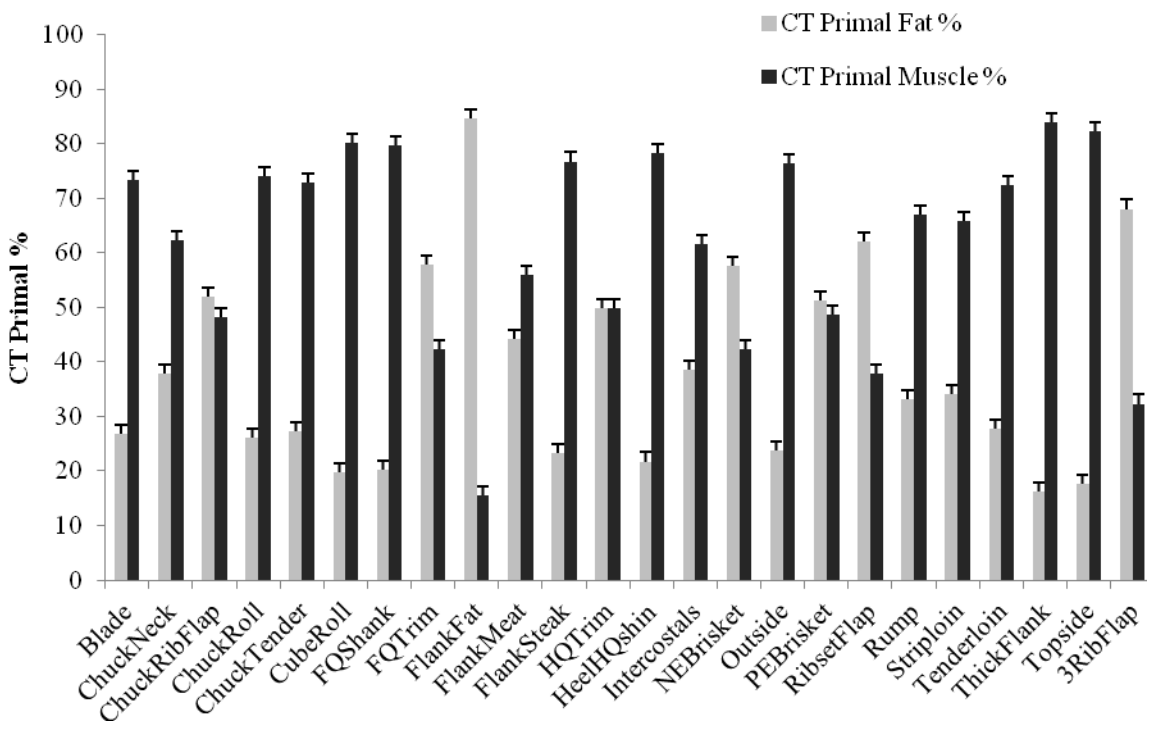

Figure 6: Percentage of fat and muscle in various primal cuts as measured by CT scan.

Table 9: Treatment group least squared means (± standard error) for carcass and yield measurements

	Treatment Group						
	Group 1	Group 2	Group 3	Group 4	Group 5	Group 6	
	Control	Peptide 1	Peptide 2	Peptide 3	Peptide 4	Nil response	Statistical Sig.
N	14	8	4	6	8	10	
Rib fat (mm)	13.0±1.05	13.6±1.30	15.1±1.73	13.5±1.47	14.4±1.30	13.5±1.20	NS
Rump fat (mm)	12.3±1.18	12.4±1.39	13.3±1.76	12.1±1.54	12.1±1.39	11.7±1.31	NS
HSCW (kg)	243±9.41	246±10.4	230±12.3	241±11.1	231±10.4	229±10.0	NS
Ossification	139±1.59	145±2.09	142±2.94	140±2.41	143±2.09	140±1.88	NS
MSA Marble score	353±4.70^{c}	340±5.95^{ab}	339±8.07^{abc}	345±6.79^{bc}	330±5.95^{a}	346±5.44^{bc}	P<0.05
Chemically determined IMF%	4.12 ± 0.47	3.96 ± 0.57	3.06 ± 0.76	4.23 ± 0.65	3.63 ± 0.58	4.48 ± 0.53	NS
EMA (cm^2)	62.7±2.66	65.7±3.18	57.1±4.09	64.7±3.54	62.9±3.18	59.8±2.97	NS
MSA Index	62.42 ± 0.2	61.79 ± 0.25	62.03 ± 0.34	62.14 ± 0.29	61.8 ± 0.25	62.17 ± 0.23	NS
Dressing %	53.0±0.37	52.8±0.45	51.4±0.58	53.6±0.50	52.9±0.45	53.1±0.41	NS
Bone %	20.8±0.32	20.7±0.38	21.2±0.50	20.7±0.43	20.8±0.38	20.9±0.36	NS
KKCF %	2.27±0.16	2.13±0.21	2.09±0.30	2.42±0.24	2.25±0.21	2.30±0.19	NS
Primal CT Muscle %	62.0 ± 1.4^{x}	63.45 ± 1.4^{y}	61.5 ± 1.57^{x}	60.59 ± 1.51^{x}	61.22 ± 1.49^{x}	61.08 ± 1.44^{x}	P<0.05
Primal CT Fat %	37.99 ± 1.41^{x}	36.57 ± 1.41^{y}	38.51 ± 1.58^{x}	39.43 ± 1.52^{x}	38.81 ± 1.51^{x}	38.99 ± 1.46^{x}	P<0.05
Cooking Loss (%)	23.4 ± 0.6	23.4 ± 0.8	24.1 ± 1.1	23.4 ± 0.9	24.5 ± 0.8	23.5 ± 0.7	NS
Shear Force (N)	32.88 ± 2.44	29.29 ± 3.2	36.75 ± 4.56	39.86 ± 3.75	35.24 ± 3.23	37.58 ± 2.89	NS

^{a,b,c} Groups within a row with different letters are different (P<0.1)
^{x,y} Groups within a row with different letters are different (P<0.05)

5.0 Discussion

5.1 Live measurements

The live animal measurements of feed efficiency, growth, and muscle and fat scans of the heifers in this study showed no differences between the control group and those treated with conjugated myostatin peptide vaccines.

5.2 Treatment group effect on carcass fatness and muscling

The results of the present study showed that the rump or rib fat (subcutaneous fat depots) were not affected by the myostatin suppressing vaccines, however there were differences between treatment groups for MSA marbling score, with two treatment groups (2 and 5) being significantly ($P=0.07$ and $P<0.01$ respectively) lower than the control. These 2 treatment groups were the ones with the higher number of animals (8 out of 9) presenting a moderate or strong immune response to candidate vaccines. When IMF was measured chemically there was no significant difference between the treatment groups. Relationship between marbling score and IMF% can be variable due to the random nature of visual assessment. Marble score can be affected by a number of factors such as assessor, temperature, fat distribution and fatty acid composition (Tume 2001). Given that the marble score of all carcasses in this study were done by the same assessor, at the same time and carcass temperature were similar it can be suggested that in this instance fat distribution and fatty acid composition may be the cause of the inconsistency between measurements. Stearic acid, a saturated fatty acid with high melting point has major influence on the appearance of marbling (Tume 2001). Interestingly it has been reported that carcasses from cattle with myostatin mutation have increased polyunsaturated fatty acids (PUFA) and a decreased concentration of saturated fatty acids (SFA) (Fiems 2012). This will certainly impact the visual appearance of marbling but not alter the percentage when determined chemically. No other differences were found between groups in other standard carcass measurements.

Primal CT scan however showed that carcasses from treatment group 2 had significantly higher muscle % and lower fat %. Group 2 was the group with the best immune response suggesting a relationship between immune and physiological responses to myostatin. Group 2 was also the same as that used by Liang *et al.* 2007 which caused an increase in muscle mass so the results are not surprising. In a long term study, where herds were visually selected for muscle divergence, high muscling myostatin heterozygous steers had 3.5% higher meat yield (estimated from commercial boneout) than low muscling homozygous normal (no myostatin mutation) steers (Cafe *et a.*, 2012). Considering this, a difference of 1.45% in heifers with similar visual muscle score in an initial research study is a modest but considerable result.

In addition rib fat EBV had a significant effect on the CT scan primals percentage of muscle and fat. Interestingly, whilst in the control group, fat % increased and muscle % decreased (measured in primal CT scan) as rib fat EBV, in group 2 as rib fat EBV increased, the percentage of muscle and fat in the primals as determined by CT scans did not change significantly. This suggests that perhaps the response to myostatin blockage may differ in animals with different genetic potential for carcass attributes. It could be suggested that animals with greater genetic fatness may have lower levels of circulating myostatin, which are down regulated more readily than the higher concentrations in genetically lean animals. Serum myostatin levels were therefore measured by ELISA however it did not show correlation with neither EBVs nor immune response.

The economic impact of the difference in muscle measured in the present study would translate into approximately 3.5 kg extra saleable meat from a 240kg carcass. The economic impact of decreasing fatness in feedlot finished heifers, which are known to have problems becoming over-fat and missing meat specification, is harder to predict. A recent study presented the cost of non-compliance, estimated that for P8 fat specification alone could vary between $16 and $80 per carcass. Anecdotal evidence indicates that since the conclusion of the MLA funded Maternal Productivity Project, which demonstrated the importance of fat on fertility of beef breeding herd, the preference for positive fat EBV bulls in sales in New Zealand and southern Australia has increased. This shift in breeding selection may result in higher incidence of non-compliance in beef carcasses due to over fatness which could be alleviated with a myostatin vaccine. The vaccine formulations used in the current project was considered low cost (approximately $15/head for 2 doses). Assuming production under industrial scale can considerably reduce costs, it is feasible that that if further development can improve vaccine effectiveness without impacting significantly on manufacturing costs than a positive benefit cost ratio can be achieved.

5.3 Variation in immune response

Serum analysis for antibodies generated against the experimental peptides in treatment group 2 and 5 showed that 8 of the 9 animals generated a moderate to high immune response with group 2 generating a significantly higher response than group 5. It is reasonable to expect that a small portion of animals receiving any form of vaccine will fail to raise an adequate immune response. This may simply be due to an overall decrease in antibody response to the peptide in less responsive animals. These peptides generated the strongest and most constant (66.7%) immune response in the treated heifers with group 2 showing a significantly higher response than group 5. While the current study shows that it is feasible to generate an immune response against a self protein epitope the correlation with desired phenotypic expression is modest. This has been reported previously in the cancer arena where a number of clinical trials have shown immune responses to self antigens but the evidence of a clinical benefit and correlation to immune response has in some instances been poor (Rosenberg 2004 and Pardoll 2002). One of the hurdles identified has been the nature of the adjuvant used; interestingly in many cases the solution to this problem has been to include alum, as used here, to enhance the immune response (Harmid et al., 2007). But this does highlight the need to further consider the nature and formulation of the vaccine and in particular the peptide concentration and adjuvant.

Our approach has been to design epitope peptides that when combined with an appropriate adjuvant should induce epitope specific neutralising antibodies. Testing of a higher dose of peptide in the vaccine formulation would be justified. While the current project considered single peptides in each vaccine consideration should be given to trialling a mixture of peptides to maximise the efficiency of the immune response. Combining all of the peptides together would cover a greater proportion of the myostatin active site and may be synergistic.

The adjuvant within a vaccination plays an important role in stimulating an immune response, namely antibody titres. A wide array of adjuvants are commercially available, all of which aim to increase the immunity generated against an antigen, more so than if the antigen was delivered alone (O'Hagan, 2000).

Given the importance of the role that the adjuvant plays in generating an immune response, for future studies, it may be of benefit to seek an alternative adjuvant in an attempt to stimulate a more efficient Th2 immune response in a higher percentage of the treated heifers. Viable alternatives that are currently commercially available include QuilA and ISCOMATRIX. The QuilA adjuvant has been used in cattle to successfully increase immune response, measured by an increase in antibody levels (Geldhof *et al.*, 2002; Shu *et al.*, 2000;

Vercauteren *et al.*, 2004). ISCOMATRIX contains a mixture of cholesterol, phospholipid and saponin, without the antigen (Sun *et al.*, 2009). This adjuvant has been used with success in a number of animal models (Pearse and Drane, 2004). However currently there are no publications within the literature that compare the efficacy of Alum, QuilA and ISCOMATRIX in generating an antibody response in cattle. Importantly, this product does not contain any materials of animal origin so is an ideal product for use in production animal systems. ISCOMATRIX is a commercial product and is thus consistent in its preparation; it is distributed in Australia by CSL so is readily available.

6.0 Conclusion

Four vaccines designed to regulate the action of the myostatin protein had no significant effect on live animal or standard carcass measurements (except for MSA marbling score). Immune response was variable and in most instances did not strongly correlate with phenotypic responses. Nevertheless in treatment group 2, the group with the most consistent immune response, a significant increase in muscle % and decrease in fat% in the primals as determined by CT scans was observed. Muscle % in this group was 1.45% higher and fat % was 1.42% lower than the control group. The economic impact of the difference in muscle measured in the present study would translate into approximately 3.5 kg extra saleable meat from a 240kg carcass. In addition effect of rib fat EBV on primal CT scanned fat and muscle % was significantly different between group 2 and control.

It is possible that with further development of the vaccine design and composition, higher immune responses can be elicited and with this, a more marked desirable physiological response could be achieved. This could have significant impact not only for the beef industry but other livestock production systems around the world.

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