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BCRC 3 - The biology of marbling and fat distribution in relation to production systems

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Abstract

The experimental design and methods for this study were approved by the Beef CRC's 2008 Annual Scientific and Industry Review, Underpinning Science Committee, and Management Committees, and by NSW DPI Animal Care and Use Committee. The experiment is focused on strategic nutritional supplementation post-weaning to enhance intramuscular fat cell development and subsequent expression of marbling during feedlotting, and on understanding the biology of marbling. The experiment is applying strategic nutritional supplementation post-weaning across divergent genotypes for marbling and fat distribution to determine if there are nutrition x genotype interactions for development of marbling and other fat depots. The experiment will allow for detailed biological and metabolic studies of fat depot development using strategic post-weaning nutritional treatment and the divergent genotypes. The strategic post-weaning nutritional treatment aims to enhance adipocyte development prior to the major period of lipid filling of fat cells during feedlotting. These studies also aim to identify differential metabolism and expression of genes in fat depots, including those within muscles, at various developmental time points to allow fine-tuning of nutrition to maximise marbling, and with a view to assisting with future identification of early biological markers of marbling and potential gene markers. The study also aims to understand the influence of marbling in altering eating quality of cuts such as the 'outside' or *biceps femoris* muscle which has been shown to have unusually high eating quality in MSA experiments using Japanese Black (Wagyu) cattle. In addition, this experiment is providing resources and data to enhance development of the BeefSpecs phenotypic prediction models in Project 1.2.1, and to enhance the accuracy and validate ultrasound scanning of highly marbled cattle by AGBU staff. The methodologies for the study have been established or are being refined. This study addresses important industry and scientific issues that impact on capacity of producers to meet market specifications and the efficiency with which specifications are met, particularly given competition for resources such as grain for feedlot cattle. Given that this project commenced in February 2009, it is too early to draw conclusions from the limited results of the study that are available to date. Progress with the experiment was presented to the Beef CRC's 2009 Annual Scientific and Industry Review in May. It is recommended that the experiment continue as planned.

Executive Summary

The experimental design and study methods were approved by the Beef CRC's 2008 Annual Scientific and Industry Review, Underpinning Science Committee, and Management Committees, and by NSW DPI Animal Care and Use Committee. Progress with the experiment was presented to the Beef CRC's 2009 Annual Scientific and Industry Review in May.

The experiment is focused on strategic nutritional supplementation post-weaning to enhance expression of marbling during feedlotting, and on understanding the biology of marbling. The experiment is using strategic nutritional supplementation across divergent genotypes for marbling and fat distribution to determine if there are nutrition x genotype interactions for development of marbling and other fat depots. The experiment will allow for detailed biological and metabolic studies of fat depot development using the strategic post-weaning nutritional treatment and the divergent genotypes. The strategic post-weaning nutritional treatment aims to enhance adipocyte development prior to the major period of lipid filling of fat cells during feedlotting. These studies also aim to identify differential metabolism and expression of genes in fat depots, including those within muscles, at various developmental time points to allow fine-tuning of nutrition to maximise marbling, and with a view to future identification of early biological markers of marbling and potential gene markers. The study also aims to understand the influence of marbling in altering eating quality of cuts such as the 'outside' or *biceps femoris* muscle which has been shown to have unusually high eating quality in MSA experiments using Japanese Black (Wagyu) cattle. In addition, this experiment is also providing resources and data to enhance development of the BeefSpecs phenotypic prediction models in Project 1.2.1, and to enhance the accuracy and validate ultrasound scanning of highly marbled cattle by AGBU staff.

Weaner steers (n = 165, ~6 months old, liveweight 221 kg on average) within three genotypes differing in fat distributional characteristics were sourced from the Northern Tablelands region of NSW in February 2009 and located at Glen Innes ARAS. Suitable cattle for the experiment were identified in conjunction with AGBU. A combination of breed differences, within breed EBVs, and sire-lines with "elite" marbling were used to identify cattle suitable for the experiment. A base-line group of 15 steers was slaughtered immediately prior to commencement of the nutritional treatments in early March. Groups will also be slaughtered at the end of the nutritional treatments (n=30), prior to feedlot entry (n=30), and after short- (n=30) and long-feeding (n=60). Fat distributional measures have been obtained by ultrasound scan, chiller assessment, yield test, CT-scan and by weighing non-carcass depots. Fat depots and muscles have been sampled for cellular, gene expression and metabolic characteristics.The steers are being fed pasture (and other forage as required), or pasture plus high energy, maize-based pellets (12.3 MJME/kgDM, 11.0% CP/kgDM) at 1% of liveweight for ~4 months after weaning. Pasture-fed and energy supplemented steers are being backgrounded until feedlot entry at ~14 months of age. To date the 2 replicates of forage fed and the 2 replicates of supplemented cattle are growing at about the same rate (~0.65 kg/d). Due to the growth rates to date, the end of treatment slaughter group are scheduled tol be killed in early-August.

This study addresses important industry and scientific issues that impact on capacity of producers to meet market specifications and the efficiency with which specifications are met, particularly given competition for resources such as grain for feedlot cattle. Given that this project commenced in February 2009, it is too early to draw conclusions from the limited results of the study that are available to date. Progress with the experiment was presented to the Beef CRC's 2009 Annual Scientific and Industry Review in May. It is recommended that the experiment continue as planned.

Contents

1 Background

Our team has reviewed the literature on marbling development (Pethick *et al.* 2007) and have developed nutritional based hypotheses for increasing marbling using targeted feeding of starch plus ω-6 fatty acids from around weaning. We also see merit in testing these hypotheses in animals that have a known genetic propensity to marble at high levels and that differ in their fat distributional characteristics ('elite' marbling EBV Angus, and 'elite' marbling sire line Wagyu x 'elite' marbling EBV Angus cows) and comparing this with cattle with lower propensity to marble but with high propensity for fat deposition in other depots (breed average marbling EBV Hereford), identified in conjunction with AGBU.

Our knowledge of intramuscular fat is increasing (Pethick *et al.* 2006). To a large extent, this fat depot develops in parallel with others, but its commercial expression (% fat in muscle or marbling) is also dependant on muscle growth. It is proposed, that muscle growth and fat growth within the muscle occur at a similar rate until a total carcase fatness of about 30-35% is reached. After this point muscle growth slows while fat growth is maintained resulting in an increased expression of intramuscular fat (%). The exception is where a particular breed has been selected to express intramuscular fat such that there is a bias or shift in fat distribution toward the intramuscular site and expression can occur at lower and more efficient levels of total carcase fatness.

We have also postulated that diets that promote both: (i) maximal fermentation in the rumen to produce gluconeogenic precursors (propionate), and (ii) which maximise starch digestion in the small intestine increase intramuscular fat deposition. Such diets are usually associated with high levels of processing which increase the accessibility of the dietary starch granule to both microbial and animal amylases and so maximise the availability of glucose to the fattening animal (Rowe *et al.* 1999). The logic behind this hypothesis is that (i) such diets would promote increased levels of anabolic hormones (insulin) which are known to stimulate lipogenesis; (ii) the logic parallels the observation in humans that diets with a high glycaemic index (i.e. diets that allow rapid glucose absorption and concomitant high insulin levels) promote obesity (Ludwig 2000); (iii) Such diets will also deliver increased levels of net energy for lipogenesis (the reason why grain feeding promotes more intramuscular fat development compared with grass finishing) and (iv) there is evidence that marbling adipocytes show a preference for glucose/lactate carbon while subcutaneous adipose tissue uses mainly acetate as a source of acetyl units for lipogenesis (see above).

It has also been postulated that the modern obesity epidemic in humans affecting the developed world is partly related to the increased consumption of polyunsaturated fatty acids (PUFAs) of the n-6 series (Ailhaud *et al.* 2006). Convincing evidence has been put forward to show that the n-6 fatty acids (in contrast to n-3 fatty acids) promote adipogenesis *in vitro* and markedly favour adipose tissue development in rodents during early life. The suggested mechanism involves arachidonic acid (n-6 C20:4) stimulation via prostocyclin of preadipocytes to form adipocytes. There is a well described increase in intramuscular fat levels in beef cattle finished with cereal grain based diets compared to grass finished animals (Pethick *et al.* 2004) that is typically attributed to differences in net energy consumption. However such diets would also vary markedly in the ratio of n-6/n-3 fatty acids with grass based diets lower and grain based diets higher and it is tempting to speculate this may be related to the hypothesis developed by Ailhaud *et al.* (2006). Again it would seem warranted to test this hypothesis relatively early in life using interventions at this axis in livestock where the marbling genetics is well defined. The genetic propensity to favour adipocyte development at the intramuscular site would be important since there is no suggestion that the n-6 fatty acids affect one depot more than another.

Research based on traditional growth studies (see below) along with new evidence from gene expression experiments using microarray analyses also suggest critical developmental time periods (10 months of age or less for cattle) for intramuscular fat cells (Wang *et al.* 2005, 2008; Lehnert *et al.* 2006).

Within pasture/forage based systems, Beef CRC and other research have shown that nutrition prior to weaning has little impact on longer-term development of intramuscular fat (Greenwood *et al.* 2006; Cafe *et al.* 2006; Greenwood and Cafe 2007). Similarly, early weaning and short periods of concentrate feeding at various levels prior to finishing of lower marbling genotypes has variable effects on marbling (reviewed by Greenwood *et al* . 2005; Greenwood and Dunshea 2008).

However, recent evidence in Hanwoo cattle with high genetic capacity to marble has shown that increasing concentrate supplementation from weaning during a prolonged pasture-feeding (backgrounding) period enhances marbling, and that this effect persists through finishing (Hong *et al*. 2008). This finding also appears to be consistent with anecdotal information on production systems in which concentrates are provided from a young age to Wagyu x Holstein cattle backgrounded at pasture prior to long-fed finishing for supply of highly marbled Australian beef to Japan (http://www.securityfoods.com/faqs.php). Certainly, Beef CRC studies have shown that post-weaning, nutrition becomes increasingly important for intramuscular fat and marbling development (Robinson *et al.* 2001; McKiernan *et al.* 2009), while high energy grain feeding promotes marbling compared to forage-based feeding systems during finishing (Johnston *et al.* 2003; Reverter *et al.* 2003).

The potential for our strategy to specifically stimulate intramuscular adipocyte development is by no means certain, but as mentioned above, experiments targeted at early life (commencing from weaning) interventions at this axis appear warranted in cattle where the marbling genetics are well defined.

The study also aims to understand the influence on marbling in altering eating quality of cuts such as the 'outside' or *biceps femoris* muscle which has been shown to have unusually high eating quality in MSA experiments using Japanese Black (Wagyu) cattle. In addition, this experiment will also provide resources and data to enhance development of phenotypic prediction models in Project 1.2.1 (see Supporting Statement from Project 1.2.1), and to calibrate new ultrasound scanning equipment which has the capacity to better measure higher levels of marbling for use in industry breeding and selection programs (M. Walcott, *pers. comm.*).

In summary, this study has been designed to improve commercial phenotypic outcomes while enhancing our biological understanding of the regulation of fat distributional characteristics. Therefore, it will increase our capacity to discover key regulatory mechanisms for improvement of marbling and fat distribution.

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2 Project Objectives

- Establish whether fat distribution and marbling and can be significantly enhanced by strategic nutritional supplementation during backgrounding;
- Quantify interactions between strategic nutritional supplementation and differing genotypes for fat distribution and marbling, across fat depots (intramuscular, intermuscular, subcutaneous and internal fat) and major cuts and muscles;
- Enhance understanding of biology of fat distribution and marbling:
- Understand the influence on marbling in altering eating quality of cuts;
- Provide resources and data to improve models for prediction of commercial characteristics;
- Provide resources and data to improve ultrasound measurement of marbling for BreedPlan.

3 Methodology

One hundred and sixty five weaner steers (~ 6 months old, liveweight 221 kg on average) within three genotypes differing in fat distributional characteristics were sourced from the Northern Tablelands region of NSW in February 2009 and located at Glen Innes ARAS. Suitable cattle for the experiment were identified in conjunction with AGBU. A combination of breed differences, within breed EBVs, and sire-lines with “elite” marbling were used to identify cattle suitable for the experiment. The cattle genotypes are high IMF and higher subcutaneous fat (Angus: 6 sires over high marbling EBV cow herds from 2 herds of origin, average Angus Breedplan sire EBVs +2.0% IMF and approx. breed average for rib and rump fat), low IMF and higher subcutaneous fat (Hereford: six sires from two herds of origin, average Hereford Breedplan EBVs +0.4% IMF and approx. breed average for rib and rump fat), and high IMF and lower subcutaneous fat (Wagyu x Angus: six high marbling sire-line Wagyu sires over high marbling EBV Angus cows).

The steers were yard weaned, and are being fed pasture (and other forage as required), or pasture plus high energy, maize-based pellets (12.3 MJME/kgDM, 11.0% CP/kgDM) at 1% of liveweight for 4 months after weaning. Pasture-fed and energy supplemented steers will then be backgrounded until feedlot entry at ~14 months of age. Groups of cattle will then be short- (100 day) or long (200-300 day) fed.

A base-line group of 15 steers was slaughtered immediately prior to commencement of the nutritional treatments in early March. Groups will also be slaughtered at the end of the nutritional treatments (n=30), prior to feedlot entry (n=30), and after short- (n=30) and long-feeding (n=60). Fat distributional measures are being obtained by ultrasound scan, chiller assessment, yield test, CT-scan and by weighing non-carcass depots. Fat depots and muscles are also being sampled for cellular, gene expression and metabolic characteristics.

Measurements to be taken during the experiment, and ***progress*** include:

Commercial

- Backgrounding and feedlot growth: ***Nutritional treatments in progress***.
- Ultrasound scans, including additional scans beyond EMA, P8 and Rib Fat if appropriate: ***Ultrasound scanning commenced***.

- Chiller assessment (ossification, marbling, meat colour, pHu etc. - MSA): ***Baseline data collected***.
- HSCW, dressing %, and retail beef yield, fat trim, bone and primal cuts (full bone-out and/or CT-scan): ***Baseline data collected***.
- Objective beef quality characteristics (shear force, compression, cooking loss, pHu, colour, IMF): ***Baseline samples collected & stored***.
- Subjective (taste panel) meat quality (if warranted from objective assessments): ***Baseline samples collected & stored***.

Biological

- Metabolism in fat depots and muscles: ***Baseline whole-herd biopsy and slaughter groups samples collected & stored. Methods established***.
- Fat depot and muscle proteins: ***Baseline whole-herd biopsy and slaughter group samples collected & stored. Methods established***.
- CT-scan and/or bone-out dissection and/or dissection of fat depots: ***Baselines data collected. Methods being refined to allow for data on larger cattle to be obtained***.
- Gene expression: ***Baseline whole-herd biopsy and slaughter groups samples collected & stored. Methods (array, RT-RCR, bioinformatic) established and list of adipogenic and lipogenic/lipolytic genes for potential assay by RT-PCR established***.
- Blood metabolite, hormones and/or markers (depending on marker identification): ***Baseline whole-herd samples collected & stored***.
- Fat cell precursor number (depending on marker identification and availability): ***Baseline slaughter samples and whole-herd biopsy samples collected & stored***.
- Fat cell number and size (if warranted from other measurements): ***Baseline samples collected & stored. Methods established to assess internal, intramuscular, intermuscular and subcutaneous fat samples***.
- Connective tissue (structure, chemistry and gene expression if warranted from objective beef quality measurements i.e. marbling, compression etc.): ***Baseline samples collected & stored. Methods established***.
- Muscle fibre characteristics (if warranted from other measurements): ***Baseline samples collected & stored. Methods established***.

4 Results and Discussion

The experimental design and study methods were approved by the Beef CRC's 2008 Annual Scientific and Industry Review, Underpinning Science Committee, and Management Committees, and by NSW DPI Animal Care and Use Committee.

One hundred and sixty five weaner steers (~ 6 months old, liveweight 221 kg on average) within three genotypes differing in fat distributional characteristics were sourced from the Northern Tablelands region of NSW in February 2009 and located at Glen Innes ARAS. Suitable cattle for the experiment were identified in conjunction with AGBU. A combination of breed differences, within breed EBVs, and sire-lines with "elite" marbling were used to identify cattle suitable for the experiment. The cattle genotypes are high IMF and higher subcutaneous fat (Angus: 6 sires over high marbling EBV cow herds from 2 herds of origin, average Angus Breedplan sire EBVs +2.0% IMF and approx. breed average for rib and rump fat), low IMF and higher subcutaneous fat (Hereford: six sires from two herds of origin, average Hereford Breedplan EBVs +0.4% IMF and approx. breed average for rib and rump fat), and high IMF and lower subcutaneous fat (Wagyu x Angus: six high marbling sire-line Wagyu sires over high marbling EBV Angus cows).

A base-line group of 15 steers was slaughtered immediately prior to commencement of the nutritional treatments in early March. Groups will also be slaughtered at the end of the nutritional treatments (n=30), prior to feedlot entry (n=30), and after short- (n=30) and long-feeding (n=60). Fat distributional measures are being obtained by ultrasound scan, chiller assessment, yield test, CT-scan and by weighing non-carcass depots. Fat depots and muscles are also being sampled for cellular, gene expression and metabolic characteristics.

The steers are being fed pasture (and other forage as required), or pasture plus high energy, maize-based pellets (12.3 MJME/kgDM, 11.0% CP/kgDM) at 1% of liveweight for ~4 months after weaning. To date, the 2 replicates of forage fed and the 2 replicates of supplemented cattle are growing at about the same rate (~0.65 kg/d), which is our objective in order to minimise confounding due to growth rate of cattle on the different nutritional treatments.

Pasture-fed and energy supplemented steers will then be backgrounded together within replicates until feedlot entry at ~14 months of age.

Progress with the experiment was presented to the Beef CRC's 2009 Annual Scientific and Industry Review in May.

5 Impact on Meat and Livestock Industry – now & in five years time

This project has only recently commenced, hence its impact is yet to be determined. The experiment will allow for detailed biological and metabolic studies of fat depot development using strategic post-weaning nutritional treatment and the divergent genotypes. The strategic post-weaning nutritional treatment aims to enhance adipocyte development prior to the major period of lipid filling of fat cells during feedlotting. These studies also aim to identify differential expression of genes in fat depots, including those within muscles, at various developmental time points to allow fine-tuning of nutrition to maximise marbling, and with a view to assisting with identification of early biological markers of marbling and potential gene markers. The study also aims to understand the influence of marbling in altering eating quality of cuts such as the 'outside' or *biceps femoris* muscle which has been shown to have unusually high eating quality in MSA experiments using Japanese Black (Wagyu) cattle. In addition, this experiment is providing resources and data to enhance development of phenotypic prediction models in Project 1.2.1, and to enhance the accuracy and validate ultrasound scanning of highly marbled cattle by AGBU staff.

In 5 years time, we envisage that this project will have enabled fine-tuning of nutrition to maximise marbling, helped identify early biological markers of marbling and potential gene markers. The study will also have enhanced the level of understand of the influence of marbling in altering eating quality of cuts and enabled enhancement of BeefSpecs phenotypic prediction models and the accuracy of ultrasound scanning of highly marbled cattle.

6 Conclusions and Recommendations

This study addresses important industry and scientific issues that impact on capacity of producers to meet market specifications and the efficiency with which specifications are met, particularly given competition for resources such as grain for feedlot cattle. Given that this project commenced in February 2009, it is too early to draw conclusions from the limited results of the study that are available to date. It is recommended that the experiment continue as planned.