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Examination of the molecular control of muscle growth and development in cattle

Key external stakeholders:

Livestock farmers, Universities, Meat Processors, Animal Feed Industry, AI industry, Department of Agriculture, Food and the Marine, Irish Cattle Breeding Federation.

Practical implications for stakeholders:

- A panel of genes has been identified which may serve as potential molecular markers for muscle growth and development in cattle.
- Farmers exploit the compensatory growth phenomenon in order to reduce the cost of feeding cattle over the winter. We have shown that muscle tissue has the ability to grow rapidly during feed realimentation, following a period of restricted feed intake.
- Animals subjected to nutritional restriction exhibit higher feed efficiency when subsequently offered *ad libitum* access to feed.
- While compensatory growth had little residual effect on meat quality characteristics, there was a trend for meat flavour and tenderness to be lower in animals that experienced compensatory growth compared to animals on a continual plane of nutrition.
- Despite its potential importance, compensatory growth ability is not a trait that will be measured routinely and thus in the absence of easily measured, accurate molecular markers, will not be selected for.

Main results:

- We found significant effects of breed (Aberdeen Angus vs Belgian Blue) and sire genetic merit for carcass weight (EPD_{cwt}) on the expression of genes in the somatotrophic axis (a major regulator of skeletal muscle growth and development in cattle). Furthermore, enzymes involved in glycolysis and the citric acid cycle were increased in Aberdeen Angus compared to Belgian Blue sired-animals, and in animals of high- compared to low-genetic merit for growth rate potential.
- Muscle tissue has the potential to recover completely following a period of compensatory growth. Compensatory growth had little residual effects on meat quality characteristics.
- Many genes, particularly those of the TGF-β pathway, were differentially regulated in muscle tissue during the compensatory growth period.

Opportunity / Benefit:

- Key genes have been identified which may serve as molecular markers for muscle growth rate. Following appropriate validation, these markers could be incorporated into future cattle breeding programs to improve the accuracy of selection for muscle growth.
- This information will also aid in the understanding of genetic influences controlling muscle growth and fat accumulation, and could contribute to breeding programmes to increase lean tissue gain of beef cattle.
- Muscle tissue has the ability to fully compensate following a period of restricted growth demonstrating that feed restriction followed by a period of compensatory growth can be implemented on farm without knock on effects for carcass meat yield.
- A further advantage is that animals which underwent nutritional restriction exhibited greater feed efficiency when offered *ad libitum* access to feed.

Collaborating Institutions:

National University of Ireland Maynooth, University College Dublin, University of Bristol

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1. Project background:

Bovine skeletal muscle is a tissue of significant economic importance worldwide. In Europe and the USA approximately 17 and 37 kg of beef, respectively, is consumed per capita annually with worldwide beef production projected to increase at a rate of 0.9 to 1.4% annually over the next decade. The Irish Cattle Breeding Federation undertakes genetic evaluations for a range of performance traits across all of the main cattle breeds. Breeding value for carcass weight, an important trait reflecting lifetime growth, is estimated using a multi-trait animal model and is expressed as the EPD for carcass weight (EPD_{cwt}). Both sire breed type and EPD_{cwt} influence carcass characteristics including yield and quality of saleable meat from cattle. Previously, data from Teagasc Grange reported that muscle area were greater for Belgian Blue × Holstein Friesian compared to Aberdeen Angus × Holstein Friesian sired steers and also for Aberdeen Angus animals sired by bulls with high compared to low EPD_{cwt}. In beef cattle production, feed accounts for up to 80% of total variable costs. Thus, strategies to reduce feed costs without compromising overall animal performance are of particular interest. Compensatory growth is the ability of an animal to undergo accelerated growth after a period of restricted feeding. The exploitation of this biological phenomenon facilitates redistribution of feed supply from a time when feed is expensive (e.g. winter) to when it is cheap and plentiful (e.g. pasture in spring/summer) while maintaining overall production targets. Animals can undergo compensatory growth when offered unrestricted access to high quality feed, following a period of undernutrition. Due to its potential benefits to the economic efficiency of cattle production, the trait has been the subject of numerous studies worldwide. Many of these studies have investigated the effect of feed restriction, followed by compensatory growth, on body weight, carcass composition, meat quality, blood metabolites and hormones and metabolic organ size. Metabolic and blood hormone profiles have offered revealing insights into the physiological changes taking place in the animals body during feed restriction and compensatory growth. However, further research in now required to simultaneously elucidate all aspects of this growth phenomenon, including performance traits, feed intake, blood metabolites and hormones, and carcass characteristics together with possible interactions with maturity genotype.

2. Questions addressed by the project:

- Does sire breed (Aberdeen Angus vs. Belgian Blue) and sire EPD_{cwt} (High vs Low for carcass growth potential) affect the expression of genes and proteins in muscle tissue?
- How do crossbred steers from Aberdeen Angus and Belgian Blue sires respond to differential feeding treatments with a view to examining the potential of these two genotypes to exhibit compensatory growth following feed realimentation?
- Does compensatory growth have an effect on meat quality and sensory analysis?
- What are the key genes and pathways controlling accelerated muscle growth during feed restriction and compensatory growth?

3. The experimental studies:

The focus of this project was to investigate how skeletal muscle growth, a trait of major economic importance to beef cattle production, is controlled at a molecular level with a view to better understanding the key mechanisms involved. The approach taken was to combine key physiological and molecular analyses regulating the growth and development of muscle in an attempt to elucidate key genes, proteins and pathways that influence muscle growth in animals differing in genetic merit for growth potential as well in animals undergoing compensatory growth.

Study 1:

Animals of either high or low EPD_{cwt} were allocated to one of four groups, in a 2 (sire breed) x 2 (sire EPD_{cwt})

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factorial design with 9 animals per group. Blood plasma was collected throughout the animals' lifetime for the analysis of IGF-1 and insulin concentrations. Muscle tissue was collected at slaughter and RT-qPCR analysis was carried out to determine the effect of sire breed and sire EPD_{cwt} on the mRNA expression of genes of the somatotropic axis. Using the same tissue, the effect of sire breed and sire EPD_{cwt} on the expression of proteins in muscle of Aberdeen Angus and Belgian Blue sired cattle was examined using 2D gel difference electrophoresis and mass spectrometry.

Study 2:
Crossbred Aberdeen Angus × Holstein Friesian (n = 22) or Belgian Blue (n = 24) × Holstein Friesian steers were assigned to one of two treatment groups in a 2 (genotypes) x 2 (feeding treatments) factorial design. The Aberdeen Angus and Belgian Blue genotypes were selected because of their well documented differences in carcass conformation, muscle composition and maturation rates. Over a 99 day differential feeding period, half of the animals in each group were offered a high energy control diet whereas the other half group was offered an energy restricted diet. At the end of the differential feeding period, both groups were offered a TMR with a grass silage:concentrate ratio of 80:20, with the concentrate proportion increasing gradually over a 3 week period to the *ad libitum* high energy ration. This period, which lasted 200 d, was termed the realimentation period, and all animals were slaughtered on d 299 of the study. Muscle biopsies were collected at 2 different time points, *viz* end of the differential feeding period (d 99) and during the realimentation period (d 131), for transcriptomic analysis. This study measured the response to a period restriction and compensatory growth in a large number of performance and physiological traits across the two genotypes. We also aimed to clarify the effect of a compensatory growth feeding regime on meat tenderness and intramuscular fat content, as the literature has been equivocal on these issues. Therefore the effect of compensatory growth on meat quality and sensory analysis in meat (including temperature and pH of carcasses post slaughter, chemical composition, muscle drip loss and cooking loss, muscle and fat colour, shear force, sensory and flavour characteristics) from Aberdeen Angus and Belgian Blue sired steers was analysed. Furthermore, the transcriptional regulation of key genes and pathways controlling muscle growth during feed restriction and compensatory growth in Aberdeen Angus sired steers was examined using a novel molecular approach called RNAseq to measure global gene expression.

4. Main results:

Gene expression of *IGF-1R* and *IGFBP3* was up-regulated in Aberdeen Angus compared to Belgian Blue sired steers whereas *IGF-1* was up-regulated in high compared to low EPD_{cwt} animals. Greater gene expression of *IGF-1* and reduced transcript levels of *IGFBP3* in muscle may play a role in increased muscle growth potential in steers during the finishing period.

The analysis of muscle showed higher protein and moisture, and lower lipid concentrations for Belgian Blue compared to Aberdeen Angus sired steers. Enzymes involved in glycolysis (glycogen phosphorylase, phosphoglycerate mutase) and the citric acid cycle (aconitase-2, oxoglutarate dehydrogenase) were increased in Aberdeen Angus sired steers. Protein abundance of glucose-6-phosphate isomerase, enolase-3 and pyruvate kinase was higher in Aberdeen Angus sired steers of high compared to low EPD_{cwt}.

In the compensatory growth study, at the end of the differential feeding period (99 d), there was an average difference in weight of 82 kg between animals in the restricted and the *ad libitum* groups with average daily gains of 1.55 vs 0.63 kg for the *ad libitum* and restricted animals, respectively. During feed restriction, animals that were restricted consumed less DM, had a poorer feed conversion ratio and lower concentrations of plasma hormone and metabolites compared to steers on the *ad libitum* diet.
Restricted steers had lower muscle and fat development, as assessed ultrasonically, compared to steers on the *ad libitum* diet. During feed realimentation, there was no difference in DM intakes between feeding treatments; however, steers which had been restricted had greater live weight gain compared to steers on the *ad libitum* diet. Overall, unrestricted steers consumed more feed and had a better feed conversion ratio compared to restricted steers. Carcass weight was affected by feeding treatment with *ad libitum* animals having heavier carcasses. At slaughter, there was no difference in plasma metabolite or hormone concentrations, linear body measurements, ultrasonically scanned fat depth, carcass conformation or dressing percentage between the two groups. Furthermore, ultrasonically scanned muscle tissue was shown to recover completely.
Compensatory growth had no effect on carcass pH and temperature decline, chemical composition, drip loss, fat colour, or juiciness. However, Warner-Bratzler shear force increased and tenderness and overall flavour decreased as a result of this compensatory growth feeding strategy. For Belgian Blue sired steers, cooking loss percentage was greater in animals that experienced compensatory growth; however, this was

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not observed for Aberdeen Angus sired animals. Meat from Aberdeen Angus sired steers had better sensory flavour characteristics compared to Belgian Blue sired steers. Live weight gain prior to slaughter (d 195 to d 299) was not correlated with the meat quality characteristics measured. Overall, genotype has larger effects of meat quality than feeding treatment. These data suggest that the compensatory growth-based feeding regime applied here had little lasting effect on meat quality characteristics.

During the realimentation period, 65 differentially expressed genes were identified. TGF-βR1, a key receptor in the TGF-β signaling pathway, which plays an important role in muscle tissue growth, was down-regulated in previously restricted animals during compensatory growth. It is hypothesised that the signaling effects of the TGF-β pathway are reduced thereby promoting accelerated cell growth and proliferation in muscle tissue of animals experiencing compensatory growth.

5. Opportunity/Benefit:

- Key genes have been identified which may serve as molecular markers for muscle growth rate. Following appropriate validation, these markers could be incorporated into future cattle breeding programs to improve the accuracy of selection for muscle growth.
- This information will also aid in the understanding of genetic influences controlling muscle growth and fat accumulation, and could contribute to breeding programmes to increase lean tissue gain of beef cattle.
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6. Dissemination:

Keady, S. (2011) 'Examination of the expression of genes and proteins controlling *M. longissimus thoracis et lumborum* growth in steers'. Thesis. National University of Ireland, Maynooth, Ireland.

Keady, S.M., Kenny, D.A., Keane, M.G. and Waters, S.M. (2011) 'Effect of sire breed and genetic merit for carcass weight on the transcriptional regulation of the somatotropic axis in *M. longissimus dorsi* of crossbred steers.' *Journal of Animal Science,* 89(12):4007-4016.

Keady, S.M., Ohlendieck, K., Doyle, S., Kenny, D.A., Keane, M.G., Owens, R. and Waters, S.M. (2011) 'Effect of breed and sire genetic merit for growth on the expression of growth related genes in muscle of steers.' *Proceedings from the International Symposium for Animal Genomics*, Dublin, Ireland, 7th October, pp29.

Keady, S.M., Kenny, D.A., Keane, M.G. and Waters, S.M. (2011) 'Effect of sire breed and genetic merit for carcass weight on the transcriptional regulation of the somatotropic axis in *M. longissimus dorsi* of crossbred steers.' *62nd Annual Meeting of the European Association of Animal Production*, Stavanger, Norway, 29th August, pp30.

Keady, S.M., Ohlendieck, K., Kenny, D.A., Doyle, S., Keane, M.G. and Waters, S.M. (2011) 'Proteomic profiling of bovine *skeletal muscle* from Aberdeen Angus and Belgian Blue steers differing in sire expected progeny difference for carcass weight.' *Proceedings of the Agricultural Research Forum*, Tullamore, Ireland, 13th March, pp114.

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