Examination of the expression of genes and proteins controlling *M. longissimus thoracis et lumborum* growth in steers

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Declaration of authorship

I hereby declare that this thesis, submitted in candidature for the degree of Doctor of Philosophy of Biology with the National University of Ireland, has not been previously submitted for a degree to this, or any other university. I further declare that work embodied in this thesis is my own and any assistance is acknowledged.

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Keady, S. M., Ohlendieck, K., Doyle S., Kenny, D. A., Keane, M. G., Owens, R. and Waters, S. M. Proteomic Profiling of Bovine *M. longissimus thoracis et lumborum* from Aberdeen Angus and Belgian Blue Steers Varying in Genetic Merit for Carcass Weight. *International Journal of Biological Science* (currently in review).

Keady, S. M., Waters, S. M., Keane, M. G., O' Riordan, E. G. and Kenny, D. A. Effect of compensatory growth on: live weight, live weight gain, feed intake, linear body measurements, carcass measurements and plasma blood hormones and metabolite concentrations in crossbred steers (Manuscript in preparation).

S. M. Keady, Moloney, A. P.., Waters, S. M., Hamill, R., Dunne, P. G., Keane, M. G., le Goff, M., Richardson, R. I. and Kenny, D. A. Compensatory growth in crossbred Aberdeen Angus and Belgian Blue steers: Colour, shear force and sensory characteristics of meat (Manuscript in preparation).

Keady, S. M., Kenny, D. A., Creevey, C., Lynn, D. and Waters, S. M. Effect of diet and compensatory growth potential on the transcriptomic profile of *M. longissimus thoracis et lumborum* in steers employing RNA*seq* technology (Manuscript in preparation).

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Keady, S. M., Kenny, D. A., Keane, M. G. and Waters, S. M. (2010). Effect of breed and sire expected progeny difference for carcass weight on the expression of growth related genes in muscle of steers. *Proc British Society of Animal Science and the Agricultural Research Forum*, pp.144.

Keady, S. M., Kenny, D. A., Keane, M. G., Kelly, A. K. and Waters, S. M. (2009). Effect of breed and compensatory growth potential on plasma analytes and on lumbar muscle and fat growth in beef cattle. *Proc Agric Res Forum*, pp.61.

List of abbreviations

A

a	Redness
AA	Aberdeen Angus
AA*H*	Aberdeen Angus × Holstein Friesian of high genetic merit for growth potential
AA*L*	Aberdeen Angus × Holstein Friesian of low genetic merit for growth potential
ACN	Acetonitrite
ACO2	Aconitase-2
ACSM1	Acyl-CoA synthetase medium chain family member 1
ACTβ	β-Actin
ActRIIB	Activin type-2 receptor
ADF	Acid detergent fibre
AI	Artificial insemination
ALS	Acid-labile subunit
AMPD1	AMP deaminase 1
ALDOA	Aldolase A
APS	Ammonium persulfate
ark	Activin receptor-like kinase
ATP	Adenosine triphosphate

B

b	Yellowness
BB	Belgian Blue
BB*H*	Belgian Blue × Holstein Friesian of high genetic merit for growth potential
BB*L*	Belgian Blue × Holstein Friesian of low genetic merit for growth potential
βHB	β-hydroxybutrate
bp	Base pair
BSA	Bovine Serum Albumin

C

$CaCl_2$	Calcium Chloride
cAMP	Cyclic AMP
CAPZA2	Capping protein alpha
CDCA8	Cell division cycle associated 8
CDC20	Cell division cycle 20 homology
cDNA	Complimentary DNA

CHAPS	3-[(cholamidopropyl) dimethylammonio]propanesulfonic acid
CMMS	Central Movement and Monitoring System
CO_2	Carbon dioxide
COMP	Cartilage oligomeric matrix protein
CSO	Central Statistics Office
ct	Cycle threshold
CV	Coefficient of varition
CW	carcass weight
CYP2B6	cytochrome P450, family 2, subfamily B, polypeptide 6
CYP4F2	cytochrome P450, family 4, subfamily F, polypeptide 2

D

d	Day (s)
DAFF	Department of Agriculture, Fisheries and Food
DEPC	Diethyl pyrocarbonate
DFP	Differential feeding period
dNTP	Deoxyribonucletide triphosphate
DM	Dry matter
DMD	Dry matter digestibility
DMI	Dry matter intake
DTT	Dithiothreitol

E

EBV	Estimated breeding value
EEF1A2	Elongation factor 1 alpha 2
EGTA	Ethylene glycol tetraacetic acid
ELOVL6	Elongation of long chain fatty acids
ENO1	Enolase
EPD	Expected progeny difference
EPDcwt	EPD for carcass weight

F

F	Feeding treatment
FABP4	Fatty acid binding protein 4. adipocyte
FASN	Fatty acid synthase
FCR	Feed conversion ratio
FOS	FBJ murine osteosarcomma viral oncogene homology
FOXM1	Forkhead box protein M1

G

g	gravity
g	Grams
G	Genotype
GA	Genome Analyser
GAPDH	Glyceraldehyde 3-phosphate dehydrogenase
GASP-1	Growth and differentiation factor associated serum protein-1
GH	Growth Hormone
GHBP	GH binding protein
GHR	Growth Hormone Receptor
GLUT	Glucose transporter
GLYAT	Glycine-N-acyltransferase
GM	Genetic merit
GO	Gene ontology
GPDH	Glycerol-3-phosphate dehydrogenase
GPI	Glucose-6-phosphate isomerase
G6PDH	Glucose-6-phosphate dehydrogenase

H

h	Hour
H	High
HEPES	4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid
HF	Holstein- Friesian
HMBS	Hyrdoxymethylbilane synthase
HSF1	Heat shock factor protein 1
HSPβ1	Heat shock protein β1
H-H	*Ad libitum* access to feed

I

ICBF	Irish Cattle Breeding Federation
IGF	Insulin-like growth factor
IGFBP	Insulin-like growth factor binding protein(s)
IGF-1R	Insulin-like growth factor 1 receptor
IGF-2R	Insulin-like growth factor 2 receptor
IMF	Intramuscular fat
IPA	Ingenuity Pathway Analysis
IRS	Insulin receptor substrate

J

K

kg	Kilogram

L

L	Low
L	Litre
LDB3	LIM domain binding 3
LEP	Leptin gene
LPL	Lipoprotein lipase
LWG	Live weight gain
L-H	Restricted access to feed for 99 d followed by *ad libitum* access to feed until slaughter

M

M	Mitotic
MAPK	Mitogen-activated protein kinase
mg	Milligram(s)
$MgCl_2$	Magnesium Chloride
$MgSO_4$	Magnesium sulphate
min	Minute(s)
miRNA	Micro RNA
mL	Millilitre(s)
mo	Month(s)
mRNA	Messenger RNA
MYLPF	myosin light chain, phosphorylatable
MYL1	myosin light chain 1

N

N	Newtons
NAD	Nicotinamide adenine dinucleotide
NADPH	Nicotinamide adenine dinucleotide phosphate
NaOAc	Sodium acetate
NCBI	National Centre for Biotechnology Information
NDF	Neutral detergent fibre
NEFA	Non-esterifed fatty acids
nm	Nanometer
nmol	Nanomole(s)

O

OGDH	2-oxogluterate dehydrogenase

P

PCK	Phosphoenolpyruvate carboxylase
PCR	Polymerase chain reaction
PEPCK	Phosphoenolpyruvate carboxykinase
PGAM2	Phosphoglycerate mutase 2
PGM1	Phosphoglucomutase
pHu	Ultimate pH (48 h)
pI	Isoelectric point
PI3-K	Phosphatidylinosital 3-kinase
PIPES	Piperazine-N,N'-bis(ethanesulfonic acid)
PKA	Protein kinase A signalling pathway
PKM2	Pyruvate kinase
PLIN1	Perilipin 1
PPAR	Peroxisome proliferator activated receptor
PRDX6	Peroxiredoxin 6
PYGM	Glycogen phosphorylase
PPM1A	Protein phosphatase, Mg^{2+}/Mn^{2+} dependent, 1A
PPP2Cβ	Protein phosphatase 2, catalytic subunit, β isozyme
PPP2R1β	Protein phosphatase 2, regulatory subunit A, β

Q

R

RIA	Radioimmunoassay
RIN	RNA integrity number
rRNA	Ribosomal RNA
RT-qPCR	Real-Time reverse transcription quantitative PCR

S

SAS	Statistical Analysis Software
SBV	Suckler Beef Value
s.e.	Standard error
SED	Standard error of the difference
s.e.m.	Standard error of the means
s.d.	Standard deviation
SDS	Sodium dodecyl sulphate

SOCS2	Suppressor of cytokine signalling 2
SW	Slaughter weight

T

TEMED	Etramethylethylenediamine
TFA	Trifluoroactic acid
TGF-β	Transforming growth factor-β
TGF-βR1	Transforming growth factor-β receptor 1
TMR	Total mixed ration
TNNI2	Troponin I
tRNA	Total RNA

U

UK	United Kingdom
UMD	Ultrasonically scanned muscle depth
USA	United States of America

V

V	Volt(s)
VCL	Vinculin
vs	*Versus*
v/v	Volume per volume

W

WBsf	Warner-Bratzler shear force
w/v	Weight per volume

X

Y

Z

ZNF217	Zinc-finger protein 217

Other

°C	Degrees Celsius
µg	Microgram
µL	Microlitre

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Thesis abstract

The first study conducted investigated the effect of sire breed and genetic merit for growth potential of the transcriptional regulation of the somatotropic axis followed by a proteomic approach to assess differentially abundant proteins. Following this, a second study was set up to examine the compensatory growth phenomena in cattle which aimed to investigate the effect of feed restriction and feed realimentation on animal production and physiological variables and the residual effects on meat quality attributes. The final chapter in this thesis focused on the transcriptional regulation of compensatory growth in *M. longissimus thoracis et lumborum* in crossbred Aberdeen Angus steers. It is evident from this thesis that genetic merit for growth potential in cattle is under molecular control and chapters 3 and 4 offer revealing insight into the somatotropic axis and glucose metabolism. RNA*seq*, a highly sensitive approach to transcriptome sequencing, was used to conduct the transcriptional sequencing analysis in chapter 7. During the differential feeding period, gene pathways relating to lipid metabolism were significantly different between the two treatments and consistent with plasma leptin concentrations and ultrasonically scanned fat depth data (chapter 5). During the realimentation period, when previously restricted steers were experiencing compensatory growth, the TGF-βR1 gene involved in the TGF-β signalling pathway, a negative regulator of growth, was down-regulated in expression. The results obtained from this study offer a novel insight into key regulatory genes and pathways controlling compensatory growth in skeletal muscle of cattle which following appropriate validation may be incorporated into genomically assisted selection strategies for beef cattle. Overall, this thesis has offered significant insight into key pathways regulating growth in cattle such as the somatotropic, glycolytic and TGF-β signalling pathways.

Chapter 1

Introduction,

Background and Review of the Literature

1.1 Introduction

There are currently over 5.8 million cattle in Ireland with cattle production incorporating both dairy and beef breeds and a large amount of cross breeding (AIMS Bovine Statistics, 2010). In Ireland, cattle rearing systems are predominantly grass based with animals at pasture from March/April to November and then over-wintering indoors (Drennan and McGee, 2009; Keane, 2010). Although grazed grass is generally a cheap source of feed, feed costs still remain the largest variable cost in beef production in Ireland (Connolly *et al*., 2010; Finneran *et al*., 2010). Within beef production systems, mechanisms to increase profits on the farm involve breeding more productive and efficient animals through the exploitation of information available in breeding databases leading to the use of sires with higher profit potential (Clarke *et al*., 2009; Campion *et al*., 2009a). Another approach to increasing profitability involves more effective use of pasture. Ireland is unique in Europe with approximately 90 % of agricultural land dedicated to grassland and meadow (Drennan *et al*., 2005). Implementation of feeding strategies to more efficiently utilise the available herbage would reduce the dependency on bought feed stuffs, such as concentrates. This study aims to contribute to efficiency and profitability in beef production through the identification and examination of key genes and pathways controlling growth in cattle. Although huge advances at an *in vitro* level have been made in recent years in understanding the mechanisms underlying muscle growth and development, further research in the animal, at an *in vivo* level, is required. To improve the usefulness and accuracy of the Irish database of genetic merit traits for beef cattle, incorporation of information relating to key genes and pathways regulating growth traits at a transcriptomic or proteomic level is important.

1.2 Skeletal Muscle

1.2.1 Introduction

Skeletal muscle is a heterogeneous tissue which attaches to bones and consists of numerous bundles, called fascicles. Fascicles contain many fibres which are multinucleated and surrounded by a cell membrane, the sarcolemma. Each muscle fibre consists of myofibrils which in turn are divisible into two kinds of myofilaments: thin filaments and thick filaments. Thin filaments contain two strands of actin and two strand of regulatory protein, called troponin and tropomyosin, coiled around one another (Hooper *et al.*, 2008). Thick filaments consist of the protein myosin. Sarcomeres are repeating units found along the length of the myofibrils. The thin filaments are attached to the ends of the sarcomere, called Z lines. The thick filaments are located in the centre of the sarcomere making up the area known as the A band. When a muscle fibre is at rest, the thick and thin filaments do not overlap completely and the area near the end of the sarcomere consisting of only thin filaments is called the I band. The H zone is located in the centre of the sarcomere and contains only thick filaments as the thin filaments do not reach this area. This design and arrangement of the thin and thick filaments is key to how the whole muscle contracts (Hooper *et al.*, 2008; Figure 1.1).

1.2.2 Classification of muscle fibres

Different methods of classification and the advent of new technologies have resulted in a wide range of nomenclatures for muscle fibre types, both within and across species. In the 1970s, Peter *et al.* (1972) classified muscle fibres according to their contractile and metabolic properties based on histochemical stains for succinate dehydrogenase and ATPase (Nemeth and Pette, 1981). This resulted in the classification of three main fibre types - slow oxidative, fast glycolytic and fast oxidative-glycolytic. Previous to this,

fibres were referred to by their colour - red, intermediate and white (Gauthier, 1969). As techniques developed, fibres were classified as type I, IIa, and IIx and with the use of immuno-histochemistry IIb and IIc (a hybrid fibre) were identified at a later stage (Oury *et al.*, 2010). In bovine skeletal muscle type I, IIa, IIx, IIb, and IIc fibres have been identified and classified in detail (Vestergaard *et al.*, 1994; Bouley *et al.*, 2005; Oury *et al.*, 2010); however, it must noted that often IIx fibres were classified as IIb as the isoforms did not allow these fibres to be distinguished (Oury *et al.*, 2010).

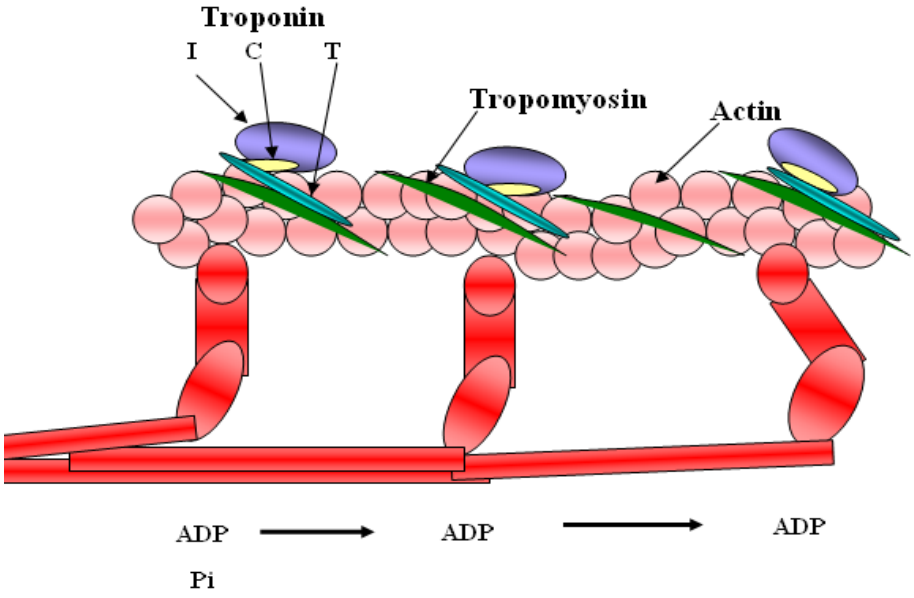


Figure 1.1 Detail of the interaction between thick and thin filaments in muscle fibres
$Ca2^{+}$ binds troponin C, allowing exposure of the myosin binding sites on actin (left-hand myosin head). Following this an inorganic phosphate is released (middle myosin head) allowing movement of the myosin head (left-hand myosin head) (adapted from Hopkins, 2006)

1.2.3 Metabolic properties of muscle cells

1.2.3.1 Glycogenesis and glycogenolysis

The formation of glycogen from glucose is known as glycogenesis. The major glycogen stores are present in skeletal muscle and liver. In brief, glucose is converted to glucose 6-phosphate by the enzymes glucokinase and hexokinase and then converted to glucose-

1-phosphate by phosphoglucomutase. Glucose-1-phosphate is then converted to glycogen, a polymer of glucose by the enzyme glycogen synthase, as reviewed by Jiang and Zhang (2003). Glycogen is converted back to glucose and is regulated by the hormone glucagon by a process called glycogenolysis which plays a critical role in maintaining glucose homeostasis. Additionally, glucagon inhibits glycogenesis by regulating glycogen synthase in the liver (Jiang and Zhang, 2003). Overall, insulin stimulates glycogenesis whereas glucagon functions antagonistically to insulin in regulating the balance between glucose and stored glycogen (Bansal and Wang, 2008).

1.2.3.2 Glycolysis and oxidation of carbohydrates

Glycolysis, meaning to split sugars, involves the formation of pyruvate or lactate (or both) from glucose. Glucose, a six carbon sugar is broken down into two three-carbon sugars and then these sugars are oxidised to form pyruvate. Overall, two adenosine triphosphate (ATP) molecules are used in glycolysis; however four ATP molecules are released with a net gain of two ATP. In skeletal muscle, energy for the contraction on fibres is supplied by ATP *via* glycolysis and oxidative phosphorylation, as recently reviewed by Ohlendieck (2010). Following glycolysis, if oxygen is present, pyruvate, first converted to Acetyl-CoA by the enzyme pyruvate dehydrogenase, enters the mitochondria where the citric acid cycle (also known as the tricarboxylic acid cycle or the Krebs cycle) takes place. The citric cycle oxidises carbohydrates which produces reducing equivalents of NAD, which results in the production of large amounts of ATP *via* oxidative phosphorylation, as reviewed by Bowtell *et al.* (2007). The citric acid cycle involves eight steps, each catalysed by a different enzyme (Figure 1.2).

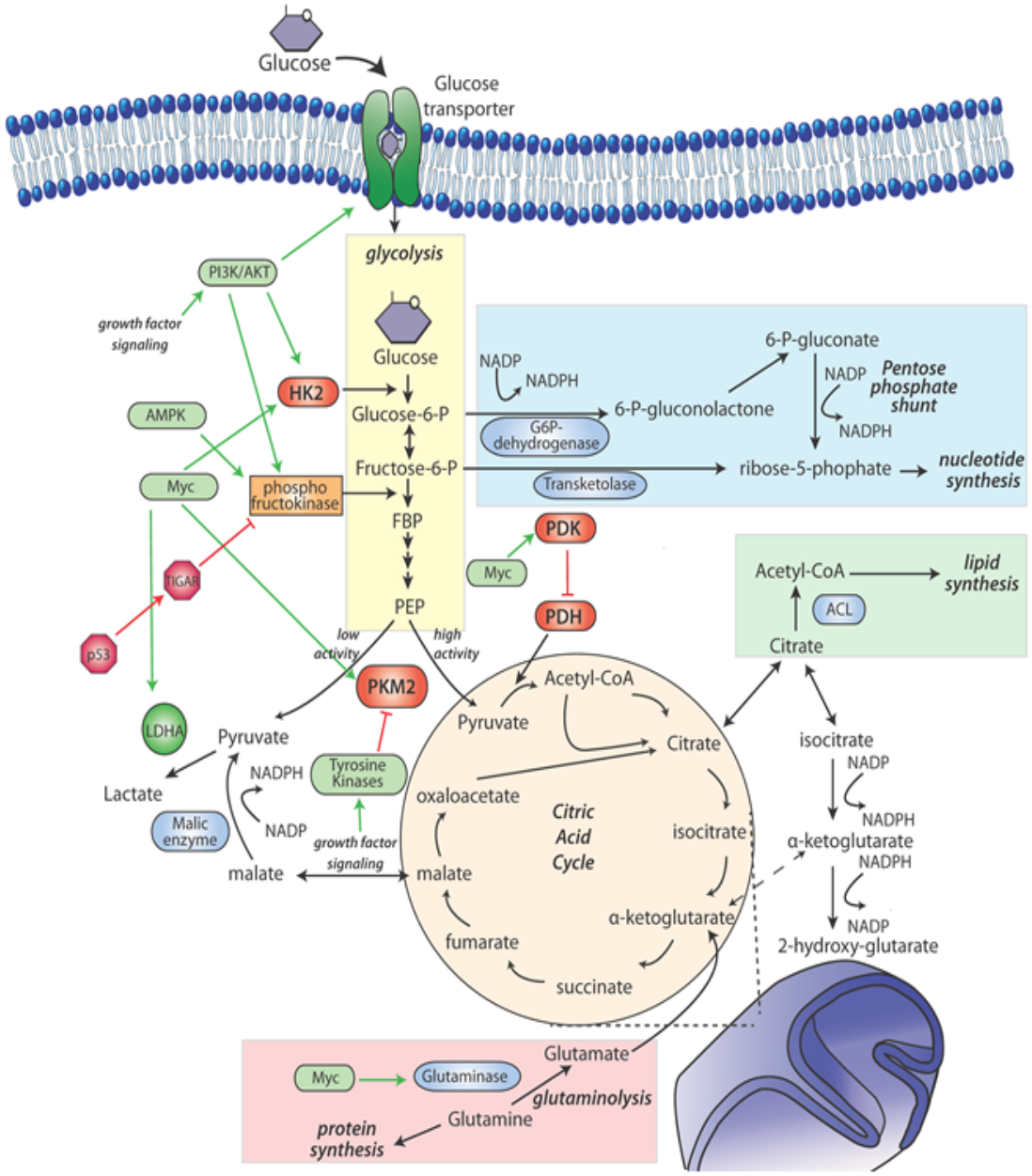


Figure 1.2 Schematic representations of glycolysis, pentose phosphate pathway, and the citric acid cycle

Glycolysis involves the formation of lactate or pyruvate from sugars. If oxygen is present in the cell, pyruvate is converted to Acetyl CoA which enters the citric acid cycle. The citric acid cycle and oxidative phosphorylation are a series of chemical reactions that convert Acetyl CoA into carbon dioxide, water and energy (Adapted from Wolf *et al*., 2010). (*Source*: http://www.impactjournals.com/oncotarget/index.php?journal=oncotarget&page=article&op=view&path%5B%5D=190&path%5B%5D=265)

1.2.3.3 Lipogenesis

Ruminants consume forages and grains of which the cell wall and soluble carbohydrates are converted to volatile fatty acids (acetate, propionate and butyrate) by fermentation in

the rumen, as reviewed by Janssen (2010). As dietary fat impairs rumen function, ruminant diets are typically low in fat (Bauman, 1976) and as a result, long chain fatty acids synthesised *de novo* are an important source for triacylglycerol synthesis in ruminant adipose tissue (Greathead *et al*., 2001), the main site of lipogenesis in non-lactating ruminants (Vernon, 1981). Acetyl-CoA, produced from the oxidation of pyruvate in the mitochondria, is the principal building block of fatty acids. NADPH is also required in large amounts for the reduction of acetyl-CoA to fatty acids. In ruminants, NAPDH is produced from two major sources, the pentose phosphate pathway and NADP isocitrate dehydrogenase (Ingle *et al*., 1972; Vernon, 1981; Laliotis *et al*., 2010).

In recent years, many genes, proteins and pathways have been highlighted as important regulatory factors in lipogenesis (Lee *et al*., 2007; Kim *et al*., 2009; Liu *et al*., 2009; Canovas *et al*., 2010). Acetyl-CoA carboxylase an important rate limiting enzyme involved in the carboxylation on Acetyl-CoA to malonyl-CoA and glucose-6-phosphate dehydrogenase, a rate limiting enzyme in the pentose phosphate pathway, are of particular interest in ruminants and warrant further investigation at a molecular level in bovine.

1.2.3.4 Intramuscular fat accumulation

The term ‘marbling’ describes the unique intramuscular fat build-up between the fibres bundles within the muscles (Smith *et al*., 2009). Greater levels of intramuscular fat accumulation are a consequence of higher numbers of intramuscular adipocytes in the muscle as well as an increase in their volume (Damon *et al*., 2006; Hocquette *et al*., 2010). In bovine, intramuscular fat marbling differ in structure and distribution depending on breed i.e. Holstein-Friesian animals have a greater number and slightly finer structure compared to German Angus or Belgian Blue (Albrecht *et al*., 2006). It

has been established by Smith and Crouse (1984) that glucose, rather than acetate, contributes a greater proportion of acetyl units for fatty acid synthesis in intramuscular fat accumulation. Further research into genes and pathways regulating intramuscular fat accumulation in bovine has been carried out (for a review see Hocquette *et al*., 2010). Intramuscular fat marbling increases flavour and juiciness of meat and this is discussed in greater detail in section 1.6.3.2

1.2.4 Muscle characteristics of cattle in Ireland

Approximately, 40% of all dairy cows in Ireland are bred to beef bulls of Aberdeen Angus (13.9%), Hereford (10.2%), Limousin (6%) Belgian Blue (2.6%), Charolais (1.8%) and other (6.2%) breeds (AIMS Bovine Statistics Report, 2010). The off-spring are reared to slaughter in Ireland as they do not meet the carcass conformation standards required for high-value live export trade to continental Europe.

1.2.4.1 Holstein-Friesian

There are 1.0 million dairy cows in Ireland with the Holstein-Friesian breed accounting for up to 95% of this figure (WHFF, 2010). Friesian Cattle were first introduced to Ireland from England and Scotland around the 1920s with Holstein cattle arriving to Ireland from Canada in 1974 (Dillon, 2008). Holstein-Friesian cows are renowned for their superior genetic merit for milk yield with Irish Holstein-Friesians producing on average 6700 litres of milk in 305 days of lactation (WHFF, 2010); however, the breed has very low muscling.

1.2.4.2 Aberdeen Angus

The Aberdeen Angus breed is an early maturing compact breed which is black in colour and naturally polled. Aberdeen Angus are a breed renowned for their easy calving

ability (McGuirk *et al*., 1998) and distinct for fat 'marbling' within the muscle. The breed was developed in north-eastern Scotland with the breed introduced to Ireland in the mid nineteenth century (Irish Aberdeen Angus Association, 2011). Aberdeen Angus cattle rank highly compared to other breeds of cattle with regards to quality and sensory analysis of the meat. This is attributed to their intramuscular fat marbling which increases sensory traits such as juiciness, and flavour (for review see Oddy *et al*., 2001). There is, however, conflicting reports whether tenderness is affected directly from intramuscular fat marbling, as reviewed by Hocquette *et al*. (2010).

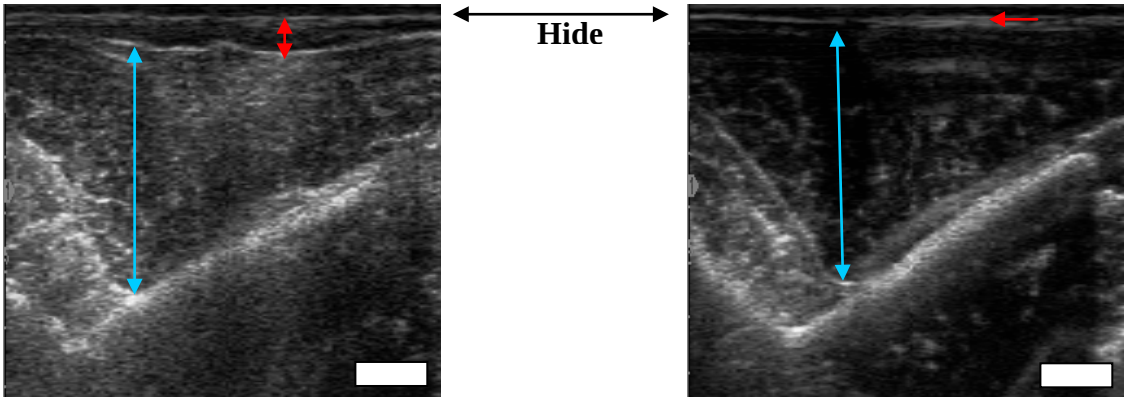
1.2.4.3 Belgian Blue

Belgian blue cattle are a late-maturing breed which accumulates more muscle compared to their early-maturing counterparts (Sadkowski *et al.*, 2009). Belgian Blue animals are renowned for their 'double muscling' effect (section 1.3.8.2.2), producing high quantities of very lean meat. Consequently, Belgian Blue is a favoured sire choice by farmers for dairy cows in Ireland due to the high monitory value of the calves. Crossbred animals that are heterozygous for the double muscle mutation are larger in size compared to their 'single' muscle equivalents (Casa *et al*., 2004). However, negative effects of double muscling include higher rates of dystocia as a result of a longer gestation period and greater birth weights of the calf (Arthur *et al*., 1988). In addition, greater muscling in the cow pelvis results in calving difficulties which require assistance to prevent calf mortality (Michaux *et al*., 1982).

1.3 Assessing animal nutrition and muscle growth

1.3.1 Ultrasound scanning measurements

Ultrasound scanning provides a relatively cheap, non invasive method of assessing muscle and fat accretion in live animals. The procedure quickly and accurately assesses back fat thickness, longissimus muscle depth, percentage intramuscular fat and *gluteus medius* (rump) fat (Greiner *et al.*, 2003). Researchers to date have used ultrasound scanning to estimate these measurements with a high degree of accuracy and repeatability (Herring and Kemp; 2001; Greiner *et al.*, 2003; Conroy *et al.*, 2010). In breed comparison studies, greater scanned muscle depth has been reported in BB animals compared to AA, while AA displayed greater back fat thickness (Campion *et al.*, 2009a) (Figure 1.3).



Aberdeen Angus ***Belgian Blue***

Figure 1.3 Ultrasonically scanned muscle and fat depth of Aberdeen Angus × Holstein-Friesian and Belgian Blue × Holstein-Friesian steers
Scanning image (↕) arrows indicates the thickness of subcutaneous fat in Aberdeen Angus (left), however this measurement is very small in Belgian Blue animals (right). Blue line (↕) indicates muscle depth. White box = 1 cm.

1.3.2 Skeletal measurements

Skeletal measurements enhance information relating to body weight, as well as ultrasonically scanned muscle/fat depth measurements, and these data are of particular

interest to the beef cattle industry. According to Albertí *et al*. (2008) a narrow pelvis indicates slow skeletal development and low muscling. In addition, Afolayan *et al*. (2006) found that chest girth predicted body weight with high precision in sheep. Differences in skeletal size may explain variation in carcass conformation at slaughter due to differences in carcass compactness (Campion *et al*., 2009a). Skeletal measurements are also obtained at slaughter including length of carcass, carcass width, and thickness, length and width of the leg (Keane *et al*., 2011).

1.3.3 Metabolites

Body weight and linear scoring can be complimented with the analysis of circulating blood metabolites to give a more comprehensive view of the nutritional and health status of an animal (Pambu-Gollah *et al*., 2000; Ndlovu *et al*., 2007). Blood glucose, urea, β-hydroxy butyrate (βHB) and non-esterified fatty acids (NEFA) are common metabolites used to assess the nutritional status of cattle.

Circulating plasma glucose and urea concentrations profiles are considered an indication of the quantity of starch and protein or the ratio of these nutrients consumed. Reduced or insufficient energy intake can lead to lower blood glucose levels in cattle (Blum *et al*., 1985; Rule *et al*., 1985; Ellenberger *et al*., 1989; Itoh *et al*., 2006). In addition, in lactating cows, glucose levels are low due to the high energy demand for milk production (van Knegsel *et al*., 2007). Plasma concentrations of NEFA indicate the degree of fat metabolism as NEFA are released into circulation as a direct result of lipid catabolism (Ndlovu *et al*., 2007). During periods of intense under nutrition or high levels of gluconeogenesis (section 1.2.4.4), blood profiles are characterised by low blood glucose levels and high concentrations of NEFA and βHB (Blum *et al*., 1985; Rule *et al*., 1985; van Knegsel *et al*., 2007). A large proportion of NEFA are directed towards ketone body synthesis in the liver. Ketogenesis represents a mechanism to

increase whole body fat utilisation by making NEFA available to the tissues in a more water soluble and easily metabolised form (Hocquette *et al*., 1998).

1.3.4 Insulin

As a result of fermentation in the rumen, very little glucose is absorbed across the gastrointestinal tract of ruminants (Harmon and McLeod, 2001). Ruminants are dependent on gluconeogenesis for their supply of glucose. Gluconeogenesis, is the formation of glucose from non-glucose molecules such as propionate and lactate and this action takes place in the liver. Although differences exist in metabolism between ruminants and monogastrics, insulin still plays an important role in glucose metabolism in ruminants. Insulin is regarded as a pancreatic hormone although it plays a supportive rather than direct role in influencing growth (Lawrence and Fowler, 1997). In humans, insulin rapidly stimulates facilitative glucose transport activity in skeletal muscle and adipocytes and a similar role for insulin have been reported in ruminants (Abe *et al*., 1994; Hocquette *et al*., 1995; Sasaki, 2002), however, the ability of insulin to stimulate glucose transport rate is greater in muscle and adipocytes in monogastrics compared to ruminants (Sasaki 1990; Hocquette *et al*., 1995).

Facilitative glucose transporter (GLUT) 1 (non-insulin-sensitive) and GLUT 4 (insulin sensitive) are present in muscle and adipocytes in bovine skeletal muscle, however, levels of GLUT 4 are greater in glycolytic and oxido-glycolytic compared to oxidative fibres (Hocquette *et al*., 1995) with concentrations decreasing gradually overtime (Abe *et al*., 1994). Insulin activates the alpha subunit of its specific receptor, the insulin receptor tyrosine kinase (IR) on the plasma membrane which results in the autophosphorylation of the beta subunit (Figure 1.4). Phosphorylation of the insulin receptor substrate (IRS) family of proteins takes place and activation on the

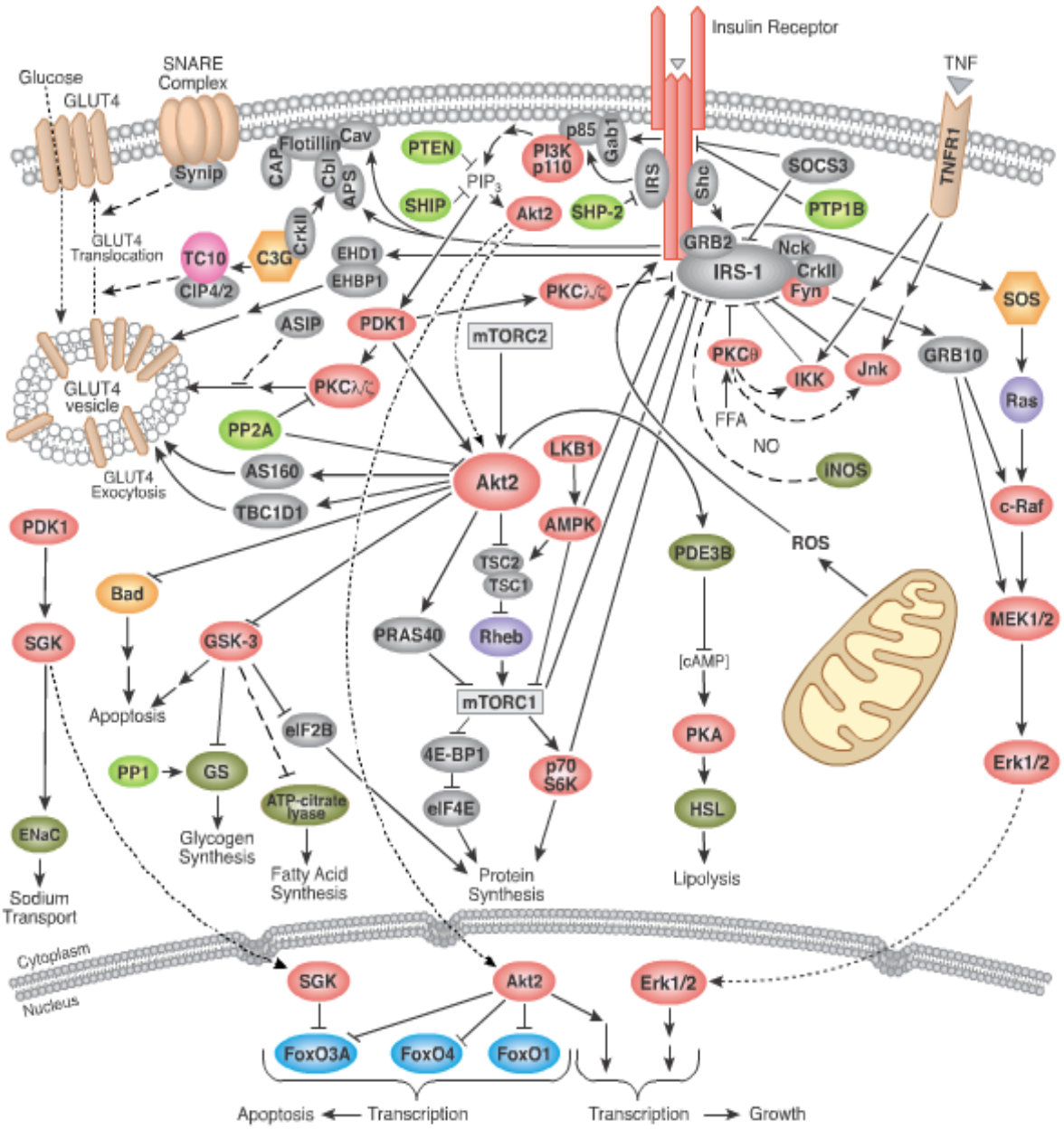


Figure 1.4 Insulin receptor signalling
Insulin activates the insulin receptor tyrosine kinase (IR) on the plasma membrane resulting in the phosphorylation of the IRS family of proteins. Consequently, intracellular vesicles containing GLUT 4 are translocated to the plasma membrane allowing glucose to enter the cell. (*Source*: http://www.cellsignal.com/reference/pathway/Insulin_Receptor.html)

phosphatidylinositol 3-kinase (PI3-K) pathway. PI3-K functions as a key mediator in the activation of down stream molecules of PI3-K to exert various metabolic effects of insulin generating the production of phosphoinositides. Consequently, intracellular vesicles containing GLUT 4 are translocated to the plasma membrane and following

fusion of GLUT 4 with the plasma membrane glucose enters the cell (Rose and Richter, 2005).

Insulin also functions to regulate gluconeogenesis and the output of glucose from the liver in ruminants (Brookman and Laarveld, 1986). The effect of insulin on substrate utilisation varies within and across species in that lactate utilisation was reduced in sheep, however, no effect of propionate utilisation was reported (Brookman, 1990). In ruminating bovine however, an increase in propionate utilisation has been observed (Donkin and Armentano, 1995).

1.3.4.1 Insulin and lipogenesis in adipocytes

Insulin is a key factor in lipogenesis and lipolysis in ruminant adipocytes (Cochrane and Rogers, 1990). As mentioned previously, insulin binds to IR located on the surface of the adipoctye which initiates the transfer of GLUT4 to the cell membrane, which allows glucose to enter the cell. Glucose is converted to glycerol-3-phosphate. The effect of insulin on lipoprotein lipase (LPL) activity is similar to humans in that insulin activates lipoprotein lipase (LPL), synthesised in adipocytes, which allows fatty acids to enter the cell through fatty acid transporters such as fatty acid binding proteins, fatty acid translocase and fatty acid transporter proteins (Faulconnier *et al*., 1994). Fatty acids and glycerol-3-phosphate are esterified into triglycerides. Refeeding underfed or fasted ruminants increases the levels lipogenesis to values on par or even greater than control animals (Bonnet *et al*., 1998). During feed restriction in ruminants, LPL activity is reduced however following feed realimentation activity is restored in both oxidative and glycolytic muscles (Bonnet *et al.*, 2000; Faulconnier *et al.*, 2001).

1.3.5 Bovine growth hormone

Growth hormone (GH) is produced in the anterior pituitary and regulates metabolism and growth in vertebrates. Its actions are mediated by GH receptor (GHR) to which it binds to form a receptor dimer as reviewed by Kopchick and Andry (2000) which initiates the transcription of many genes including the insulin like growth factor-1 gene (*IGF-1*) (Jiang *et al*., 2007) (Figure 1.5).

GH binding protein (GHBP) has also been identified in most species, including bovine (Devolder *et al*., 1993; Davis *et al*., 1994), despite previous suggestions to the contrary (Gavin *et al*., 1991). In humans, GHBP is the soluble extra cellular domain of GHR and has a similar affinity for GH as GHR. *In vivo*, GHBP act to increase the biological activity and prolong the half life of GH (Baumann *et al*., 1988; Turyn *et al*., 1997); however, contradictory findings from *in vitro* studies suggest that GHBP compete with surface GHR for GH thus inhibiting GH action (Manner *et al*., 1991). Regardless of which mechanism, GHBP may act as a reservoir keeping GH in circulation at all times as 60 % of GH is bound to GHBP (Kopchick and Andry, 2000). In cattle, the effects of GHBP on GH are still uncharacterised and warrant further investigation.

In addition to inducing growth, GH stimulates milk production postpartum in cows. In fact, recombinant bovine GH is a synthetic hormone marketed to farmers and administered subcutaneously to dairy cows to increase milk production. However, in Europe since 2000, the use of recombinant bovine GH is banned on the grounds of animal welfare issues. In addition, human health concerns relating to cancer were also considered as a reason for the ban however, no research has yet to substantiate this, possibly as a result of a 95 % loss of recombinant GH during pasteurisation (Le Breton *et al*., 2010).

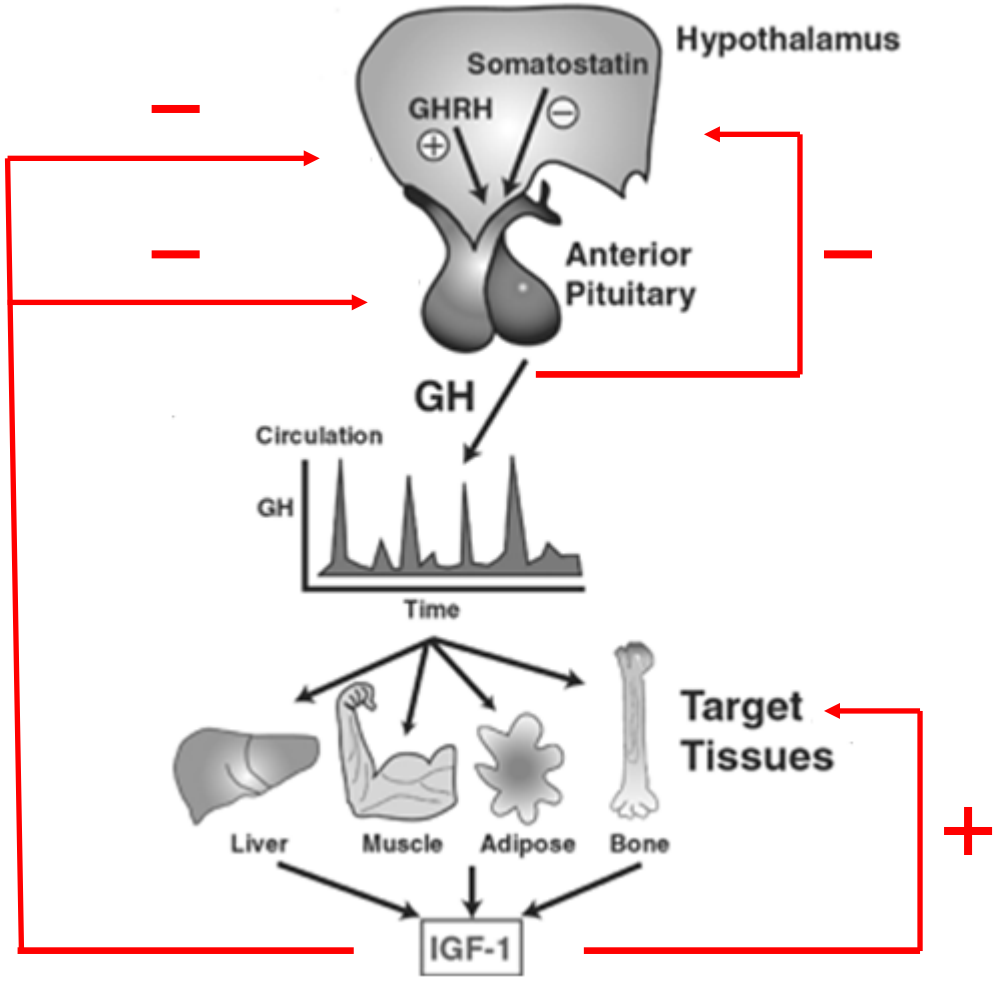


Figure 1.5 The growth hormone axis
Growth hormone (GH) is produced in the anterior pituitary gland and released into circulation. GH receptors are present in the liver, muscle, adipose and bone and in response to GH, insulin-like growth factors (IGFs) are released (adapted from Kopchick *et al*., 2002). (*Source*: http://edrv.endojournals.org/content/23/5/623/F2.expansion)

1.3.6 Circulating IGF-1

The growth promoting effect of GH is mediated by the insulin-like growth factor axis. This complex system consists of two ligands (IGF-1 and IGF-2), two receptors (IGF-1R and IGF-2R) and six IGF binding proteins (IGFBP1-6). The IGF ligands are structurally related to insulin and are critical for growth and development in vertebrates. In addition, to promoting somatic growth, IGFs are important for the development and functional maturity of the central nervous system, skeletal tissue and reproductive organs (Duan, 2005). Upon the interaction of GH with hepatic GH-receptors, IGF are produced in the liver and released into the circulatory system. IGF are unique among peptide hormones

because their receptor, IGF-1R, is present in all cell types and tissues (Clemmons, 2009). Once a ligand binds to the receptor IGF-1R, activation of multiple intracellular signal transduction cascades occurs, including the PI3K-Akt cascade. Activation of this pathway leads to increased protein synthesis and inhibition of apoptosis (Clemmons, 2009). In addition, activation on the RAS/MAP kinase pathway occurs, leading to mitogeneis (Figure 1.6). GH, through binding to GHR promotes the release of IGF-1 at a local level in various tissues, including skeletal tissue (Adam and McCue, 1998). This locally produced IGF promotes myofiber regeneration, hypertrophy and skeletal muscle regeneration, as reviewed by Philippou *et al.* (2007).

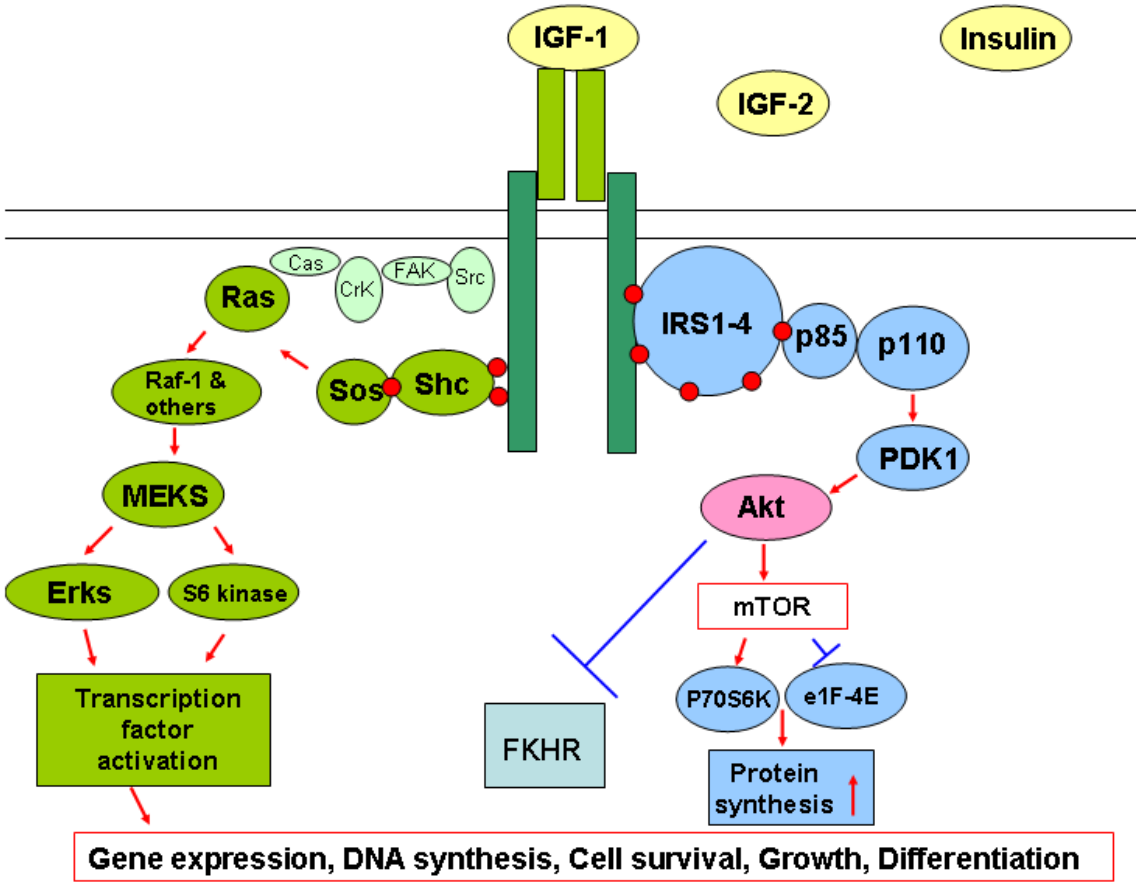


Figure 1.6 Signalling pathways activated in response to IGF-1 binding with IGF-1R
When a ligand binds to IGF-1R multiple signalling cascade are activated, including the phosphatidylinositol 3-kinase (PI3-K)-Akt cascade. Akt functions through mTOR and p70S6K to stimulate protein synthesis and inhibition of apoptosis (adapted from Clemmons, 2009).

1.3.6.1 IGFBP binding proteins

These IGFBP are a family of secreted proteins that serve to potentiate or inhibit the actions of IGFs, as reviewed by Hwa *et al.* (1999). When IGF-1 or IGF-2 is bound in an IGFBP ternary structure it is safeguarded for up to 30 minutes (min) in a controlled release mechanism, compared with a half-life of 10 to 12 min for free IGF in circulation (Guler *et al.*, 1989; Clemmons, 2009) and therefore potentiating its effect. Approximately 99 % of the IGFs in plasma are bound to high affinity IGFBP (Figure 1.7; Hossner, 2005) as IGFBP bind IGF with high affinity that is greater than those of the IGF-1R (Denley *et al.*, 2005; Duan *et al.*, 2010). However, this action prevents IGF binding to the receptor IGF-1R and therefore inhibits its bioavailability.

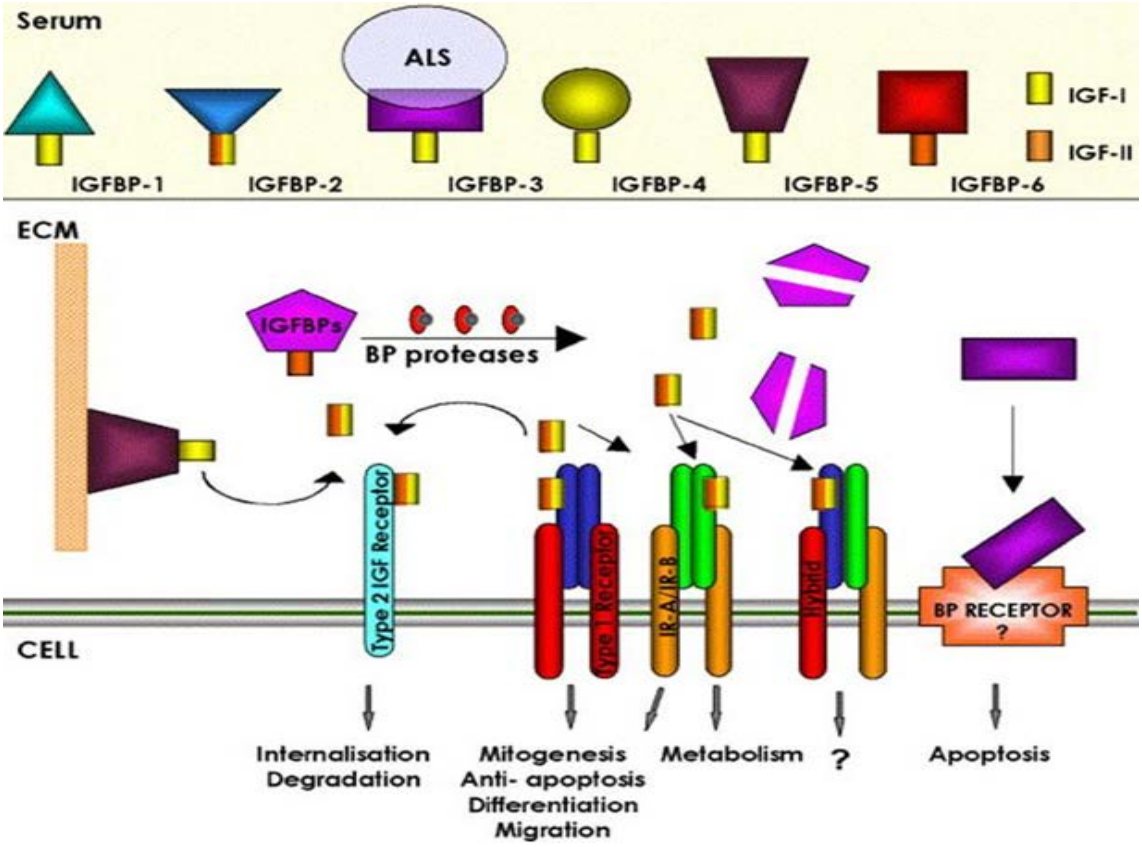


Figure 1.7 Schematic representation of the IGF system
The IGF system consists of ligands IGF-1 and IGF-2, their receptors, IGF-1R and IGF-2R, and six high affinity binding proteins, IGFBP1-6. (*Source*: http://www.igfsociety.org)

1.3.7 Leptin

Leptin, produced from the *ob* gene, is involved in the hypothalamic control of body energy homeostasis, an indicator of body fat reserves and regulator of appetite and energy expenditure (Delavaud *et al*., 2002). In both ruminants and monogastrics, research has shown a positive correlation between circulating concentrations of leptin and fat accumulation (Berg *et al*., 2003; Geary *et al*., 2003). In addition, in cattle leptin concentrations positively correlate to *M. longissimus* fat thickness, intramuscular fat marbling and kidney, pelvic and heart fat (Geary *et al*., 2003). In addition, plasma leptin is positively regulated by energy intake in the cow as mild feed restriction decreased plasma leptin concentrations without any change in adipose cell size or body condition score (Delavaud *et al*., 2002). The authors suggest that the medium-term effects of feeding levels on plasma leptin are independent of long term regulation by changes in adiposity. Other variables reported to alter leptin concentrations include cold exposure, insulin, glucose and oestrogen, as reviewed by Margetic *et al*. (2002).

1.3.8 Mechanisms regulating muscle growth

1.3.8.1 Myostatin signalling and muscle atrophy

Myostatin, a member of the transforming growth factor (TGF) ß superfamily, regulates muscle mass by the inhibition of myogenesis (Figure 1.8; McPherron *et al*., 1997). Active myostatin ligand binds to ActRIIB, the type II receptor (Lee and McPherron, 2001), complexes with a second cell receptor, either activin receptor-like kinase (Ark) 4 or Ark 5, which initiates signalling though the Smad signal pathway (Rebbapragada *et al*., 2003; Zhu *et al*., 2004). The interaction between myostatin and ActRIIB appears to be similar to the interaction of TGF-ß and its receptors. Smad2 and Smad3 form a complex with Smad4, a co-Smad which translocates into the nucleus where it regulates

expression of genes such as MyoD (Ríos *et al.*, 2002; Zhu *et al.*, 2004). As a result, muscle growth is controlled by regulating cell proliferation and differentiation. Myostatin can also bind with follistatin, follistatin related gene and growth and differentiation factor associated serum protein-1 (GASP-1) to prevent its activity and inhibit muscle atrophy (Lee and McPherron, 2001; Hill *et al.*, 2002a,b; Cassano *et al.*, 2009).

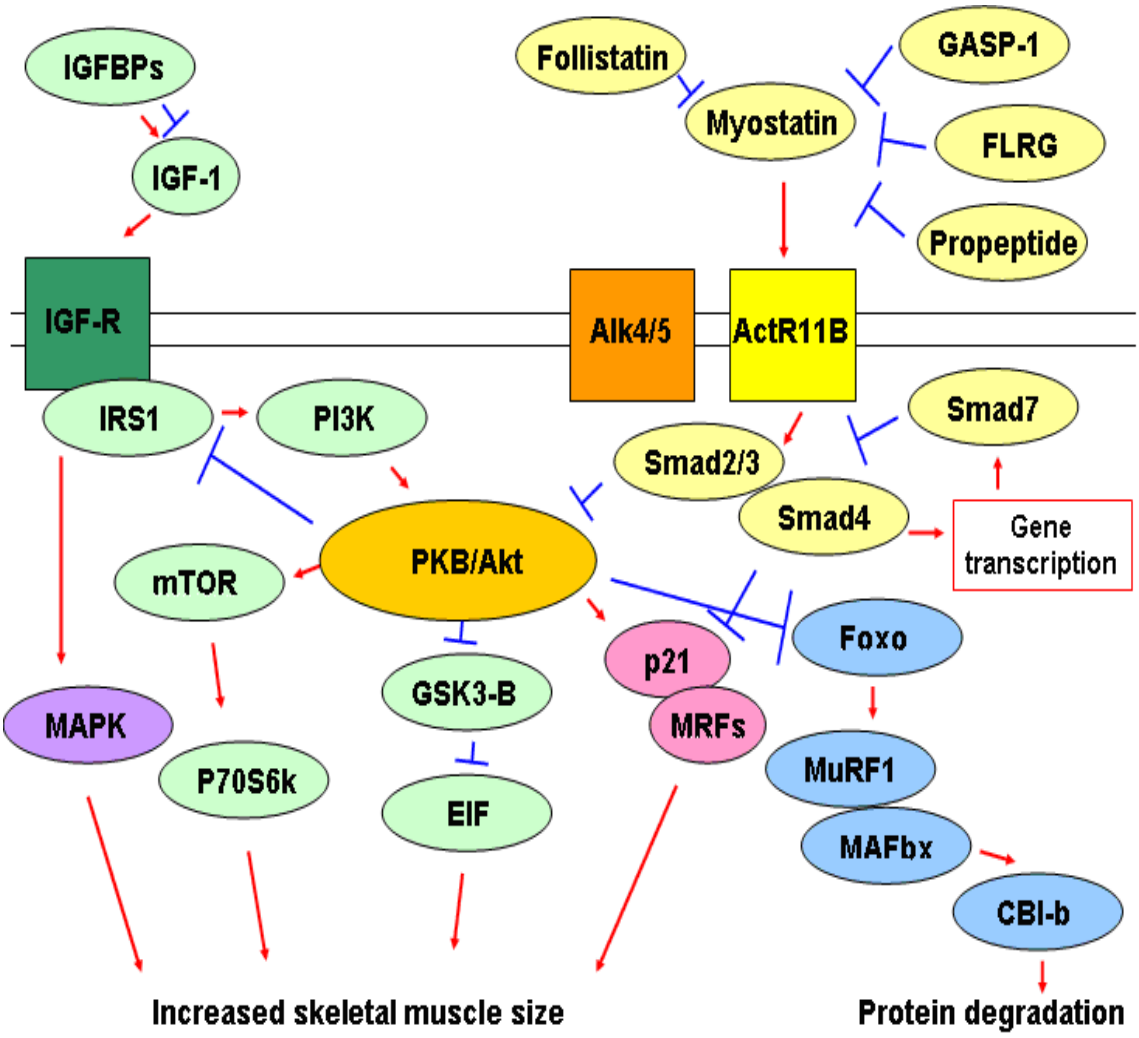


Figure 1.8 Schematic displaying the main proteins involved in myostatin signalling pathways
Myostatin ligand binds to ActRIIB which initiates signalling though the Smad signal pathway. This leads to protein degradation and muscle atrophy (Adapted from Otto and Patel, 2010)

1.3.8.2 Myostatin mutation

1.3.8.2.1 Muscle hypertrophy

Muscle hypertrophy, termed double muscling (DM), is a heritable condition which involves intensified muscle development due to general skeletal-muscle cell hyperplasia and hypertrophy (McPherron *et al*., 1997). This phenotype has been identified in many species including humans, sheep and whippets (Schuelke *et al*., 2004; Clop *et al*., 2006; Mosher *et al*., 2007). In addition, muscle hypertrophy has been reported in many European cattle breeds including Friesian, Shorthorn (UK), Belgian Blue (Belgium) (Figure 1.9), Charolais and Blonde d'Aquitaine (France), Piedmontese (Italy), Rotbunt (Germany) and Rubia Gallaga (Spain) (Vissac, 1982). Double muscling in cattle is often considered undesirable due to increased dystocia, poor calf viability, slowness of females to reach sexual maturity and unfavourable meat attributes due to paler meat colour and a lower intramuscular fat content (Kieffer and Cartwright, 1980; Arthur *et al*., 1988; Arthur *et al*., 1989; Arthur, 1995; Cuvelier *et al*., 2006a,b).

Figure 1.9 Double muscled Belgian Blue bull
This double muscled Belgian Blue bull is homozygous for the *nt821del(11)* deletion in the myostatin gene (Soure: http://www.cellbiol.net/ste/bookimages.php)

1.3.8.2.2 Muscle hypertrophy due to myostatin mutation

The muscle hypertrophy phenotype in cattle arises from a failure to produce a functional myostatin protein (McPherron and Lee, 1997). There are six identified mutations in the myostatin sequence that result in muscle hypertrophy due to alterations in the sequence code. The Belgian Blue and Piedmontese breeds both exhibit muscle hypertrophy due to mutations in the coding region of the myostatin coding sequence; however, the mutations differ across the two breeds, with Belgian Blue having an 11 bp deletion of nucleotides in the third exon, referred to as *nt821(del11)*, whereas Piedmontese exhibit a missense mutation resulting in an amino acid change in exon 3 (Grobet *et al.*, 1997; Bellinge *et al.*, 2005) (Figure 1.10). The *nt821(del11)* mutation in the sequence of myostatin in Belgian Blue results in a truncation of the bioactive C-terminal domain of the protein.

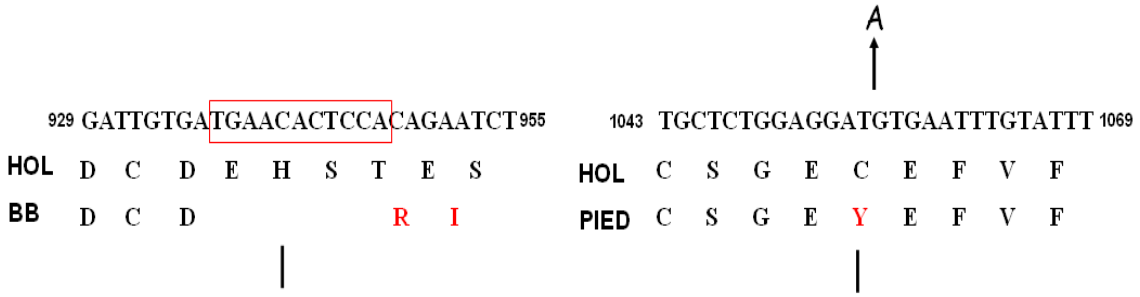


Figure 1.10 Representation of myostatin mutations in Belgian Blue and Piedmontese cattle compared with wild type Holstein cattle
Myostatin mutations in Belgian Blue (left) and Piedmontese (right) cattle compared with wild type Holstein cattle. Red letters indicate changes in nucleotide and amino acid sequence (adapted from McPherron and Lee, 1997).

1.3.8.2.2.1 Muscle hypertrophy and muscle fibre type

Bouley *et al.* (2005) studied the effect of the myostatin null mutation on the muscle fibre composition in young Belgian Blue bulls and found that double muscled bulls had lower levels of slow-twitch oxidative and a greater level of fast twitch glycolytic fibres compared to conventional muscle types. Bulls heterozygous for the myostatin null mutation were intermediate in their composition. Due to greater amount of fast twitch

glycolytic fibres and reduced blood circulation, Belgian Blue cattle experience fatigue earlier during forced exercise (Holmes *et al*., 1973).

1.4 Genetic merit for beef production traits

1.4.1 Introduction

The main organisation generating genetic information for cattle breeding in Ireland is the Irish Cattle Breeding Federation (ICBF). The Cattle Movement and Monitoring System (CMMS) is a national computer database operated by the Department of Agriculture, Fisheries and Food (DAFF), which records calf registrations and cattle mortality, movement, exports and slaughter date. The ICBF breeding database is updated daily with information from the CMMS. In addition, the ICBF collects data from abattoirs, cattle marts, AI companies and directly from farms. This information allows the ICBF to carry out genetic evaluations and develop breeding schemes for the benefit of the cattle industry.

1.4.2 Genetic evaluations

As previously mentioned, the ICBF computes genetic evaluations for Irish dairy and beef cattle. In brief, data are collected, added to the cattle breeding database and sire expected progeny differences (EPD) are calculated across a range of performance traits such as animal growth, carcass traits, reproduction and calving attributes. Sire EPD is an estimate of half the estimated breeding value (EBV) of a sire i.e. the breeding value of a particular trait that which will be passed on to its potential progeny and sire EPDs are the unit indicator of genetic merit in Ireland. The breeding value of an animal being the cumulative sum of the additive effects of its genes on a given trait. A sire EPD value can be positive or negative as it is relative to a given genetic base. In addition, each sire

EPD is assigned a reliability score (0-100%) which indicates the confidence level of the measure. The higher the reliability score, the lower the chance that the estimate will change as more information is collected on the animal, its progeny and relatives. Sire EPDs across a range of traits are examined and genetic merit for carcass weight are undertaken in a 15 x 15 multi-trait animal model which include information for: carcass weight, carcass conformation class, carcass fat class, cull cow weight, weaning weight, live weight, feed intake, hindquarter development, height at withers, length of back, length of pelvis, loin development, width at withers, width behind withers and calf quality. EPDs are also generated for a range of maternal traits.

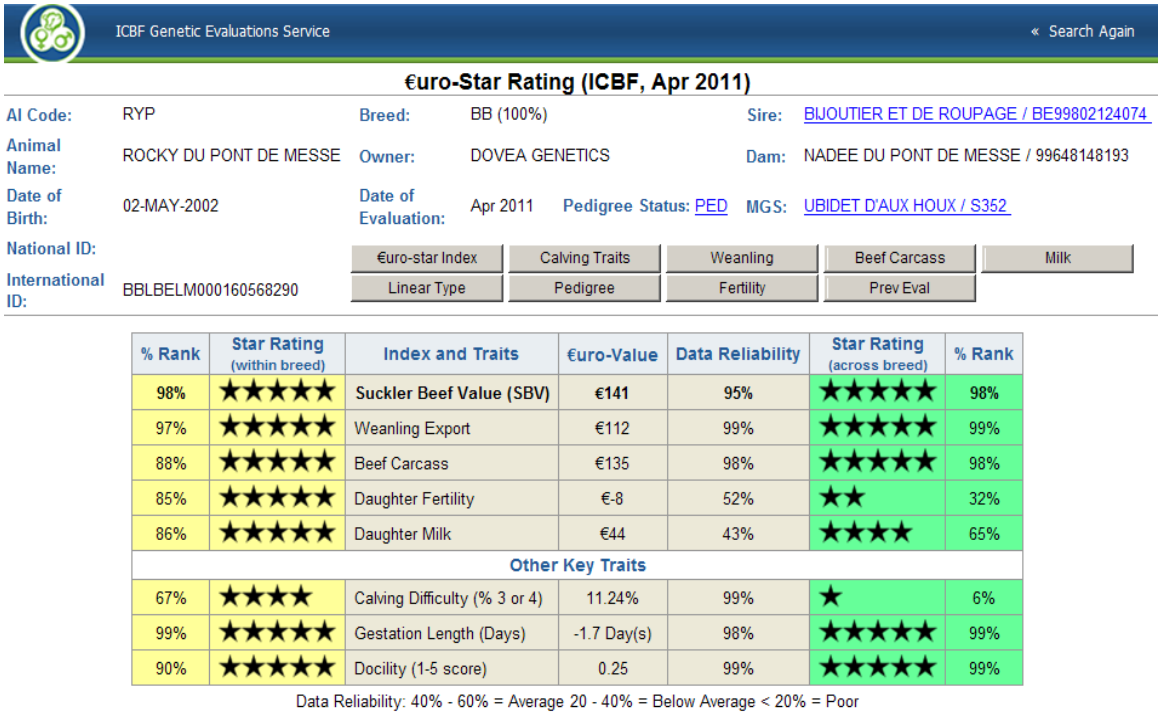
1.4.3 Economic and €uro-Star indices

From the individual trait EPDs, an economic index and €uro-Star indices are estimated to help simplify breeding decisions. The development of these economic values is described by Amer *et al*. (2001). In total, 16 traits are assigned to five sub-indices, and finally one overall Suckler Beef Value (SBV) index (incorporating the 5 sub-indices) is estimated reflecting the overall economic value of a bull (Cromie, 2008). The five sub-indices are calving traits, weanling export value, beef carcass value, milk and fertility traits and calf quality. Animals are then ranked, both within their own breed and across breeds, based on a star rating. The €uro-star rating ranges from 1 star (poor) to 5 stars (excellent). An example of a €uro-star index for a bull with AI code RYP is shown in Figure 1.11. In this example, the bull has a SBV of €141 suggesting that his progeny should return €141 more profit compared to progeny of a sire with a SBV of €0.

1.4.4 Physiological regulation of genetic merit for growth

Animals of higher genetic merit for carcass weight grow faster and produce heavier carcasses (Campion *et al*., 2009a). However, the mechanisms responsible for this higher

growth rate are still at an early stage of elaboration. Many studies have evaluated the effect of genetic merit for growth (Crews *et al*., 2004; Keane and Diskin, 2007; Keane *et al*., 2011); however, few have examined the physiological mechanisms regulating this higher growth potential. Both Campion *et al*. (2009b) and Clarke *et al*. (2009) examined the circulating glucose profile from animals of high or low growth potential and reported no difference in glucose levels at any stage throughout their lifetime. Interestingly, Clarke *et al*. (2009) reported no difference in IGF-1 concentrations between animals of either high or low growth potential; however, low animals had greater insulin concentrations supporting the theory that muscle development and rapid growth are associated with decreased plasma concentrations of insulin (Hocquette *et al*., 1998). Further research is required into potential molecular mechanisms regulating genetic merit for growth, for example the local regulation of IGF-1 in tissues and other key genes of the somatotropic axis.



ICBF Genetic Evaluations Service « Search Again

€uro-Star Rating (ICBF, Apr 2011)

AI Code:	RYP	Breed:	BB (100%)		Sire:	BIJOUTIER ET DE ROUPAGE / BE99802124074	
Animal Name:	ROCKY DU PONT DE MESSE	Owner:	DOVEA GENETICS		Dam:	NADEE DU PONT DE MESSE / 99648148193	
Date of Birth:	02-MAY-2002	Date of Evaluation:	Apr 2011	Pedigree Status: PED	MGS:	UBIDET D'AUX HOUX / S352	
National ID:		€uro-star Index	Calving Traits	Weanling	Beef Carcass	Milk	
International ID:	BBLBELM000160568290	Linear Type	Pedigree	Fertility	Prev Eval		

% Rank	Star Rating (within breed)	Index and Traits	€uro-Value	Data Reliability	Star Rating (across breed)	% Rank
98%	★★★★★	**Suckler Beef Value (SBV)**	**€141**	**95%**	★★★★★	98%
97%	★★★★★	Weanling Export	€112	99%	★★★★★	99%
88%	★★★★★	Beef Carcass	€135	98%	★★★★★	98%
85%	★★★★★	Daughter Fertility	€-8	52%	★★	32%
86%	★★★★★	Daughter Milk	€44	43%	★★★★	65%
		Other Key Traits				
67%	★★★★	Calving Difficulty (% 3 or 4)	11.24%	99%	★	6%
99%	★★★★★	Gestation Length (Days)	-1.7 Day(s)	98%	★★★★★	99%
90%	★★★★★	Docility (1-5 score)	0.25	99%	★★★★★	99%

Data Reliability: 40% - 60% = Average 20 - 40% = Below Average < 20% = Poor

Figure 1.11 Example of a €uro-star index for an AI bull, RYP
This €uro-Star rating for Rocky Du Pont De Messe (RYP) highlights his star rating both within and across breed. RYP has a SBV of €141 suggesting that his progeny should return €141 extra profit compared to progeny of a sire with a SBV of €0. (*Source*: http://www.icbf.ie/taurus/bull_search/index.php?search_type=num&search=ryp).

1.4.5 Molecular mechanisms regulating genetic merit for growth

Researchers in France (Bernard *et al*., 2009) examined the molecular mechanisms regulating growth in 15- and 19-month-old Charolais bulls. Using microarray technology, the authors reported that genes relating to the glycolytic pathway were upregulated in animals classified as high for muscle growth potential. In addition, gene expression of fibroblast growth factor 6 (FGF6) was down-regulated in animals with high growth potential. Fibroblast growth factors inhibit skeletal muscle differentiation (Kudla *et al*., 1995). Further investigation is required into the physiological and molecular mechanisms regulating high and low growth potential.

1.5 Compensatory growth

1.5.1 Introduction

Following a period of restricted development usually due to reduced feed intake an organism has the potential to undergo enhanced growth upon realimentation to a higher energy diet, thus enabling it to achieve its pre-determined inherent size (Figure 1.12) (Hornick *et al*., 2000). Bohman (1955) first termed this abnormally rapid growth period as 'compensatory growth'. The fact that animals display compensatory growth indicates that growth rate is usually below the potential maximum (Jobling, 2009) and therefore compensatory growth models have been of keen interest to researchers studying growth and efficiency. There are a vast number of publications investigating feed restriction and feed realimentation in humans, ruminants, chickens, fish, and swine (Leeson and Zubair, 1997; Ritacco *et al*., 1997; Johansen and Overturf, 2000; Tsintas *et al*., 2000; Tolla *et al*., 2003).

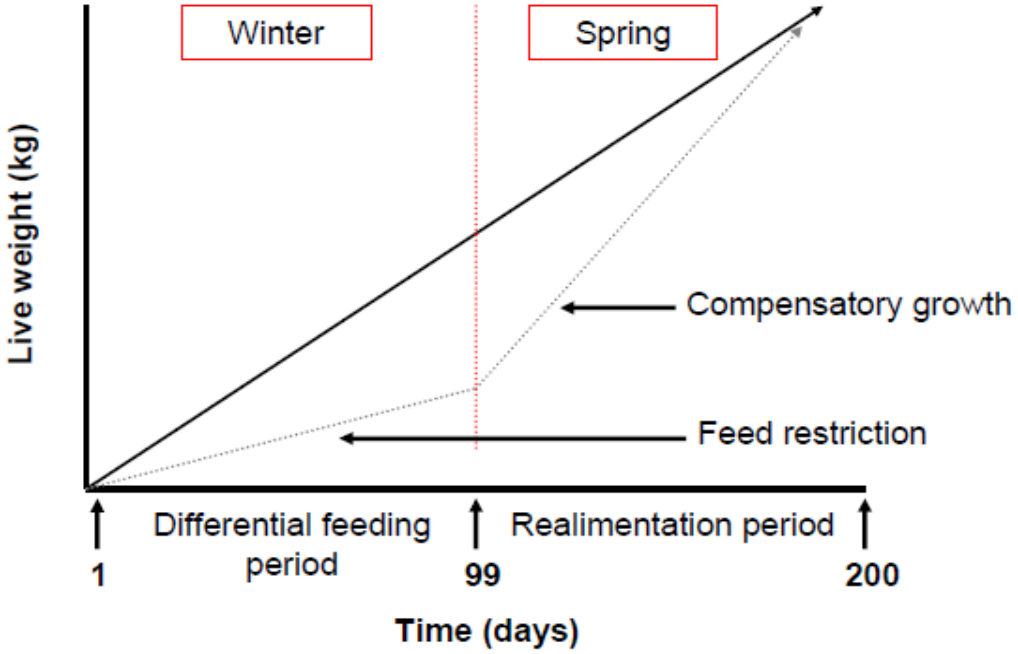


Figure 1.12 Schematic representation of compensatory growth in cattle
Animals that undergo feed restriction during the differential feeding period (dotted line) experience compensatory growth when a higher energy diet is offered. Animals are capable of compensating 100 % to reach their predetermined weight (dark line).

1.5.2 Compensatory growth in cattle

Compensatory growth in cattle has been widely shown to occur, with animals compensating up to 100 % in some instances during the realimentation period (Ryan *et al*., 1990). When animals move from a predominantly forage diet during restriction to a predominantly concentrate diet during realimentation a period of adaptation is necessary whereby the animal adjusts to the higher plane of nutrition. In cattle this is normally three to four weeks and this adaptation process needs to be monitored carefully to avoid acidosis (a sudden pH drop in the rumen). Compensatory growth is greatest when animals are relatively mature, when the period of restriction is short, (usually about three months in cattle) and when the dietary restriction is not too severe (Coleman and Evans, 1986; Hornick *et al*., 2000). This compensatory growth phenomenon has attracted the attention of many producers due to the fact it can off-set the effects of feed restriction and therefore reduce the costs of production (Table 1.1). Thus, farmers have

the flexibility to reduce feed demand at a time when feed is expensive (winter time) and allow the animals to compensate when feed is cheap and plentiful (grazing season).

1.5.3 Benefits of compensatory growth-based feeding strategy

As mentioned previously, the key benefit of a compensatory growth-based feeding strategy is a reduction in total feed costs over the lifetime of the animal. In Irish studies, a saving in feed costs of up to €90 per animal has been achieved using a compensatory growth-based feeding regime (Keane and Drennan, 1994; Keane, 2011, personal communication).

During the winter when animals are housed in slatted floor sheds the slurry accumulates in a storage facility underneath the shed. Slurry disposal is expensive, and in addition, under the Nitrates Directives (91/676/EEC), herd owners are required to limit the amount of nitrogen from manure to 170 kg/hectare/year. As feed input is lowered during the winter period in compensatory growth-based production systems, this results in a reduction of faecal output with consequent reductions in handling costs and organic nitrogen production.

A compensatory growth feeding strategy has been shown to improve meat quality including tenderness in both swine and bovine studies in many instances (Hansen *et al.*, 2006; Stolzenbach *et al.*, 2009; Therkildsen *et al.*, 2011), however, not all (Sinclair *et al.*, 2001; Kristensen *et al.*, 2004). This topic is discussed in further detail in section 1.4.3.

Ireland is coming under increasing pressure from international agreements such as the Kyoto Protocol and EU2020, to substantially reduce greenhouse gas emissions including those from agriculture. It is envisaged that a compensatory growth-based feeding regime would lead to a reduction in such emissions as cattle become more feed

Table 1.1 Summary of recent studies involving compensatory growth

Breed[1]	Sex[2]	Initial Weight	Restriction period (day)	Realimentation period (day)	Main effects of CG	Reference
F	S	460 kg	56 days	70 days	• No difference between feeding treatments for carcass characteristics including carcass weight, fat class or conformation score • Mean fat depth, *M. longissimus* area and pistola weight were similar for both treatments	Moloney *et al*. (2008)
BR	S	202 kg	120 days	316 days	• Animals that experienced weight loss during the restriction period did not compensate fully • No difference in live weight at the end of the finishing period between slow growing animals and control group • At slaughter no difference in carcass characteristics, fat depth or eye muscle area	Tomkins *et al*. (2006)
HF	B	159 kg	97 days	140 days	• No difference in absolute feed consumption during realimentation period • Circulating IGF-1 concentrations increased and remained high	Therkildsen *et al*. (2005)
AA, CH, H	S	402 kg	70 days	70 days	• No difference between treatments for carcass weight, dressing percentage, eye muscle area, fat class or conformation score	Sinclair *et al*. (2001)
BB	B	310 kg	115 days 239 days 411 days	147 days 120 days 112 days	• No significant difference in slaughter weight and carcass weights between treatments except for animals restricted for 411 days whose slaughter weight and carcass weights were greater • Greater carcass connective and adipose tissue	Hornick *et al*. (1998a)
HE	S	250 kg	89 days	330 days	• No difference in feed intakes corrected for body weight between control and compensating animals for first 12 weeks of realimentation	Ryan *et al.* (1993)

[1]F = Friesian; BR = Belmont Red; AA = Aberdeen Angus; CH = Charolais; HF = Holstein-Friesian; BB = Belgian Blue; HE = Hereford; [2]S = Steer; B = Bull;

efficient following feed restriction and realimentation and therefore produce less methane (Hegarty *et al*., 2007; Nkrumah *et al*., 2006). However, in many instances animals offered a restricted ration have access to more forage in their diet and therefore this offsets the reduction in methane outputs during the realimentation period as animals on forage based diets produce more methane (Boadi and Wittenberg, 2001).

1.5.4 Compensatory growth and body composition

Compensatory growth in cattle may affect not only live weight gain but also feed intake, viscera size, lean tissue growth, and muscle fibre type (Ryan *et al*., 1993; Yambayamba *et al*., 1996a,b; Lehnert *et al*., 2006). Hornick *et al*. (2000) states that when an animal is fed at maintenance, muscle growth is close to zero, however, fat mobilisation continues. Frequently, but not always, adipose tissue develops rapidly in cattle during compensatory growth following feed restriction and realimentation (Hornick *et al*., 1998a). These authors reported higher percentages of connective and adipose tissues at slaughter in BB bulls undergoing compensatory growth following a period of feed restriction compared to bulls offered a high energy diet throughout the study.

Several studies have investigated the effect of compensatory growth on viscera weights including the liver (Carstens *et al*., 1991; Yambayamba *et al*., 1996a). The liver is a metabolically active tissue responsible for between 17-26% of total oxygen consumption in beef steers (Baldwin *et al*., 2004). Yambayamba *et al*. (1996a) reported that, during feed restriction, liver and spleen relative to live weight were lighter in restricted animals compared to control animals suggesting that feed restriction decreases the metabolic activity and size of the liver. However, during the realimentation period when the restricted animals were compensating the liver and spleen weights were similar to control animals by day 29 of realimentation, and heavier than control animals by day 50 of the study. The liver and spleen weights had returned to weights similar to

the control weights by day 134 post realimentation. Many studies reported greater feed intake in compensating animals following feed realimentation (Sainz *et al*., 1995), often resulting in greater gut fill proportion (Yambayamba *et al*., 1996a). In ruminants, especially large ruminants such as cattle, gut fill fluctuations can account for up to 40 kg of bodyweight in mature animals (Phillips, 2010). Additional gain in weight due to increased gut contents, often mistakenly recognised as compensatory growth, must be adjusted for in compensatory growth models when calculating actual live weight gains during the realimentation period.

Myofibre classification was investigated in association with compensatory growth (Lehnert *et al*., 2006). These authors reported a shift from fast glycolytic to slow-twitch oxidative fibres in the *M. longissimus* during the restriction period. Following feed realimentation, fibre type percentages returned to values similar to prior to feed restriction.

1.5.5 Physiological mechanisms regulating compensatory growth

The nature of the restriction diet, length of restriction period, and the stage of development and body condition of the animal prior to restriction affect the degree of compensation. Therefore mechanisms controlling compensatory growth are difficult to elucidate.

A rise in circulating levels of IGF-1 is sometimes considered a regulator of accelerated growth; however, reports in the literature are equivocal on this point. Yambayamba *et al*. (1996b) reported a reduction in circulating levels of IGF-1 in heifers during a restriction period with IGF-1 concentrations rising to the same concentrations as control animals upon realimentation, suggesting that compensatory growth was not due to elevated levels of circulating IGF-1. In addition, Ritacco *et al*. (1997) concluded that compensatory growth was not mediated by IGF-1 in runt piglets. However,

Ellenberger *et al*. (1989) and Hornick *et al*. (1998b) observed greater plasma concentrations of IGF-1 in compensating animals compared to control animals during the realimentation period. An in-depth investigation of the somatotropic axis including the involvement of the IGFBP would provide more knowledge of the somatotropic axis control over compensatory growth.

The reduction in circulating IGF-1 during a feed restriction period, *via* negative feedback mechanisms, results in an increase in plasma GH concentrations (Hornick *et al*., 1998b; Martínez-Ramreíz *et al*., 2009). Yambayamba *et al*. (1996b) noted that although IGF-1 rose to the same concentration as control animals by day 10 of the realimentation period, GH was still elevated by day 31 suggesting that GH is possibly involved in regulating compensatory growth. However, Blum *et al*. (1985) reported that GH concentrations were slightly higher, but not significantly above those of the control animals during the realimentation period.

1.5.6 Molecular mechanisms regulating compensatory growth

Researchers in Australia (Lehnert *et al*., 2006) attempted to unveil the molecular mechanisms regulating compensatory growth in cattle; however, only one gene, relating to fibre type, was revealed as being differentially expressed between control and compensating animals during the realimentation period. It must be noted however that gene expression profiles during the realimentiation period were obrtained 84 days post realimentation and by then the animals had entered a normal growth trajectory. None the less, this study offers huge insight into genes and pathways differentially expressed during the differential feeding period between control and restricted animals.

At a proteome level, Lametsch *et al*. (2006) reported that during compensatory growth in pigs, seven proteins were found differentially expressed between control and compensating pigs. The seven proteins included heat shock cognate 70 (HSC70), heat

shock protein 27 (HSP27), enolase 3, glycerol-3-phosphate dehydrogenase (GPDH), aldehyde dehydrogenase E2, aldehyde dehydrogenase E3, and biphosphoglydrtae mutase. The heat shock proteins, HSC70 and HSP27, have been associated with muscle development. HSC70 plays a role in the regulation of proteolytic pathways during muscle development and regulation while HSP27 is involved in stabilisation of microfilaments and cytokine signal transduction (Liu and Steinacker, 2001). Additionally, enolase 3 and GPDH are involved in glycolysis and lipogenesis, respectively. Aldehyde dehydrogenase catalyses the oxidation of aldehydes to carboxylic acids (Purich and Allison, 2000), however, the role of aldehyde dehydrogenase E2, aldehyde dehydrogenase E3, and biphosphoglydrtae mutase enzymes in compensatory growth is unclear. Again, muscle sampling time was late into the realimentation period (day 60), suggesting that key regulatory genes and pathways were missed in the proteome profiling.

More recently, Connor *et al*. (2009) investigated the effect of compensatory growth on hepatic gene expression at day -14, +1, and +14 relative to feed realimentation following feed restriction, using micro-array technology. The authors reported increases in hepatic genes relating to the mitochondrial complex and electron transport in animals experiencing compensatory growth. It has been hypothesised that differences in mitochondrial activity may be associated with differences in feed efficiency (Kolath *et al*., 2006; Connor *et al*. 2009).

1.6 Meat Quality

1.6.1 Introduction

Meat quality is a term used to describe a range of traits that the consumer and/or processor perceives as desirable. These traits are divided into visual (colour and texture

of the meat, quantity and colour of fat, water holding capacity), sensory (tenderness, flavoursome, juiciness), credence traits (safety and health concerns) and more subjective traits ('green' image, production environment or welfare status) (Becker, 2000; Warner *et al*., 2010).

1.6.2 National and international meat quality standards

In Ireland, the Bord Bia Quality assurance mark (Figure 1.13), introduced in 1989, is used to certify that the product is produced to a particular set of standards and that the producer/processor is inspected regularly to ensure that these requirements are met (Bord Bia, 2011). In addition, Bord Bia operates the Beef Quality Assurance scheme (BQAS) which sets out additional strict guidelines relating to traceability, welfare, chilling and hygiene at both the farm and slaughter house. Although these schemes ensure high levels of meat quality and safety, this mark does not guarantee tenderness or flavour, key issues that consumer research indicated are important elements of eating quality (Becker *et al*., 1998: Moloney *et al*., 2001). In Australia, an innovative grading scheme (Meat Standards Australia, MSA) was adopted in 2000 which guaranteed quality score is assigned to meat. Cuts of meat are labelled with a grade: 3 stars (tenderness guaranteed), 4 stars (premium tenderness) or 5 stars (superior tenderness) as well as a recommended cooking method. Consumers are willing to pay considerably more for MSA-graded cuts than non-graded cuts (Lyford *et al*., 2010). Although there is no Irish or European standard for assurance of tenderness, flavour and consistency, many large commercial outlets are introducing 'benchmark' standards outlining key requirements from both the farm and the processor based on perceived consumer needs. These include choosing specific breeds, slaughter age, dry aging and an extended aging period of 28 days to improve flavour and tenderness in beef.

Figure 1.13 Bord Bia quality symbol
Bord Bia 'Quality' assurance mark. (*Source*: http://bordbia.ie)

1.6.3 Factors affecting meat quality

1.6.3.1 Genotype, feeding level and environment

Genotype is an important matter affecting meat quality (Monsón, 2004). However, it is difficult to compare the effect of genotype in isolation as other factors relating to the chosen breeds may differ i.e. early *vs.* late maturing breeds, intramuscular fat accumulation, age at slaughter, production system (grass or concentrates) or mutations in their DNA sequence (Piedmontese and Belgian Blue breeds).

Many researchers to date (Keane and Allen, 1998; French *et al*., 2001; Moloney *et al*., 2001; 2008) have reported that pre-slaughter feeding level and growth rate had no effect on many meat quality traits including tenderness, meat colour, shear force, and muscle drip loss percentage. However, other researchers including Hornick *et al*. (1998a), Carrasco *et al*. (2007) and Therkildsen *et al*. (2008) reported that muscle and fat colour and drip loss percentage were affected by feeding level suggesting further study is required to fully elucidate the effects of feeding level on meat quality traits.

The environment prior to slaughter affects meat quality (although much of this research relates to swine rather than bovine) and therefore transport time to abattoir, resting time after transport, amount of time spent in the lairage area (holding pens), opportunities to mix in large unfamiliar groups and availability of food and water

(Warriss, 1990; Lahucky *et al*., 1998; Honkavaara *et al*., 2003; Villarroel *et al*., 2003) should be considered also for cattle in order to reduce stress levels.

1.6.3.2 Intramuscular fat

Intramuscular fat and its effect on meat quality traits, especially sensory traits, has evoked much research and debate (Nishimura *et al*., 1999; Liu *et al*., 2009; Hocquette *et al*., 2010). In many instances intramuscular fat is positively associated with some sensory palatability characteristics including increased juiciness and improved flavour in beef and pork (Oddy *et al*., 2001). Hocquette *et al*. (2010) reported that intramuscular fat marbling directly affects juiciness and flavour but that tenderness was influenced indirectly. In a recent report by Hocquette *et al*. (2011), the authors describe a curvilinear relationship between intramuscular fat and flavour. In addition, it has been suggested that the relationship between intramuscular fat and tenderness is dependent on other factors including muscle type (Nishimura *et al*., 1999; Oddy *et al*., 2001).

1.6.3.3 Temperature and pH of carcass post mortem

As muscle is converted to meat, anaerobic glycolysis takes place and glycogen stores in the muscle are depleted (for a review see Pearce *et al*., 2011). The pH of the muscle falls as lactic acid is produced as an end product of glycolysis. The rate of pH fall and the ultimate pH (pHu) of the meat effects many aspects of meat quality (i.e. colour and water holding capacity).

Temperature of the cold room has a significant effect on cooling rate (Hannula and Puolanne, 2004). If the carcass temperature falls too fast, and glycolysis is too slow (i.e. a high muscle pH), sarcomere length is reduced, and as a result toughening of the meat occurs (for a review see Maltin *et al*., 2003; Warner *et al*., 2010). Alternatively, if

the rate of temperature fall is slow, and glycolysis is fast, toughening of the meat can also occur (Figure 1. 14).

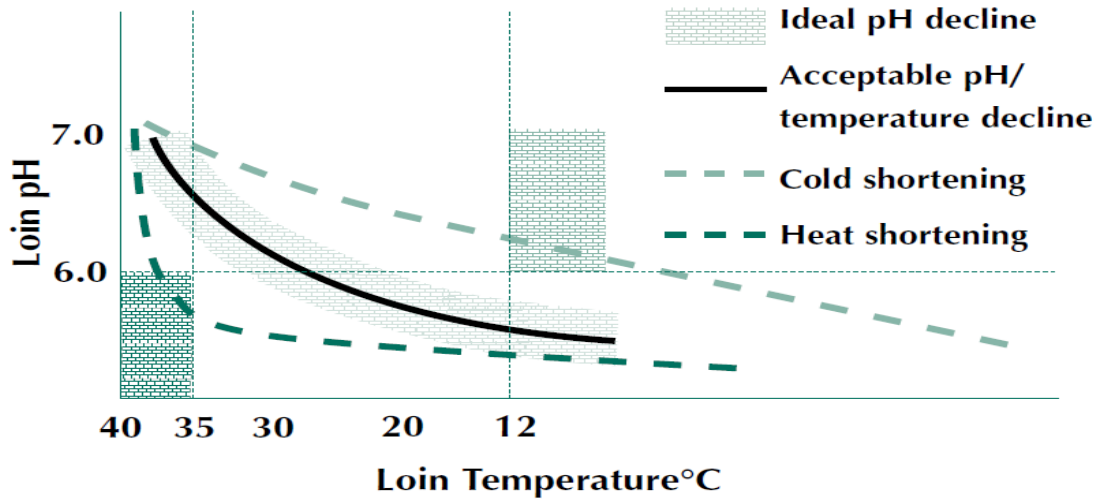


Figure 1.14 Ideal pH and temperatre decline in meat post slaughter
(*Source*: http://www.shorthorn.com.au/assets/files/PH_temperature_decline_and_Beef_eating_quality.pdf

1.6.3.4 Compensatory growth

Intensive, but often conflicting, research has been undertaken to assess if compensatory growth prior to finishing has an effect on meat colour, shear force and organoleptic properties of meat (Table 1.2). Many studies to date in swine (Kristensen *et al*., 2004; Wood *et al*., 2004) state that compensatory growth affects meat quality attributes, with an increase in meat tenderness and meat juiciness. However, it must be noted that this may be a consequence of differences in intramuscular fat marbling rather than the direct effect of compensatory growth. Compensatory growth prior to slaughter improved meat quality characteristics in many studies (Stolzenbach *et al*., 2009; Therkildsen *et al*., 2011) but not all (Kristensen *et al*., 2004; Hansen *et al*., 2006). In addition, many researchers reported that the majority of the effects related to genotype of the animal rather than feeding treatment (Lobley *et al*., 2000; Sinclair *et al*., 2001; Moloney *et al*., 2001; 2008) and this warrants further investigation.

Table 1.2 Summary of studies involving compensatory growth and the effect on meat quality traits

Breed[1]	Sex[2]	Length of restriction period (day)	Length of realimentation period	Muscle type	Effect of compensatory growth (CG) on meat quality	Reference
HF	C	17 days	17 days	*M. longissimus thoracis et lumborum* *M. semimembranosus*	• Lower shear force in both muscle types following CG feeding regime • No effect on *L*, *a* or *b* measurements • Improved sensory and flavour • Improved flavour as a result of CG feeding regime	Therkildsen *et al*. (2011)
F	S	56 days	70 days	*M. longissimus thoracis et lumborum*	• No effect on shear force at day 2, 7 or 14 days of ageing • No effect on *L*, *a* or *b* measurements at day 2, 7 or 14 days of aging • No effect on cook loss • No effect on sarcomere length	Moloney *et al*. (2008)
BR	S	120 days	316 days	*M. longissimus thoracis et lumborum* *M. semitendenosis*	• No effect on shear force measurements • No effect on *L*, *a* or *b* measurements • No effect on cook loss	Tomkins *et al*. (2006)
HF	B	97 days	140 days	*M. longissimus thoracis et lumborum* *M. semimembranosus* *M. supraspinatus*	• Texture was muscle type specific • Flavour was muscle specific as LD developed off flavour when derived from CG-based feeding regime	Hansen *et al*. (2006)
BB	B	115 days 239 days 411 days	147 days 120 days 112 days	*M. longissimus thoracis*	• Cattle exhibiting CG had lower shear force • Cattle exhibiting CG had greater redness, yellowness, cooking losses and drip losses	Hornick *et al*. (1998a)

[1] HF = Holstein-Friesian; F = Friesian; BR = Belmont Red; BB = Belgian Blue. [2] B = Bull; S = Steer C = Cow.

1.7 Transcriptomics as a tool to understanding muscle growth

1.7.1 Introduction

The study of the transcriptome is referred to as transcriptomics. The transcriptome consists of all RNA transcripts including: mRNA, rRNA, tRNA, and non coding RNA (miRNA and other small RNAs). Transcription is the synthesis of RNA under the direction of DNA in the nucleus of the cell. In brief, DNA provides a template for assembling a sequence of pre-messenger RNA which contains both introns and exons. The introns are removed from the pre-mRNA before the mRNA can leave the nucleus for translation to a polypeptide, as reviewed by Witten and Ule (2011). In the past decade there have been dramatic advances in our understanding of the bovine genome and transcriptome. The bovine genome, version 4.0, was sequenced and annotated by the Bovine Genome Sequencing and Analysis Consortium and released in 2007. This work was carried out by over 300 researchers across 25 different countries. The 7.1 fold coverage bovine genome sequence represents primarily DNA from a Hereford female animal. The *Bos Taurus* genome contains approximately 22,000 protein-coding genes with 14,350 orthologs shared among seven mammalian species (Elsik *et al*., 2009). The Btau 4.0 genome is made up of 29 autosomes and the X chromosome with 90% of the genome aligning to these chromosomes.

1.7.2 Polymerase chain reaction

Polymerase chain reaction (PCR) was first developed in 1984 by Kary Mullis. This process allows the amplification of DNA, without cloning, and this has revolutionised the field of molecular biology. This process was made possible through the discovery of *Thermus aquaticus*, a thermophilic bacterium living in hot springs (Brock and Freeze, 1969). A heat resistant enzyme, *Taq* polymerase, was isolated from *Thermus*

Aquaticus in 1976 (Chien *et al*, 1976). A PCR reaction involves three main temperature adjustments. Firstly, a high temperature (95 ℃) is applied for approximately 30 seconds and the DNA double helix, consisting of 4 different nucleotide types, is separated into two strands. The temperature is then reduced to approximately 55 ℃-60 ℃, allowing primers to anneal to the 5' region of the DNA strand. The temperature then rises to 72 ℃ to optimise the activity of *Taq* Polymerase. The polymerase then extends the complimentary strand of the DNA resulting in the formation of the double helix structure again.

1.7.3 Real-time reverse transcription PCR

Real-time reverse transcription quantitative PCR (RT-qPCR) provides a sensitive and accurate method of measuring the amount of target sequence or gene expression in a sample. RT-qPCR has the capacity to detect and measure fluorescence which correlates with PCR product concentration. In the initial stages of RT-qPCR, there is little change in fluorescence; therefore, a baseline is set which acts to subtract the background fluorescence from the overall fluorescence. A threshold line is set above the baseline yet low enough to be within the exponential growth region (VanGuilder *et al*., 2008). A reporter dye, for example SYBR green, binds double-stranded DNA and the increase of fluorescence signals indicates amplification. A cycle threshold (Ct) is calculated and this refers to the cycle number at which the amplification curve (fluorescence) crosses the threshold line. In addition, a passive reference dye provides an internal fluorescence reference to which the reporter dye can be normalised during data analysis. qPCR has many applications, such as in the quantification of gene expression, microRNA detection, viral and bacterial quantification, gene knockout studies and validation of microarray studies (Jiang *et al*., 2005). For the detection of differentially expressed genes, data is normalised to a reference gene, also referred to a house-keeping gene in

the past. A reference gene is a gene tested for expression stability, in that the expression varies very little (Peréz *et al*., 2008; Bustin *et al*., 2009).

1.7.4 Next generation RNAseq

RNA*seq* is a recently developed, highly sensitive, approach to transcriptome sequencing (Bullard *et al.*, 2010). RNAseq employs deep-sequencing of the transcriptome and quantification by counting the number of reads which align to a given transcript. RNAseq has many advantages and novel opportunities over earlier technologies in that it is not limited to detecting transcripts corresponding to existing genomes, alternative splicing can be identified and sequence variation on a nucleotide bases can be revealed (Mortazavi *et al*., 2008). Furthermore, RNAseq has shown high levels of reproducibility, both for technical and biological replicates (Nagalakshmi *et al*., 2008). In a short period of time, RNAseq has become the method of choice for transcriptome analyses; however, as with all technologies RNAseq is not bias-free. PCR amplification during the cDNA library preparation steps introduces bias, although this effect can be lessened with the advent of on-flow cell reverse transcription sequencing (FRTseq) (Mamanova *et al*., 2010) and the introduction of fewer PCR cycles. In addition, a gene length bias exists in that there is a greater statistical ability to detect longer genes as differential expressed (Oshlack and Wakefield, 2009). At present, the above issue is addressed by careful statistical modelling with the advent of Goseq software (Young *et al*., 2010) which assesses GO and pathways for a preponderance of long genes and corrects accordingly.

1.7.4.1 Illumina sequencing by RNAseq

The Illumina method of RNAseq involves the sequencing of cDNA libraries, synthesised from mRNA. Universal adapters, attached onto the ends of each cDNA

library bind PCR primer which are immobilised onto a glass slide, referred to as a flowcell. The PCR primers then extend to form a complement of the DNA library (Figure 1.15 a,b). The original template is removed and the next step is quite unique in that the single stranded DNA bends to allow the adapter at the adjacent end of the strand to bind with a neighbouring PCR primer (Figure 1.15 c,d). Polymerase extends the PCR primer, forming a double stranded bridge (Figure 1.15e). This bridge is then denatured to form two single stranded templates. This process, known as bridge amplification, is repeated numerous times (Fuller *et al*., 2009) and allows the generation of million of clusters. The reverse strands are then cleaved and washed away leaving only the forward stranded templates.

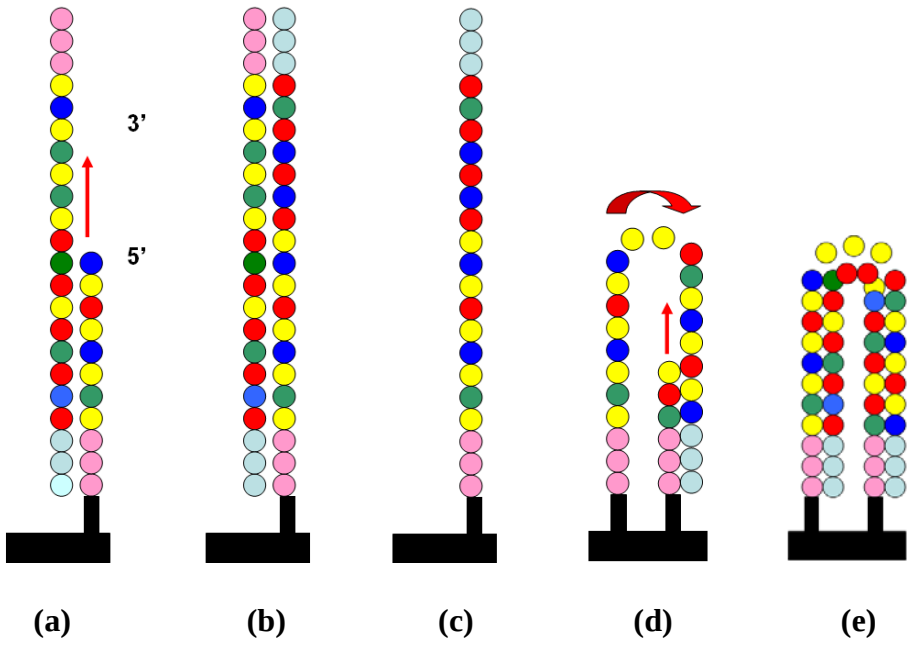


Figure 1.15 Schematic representation of mRNA*seq* workflow
cDNA libraries are hybridised onto the flowcell. DNA polymerase extend the length of the template making a complement strand (a-b). The original strand is washed away (c) and bridge amplification occurs (d-e). The reverse strand is cleaved away and the templates are ready for sequencing by synthesis.

The above process takes place on a cluster station and from this the flowcell is transferred to the Illumina Genome Analyzer (GA_{2x}) for sequencing. A sequencing primer is added to the flowcell which binds the adapter sequence at the 3' end of each

transcript. Following this, the flowcell is flooded with dNTPs and DNA polymerase. A single nucleotide attaches to each strand and a laser and camera are used to detect and capture the fluorescence at four different wave lengths. Once the signal has been documented, the fluorophore, responsible for the fluorescence, is removed and the process is repeated again with a new nucleotide. This process is referred to as sequencing by synthesis. The GA_{2x} assembles all sequences for each cluster and are then exported for bioinformatic analysis.

1.7.4.2 Bioinformatic analysis of RNAseq data

Paulien Hogeweg and Ben Hesper coined the term "bioinformatics" in the 1970's for the study of informatic processes in biotic systems. However, since the late 1980's the term bioinformatics refers to the computational methods for comparative analysis of genome data (Hogeweg, 2011). Bioinformatics allow the organisation, filtering and decoding of very large datasets generated from genomic, transcriptomic and proteomic analysis. For the analysis of RNAseq transcriptomic data, packages including Bowtie (Langmead *et al*, 2009), Tophat (Trapnell *et al*, 2009) and EdgeR (Robinson *et al*., 2010) are utilised for the alignment of the transcriptome to the genome and differential gene expression analysis.

1.7.4.3 Gene ontology and pathway analysis

Gene ontology (GO) is a standardised and consistent method of describing gene and gene products in different databases and was introduced in 2000 by the Gene Ontology Consortium. The aim of the Consortium was to provide a controlled vocabulary for describing the roles of genes and gene products in any organism (Ashburner *et al*., 2000). Gene Ontology's describe gene products in terms of three domains: biological processes, molecular functions, and cellular components (Ashburner *et al*., 2000). There

are many tools available for performing gene ontology (GO) analysis including DAVID (Dennis *et al*, 2003), EasyGO (Zhou and Su, 2007), and GSEA (Subramanian *et al*., 2005). Standard methods of gene ontology assume that each gene is independent and has equal probability of being detected as differentially expressed (Young *et al*., 2010). However, in RNAseq experiments, the expression level of genes correspond to the number of reads that align to that transcript and therefore differences in gene length will yield differing numbers of total reads. This makes standard methods of GO unsuitable for RNAseq data analysis. New GO software was needed for RNAseq data that accounted for selection bias due to gene length resulting in the development of GOseq (Young *et al*., 2010) which accounts for the bias relating to transcript length or total read count.

Pathway analysis may also be performed and there are many softwares available for this procedure, for example Ingenuity Pathway Analaysis (IPA; Ingenuity Systems, www.ingenuity.com), InnateDB (Lynn *et al*., 2008), and Kyoto Encyclopaedia of Genes and Genomes (KEGG; Kanehisa and Goto, 2000). As mentioned above however, there is a gene length bias occurring with RNAseq data and therefore caution is required when carrying out pathway analysis (Oshlack and Wakefield, 2009).

1.7.5 Proteomics

1.7.5.1 Introduction

The word 'proteome' was coined by Dr. Marc Wilkins in 1994 and comes from the words '*prote*in' and 'gen*ome*'. Proteomics refers to study of the proteome. Proteomic-based approach offers the researcher a snapshot of the proteins of the cells been studied (Guo *et al*., 2008). Translation occurs in ribosomes whereby proteins are formed when base sequences in mRNA are converted into amino acid sequences of a polypeptide. In

brief, three base pairs of DNA make up a codon, with each codon representing an amino acid i.e. the base triplet UCA on an mRNA strand results in the placement of the amino acid serine of the polypeptide. Many mechanisms are available to expand the coding capacity of the genome with approximately 22,000 human genes (Pertea and Salzberg, 2010), similar to the bovine genome (Elsik *et al*., 2009), coding for tens if not hundreds of different proteins (Saghatelian and Cravatt, 2005) although exact quantities are not available. Firstly, diversification of proteins occurs at the transcriptional level by mRNA splicing (Hampson and Rottmann, 1987), including tissue-specific alternate splicing (Fürbass *et al*., 1997). Alternative splicing allows multiple transcripts to be produced from a single gene. Secondly, post-translational modifications of proteins at one or more sites may results. There are two types of post-translational modifications; covalent modification and cleavage of the protein backbone (Walsh *et al*., 2005). Post-translational modifications can determine a protein activity state, turnover and interactions with other proteins (Mann and Jensen, 2003). These mechanisms offer the organism a diverse range of proteins and their functions.

The transcriptome and the proteome are irrevocably linked as the transcriptome covers all protein-coding genes. One may argue that analysis of the proteome is more important for identifying the functional nature of the transcriptome. That said, the current generation of proteomic tools is yet to catch up to the rapidly evolving transcriptomic tools currently available.

1.7.5.2 2D-gel electrophoresis and protein identification

A 2D-gel based approach separates proteins firstly based on their isoelectric point (p*I*), followed then by separation based on their molecular mass. The p*I* of a protein is the point on the pH scale at which the net charge of the protein is zero. This technique

allows the separation of hundreds of proteins on one 2D gel. The protein spot can be excised from the gel and its identification obtained using mass spectrometry.

Frederick Sanger (with Hans Tubby) was the first to discover a method to sequence proteins when he detailed the complete amino acid sequence of the two polypeptide chains of bovine insulin (Sanger and Tubby, 1951; Sanger and Thompson, 1953a,b). This sparked an interest in protein sequences in that it was now known there was a well defined genetic code. Most proteome-based experiments incorporate mass spectrometry for the identification of proteins. Proteins are digested to peptides using a trypsin enzyme and the peptides are fragmented to a 'MS/MS' spectra which can then be used to identify the protein. Recent developments have resulted in a quantitative method to assess protein abundance levels (Ong and Mann, 2005).

1.8 Hypothesis and objectives of thesis

Based on a review of the literature relating to the physiological and molecular mechanisms regulating muscle growth in cattle, further research was required to understand mechanisms regulating higher growth potential in cattle. In addition, further research was required into the elucidation of key mechanisms driving compensatory growth at a physiological and molecular level.

It was hypothesised that key genes and proteins regulating muscle growth and development could be identified between animals of high and low genetic merit for growth. Additionally, by selecting all animals of one growth potential, further elucidative techniques could unveil key pathways controlling the compensatory growth phenomenon in crossbred Aberdeen Angus steers.

To test this hypothesis, two studies were undertaken, with the following five objectives:

1. To examine the effect of sire breed and sire EPD_{cwt} on the mRNA expression of genes of the somatotropic axis in *M. longissimus thoracis et lumborum* in Aberdeen Angus × Holstein-Friesian (AA) and Belgian Blue × Holstein-Friesian (BB) cattle using qRT-PCR (chapter 3).

2. To examine the effect of sire breed and sire EPD_{cwt} on the expression of proteins in *M. longissimus thoracis et lumborum* in AA and BB cattle using 2D gel electrophoresis and mass spectrometry (chapter 4).

3. To study the response of crossbred AA and BB steers, to differential feeding treatments with a view to examining the potential of these two genotypes to exhibit compensatory growth following feed realimentation (chapter 5).

4. To examine the effect of compensatory growth on meat quality and sensory analysis in *M. longissimus thoracis et lumborum* from AA and BB steers (chapter 6).

5. To examine the transcriptome for key regulatory pathways controlling *M. longissimus thoracis et lumborum* growth during feed restriction and compensatory growth in AA steers using RNA*seq* analysis (chapter 7).

Chapter 2

Materials and Methods

2.1 Materials

2.1.1 Experimental subjects

The experimental subjects used in each chapter are described in Table 2.1. Examples of AA and BB genotypes are shown in Figure 2.1.

Table 2.1 Description of the experimental subjects used in this thesis

Chapter	Sex	n	Breed (n)	Trial[1]	Tissue collection
Chapter 3	Steer	32	AA[2] × HF[3] (17) BB[4] × HF (16)	*H*[5] (16) *L*[6] (17)	Slaughter
Chapter 4	Steer	20[7]	AA × HF (10) BB × HF (10)	*H* (10) *L* (10)	Slaughter
Chapter 5 and 6	Steer	46	AA × HF (22) BB × HF (24)	H-H[8] (23) L-H[9] (23)	Slaughter
Chapter 7	Steer	12[10]	AA × HF (12)	H-H (6) L-H (6)	Biopsy[11]

[1]Genetic merit (*H* and *L*) or compensatory growth (H-H and L-H) study, [2]Aberdeen Angus, [3]Holstein-Friesian, [4]Belgian Blue, [5]High for genetic merit for growth, [6]Low for genetic merit for growth, [7]Subset of animals used in chapter 3, [8]*ab libitum* access to feed throughout the study, [9]Restricted feeding for 99 days (d) followed by *ad libitum* access to feed until slaughter (d 299), [10]Subset of Aberdeen Angus animals used in chapter 5 and 6, [11]Biopsy at two time points throughout study [end of differential feeding period (d 97) and 32 days post feed realimentation (d 131)].

Figure 2.1 Crossbred Belgian Blue (left) and Aberdeen Angus (right) steers used in the compensatory growth model (chapter 5, 6 and 7)

2.1.2 RT-qPCR analyses (chapter 3)

2.1.2.1 Nanopure water

Nanopure water was obtained using the Barnstead NANOpure Water purification Systems (Thermo Fisher Scientific Inc., Dublin, Ireland). The water was deionised, passed through a total organic carbon analyser, treated with dual band (185 and 254 nm) UV light and passed through a 0.2 µm filter.

2.1.2.2 Diethyl pyrocarbonate (DEPC) water

DEPC solution was obtained from Sigma-Aldrich Ireland Ltd., Wicklow, Ireland (catalogue number D5758) and stored at 4 °C. In brief, DEPC inactivates RNase from water and other laboratory equipment. To deactivate DEPC the solution is autoclaved as this hydrolysis Diethyl pyrocarbonate releasing ethanol and carbon dioxide.

2.1.2.3 TRI reagent

TRI reagent (Sigma-Aldrich Ireland Ltd., Wicklow, Ireland, catalogue number T9424) was ready to use from the bottle and stored at 4 °C. TRI reagent is a mixture of guanidine thiocyanate and phenol. It dissolves DNA, RNA, and protein on homogenisation or lysis of tissue sample. Once chloroform is added, and centrifuging begins the mixture separates into 3 phases: an aqueous phase containing the RNA, the interphase containing DNA, and an organic phase containing proteins. The aqueous phase can then be removed for subsequent RNA isolation.

2.1.2.4 RQ1 RNase-Free DNase kit

RQ1 RNase-Free DNase kit was obtained from Promega, Southampton, UK (catalogue number 9PIM610) and the regents are listed in Table 2.2. Extracted RNA from tissue

will undoubtedly contain DNA. Prior to RT-qPCR, this DNA must be removed as the intrigrity of RNA must be maintained. RQ1 RNase-Free DNase degrades double stranded and single stranded DNA.

Table 2.2 Reagent composition for RQ1 RNase-Free DNase

Reagent	Composition and initial concentration of solution	
Reaction Buffer	Tris-HCl (pH 8.0)	400 mM
	Magnesium sulphate ($MgS0_4$)	100 mM
	Calcium Chloride (CaCl2)	10 mM
Enzyme buffer	HEPES (pH 7.5)	10 mM
	Glycerol	50 %(v/v)
	Calcium Chloride (CaCl2)	10 mM
	Magnesium Chloride ($MgCl_2$)	10 mM
Stop solution	EGTA (pH 8.0).	20 mM

2.1.2.5 Agilent Bioanalyzer and RNA 6000 Nano kit

RNA quality must be assessed prior to RT-qPCR to determine the degree of RNA degradation. An RNA integrity Number (RIN) number, developed by Agilent, classifies RNA based on the entire electrophoretic trace, including the 18s:28s ratio. The RIN value is a numbering system ranging from 1 (highly degraded) to 10 (intact RNA) and was assessed using the Agilent Bioanalyzer 2100 (Agilent Technologies Ireland Ltd., Cork, Ireland, catalogue number G2939AA) and the RNA 6000 Nano reagents supplied from Agilent (Agilent Technologies Ireland Ltd., Cork, Ireland, catalogue number 5067-1511). The Agilent Bioanalyzer 2100 is based on a combination of microfluidic chips, voltage-induced size separation in gel filled channels and laser-induced fluorescence (LIF) detection on a miniaturised scale (Schroeder *et al.*, 2006). The reagents are stored at 4 °C and allowed to equilibrate to room temperature prior to use.

2.1.2.6 Other reagents

Dulbecco's Phosphate Buffered Saline (DPBS) was obtained through Bio Sciences Ltd., Dublin, Ireland (catalogue number 14190-086).

2.1.2.7 High capacity cDNA reverse transcription kit

DNAse-treated RNA is reverse transcribed using the High Capacity cDNA Reverse Transcription kit supplied by Applied Biosystems (Life Technologies Ltd., Paisley, UK, catalogue number 4368814). The kit comprised of 5 reagents, and the concentrations of each are listed in Table 2.3. All reagents were stored at -20 °C and ready to use from the kit once defrosted on ice.

Table 2.3 Reagent composition of High Capacity cDNA Reverse Transcription kit

Reagents	Initial concentration	Working Volume
10x RT buffer	1.0 mL	2 µL
10x RT Random Primers	1.0 mL	2 µL
25x dNTP Mix	100 mM	0.8 µL
Multiscribe Reverse Transcription	50 U/µl	1 µL

2.1.2.8 Oligo primers

Primers for real-time RT-qPCR (Table 2.4) were commercially synthesised (Sigma-Aldrich Ireland Ltd., Wicklow, Ireland). The PCR products generated by amplification were sequenced to verify their primer specific identity (Biochemistry DNA Sequencing Facility, University of Cambridge, UK).

2.1.2.9 Fast SYBR Green Mastermix

Fast SYBR Green Mastermix was obtained from Applied Biosystems (Life Technologies, Paisley, UK, catalogue number 4385616). The composition of Fast SYBR Green mastermix were buffer, ampliTaq FAST DNA polymerase ultra pure, SYBR Green 1 dye, dNTP, uracil-DNA glycosylase and ROX dye; however, due to patenting issue initial concentration are unavailable at present. The SYBR Green dye binds to all double-stranded DNA in the sample. SYBR Green dye is stored at -20 °C and ready to use once defrosted on ice.

Table 2.4 Bovine oligonucleotide primers

Gene	Sequence	Accession number
IGF-1	F: 5'- AGTTGGTGGATGCTCTCCAGT	NM_001077828
	R: 3'- CACTCATCCACGATTCCTGTC	
IGF-2	F: 5'- ACCAAGGACGAGGAACACAC	NM_174087
	R: 3'- AGGCTCCACTCTCCACTCAA	
IGF-1R[1]	F: 5'- AGGTCCTTCGCTTGGTCAT	XM_002696504
	R: 3'- GCATCTGGGGTTGTACTGC	
IGF-2R[2]	F: 5'- CTACGTCAACGGGGACAAGT	NM_174352[9]
	R: 3' - TCGTTCTGGAGCTGAAAGGT	
IGFBP1	F: 5'- ACCAGCCCAGAGAATGTGTC	NM_174554
	R: 3'- GCTCCTTCCACTTCTTGACG	
IGFBP2	F: 5'- CACATCCCCAACTGTGACAA	NM_174555
	R: 3' GATCAGCTTCCCGGTGTTAG	
IGFBP3	F: 5'- GGAACTCTGGAAACCGACAA	AF305199
	R: 3' - ATGGCTGAGTGGGAAAACAC	
IGFBP4	F: 5'- ATGTGCCTGATGGAGAAAGG	NM_174557
	R: 3'- GCCATCCTGTGACTTCCTGT	
IGFBP5	F: 5'- AGCAAGCCAAGATCGAAAGA	BC149394
	R: 3'- GGCAGTGTTCTCAGCTCCTC	
IGFBP6	F: 5'- GGGAGAGAATCCCAAGGAGA	NM_001040495
	R: 3' - AGTGGTAGAGGTCCCCGAGT	
ALS[3]	F: 5'- TGCTACGCTAGACCACAAC	NM_001146006
	R: 3'- CGGAGACAGTTCCCAGAGAG	
GHR[4]	F: 5'- ATGGCGGTATTGTGGATCAT	NM_176608
	R: 3'- GGATGTCGGCATGAATCTCT	
GAPDH[5]	F: 5'- GGGTCATCATCTCTGCACCT	NM_001034034
	R: 3'- GGTCATAAGTCCCTCCACGA	
EEF1A2[6]	F: 5'- AGGTGAAGTCGGTGGAGATG	BC108110[10]
	R: 3'- GATGACCTGGGACGTGAACT	
HMBS[7]	F: 5'- CCAGCTGCAGAGAAAGTTCC	NM_001046207[10]
	R: 3'- GACCCACAGCATACATGCAC	
ACTB[8]	F: 5'- ACTTGCGCAGAAAACGAGAT	NM_173979
	R: 3'- CACCTTCACCGTTCCAGTTT	

[1]Insulin-like growth factor -1 receptor, [2]Insulin-like growth factor -2 receptor, [3]Acid-labile subunit, [4]Growth hormone receptor,[5]Glyceraldehyde 3-phosphate dehydrogenase. [6]Elongation factor 1 alpha 2, [7]Hyrdoxymethylbilane synthase, [8]β-Actin, [9]M[c]Carthy *et al*. (2009), [10]Perez *et al*. (2008).

2.1.2.10 Other reagents

Nuclease-free water was obtained from Sigma-Aldrich Ireland Ltd., Wicklow, Ireland (catalogue number W4502-1L), aliquoted into vials and stored at -20 °C until use. RNaseZAP was obtained from Ambion (Life Technologies Ltd., Paisley, UK, catalogue number AM9780).

2.1.3 Proteome analyses (chapter 4)

All chemicals were obtained from Sigma-Aldrich Ireland Ltd., Wicklow, Ireland unless other stated.

2.1.3.1 Lysis buffer

7 M urea, 2 M thiourea, 1 %(w/v) dithiothreitol (DTT), 4 %(w/v) 3-[(cholamidopropyl) dimethylammonio] propanesulfonic acid (CHAPS) and 8 %(v/v) ampholytes (pH 3-10; Bio-Rad Laboratories, Hertfordshire, UK, catalogue number 163-1113).

2.1.3.2 Bradford assay

A Bradford assay determines the concentration of solubilised proteins in a sample. Bradford solution (Bio-Rad Laboratories, Hertfordshire, UK, catalogue number 500-002) contains Coomassie Blue G-250 dye, phosphoric acid and methanol. In brief, Coomassie Brilliant Blue G-250 dye, shifts from 465 nm to 595 nm when binding to proteins occur. The Bradford kit contains Bradford solution and a bovine serum albumin (BSA) standard. One part Bradford solution is mixed with four parts deionised water and the solution is stored at 4 °C.

2.1.3.3 Immobiline Drystrip gels

Immobiline Drystrip gels pH 3-10, 24 cm were obtained from GE Healthcare UK Ltd., Buckinghamshire, UK (catalogue number 17-6002-44). Immobiline Drystrip gels contain a preformed pH gradient immobilised in polyacrylamide gels. Proteins migrate through this gradient until they reach their p*I*. The strips are stored at -20 °C and prior to use, the strips are allowed to equilibrate to room temperature, the protective strip is removed and the strips are rehydrated in rehydration solution (section 2.1.3.4) over night.

2.1.3.4 Rehydration solution

8 M urea, 0.5 % CHAPS, 0.2 %(w/v) DTT, 0.2 %(v/v) ampholytes (pH 3-10; Bio-Rad Laboratories, Hertfordshire, UK; catalogue number 163-1113), 12 µL Destreak (GE Healthcare UK Ltd., Buckinghamshire, UK; catalogue number 17-6003-18) and Bromophenol Blue. DeStreak reagent (GE Healthcare Biosciences, UK) was added to the sample solution to stabilise thiol groups and prevent non-specific oxidation.

2.1.3.5 12 % polyacrylamide gel

40 %(v/v) Ultrapure Protogel (National Diagnostics USA, Georgia, USA; catalogue number EC-890), 26 %(v/v) Protogel buffer (National Diagnostics USA, Georgia, USA; catalogue number EC-892), 0.4 %(w/v) ammonium persulfate (APS), 0.04 %(v/v) etramethylethylenediamine (TEMED).

2.1.3.6 Equilibration buffer

6 M urea, 30 %(v/v) glycerol, 2 %(w/v) sodium dodecyl sulphate (SDS), 1.5 M Tris buffer (pH 8.8) and Bromophenol Blue.

2.1.3.7 Ruthenium II Bathophenathroline Disulfonate Chelate 20 mM (RuBPs) dye

Potassium pentachloro aquo ruthenate (0.2 g) was added to 20 mL boiling water and kept under reflux. Bathophenanthroline disulfonate (3M, 0.9 g) was added and reflux allowed to continue for 20 minutes (min) until a greenish-brown solution occurred. Sodium ascorbate solution (500 mM, 5 mL) was the added and reflux was allowed to continue for 20 min until a deep orange-brown solution was formed. The dye was allowed to cool and then the pH adjusted to 7 with sodium hydroxide. The solution was stored at 4 °C in the dark.

2.1.3.8 Coomassie stain

45 %(v/v) methanol, 10 %(v/v) acetic acid and 0.2 %(w/v) Coomassie Brilliant Blue.

2.1.3.9 Sequencing grade modified trypsin

Sequencing grade modified trypsin was obtained from Promega, Southampton, UK (catalogue number V5111). In brief, trypsin is a serine protease that specifically cleaves at the carboxylic side of lysine and arginine. The kit comprised 2 reagents; trypsin enzyme [lyophilised state, previously dissolved in 50 mM Tris-HCl, 1 mM $CaCl_2$ (pH 7.6)] and trypsin reconstitution buffer (50 mM acetic acid). Reconstitution buffer (100 µL) was added to 20 µg trypsin. The solution was aliquoted into 10 x 10 µL and placed at -20 °C.

2.1.3.10 Other reagents

LC-MS chromasolv water (Fluka, Milwaukee, WI, USA; catalogue number 39253) was obtained through Sigma-Aldrich Ireland Ltd, Wicklow, Ireland. Protease "Complete Mini" inhibitor cocktail was obtained from Roche, Clare, Ireland (catalogue number 04693116001).

2.1.4 Animal growth and performance (chapter 3 and chapter 5)

2.1.4.1 Linear body measurements and ultrasonically scanned muscle and fat depth

A calliper was used to measure height at withers, chest depth and width of pelvis while a measuring tape was used to record chest circumference and length of back. To obtain *M. longissimus thoracis et lumborum* and fat depth measurements, an ultrasound scanner (Concept MCV Veterinary Ultrasound scanner with 3.5 MHz probe; Dynamic Imaging, Livingston, Scotland) was used.

2.1.4.2 Area of M. longissimus thoracis et lumborum

A digital planimeter (Placom KP-90N, Sokkisha, Japan) was used to measure the *M. longissimus thoracis et lumborum* area.

2.1.4.3 Clinical biochemistry

2.1.4.3.1 Glucose

Circulating concentrations of glucose were determined using the hexokinase method on an automatic clinical analyser (Olympus AU400 Clinical Analyser, Tokyo, Japan) using reagents supplied by Olympus (catalogue number OSR6121). In brief, glucose is phosphorylated by hexokinase in the presence of ATP and Mg^{2+} to produce glucose-6-phosphate and ADP. Glucose-6-phosphate dehydrogenase (G6PDH) oxidises glucose-6-phosphate to gluconate-6-phosphate with the reduction of NAD^+ to NADH. The increase in absorbance at 340 nm is proportional to the glucose concentration in the sample. Final concentration of reactive ingredients include: Piperazine-N,N'–bis(ethanesulfonic acid (PIPES) buffer (pH 7.6; 24.0 mmol/L), ATP (≥ 2.0 mmol/L), NAD^+ (≥ 1.32 mmol/L), Mg^{2+} (2.37 mmol/L), hexokinase (≥ 0.59 kU/L), G6PDH (≥ 1.58 kU/L). The reagents were ready for use and can be placed directly on board the instrument.

2.1.4.3.2 Urea

Circulating concentrations of urea were determined on an automatic clinical analyser (Olympus AU400 Clinical Analyser, Tokyo, Japan) using reagents supplied by Olympus (catalogue number OSR6134). Briefly, urea is hydrolysed in the presence of water and urease and produces ammonia and carbon dioxide. The ammonia produced combines with 2-oxogluterate and NAPD in the presence of glutamate-dehydrogenase to yield glutamate and NAD^+. The decrease in NADH absorbance per unit time is

proportional to the urea concentration. Final concentration of reactive ingredients include: Tris buffer (100 mmol/L), NADH (≥ 0.26 mmol/L), tetra-sodium diphosphate (10 mmol/L), ethylenediaminetetraacetic acid (EDTA) (2.65 mmol/L), 2-oxogluterate, urease (≥ 17.76 mmol/L), ADP (≥ 2.6 mmol/L), glutamate dehydrogenase (≥ 0.16 kU/L). The reagents required no preparation prior to placement on the instrument.

2.1.4.3.3 NEFA

Circulating concentrations of NEFA were determined on an automatic clinical analyser (Olympus AU400 Clinical Analyser, Tokyo, Japan) using reagents supplied by Randox Laboratories, Co. Antrim, NI (catalogue number FA115). The kit comprised 5 reagents and the composition of each is described in Table 2.5. Reagents R1a and R2a were ready to use from the kit. The enzyme/coenzyme reagent (R1b) was reconstituted with 10 mL of buffer (R1a). Maleimide (R2b) was reconstituted with enzyme diluent (R2a) making sure that maleimide was completely dissolved. This solution was then used to reconstitute the enzyme reagent (R2c) and placed on board the instrument. All reagents were kept at 4 °C. In brief, NEFA is converted to Acyl CoA adenosine monophosphate

Table 2.5 Reagent composition for quantitative NEFA determination

Reagent Name	Composition and initial concentration of solution	
R1a -Buffer	Phosphate buffer (pH 6.9)	0.04 mol/L
	Magnesium chloride	3 mmol/L
	Surfactant	
R1b - Enzyme/coenzyme	Acyl Coenzyme A synthesase	≥0.3 U/mL
	Ascorbate oxidase	≥1.5 U/mL
	Coenzyme A	0.9 mmol/L
	ATP	5.0 mmol/L
	4-aminoantipyrine	1.5 mmol/L
R2a - Enzyme diluent	Phenoxyethanol	0.3 %(w/v)
	Surfactant	
R2b - Malaimide		10.6 mmol/L
R2c – Enzyme reagent	Acyl coenzyme A oxidase	≥10 U/mL
	Peroxidase	7.5 U/mL
	TOOS (N-ethyl-N-(2hydroxy-3-sulphopropyl)m-toluidine	1.2 mmol/L

(AMP) and pyrophosphoric acid (PPi) by Acyl CoA synthetase in the presence of ATP and Co enzyme A. Acyl-CoA is then oxidised to 2,3,-trans-Enoyl-CoA and hydrogen peroxide in the presence of Acyl-CoA oxidase. In the presence of peroxidise, hydrogen peroxide forms a purple adduct by oxidative condensation with N-ethyl-N-(2hydroxy-3-sulphopropyl)m-toluidine (TOOS). The absorbance of the purple pigment at 550 nm is proportional to the concentrations of NEFA.

2.1.4.3.4 β-hydroxybutrate (βHB)

Circulating concentrations of βHB were determined on an automatic clinical analyser (Olympus AU400 Clinical Analyser, Tokyo, Japan) using reagents supplied by Randox Laboratories, Co. Antrim, NI (catalogue number RB 1007). The kit comprised 2 reagents and the composition of each is described in Table 2.6. The buffer was ready to use from the kit following gently inversion. The enzyme/coenzyme reagent was reconstituted with 10 mL of buffer (a). Reagents were stored a 4 °C prior, to and after preparation, and placed onboard the instrument. Briefly, βHB is oxidised to acetoacetate by the enzyme 3-hydroxybutyrate dehydrogenase. Associated with this oxidation, NAD^+ is reduced to NADH and the change of absorbance that accompanies this can be directly correlated with the βHB concentration.

Table 2.6 Reagent composition for quantitative βHB determination

Reagent	**Composition and initial concentration of solution**	
Buffer	Tris buffer (pH 8.5)	100 mmol/L
	EDTA	2 mmol/L
	Oxalic acid	20 mmol/L
Enzyme/Coenzyme	NAD	2.5 mmol/L
	3-hydroxybutyrate dehydrogenase	0.12 U/mL

2.1.5 Meat quality and sensory analysis

2.1.5.1 Temperature and pH of the carcasses

A glass electrode attached to a portable pH meter (Knick Portamess 913 pH meter, GmbH & Co., Berlin, Germany) was used. A thermometer probe (Knick Portamess 913 thermometer, GmbH & Co., Berlin, Germany) was used to measure carcass temperature. A portable pH meter (Model no 250A, Orion Research Inc., Boston, USA) was used to record ph at 48 hours (h) post slaughter.

2.1.5.2 Chemical composition of M. longissimus thoracis et lumborum

The Smart System 5 microwave moisture drying oven and NMR Smart Trac Rapid Fat analyser (CEM Corporation, North Carolina, USA) was used to measure the intramuscular fat and moisture concentrations. Protein concentration was determined using a LECO FP328 (LECO Corp., MI, USA) protein analyser.

2.1.5.3 Muscle and adipose colour

Samples of *M. longissimus thoracis et lumborum* and trimmed adipose colour were measured using a Hunterlab UltraScan XE colorimeter (Hunter Associates Laboratory, Inc., Reston, VA, USA).

2.1.5.4 Warner-Bratzler shear force (WBsf)

An Instron universal testing machine (Model no. 5543, Instron Europe, High Wycombe, Bucks, UK) equipped with a Warner Bratzler shearing device was used to measure shear force. The crosshead speed was 5 cm/min. For analysis of the data, Instron Series IX Automated Materials Testing System software for Windows (Instron Corporation, Bucks, UK) was employed.

2.1.5.5 Sensory and flavour analysis

A computerised sensory system (Fizz, version 2.10, Biosystems, France) was used for direct entry of sensory responses.

2.1.6 RNAseq library preparation

2.1.6.1 Dynabeads Oligo (dT)

Messenger RNA was isolated from total RNA using Dynabeads Oligo (dT) from Bio Sciences Ltd., Dublin, Ireland (catalogue number 610-05). The kit contains 4 reagents and the composition of each are listed in Table 2.7. In brief, the Dynabeads oligo (dT) bind the poly A tail of mRNA, isolating the mRNA from total RNA.

Table 2.7 Composition of Dynabeads Oligo (dT) beads

Reagent	**Composition and initial concentration of solutions**	
Dynabeads Oligo (dT) $_{25}$	Dynabeads Oligo (dT) $_{25}$	5 mg/ml (density 1.6g/cm^3)
	Phosphate –buffered saline (pH 7.4)	
	Tris HCl (pH 7.5)	250 mM
	EDTA	20 mM
	Tween-20	1 %(v/v)
	Sodium azide (NaN_3)	0.02 %(w/v)
Binding Buffer	Tris-HCl, (pH 7.5)	20 mM
	Lithium Chloride (LiCl),	1.0 M
	EDTA	2.0 mM
Washing Buffer	Tris-HCl, (pH 7.5)	10 mM
	LiCl	0.15 M
	EDTA	1 mM
Tris-HCl	Tris-HCl	20mM

2.1.6.2 Fragmentation reagent

The isolated mRNA was fragmented using a fragmentation reagent kit supplied by Ambion (Life Technologies Ltd., Paisley, UK, catalogue number AM8740). The fragment reagent cleaves RNA to sizes between 60-200 nucleotides. The kit contains fragmentation reagent in a buffered zinc solution and a stop solution containing 200

mM EDTA (pH 8). The solutions are stored at room temperature and are ready to use from the kit. The reaction is stopped by the addition of the stop solution.

2.1.6.3 cDNA synthesis

See Table 2.8 for a list of chemicals required for 1st and 2nd strand cDNA synthesis, their concentrations and catalogue numbers.

Table 2.8 Reagents and in cDNA synthesis for RNA*seq*

cDNA synthesis	Reagent	Conc.	Company	Code
1st Strand	Random primers	3 µg/µL	Bio Sci	48190-011
	dNTP Mix	10 mM	Bio Sci	18427-013
	RNaseOUT Recombinant Ribonuclease Inhibitor	40 U/µL	Bio Sci	10777-019
	Superscript II RT		Bio Sci	18064-014
	First strand buffer	5x	-	-
	DTT	100 mM	-	-
2nd Strand	Second Strand buffer	5x	Bio Sci	10812-014
	dNTP Mix	10 mM	Bio Sci	18427-013
	Ribonuclease H	2 U/µL	Bio Sci	18021-071
	E. coli DNA Polymerase 1	10 U/µL	Bio Sci	18010-025

2.1.6.4 End repair, adaptor ligation, gel purification and PCR enrichment

The reagents required for end repair, adaptor ligation, gel purification and PCR enrichment, their initial concentrations and supplier are listed in Table 2.9.

2.1.6.5 2% Agarose gel

Low range ultra agarose (8 g) (Bio-Rad Laboratories, Hertfordshire, UK; Cat no. 161-3106) was weighed out and placed in a 400 mL Duran bottle. Tris-actetate EDTA (200 mL) (Sigma-Aldrich Ireland Ltd., Wicklow, Ireland, catalogue number T9650-1L) was added to the agarose. The mixture was heated in a microwave for approx 5-8 min making sure that the agarose was fully dissolved. In a fume hood, 16 µL of ethidium

bromide (Sigma-Aldrich Ireland Ltd., Wicklow, Ireland, catalogue number E1510) was added to the cooling agarose. The agarose was poured into a gel tray and allowed to set.

2.1.6.6 QIAquick PCR purification kit

The QIAquick PCR purification kit was obtained from Qiagen Ltd., Sussex, UK (catalogue number 28106). The kit contains 3 buffer reagents (buffer PB, buffer PE and buffer EB) and QIAquick spin columns. Buffer PB contains guanidine hydrochloride and isopropanol. Buffer PE contains sodium perchlorate and isopropanol. Buffer EB contains 10 mM Tris Cl (pH 8.5). All components are stored at room temperature.

Table 2.9 Reagents, concentrations and supplier for end repair, adaptor ligation, gel purification and PCR enrichment

	Reagent	Conc.	Company	Code
End Repair	T4 DNA Ligase Buffer with 10mM ATP	10x	NEB[1]	B0202S
	dNTP Mix	10 mM	Bio Sci[2]	18427-013
	T4 DNA Polymerase	3 U/µL	NEB[1]	M0203L
	Klenow DNA Polymerase	5 U/µL	NEB[1]	M0210S
	T4 PNK	10 U/µL	NEB[1]	M0201L
Addition 'A' Base	Klenow buffer	6 ml	NEB[1]	B7002S
	dATP	10 mM	Bio Sci[2]	18252-015
	Klenow 3'-to-5' exo-	5 U/µL	NEB[1]	M0212S
	MinElute Gel extraction kit	-	Quigen[3]	28604
Adaptor Ligation	Quick Ligation Kit		NEB[1]	M2200S
	Ligase buffer	2x	-	-
	T4 DNA Ligase	-	-	-
Gel Purification	Agarose (Low Range Ultra)	2 %(w/v)	Bio-Rad[4]	161-3106
	Ethidium bromide	400 ng/mL	Sigma[5]	E1510
	DNA ladder	500 µg/ml	NEB[1]	N3233L
	TAE buffer	10x	Sigma[5]	T9650-1L
	Disposable Gel Excision Tip (GeneCatcher)	-	Web Scientific[6]	PKB6.5
PCR enrichment	Phusion HF polymerase	2 U/µL	NEB[1]	F530S
	dNTP Set	100 mM	Bio Sci[2]	10297-018
	PCR primer 1.1	25 µM	Illumina[7]	1000537
	PCR primer 2.1	25 µM	Illumina[7]	1000538

[1]New England Biolabs Ltd., Hertfordshire, UK; [2]Bio Sciences Ltd., Dublin, Ireland; [3] Qiagen Ltd., Sussex, UK; [4]Bio-Rad Laboratories, Hertfordshire, UK; [5]Sigma-Aldrich Ireland Ltd., Wicklow, Ireland; [6]WebScientific, Cheshire, UK; Illumina United Kingdom, Essex, UK.

2.1.6.7 Qubit fluorometer and HS dsDNA kit

The Qubit fluorometer and HS dsDNA kit were obtained for Bio Sciences Ltd., Dublin, Ireland (catalogue number Q32866). This kit contains 4 reagents: Qubit dsDNA HS reagent, Qubit dsDNA HS buffer, Qubit dsDNA HS standard 1 and Qubit dsDNA HS standard 2. The reagent and buffer were stored at room temperature and both standards were stored at 4 ℃. The reagent contained 200x in DMSO solution. Standard 1 contains 0 ng/L and standard 2 contains 10 ng/µL in TE buffer.

2.1.6.8 Other reagents

Glycogen (5 mg/mL) was obtained from Ambion (Life Technologies Ltd., Paisley, UK, catalogue number AM9510).

2.2 Methods

2.2.1 Experimental licence

All animal procedures performed in this study were conducted under experimental licence from the Department of Health and Children, in accordance with the Cruelty of Animals Act 1876 and the European Communities Regulations 2002 and 2005. Licence Number B100-3984. In addition, ethics approval was granted from the Animal Research Ethics Committee, University College Dublin, Belfield, Dublin, Ireland. Application No. AREC-P-09-66-Keady-Kenny. Animals were slaughtered in a licensed abattoir, Meadow Meats, Rathdowney, Co. Laois, Ireland.

2.2.2 Experimental design

The experimental design, animal management prior to and during the study and sample collection for chapters 3 to 7 are described in detail below.

2.2.2.1 Experimental design and animal management (chapter 3)

This study utilised muscle samples harvested at slaughter from a larger study by Campion *et al*. (2009a). Briefly, in that study, male progeny (n = 114) of Holstein-Friesian dairy cows and sired through artificial insemination (AI), by bulls of 2 contrasting beef breeds, *viz.* AA (n = 56) and BB (n = 58) were used. Within breed, progeny were classified as from sires of either high (*H*) or low (*L*) EPD_{cwt}. For AA sired animals 32 were of *H* EPD_{cwt} and 24 of *L* EPD_{cwt}, while for *BB* 31 were of *H* and 27 of *L* EPD_{cwt}. There was no difference between the dams of the various genetic groups in estimated genetic merit for beef production and carcass weight (Campion *et al*., 2009b). The finishing diet consisted of a total mixed ration having a grass-silage:concentrate ratio of 30:70 on a dry matter (DM) basis (Campion *et al*., 2009a). For the current

study, a representative subset of 33, AA (n = 17) and BB (n = 16), sired steers with either *H* or *L* EPD_{cwt} were selected. To determine the number of animals to allocate to each group a sample size calculation was performed which used gene expression variance estimates from existing work from this laboratory (Kelly *et al*., 2011) as well as other published estimates. Within breed, these animals were allocated to one of 4 groups, in a 2 (sire breed) x 2 (sire EPD_{cwt}) factorial design, based on the original blocking criteria of Campion *et al*. (2009a). The subgroups were as follows (i) AA*H* (n = 8), (ii) AA*L* (n = 9), (iii) BB*H* (n = 8), and (iv) BB*L* (n = 8). Animals represented the progeny of 16 different sires (AA; n = 7 and BB; n = 9) and had a mean weight and age at slaughter of 591 kg (SD 62 kg) and 764 days (d) (SD 37 d), respectively. Mean values for the subgroups used in this study for the main outcome traits were similar to that of Campion *et al*. (2009a) and there was no over dominance of any particular sires within group.

2.2.2.1.1 Sample collection (chapter 3)

Animals were blood sampled (10 mL) for the analysis of plasma concentrations of IGF-1 and insulin *via* jugular venipuncture at approximately 7, 14, and 18 months of age and again at 2 d before slaughter (24 months of age). Samples of *M. longissimus thoracis et lumborum* were harvested from animals within 30 min of slaughter, washed in sterile DPBS and snap-frozen in liquid nitrogen. Samples were stored in dry ice for 1 h before being transferred to a -80 ^{o}C freezer for long term storage.

2.2.2.2 Experimental design and animal management (chapter 4)

A representative subset of 20 steers from the larger study by Campion *et al*. (2009a) were selected at slaughter in a 2 (sire breed) x 2 (sire EPD_{cwt}) factorial design (n=5 per treatment), giving 4 genetic groups of AA*H*, AA*L*, BB*H* and BB*L*, based on the original

blocking criteria of Campion *et al*. (2009a). The 20 animals represented the progeny of 12 sires (AA; n=6 and BB; n=6) with no over dominance of any particular sire within group. Mean values for the subgroups used in this study for the main outcome traits were similar to that of Campion *et al*. (2009a).

2.2.2.2.1 Sample collection (chapter 4)

Samples of *M. longissimus thoracis et lumborum* were harvested from animals within 30 min of slaughter, washed in sterile DPBS (section 2.1.2.6) and snap-frozen in liquid nitrogen. Samples were stored in dry ice for 1 h before being transferred to a -80 ^{o}C freezer for long term storage. At 48 h post slaughter, steaks were cut from the *M. longissimus thoracis et lumborum* located at the 6th and 10th rib, vacuum packaged immediately, frozen and used for subsequent chemical analysis.

2.2.2.3 Experimental design and animal management (chapter 5, 6, 7)

Spring-born male progeny (n = 46) of Holstein-Friesian dams and sired by AA or BB bulls were identified and sourced from Irish commercial herds in autumn 2009. After arrival at Grange Beef Research Centre, the calves were vaccinated against bovine respiratory syncytial (BRS) virus, parainfluenza 3 virus and pasturella haemolytica using Bovipast RSP (Intervet, Schering-Plough Ltd., Wicklow, Ireland) containing inactivated BRS-Virus strain EV908, Parainfluenza-3-virus and pasturella haemolytica A1. Animals were also vaccinated against infectious bovine rhinotracheitis (IBR) using Rispoval IBR marker live (Pfizer Animal Health, Cork, Ireland) containing Bovine herpes virus type 1. In addition, animals were vaccinated for protection against the clostridial disease, Blackleg using a vaccine containing *C. chauvoei* (Intervet, Schering-Plough Ltd., Wicklow, Ireland). The animals were treated for internal and external parasites using Closamectin (Norbrook Laboratories Ltd., Monaghan, Ireland)

containing 0.5 % Ivermectin and 12.5 % Closantel as active ingredients. Animals were castrated using the burdizzo method (Pang *et al.*, 2009) within 1 month of arrival. They were subjected to a 3 month common feeding period of grass silage *ad libitum* plus 1 kg of concentrates per head per day before commencing the study to acclimatise the animals to their environment, reduce any latent influence of previous environment and facilitate recovery from castration. Mean age at the commencement of the study was 362 (SD 15.5) and 369 (SD 19.4) d for AA and BB steers, respectively. Mean weights were 295 (SD 30.0) and 287 (SD 48.6) kg for AA and BB, respectively. Within genotype, animals were blocked by weight and randomly assigned to 1 of 2 treatment groups in a 2 (genotypes) x 2 (feeding treatments) factorial design. Over a 99 d period, designated as the differential feeding period, 1 group (11 AA and 12 BB) was offered a high energy control diet (H-H) consisting of concentrates *ad libitum* (DM 825 g/kg, *in vitro* DM digestibility (DMD) 862 g/kg, crude protein (CP) 120.9 g/kg, ash 43 g/kg, neutral detergent fibre (NDF) 557 g/kg and acid detergent fibre (ADF) 351 g/kg) and 7 kg of grass silage per head daily (DM 228 g/kg, *in vitro* DM digestibility 677 g/kg, CP 112 g/kg, ash 80 g/kg, NDF 557 g/kg, ADF 351 g/kg and pH 3.6). The second group (11 AA and 12 BB) was offered an energy restricted diet (L-H) consisting of grass silage *ad libitum* plus 0.5 kg of the same concentrate feed as that offered to H-H per head per day. From the end of the differential feeding period (99 d), both groups of animals were then offered a total mixed ration (TMR) having a grass silage:concentrate ratio of 80:20. The concentrate proportion increased gradually over a 3 week period until the animals had *ad libitum* access to concentrate and 7 kg grass silage per head per day. All animals were offered this TMR to facilitate more accurate appraisal of live weight and live weight gain and to ensure normal rumen function. This period, which lasted 200 d, was known as the realimentation period, and all animals were slaughtered together on d 299 of the study.

Animals were weighed on 2 consecutive days at the start of the study, at the end of the differential feeding period and again at slaughter. Additionally, throughout the study, animals were weighed regularly at 2 to 3 week intervals. Weighing was conducted at the same time each morning before fresh feed was offered.

2.2.2.3.1 Sample collection (chapter 5)

Live weights were recorded every 2-3 weeks and live weight gains determined. Feed intakes were recorded daily. Linear body measurements, ultrasonically scanned *M. longissimus thoracis et lumborum* and subcutaneous fat depth were recorded on 4 separate occasions throughout the study: start (d 0), end of differential feeding period (d 99), 32 d following the commencement of realimentation (d 131) and 2 d prior to slaughter (d 297). Blood samples for the analyses of IGF-1, insulin and leptin were collected at these time points also. For the analyses of circulating metabolites, animals were blood sampled on 8 occasions throughout the study: start, (d 0); middle of the differential feeding period (d 55); end of differential feeding period (d 99); early in the realimentation period (d 131); another 2 times during the realimentation period (d 233, d 273) and again before slaughter (d 299).

Following slaughter, cold carcass weight, dressing percentage, carcass conformation, fat class, visceral weights, linear carcass measurements, *M. longissimus thoracis et lumborum* outline and rib joint composition was recorded.

2.2.2.3.2 Sample collection (chapter 6)

The right side of each carcass was cold-boned at 24 h post slaughter. Immediately, a section of *M. longissimus thoracis et lumborum* thirty centimetres distal to the 10^{th} rib was chosen and transferred on ice approximately 2 h to the Teagasc Ashtown Food Research Centre, Dublin, Ireland. Three steaks were cut, each 2.5 cm in thickness. The

adhering fat was removed from the steaks and subsequently used for fat colour analysis, described later (section 2.2.8.4). The first steak was used immediately for drip loss measurement while the second steak was used for colour assessment. Following this the steak used for colour assessment was vacuum packed, aged for 14 days at 2 °C, frozen at -20 °C and subsequently used in the WBsf assessment (section 2.1.5.4). The third steak was vacuum packed, frozen at -20 °C and subsequently used for chemical composition assessment as described below. The remaining muscle, with subcutaneous fat intact, was vacuum packed immediately, aged for 14 days, frozen at - 20 °C and forwarded to the Division of Farm Animal Science, University of Bristol for sensory analysis assessment.

2.2.2.3.3 Sample collection (chapter 7)

The hair was clipped from an area along the back between the 12th and 13th rib (*M. longissimus thoracis et lumborum*). The clipped area was scrubbed and disinfected with alcohol. A local anesthesia (Lidocaine injection) was administrated, with the volume calculated based on the weight of the animal. A small incision of the skin was made with a blade and any blood was cleaned with a sterile swab. A biopsy (~ 0.5 g to 1 g) of muscle was taken with a trochar and cannula instrument (Figure 2.2). The incision was closed with 2 staples and the area was sprayed with antiseptic spray. The staples were removed 10 days later. The muscle was washed in DPBS and snap frozen in liquid nitrogen. Samples were stored at - 80 °C until total RNA was extracted (section 2.2.7.1.1).

Figure 2.2 Trochar and cannula instrument

2.2.3 RT-qPCR analyses (chapter 3)

2.2.3.1 RNA extraction, quantification and qualification

2.2.3.1.1 RNA extraction from tissue (chapter 3 and chapter 7)

In a fume hood, approximately, 100 mg of frozen muscle tissue was placed in a 10 mL glass bottle containing 3 mL of TRI reagent (section 2.1.2.3). After homogenising for 2 min, the samples were allowed to stand for 5 min at room temperature to ensure complete dissociation following homogenisation. The mixture was then evenly divided into 3 Eppendorf tubes and 200 µL of chloroform were added. The tubes were tightly closed and shaken vigorously for 15 sec. The samples were allowed to stand for 2 min at room temperature. The resulting mixture was centrifuged at 12,000 × *g* for 15 min at 4 °C. The centrifugation step separates the mixture into 3 phases: a red organic phase (containing protein), an interphase (containing DNA), and a colourless upper aqueous phase (containing RNA). The aqueous phase was transferred to a fresh 1.5 mL tube and isopropanol was added to each sample at a ratio of 0.6 isopropanol to supernatant (~360 µL). The tubes were vortexed for 10 sec and the tubes transferred to a pre-chilled centrifuge. Samples were centrifuged at 12,000 × *g* for 10 min at 4 °C. The RNA precipitate formed a small white pellet on the bottom of the tube. The supernatant was removed and the 1 mL of 75 % ethanol was added to the pellet. The sample was vortexed and then centrifuged at 7,500 × *g* for 5 min at 4 °C. Following centrifugation, the ethanol was carefully removed and the pellet air-dried briefly ensuring that the pellet did not dry out completely. Nuclease-free water (20 µL) was added to each tube and the pellet dissolved completely by gently pipetting. The contents of each tube were pooled and added to a sterile 1.5 mL tube. To determine the quantity of total RNA in the sample a Nanodrop spectrophotometer was utilised. Before the RNA concentration of

the samples could be read, a blank measurement was required. Nuclease-free water (1.2 µL, section 2.1.2.10) was pipetted onto the end of the fibre optic cable of the spectrophometer. A second fibre optic cable was then brought into contact with the sample causing the sample to bridge the gap between cables. Once the fluorescence of the blank sample was measured at 260 nm the total RNA concentration of the samples could be measured. The concentration in ng/µL were recorded for each sample in triplicate and the results were averaged. The A260/280 ratio, an indicator of protein contamination was also recorded. Samples with ratios between 1.8 and 2.0 were accepted.

2.2.3.1.2 RQ1 RNase-Free DNase kit

RQ1 RNase-Free DNase degrades double and single stranded DNA (section 2.1.2.4) Total RNA and reagents were allowed to defrost on ice. Into a 0.5 mL safe-lock Eppendorf tube, 2 µg of total RNA was added. RQ1 RNase-Free DNase 10X Reaction buffer (1 µL) was added followed by the RQ1 RNase-Free DNase enzyme (2 µL) was then added. Nuclease-free water (to a final volume of 10 µL) was added and the mixture was mix-pipetted. The solution was allowed to incubate at 37 °C for 30 min. Following this, 1 µL of RQ1 DNase stop solution was added to terminate the reaction and the tubes were incubated at 65 °C for 10 min to deactivate the DNase.

2.2.3.1.3 Agilent Bioanalyzer and RNA 6000 Nano kit

RNA quality must be assessed prior to RT-qPCR to determine the degree of RNA degradation. An RNA integrity Number (RIN) number, developed by Agilent, classifies RNA based on the entire electrophoretic trace, including the 18s:28s ratio. To determine the RNA integrity number (RIN) of the total RNA, an Agilent 2100 Bioanalyzer was used (section 2.1.2.5). Prior to use an electrode cleaner containing 350 µL of

RNaseZAP was placed in the instrument. Following, a second electrode cleaner containing 350 µL of nuclease-free water was used. The RNA 6000 Nano kit was used according to the manufacturer's guidelines (section 2.1.2.5). In brief, all reagents were allowed to equilibrate to room temperature for 30 min prior to use. Agilent RNA 6000 Nano gel matrix (red) (550 µL) was pipetted onto the top receptacle of a spin filter. The spin filter was placed in a centrifuge and spun for 10 min at 1500 × *g*. Aliquots (65 µL) of the filtered gel were placed in 0.5 mL RNase-free eppendorf tubes. The aliquots were stored at 4 °C. The RNA 6000 Nano dye concentrate (blue) was vortexed and spun down. RNA 6000 Nano dye concentrate (blue) (1 µL) of was added to a 65 µL aliquot of filtered gel, vortexed thoroughly and visually inspected to ensure proper mixing of gel and dye. This mixture was spun for 10 min at room temperature at 13,000 × *g*. A RNA Nano chip was placed on the chip priming station and 9.0 µL of the gel-dye mix was pipetted carefully into the well marked 'G'. The plunger was placed at the 1 mL position and then the chip priming station was closed. The plunger was pressed until it was held by the clip. After exactly 30 seconds, the plunger was released. After 5 seconds, the plunger was slowly pulled back to the 1 mL position. Carefully, 9.0 µL of the gel-dye mix was then pipetted in the 2 wells above 'G'. RNA 6000 Nano marker (green) (5 µL) was pipetted into the well marked with the ladder symbol and each of the 12 sample wells. One aliquot of the RNA ladder mixture was allowed to thaw on ice. The RNA ladder and the total RNA samples were heat denatured for 2 min at 70 °C. The RNA ladder (1 µL) was pipetted into the appropriate well. Carefully, 1 µL of each sample was placed into each of the 12 sample wells. The chip was vortexed for 60 seconds and placed into the instrument. Samples with RIN values equal to or greater than 8 were deemed acceptable.

2.2.3.2 cDNA synthesis

cDNA was synthesised from total RNA using the High Capacity cDNA Reverse Transcription kit (section 2.1.2.7). In brief, RNA (1 µg) up to a volume of 10 µL was added into a cDNA mastermix. This contained 2.0 µL 10X RT buffer, 0.8 µL 25X dNTP Mix (100 mM), 2.0 µL 10X RT random primers, 1.0 µL Multiscribe Reverse Transcriptase and 4.2 µL nuclease-free water per reaction. The tubes were placed in a Mastercycler Thermal Cycler Gradient set at 25 °C for 10 min, 37 °C for 120 min followed by 85 °C for 5min.

2.2.3.3 Primer design and RT-qPCR

Primers for real-time RT-qPCR (section 2.1.2.8) were designed to measure expression of the candidate and reference genes using the Primer3 software program (Rozen and Skaletsky, 2000). Primer specificity was established using the Basic Local Alignment Search Tool (BLAST) from the National Center for Biotechnology Information (http://www.ncbi.nlm.nih.gov/BLAST/). To determine the relative gene expression levels, a suitable highly stable reference gene was required. Reference genes tested across all samples using RT-qPCR included glyceraldehyde 3-phosphate dehydrogenase (*GAPDH*), β-actin (*ACTB*), hydroxymethylbilane synthase (*HMBS*) and elongation factor 1 alpha 2 (*EEF1A2*) (Byrne *et al.*, 2005; Pérez *et al.*, 2008). Data were analysed using NormFinder, a model-based approach software programme (Andersen *et al.*, 2004; accessible through MultiD Analyses AB, Gothenburg, Sweden) to measure the overall stability of the tested reference genes. The software calculates the intra- and intergroup CV and combines both coefficients to give a stability value - a lower value implying a higher stability in gene expression. Results showed that both *EEF1A2* and *HMBS*, previously reported to be highly stable in bovine *M. longissimus thoracis et lumborum* (Perez *et al.*, 2008), were unsuitable for use in this study, having stability

measures of 0.87 and 0.75 respectively. *GAPDH* had a stability value of 0.54, and as a result was chosen as the reference gene in this study. RT-qPCR reactions were carried out using SYBR FAST Green Mastermix (section 2.1.2.9). Real-time PCR measurements were performed in triplicate using the Applied Biosystems Fast 7500 v2.0.1 instrument with the following cycling parameters: 95 °C for 20s; 40 cycles of 95 °C for 3s; 60 °C for 30s, followed by amplicon dissociation (95 °C for 15s; 60 °C for 60s; 95 °C for 15s and 60 °C for 15s). Primer concentrations were optimised for each gene and disassociation curves were examined for the presence of a single PCR product. The efficiency of the reaction was calculated using a 2-fold serial dilution of cDNA and generation of a standard curve. All PCR efficiency coefficients were between 0.9 and 1.0 and therefore deemed acceptable. The software package GenEx 5.2.1.3 (MultiD Analyses AB, Gothenburg, Sweden) was used for efficiency correction of the raw cycle threshold (Ct) values, interplate calibration based on a calibrator sample included on all plates, averaging of replicates, normalisation to the reference gene and the calculation of quantities relative to the highest Ct.

2.2.4 Proteomic analysis (chapter 4)

2.2.4.1 Protein extraction and quantification

2.2.4.1.1 Crude protein extraction

Samples of *M. longissimus thoracis et lumborum* were harvested from the animals at slaughter, washed in sterile DPBS (section 2.1.2.6) and snap frozen in liquid nitrogen. Muscle samples were stored at -80 °C until analysis. Frozen muscle (100 mg) was weighed and crushed into a fine powder using a mortar and pestle and placed in lysis buffer (section 2.1.3.1). To avoid protein degradation, a protease inhibitor cocktail was

added (section 2.1.3.10). Samples were shaken vigorously at 4 ^{o}C for 150 min before centrifugation at 10,000 × *g* for 20 min at 4 ^{o}C.

2.2.4.1.2 Bradford assay

Bradford colorimetric protein assays were performed to determine protein concentration using BSA as a standard (section 2.1.3.2). A dilution series including a blank ranging from 0 to 100 µg/mL was produced. Standards were allowed to incubate at room temperature for 10 min and then the absorbance was read on a spectrophotometer at 595 nm and a standard curve generated. The samples of interest were then incubated at room temperature for 10 min also and the absorbance read on a spectrophotometer as described above. The readings were compared to the standard curve and a relative measurement of the concentration of protein was determined.

2.2.4.2 2D gel electrophoresis

Immobiline DryStrips (section 2.1.3.3), 24 cm, covering a pH range of 3 - 10 were re-hydrated overnight in 500 µg of sample up to a volume of 450 µL of rehydration buffer (section 2.1.3.4). Isoelectric focusing was carried out in an Ettan IPGphor 3 Isoelectric Focusing Unit (Geheathcare Biosciences, UK) and the following voltage/time programme was used: 100 V for 120 min, 500 V for 90 min, 1000 V 60 min, 2000 V for 60 min, 4000 V for 60 min, 6000 V for 120 min, 8000 V for 150 min, 500 V for 240 min and 8000 V for 300 min. Following focusing, strips were equilibrated for 15 min in 1 %(w/v) DTT followed by 15 min in 2.5 %(w/v) iodoacetamide. After equilibration proteins were separated on a 12 % polyacrylamide gel (section 2.1.3.5) using the DALT*twelve* separation unit (GE Healthcare Biosciences, UK).

2.2.4.3 Protein staining

RuBPS (section 2.1.3.7) a well-established fluorescent dye for protein staining, was applied for 6 h followed by de-staining using 40 %(v/v) ethanol and 10 %(v/v) acetic acid for 15 h. Imagemaster platinum analysis software v5.0 was used for imaging gels using a Typhoon variable mode image scanner (GE Healthcare Biosciences, UK). Progenesis Samespots V3.2.3 software was used for the detection of protein spots, background subtraction and detection of proteins with statistically significant differences across groups. Differentially expressed protein spots ($P < 0.05$) between AA and BB were classified as biologically significant with a fold change greater than 1.9 and were selected for tryptic digestion from gels stained with Coomassie Brilliant Blue G-250. At a fold change of greater than 1.9, no difference in protein abundance was identified between *H* and *L* steers. However, normal biological systems are characterised by larger number of small changes rather than a small number of large changes (Amaral *et al*., 2004). Therefore, a 1.5 fold induction in protein abundance was selected between *H* and *L* steers for EPD_{cwt} (AA*H vs.* AA*L*; BB*H vs.* BB*L*) which is consistent with findings of previous research in relation to the *H* and *L* for growth rate (Bernard *et al*., 2009).

2.2.4.4 Mass spectrometry

Protein spots were carefully excised from 2D gels using a sterile pipette tip and digested using trypsin (section 2.1.3.9) at 37 °C overnight. The solution was removed and 30 %(v/v) acetonitrite (ACN) / 0.2 %(v/v) trifluoroacetic acid (TFA) was added to each gel plug and incubated for 10 min at 37 ^{o}C, followed by 60 %(v/v) ACN / 0.2 %(v/v) TFA for 10 min at 37 ^{o}C. The solution was dried in a speedy-vac overnight and 10 µL 0.1 %(v/v) formic acid was added to the protein pellet. Proteins were identified from their peptide sequence, searching on the NCBI (National Centre for Biotechnology

Information, http://www.ncbi.nlm.nih.gov) and Swiss-prot databases (www.expasy.org). These software programmes were checked using MASCOT search engine (http://www.matrixscience.com; v20100212 Matrix Science, London, UK) with comparison to mammalian databases allowing up to one single trypsin missed cleavage.

2.2.5 Physical measurements (chapter 5)

2.2.5.1 DM intakes and feed analysis

Animals were individually fed in tie-up stalls during the differential feeding period and subsequently moved to individual slatted floor pens for the realimentation period. Fresh feed was offered daily and feed refusals were removed and weighed twice weekly. Two samples of offered silage were collected weekly and stored at -20 °C. One was used to determine DM content whereas the other sample was used for chemical analysis. To determine the DM content, samples were weighed and dried at 40 °C for 48 h in a preheated oven with forced air circulation. For chemical analysis, samples were dried at 40 °C for 48 h, pooled on a monthly basis and milled. The pooled samples were chemically analysed for DM digestibility (DMD) using the method of Tilley and Terry (1963) with the modification that the final residue was isolated by filtration. The CP (nitrogen x 6.25) concentration was determined using a Leco FP-528 N analyser based on the methods of the Association of Analytical Chemists (AOAC, 1990). An Ankom fibre analyser was used to determine NDF values by the method of Van Soest *et al.* (1991) and the filter bag technique of Ankom (2006) was used to calculate ADF concentrations. In addition, volatile fatty acids (VFA) and ash were measured as described by Cummins *et al*. (2007). A sample of juice from the silage was obtained for the determination of pH and ammonia nitrogen (NH_3N) concentration as also described by the method of Cummins *et al*. (2007). Two samples of concentrates were also

collected weekly for laboratory analyses. To determine DM, 1 sample was dried at 98 °C for 16 h. The second sample was dried at 40 °C for 16 h, pooled on a monthly basis, milled and subsequently subjected to chemical analysis (DMD, CP, ash, NDF, ADF and NE) as described for forage

2.2.5.2 Live weight

Animals were weighed using a scales (TRU-Test, Co. Cork, Ireland) every 2 to 3 weeks at the same time in the morning before fresh feed was offered. Prior to slaughter, animals were weighed the day before and again on the morning of slaughter. These weights were then averaged to give a final live SW.

2.2.5.3 Ultrasonically scanned muscle and fat depths

Animals were ultrasonically scanned on their right side at the 3rd lumbar vertebra to obtain *M. longissimus thoracis et lumborum* depth using a Dynamic Imaging ultrasound scanner (Concept MCV Veterinary Ultrasound scanner with 3.5 MHz probe; Dynamic Imaging, Livingston, Scotland). Measurements were obtained 5 times throughout the study: start (d 0); middle of the differential feeding period (d 55); end of differential feeding period (d 99); early in the realimentation period (d 131) and again before slaughter (d 299), as described by Campion *et al*. (2009a). In addition, at each muscle scan occasion, 3 ultrasonically measured subcutaneous fat depths were taken at the third lumbar vertebra with another 4 fat depth measurements taken at the thirteenth rib. These measurements were averaged to give a single mean value for fat depth (Campion *et al*., 2009a).

2.2.5.4 Skeletal measurements

Linear body measurements (Campion *et al*., 2009a) were recorded on 4 separate occasions: start (d 0); end of differential feeding period (d 99); early in the realimentation period (d 131) and again before slaughter (d 299). A calliper was used to measure height at withers, chest depth and pelvic width and a measuring tape was used to record chest girth and back length. These measurements were expressed relative to live weight on the day of measurement.

2.2.6 Physiological measurements (chapter 5)

2.2.6.1 Glucose

Blood samples were collected by jugular venipuncture into vacutainer tubes containing sodium fluoride/potassium oxalate as anticoagulant. Immediately after collection, tubes were gently inverted 3 times to prevent blood clotting. Following centrifugation for 10 min at 2000 × *g* at 4 °C, plasma was collected and frozen at -20 °C until analysis. Circulating concentrations of glucose were determined using an automatic clinical analyser (Olympus AU400 Clinical Analyser, Tokyo, Japan) using a enzymatic UV test and reagents supplied by Olympus (section 2.1.4.3.1)

2.2.6.2 Urea

Blood samples were collected by jugular venipuncture into vacutainer tubes containing lithium heparin oxalate as an anticoagulant. Tubes were inverted gently 3 times to prevent blood clot formation. Following centrifugation for 10 min at 2000 × *g* at 4 °C, plasma was collected and frozen at -20 °C until analysis. Circulating concentrations of urea were determined using an automatic clinical analyser (Olympus AU400 Clinical

Analyser, Tokyo, Japan) using a UV kinetic test and reagents supplied by Olympus (section 2.1.4.3.2).

2.2.6.3 NEFA

Blood samples were collected by venipuncture into vacutainer tubes containing sodium citrate as an anticoagulant. Tubes were immediately inverted 3 times to prevent clotting of the blood. Following centrifugation for 10 min at 2000 × *g* at 4 ℃, plasma was collected and frozen at -20 ℃ until analysis. Circulating concentrations of non-esterified fatty acids were determined using an automatic clinical analyser (Olympus AU400 Clinical Analyser, Tokyo, Japan) using reagents supplied by Randox Laboratories (section 2.1.4.3.3). Samples and reagents were mixed and incubated for exactly 10 min at 37 ℃ and absorbance was read.

2.2.6.4 βHB

Blood samples were collected by venipuncture into vacutainer tubes containing lithium heparin as an anticoagulant. Immediately after collection, tubes were gently inverted 3 times to prevent blood clotting. Following centrifugation for 10 min at 2000 × *g* at 4 ℃, plasma was collected and frozen at -20 ℃ until analysis. Circulating concentrations of βHB were determined using an automatic clinical analyser (Olympus AU400 Clinical Analyser, Tokyo, Japan) using reagents supplied by Randox Laboratories (section 2.1.4.3.4).

2.2.6.5 IGF-1 (chapter 3 and chapter5)

Blood samples were collected by venipuncture into vacutainer tubes containing lithium heparin as an anticoagulant. Immediately after collection, tubes were gently inverted 3 times to prevent blood clotting. Following centrifugation for 10 min at 2000 × *g* at 4 ℃,

plasma was collected and frozen at -20 °C until analysis. Circulating concentrations of IGF-1 were determined by radioimmunoassay (RIA) after an acid-ethanol extraction procedure, as described by Spicer *et al*. (1988). Intraassay CV values for IGF-1 quantification were 9.8, 8.2, and 15.9 % for low, medium, and high standards, respectively for chapter 3. Interassay CV for IGF-1 were 5.4, 5.2 and 3.3 % for low, medium and high standards, respectively for chapter 3. The intraassay CV values for IGF-1 quantification in chapter 5 were 14.3, 12.7 and 13.6 % for low, medium, and high standards, respectively with the interassay CV for IGF-1 10.8, 3.2 and 8.6 for low, medium and high standards, respectively.

2.2.6.6 Insulin (chapter 3 and chapter 5)

Blood samples were collected by venipuncture into vacutainer tubes containing lithium heparin as anticoagulant. Immediately, tubes were gently inverted 3 times to prevent blood clotting. Following centrifugation for 10 min at 2000 × *g* at 4 °C, plasma was collected and frozen at -20 °C until analysis. Insulin concentrations were quantified by fluoroimmunoassay (AutoDELFIA, PerkinElmer Life and Analytical Sciences, Turku, Finland) and validated for bovine plasma (Ting *et al*., 2004). Intraassay CV for insulin was 5.9, 3.5, and 3.1 % for low, medium, and high standards, respectively for chapter 3 while the interassay CV was 11.5, 8.1, and 7.0 % for low, medium and high standards, respectively. The intraassay CV for insulin quantification in chapter 5 were 10.0, 8.8 and 9.9 % for low, medium, and high standards, respectively with the interassay CV for insulin 5.9, 13.4 and 7.2 % for low, medium and high standards, respectively.

2.2.6.7 Leptin

Blood samples were collected by venipuncture into vacutainer tubes containing lithium heparin as anticoagulant. Immediately, tubes were gently inverted 3 times to prevent

blood clotting. Following centrifugation for 10 min at 2000 × *g* at 4 °C, plasma was collected and frozen at -20 °C until analysis. Leptin analysis was carried out using a double antibody RIA as described by Wylie *et al*. (2008). In brief, the primary antibody (GP-OL3) was raised in guinea pigs against recombinant ovine leptin. The second antibody was goat anti-guinea pig IgG. The mean intraassay CV for leptin was 6.9 and 6.7 % for high and low standards, respectively while the mean interassay CV for leptin was 16.5 and 19.9 % for high and low standards, respectively.

2.2.7 Slaughter measurements (chapter 5)

2.2.7.1 Carcass characteristics and non-carcass components

Animals were weighed the day before slaughter and again on the morning of slaughter. These weights were averaged to give final live weight at slaughter (SW). The steers were transported 130 km to Meadow Meats commercial slaughter facility in Rathdowney, Co. Laois and slaughtered within 1 h of arrival.

Carcass conformation class and fat class were automatically recorded on a 15 point scale using video imaging analysis equipment (VBS2000, E + V, Oranienburg, Germany) as described by Hickey *et al*. (2007). Non-carcass components were weighed for each animal separately, namely the heart, lungs, gall bladder, liver, spleen, intestines (full), rumen and reticulum (but excluding abomasums) and omasum (full and empty), fore and hind feet, hide, kidneys, head, and perinephric plus retroperitoneal fat. The former measurments were subsequently scaled to SW while perinephric plus retroperitonneal fat was scaled to carcass weight. Following slaughter cold carcass weight (CW; Hot CW × 0.98) was recorded and dressing percentage calculated as a proportion of CW to SW.

2.2.7.2 Linear carcass measurements

Linear carcass measurements (Campion *et al*., 2009a) were recorded at 3 h post slaughter on the right side of each carcass. A measuring tape was used to record the carcass length, leg length and chest depth, and callipers used to measure the maximum leg width and the leg thickness (width of leg from the medial splitting surface of the symphysis pubis). Measurements wers subsequently scaled to CW.

2.2.7.3 Area of M. longissimus thoracis et lumborum and rib dissection

After 24 h at 4 °C, the right side of each carcass was quartered between the fifth and sixth ribs into a pistola hind quarter (without the flank) and a fore quarter that included the flank as described by Keane and Allen (1998). The pistola was separated by cutting between the 10th and 11th ribs. The *M. longissimus thoracis et lumborum* outline at the 10th rib was traced on to translucent paper and the area was subsequently measured using a digital planimeter (Placom KP-90N, Sokkisha, Japan). Area of *M. longissimus thoracis et lumborum* was expressed relative to carcass weight. The sixth to 10th rib joint (5-rib joint) was weighed and dissected into *M. longissimus thoracis et lumborum*, other muscle, muscle trim, total fat and bone plus ligamentum nuchae/supraspinale.

2.2.8 Meat quality (chapter 6)

2.2.8.1 Temperature and pH of carcass at slaughter

One hour following slaughter, temperature measurements were recorded by making a 3 cm incision with a scalpel blade between the 10th and 11th rib and inserting a thermometer probe (Knick Portamess 913 thermometer, GmbH & Co., Berlin, Germany). Additionally, the pH of the *M. longissimus thoracis et lumborum* was measured by insertion of a glass electrode attached to a portable pH meter (Knick

Portamess 913 pH meter, GmbH & Co., Berlin, Germany), close to the insertion point of the temperature probe. The pH reading was automatically adjusted for carcass temperature. This was repeated periodically for the first 8 h post slaughter. The ultimate pH was recorded at 48 h post slaughter (pHu) using a portable pH meter (Model no 250A, Orion Research Inc., Boston, USA) (section 2.1.5.1).

2.2.8.2 Chemical composition of M. longissimus thoracis et lumborum

Intramuscular fat and moisture concentrations were determined from thawed *M. longissimus thoracis et lumborum* using the Smart System 5 microwave moisture drying oven and NMR Smart Trac Rapid Fat analyser (CEM Corporation, North Carolina, USA) according to AOAC Official Methods 985.14 (AOAC, 1995a) and 985.26 (AOAC, 1995b). Protein concentration was determined using a LECO FP328 (LECO Corp., MI, USA) protein analyser based on the Dumas method in accordance with AOAC Official Method 992.15 (AOAC, 1995c).

2.2.8.3 Drip loss

Drip loss was measured using the hanging bag method (Honikel, 1998). In brief, samples of *M. longissimus thoracis et lumborum* of a standard size (4 cm × 4 cm × 2 cm) and weight (100 g) were cut and weighed at 48 h post slaughter. Samples were suspended in plastic bags without any contact with the inside of the bag, stored at 4 °C and were reweighed after 72 h of hanging. Drip loss was calculated as the percentage of weight lost from the sample over the 72 h period.

2.2.8.4 Muscle and fat colour

A freshly cut sample of *M. longissimus thoracis et lumborum* (25 mm) was trimmed of adhering adipose tissue at 48 h post slaughter, wrapped with oxygen-permeable PVC

film and permitted to bloom in darkness at 4 ^{o}C, for 4 h to permit oxygenation of the myoglobin. Readings of '*L*' (lightness), '*a*' (redness) and '*b*' (yellowness) values were measured and muscle hue angle ('*H*') and saturation ('*C*') were calculated as $\tan^{-1}$ (*b/a*) and $[(a)^{2} + (b)^{2}]^{0.5}$, respectively, on both the muscle and the trimmed adipose tissue using a Hunterlab UltraScan XE colorimeter (Hunter Associates Laboratory, Inc., Reston, VA, USA). Final conversion of hue angle from radians to degrees was achieved by multiplying $\tan^{-1}$ (b/a) by 180/π (Liu *et al*., 1996). Four readings were made on non-overlapping areas of each sample using the optical port (Ø 2.54cm) and average values were reported as final readings. Diffuse illumination (D_{65}, 10°) with an 8° viewing angle was used. The spectrocolorimeter was used in reflectance mode and the specular component was excluded.

2.2.8.5 WBsf and cooking loss

WBsf was measured according to the procedure of Shackelford *et al*. (1994). In brief, steaks were trimmed of external fat, weighed and cooked in open vacuum pack bags in a circulating water bath (Grant instruments Ltd., UK) set at 72 °C, until their internal temperature reached 70 °C (assessed using a Minitherm H18751 temperature probe, Hanna Instruments Ltd., UK). Steaks were cooled to room temperature, reweighed for determination of cooking loss percentage and tempered at 4 °C overnight. Cooking loss percentage was determined as the difference between the weight of the steak after cooking and its initial weight prior to cooking, expressed as a percentage. Seven cores (1.25 cm diameter) parallel to the direction of the muscle fibres were removed from each steak and each core was sheared using an Instron Universal testing machine (Model no. 5543, Instron Europe, High Wycombe, Bucks, UK) equipped with a Warner Bratzler shearing device. The crosshead speed was 5 cm/min. The highest and lowest shear force measurements were excluded in calculation of means. For analysis of the

data, Instron Series IX Automated Materials Testing System software for Windows (Instron Corporation, Bucks, UK) was employed and results were expressed in Newtons (N).

2.2.8.6 Sensory and flavour analysis

On the day before sensory assessment, samples of *M. longissimus thoracis et lumborum* were thawed and steaks 1.9 cm thick were prepared. Steaks were cooked under a conventional grill, turning every 3 min until the internal temperature of the muscle reached 74 ^{o}C as measured by a thermocouple probe. Samples, approximately 2 cm x 2 cm x 1.9 cm were then cut from the approximate centre of the steaks, avoiding areas of connective tissue, and served hot to the 10 member trained sensory panel. Samples were assessed for sensory and flavour characteristics. Each booth contained a computer screen and optical mouse as part of the computerised sensory system (Fizz, version 2.10, Biosystems, France) for direct entry of sensory responses. See Appendix Table A.1 for list of sensory and flavour terms and definitions derived.

2.2.9 RNAseq library preparation (chapter 7)

2.2.9.1 Extraction of mRNA from total RNA

Total RNA was extracted from the muscle biopsy as described in section 2.2.3.1.1. Total RNA (10 µg) was diluted in nuclease-free water (section 2.1.2.10) up to a volume of 50 µL in a 1.5 mL RNase free nonsticky Eppendorf tube. The sample of total RNA was heated at 65 °C for 5 min to disrupt the secondary structures. The tube was then stored on ice. Dynabeads oligo(dT) (100 µL, section 2.1.6.1) were aliquoted into a 1.5 mL RNase free non-sticky Eppendorf tube. Dynabeads oligo (dT) bind the poly A tail of mRNA, isolating the mRNA from total RNA. The beads were washed twice with 100

µL binding buffer, vortexed briefly and the tubes placed on the magnetic rack. The beads accumulated at the back wall of the tube close to the magnet. The supernatant was removed and the procedure repeated, making sure to work quickly to avoid the beads drying out. The beads were then resuspended in 50 µL binding buffer. Heated total RNA (50 µL) was added to the beads and the tubes were then rotated for 5 min at room temperature. The tubes were then placed on the magnetic stand and the supernatant was removed. The mRNA was now bound to the beads. Washing buffer (100 µL) was added to the beads, vortexed briefly and the supernatant removed. This washing step was repeated and then 20 µL of 10 mM Tris-HCl was added to the beads following removal of the washing buffer. The mRNA and beads were heated at 80 °C for 2 min which allowed the removal of the mRNA from the beads. The tube was placed on the magnetic stand and the supernatant, containing the mRNA was transferred to a 1.5 mL non-sticky Eppendorf tube containing 80 µL of binding buffer. The tube was heated to 65 °C for 5 min and then placed on ice. The beads were washed with 100 µL of washing buffer, to prepare the beads for the second round of mRNA binding. This was required to ensure minimal carry-over of rRNA contamination. To the washed beads, the 100 µL of mRNA was added and the tubes rotated for 5 min at room temperature. The supernatant containing rRNA was removed. The beads were washed twice with 100 µL of washing buffer and 10 µL of 10 mM Tris-HCl was added and mixed well. The mRNA and beads were heated at 80 °C for 2 min and the supernatant (~9 µL) containing the mRNA was transferred to a 200 µL thin wall PCR tube.

2.2.9.2 Fragmentation of mRNA

Fragmentation reagent (1 µL, section 2.1.6.2) was added to 9 µL of mRNA. The tube was incubated in a PCR thermocycler at 70 °C for exactly 5 min. Stop buffer (1 µL) was added and the tubes were placed on ice. The solution was transferred to a 1.5 mL

microcentrifuge tube and 1 µL of 3 M sodium acetate (NaOAc; pH 5.2), 2 µL of glycogen (5 ug/µL) and 30 µL of 100 % ethanol was added. The tubes were placed in -80 °C freezer for 30 min. The tubes were centrifuged at 14,000 × *g* for 25 min in a pre-chilled (4 °C) microcentrifuge. Carefully, the supernatant was removed and the pellet was washed with 200 µL of 80 % ethanol. The ethanol was removed and the pellet was air-dried. The RNA pellet was resuspended in 10.5 µL of RNase-free water.

2.2.9.3 First and second strand cDNA sysnthesis

The fragmented mRNA (10.5 µL) was transferred to a 200 µL thin wall PCR and 1 µL of random hexamer primers (3 ug/µL) were added. The mixture was incubated in a PCR thermocycler at 65 °C for 5 min. The tube was placed on ice. To the solution, 4 µL of first strand buffer, 2 µL 100 mM DTT, 1 µL dNTP mix (10 mM), 0.5 µL RNaseOUT (40 U/µL) were added and the solution mixed well. The tube was heated at 25 °C for 2 min. SuperScriptII (1 µL) was added to the sample. The tube was incubated in a thermocycler with the following program: 25 °C for 10 min, 42 °C for 50 min, 70 °C for 15 min. The tube was placed on ice. Nuclease free water (51 µL) was added to the first strand cDNA synthesis mix. Second strand buffer (20 µL) was added, followed by 3 µL of dNTP mix (10 mM). The solution was mixed well and placed on ice for 5 min. RNaseH (1 µL; 2 U/µL) and 5 µL of DNA Pol I (10 U/µL) were added and the mixture was incubated at 16 °C for 2.5 h. The DNA was purified with a *QIAquick PCR* spin column (section 2.1.6.6). The cDNA was eluted in 30 µL of EB solution.

2.2.9.4 End repair

Nuclease-free water (45 µL) was added to the eluted cDNA. To the mixure, 10 µL of T4 DNA Ligase buffer with 10 mM ATP, 4 µL dNTP mix (10 mM), 5 µL T4 DNA Polymerase (3 U/µL), 1 µL Klenow DNA Polymerase (5 U/µL) and 5 µL T4 PNK (10

U/µL) were added. The sample was incubated at 20 °C for 30 min. The DNA was purified using a *QIAquick PCR* spin column. The cDNA was eluted in 32 µL of EB solution.

2.2.9.5 Addition of a single 'A' base and adapter ligation

This step adds an 'A' base to 3' end of blunt phosphorylated DNA fragments, using the polymerase activity of Klenow (3'-to-5' exo minus) prepares ends for ligation to the adapters which have a single 3'-T overhang. To the 32 µL eluted DNA, 5 µL Klenow buffer, 10 µL dATP (1 mM), 3 µL Klenow 3'-to-5' exo- (5 U/µL) were added. The samples were incubated at 37 °C for 30 min. The DNA was purified using a *QIAquick MinElute* column. The cDNA was eluted in 23 µL of EB solution. This protocol ligates adapters to the ends of the cDNA fragments, preparing them to be hybridised to a flow cell. In brief, 25 µL 2X Quick DNA Ligase buffer, 1 µL adapter-oligo mix and 1 µL T4 DNA Ligase was added to 23 µL eluted DNA and the solution mixed well. The tube was incubated at room temperature for 15 min. Following this, the cDNA with ligated adapters was purified with a *QIAquick MinElute* column and the cDNA were eluted in 10 µL of EB solution.

2.2.9.6 Gel purification of adapter ligated DNA templates

DNA ladder (8 µL) was loaded on an agarose gel. Loading buffer (10 µL) was added to the eluted adapter ligated DNA and the mixture was loaded onto the gel. To prevent cross contamination, 2 wells between samples were left empty. Gel electrophoresis was performed at 120 V for 90 min. On a Dark Reader Transilluminator, the gel was visualised. A clean GeneCatcher disposable gel excision tip was used to cut a gel slice (300 bp) at the position of the sample using the ladder to orientate the sample. The gel

slice was weighed and a QIA*quick* Gel Extraction kit was used to purify the sample. The DNA was eluted with 30 µL of EB solution.

2.2.9.7 PCR enrichment of purified adapter ligated DNA templates

The following PCR master mix was prepared in a thin-walled PCR tube: 10 µL cloned Phusion HF buffer, 1 µL PCR primer 1.1, 1 µL PCR primer 2.1, 0.5 µL 25 mM dNTP mix, 0.5 µL Phusion polymerase (2 U/µL), and 7 µL nuclease-free water. The 30 µL of gel purified adapter ligated material was added to the mastermix. The following PCR programme was used to amplify/enrich the library. Step 1: 98 °C for 30 sec, Step 2: 98 °C for 10 sec, Step 3: 65 °C for 30 sec, Step 4: 72 °C 30 sec: Step 5: Repeat step 2 and 3 15 times: Step 6: 72 °C for 5 min. The DNA was purified using a *QIAquick PCR* spin column. The DNA was eluted in 30 µL of EB solution.

2.2.9.8 Quality control and sequencing of cDNA libraries

The DNA was quantified using a Qubit Fluorometer and HS dsDNA kit. In brief, 10 µL of Qubit reagent was added to 1,990 µL of Qubit buffer to generate a working solution. This working solution (190 µL) was added to each of 2 thin-walled, clear 0.5 mL Qubit assay tubes. 10 µL of each Qubit standard (0ng/µL and 10n/µL) was added to the appropriate tube and the samples were mixed by vortexing. 199 µL of the working solution was added to thin-walled tube and 1 µL of the DNA sample was added. The final volume in each tube was 200 µL. The tubes were allowed to equilibrate at room temperature for 2 min. Standard 1 was inserted into the Qubit 2.0 fluorometer, the lid closed and the absorbance read. The same procedure was carried out for standard 2. The fluorometer was now calibrated. The tubes containing the samples were then inserted into the fluometer and the absorbance read as before. A DNA chip (Agilent) was used to assess the DNA size and quality. The sequencing of the RNA*seq* libraries was

performed on the Illumina Genome Analyser II at the Conway Institute, University College Dublin, Dublin, Ireland using 40bp, paired-end, version 4 kits, according to the manufacturer's instructions.

2.2.10 Data mining and analysis

2.2.10.1 Quality check of reads

FastQC (version 0.9.2) (http://www.bioinformatics.bbsrc.ac.uk/projects/fastqc/) was used to assess the quality of the reads. Such assessments included per base sequence quality, per base N count, per sequence quality and over represented sequences. The per base sequence quality assigns an average Phred score to each lane of the flow cell which relates to the possibility of an error in calling that base i.e, if a Phred score of 30 is assigned to a base, there is a 1 in 1000 chance of that base being called incorrectly.

2.2.10.2 Aligning of sequenced reads to the bovine genome

Following FastQC, Tophat (version 1.3.1) was used to align the reads to the bovine genome (version Btau_4.0). The Tophat script (Figure 2.3) used allowed an inner mate distance (-r) of 250bp plus a mate standard deviation of 30bp, a segment length of 20 (therefore 2 x 20bp sequences per 40 bp reads) with 1 mismatch allowed per segment length. Additionally, a single read (-g 1) was retained for each location and the other 'duplicates' removed. This is to avoid any potential PCR bias (putative PCR duplication) introduced during the library preparation. Furthermore, library type was unstranded as the sequence reads were not strand specific. Following alignment, the BAM output file was converted to a SAM file and sorted by read name, rather than chromosomal location.

```
tophat -r 250 --mate-std-dev 35 --segment-length 20 --segment-
mismatches 1 -g 1 -o "/home2/skeady/data/flowcell1/lane1" --
solexa1.3-quals --library-type fr-unstranded Btau_4.0
/data/skeady/run_110218/s_1_1_sequence.txt
/data/skeady/run_110218/s_1_2_sequence.txt
```

Figure 2.3 Tophat script used for aligning reads to genome

2.2.10.3 Gene counts and identification of differentially expressed genes

Hts*eq*-count (version 0.5.3; http:///www-huber.embl.de/users/anders.HTSeq) was used to convert aligned reads into counts per gene using the union model and the Ensembl (version 61) annotation of the bovine genome (ftp:ftp.ensembl.org/pub/release-61/fasta/bos_taurus/dna/) (Figure 2.4). To identify differentially expressed genes bases on read counts assigned by Hts*eq*, the R (version 2.12.1) Bioconductor package EdgeR (version 1.6.12) (Robinson *et al*., 2010) was employed. EdgeR models data as a negative binomial distribution to account for biological and technical variation using a generalisation of the Poisson distribution model. Firstly, data was normalised across library size using the trimmed mean of M-values normalisation method (Robinson and Oshlack, 2010). Genes were then classified as differentially expressed with a Benjamini-Hochberg false discovery rate (FDR) corrected *P* - value of < 0.1 with a fold change of > 2.0 (Benjamini and Hochberg, 1995).

```
htseq-count -m union -t exon -i gene_id -s no
accepted_hits_sorted.sam
/data/shared/genomes/Btau_4.0/Bos_taurus.Btau_4.0.62.gtf >
htseq38.txt
```

Figure 2.4 Hts*eq*-count script used for generating gene counts

2.2.10.4 Identification of over-represented GO terms and pathways

There is greater statistical power to detect longer genes as significantly differentially expressed compared to shorter genes, as differential gene expression in RNA*seq* is based on the number of reads aligning to that gene location (Oshlack and Wakefield, 2009). To correct for this bias with analysing RNA*seq* data and to identify GO terms which were significantly more represented than expected by chance, the R package GO*seq* (version 1.1.7) which corrects for gene length bias was used (Young *et al*., 2010). GO terms were considered statistically significant with a FDR < 0.1.

Bovine Ensembl genes were converted to human Ensembl orthologs prior to pathway analysis using InnateDB pathway analysis tool (Lynn *et al*., 20080). InnateDB identifies significantly over-represented biological pathways using a hypergeometric test and incorporates pathway annotations from many publicly available databases including: Kyoto Encyclopedia of Genes and Genomes (KEGG) database, the NCI-Nature Pathway Interaction Database (PID), Integrating Network Objects with Hierarchies (INOH) database, Netpath and Reactome databases. Pathways were considered statistically significant with a FDR of < 0.1.

2.2.11 Statistical analysis

2.2.11.1 Statistical analysis (chapter 3)

Data were checked for normality using the UNIVARIATE procedure of statistical analysis software (SAS, 2008). Relative gene expression data for *IGF-2R*, *IGFBP5*, and *IGFBP6* were transformed as appropriate by raising to the power of λ (TransReg procedure, SAS, 2008). The remaining non-normally distributed gene expression values were log transformed using $\log_2$. Plasma analyte data were analysed using repeated measures ANOVA (PROC MIXED, SAS) with sire breed and sire EPD_{cwt}, and day of

sampling included as fixed effects together with their interaction term as appropriate. Day of sampling was included as the repeated term and an unstructured variance-covariance structure was selected. Gene expression data were analysed using mixed models ANOVA (PROC MIXED). Sire breed and sire EPD_{cwt} were included as fixed effects in the statistical model together with the interaction term, where appropriate. Sire was included as a random effect. The Tukey critical difference test was performed to determine the existence of statistical differences between treatment mean values. Spearman correlation coefficients amongst gene expression values and production traits were determined using the CORR procedure of SAS. The Spearman correlation procedure was the chosen method due to the non-parametric nature of the data.

2.2.11.2 Statistical analysis (chapter 4)

Data relating to the chemical composition of the muscle and key performance data from Campion *et al*. (2009a,b) were checked for adherence to normality using the UNIVARIATE procedure of statistical analysis software (SAS, 2008). Non-normally distributed data were transformed as appropriate by raising to the power of λ (TransReg procedure). Data were analysed using mixed models ANOVA (PROC MIXED). Sire breed and genetic merit for growth potential were included as fixed effects in the statistical model together with the interaction term, where appropriate. Sire was included as a random effect. The Tukey critical difference test was performed to determine the existence of statistical differences between treatment mean values.

Using Progenesis Samespots V3.2.3, the gel images were placed into groups (AA, BB, AA*H* and AA*L*) and analysis was carried as follows: AA *vs.* BB; AA*H vs.* AA*L*; BB*H vs.* BB*L*. The Progenesis Samespots software uses a one way ANOVA test and therefore the interaction term could not be tested. For sire breed comparison, gel images from both breeds were matched to a single reference gel image. Power analysis

was performed independently for each protein spot with changes displaying a power of < 0.8 being removed from the analysis. Statistical analysis of the relative abundance of each matched protein spot across the data sets was carried out using student *t*-test. Additionally, *t*-tests between mean protein differences, with a *P*-value of > 0.05 were removed from analysis.

2.2.11.3 Statistical analysis (chapter 5)

The MEANS procedure of Statistical Analysis Software (SAS; 2008) was used to determine the average ages and weights of the steers. Data collected from the study were checked for normality using the UNIVARIATE procedure (SAS, 2008). Where appropriate, data were transformed by raising to the power of λ using the TransReg procedure (SAS, 2008). Data were analysed using mixed models methodology (PROC MIXED, SAS). Within genotype, animals were blocked by weight to treatment. Block, genotype, feeding treatment (H-H or L-H) and their interaction were included as main effects and sire was included as a random effect in the statistical model. Where no interactions were observed, the data were reanalysed for main effects only. The Tukey critical difference test was performed to determine the existence of statistical differences between treatment mean values. For data with repeated measures (live weight, blood metabolites and hormones, linear body measurements and muscle and fat scans), sample day was included as a repeated effect with an unstructured or compound symmetry covariance structure assumed amongst records within animal, employed as appropriate. The choice of residual covariance structure was based on the magnitude of the Akaike Information Criterion (lower is better).

2.2.11.4 Statistical analysis (chapter 6)

Data were checked for normality using the UNIVARIATE procedure of statistical analysis software (SAS Institute, 2008). Where appropriate, data were transformed by raising to the power of λ using the TransReg procedure (SAS, 2008). Data were subsequently analysed using mixed model methodology within the MIXED procedure of SAS. Within genotype, animals were blocked by weight to treatment. Block, genotype (AA *vs* BB), feeding treatment (H-H or L-H) and their interaction were included as main effects and sire of the animal was included as a random effect in the statistical model. Where no statistically significant interactions were observed, the data were reanalysed for main effects only. The Tukey critical difference test was performed to determine the existence of statistical differences between treatment mean values. For data with repeated measures (pH and temperature of carcasses at slaughter), sample time was included as a repeated effect with an unstructured or compound symmetry covariance structure assumed among records within animal as appropriate. The choice of residual covariance structure was based on the magnitude of the Akaike Information Criterion (lower is better). To assess the contribution of intramuscular fat to tenderness and sensory and flavour characteristics, data relating to the sensory and flavour characteristics, as well as WBsf, were reanalysed and adjusted for intramuscular fat, with the same traits as mentioned previously transformed to ensure normality. Additionally, Spearman correlation coefficients amongst meat quality values and production traits were determined using the CORR procedure of SAS.

Chapter 3

Effect of Sire Breed and Genetic Merit for Carcass Weight on the Transcriptional Regulation of the Somatotropic Axis in *M. longissimus thoracis et lumborum*

3.1 Introduction

Bovine skeletal muscle is a tissue of significant economic importance to the global economy. Worldwide, beef production is projected to increase at a rate of 0.9 - 1.4% annually over the next decade (European Commission, 2010). 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 expected progeny difference for carcass weight (EPD_{cwt}) (Campion *et al*., 2009a). Similar genetic evaluations for carcass characteristics are routinely conducted worldwide (Crews *et al*., 2004; Van Groningen *et al*., 2006) and sires can be ranked based on their EPD_{cwt}.

Bernard *et al*. (2009) examined the effects of genetic selection in favour of high muscle growth on gene expression in muscle of young bulls. These authors reported that many genes of the somatotropic axis were differentially expressed between bulls selected for high compared with low growth potential. *In vivo* and *in vitro* studies have shown that both IGF-1 and IGF-2 stimulate proliferation and differentiation of muscle cells, through their interaction with IGF receptors (Jones and Clemmons, 1995; Oksbjerg *et al*., 2004). Consequently, the somatotropic axis is likely to be a promising target for candidate genetic markers for improving meat yield in cattle. To the author's knowledge, there is little published information available on comparisons of different breeds and within breed genetic merit for carcass growth on the expression of component genes of the somatotropic axis in bovine muscle.

Therefore, the objective of this study was to determine the effect of (i) sire breed and (ii) sire EPD_{cwt} on the gene expression within the somatotropic axis system in *M. longissimus thoracis et lumborum* in Aberdeen Angus (AA) and Belgian Blue (BB) cattle. In brief, animals of either high (*H*) or low (*L*) EPD_{cwt} were allocated to 1 of 4

groups, in a 2 (sire breed) x 2 (sire EPD_{cwt}) factorial design, based on the original blocking criteria of Campion *et al*. (2009a). The subgroups were as follows (i) AA*H* (n = 8), (ii) AA*L* (n = 9), (iii) BB*H* (n = 8), and (iv) BB*L* (n = 8). Blood plasma was collected throughout the animals' lifetime for the analysis of IGF-1 and insulin concentrations. At slaughter, *M. longissimus thoracis et lumborum* was collected due to its high commercial value and RT-qPCR analysis was carried out to determine gene expression levels within the somatotropic axis. In addition, correlation analysis was conducted to determine possible positive or negative relationships between genes of the somatotropic axis and animal production variables.

3.2 Effect of sire breed and genetic merit for carcass weight on physiological measurements and gene expression in *M. longissimus thoracis et lumborum*

3.2.1 Plasma concentrations of IGF-1 and insulin

See section 2.2.2.1 and 2.2.2.1.1 for full details of experimental design and animal selection. No effect ($P > 0.05$) of sire breed or EPD_{cwt} or their interaction was observed for plasma concentrations of IGF-1 or insulin (Table 3.1). Plasma concentrations of IGF-1 increased linearly between 7 and 24 mo of age. A similar trend was observed for circulating concentrations of insulin with levels increasing linearly overtime throughout the lifetime of the animal.

3.2.2 Gene expression of the somatotropic axis

Despite a sire breed × EPD_{cwt} interaction being detected for *IGFBP3*, this interaction was found to be non significant ($P > 0.05$). Consequently, the effects of breed and EPD_{cwt} are reported below (Table 3.2). There was a statistically significant difference ($P < 0.001$) in transcript levels for *IGFBP3* between the breeds with expression greater in AA compared with BB; however, no effect ($P > 0.05$) of EPD_{cwt} was observed in expression levels of *IGFBP3*. Similarly, transcript levels of *IGF-1R* were greater ($P < 0.001$) in AA animals compared with BB. No difference ($P > 0.05$) was observed in gene expression levels for *IGF-1R* across EPD_{cwt} groups. A difference in mRNA expression levels for *IGF-1* was observed between EPD_{cwt} groups with transcript levels up-regulated ($P < 0.01$) in *H* compared to *L* animals. There was no difference ($P > 0.05$) in gene expression levels across breed for *IGF-1*. Neither was an effect of sire breed or EPD_{cwt} evident ($P > 0.05$) for gene expression levels of *IGF-2*, *IGF-2R*, *IGFBP4*, *IGFBP5*, *IGFBP6* or GH receptor (*GHR*). Even after 40 amplification cycles, gene

expression of *IGFBP1*, *IGFBP2*, and *ALS* in *M. longissimus thoracis et lumborum* remained undetected.

Table 3.1 Effect of sire breed and EPD for carcass weight (EPD_{cwt}) on the plasma concentrations of IGF-1 and insulin[1]

	Breed[2]			EPD_{cwt}[3]			Time (T)					*P*-Values[4]		
Trait	AA	BB	SED	*H*	*L*	SED	7 mo	14 mo	18 mo	24 mo	SEM	B	EPD_{cwt}	T
IGF-1, ng/mL	249.5	275.3	40.54	255.7	268.9	40.61	132.8[a]	271.9[b]	288.2[b]	309.7[b]	28.5	0.579	0.747	< 0.001
Insulin, µIU/mL	12.3	14.5	1.54	13.5	13.3	1.55	3.48[a]	5.98[b]	7.52[b]	19.43[c]	1.32	0.175	0.877	< 0.001

[a-c]Least squares means within a row without a common superscript differ ($P < 0.05$).

[1]Animals were blood sampled by jugular venipuncture at approximately 7 mo, 14 mo, and 18 mo of age, and again 2 d before slaughter at 24 mo of age. [2]AA = Aberdeen Angus; BB = Belgian Blue. [3]H = high for $EPD_{cwt;}$ *L* = low for $EPD_{cwt.}$ [4]No statistically significant interactions (B × EPD_{cwt}; B × T; EPD_{cwt} × T) were observed.

Table 3.2 Effect of sire breed (B) and expected progeny difference for carcass weight (EPD_{cwt}) on the relative expression of genes[1] of the somatotropic axis

Gene[7]	Breed[2]			EPD_{cwt}[3]			*P*- values		
	AA	BB	SED	*H*	*L*	SED	B	EPD_{cwt}	B × EPD_{cwt}
IGF-1	6.83	5.12	0.948	7.60	4.36	0.973	0.091	0.004	0.154
IGF-1R[4]	17.2	2.81	2.833	8.41	11.6	2.883	0.0003	0.781	0.496
IGF-2	16.1	20.9	3.407	15.8	21.3	3.524	0.183	0.139	0.064
IGF-2R[5]	1.72	5.42	1.799	4.09	3.06	1.782	0.197	0.248	0.463
IGFBP3	57.9	4.61	10.231	41.4	21.2	10.231	< 0.0001	0.239	0.07
IGFBP4	6.26	9.61	4.175	9.63	6.23	4.175	0.359	0.964	0.411
IGFBP5	22.1	24.1	8.496	23.8	22.4	8.496	0.314	0.814	0.403
IGFBP6	3.59	3.68	1.146	4.57	2.71	1.146	0.822	0.167	0.327
GHR[6]	13.7	7.81	3.128	9.41	12.1	3.128	0.074	0.077	0.112

[1]Gene expression values were normalised to the reference gene after adjustment for efficiencies and interplate variation and converted to values relative to the greatest cycle threshold (Ct) within each data set. [2]AA = Aberdeen Angus; BB = Belgian Blue. [3]H = high for $EPD_{cwt;}$ *L* = low for $EPD_{cwt.}$ [4]*IGF-1R* = IGF-1 receptor. [5]*IGF-2R* = IGF-2 receptor. [6]*GHR* = GH receptor. [7]Gene expression of *IGFBP1*, *IGFBP2*, and acid-labile subunit (*ALS*) remained undetected.

3.3 Correlation analyses

3.3.1 Correlation between expressions of genes in the somatotropic axis

Correlation analysis was carried out to examine potential associations between genes of the somatotropic axis (Table 3.3). In summary, *IGF-1* gene expression values were positively correlated with expression of *IGFBP3*, *IGFBP4*, *IGFBP5*, and *IGFBP6*, whereas *IGF-1R* gene expression was negatively associated with *IGFBP5* and *IGFBP6* but positively associated with *IGFBP3* and *GHR*. Gene expression of *IGF-2* was positively associated with gene expression of *IGFBP4* and *GHR* while negatively correlated with *IGFBP6*. Gene expression of *IGF-2R* was negatively correlated with expression of *IGFBP3* and *IGFBP5* but positively correlated with expression of *IGFBP6*. Finally, gene expression of *IGFBP5* was positively correlated with expression of *IGFBP3*.

Table 3.3 Associations[1] between expression of genes of the somatotropic axis in *M. longissimus thoracis et lumborum*

Gene	*IGF1*	*IGF-2*	*IGF1R*[2]	*IGF2R*[3]	*IGFBP3*	*IGFBP4*	*IGFBP5*	*IGFBP6*
IGF2	0.11							
IGF1R[2]	0.09	0.24						
IGF2R[3]	-0.33	0.17	0.14					
IGFBP3	0.54 ***	-0.24	0.48 **	-0.47 **				
IGFBP4	0.45 **	0.60 **	0.09	-0.01	0.05			
IGFBP5	0.44 **	-0.21	-0.35 *	-0.69 ***	0.47 **	0.05		
IGFBP6	0.47 **	-0.52 ***	-0.48 **	0.39 *	0.32	0.03	0.03	
GHR[4]	0.31	0.57 ***	0.39 *	-0.26	0.31	0.24	0.18	-0.23

[1]Values presented are Spearman correlation coefficients r from unadjusted data (n = 33). [2]*IGF-1R* = IGF-1 receptor. [3]*IGF-2R* = IGF-2 receptor. [4]*GHR* = GH receptor. **P* < 0.05; ***P* < 0.01; ****P* < 0.001.

3.3.2 Correlation between expression of genes and animal production variables.

Correlation analysis was carried out to examine the potential associations between expression of genes of the somatotropic axis and relevant accompanying animal production traits, recorded as part of the studies of Campion *et al*. (2009a,b). The Spearman correlation coefficients for these associations are presented in Table 3.4. In brief, weight at slaughter and carcass weight were negatively correlated with expression of *IGF-2* and *GHR*, whereas both measures of animal weight were positively correlated with expression of *IGF-2R*. Pre-slaughter ultrasonically scanned *M. longissimus thoracis et lumborum* depth and area were negatively associated with expression of *IGFBP3* and *GHR* whereas *M. longissimus thoracis et lumborum* area per kilogram of carcass weight correlated negatively with expression of *IGF-1R* and *IGFBP3* but was positively associated with expression of *IGFBP4*.

Table 3.4 Associations[1] between expression of genes of the somatotropic axis in *M. longissimus thoracis et lumborum* and production variables

Gene	Slaughter weight, kg	Carcass weight, kg	UMD[2], mm	*M. longissimus thoracis et lumborum* area, cm^2	*M. longissimus thoracis et lumborum* area[3], cm^2/kg
IGF-1	0.01	-0.06	-0.02	-0.09	0.15
IGF-2	-0.46 **	-0.43 **	-0.23	-0.17	0.29
IGF- 1R[4]	-0.12	-0.25	-0.25	-0.55 ***	-0.41 **
IGF-2R[5]	0.37 *	0.40 *	0.26	0.41 *	0.01
IGFBP3	-0.11	-0.25	-0.37 *	-0.63 ***	-0.40 *
IGFBP4	-0.19	-0.15	0.05	0.06	0.37 *
IGFBP5	-0.18	-0.23	-0.27	-0.22	0.11
IGFBP6	0.33	0.36 *	0.34	0.28	0.07
GHR[6]	-0.67 ***	-0.71 ***	-0.41 *	-0.52 **	0.21

[1]Values presented are Spearman correlation coefficients r from unadjusted data (n = 33). [2]UMD = Pre-slaughter ultrasonically scanned *M. longissimus thoracis et lumborum* depth. [3]Expressed per kilogram of carcass weight. [4]*IGF-2R* = IGF-2 receptor. [5]*IGF-1R* = IGF-1 receptor. [6]*GHR* = GH receptor. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

3.4 Chapter summary, discussion and conclusion

3.4.1 Chapter summary

The somatotropic axis plays an important role in postnatal growth, development, and differentiation of skeletal muscle. The aim of this study was to examine the effect of sire breed and sire EPD for carcass weight (EPD_{cwt}) on the expression of components of the somatotropic axis in *M. longissimus thoracis et lumborum* of beef cattle at slaughter. Crossbred Aberdeen Angus (AA; n = 17) and Belgian Blue (BB; n = 16) steers born to Holstein-Friesian dams and sired by bulls with either high (*H*) or low (*L*) EPD_{cwt} were employed in the study. Thus, there were 4 genetic groups *viz. BBH* (n = 8), *BBL* (n = 8), *AAH* (n = 8), and *AAL* (n = 9). Blood samples were collected *via* jugular venipuncture at regular intervals for analysis of plasma concentrations of IGF-1 and insulin. Total RNA was isolated from *M. longissimus thoracis et lumborum* collected at slaughter and the mRNA expression of *IGF-1*, *IGF-2*, cognate receptors (*IGF-1R*; *IGF-2R*), 6 *IGFBP*, acid labile subunit (*ALS*), and GH receptor (*GHR*) was measured by RT-qPCR. There was no effect of either sire breed or EPD_{cwt} on concentrations of circulating IGF or insulin ($P > 0.05$). Gene expression of *IGF-1R* and *IGFBP3* was up-regulated in AA ($P < 0.001$) compared to BB whereas *IGF-1* was up-regulated in *H* compared to *L* animals ($P < 0.01$). Correlation analysis indicated moderate positive associations between gene expression of *IGFBP3* and *IGF-1* (r = 0.54; $P < 0.001$) and *IGF-1R* (r = 0.48; $P < 0.01$). In addition, correlation analysis revealed that mRNA expression of *IGFBP3* was moderately negatively associated with *M. longissimus thoracis et lumborum* area per kilogram carcass weight (r = -0.40; $P < 0.05$). 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. These data will contribute to a better understanding of the molecular control of muscle growth at a tissue level in cattle.

3.4.2 Chapter discussion

The main objective of this study was to examine the effects of both sire breed and sire EPD_{cwt} on the expression of constituent genes of the somatotropic axis in muscle tissue. There is clear evidence that breed type influences carcass characteristics including both yield and quality of saleable meat from cattle (Keane and Moloney, 2010). The AA and BB sire breeds were selected because of their well documented differences in carcass conformation, muscle composition and maturation rates (early *versus* late) (Bellinge *et al*., 2005; Keane and Drennan, 2008; Dinh *et al*., 2010). Campion *et al*. (2009a) reported that *M. longissimus thoracis et lumborum* area and *M. longissimus thoracis et lumborum* area adjusted for carcass weight were greater for BB compared to AA sired steers and also for AA animals sired by bulls with *H* compared to *L* breeding values for carcass weight. As *M. longissimus thoracis et lumborum* was obtained from a subset of AA and BB animals from Campion *et al*. (2009a), the following briefly summarises the main findings. There was no effect of EPD_{cwt} on daily DMI or DMI per kilogram live weight during the finishing period. Additionally, progeny from AA sires of *H* EPD_{cwt} had greater growth rates and produced heavier carcasses compared to progeny from AA sires of *L* EPD_{cwt}. However, there was no detectable effect of sire EPD_{cwt} on growth rate or carcass weight for BB. At slaughter, BB had heavier carcasses and greater dressing percentage compared with AA cattle. Pre-slaughter ultrasonically scanned *M. longissimus thoracis et lumborum* depth, carcass *M. longissimus thoracis et lumborum* area, *M. longissimus thoracis et lumborum* area per kilogram carcass weight were all greater for BB compared to AA animals. In addition, ultrasonically scanned *M. longissimus thoracis et lumborum* depth was greater in *AAH* compared to *AAL*.

The somatotropic axis, also known as the GH-IGF system, consists of peptide hormones, cell surface receptors, and binding proteins (Denley *et al*., 2005). The axis is critical in regulating postnatal growth, development and differentiation of skeletal

muscle (Clemmons, 1997; Duan and Xu, 2005; Duan *et al*., 2010). Muscle growth is mediated by the activation, proliferation, and differentiation of muscle cells and appears to also be modulated by mitotic and myogenic activity of locally produced IGF-1 (Philippou *et al*., 2007). Furthermore, muscle cell cultures have been shown to produce IGF and IGFBP.

The action of GH in regulating growth and development is mediated by its plasma-membrane bound receptor, GHR (Isaksson *et al*., 1985). Dauncey *et al*. (1994) and Katsumata *et al*. (2000) reported that mRNA expression of *GHR* in *M. longissimus* was up-regulated in pigs growing at a slower rate than pigs with faster growth rates. In the current study, gene expression of *GHR* was not different across either sire breed or EPD_{cwt}. Spurlock *et al*. (2006) found that after administration of clenbuterol, a ß2-adrenergic receptor agonist, male mice experienced increased body weight. Consequently, gene expression of *GHR* in muscle was down-regulated compared to control mice. In addition, Castigliego *et al*. (2010) reported that expression of *GHR* was down-regulated in bovine muscle after administration of recombinant bovine GH. The results of the correlation analysis support these findings (Dauncey *et al*., 1994; Katsumata *et al*., 2000; Spurlock *et al*., 2006) in that moderate negative associations were observed between expression of *GHR* and weight at slaughter, carcass weight and *M. longissimus thoracis et lumborum* area per kilogram of carcass weight.

In addition, to circulating in blood, IGF concentrations are also regulated in tissues. In muscle cell lines and cultures, IGF-1, and IGF-2 were produced and secreted into the culture medium (Jones and Clemmons, 1995; Oksbjerg *et al*., 2004). Local production of IGF-1 in skeletal muscle is thought to play a predominant role in supporting normal muscle growth through autocrine or paracrine (or both) mechanisms (Sjögren *et al*., 1999; Dayton and White, 2008). In addition, it was observed that locally produced IGF-1 in muscle, plays a key role in myofiber regeneration and hypertrophy

(Isgaard, 1992; Philippou *et al*., 2007). For example, Chen *et al*. (2011) reported that gene expression of *IGF-1* was significantly lower in *M. longissimus* tissue of growth restricted piglets compared to control animals. In the current study, a statistically significant difference in mRNA transcript levels for *IGF-1* between *H* and *L* groups for EPD_{cwt} was consistent with the findings of Campion *et al*. (2009a) who reported that animals of *H* EPD_{cwt} produced heavier carcasses with greater *M. longissimus thoracis et lumborum* area compared to animals of *L* EPD_{cwt}. This increased muscle growth can possibly be attributed to the greater local *IGF-1* gene expression observed, as no statistically significant difference in plasma concentrations of IGF-1 or insulin was detected across sire breed or EPD_{cwt}.

In addition, 6 different IGFBP have been identified and these play a critical role in the somatotropic axis by regulating IGF/IGF-R activity by potentiating or inhibiting the role of IGF action in muscle (Jones and Clemmons, 1995; Duan *et al*., 2010). When IGF-1 or IGF-2 is bound in an IGFBP ternary structure it is safeguarded for up to 30 min in a controlled release mechanism, compared with a half-life of 10 to 12 min for free IGF in circulation (Guler *et al*., 1989; Clemmons, 2009). At a tissue level, IGFBP can both inhibit and potentiate IGF action by either preventing IGF from binding with IGF-1R or by releasing IGF to bind IGF-1R (Denley *et al*., 2005). Both IGF-1 and IGF-2 have a greater affinity for IGFBP3 compared to their receptor, IGF-1R (Jones and Clemmons, 1995). Consequently, IGFBP3 binds IGF-1 resulting in a decrease in the bioavailability of IGF-1 (Jones and Clemmons, 1995; Sadkowski *et al*., 2009). For example, Tilley *et al*. (2007) found that mRNA transcript levels of *IGFBP3* were greater in porcine foetuses which were small in size for their gestational age compared with foetuses of normal size. In the current study, AA had greater levels of *IGFBP3* in muscle tissue compared to BB. The suggestion that IGFBP3 may function to inhibit growth is supported by the fact, that, in this study the BB animals had consistently

larger scanned *M. longissimus thoracis et lumborum* depth than AA at all times throughout their lives (Campion *et al*., 2009a) and at slaughter had larger *M. longissimus thoracis et lumborum* area per kilogram of carcass weight (Campion *et al*., 2009b). Furthermore, Clemmons (2009) suggests that lower concentrations of IGFBPs, including IGFBP3 alter the equilibrium between IGF-1 and IGF-1R to enhance IGF-1 effects, further supporting this theory.

The endocrine, autocrine, and paracrine functions of IGF-1 are mediated through binding to IGF receptors (IGF-1R and IGF-2R). IGF-1 and IGF-2 bind to IGF-1R with varying degrees of affinity and the IGF-1R-activated intracellular processes can affect cell proliferation and differentiation (Phillippou *et al*., 2007; Duan *et al*., 2010). An effect of sire breed was recorded for *IGF-1R*, with AA having greater mRNA expression compared to BB. However, both systemic IGF-1 concentrations as well as local *IGF-1* gene expression in muscle were not different between sire breeds. In addition, there was no association between *IGF-1R* expression and circulating IGF-1 concentrations at slaughter (data not shown). Tilley *et al*. (2007) reported that *IGF-1R* mRNA levels were greater in small foetuses compared with foetuses of average size. In addition, Micke *et al*. (2011) found that expression of *IGF-1R* was up-regulated at slaughter in muscle of cattle that were smaller at birth and suggested that this increase in gene expression acts as a compensatory effect in lighter animals to promote muscle growth. The positive association between *IGFBP3* and *IGF-1R* expression reported in the current study supports that finding.

Myostatin, a member of the TGF-ß superfamily is a negative regulator of muscle mass (McPherron *et al*., 1997). However, in the current study there was no value in measuring gene expression of myostatin to investigate its regulatory effect on muscle growth as mutations in the myostatin coding sequence result in a truncated protein and consequently muscle hypertrophy or ‘double’ muscling occurs (McPherron *et al*., 1997;

Fahrenkrug *et al*., 1999). The Piedmontese and BB are two such breeds in which this phenomenon is observed (McPherron *et al*., 1997). The myostatin mutation phenomenon and its effect on muscle growth and the somatotropic axis must be considered when attempting to address potential effects of sire breed or EPD_{cwt} for crossbred BB animals. Crossbred BB animals that are heterozygous for the double muscling myostatin mutation have increased muscle mass compared to their conventional counterparts (Casas *et al*., 2004). At slaughter, BB had heavier carcasses, greater dressing percentage and greater muscle size compared with AA. The IGF-1 and myostatin signaling pathways work simultaneously to achieve a controlled but flexible system for controlling muscle growth (Otto and Patel, 2010). Kamanga-Sollo *et al*. (2003) reported that myostatin caused increased production (doubling) of *IGFBP3* mRNA in porcine embryonic cells compared to control cultures which consequently resulted in reduced cell proliferation. It has been reported that free IGFBP3 which is not bound to IGF, affects cells *via* IGF-independent methods (Hwa *et al*., 1999; Baxter, 2000). A number of mechanisms have been proposed which characterise the relationship between IGFBP3 and myostatin (Dayton and White, 2008); however, the full process has yet to be elucidated. A potential mechanism derived from the work of Dayton and White (2008) postulated that IGFBP3 mediates the proliferation-suppression actions of myostatin by down-regulating the production of co-repressors, Ski and SnoN. In the current study, as BB animals had lower mRNA transcript levels of *IGFBP3* compared to AA animals, we suggest that myostatin produced in the AA animals is fully functioning resulting in greater mRNA transcript levels of *IGFBP3* in AA compared to the BB animals. In contrast, all of the BB cattle employed in this study, should have been heterozygous for the myostatin mutation resulting in smaller levels of active myostatin and therefore *IGFBP3* expression remained low.

Consequently, the lower levels of *IGFBP3* may have contributed to the greater muscle mass in BB animals.

The main IGFBP secreted by skeletal muscle is IGFBP5 (Duan *et al*., 2010). Depending on tissue type and circumstance, IGFBP5 has the ability to inhibit or potentiate IGF action (Clemmons, 1997; Ewton *et al*., 1998; Schneider *et al*., 2002). In skeletal muscle, Mukherjee *et al*. (2008) concluded that IGFBP5 inhibited IGF-1 action. They noted that the majority of other studies analyzing IGFBP5 action on IGF-1 in muscle reported similar findings, however, they emphasised that many of these case studies involved over-expression of *IGFBP5*. Surprisingly, in the current study *IGFBP5* was not differentially expressed across sire breed or EPD_{cwt}. Lehnert *et al*. (2007) highlighted the gene expression pattern of *IGFBP5* in *M. longissimus* of developing bovine foetuses, as well as new born calves. The authors reported that expression of *IGFBP5* was significantly reduced in new born calves compared with d 60, d 135, and d 195 of fetal development, suggesting that IGFBP5 may play a role in early muscle development in the bovine. The animals in the current study had a mean slaughter age of 764 d. To examine the potentiating or inhibitory effects of IGFBP5, muscle sampling by biopsy collection would be required at key growth periods throughout the animals' life, starting at an earlier age. Alternatively, Ning *et al*. (2007) proposed that IGFBP3 can compensate for the loss of a functioning IGFBP5 protein in mammary tissue. In that study, *IGFBP5* knockout mice exhibited normal growth and body composition. In the current study, although *IGFBP5* was highly expressed in both breeds, increased *IGFBP3* expression in AA was possibly compensating for the lack of a change in gene expression of *IGFBP5* in these animals. However, this theory warrants further investigation in bovine skeletal muscle as correlation analyses detected a positive relationship between gene expression of *IGFBP5* and expression of *IGFBP3*.

3.4.3 Chapter conclusion

This is the first study to examine and report differences in the expression of key somatotropic genes in the muscle of cattle of *H* or *L* EPD_{cwt} and across breeds of such contrasting morphology and maturity type. We have demonstrated that elevated *IGF-1* expression in muscle tissue may serve to promote growth *in vivo* supporting many other research findings (Powell-Braxton *et al*., 1993; Clemmons, 2009). The current study supports the findings of previous research, whereby IGFBP3 was proposed to mediate the equilibrium between IGF-1 and IGF-1R to enhance IGF-1 effects, thus promoting growth. Together an increase in gene expression of *IGF-1* and a reduction in transcript levels of *IGFBP3* in muscle may play a role in greater muscle growth potential in steers during the finishing period. Consequently, *IGFBP3* and *IGF-1* may serve as potential candidates for future investigation of molecular markers for muscle growth including exploration of small RNA regulation, transcription factors, and copy number and SNP variation. Indeed, recent data has shown that a SNP in the promoter region of *IGF-1*, predicted to introduce binding sites for transcription factors Heat Shock Factor 1 (HSF1) and Zinc finger protein 217 (ZNF217) was associated with increased cow carcass weight (Mullen *et al*., 2011). Future studies should focus on sequencing the entire *IGF-1* and *IGFBP3* genes and regulatory regions in large numbers of animals divergent in growth performance for SNP discovery and subsequent association studies. Following appropriate validation, such markers could be incorporated into future cattle breeding programs to improve the accuracy of selection for muscle growth. However, as this study clearly demonstrates, the expression of these key genes varies between breeds, thus emphasizing the necessity to validate all markers for growth across breed.

Chapter 4

Proteomic Profiling of *M. longissimus thoracis et lumborum* from Aberdeen Angus and Belgian Blue Steers Varying in Genetic Merit for Carcass Weight

4.1 Introduction

Bovine skeletal muscle is a tissue of significant economic importance worldwide. Approximately 17 kg in Europe and up to in 37 kg in USA of beef is consumed per capita annually (USDA, 2011). Genetic evaluations for a range of performance traits across all of the main cattle breeds in Ireland are undertaken by the Irish Cattle Breeding Federation. Breeding value for growth rate, an important commercial trait reflecting lifetime growth, is estimated using a multi-trait animal model and is expressed as the expected progeny difference for carcass weight (EPD_{cwt}) (Campion *et al*., 2009a). Similar genetic evaluations for carcass characteristics are routinely conducted worldwide (Crews *et al*., 2004; Van Groningen *et al*., 2006).

Sire breed type and sire EPD_{cwt} influence carcass characteristics including both yield and quality of saleable meat from cattle (Campion *et al*., 2009a,b; Keane and Moloney, 2010; Keane *et al*., 2011). Previously, Campion *et al*. (2009a) reported that *M. longissimus thoracis et lumborum* area and *M. longissimus thoracis et lumborum* area per unit carcass weight were greater for Belgian Blue × Holstein Friesian (BB) compared to Aberdeen Angus × Holstein Friesian (AA) sired steers and also for AA animals sired by bulls with high (*H*) compared to low (*L*) breeding values for carcass weight.

BB is a late-maturing breed which accumulates more muscle compared to its early-maturing counterparts whereas AA, an early-maturing breed, are renowned for higher levels of marbling fat in muscle, which is favourably associated with tenderness and flavour of beef (Kuber *et al*., 2004). Bovine skeletal muscle is a heterogeneous tissue comprised of several fibre types, type I, IIa, IIb, IIc, IIx (Bouley *et al*., 2005; Oury *et al*., 2010) influenced by genotype of the animal (Bouley *et al*., 2005; Chaze *et al*., 2008). Additionally, its properties evolve during postnatal life and can be modified by environmental conditions (Therkildsen, 2005; Shibata *et al*., 2009).

Studies to date have investigated the physical and physiological differences in animals varying in EPD_{cwt} (Crews *et al*., 2004; Keane *et al*., 2011). In addition, Bernard *et al*. (2009) examined the effects of genetic selection in favour of high muscle growth on gene expression in muscle of young Charolais bulls using microarray technology. These authors showed that many genes of the glycolytic pathway were differentially expressed in bulls selected for *H* or *L* growth potential. Research has also been undertaken to understand the molecular difference in muscle characteristics of various cattle breeds (Lehnert *et al*., 2007; Sadkowski *et al*., 2009). However, to the authors' knowledge, few data exist on the effect of either breed or genetic merit for carcass growth on global protein abundance in bovine muscle. Therefore, the objective of this study was to determine the effect of (i) sire breed and (ii) sire EPD_{cwt} on the expression of proteins in *M. longissimus thoracis et lumborum* in AA and BB cattle. Proteomic-based approaches offer researchers a snapshot of the proteins of the tissue being studied (Guo *et al*., 2008) and the *M. longissimus thoracis et lumborum* was selected due to its high commercial value.

4.2 Effect of sire breed and genetic merit for carcass weight on the chemical analysis and protein abundance of *M. longissimus thoracis et lumborum*

4.2.1 Chemical analysis of M. longissimus thoracis et lumborum

See section 2.2.2.2 and 2.2.2.2.1 for full details of experimental design and animal selection. There was a difference in the chemical composition of the muscle between breeds (Table 4.1) with BB having higher ($P < 0.001$) protein and moisture ($P < 0.02$) content and a lower ($P < 0.001$) lipid concentration compared with AA. There was no effect ($P > 0.05$) of EPD_{cwt} or sire breed × EPD_{cwt} interaction on *M. longissimus thoracis et lumborum* composition following chemical analysis.

Table 4.1 Effect of sire breed (B) and expected progeny difference for carcass weight (EPD_{cwt}) on the chemical composition of *M. longissimus thoracis et lumborum* at slaughter

Variable, %	Breed[1]			EPD_{cwt}[2]			*P*-value		
	AA	BB	SED	*H*	*L*	SED	B	EPD_{cwt}	B × EPD_{cwt}
Protein	22.22	23.12	0.16	22.76	22.57	0.16	0.001	0.25	0.88
Moisture	71.39	73.29	0.27	72.39	72.30	0.27	0.02	0.73	0.64
Fat	6.01	2.99	0.37	4.39	4.62	0.37	0.001	0.53	0.84

[1]AA = Aberdeen Angus × Holstein Friesian; BB = Belgian Blue × Holstein Friesian. [2]*H* = high for $EPD_{cwt;}$ *L*= low for $EPD_{cwt.}$ **P* < 0.05, ***P* < 0.01, ****P* < 0.001.

4.2.2 Effect of sire breed on protein abundance

Twenty-one protein spots, relating to sixteen protein products, were identified as different ($P < 0.01$) in their abundance across sire breed with fold changes larger than 1.9 (Figure 4.1 and Table 4.2). To investigate the functional significance of the identified differentially expressed proteins, Ingenuity Pathway Analysis (IPA) software (IPA V9.0; Ingenuity Systems, Mountain View, CA http://www.ingenuity.com) was used. Overall the top canonical pathway identified was glycolysis/gluconeogenesis with three proteins [glycogen phosphorylase (PYGM), phosphoglycerate mutase 2 (PGAM2)

and aldolase A (ALDOA)] within the pathway activated. The second canonical pathway identified as activated was the citric cycle with enzymes aconitase-2 (ACO2) and 2-oxoglutarate dehydrogenase (OGDH) identified as greater in abundance in AA compared to BB. The third canonical pathway was the protein kinase A signalling (PKA) pathway with proteins; myosin light chain 1 (MYL1), myosin light chain, phosphorylatable (MYLPF), PYGM, and troponin I (TNNI2) differing in abundance across breed. Finally, the fourth canonical pathway identified was the pentose phosphate pathway with ALDOA and phosphoglucomutase (PGM1) activated within the pathway. Other proteins identified as greater in abundance in AA compared to BB include AMP deaminase 1 (AMPD1), LIM domain binding 3 (LDB3), vinculin (VCL), capping protein alpha (CAPZA2), heat shock protein beta-1 (HSPβ1) and peroxiredoxin 6 (PRDX6).

4.2.3 Effect of EPD_{cwt} on protein abundance

No difference ($P > 0.05$) in protein spot abundance was detected between BB*H* and BB*L* animals. For AA however, a difference ($P < 0.05$) in protein abundance of three glycolytic enzymes was observed between *H* and *L* EPD_{cwt} groups. These three proteins glucose-6-phosphate isomerase (GPI), enolase (ENO1) and pyruvate kinase (PKM2), were identified in the top canonical pathway, glycolysis/gluconeogenesis. These three proteins were higher in abundance in AA*H* compared to AA*L* steers (Table 4.3 and Figure 4.2).

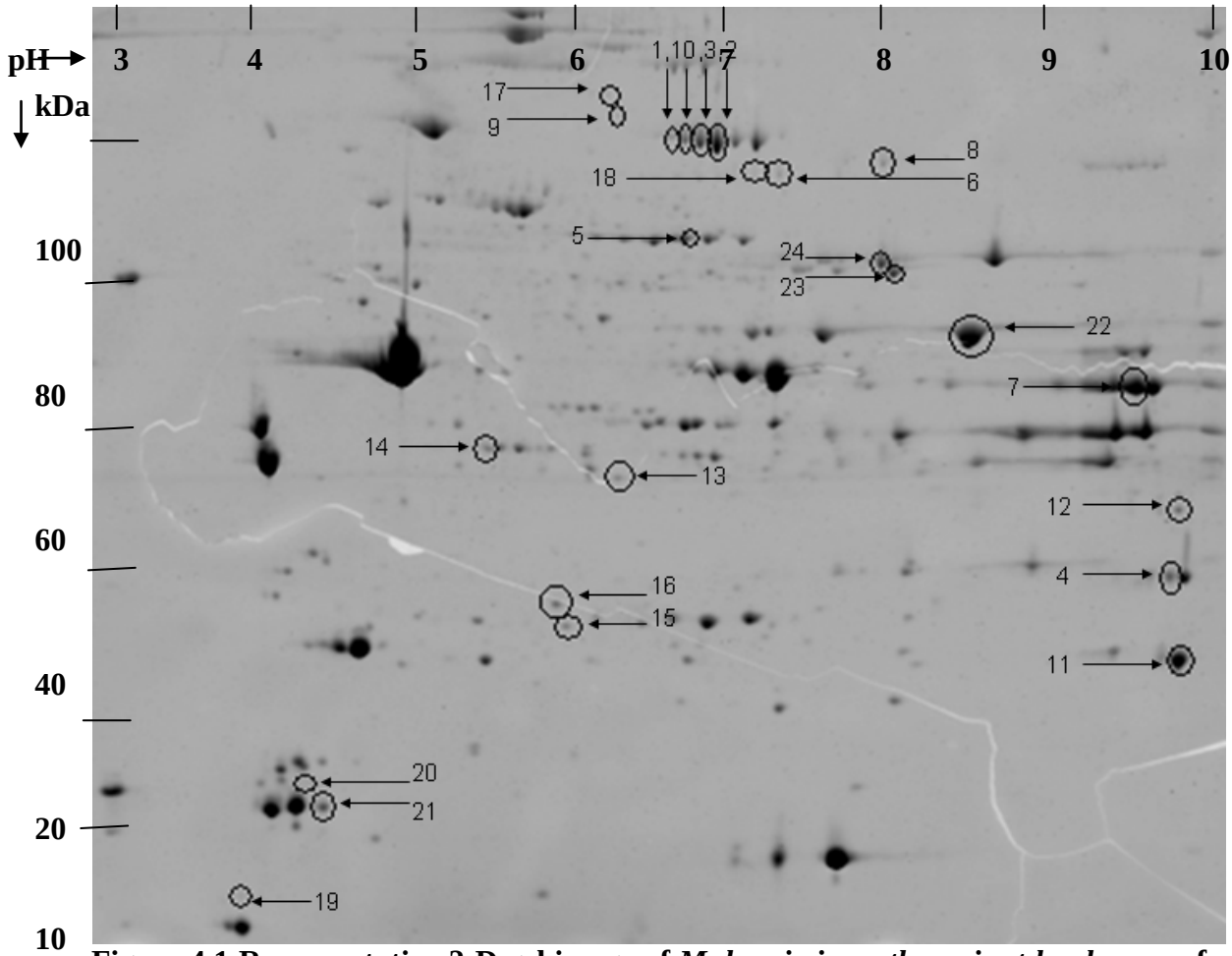


Figure 4.1 Representative 2-D gel image of *M. longissimus thoracis et lumborum* of crossbred steers

The proteins are indicated by spot number which correspond to those identified as varying with statistical significance between the different groups (see Table 4.2 and 4.3).

Table 4.2 Proteins differentially expressed between Belgian Blue (BB) and Aberdeen Angus (AA) steers[1]

No.	Identified Protein	NCBI accession No. (Source)[1]	Mascot score	Fold Change[2]	*P* - value	Matched peptides / sequence coverage %	Theoretical p*I* / M_r (kDa)
	Metabolic						
1	PYGM[3]	gi|73983205 (Canine)	224	5.7↑	<0.0001	6/10	6.6/ 97.5
2		gi|154426116	1652	2.9↑	<0.0001	34/47	6.7/97.5
3		gi|28461197	1374	2.8↑	<0.0001	31/37	6.7/97.6
6			609	2.0↑	0.002	18/24	
4	PGAM2[4]	gi|84000195	643	2.9↑	0.004	13/49	9.0/28.8
5	PGM1[5]	gi|116004023	1029	2.2↓	0.0001	21/52	6.4/61.8
7	ALDOA[6]	gi|156120479	804	2.8↑	<0.0001	15/53	8.5/39.9
8	ACO2[7]	gi|74268076	816	5.3↑	0.0006	17/30	8.0/85.9
9	OGDH[8]	gi|115496742	491	3.9↑	0.0001	12/12	6.3/116.8
18	AMPD1[9]	gi|154152079	279	4.0↑	0.0002	6/10	6.9/87.2
	Contractile apparatus						
10	TNNT1[10]	gi|21039010	219	3.9↑	<0.0001	5/23	6.2/30.1
11	TNNI2[11]	gi|76658412	213	3.5↑	<0.0001	4/17	8.9/21.5
19	MYL1[12]	gi|1181841	451	4.6↓	<0.0001	8/52	4.7/18.8
20	MYLPF[13]	gi|115497166	206	2.8↓	0.003	5/31	4.9/19.1
21			497	2.1↓	0.005	9/61	
	Cell structure						
12	LDB3[14]	gi|78369256	362	4.0↑	0.003	7/33	9.3/35.5
13	VCL[15]	gi|194679459	440	2.3↑	0.006	10/13	5.9/11.7
17		gi|194679457	432	1.9↑	0.003	10/10	5.6/124.3
14	CAPZA2[16]	gi|433308 (Human)	382	2.2↑	<0.0001	8/44	5.6/32.9
	Cell defence						
15	HSPβ1[17]	gi|85542053	750	2.2↑	0.0006	15/76	6.0/22.4
16	PRDX6[18]	gi|27807167	626	1.9↑	0.0003	13/60	6.0/25.1

[1]AA = Aberdeen Angus × Holstein Friesian; BB = Belgian Blue × Holstein Friesian. [2]↑ = Increased in AA *vs.* BB; ↓ = Decreased in AA *vs.* BB. [3]Phosphorylase, glycogen, muscle; [4]Phosphoglycerate mutase 2; [5]Phosphoglucomutase; [6]Aldolase A; [7]Aconitase-2; [8]2-oxoglutarate dehydrogenase; [9] AMP deaminase 1; [10]Slow Troponin T (slow); [11]Troponin I type 2 (fast); [12]Myosin light chain 1, skeletal, fast; [13]Myosin light chain, phosphorylatable skeletal, fast; [43]LIM domain binding 3; [15]Vinculin isoform2, isoform 1; [16]Capping protein alpha; [17]Heat shock protein beta-1; [18]Peroxiredoxin 6.

Table 4.3 Proteins differentially expressed between Aberdeen Angus[1] steers of either high (*H*) *vs.* low (*L*) for sire expected progeny difference for carcass weight (EPD_{cwt})

No.	Identified Protein	NCBI Accession No. (Source)[1]	Mascot score	Fold Change[2]	*P* - value	Matched peptides/ sequence coverage%	Theoretical p*I* / M_r (kDa)
	Metabolic						
22	ENO1[3]	gi|87196501 (Canis)	1117	1.5↑	<0.0001	21/50	7.6/47.5
23	GPI[4]	gi|94966765	646	1.8↑	0.003	13/24	7.3/63.0
24	PKM2[5]	gi|73587283	894	1.7↑	0.012	14/34	8.6/62.0

[1]AA = Aberdeen Angus × Holstein Friesian; [2]↑ = Increased in *H* compared to *L* for EPD_{cw}; [3]Enolase-3; [4]Glucose-6-phosphate isomerase; [5]Pyruvate Kinase.

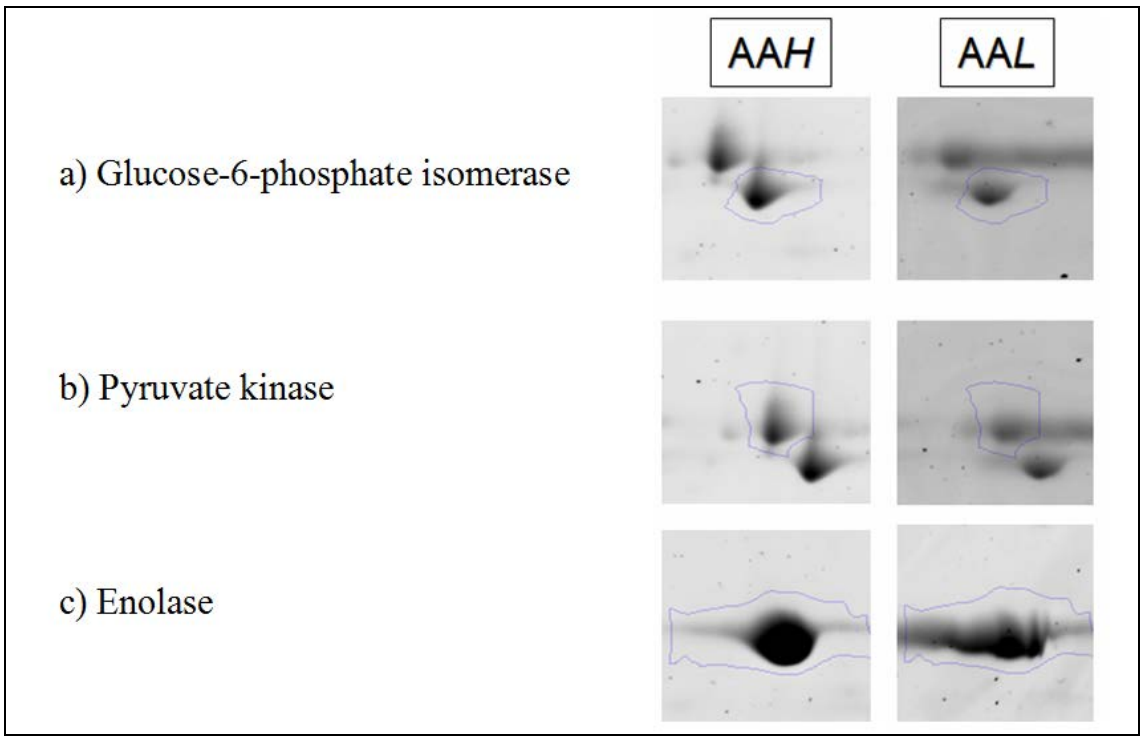


Figure 4.2 Representattion of 2-D gel 'spots' showing the protein abundance differences between AA*H* and AA*L* genotypes
Clear visual differences in protein spot abundance can be identified for proteins a) glucose-6-phosphate b) pyruvate kinase and c) enolase between AA*H* and AA*L* steers.

4.3 Chapter summary, discussion and conclusion

4.3.1 Chapter summary

Bovine skeletal muscle is a tissue of significant value to the beef industry and global economy. Through proteome analysis, it may be feasible to detect potential molecular mechanisms regulating muscle growth and intramuscular fat accumulation. The current study aimed to investigate differences in protein abundance in skeletal muscle tissue of two cattle breeds of contrasting maturity (early *vs* late maturing), adiposity and muscle growth potential, Belgian Blue × Holstein Friesian (BB) and Aberdeen Angus × Holstein Friesian (AA). Twenty AA (n=10) and BB (n=10) steers, the progeny by artificial insemination of sires of either high (*H*) or low (*L*) genetic merit, expressed as expected progeny difference for carcass weight (EPD_{cwt}), were evaluated as four genetic groups viz. BB*H*, BBL, AA*H*, and AA*L* (n=5 per treatment). Chemical composition analysis of *M. longissimus thoracis et lumborum* showed higher protein and moisture, and lower lipid concentrations for BB compared to AA. To investigate the effect of sire breed and EPD_{cwt} on *M. longissimus thoracis et lumborum*, proteomic analysis was performed using 2-D gel electrophoresis followed by mass spectrometry. Proteins were identified from their peptide sequences, using the NCBI and Swiss-prot databases. In terms of sire breed, metabolic enzymes involved in glycolysis (glycogen phosphorylase, phosphoglycerate mutase) and the citric acid cycle (aconitase-2, oxoglutarate dehydrogenase) were increased in AA whereas myosin light chain isoforms were decreased in AA compared to BB animals. Protein abundance of glucose-6-phosphate isomerase, enolase-3 and pyruvate kinase was higher in AA*H* compared to AA*L* animals. No difference in protein spot abundance was detected between BB*H* and BB*L* animals. This information will aid in the understanding of genetic influences controlling

muscle growth and fat accumulation, and could contribute to future breeding programmes to increase lean tissue gain of beef cattle.

4.3.2 Discussion

The current study examined the differences in protein abundance in *M. longissimus thoracis et lumborum* across two contrasting breeds of cattle (AA and BB) divergent in EPD_{cwt}. These breeds were selected based on their well documented differences in (i) maturation rates (early *vs.* late) and (ii) intramuscular fat accumulation as well as perceived meat quality differences (Kuber *et al*., 2004; Sadkowski *et al*., 2009).

4.3.2.1 Effect of sire breed on protein abundance

The top canonical pathway identified as significantly different between sire breed was glycolysis/gluconeogenesis with three proteins, PYGM, PGAM2 and ALDOA within the pathway activated. Bernard *et al.* (2009) found that glycolytic enzymes were increased in bulls divergently selected for high, compared with low muscle growth. However, in the current study, AA had higher abundance of proteins involved in glycolysis and the citric cycle compared to BB cattle. Results from this study and those of Bernard *et al*. (2009) may differ due to the difference in sire breed genetics and age of the animals employed in the study. Purebred and crossbred AA animals are renowned for having large amounts of intramuscular adipose tissue content within the muscle which increases with age (Hocquette *et al*., 1998; Dinh *et al*., 2010). In the current study, chemical analysis of *M. longissimus* revealed, that at slaughter, AA animals had higher lipid concentrations, thereby supporting this theory. A possible explanation for higher abundance of glycolytic enzymes in AA may be due to 'marbling' of fat within the muscle. In support of the current findings, Murigiano *et al*. (2010) compared *M. longissimus* from two distinct pig breeds, Casertana (high lipid deposition) and Large

White (lean meat production) and reported that the Casertana breed had higher abundance of glycolytic enzymes compared to Large White animals. Additionally, consistent with the current findings, Sieczkowska *et al*. (2010) reported that high PKM2 protein abundance, an enzyme involved in the glycolytic pathway, was linked with an increase in intramuscular fat content in *M. longissimus* in pigs. In fatty acid synthesis, NADPH is required to support lipogenesis. In ruminants, 50 - 80% of NADPH required for fatty acid synthesis in adipose tissue is produced by glucose oxidation *via* the pentose phosphate pathway (Vernon, 1981). Up to 50% of NADPH used in fatty acid synthesis is also generated *via* decarboxylation of isocitrate to α-ketoglutarate (isocitrate dehydrogenase pathway) (Vernon, 1981; Nafikov and Beitz, 2007). This action is preceded by the activity of the enzyme ACO2 and followed by a reaction involving the enzyme OGDH. These enzymes are involved in the citric cycle, the third canonical pathway identified, which were increased in AA compared to BB steers in the current study. This suggests that greater protein abundance of enzymes relating to oxidative metabolism may be associated with intramuscular fat accumulation in muscle, however, there are greater quantities of mitochondria, where the citric acid cycle takes place, in slow-twitch compared to fast-twitch fibre and therefore this warrants further investigation. This study reveals that different metabolic actions are taking place in the muscle tissue which directly relates to sire breed type in cattle. Intramuscular fat accumulation may be associated with increased abundance of enzymes relating to glycolysis and the current research supports these findings (Murgiano *et al*, 2010; Sieczkowska *et al*, 2010).

Glucose-1-phosphate is converted to glucose-6-phosphate during glycogen breakdown by phosphoglucomutase (PGM1) allowing it entry into the glycolytic pathway (McMurry and Begley, 2005). Alternatively, this enzyme functions in reverse also, facilitating glycogen synthesis. A 2.2 fold increase was observed in expression of

PGM protein for BB compared to AA cattle. Consistent with the current results, Hamelin *et al*. (2006) reported that the protein abundance of PGM and PGM2 was increased in longissimus muscle of rams with muscular hypertrophy compared to conventional genotypes. Muscular hypertrophy or 'double' muscling occurs from mutations in the myostatin coding sequence result in a truncated protein (McPherron *et al*., 1997). Consequently, muscle fibre type is altered with double-muscle breeds having greater fast-twitch glycolytic and reduced slow-twitch oxidative fibres (Bouley *et al*., 2005). Fast-twitch glycolytic, also referred to as white fibres, store high quantities of glycogen (Sherwood, 2006) and therefore it is hypothesised that in the current study PGM functions in glycogen synthesis rather than glycogen breakdown.

The second canonical pathway identified in the current study was the PKA signalling pathway which incorporated proteins such as MYL1, MYLPF and TNNI2. This pathway is central to many functions in the cell as well as plays a role in cytoskeleton regulation. In the study of Murigiano *et al*. (2010), *M. longissimus* from two distinct pig breeds, Casertana and Large White, was compared. Proteins related to MYL1 were increased in Large White, a breed which excels in growth of lean muscle tissue, consistent with the data for BB cattle.

4.3.2.2 Effect of EPD_{cwt} on protein abundance

The glycolytic pathway provides cells with metabolic precursors and a rapid source of energy (Murgiano *et al*., 2010). The results of the current study show that within AA, a statistically significant difference in enzymes relating to glycolysis and gluconeogenesis were observed between animals of *H* compared with *L* EPD_{cwt}, with GPI, ENO3 and PKM2 exhibiting increased protein expression in AA*H* compared to AA*L* animals. Bernard *et al*. (2009) examined the effects of genetic selection in favour of high muscle growth on gene expression in the muscle of young Charolais bulls using microarray

technology. Consistent with the current findings, the authors reported that gene expression of *GPI* and *ENO3* was increased in bulls of *H* compared to *L* muscle growth. In addition, it has been established that animals undergoing accelerated muscle growth (e.g. compensatory growth) exhibit greater gene and protein abundance of GPI, ENO3 and PKM2 (Lametsch *et al*, 2006; Lehnert *et al*., 2006). Teltathum and Mekchay, (2009) and Doherty *et al*. (2004) reported that during *pectoralis* muscle growth in chickens, enzymes relating to the glycolytic pathway (ENO3 and PKM2) were greater in abundance during a growth phase compared to at hatching. In ovine studies, Hamelin *et al*. (2010) reported that the protein abundance of ENO3 and PKM2 was greater in the *M. longissimus* of fast compared to slow growing rams. These findings indicate that high muscle growth potential may be associated with increased glycolysis, and in the case of Bernard *et al*. (2009), decreased oxidative metabolism.

In contrast to the findings for AA, no effect of EPD_{cwt} on protein abundance was detectable for BB animals. This result is consistent with the performance data for these animals, reported by Campion *et al*. (2009a), where no difference in growth rate or carcass weight between BB steers of *H* or *L* EPD_{cwt} was observed. The authors (Campion *et al*., 2009a) proposed that this absence of a genetic merit effect on growth rate in BB steers may be associated with the fact that these animals are crossbred from Holstein-Friesian cows and heterozygous for the mutation in the myostatin gene, whereas EPD_{cwt} values are calculated based on performance data from both crossbred and purebred (heterozygous and homozygous for the mutation in the myostatin gene, respectively) animals.

4.3.3 Chapter conclusion

We have provided evidence for different metabolic processes taking place in muscle of crossbred AA and BB steers which are specific to breed type. AA, an early maturing

breed lays down intramuscular fat at an earlier age while late maturing breeds like BB continue growing to a heavier mature weight. Proteins related to fibre type were increased in BB steers, with proteins involved in glycolysis and the citric cycle, in greater abundance in the muscle of AA animals. In addition, this study facilitated greater insight into differential muscle proteome expression across bovine breeds divergently selected for muscle growth rate potential, with proteins involved in glycolysis increased in AA*H* compared to AA*L* steers. Data from this study will aid in the understanding of genetic influences controlling muscle growth and fat accumulation and future work will continue to examine the potentiating or inhibitory effects of sire breed and EPD_{cwt} at more critical time points during the growth phase of the animal to elucidate key proteins regulating muscle growth. Glycolytic enzymes are potential candidates for future investigation including exploration of single nucleotide polymorphisms. Following appropriate validation, these markers could be incorporated into future cattle breeding programs to improve the accuracy of selection for muscle growth.

Chapter 5

Live Weight Gain, Feed Intake, Linear Body Measurements, Carcass Measurements and Plasma Hormones and Metabolite Concentrations in Steers Undergoing Compensatory Growth

5.1 Introduction

In beef cattle production, feed accounts for approximately 75% of total variable costs (Connolly *et al*., 2010; Finneran *et al*., 2010). Thus, strategies to reduce costs without compromising overall feed efficiency or animal performance are of particular interest to the sector. Compensatory growth is the ability of an animal to undergo accelerated growth after a period of restricted feeding, as reviewed by Hornick *et al*. (2000). The exploitation of this biological phenomenon facilitates redistribution of feed supply from a time when feed is expensive (e.g. wintertime) to when it is cheap and plentiful (e.g. pasture in spring/summer) while still maintaining overall production targets through utilisation of compensatory growth potential. Animals undergo compensatory growth when previous growth rates are below the potential maximum (Jobling, 2010). Due to its potential benefits to the economic efficiency of cattle production, the trait has been the subject of numerous studies worldwide (Ritacco *et al*., 1997; Leeson and Zubair, 1997; Tolla *et al*., 2003; Johansen and Overturf, 2006). 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 (Coleman and Evans, 1986; Keane and Drennan, 1994; Sainz *et al*., 1995; Yambayamba *et al*., 1996a,b; Hornick *et al*., 1998a,b; Tolla *et al*., 2003; Lehnert *et al*., 2006; Fiems *et al*., 2007; Connor *et al*., 2009). 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 (Yambayamba *et al*., 1996a,b). However, further research in now required to stimultaneously elucidate all aspects of this growth phenomena including performance traits, feed intake, blood metabolites and hormones, and carcass characteristics together with possible interations with maturity genotype. The objective of this study therefore was to eluicidate the response to realimentation following dietary restriction in growth

rate, feed efficiency, metabolite and metabolic hormone profiles and body and carcass characteristics across two beef cattle genotypes representing contrasting maturity types.

5.2 Effect of genotype and feeding treatment on the live weight, live weight gain, feed intake, linear body measurements, carcass measurements and plasma blood hormones and metabolites

5.2.1 Dry matter intake (DMI) and feed conversion ratios (FCR)

See section 2.2.2.3 and 2.2.2.3.1 for full details of experimental design and animal selection. The effect of both genotype and feeding treatment on DMI and feed conversation ratio (FCR) is presented in Table 5.1. A genotype × feeding treatment interaction was observed during the differential feeding period with AA/L-H consuming more silage DM compared to BB/L-H. As expected, there was a feeding treatment effect ($P < 0.001$) for both silage and total DMI with L-H consuming more silage but less total DM compared to H-H steers during the differential feeding period. Interestingly, during the feed realimentation period, there was no difference ($P > 0.05$) in total DMI between groups. Overall, for the entire period there was no effect of genotype ($P > 0.05$) on DMI; however, L-H consumed 20 % less feed on a DM basis compared to H-H steers ($P < 0.001$).

FCR was not affected by genotype ($P > 0.05$) at any time throughout the study. There was an effect of feeding treatment, however, with L-H animals having a greater FCR ($P < 0.05$) during the differential feeding period. During the realimentation period though, the converse was true, with H-H having a greater FCR ($P < 0.001$). For the study overall, there was no effect of genotype on FCR but FCR was better for L-H compared to H-H steers.

5.2.2 Live weight and live weight gain

Live weight changes and live weight gains as affected by both genotype and feeding treatment are reported in Table 5.2. No genotype × period interaction ($P > 0.05$) was observed for live weight with both genotypes having similar live weights across the

Table 5.1 Effect of genotype (G) and feeding treatment (F) on total DM intakes (DMI) and feed conversion ratio (FCR)

Trait	Genotype[1] (G)			Feeding treatment[2] (F)			*P*-value	
	AA	BB	SED	H-H	L-H	SED	G	F
DMI, kg/d								
Differential feeding period, d 0 to 99								
Silage[3]	3.07	3.04	0.02	2.14	3.96	0.02	0.09	<0.0001
Total	6.92	6.96	0.06	9.48	4.41	0.08	0.99	<0.0001
Early realimentation period, d 99 to 131								
Total	10.27	10.32	0.09	10.34	10.25	0.09	0.99	0.89
Final period to slaughter, d 131 to 253								
Total	10.24	10.21	0.09	10.12	10.33	0.09	0.99	0.20
Entire period, d 0 to 253								
Total	8.91	8.92	0.07	9.93	7.89	0.19	0.82	<0.0001
Feed Conversion Ratio[4]								
Differential feeding period, d 0 to 99	7.44	6.99	0.58	6.51	7.92	0.56	0.45	0.02
Early realimentation period, d 99 to 131	6.92	7.42	0.62	8.72	5.63	0.61	0.44	<0.0001
Finishing period, d 131 to 253	6.29	5.78	0.34	6.45	5.61	0.33	0.14	0.01
Entire period, d 0 to 253	6.44	6.15	0.18	6.65	5.94	0.18	0.13	0.0005

[1]AA = Aberdeen Angus × Holstein Friesian; BB = Belgian Blue × Holstein Friesian. [2]H-H = *ab libitum* access to feed throughout the study; L-H = Restricted feeding for 99 d followed by *ad libitum* access to feed until slaughter. [3]Interaction, there was a G × F interaction ($P < 0.05$) with values for AA/H-H, AA/L-H, BB/H-H and BB/L-H 2.14, 3.99, 2.15 and 3.93 kg/day, respectively. [4]Live weight gain ÷ total DMI.

Table 5.2 Effect of genotype (G) and feeding treatment (F) on mean live weight and live weight gain

Trait	Genotype[1] (G)			Feeding treatment[2] (F)			*P*-value	
Live weight, kg	AA	BB	SED	H-H	L-H	SED	G	F
Start, d 0	307	288	7.01	296	298	6.89	0.79	1.00
End of differential feeding period, d 99	404	390	7.01	438	356	6.89	0.99	<.0001
Realimentation, d 131	452	438	7.01	474	416	6.89	0.99	<.0001
Slaughter, d 299	655	644	7.10	669	630	6.99	1.00	0.04
Live weight gain, kg/d								
Differential feeding period, d 0 to 99	1.06	1.12	0.052	1.55	0.63	0.050	0.28	<.0001
Realimentation period, d 99 to 131	1.50	1.50	0.097	1.26	1.74	0.093	0.98	<.0001
Realimentation period, d 131 to 195	1.65	1.90	0.07	1.63	1.91	0.06	0.001	0.0001
Realimentation period, d 195 to 253	1.34	1.33	0.09	1.34	1.33	0.09	0.89	0.87
Realimentation period, d 253 to 299	0.91	0.64	0.18	0.84	0.71	0.17	0.14	0.47
Entire period, d 0 to 299	1.25	1.26	0.038	1.33	1.18	0.036	0.81	0.0004

[1]AA = Aberdeen Angus × Holstein Friesian; BB = Belgian Blue× Holstein Friesian. [2]H-H = *ad libitum* access to feed; L-H = restricted access to feed for 99 d followed by *ad libitum* access to feed until slaughter. There was no G × F interaction.

various measurement timepoints. No difference for live weight ($P < 0.001$) was observed between feeding treatments observed at the start (d 0); however, H-H were heavier compared to L-H steers at the end of the differential feeding period (d 199; $P < 0.001$) which sustained until slaughter (d 299; $P < 0.05$).

There was no effect of genotype on live weight gain for any period throughout the study with the exception of between d 131 to d 195 (middle of the realimentation period) when BB had greater gains compared to AA steers ($P < 0.001$). There was an effect of feeding treatment during the differential feeding period ($P < 0.001$) with live weight gains greater in H-H compared to L-H steers; however, from the end of the differential feeding period (d 99) to early in the realimentation period (d 131) live weight gain was greater ($P < 0.001$) for the L-H compared to the H-H steers. This greater ($P < 0.001$) live weight gain of L-H steers continued up to d 195 i.e. 96 d post commencement of the realimentation period. There after, there was no difference ($P > 0.05$) between feeding treatments in live weight gain for the remainder of the study.

5.2.3 Linear measurements and ultrasonically scanned muscle and fat depth

The effect of genotype and feeding treatment on carcass linear measurements and ultrasonically scanned muscle and fat depths are reported in Table 5.3. No genotype × period ($P > 0.05$) interaction was observed for height at withers, chest girth, back length or chest depth throughout the study; however, there was an overall effect of genotype with height at withers (tendency; $P = 0.06$), back length ($P < 0.01$) and chest depth ($P < 0.05$) greater in BB compared to AA steers. For pelvic width measurements scaled to live weight, BB had greater ($P < 0.01$) values compared to AA steers at the end of the differential feeding period (d 99) with a strong tendency for greater pelvic width proportions in BB compared to AA early in the realimentation period (d 131; $P = 0.06$).

Table 5.3 Effect of genotype (G) and feeding treatment (F) on linear body measurements and ultrasonically scanned muscle and fat depths

Trait	Genotype[1] (G)			Feeding treatment[2] (F)			*P*-value	
	AA	BB	SED	H-H	L-H	SED	G	F
Linear body measurement								
Height at withers, mm/kg								
Start, d 0	3.69	3.86	0.102	3.73	3.82	0.101	0.76	0.99
End of DFP[3], d 99	3.03	3.11	0.056	2.79	3.34	0.055	0.81	<.0001
Realimentation, d 131	2.65	2.69	0.050	2.55	2.79	0.049	0.97	<.0001
Slaughter, d 299	2.01	2.06	0.039	1.98	2.09	0.038	0.85	0.08
Chest girth, mm/kg								
Start, 0 d	4.78	4.72	0.082	4.75	4.75	0.081	0.99	1.00
End of DFP[3], d 99	4.34	4.39	0.059	4.13	4.59	0.059	0.99	<.0001
Realimentation, d 131	3.91	3.90	0.053	3.79	4.02	0.052	1.00	0.001
Slaughter, d 299	3.29	3.24	0.103	3.26	3.27	0.102	0.99	1.00
Length of back, mm/kg								
Start, 0 d	3.27	3.45	0.063	3.38	3.31	0.063	0.22	0.94
End of DFP[3], d 99	2.71	2.72	0.044	2.50	2.93	0.044	1.00	<.0001
Realimentation, d 131	2.27	2.33	0.045	2.23	2.37	0.044	0.94	0.03
Slaughter, d 299	1.81	1.88	0.048	1.78	1.90	0.048	0.75	0.21
Chest depth, mm/kg								
Start, d 0	1.85	1.93	0.039	1.87	1.90	0.039	0.47	0.99
End of DFP[3], d 99	1.56	1.59	0.026	1.47	1.69	0.026	0.94	<.0001
Realimentation, d 131	1.37	1.39	0.022	1.33	1.43	0.022	0.93	<.0001
Slaughter, d 299	1.10	1.13	0.021	1.10	1.14	0.021	0.88	0.63
Pelvic width, mm/kg								
Start, d 0	1.29	1.44	0.079	1.43	1.29	0.079	0.63	0.71
End of DFP[3], d 99	1.08	1.13	0.015	1.04	1.17	0.015	0.01	<.0001
Realimentation, d 131	0.94	0.99	0.018	0.93	1.01	0.017	0.06	0.001
Slaughter, d 299	0.82	0.85	0.049	0.82	0.84	0.023	0.21	0.98
Ultrasound measurement								
Muscle depth[4], mm								
Start, d 0	44.26	46.64	1.389	45.90	45.01	1.367	0.78	0.99
Middle of DFP[3], d 99	49.51	51.85	1.389	53.12	48.25	1.367	0.81	0.02
End of DFP[3], d 99	50.92	54.14	1.389	56.02	49.05	1.367	0.38	<.0001
Realimentation, d 131	55.65	60.43	1.389	59.04	57.04	1.367	0.03	0.90
Slaughter, d 299	58.77	67.06	1.417	62.49	63.33	1.393	<.0001	0.99
Fat Depth[5], mm								
Start, d 0	0.79	0.61	0.065	0.74	0.67	0.065	0.15	0.97
Middle of DFP[3], d 99	1.06	0.70	0.085	0.98	0.79	0.085	0.001	0.48
End of DFP[3], d 99	1.41	0.79	0.136	1.53	0.67	0.136	0.001	<.0001
Realimentation, d 131	3.51	2.51	0.287	3.42	2.60	0.286	0.021	0.12
Slaughter, d 299	7.41	5.14	0.481	6.77	5.78	0.48	0.001	0.55

[1]AA = Aberdeen Angus × Holstein Friesian; BB = Belgian Blue × Holstein Friesian. [2]H-H = *ab libitum* access to feed throughout the study; L-H = Restricted feeding for 99 d followed by *ad libitum* access to feed until slaughter. [3]DFP = Differential feeding period. [4]*M. longissmus thoracis et lumborum*. [5]Average 13th rib and lumber fat measurements. There was no G × F interactions.

A feeding treatment × period interaction ($P < 0.001$) existed for all body linear measurements. These were generally due to there being no difference between the feeding treatments at the start and large differences at the end of the differential feeding period which subsequently declined in magnitude during the realimentation period. For height at withers, chest girth, back length and chest depth H-H animals having lower proportions relative to bodyweight at the end of the differential feeding period (d 99; $P < 0.001$) and early in the realimentation period (d 131; $P < 0.05$) whereas, there was no difference between feeding treatments at the start (d 0; $P > 0.05$) or at the end of the study (d 299; $P > 0.05$). For pelvic width, H-H and L-H animals had similar values at start of the study (d 0; $P > 0.05$) and again at slaughter (d 299; $P > 0.05$); however, H-H had greater proportions relative to bodyweight when compared to L-H animals at the end of the differential period (d 99; $P < 0.001$) and beginning of the realimentation period (d 131; $P < 0.001$).

There was no difference for muscle depth between genotypes at the start of the study (d 0; $P > 0.05$), during the differential feeding period (d 55; $P > 0.05$) or at the end of the differential feeding period (d 99; $P > 0.05$); however, BB had greater muscle depth during the realimentation period (d 131; $P < 0.05$) to slaughter compared to AA steers (d 299; $P < 0.001$). Across feeding treatment, L-H had lower muscle depth during the differential feeding period (d 55; $P < 0.05$) and at the end of the differential feeding period (d 99; $P < 0.001$) compared to H-H steers; however, early into the realimentation period this difference was negated (d 131; $P < 0.05$).

Both genotypes had similar fat depth values at the start of the study (d 0; $P > 0.05$); however, AA had greater fat depths compared to BB animals at the end of the differential feeding period (d 99; $P < 0.01$), during the realimentation period (d 131; $P < 0.05$) and at slaughter (d 299; $P < 0.001$). L-H steers had lower fat depth values at the end of the differential feeding period (d 99; $P < 0.001$) compared to H-H steers,

however early into the realimentation period this difference was absent (d 131; $P > 0.05$) which continued until slaughter (d 299; $P > 0.05$).

5.2.4 Metabolic hormones and metabolites

The effect of feeding treatment on plasma concentrations of IGF-1 is reported in Figure 5.1a. No genotype × period interaction ($P > 0.05$) was observed for concentrations of IGF-1. L-H animals had lower values for plasma concentrations of IGF-1 compared to the H-H steers at the end of the differential feeding period (d 99; $P < 0.001$) but similar values at all other times throughout the study.

A similar profile to IGF-1 was observed for concentrations of insulin. No effect of genotype or no genotype × period interaction ($P > 0.05$) was observed with similar values for AA and BB steers at all stages throughout the study. Plasma concentrations of insulin with similar for H-H and L-H animals at the start of the study (d 0; $P > 0.05$), during the realimentation period (d 131; $P > 0.05$) and at slaughter (d 299; $P > 0.05$); however, lower values were detected in L-H steers at the end of the differential feeding period (d 99; $P < 0.001$), compared to H-H steers (Figure 5.1b).

Leptin concentrations were greater in AA at slaughter (d 299; $P < 0.01$) but similar values at the start of the study (d 0; $P > 0.05$), at the end of the differential period (d 99; $P > 0.05$) and during the realimentation period (d 131; $P > 0.05$) compared to BB steers. L-H animals had lower plasma concentrations of leptin at the end of the differential feeding period (d 0; $P < 0.001$) compared to the H-H steers but similar values at all other times (Figure 5.1c).

No effect of genotype or interaction involving genotype ($P > 0.05$) was observed for blood glucose concentrations. Blood glucose concentrations were similar for H-H and L-H steers at the start of the study (d 0; $P > 0.05$), late in the realimentation period (d 233 and d 273; $P > 0.05$) and at slaughter (d 299; $P > 0.05$); however, L-H had lower

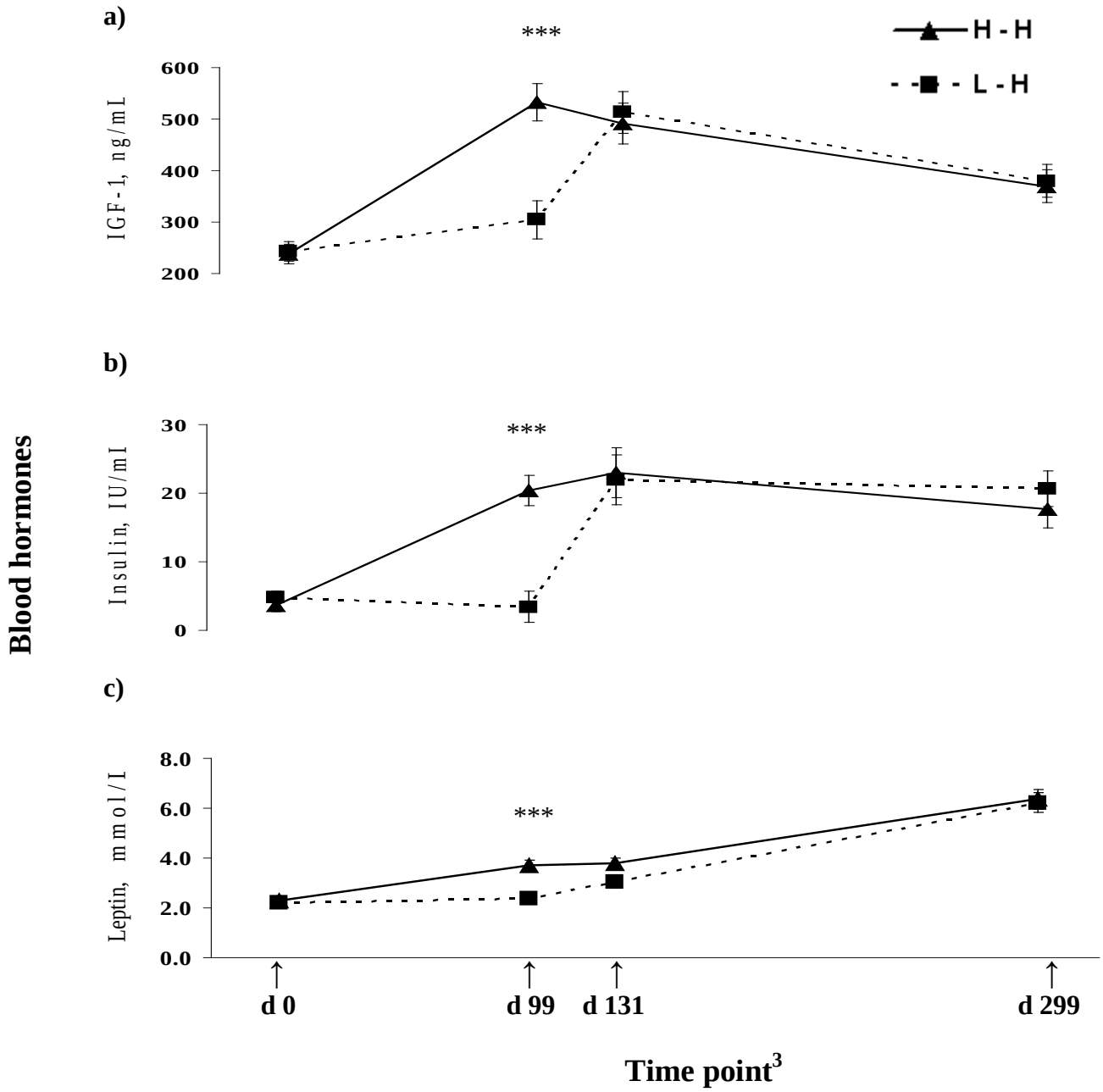


Figure 5.1 Effect of feeding treatment[1,2] on plasma concentrations of blood hormones

***P < 0.001. Error bars equal standard error (s.e.). [1]H-H = ab libitum access to feed throughout the study; L-H = Restricted feeding for 99 d followed by *ad libitum* access to feed until slaughter. [2]There was no differences observed between genotypes at any time so feeding treatments effect reported only. [3]d 0 = Start; d 99 = End of differential feeding period; d 131 = Realimentation period; d 299 = Slaughter.

values during, and at the end of the differential feeding period (d 55 and d 99, respectively; $P < 0.001$) compared to the H-H steers with the opposite effect evident early in the realimentation period (d 131; $P < 0.01$). During the realimentation period the differences narrowed and by d 233 of the study (after 134 d post commencement of realimentation) there was no difference in glucose concentrations between feeding treatments (Figure 5.2a).

Plasma urea concentrations ($P < 0.001$) were greater for AA compared to BB (5.46 *vs.* 4.34, respectively) at d 233 of the study, after 134 d post realimentation but similar values at all other times throughout the study. Additionally, blood urea concentrations for H-H and L-H steers were similar at the start (d 0; $P > 0.05$), end of the realimentation period (d 233 and d 273; $P > 0.05$) and at slaughter (d 299; $P > 0.05$); however, during the differential feeding period and early in the realimentation period, L-H had lower values ($P < 0.05$) compared to H-H animals (Figure 5.2b).

No effect of genotype or interaction involving genotype ($P > 0.05$) was observed for βHB concentrations. Plasma concentrations of βHB were lower for L-H animals during (d 55) and at the end of the differential feeding period (d 99; $P < 0.001$) and early in the realimentation period (d 131; $P < 0.05$) compared to H-H animals but no difference thereafter (Figure 5.2c).

Similarly, no effect of genotype or feeding treatment or interaction ($P > 0.05$) or interaction between them was observed for plasma concentrations of NEFA (Figure 5.2d).

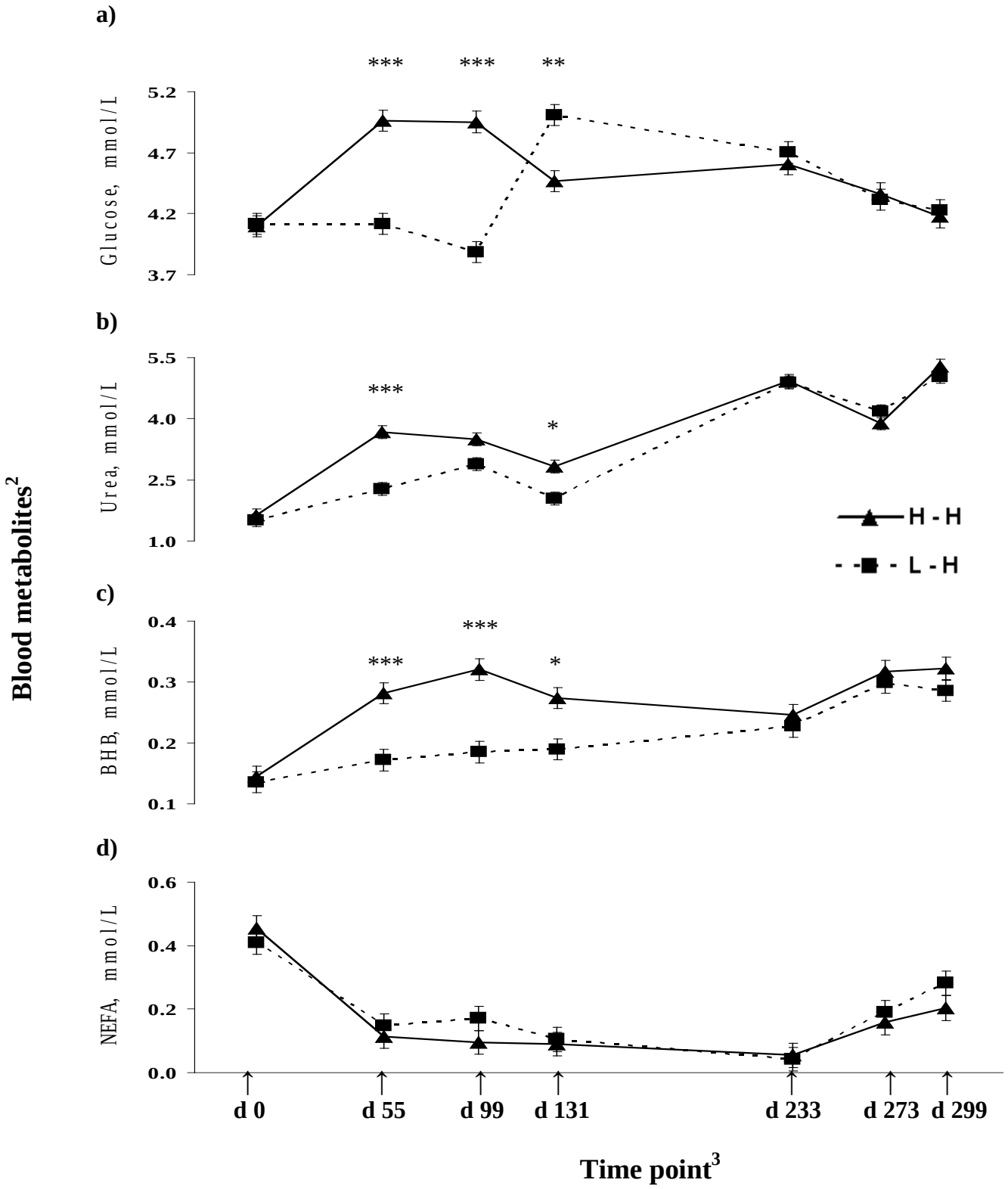


Figure 5.2 Effect of feeding treatment[1] on plasma concentrations of blood metabolites

*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$. [1]H-H = *ab libitum* access to feed throughout the study; L-H = Restricted feeding for 99 d followed by *ad libitum* access to feed until slaughter. [2]Betahydroxybutyrate = βHB. [3]d 0 = Start; d 55 = Middle of differential feeding period; d 99 = End of differential feeding period; d 131 = Realimentation period; d 233 = Realimentation period; d 273 = Realimentation period; d 299 = Slaughter.

5.2.5 Carcass traits and non-carcass components

The effect of genotype and feeding treatment on carcass traits, 5-rib joint weight, *M. longissimus thoracis et lumborum* area, rib joint tissue proportion and non-carcass components are summarised in Table 5.4. No genotype × feeding treatment interaction was recorded for any of these traits. There was an effect of genotype ($P < 0.05$) and feeding treatment ($P < 0.001$) for CW with H-H and BB animals having heavier carcasses compared to L-H and AA animals, respectively.

An effect of genotype ($P < 0.001$) was observed for dressing percentage with BB having greater values compared to AA steers. There was no effect of feeding treatment ($P < 0.05$) on dressing percentage.

There was an effect of genotype ($P < 0.001$) on carcass conformation class with BB carcasses having greater values compared to AA carcasses. However, there was no effect ($P > 0.05$) of feeding treatment on carcass conformation class.

An effect of genotype ($P < 0.001$) for carcass fat class was also observed with AA having greater values compared to BB carcasses. There was no effect of feeding treatment on carcass fat class, though, L-H tended ($P = 0.08$) to have a lower carcass fat class value compared to H-H carcasses.

No genotype × feeding treatment interaction was observed for *M. longissimus thoracis et lumborum* area, weight of 5-rib joint or 5-rib joint composition. There was no effect of genotype ($P > 0.05$) on 5-rib joint weight however, there was an effect of feeding treatment ($P < 0.001$) with H-H having a heavier 5-rib joint compared to L-H carcasses. There was an effect of genotype ($P < 0.001$) on *M. longissimus*, total muscle and fat proportions with greater values for the first two traits for BB and a greater value for the latter for AA carcasses. There was no effect of genotype on the proportion of other muscle ($P > 0.05$), muscle trim ($P > 0.05$) or bone plus ligamentum total fat proportions ($P < 0.05$) with nuche/supraspinale proportions ($P > 0.05$).

Table 5.4 Effect of genotype (G) and feeding treatment (F) on slaughter traits, 5-rib joint weight, *M. longissimus thoracis et lumborum* area, rib joint dissection and selected non-carcass components and carcass measurements

Trait	Genotype[1] (G)			Feeding treatment[2] (F)			*P*-value	
	AA	BB	SED	H-H	L-H	SED	G	F
Cold carcass weight, kg	354	369	5.860	373	350	5.538	0.02	0.0003
Dressing percentage, %	52.7	56.6	0.409	54.7	54.6	0.398	<.0001	0.75
Carcass conformation[3]	7.25	9.08	0.411	8.33	8.02	0.402	<.0001	0.44
Fat class[4]	10.39	7.96	0.478	9.59	8.75	0.466	<.0001	0.08
Perinephric plus retroperitoneal fat, kg	11.69	11.41	0.798	12.45	10.65	0.778	0.72	0.03
Perinephric plus retroperitoneal fat[5], g/kg	34.24	31.20	2.246	34.24	31.20	2.191	0.18	0.17
5-rib joint, kg	8.39	8.58	0.232	8.91	8.06	0.219	0.42	0.0006
M. longissimus area[5], cm^2/kg	0.24	0.28	0.012	0.25	0.27	0.012	0.004	0.29
Ribs joint composition[6], g/kg								
M. longissimus	226.82	275.30	10.869	245.52	256.60	10.59	<.0001	0.31
Other muscle	278.12	284.49	14.205	271.83	290.78	13.61	0.66	0.18
Muscle trim	76.48	91.10	8.160	86.74	80.85	7.957	0.08	0.47
Bone and other tissue	236.89	236.31	5.811	232.62	240.58	5.535	0.92	0.16
Fat	185.70	115.84	13.070	166.59	134.96	12.74	<.0001	0.02
Total muscle	569.64	641.95	11.866	594.41	617.18	11.57	<.0001	0.06
Linear carcass measurements[5]								
Length of carcass, mm/kg	3.84	3.64	0.068	3.65	3.82	0.064	0.006	0.01
Carcass depth, mm/kg	1.39	1.32	0.030	1.31	1.40	0.029	0.04	0.004
Leg width, mm/kg	1.22	1.21	0.023	1.17	1.25	0.022	0.75	0.001
Leg thickness, mm/kg	0.83	0.82	0.014	0.80	0.84	0.014	0.49	0.005
Leg length, mm/kg	2.07	1.98	0.038	1.97	2.08	0.036	0.03	0.006
Non-carcass components[6]								
Heart, g/kg	4.27	4.05	0.167	4.03	4.28	0.163	0.21	0.13
Lungs, g/kg	11.48	11.01	0.551	11.11	11.38	0.529	0.40	0.61
Gall bladder, g/kg	1.14	1.16	0.088	1.14	1.16	0.088	0.75	0.74
Liver, g/kg	10.56	10.58	0.383	10.45	10.69	0.373	0.95	0.53
Spleen, g/kg	2.00	1.70	0.199	1.96	1.74	0.169	0.15	0.21
Intestines, g/kg	41.76	38.54	1.33	40.63	39.67	1.29	0.02	0.46
Rumen full, g/kg	58.41	54.39	2.627	53.88	58.90	2.468	0.14	0.05
Rumen empty, g/kg	17.44	16.66	0.637	16.34	17.75	0.622	0.23	0.03
Fore feet, g/kg	9.21	9.61	0.263	9.34	9.48	0.257	0.13	0.61
Hind feet, g/kg	9.77	9.94	0.234	9.68	10.03	0.222	0.48	0.13
Hide, g/kg	96.77	81.55	1.865	88.47	89.85	1.818	<.0001	0.45
Kidney, g/kg	1.98	1.99	0.059	1.93	2.03	0.058	0.85	0.09
Head, g/kg	28.95	30.23	0.548	28.94	30.24	0.534	0.03	0.02

[1]AA = Aberdeen Angus × Holstein Friesian; BB = Belgian Blue × Holstein Friesian. [2]H-H = ab libitum access to feed throughout the study; L-H = Restricted feeding for 99 d followed by *ad libitum* access to feed until slaughter. [3]Scaled 1 (poorest) to 15 (best). [4]Scaled 1 (leanest) to 15 (fattest). [5]*M. longissimus thoracis et lumborum* expressed per kilogram carcass weight; [6]Expressed per kilogram of total 5-rib joint weight. [7]Expressed per kilogram slaughter weight.

There was an effect of feeding treatment on H-H having greater values compared to L-H carcasses. However, there was no effect of feeding treatment on the proportions of *M. longissimus thoracis et lumborum* ($P > 0.05$), other muscle ($P > 0.05$), muscle trim ($P > 0.05$) or bone plus ligamentum nuche/supraspinale ($P > 0.05$). A strong tendency ($P = 0.06$) for L-H animals to have greater total muscle proportion compared to H-H animals was also detected. There was an effect of genotype ($P < 0.01$) on scaled *M. longissimus thoracis et lumborum* area with values greater for BB compared to AA carcasses however there was no effect of feeding treatment ($P > 0.05$) on scaled *M. longissimus thoracis et lumborum* area.

An effect of genotype was observed for scaled measurements of carcass length ($P < 0.01$), carcass depth ($P < 0.05$) and leg length ($P < 0.05$) with proportions in BB lower compared to AA carcasses. There was an effect of feeding treatment ($P < 0.01$) on all carcass measurements, when scaled for carcass weight, in that proportions were greater for L-H compared to H-H animals.

There was an effect of genotype on weight as a proportion of SW of full intestines ($P < 0.05$), hide ($P < 0.001$) and head ($P < 0.05$) with greater values for the former two offal components for AA and a greater value for the latter for BB. There was no effect of genotype ($P > 0.05$) on the proportional weights of heart, lungs, gall bladder, liver, spleen, full rumen, empty rumen, feet (fore or hind), kidney, perinephric plus retroperitoneal and perinephric plus retroperitoneal fat scaled for carcass weight. When scaled for SW, there was an effect of feeding treatment on the weight of the rumen both full ($P < 0.05$) and empty ($P < 0.05$), and on the head ($P < 0.05$) with values greater in L-H compared to H-H steers. There was no effect of feeding treatment ($P > 0.05$) on scaled measurements of weight for heart, lungs, gall bladder, liver, spleen, intestines full, feet (fore or hind), hide or kidney. There was an effect ($P < 0.05$) of feeding treatment on weight of perinephric plus retroperitoneal fat with H-H having

greater values compared to L-H carcasses; however, when scaled for carcass weight this effect was not statistically significant ($P > 0.05$).

5.3 Chapter summary, discussion and conclusion

5.3.1 Chapter summary

Compensatory growth is the ability of an animal to undergo accelerated growth after a period of restricted feeding. Crossbred Aberdeen Angus × Holstein Friesian (AA; n = 22) or Belgian Blue (BB; n = 24) × Holstein Friesian steers were assigned to one of two treatment groups in a 2 (genotypes) x 2 (feeding treatments) factorial design. Over a 99 d differential feeding period, one group (11 AA and 12 BB) was offered a high energy control diet (H-H) whereas the second group (11 AA and 12 BB) was offered an energy restricted diet (L-H). At the end of the differential feeding period (99 d), both groups of animals were then offered the H-H ration. This period, which lasted 200 d, was known as the realimentation period, and all animals were slaughtered on d 299 of the study. During feed restriction, L-H consumed less DM, had a poorer feed conversion ratio (FCR) and lower concentrations of plasma hormone and metabolites compared to H-H steers. Additionally, L-H had lower muscle and fat development, as accessed ultrasonically, compared to H-H steers. During feed realimentation, there was no difference in DM intakes (DMI) between feeding treatments; however, L-H had greater live weight gain compared to H-H steers. Overall, H-H consumed greater quantities on a DM basis, however these animals had a better FCR compared to L-H steers. Following slaughter, carcass weight was affected by feeding treatment with H-H having heavier carcasses than L-H steers. Additionally, 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 H-H and L-H steers. Overall, L-H had a compensatory growth (or recovery) index of 52 % and did not fully compensate for the loss of gains during the differentially feeding period; however,

ultrasonically scanned *M. longissimus thoracis et lumborum*, a tissue of high economic value, recovered completely making it a target of interest for further investigation.

5.3.2 Chapter discussion

The objective of this study was to examine the response in a large number of performance and physiological traits to a period of restriction feeding across two genotypes varying in age of maturity, on their potential to exhibit compensatory growth following feed realimentation. The AA and BB genotypes were selected because of their well documented differences in carcass conformation, muscle composition and maturation rates (early *vs.* late; Bellinge *et al*., 2005; Keane and Drennan, 2008; Dinh *et al*., 2010). This study offers revealing insight into the compensatory growth phenomena in cattle while further elucidating the mechanisms regulating its control.

5.3.2.1 DMI and FCR

Although AA consumed greater intakes of silage compared to BB when offered the restricted energy diet, this difference was not observed between breeds allowed *ad libitum* feed intake during the same period. However, H-H animals were offered concentrates *ad libitum* with silage offered restrictively to ensure high dietary energy consumption and therefore this effect of genotype may not have been observed for forage intake.

Sainz *et al*. (1995) reported that *ad libitum* feeding following feed restriction in beef steers resulted in greater feed intakes during the realimentation period. However, this was not observed in the current study, with no difference in DMI between feeding treatments during the realimentation period. This is consistent with Hornick *et al*. (1998a) who reported no difference in DMI during feed realimentation in BB double muscled bulls following feed restriction. Although no difference was observed in feed

intakes between feeding treatments during the realimentation period, live weight gain was increased in L-H compared to H-H steers which resulted in a better FCR for L-H. An overall increased efficiency was also observed in some compensatory growth studies reported in the literature (Yambayamba *et al*., 1996; Ritacco *et al*., 1997) but not in others (Coleman and Evans, 1986; Sainz *et al*., 1995; Hornick *et al*., 1998a; Vasconcelos *et al*., 2009).

5.3.2.2 Live weight and live weight gain

Greater mature live weight and live weight gain in late maturing compared to early maturing cattle breeds have been reported in some (Coleman and Evans, 1986) but not all (Cuvelier *et al*., 2006a; Albertí *et al*., 2008) studies, with both traits dependent on feeding intensity and age (Coleman and Evans, 1981). In the current study, all steers were crossbreds, born to Holstein-Friesian cows and therefore the absolute effect of breed on live weight differences was diluted, and reflected the overall absence of breed differences for weight gain. This is in in agreement with both Campion *et al*. (2009a) and Keane *et al*. (2011) who also compared crossbred AA and BB steers. Overall, AA and BB steers responded similarly to feed restriction and feed realimentation with a difference only observed in live weight gains during the middle of the realimentation period (d 131 to d 195 of the study). In a similar study involving early and late maturing breeds subjected to differential feeding followed by realimentation on a high energy diet, Coleman and Evans (1981) reported compensatory growth in Charolais steers but not in Angus steers during the finishing period. This may have been due to the immaturity of the Angus at the start of that study and also to the length of the differential feeding period (306 d). Indeed Hornick *et al*. (2000) states that compensatory growth is enhanced when the duration of the restriction period is short (approximately 3 months in cattle). In the current study, greater compensatory growth

was observed early into the realimentation period in L-H compared to H-H steers. This is consistent with the literature which generally states that the phenomenon is typically expressed mainly between d 30 and d 60 of realimentation after adaptation to a new diet (for review see Hornick *et al*., 2000). Although considerable compensatory gain was observed in the L-H animals it was not complete. This may have been due to the levels of feeding compared during the differential feeding period as Neel *et al*. (2007) reported greater compensatory gains in crossbred steers that had a growth rate of 0.23 kg/d compared with growth rates of 0.45 and 0.68 kg/d during the restriction period. The growth rate of the L-H group during the differential feeding period in the present study was 0.63 kg/d and the realimentation period was continued until compensation had completely ceased. All animals were adequately finished for market, when slaughtered.

5.3.2.3 Linear body measurements and ultrasonically scanned muscle and fat depth

Campion *et al*. (2009a) reported an effect of genotype on chest girth, back length and chest depth scaled for live weight with BB having lower scaled measurements throughout their lifetime compared to AA steers. In the current study, and contradictory to those findings, no difference was observed between genotypes for chest girth, back length and chest depth. According to Albertí *et al*. (2008) a narrow pelvis indicates slow skeletal development and low muscularity. This observation in respect of muscling is confirmed in the present study in which AA had a relatively smaller pelvic width than BB at the end of the differential feeding period (d 99) and early in the realimentation period (d 131) in addition to a lower *M. longissimus thoracis et lumborum* depth, smaller *M. longissimus thoracis et lumborum* area and a lower proportion of total muscle in the 5-rib joint. By the time of slaughter, the differences in scaled body measurements between feeding treatments were absent which is consistent with studies in sheep and rabbits (Kamalzadeh *et al*., 1998; Yakubu *et al*., 2007). As no feeding

treatment or genotype × feeding treatment interaction existed for linear body measurements at slaughter it would appear that relatively mild growth retardation followed by expression of compensatory growth has no latent effects on skeletal development.

Scanned *M. longissimus thoracis et lumborum* depth was greater for BB animals from the early realimentation period up until slaughter, which reflects the well documented greater muscularity and better carcass conformation of this breed (McPherron and Lee, 1997; Hickey *et al*., 2007; Keane and Moloney, 2010; Keane *et al*., 2011). Ultrasonically scanned muscle depth was greater, as expected, in H-H during the differential feeding period compared to L-H animals. However, as L-H experienced compensatory growth, this difference in muscle depth between dietary groups disappeared early into the realimentation period. This supports the findings of Schoonmaker *et al*. (2004) who also reported a difference in ultrasonically scanned muscle depth in steers offered different planes of nutrition but no difference in ultrasonically scanned muscle depth at slaughter after a period of feed realimentation. Importantly, it can be concluded that a feeding regime designed to exploit compensatory growth similar to that employed in the current study, has no residual effect on the growth of the economically important *M. longissimus thoracis et lumborum*.

The subcutaneous fat depth for BB animals compared with AA was expected as late maturing breeds, and particularly BB animals, deposit less fat compared to early maturing beef breeds, when compared at the same age or weight (Sadkowski *et al*., 2009). Greater fat depth was observed for H-H at the end of the differential feeding period (d 99) compared to L-H steers which stands to reason and is consistent with the findings of previous reports (Vasconcelos *et al*., 2009). Similarly for muscle depth, the difference in fat depth between dietary groups disappeared early into the realimentation period (d 131). Again, this was also observed by Vasconcelos *et al*. (2009) in that

scanned fat depths were similar for steers offered a low compared to a high energy ration before the finishing period.

5.3.2.4 Metabolic hormones and metabolites

Circulating IGF-1 is critical to regulating postnatal growth, development and differentiation of skeletal muscle (Clemmons, 1997; Duan and Xu, 2005; Duan *et al*., 2010) through its interaction with IGF receptors (Jones and Clemmons, 1995; Oksbjerg *et al*., 2004). Yambayamba *et al*. (1996b) reported a reduction in circulating levels of IGF-1 in heifers during a dietary restriction period with IGF-1 concentrations rising to the same level as control animals upon realimentation on a higher energy ration. A similar outcome was observed in the current study with L-H steers exhibiting lower plasma levels of IGF-1 during the restricted period. Upon realimentation, however, plasma levels of IGF-1 in L-H rose to, but did not exceed those of H-H animals, suggesting that compensatory growth is not a direct consequence of elevated systemic availability of IGF-1. Similarly, in pigs during compensatory growth, system concentrations of IGF-1 rose to levels comparable with that of control animals but did not surpass the control concentrations. In fact, Ritacco *et al*. (1997) concluded that compensatory growth was not mediated by IGF-1 in runt piglets. However, although L-H did not have IGF-1 concentrations greater than H-H animals, local production of IGF-1 within tissues or variation in expression levels of key genes in the somatotropic axis, for example IGF-1 receptors may have induced compensatory growth and therefore this warrants further investigation.

In ruminants, post-prandial absorption of VFA from the gastrointestinal tract after feeding trigger the secretion of insulin from the pancreas (de Jong, 1982; Hornick *et al*., 1998b). Insulin stimulates facilitative glucose transport activity in skeletal muscle and adipocytes (Hocquette *et al*., 2000; Sasaki, 2002). Yambayamba *et al*. (1996b)

reported that insulin concentrations decreased in Hereford heifers offered a restricted ration, however by d 10 of feed realimentation insulin had increased to the same concentration as control animals. A comparable trend was observed in the current study with no difference in insulin concentrations between feeding treatments by d 131 of the study, 32 d post commencement of realimentation. The greater intake of energy and protein in the diet during the differential feeding period resulted in greater plasma glucose and potentially greater portal plasma amino acids and therefore greater concentrations of insulin in the H-H animals (Hornick *et al*., 1998b). In the L-H steers, the increase in insulin concentrations during the realimentation period is associated with the consumption of the higher energy ration. Whereas glucose declined linearly during the realimentation period, insulin concentrations remained high during this period and through to slaughter. Insulin stimulates glucose uptake by initiating the translocation of glucose transporters to the plasma membrane, allowing the uptake of glucose into the cell (for review see Hocquette and Abe, 2000) and therefore lowering peripheral blood glucose levels (Dunshea *et al*., 1995). In addition, insulin promotes lipogenesis and inhibits lipolysis (Beeby *et al*., 1988; Istasse *et al*., 1990) by directing long chain fatty acids towards triglyceride storage rather than oxidation in muscle (Hocquette *et al*., 2000; Daix *et al*, 2008). In fact, Matsuzaki *et al*. (1997) reported a positive relationship between plasma insulin concentrations and carcass fat proportions. However, no difference in insulin concentrations was detected between genotypes although AA had greater scanned fat depths, greater carcass fat class (tendency) and greater rib joint fat proportion compared to BB steers.

Leptin is involved in the hypothalamic control of body energy homeostasis; is an indicator of body fat reserves and regulator of appetite and energy expenditure (Delavaud *et al*., 2002). In ruminants, research has shown a positive correlation between circulating concentrations of leptin and fat accumulation (Berg *et al*., 2003; Geary *et al*.,

2003). The results of the current study support this finding in that leptin concentrations were lower in L-H animals at the end of restriction which corresponds with lower ultrasonically scanned fat depths. Following realimentation, leptin concentrations rose to similar values as H-H, coinciding with increases in scanned fat depths. Across genotype however, leptin concentrations did not correspond with fat reserves. Indeed, AA had great scanned subcutaneous fat depths from early into the study; however, a difference in leptin concentrations was only observed at slaughter and this warrants further investigation.

Reduced plasma concentrations of glucose during the differential feeding period compared to H-H steers was expected due to the lower availability of substrates in the diet. Consistent with the current results, Yambayamba *et al*. (1996) and Hornick *et al*. (1998b) all reported lower blood glucose concentrations in restricted bulls and heifers compared to animals offered a high energy ration. It is unclear why glucose concentrations dropped in the H-H steers at d 131 of the study, 32 d post commencement of realimentation. In the current study, after realimentation, glucose concentrations in L-H increased considerably with values greater than H-H steers during this period. In fact, concentrations were equal to values for the H-H steers during the differential feeding period. Similarly, Yambayamba *et al*. (1996b) and Hornick *et al*. (1998b) all reported that plasma glucose levels rose in bulls, heifers and steers during similar realimentation periods with concentrations of glucose never rising about that of animals on a continual plane of nutrition throughout the study. As glucose concentrations in L-H never exceeded values observed for H-H animals during the differential feeding period, it appears that greater systemic availability of glucose is not a major driver of compensatory growth; however, greater utilisation potential at a local level must be considered and warrants further investigation.

Both Ellenberger *et al*. (1989) and Fiems *et al*. (2007) observed no difference in blood urea nitrogen concentrations in steers and cows during feed restriction compared to animals offered a control ration. Yambayamba *et al*. (1996b) reported a decrease in urea concentrations during feed restriction and in the current study L-H animals had greater urea concentrations during the differential feeding period. Overall, AA had greater urea concentrations compared to BB animals throughout the study. Consistent with this, Campion *et al*. (2009b) also observed greater systemic levels of urea in crossbred AA compared to BB steers at slaughter. In addition, Beeby *et al*. (1988) reported greater urea concentrations in early maturing compared to late maturing cattle. The authors attributed this result to the early maturing steers requiring less of their protein intake for muscle growth with the excess being deaminated.

Concentrations of plasma βHB were lower in L-H during the differential feeding period compared to H-H animals which supports the findings of both Thorp *et al*. (1999) and Cummins (2009) who reported that animals offered a silage-based diet had lower glucose and βHB concentrations compared to animals fed a ration consisting mainly of concentrates due to the lower DMI of the animals. Campion *et al*. (2009b) noted that circulating levels of βHB were similar in crossbred AA and BB steers throughout life with only a genotype effect evident at 10 mo of age. Similarly, no effect of genotype was detected in the current study.

An increase in circulating NEFA concentrations in steers and heifers following feed restriction was noted by Blum *et al*. (1985) and Yambayamba *et al*. (1996b). NEFA concentrations subsequently decreasing to levels equivalent with animals offered the control ration within 10 d of increasing their plane of nutrition (Blum *et al*. 1985; Yambayamba *et al*., 1996b). In the current study, all animals were in a positive energy balance and not catabolising body tissue. Therefore, NEFA concentrations were not altered by genotype or feeding treatment. In support of these findings, Ellenberger *et al*.

(1989) reported that moderate feed restriction had no effect on NEFA levels in Angus × Hereford steers.

5.3.2.5 Carcass traits and non-carcass components

As expected, BB had greater CW compared to AA which was a contribution from their greater SW and greater dressing percentage. Keane and Moloney (2010) reported a similar result with crossbred BB steers having greater carcass weights and dressing percentages compared to AA animals. Although compensatory gain occured in the L-H animals it was not sufficient to offset their previous lower gains during the differential feeding period, with an average difference between H-H and L-H of 40 kg and 23 kg in SW and CW, respectively. The absence of a difference in dressing percentage between feeding treatments was surprising as dressing percentage generally increases with increasing weight (Patterson *et al*., 1994). Yaqoub and Babiker (2008) reported no difference in dressing percentage after compensatory growth in chickens. This lack of a difference, in the current study, between feeding treatments suggests that mild feed restriction followed by compensatory growth does not have residual effects on dressing percentage following 200 d of feed realimentation.

The superior carcass conformation of BB compared with AA agrees with many reports in the literature (Keane and Drennan, 2008; Campion *et al*., 2009a; Keane and Moloney, 2010). Due to the association of carcass conformation with CW (Keane *et al*., 2006) the absence of a difference between the two feeding treatments was surprising given that carcasses were heavier for H-H compared to L-H. Schiavon *et al*. (2010) reported that following compensatory growth in bulls no difference in conformation score was evident between treatments. In addition, Keane (2010) reported no difference in carcass conformation scores in steers, with a live weight of 620 kg, following a compensatory growth feeding regime. Thus, it may be concluded that a period of mild

feed restriction followed by a sufficient period of compensatory growth does not affect carcass conformation in cattle of either early or late maturing genotypes.

As BB is a late maturing breed type which preferentially partitions nutrients to muscle rather than fat accretion compared to early maturing counterparts (Campion *et al*., 2009a,b; Sadkowski *et al*., 2009; Keane, 2010), and especially as BB animals have particularly low carcass fat, the difference between the genotypes in fat class was unsurprising. Interestingly, Hornick *et al*. (1998a) reported greater fat deposition in animals experiencing compensatory growth compared to control bulls. However, in a study carried out by Keane (2010), crossbred BB steers offered grass silage for 84 d followed by concentrates *ad libitum* had similar carcass fat class values to steers offered concentrates *ab libitum* throughout. Just a tendency for a difference in fat class was observed in the current study with L-H having lower fat class suggesting that mild feed restriction for a short period of time followed by an adequate realimentation period does not adversely affect fat class.

Once scaled for CW, the difference in weight of perinephric plus retroperitoneal fat between the dietary treatments disappeared. Yambayamba *et al*. (1996a) reported that abdominal fat was lower in animals subjected to a feed restriction period compared to animals offered *ad libitum* access to feed. However, Moloney *et al*. (2008) reported that growth pattern before slaughter did not affect either the weight of perinephric plus retroperitoneal fat or weight of perinephric plus retroperitoneal fat proportional to CW in Friesian steers, which supports the current findings. Additionally, there was no effect of genotype on perinephric plus retroperitoneal fat between crossbred AA and BB steers which is in line with similar findings including Keane and Moloney (2010) and Keane *et al*. (2011). However, Keane and Drennan, (2008) reported a greater proportion of perinephric plus retroperitoneal fat in crossbred AA compared to BB animals. Generally, fat is partitioned in the order perirenal, intermuscular and subcutaneous

adipose depots with intramuscular being the last adipose depot to develop (Hammond, 1955; Sainz and Hasting, 2000). However, certain breeds of bovine (Wagyu and Angus) have the potential to partition energy to muscle adipocytes at an earlier growth stage promoting marbling. Indeed, higher levels of intramuscular fat were observed in meat from AA compared to BB (chapter 4).

As there was a difference in CW between genotypes, a variation between the genotypes in the weight of the 5-rib joint would also be expected. However, only a numerical difference in ribs joint weight proportional to the difference in CW was observed. This is in agreement with Keane and Moloney (2010) who also found a numerical but non-significant difference in the weight of the ribs joint between crossbred AA and BB steers. The difference in the weight of the 5-rib joint between H-H and L-H steers, with greater values for the former, is broadly proportional to the difference in CW between the feeding treatments.

Generally, the literature shows that crossbred BB animals have greater *M. longissimus thoracis et lumborum* area compared to AA animals (Keane and Drennan, 2008; Campion *et al*., 2009b; Keane and Moloney, 2010; Keane *et al*., 2011). Additionally, crossbred BB animals heterozygous for the double muscling myostatin mutation have increased muscle mass compared to their conventional counterparts (Casas *et al*., 2004). The current results support these findings with greater muscle proportions observed in BB compared to AA carcasses. Steen and Kilpatrick (2000) reported no difference in *M. longissimus* area at slaughter between steers offered either a restricted or *ad libitum* access to feed before finishing. This is consistent with the findings of the present study, with no difference in *M. longissimus thoracis et lumborum* area between H-H and L-H animals. This, in turn, supports the ultrasonically scanned muscle depth observations.

M. longissimus thoracis et lumborum and total muscle values in the 5-rib joint were greater in BB steers compared to AA with no difference in bone plus ligamentum nuchae/supraspinale proportions between genotypes, which is consistent with Keane *et al*. (2011) who found similar results for these genotypes. In the current study, other muscle was not affected by genotype; however, Keane *et al*. (2011) reported greater values in BB compared to AA steers. Typically, an increase in total fat proportion of the ribs joint for AA animals compared to BB is observed (Keane and Moloney, 2010; Keane *et al*., 2011) as AA are an early maturing breed which lay down fat at an earlier age and lighter weight. The current study supports this with AA having a greater proportion of fat compared to BB. Total fat proportion was greater in H-H compared to L-H carcasses. This is contrary to Hornick *et al*. (1998a) who reported greater percentages of connective and adipose tissue at slaughter in bulls exhibiting compensatory growth after a period of feed restriction compared to bulls offered a high energy diet throughout the study; however in that study, animals were younger and exposed to a longer restriction period and shorter realimentation period. Yambayamba and Price (1991) reported that heifers experiencing compensatory growth after a period of feed restriction had similar fat proportions compared to animals offered *ad libitum* feed throughout the study. Again, these animals were younger which may account for similar fat proportions in that study, not observed in the present study. In the current study, differences relating to fat proportions in the 5-rib joint between feeding treatments maybe due to ration rather than feeding treatment as the H-H animals had access to the greater energy diet for a longer period of time with is consistent with Wilkinson and Prescott (1970).

Keane and Moloney (2010) reported that carcass length and carcass depth (scaled for carcass weight) were greater in crossbred AA steers compared to crossbred BB with no difference found between genotypes for leg width and leg thickness. In

support of these findings, AA animals in the current study had greater scaled carcass length and depth with no difference in leg width and leg thickness. Additionally, Campion *et al*. (2009a) and Keane *et al*. (2011) found that crossbred AA steers had greater relative leg length compared to BB steers supporting the current results. Greater carcass measurements for AA indicate a greater body size relative to live weight which parallels their poorer conformation. The smaller linear carcass values for BB indicate greater carcass compactness in this genotype (Keane *et al*., 2011). Patterson and Steen (1995) reported that plane of nutrition before finishing did not affect carcass measurements in Friesian steers; however, feeding treatment had an effect on all carcass measurements in the current study with H-H having reduced proportions compared to L-H animals. Greater carcass compactness in the H-H animals was observed although this was not reflected in conformation with, as discussed earlier, no difference observed in carcass conformation between H-H and L-H animals.

BB had lighter intestinal weight when scaled for SW which contributed to their greater dressing percentage. This supports the findings of McPherron and Lee (1997) who reported that a myostatin null mutation in cattle (BB breed) is associated with a reduction in size of internal organs. Interestingly, L-H animals showed greater rumen (empty and full) proportions compared to H-H animals. The greater full rumen weight would suggest greater feed intakes in L-H animals however, this was not observed with no difference in DMI between feeding treatments during the realimentation period. Empty rumen proportion wease greater in L-H compared to H-H steers which is in agreement with Yambayamba *et al*. (1996a) who reported that beef heifers undergoing compensatory growth during a realimentation period also had greater empty stomach proportions. They suggested that this indicated a greater metabolic activity in that organ resulting in a greater capacity for recovery. However, a larger rumen would consume more energy and reduce feed efficiency. Liver and gastrointestinal tract weights appear

to increase and decrease proportionally to feed intake and consequently the energy required to sustain these organs increases proportionally also (Johnson *et al*., 1990).

The greater hide weight for AA compared to BB animals when expressed per unit of live weight supports the findings of Campion *et al*. (2009a) for animals of the same genotype. The difference may be attributed to breed effects in skin thickness (Tulloh, 1961). Ansay and Hanset (1979) reported that hide plus internal organ weights from double muscled cattle were only 81% of the corresponding value for their conventional counterparts. Although the present BB animals were not homozygous for the double muscling myostatin mutation the presence of 1 myostatin allele with the myostatin mutation for muscle hypertrophy contributes a proportion of the 2 allele effect (Short *et al*., 2002; Casas *et al*., 2004). Overall, a lack of interactions indicates compensatory growth influenced carcass traits and non-carcass components similarly across both genotypes.

A simple economic appraisal was carried using current Irish input and output costs and values for initial live weight value (CSO, 2010a), silage cost (Finneran *et al*., 2011), concentrates cost (CSO, 2010b), and carcass value (Bord Bia, 2010). The L-H feeding model set up to exploit compensatory growth yielded on average of €35 premium per head over the continual feeding regime. This figure is based on average gross margin over feed costs and does not consider overhead costs such as labour, veterinary aid and electricity; these were assumed to be constant across the two feeding treatments.

5.3.3 Chapter conclusion

It is concluded that the degree of feed restriction applied in this study had an effect on live weight, live weight gain, blood metabolite and hormone concentrations, ultrasonically scanned muscle and fat depths and linear body measurements. After

realimentation, many of these traits including blood metabolite and hormone concentrations, ultrasonically scanned muscle depths and linear body measurements returned to levels detected in the unrestricted steers. Although compensatory gain was exhibited by the restricted animals it did not fully offset the loss in potential gains during the differential feeding period with a difference of 23 kg difference in CW. However, measurements of muscle such as ultrasonically scanned muscle depth and muscle proportions in the rib joint returned to their inherent size, as observed in animals on a continual plane of nutrition. In fact, total muscle from the 5-rib joint had a tendency to be greater in L-H steers suggesting that the differences in CW were partly due to fat. Indeed, fat class was greater in unrestricted compared to previously resticted stters. In the current study, this absence of total compensation may be due to the level of dietary restriction not being severe enough as discussed earlier. In brief therefore the mild feed restriction applied in this study followed by compensatory growth during a 200 d realimentation period had no lasting effects (positive or negative) at slaughter on plasma metabolite or hormone concentrations, linear body measurements, ultrasonically scanned fat depth, carcass conformation or dressing proportion in crossbred AA and BB steers. Although the restricted animals had a compensatory index (Hornick *et al*., 1998a) of just 52 %, *M. longissimus thoracis et lumborum* a muscle of high economic value recovered completely as indicated by the ultrasonic scanning. The processes regulating compensatory growth in this tissue may also offer revealing insights into the compensatory growth process. Therefore, *M. longissimus thoracis et lumborum* makes an interesting tissue of choice for investigating the molecular mechanisms regulating compensatory growth in bovine in the future.

Chapter 6

Effect of Genotype and Compensatory Growth on Colour, Tenderness and Sensory Characteristics of Beef

6.1 Introduction

Compensatory growth is the ability of an animal to undergo accelerated growth following a period of restricted feed intake (Hornick *et al*., 2000). In grass based systems, compensatory growth allows the realignment of feed demand from a time when feed is expensive to a time when feed is plentiful and cheap (Keane and Drennan, 1994). Exploitation of the compensatory growth phenomena can facilitate a reduction in feed costs, which account for the majority of variable costs in beef production (Finneran *et al*., 2010) as well as leading to increased feed efficiency and profitability (Keane and Drennan, 1994; Yambayamba *et al*., 1996; Ritacco *et al*., 1997). However, in both bovine and porcine models there is extensive, but oftentimes conflicting data, as to the effect of compensatory growth on meat quality, particularly its effect on meat tenderness (Sinclair *et al*., 2001; Kristensen *et al*., 2004; Hansen *et al*., 2006; Therkildsen *et al*., 2008; Stolzenbach *et al*., 2009; Therkildsen *et al*., 2011). The literature has also been equivocal on whether a compensatory growth feeding regime influences intramuscular fat concentrations with many researchers reporting that intramuscular fat increases (Therkildsen *et al*., 2011) while others finding no difference or less intramuscular fat compared to animals on a continual plane of nutrition (Yambayamba *et al*., 1991; Hornick *et al*., 1998; Tomkins *et al*., 2006; Moloney *et al*., 2008). Additionally, information on whether the effects of compensatory growth on aspects of meat quality are consistent across different cattle breeds is lacking. In chapter 5, it was concluded that compensatory growth resulted in no negative residual effects on growth of skeletal muscle. Therefore, this study sought to clarify whether animals that underwent a compensatory growth feeding regime produced meat of a more tender nature with higher intramuscular fat or whether the opposite is the case, as the literature has been equivocal on these issues.

6.2 Effect of compensatory growth on meat quality characteristics

6.2.1 Live weight and live weight gain

Results relating to live weight and live weight gain have been described in detail in chapter 5. In brief, H-H steers were heavier than L-H steers at the end of the differential feeding period (d 199; $P < 0.001$) and this difference remained at slaughter (d 299; $P < 0.05$) (Table 6.1). Compensatory growth was evident early into the realimentation period in the L-H animals; however, animals failed to compensate completely with a compensatory growth recovery index (Hornick *et al.*, 2000) of 52 %. Sometime between d 195 and d 253 of the study, during the realimentation period, compensatory growth ceased and for a substantial period prior to slaughter, there was no difference in live weight gain between treatments (chapter 5). Live weight was not difference between the two genotypes at any stage during the study; however, between d 131 and d 195 of the study, BB had greater live weight gains compared to AA animals.

6.2.2 Temperature and pH of carcasses post slaughter

The pH and temperature data are reported in Figure 6.1. There was no feeding treatment × time interaction ($P > 0.05$) for *M. longissimus thoracis et lumborum* temperature but mean temperature was greater for the H-H compared to the L-H carcasses ($P < 0.01$). There was no effect of genotype ($P > 0.05$) or interaction involving genotype ($P > 0.05$) for temperature measurements post slaughter. There was no effect of feeding treatment ($P > 0.05$), genotype ($P > 0.05$) or their interaction on pH at 1.5 h, 3 h, 4.5 h post slaughter or on pHu (48 h). Data were not collected at 6 and 8 h post slaughter due to instrument malfunction.

Table 6.1 Effect of genotype (G) and feeding treatment (F) on selected production and meat quality attributes

Variable	Genotype[1] (G)			Feeding treatment[2] (F)			*P*-value	
	AA	BB	SED	H-H	L-H	SED	G	F
Live weight, kg								
Start, d 0	307	288	7.010	296	298	6.894	0.79	1.00
End of differential feeding period, d 99	404	390	7.010	438	356	6.894	0.99	<.0001
Realimentation, d 131	452	438	7.010	474	416	6.894	0.99	<.0001
Slaughter, d 299	655	644	7.103	669	630	6.989	1.00	0.04
Live weight gain, kg/d								
Differential feeding period, d 0 to 99	1.06	1.12	0.052	1.55	0.63	0.050	0.28	<.0001
Realimentation period, d 99 to 131	1.50	1.50	0.097	1.26	1.74	0.093	0.98	<.0001
Realimentation period, d 131 to 195	1.65	1.90	0.07	1.63	1.91	0.06	0.0007	0.0001
Realimentation period, d 195 to 253	1.34	1.33	0.09	1.34	1.33	0.09	0.89	0.87
Realimentation period, d 253 to 299	0.91	0.64	0.18	0.84	0.71	0.17	0.14	0.47
Entire period, d 0 to 299	1.25	1.26	0.038	1.33	1.18	0.036	0.81	0.0004
Carcass weight	354	369	5.860	373	350	5.538	0.02	0.0003
Carcass conformation	7.25	9.08	0.411	8.33	8.02	0.402	<.0001	0.44
Fat class	10.39	7.96	0.478	9.59	8.75	0.466	<.0001	0.08
Muscle[3] Composition								
Protein, %	21.69	22.41	0.327	21.87	22.24	0.318	0.04	0.25
Moisture, %	70.35	73.37	0.684	71.67	72.04	0.665	0.0001	0.58
Fat, %	7.45	3.64	0.787	5.90	5.18	0.767	<.0001	0.35
Ash, %	1.09	1.11	0.030	1.08	1.12	0.030	0.77	0.27
Drip loss, %	1.41	2.07	0.209	1.67	1.81	0.200	0.004	0.48
Muscle[3] colour								
L (lightness)	35.18	37.37	0.491	36.30	36.25	0.479	0.0001	0.90
a	15.09	14.19	0.329	15.04	14.24	0.321	0.01	0.02
b	8.51	9.01	0.197	8.91	8.61	0.192	0.02	0.13
Hue[4]	29.62	32.60	0.471	30.84	31.38	0.448	<.0001	0.24
Chroma[5]	17.50	16.94	0.358	17.63	16.82	0.348	0.13	0.03
Fat colour								
L (lightness)	68.07	67.65	0.815	67.84	67.87	0.794	0.61	0.97
a	7.73	7.35	0.658	7.75	7.34	0.640	0.57	0.52
b	14.82	15.37	0.346	15.14	15.06	0.336	0.12	0.81
Hue	62.77	64.75	1.626	63.14	64.39	1.585	0.23	0.44
Chroma	16.66	17.02	0.566	16.96	16.72	0.546	0.53	0.66
WBsf[6], N	25.29	32.63	2.801	25.09	32.83	2.731	0.014	0.009
WBsf[7], N	*27.94*	*30.59*	*3.629*	*25.84*	*32.69*	*2.651*	*0.47*	*0.02*
Cooking loss[8], %	28.71	28.37	0.572	27.89	29.19	0.559	0.56	0.03

[1]AA = Aberdeen Angus × Holstein Friesian; BB = Belgian Blue × Holstein-Friesian. [2]H-H = *ab libitum* access to feed throughout the study; L-H = Restricted feeding for 99 days (d) followed by *ad libitum* access to feed until slaughter (d 299). [3] *M. longissimus thoracis et lumborum* [4]Hue = $[\tan^{-1}(b/a)] \times [180/\Pi]$. [5]Chroma = $[(a^2 + b^2)]^{0.5}$. [6]Warner-Bratzler shear force. [7]Warner-Bratzler shear force adjusted for intramuscular fat. [8]Genotype × feeding treatment interaction ($P < 0.05$).Values equal 28.70, 28.72, 27.07, 29.67 for AA/H-H, AA/L-H, BB/H-H, BB/L-H, respectively.

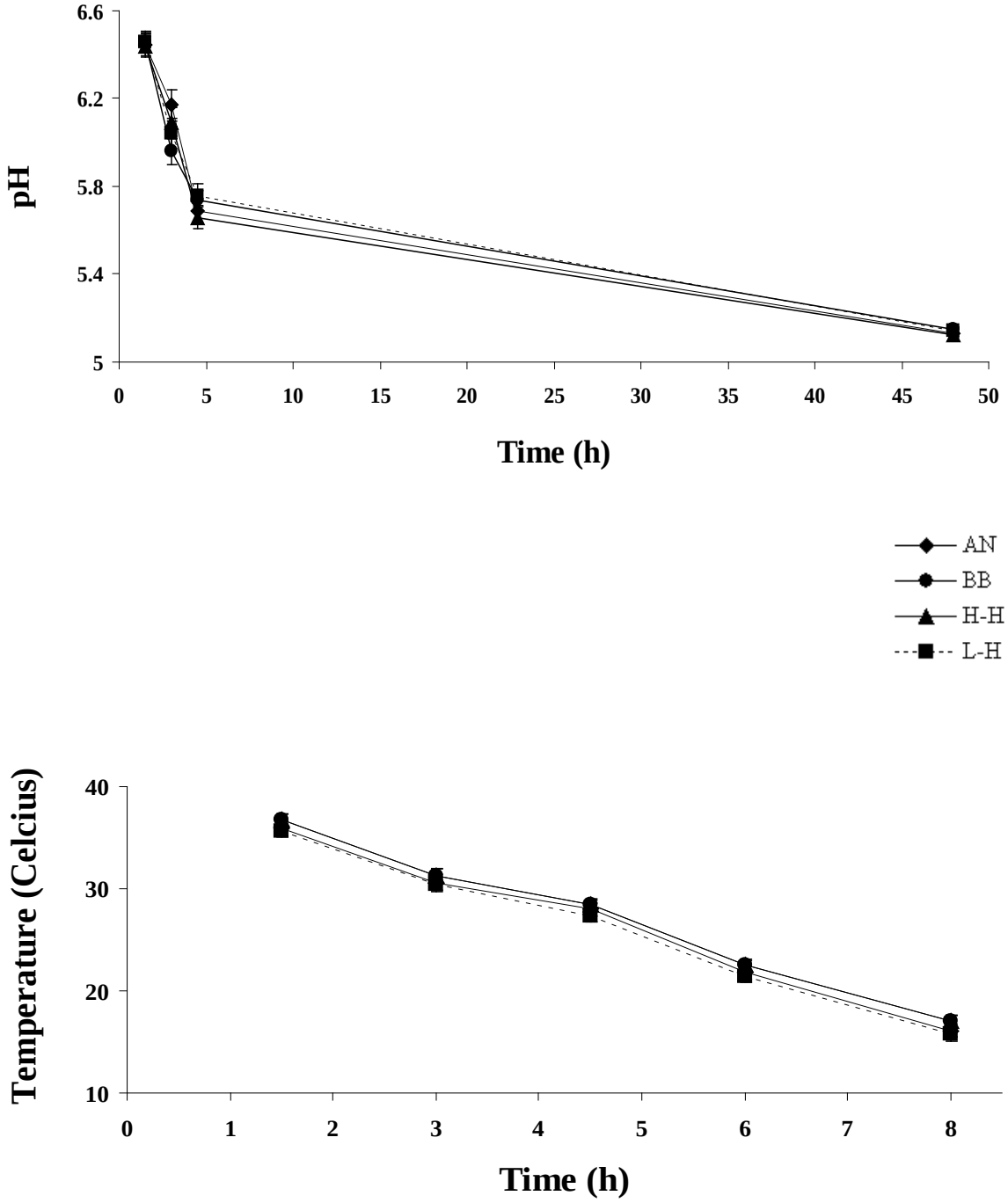


Figure 6.1 Effect of genotype (G) and feeding treatment (F) on carcass pH and temperature decline post slaughter

Carcass pH measurements at 6 h and 8 h were unattainable due to instrument malfunction. AA = Aberdeen Angus × Holstein Friesian; BB = Belgian Blue × Holstein Friesian. H-H = *ab libitum* access to feed throughout the study; L-H = Restricted feeding for 99 days (d) followed by *ad libitum* access to feed until slaughter (d 299).

6.2.3 Chemical composition of M. longissimus thoracis et lumborum

There was no effect of feeding treatment or interaction involving feeding treatment on the chemical composition of the *M. longissimus thoracis et lumborum*. Protein and moisture proportions were greater ($P < 0.05$) and fat proportions lower ($P < 0.001$) for BB compared to AA (Table 6.2).

6.2.4 Muscle drip loss and cooking loss

Drip loss together with muscle and fat colour values are presented in Table 6.2. No feeding treatment × genotype interaction ($P > 0.05$) was detected for drip loss. In addition, there was no effect of feeding treatment but drip loss was greater ($P < 0.01$) for BB compared to AA. Cooking loss percentage was greater ($P < 0.05$) in meat from L-H animals compared to H-H animals. However, there was no effect of genotype on cooking loss percentage.

6.2.5 Muscle and fat colour

There was no feeding treatment × genotype interaction ($P > 0.05$) for any of the muscle colour characteristics. Both redness (a) ($P < 0.05$) and chroma ($P < 0.05$) values were lower in L-H compared to H-H animals. Lightness (L) ($P < 0.001$), yellowness (b) ($P < 0.01$) and hue ($P < 0.001$) values were lower for AA compared to BB. The a ($P < 0.01$) value was lower for BB compared to AA animals. There was no effect of either genotype ($P > 0.05$) or feeding treatment ($P > 0.05$) or their interaction ($P > 0.05$) of any of the fat colour variables measured.

Table 6.2 Effect of genotype (G) and feeding treatment (F) on the sensory characteristics of *M. longissimus thoracis et lumborum*

Trait	Genotype[1] (G)			Feeding treatment[2] (F)			*P*-value	
	AA	BB	SED	H-H	L-H	SED	G	F
Attributes[3]								
Tenderness	4.58	4.21	0.231	4.60	4.19	0.225	0.12	0.08
Juiciness	5.23	4.95	0.109	5.12	5.06	0.107	0.02	0.60
Beef	4.60	4.23	0.079	4.43	4.40	0.078	<.0001	0.72
Abnormal	2.34	2.60	0.088	2.40	2.54	0.085	0.005	0.12
Hedonic[3]								
Flavour liking	5.13	4.67	0.100	4.99	4.81	0.098	0.0001	0.07
Overall liking	4.81	4.35	0.14	4.71	4.45	0.13	0.002	0.09
Cutting[4]								
Ease of cutting	50.02	44.51	3.301	50.85	43.69	3.178	0.11	0.03
Cleanness of cut	60.69	57.27	2.398	59.05	58.05	2.328	0.16	0.43
Initial Bite[4]								
Toughness	46.48	53.89	3.338	47.34	53.03	3.231	0.04	0.09
Juiciness	55.38	49.40	1.448	52.42	52.37	1.412	0.0003	0.97
Sponginess	23.42	23.03	1.326	24.19	22.26	1.256	0.77	0.14
Crunchy	29.23	28.54	1.831	28.11	29.66	1.749	0.71	0.38
Eating[4]								
Toughness	44.32	51.50	3.315	45.20	50.63	3.231	0.04	0.10
Moisture	55.91	49.73	1.665	53.44	52.20	1.624	0.001	0.45
Pulpy	61.65	55.25	1.811	58.63	58.27	1.766	0.0015	0.84
Chewiness	42.13	48.18	3.349	42.73	47.59	3.267	0.08	0.15
Gristle	8.23	7.61	1.391	7.66	8.18	1.356	0.66	0.70
Fibres	46.38	50.19	2.021	48.56	48.01	1.970	0.07	0.78
Greasiness	18.52	14.59	1.335	16.38	16.73	1.301	0.007	0.79
Dissoluble	43.13	40.41	2.86	43.58	39.95	2.736	0.35	0.20
Residue[4]								
Greasy	20.07	15.25	1.619	17.12	18.20	1.579	0.006	0.50
Swallow	54.71	48.09	2.727	54.15	48.65	2.598	0.022	0.04
Particles	43.65	46.33	1.120	45.53	44.45	1.069	0.024	0.32
Mouth feel	59.27	52.77	1.577	55.87	56.17	1.538	0.0003	0.85
Pulpy	61.85	56.00	1.491	59.87	57.99	1.454	0.0006	0.21
Flavour[4]								
Greasy	18.00	11.31	1.349	14.51	14.81	1.315	<.0001	0.82
Bloody	5.62	5.60	0.733	5.80	5.42	0.714	0.97	0.59
Livery	6.09	6.24	0.75	6.45	5.88	0.73	0.70	0.53
Metallic	9.22	10.77	1.033	10.42	9.57	1.007	0.15	0.40
Bitter	4.14	5.66	0.59	4.52	5.28	0.58	0.007	0.17
Sweet	16.95	11.91	1.383	14.71	14.16	1.332	0.001	0.68
Rancid	0.60	0.59	0.21	0.59	0.60	0.20	0.82	0.69
Fishy	2.65	2.51	0.199	2.51	2.66	0.187	0.49	0.43
Acidic	5.65	8.59	0.628	6.88	7.36	0.602	<.0001	0.43
Cardboard	13.02	17.29	1.108	14.32	15.98	1.079	0.0007	0.14
Vegetable	12.25	14.24	0.762	13.31	13.18	0.742	0.01	0.86
Dairy	24.40	16.34	1.500	21.76	18.97	1.461	<.0001	0.07
Overall	51.93	42.41	1.732	49.15	45.20	1.688	<.0001	0.03

[1]AA = Aberdeen Angus × Holstein Friesian; BB = Belgian Blue × Holstein Friesian. [2]H-H = *ab libitum* access to feed throughout the study; L-H = Restricted feeding for 99 days (d) followed by *ad libitum* access to feed until slaughter (d 299). [3]Eight point scale. [4]One hundred point scale.

6.2.6 WBsf

No feeding treatment × genotype interaction ($P > 0.05$) was detected for WBsf. However, the *M. longissimus thoracis et lumborum* from L-H animals had higher WBsf values ($P < 0.01$) compared to H-H animals while BB animals had greater ($P < 0.05$) WBsf values compared to AA animals. When adjusted for intramuscular fat, the genotype effect disappeared ($P > 0.05$); however, the effect of feeding treatment was still evident ($P < 0.05$).

6.2.7 Sensory and flavour characteristics of M. longissimus thoracis et lumborum

The results of the sensory analysis are presented in Table 6.3. There was no feeding treatment × genotype interaction ($P > 0.05$) for any of the characteristics investigated. H-H had greater values for ease of cutting ($P < 0.05$), swallow ($P < 0.05$) and overall flavour ($P < 0.05$). Additionally, there was a tendency for tenderness (tendency; $P = 0.08$), flavour liking (tendency; $P = 0.07$), overall liking (tendency; $P = 0.09$) and dairy (tendency; $P = 0.07$) to be lower in L-H while toughness ($P = 0.09$) to be higher in L-H compared to H-H steers. AA had greater values for juiciness ($P < 0.05$), beef ($P < 0.001$), flavour liking ($P < 0.001$), overall liking for sensory characteristics ($P < 0.01$), juiciness on biting ($P < 0.001$), moisture ($P < 0.001$), pulpy ($P < 0.01$), greasiness on eating ($P < 0.01$), greasy residue ($P < 0.01$), swallow ($P < 0.05$), mouth feel ($P < 0.001$), pulpy residue ($P < 0.001$), greasy flavour ($P < 0.0001$), sweet ($P < 0.001$), dairy ($P < 0.001$) and overall flavour ($P < 0.001$). BB had greater values for abnormal ($P < 0.01$), toughness on biting ($P < 0.05$), toughness on eating ($P < 0.05$), chewiness (tendency; $P = 0.08$), fibres (tendency; $P = 0.07$), particles ($P < 0.05$), bitter ($P < 0.01$), acidic ($P < 0.001$), cardboard ($P < 0.001$) and vegetable ($P < 0.01$). When adjusted for intramuscular fat concentration the effect of genotype for many of sensory characteristics disappeared. However, regardless of intramuscular fat, overall flavour

Table 6.3 Effect of genotype (G) and feeding treatment (F) on the sensory characteristics of *M. longissimus thoracis et lumborum* with intramuscular fat as a covariant

Trait	Genotype[1] (G)			Feeding treatment[2] (F)			*P*-value	
	AA	BB	SED	H-H	L-H	SED	G	F
Attributes[3]								
Tenderness	4.46	4.31	0.313	4.57	4.19	0.228	0.63	0.12
Juiciness	5.08	5.06	0.133	5.08	5.07	0.096	0.85	0.93
Beef	4.53	4.28	0.104	4.41	4.40	0.076	0.03	0.95
Abnormal	2.35	2.59	0.121	2.41	2.54	0.088	0.06	0.14
Hedonic[3]								
Flavour liking	5.04	4.75	0.130	4.97	4.82	0.095	0.03	0.12
Overall liking	4.69	4.45	0.17	4.68	4.46	0.12	0.002	0.09
Cutting[4]								
Ease of cutting	49.79	45.84	4.474	51.19	44.45	3.275	0.39	0.05
Cleanness of cut	60.66	57.81	3.293	60.08	58.38	2.406	0.39	0.48
Initial Bite[4]								
Toughness	47.95	51.33	4.419	47.25	52.02	3.228	0.45	0.15
Juiciness	53.31	50.99	1.707	51.83	52.47	1.247	0.18	0.61
Sponginess	23.22	23.54	1.762	24.26	22.51	1.296	0.86	0.19
Crunchy	29.30	28.46	2.471	28.12	29.64	1.814	0.73	0.41
Eating[4]								
Toughness	46.61	49.63	4.417	45.81	50.43	3.227	0.49	0.16
Moisture	53.70	51.44	2.010	52.81	52.32	1.469	0.27	0.74
Pulpy	60.15	56.41	2.382	58.21	58.35	1.740	0.12	0.93
Chewiness	43.46	47.16	4.576	43.10	47.52	3.343	0.42	0.19
Gristle	8.37	7.50	1.919	7.69	8.17	1.402	0.66	0.74
Fibres	47.14	49.60	2.763	48.77	47.96	2.018	0.38	0.69
Greasiness	16.97	16.60	1.517	16.23	17.34	1.112	0.80	0.32
Dissoluble	42.33	43.00	3.739	44.05	41.27	2.738	0.85	0.31
Residue[4]								
Greasy	19.40	18.75	1.795	17.98	20.17	1.319	0.72	0.11
Swallow	53.98	50.11	3.584	54.46	49.64	2.631	0.29	0.08
Particles	43.67	46.36	1.509	45.55	44.48	1.109	0.09	0.34
Mouth feel	58.35	54.93	1.959	56.12	57.16	1.437	0.09	0.47
Pulpy	60.06	57.39	1.851	59.37	58.08	1.352	0.16	0.35
Flavour[4]								
Greasy	15.39	13.55	1.294	13.79	15.14	0.935	0.17	0.16
Bloody	5.69	5.54	1.028	5.82	5.41	0.743	0.88	0.58
Livery	6.44	5.94	1.03	6.55	5.83	0.75	0.63	0.40
Metallic	10.19	9.93	1.356	10.68	9.44	0.980	0.85	0.22
Bitter	4.59	5.28	0.80	4.64	5.21	0.58	0.40	0.31
Sweet	16.47	14.11	1.717	15.19	15.38	1.236	0.18	0.87
Rancid	0.60	0.59	0.29	0.59	0.60	0.21	0.99	0.74
Fishy	2.69	2.44	0.270	2.50	2.63	0.195	0.38	0.53
Acidic	5.87	7.69	0.791	6.69	6.87	0.569	0.03	0.76
Cardboard	14.67	15.87	1.287	14.77	15.77	0.930	0.35	0.29
Vegetable	12.54	13.99	1.058	13.39	13.14	0.765	0.18	0.75
Dairy	23.61	18.78	1.848	22.16	20.23	1.332	0.02	0.16
Overall	50.36	43.75	2.286	48.72	45.40	1.652	0.008	0.06

[1]AA = Aberdeen Angus × Holstein Friesian; BB = Belgian Blue × Holstein Friesian. [2]H-H = *ab libitum* access to feed throughout the study; L-H = Restricted feeding for 99 days (d) followed by *ad libitum* access to feed until slaughter (d 299). [3]Eight point scale. [4]Adjusted data - values in parenthesis represent back transformed means. [5]One hundred point scale.

(P < 0.01) was still greater in AA compared to BB steers with beef ($P = 0.03$), flavour liking ($P < 0.05$), overall liking ($P < 0.01$), mouth feel (tendency; $P = 0.09$) and dairy ($P < 0.05$) greater with values for abnormal (tendency; $P = 0.06$), particles (tendency; $P = 0.09$) and acidic ($P < 0.05$) lower in AA compared to BB.

6.3 Correlation analysis

6.3.1 Association between production and meat quality variables

Correlation analysis between meat quality characteristics and production variables are presented in Table 6.4. In summary, CW was not associated with any meat quality traits with the exception of cook loss whereby a negatively correlation was observed (r = -0.33; $P < 0.05$). Similarly, live weight gain prior to slaughter (d 195 to d 299) was not correlated with the meat quality characteristics measured. WBsf was negatively correlated with both intramuscular fat (r = -0.41; $P < 0.01$) and sensory tenderness (r = -0.45; $P < 0.01$) while intramuscular fat was negatively correlated with drip loss percentage (r = -0.58; $P < 0.001$) and positively correlated with sensory tenderness (r = 0.32; $P < 0.05$). Additionally, drip loss was negatively correlated with both sensory tenderness (r = -0.45; $P < 0.01$) and cook loss percentage (r = -0.43; $P < 0.01$). No statistically significant correlations were observed between pHu and production and meat quality variables.

Table 6.4 Association[1] between production and meat quality variables

Variable	CW[2]	LWG[3]	WBsf[4]	pHu[5]	IMF[6]	Drip loss	Tenderness[7]
LWG[3]	0.10						
WBsf[4]	-0.28	0.19					
pHu[5]	-0.24	0.13	0.13				
IMF[6]	-0.05	0.20	-0.41**	-0.02			
Drip loss	0.27	0.07	0.24	-0.13	-0.58***		
Tenderness[7]	-0.07	-0.12	-0.45**	-0.15	0.32*	-0.45**	
Cook loss	-0.33*	-0.24	0.18	0.006	0.007	-0.43**	0.13

[1]Values presented are Spearman correlation coefficients *r* from unadjusted data. [2]Cold carcass weight. [3]Live weight gain prior to slaughter (d 195 to d 299). [4]Warner-Bratzler shear force. [5]Ultimate pH at 48 h. [6]Intramuscular fat percentage. [7]Sensory tenderness. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

6.4 Chapter summary, discussion and conclusion

6.4.1 Chapter summary

The objective of this study was to examine the effect of feed restriction followed by compensatory growth on meat quality and sensory characteristics in meat from Aberdeen Angus × Holstein-Friesian (AA) and Belgian Blue × Holstein-Friesian (BB) steers. Compensatory growth during a 200 day realimentation period following 99 d of feed restriction 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. Within BB, cooking loss percentage was greater in animals that experienced compensatory growth; however, this was not observed in AA animals. Meat from AA had greater sensory flavour characteristics compared to BB steers. Correlation analysis was carried out to assess potential relationships between traits. In brief, live weight gain prior to slaughter (d 195 to d 299) was not correlated with the meat quality characteristics measured. Additionally, WBsf was negatively correlated with both intramuscular fat ($r = -0.41$) and sensory tenderness ($r = -0.45$) while intramuscular fat was negatively correlated with drip loss percentage ($r = -0.58$) and positively correlated with sensory tenderness ($r = 0.32$). Overall, genotype has greater effects of meat quality that feeding treatment, supporting other research findings in this area. These data suggest that the compensatory growth-based feeding regime applied here had little permanent effect on meat quality characteristics. However, the fact that animals who exhibited compensatory growth had reduced tenderness and flavour cannot be ignored and this area warrants further investigation.

6.4.2 Chapter discussion

The objective of this study was to examine the effect of restricted feeding followed by compensatory growth during a realimentation period, on meat quality and sensory and flavour characteristics in AA and BB genotypes. Due to the overall lack of statistical and in particular biological significant interactions between genotype and feeding treatment, the two factors are discussed separately.

6.4.2.1 Effect of feeding treatment on meat quality attributes

6.4.2.1.1 Temperature and pH of carcasses post slaughter

Meat quality traits are affected by the rate of pH and temperature fall, however many of these relationships are still not fully understood (Maltin *et al*., 2003; Van Laack *et al*., 2011). If the carcass cools too quickly, and glycolysis occurs too slowly, resulting in a high pH and a reduced sarcomere length, meat toughening occurs (for a review see Maltin *et al*., 2003; Warner *et al*., 2010). Alternatively, if the temperature decline is slow, and glycolysis fast, toughening of the meat can also occur. The rate of decline in pH and temperature was similar for both genotypes and feeding treatments, indicating that anti-mortem glycogen stores were equivalent in all groups (Moloney *et al*., 2008). Additionally, Moloney *et al*. (2008) reported no difference in the pattern of temperature or pH decline or pHu between steers that experienced compensatory growth following 8 weeks feed restriction and those on a continual plane of supporting the results of the current study.

6.4.2.1.2 Chemical composition of M. longissimus thoracis et lumborum

Although a difference was found in the weight of fat in the 5 rib joint with H-H having greater proportions (chapter 5), no difference was found in intramuscular fat

concentration between feeding treatments. This supports the findings of both porcine (Kristensen *et al*., 2004; Heyer and Lebret, 2007) and bovine based (Moloney *et al*., 2008) studies. Additionally, Moloney *et al*. (2008) reported no difference in moisture and protein proportions in animals offered a continuous plane of nutrition or animals subjected to L-H feeding regime supporting the current findings.

6.4.2.1.3 Muscle drip and cook loss

Appearance is often the only criterion available to consumers to appraise meat prior to purchase (Andersen, 2000; Otto *et al*., 2006). According to Andersen (2000), drip loss, marbling and colour are the key factors when evaluating meat prior to purchase. Hornick *et al*. (1998) reported that meat from animals which experienced compensatory growth prior to slaughter resulted in greater drip loss when the restriction period was extended from 115 days to 239 days. However, the authors report that this finding may be related to the fat content of the muscle rather than accelerated growth prior to slaughter as a low fat content in meat is associated with higher water content (Hornick *et al*., 1998). Keane and Allen (2009) reported that feeding level prior to slaughter had no effect on muscle drip loss percentage in steers. The results of the current study support this finding with no effect of feeding treatment on drip loss percentage in H-H and L-H animals. Therefore, it can be concluded that a compensatory growth feeding regime applied in this study has no direct effect on percentage meat drip loss at slaughter.

As a result of cooking, meat loses a large quantity of its mass as meat juices, which are typically 90 % water (Oillic *et al*., 2011). Within BB animals, meat from L-H animals (BB/L-H) had a greater cooking loss percentage compared to meat from the BB/H-H animals; however, this was not observed in meat from the AA animals. Hornick *et al*. (1998) reported that BB bulls that exhibited compensatory growth had

greater cooking loss supporting the finding in the current study. In contrast, Moloney *et al*. (2008) reported no difference in cooking loss in Friesian steers suggesting that perhaps differences in cooking loss resulting from compensatory growth are genotype specific resulting from the BB animals being heterozygous for the myostatin mutation although this theory warrants further elucidation.

6.4.2.1.4 Muscle and fat colour

Muscle and fat colour have a large influence on consumer selection of meat at the retail outlet (Brugiapaglia and Destefanis, 2009). Moloney *et al*. (2008) reported no difference in *M. longissimus thoracis et lumborum* colour variables between steers offered different levels of feeding before slaughter which supports the results from the current study for lighness, *b* and hue variables. However, *a* and chroma values were lower for L-H compared to H-H steers. Hornick *et al*. (1998) reported that compensatory growth in BB bulls resulted in greater values for *a*; however, these difference in *a* were dependent on the length of the restriction and realimentation periods. Similarly, the difference in chroma values between H-H and L-H animals reflects the difference in *a* values from which it was calculated. Researchers in Australia (Lehnert *et al*., 2006) reported that nutritional restriction in beef steers resulted in lower concentrations of type 2 (fast glycolytic) myofibers and consequently higher levels of type 1 (slow oxidative) in *M. longissimus thoracis et lumborum*. However, during realimentation fibre distribution returned to normal. The authors suggest that under-nutrition and weight loss in cattle result in a mechanism that preserves slow-twitch fibres (Lehnert *et al*., 2006). Greater concentrations of slow-oxidative fibres results in lower *a* measurements suggesting that perhaps the compensatory growth-based regime implemented here had permanent effects on fibre type however further investigation is required.

Research has shown that β-carotenes in the diet of cattle accumulate in subcutaneous adipose which results in the tissue acquiring a yellow colour (for review see Dunne *et al*., 2009). Despite the major differences in diet during the differential feeding period, no residual effect on fat colour was observed between feeding treatments suggesting that the feeding regime implemented had no lasting effects on subcutaneous fat colour. It is likely that the length of the realimentation period (200 d), when all animals were offered ad libitum access to a high concentrate based diet 'diluted' any residual effects on fat colour introduced during the differential feeding period.

6.4.2.1.5 Tenderness and sensory and flavour analysis

The most important and critical assessment of beef occurs in the mouth of the consumer. Tenderness and flavour are important factors determining eating quality as indicated by consumer research (Becker *et al*., 1998: Moloney *et al*., 2001). Sinclair *et al*. (2001) and Moloney *et al*. (2008) reported that pre-slaughter growth rate had no effect on meat tenderness with the major effects appearing to relate to genotype. Therkildsen *et al*. (2008; 2011) reported that a compensatory growth feeding regime may improve tenderness in meat from Friesian cows, however this was muscle-type specific in bulls. The higher WBsf in *M. longissimus thoracis et lumborum* from the L-H steers was not consistent with findings by Moloney *et al*. (2008) which stated that at slaughter WBsf values were similar between animals that exhibited compensatory growth and those on a continuous plane of nutrition throughout the study. Additionally, Chaosap *et al*. (2011) reported that feed restriction followed by *ad libitum* access to feed during the finishing period had no effect on WBsf measurements in meat aged for 8 days from crossbred female pigs. Additionally, when data were adjusted for intramuscular fat the difference between treatments was still present. The WBsf data are

consistent with findings from the sensory analyses in that tenderness (tendency towards statistical significance) and ease of cutting were both lower, while toughness on biting was higher, in meat from L-H compared to H-H steers. When using a mechanical methodology to assess tenderness a significant difference was observed between treatments while at a sensory level a tendency for a difference was also observed. Additionally, it must be noted that although differences were observed between feeding treatments even the higher average value of 33 N recorded for the L-H would be considered tender (AFRC, 2008). In the current study, both H-H and L-H had similar growth rates prior to slaughter, were exposed to similar pre-slaughter conditions, pH and temperature fell at similar rates and the chemical composition, including fat percentage, within the muscle was comparable across treatment. These pre- and post-slaughter variables are considered as potentially important in determining meat tenderness (Maltin *et al*. 2003). Perhaps live weight gain during the differential feeding period or early realimentation period had a residual effect on tenderness, as a result of fibre type composition (meat colour was also affected as discussed earlier), resulting in higher shear force measurement in the L-H compared to H-H animals noticeable at slaughter; however, this warrants further investigation.

Flavour characteristics were also affected by feeding treatment with dairy (tendency) and overall flavour lower in meat from L-H compared to H-H animals. This suggests that a compensatory growth-based feeding model affects not only mechanical and sensory tenderness but other attributes relating to flavour. However, ease of cutting and overall flavour (tendency) were greater in meat from H-H compared to L-H steers suggesting a compensatory growth-based feeding regime has an effect of meat quality which is not explainable by differences in fat content. However, it must be noted that a difference of between 2 and 6 on a 100 point scale was identified and while statistically

significant, the untrained consumers' palate may not be sensitive enough to detect these subtle differences.

6.4.2.2 Effect of genotype on meat quality attributes

6.4.2.2.1 pH and temperature of carcass at slaughter

Kuber *et al*. (2004) reported a difference in rate of decline of pH across genotypes (Wagyu, Limousin and their F_1 hybrid); however, the Wagyu genotype typically has very high concentrations of intramuscular fat which may alter the pH decline (Bendall, 1978). Despite AA having double the muscle fat content of BB and scoring nearly 25 % higher in carcass fat class there was no effect of genotype, which supports the findings of both Sinclair *et al*. (2001) and Cuvelier *et al*. (2006a). These authors detected no difference in pH decline in steers and bulls across very different genotypes also. In addition, the authors reported no difference in pHu at 48 h post slaughter which is consistent with the current study. However, Page *et al*. (2001) examined the pHu of longissimus muscle from 1062 beef carcasses varying in breed and gender and found the mean pHu to be 5.50. The pHu in this study is lower than expected and the reason behind this warrants further investigation. Perhaps, *ad libitum* access to concentrates over a 200 day period resulted in high glycogen stores within the muscle and consequently a low pH post slaughter; however, this observation was not observed with pigs (Chaosap *et al*., 2011). It has been suggested that a low pHu results in meat of poorer eating quality and reduced tenderness (Maltin *et al*., 2003); however, the sensory characteristics analysis and WBsf measurements provided no evidence for this.

Carcasses with greater levels of subcutaneous and intramuscular fat often cool more slowly compared to leaner carcasses (Lochner *et al*., 1980); however, this was not detected in this study with AA and BB carcasses. On the other hand, Cuvelier *et al*.

(2006a) reported a temperature difference in carcasses from AA and BB bulls 1 h post slaughter with AA having greater temperature loss compared to BB. The authors suggest that this may be associated with the lower CW, although the greater carcass fat in AA should have limited this from happening.

6.4.2.2.2 Chemical composition of M. longissimus thoracis et lumborum

Genotype had an effect on the chemical composition of the muscle which supports the findings of Keane *et al*. (2011) for similar genotypes. Generally, intramuscular fat in muscle is perceived as important as intramuscular fat correlates strongly with flavour and juiciness (Hocquette *et al*. 2010). Despite this however, consumers are also becoming more health conscious, choosing leaner cuts of meat (Resurreccion, 2003). Therefore, a demand exists in the market for cuts of meat with varying levels of intramuscular fat content.

6.4.2.2.3 Muscle drip and cook loss

A low pHu is associated with increased drip loss (Maltin *et al*., 2003); however, in the current study the difference in drip loss between genotypes could not be explained by pHu, which was similar across both genotypes. Additionally, no statistically significant correlation was observed between pHu and drip loss. Cuvelier *et al*. (2006b) suggested that BB bulls have greater drip losses due to their greater meat water content. Greater moisture content in meat from BB was observed in the current study and in further agreement Keane *et al*. (2011) also reported that crossbred BB steers had greater moisture content in *M. longissimus thoracis et lumborum* compared to AA steers supporting this theory. The effect of genotype on cook loss was described previously.

6.4.2.2.4 Muscle and fat colour

Kim *et al*. (2004) reported that myoglobin gene expression was greater in red muscle compared to white muscle. Double muscled animals have a greater percentage of white muscle fibres compared to their conventional counterparts (West, 1974). Consequently, BB animals being heterozygous for double muscling are likely to have lower myoglobin levels in their muscle and this explains the greater *L* and lower *a* values for BB compared to AA steers. The divergence in lightness values observed here would be a perceivable difference by the eye (Zhu and Brewer, 1999) and therefore this meat colour difference would be noticeable to the consumer. The difference in *a* values between genotypes support the findings of Keane *et al*. (2011); however, Campion *et al*. (2009) found no difference in *a* value between similar AA and BB genotypes. Additionally, AA had lower *b* values compared to BB steers. This result is at variance with Campion *et al*. (2009), Cuvelier *et al*. (2006a) and Keane *et al*. (2011) who all found no difference in *b* values in muscle from AA and BB bulls or steers and this warrants further investigation. The difference in hue values across genotypes reflects the differences in *a* and *b* values from which it was calculated.

Differences in fat colour across genotypes have been noted in the past with dairy breeds having more yellow subcutaneous fat than British or European beef breeds when offered similar diets (Barton and Pleasants, 1993; Dunne *et al*., 2004; Dunne *et al*., 2009). Boom and Sheath (1997) suggest that subcutaneous fat thickness affects fat colour as carotenes which accumulate in subcutaneous fat are diluted or concentrated as the fat increases or decreases in thickness, respectively. However, in the current study, no difference in *b* values were observed despite AA having greater fat thickness (ultrasonically assessed) and carcass fat class (chapter 5).

6.4.2.2.5 Tenderness and sensory and flavour analysis

While in the current study, the meat was aged for 14 days, Cuvelier *et al*. (2006a,b) reported no effect of genotype on WBsf measurements in meat from AA and BB bulls aged for 2 days and 8 days, respectively. Alternatively in this study, greater WBsf values were observed in meat from BB compared to AA animals. Again, this finding is supported by the sensory characteristics in that toughness on biting, toughness while eating and chewiness (tendency) were higher in meat from BB compared to AA steers. Interestingly, no difference in tenderness measured at a sensory level was detected between genotypes. Correlation analysis indicated just moderate negative associations between the two measures of tenderness which is similar to the findings in many other studies (Caine *et al*., 2003; Peachey *et al*., 2002). When adjusted for intramuscular fat, the effect of genotype disappeared supporting findings that intramuscular fat affects tenderness (Hocquette *et al*., 2010)

There is extensive and often conflicting, research into the contribution of intramuscular fat to the sensory characteristics of meat. Hocquette *et al*. (2010) reported that intramuscular fat directly affects juiciness and flavour but that tenderness was influenced indirectly. In addition, Sinclair *et al*. (2001) reported that intramuscular fat had no effect on tenderness of meat from AA steers with similar values being reported for Charolais steers. The authors did however report that juiciness, flavour and overall acceptability were greater in *M. longissimus thoracis et lumborum* from AA steers compared to Charolais. However, when sensory analysis was carried out on *M. biceps femoris* from the same animals (Sinclair *et al*., 2001) there was no difference in juiciness or beef flavour between the genotypes suggesting that the relationship between intramuscular fat and many of the traits associated with meat quality may be related to muscle type. In the current study, juiciness and moisture were greater in AA which are a result of greater intramuscular fat (indeed when data were adjusted for intramuscular fat

these differences disappear) which is in agreement with Sinclair *et al*. (2001); however, in contrast, overall flavour, when adjusted for intramuscular fat, remained greater in AA compared to BB.

6.4.2.3 Association between production and meat quality variables

Wbsf was just moderately negatively correlated with sensory tenderness. Research in this area has shown a high variation between association studies (Shackelford *et al*., 1994; Caine *et al*., 2003) suggesting that WBsf may not always be a reliable indicator of tenderness as perceived in the mouth of the consumer. Live weight gain in the preceding 104 days prior to slaughter was not correlated with any meat quality trait which is surprising as research in this area would indicate otherwise (Zgur *et al*., 2003). Intramuscular fat was just moderately positively correlated with sensory tenderness supporting the findings of a number of previous studies (Seideman *et al*., 1989; Hocquette *et al*., 2010).

6.4.3 Chapter conclusion

Nutritional restriction followed by compensatory growth during a 200 day realimentation period had no lasting effects, either positive or negative, on carcass pH and temperature decline, chemical composition, drip loss, fat colour or many of the sensory characteristics including juiciness measurements. However, this feeding regime did affect cooking loss (in BB steers only), overall flavour and both mechanical and sensory measurements of tenderness resulting in tougher meat of poorer flavour. Further research is now required to explain the basis behind these findings to minimise any potential negative effects of this widely practiced management regimen on consumer perceptions of the resulting beef.

Although animals employed in this study were crossbred, many of the meat quality differences observed between purebred AA and BB genotypes for chemical composition of the *M. longissimus thoracis et lumborum*, muscle and fat colour varaibles, tenderness and sensory and flavour attributes were still obvious. Overall, meat from AA had greater sensory and flavour characteristics compared to BB steers. This was apparently mainly driven by the intramuscular fat which directly affects flavour and juiciness of the meat. Overall, it can be concluded that genotype has greater effects of meat quality that feeding treatment, supporting other research findings in this area.

Chapter 7

Transcriptional Regulation of *M. longissimus thoracis et lumborum* during Nutritional Restriction and Compensatory Growth in Aberdeen Angus Steers Employing RNA*seq* Technology

7.1 Introduction

Compensatory growth is the ability of an animal to undergo accelerated growth following a period of restricted feeding. The fact that compensatory growth is achievable indicates that growth rate in cattle is usually below the inherent maximum potential (Jobling, 2010) and therefore the mechanisms regulating this accelerated growth rate warrant further elucidation. To date, studies have attempted to elucidate the mechanisms controlling compensatory growth in a number of livestock species including cattle (Lehnert *et al*., 2006; Connor *et al*., 2009) and pigs (Lametsch *et al*., 2006); however, key genes and pathways underlying this mechanism in muscle have yet to be sufficiently characterised in any species. Data from chapter 5 of this thesis has shown that *M. longissimus thoracis et lumborum*, a tissue of high economic value, rapidly recovered following realimentation in cattle which had previously experienced nutritional restriction for 99 d and therefore chosen for transcriptomic analysis in this chapter. There is a dearth of information regarding the molecular mechanisms governing this period of accelerated growth in muscle tissue. Transcriptomic analysis of muscle tissue, harvested during the peak of compensatory growth, provides a detailed view of numerous molecular processes simultaneously, which greatly increases the understanding of accelerated muscle growth.

The experimental design is described in detail in section 2.2.2.2 and 2.2.2.3.1. In brief, crossbred Aberdeen Angus × Holstein Frisian (AA; n = 22) or Belgian Blue × Holstein Frisian (BB; n = 24) steers were assigned to 1 of 2 treatment groups. Over a 99 d differential feeding period, 1 group (11 AA and 12 BB) was offered a high energy control diet (H-H) whereas the second group (11 AA and 12 BB) was offered an energy restricted diet (L-H). At the end of the differential feeding period (99 d), both groups of animals were then offered a TMR having a grass silage:concentrate ratio of 80:20, with the concentrate proportion increasing gradually over a 3 week period to H-H ration.

This period, which lasted 200 d, was termed the realimentation period (d 99 – d 299), and all animals were slaughtered on d 299 of the study.

It was concluded in chapter 5 of this thesis that compensatory growth was evident in both Aberdeen Angus and Belgian Blue steers following nutritional restriction and feed realimentation and that very few biological interactions were observed. For this reason, and in an effort to reduce costs, a representative subset of 12 AA steers, H-H (n = 6) and L-H (n = 6), were selected and *M. longissimus thoracis et lumborum* biopsies were collected at the end of the differential feeding period (d 97) and again 32 days post realimentation (d 131) (section 2.2.2.3.5). Additionally, muscle tissue harvested from AA steers was chosen as to avoid confounding effects of the presence of myostatin mutation in the BB steers. RNA was extracted from the muscle tissue (section 2.2.3.1.1) and an RNA*seq* analysis was performed (section 2.2.9) as this technology offers a highly sensitive approach to examining differential gene expression.

7.2 Transcriptional profiles of *M. longissimus thoracis et lumborum* during nutritional restriction and compensatory growth in steers

7.2.1 Raw RNAseq data

Twenty-four cDNA libraries were sequenced using RNA*seq* technology. These libraries represented 12 animals (H-H, n=6; L-H, n=6) at 2 different time points (end of the differential feeding period and during the realimentation period). Overall, approximately 14,222 genes were expressed in bovine skeletal tissue with at least one reported read sequence per gene. On average 33,209,342 reads were sequenced per lane with 20,479,312 reads per lane aligning to the bovine genome (Btau4.0) (see Appendix Table A.2). This resulted in approximately 36 % of reads failing to align. A reason for this high percentage of reads failing to align includes removal of multiple reads that align to the same position on a chromosome. A strict approach was taken (allowing only 1 read per genomic location with the remaining reads discounted) to ensure that putative PCR duplication introduced during the library preparation steps is reduced. PCR duplication may bias sequencing results and therefore this strep was taken for qualitative reasons.

7.2.2 L-H vs. H-H steers at the end of the differential feeding period

At the end of the differential feeding period (d 97), there were 86 genes differentially expressed between L-H and H-H steers with human orthologs identified for 77 of these genes. Of these 86 genes, 15 were up-regulated and 71 down-regulated in L-H compared to H-H. InnateDB pathway analysis software (Lynn *et al.*, 2008) identified 40 over-enriched pathways (see Appendix Table A.3). For this study, 4 key pathways of interest (Table 7.1) were chosen for further discussion which includes: Peroxisome proliferator activated receptor (PPAR) signalling, adipocytokine signalling, insulin

Table 7.1 Statistically significant over-enriched pathways between L-H and H-H steers at the end of the differential feeding period (d 97)

Pathway	No. of genes in pathway	No. of genes identified	Gene name and Log fold change[1,2,3]
PPAR signalling	70	6	*ADIPOQ* (-2.6), ***ANGPTL4* (1.8)**, *FABP4* (-3.1), PCK1 (-2.4), *PCK2* (-4), *PLIN1* (-2)
Adipocytokine signalling	68	4	*ADIPOQ* (-2.6), *LEP*(-4.7), *PCK1* (-2.4), *PCK2* (-4)
Insulin signalling	135	4	*FASN* (-2), *PCK1* (-2.4), *PCK2* (-4), ***SOCS2* (1.5)**
Metabolism of lipids and lipoproteins	231	4	*ELOVL6* (-2.8), *FABP4* (-3.1), *FASN* (-2), *PLIN1* (-2)

[1]Up-regulated genes in L-H versus H-H steers denoted in **RED** font; down-regulated genes in L-H versus H-H steers denoted in GREEN font. [2]Numbers in parenthesis represent Log fold changes (i.e. Log 1 is a 2-fold change). [3]*ADIPOQ* = adiponectin, C1Q and collagen domain containing; *ANGPTL4* = angiopoietin-like 4; *FABP4* fatty acid binding protein 4, *PCK* = phosphoenolpyruvate carboxylase; *PLIN1* = perilipin 1; *LEP* = leptin; *FASN* = fatty acid synthase; *SOCS2* = suppressor of cytokine signalling 2; *ELOVL6* = elongation of long chain fatty acids; adipocyte.

signalling and metabolism of lipids and lipoproteins. These pathways were chosen as they represent the most statistically significant pathways with the highest proportion of differentially expressed genes. The PPAR signalling pathway consists of 70 genes with 6 genes associated with this pathway identified as differentially expressed in the current study. Of these 6 genes, 5 were down-regulated and 1 gene up-regulated in L-H compared to H-H steers. The second pathway identified was adipocytokine signalling with 4 genes in this pathway identified as down-regulated in L-H compared to H-H steers. These genes were adiponectin, C1Q and collagen domain containing (*ADIPOQ*), leptin (LEP), phosphoenolpyruvate carboxylase (PCK) 1 and PCK2. The third pathway of interest was insulin signalling pathway with 4 genes identified as differing in expression. These genes include fatty acid synthase (*FASN*), *PCK1*, *PCK2* and suppressor of cytokine signalling 2 (*SOCS2*). Expression of *SOCS2* was up-regulated in L-H with the remaining identified genes down-regulated in the pathway. Four genes associated with the pathway involved with metabolism of lipids and lipoproteins were identified as differentially expressed with all genes down-regulated in L-H animals. These genes included elongation of long chain fatty acids (*ELOVL6*), fatty acid binding protein 4, adipocyte (*FABP4*), FASN and perilipin 1 (*PLIN1*).

In addition, GO*seq* analysis identified 53 over-enriched GO terms between L-H and H-H. The most significant GO terms of interest returned included terms for “extracellular region part”, “lipid metabolic process” and “cell differentiation” (Figure 7.1). The most statistically significant GO term, extracellular region part, comprised of 9 genes in total with 1 gene up-regulated and 8 genes down-regulated in L-H compared to H-H steers. For a full representation of GO terms identified as statistically significant see appendix Table A.4.

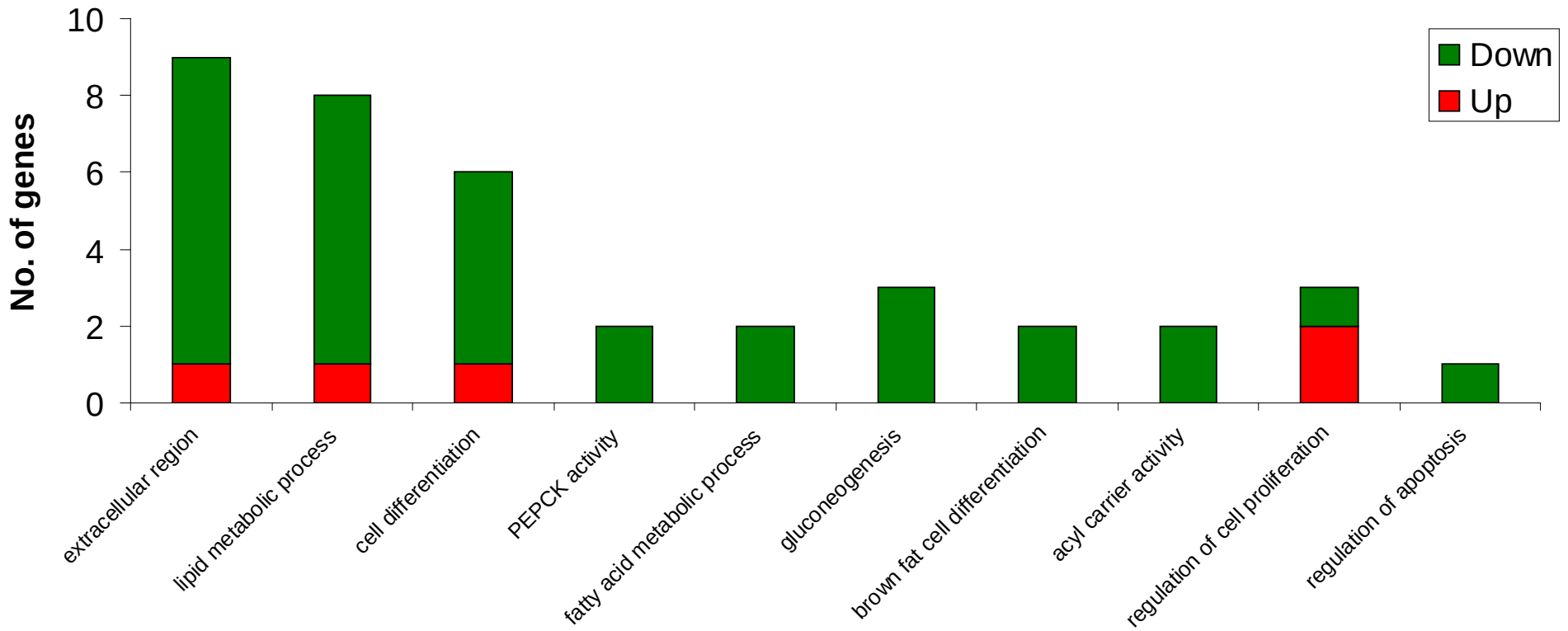


Figure 7.1 Statistically significant over-enriched GO terms identified between L-H and H-H steers at the end of the differential feeding period (d 97)

7.2.3 L-H vs H-H steers during the realimentation period

During the realimentation period, when previously restricted animals (L-H) were experiencing compensatory growth (day 131), there were 65 genes differentially expressed between L-H and H-H steers, with human orthologs identified for 49 of these genes. Of these 65, 40 genes were up-regulated and 25 genes down-regulated in L-H compared to H-H. InnateDB pathway analysis software (Lynn *et al*., 2008) identified 19 pathways (see appendix Table A.5) as over-enriched. For the purpose of this study, 4 key pathways of interest (Table 7.2) were chosen which include: Transforming growth factor-β (TGF-β) signalling, mitogen-activated protein kinase (MAPK) signalling, Forkhead box protein M1 (FOXM1) transcription factor network and mitotic (M) phase. Again, these pathways were chosen as they represented the highest proportion of differentially expressed genes and were selected based on their biological relevance to growth and differentiation.

The TGF-β signalling pathway comprises 84 genes with 4 of these genes identified as differentially expressed (Table 7.2). These genes were *COMP* (cartilage oligomeric matrix protein) and *PPP2Cβ* (protein phosphatase 2, catalytic subunit, β isozyme) which were up-regulated in L-H compared to H-H and *PPP2R1β* (protein phosphatase 2, regulatory subunit A, β) and *TGF-βR1* (transforming growth factor-β receptor 1) which were down-regulated in L-H compared to H-H steers. The MAPK signalling pathway is a key regulator of skeletal muscle development and comprises 268 genes, with 3 genes in the current study down-regulated in L-H steers: *FOS* (FBJ murine osteosarcomma viral oncogene homology), *PPM1A* (protein phosphatase, Mg^{2+}/Mn^{2+} dependent, 1A) and *TGF-βR1*. The FOXM1 transcription factor network was identified as over-enriched with 2 genes (*FOS* and *FOXM1*) that encode transcription factors differentially expressed between treatments. Finally, the M phase signalling pathway was identified as over-enriched with 2 genes up-regulated in L-H

during compensatory growth. These genes, *CDC20* (cell division cycle 20 homology) and *CDCA8* (cell division cycle associated 8) play key roles in cell division during mitosis. No significant over-enriched GO terms were returned from GO*seq* analysis. Although not correcting for gene length bias in its analysis, Innatedb software (Lynn *et al*., 2008) was used for GO term analysis and 1 GO term, "positive regulation of cell growth" was identified as over-enriched.

Table 7.2 Statistically significant over-enriched pathways between L-H and H-H steers during the realimentation period (d 131) when L-H animals were exhibiting compensatory growth

Pathway	No. of genes in pathway	No. of genes identified	Genes name and Log fold change[1,2,3]
TGF-β signalling	84	4	***COMP* (2.5)**, **PPP2CB (2.2)**, *PPP2R1B* (-2),*TGF-βR1* (-2.9)
FOXM1 transcription factor network	39	2	*FOS* (-1.9), ***FOXM1* (2.7)**
M phase	83	2	***CDC20* (1.8)**, ***CDCA8* (2.3)**
MAPK signalling,	268	3	*FOS* (-1.9), *PPM1A* (-1.7), *TGF-βR1* (-2.9)

[1]Up-regulated genes in L-H versus H-H steers denoted in **RED** font; down-regulated genes in L-H versus H-H steers written in GREEN font. [2]Numbers in parenthesis represent Log fold changes (i.e. Log 1 is a 2-fold change). [3]*COMP* = cartilage oligomeric matrix protein; *PPP2Cβ* = protein phosphatase 2, catalytic subunit, β isozyme; *PPP2R1β* = protein phosphatase 2, regulatory subunit A, β; *TGF-βR1* = transforming growth factor, β receptor 1; *FOS* = FBJ murine osteosarcomma viral oncogene homology; *PPM1A* = protein phosphatase, Mg2+/Mn2+ dependent, 1A; *FOXM1* = Forkhead box protein M1; *CDC20* = cell division cycle 20 homology; *CDCA8* = cell division cycle associated 8.

7.3 Chapter summary, discussion and conclusion

7.3.1 Chapter summary

Compensatory growth is the ability of an animal to undergo accelerated growth after a period of restricted feeding, as reviewed by Hornick *et al.* (2000). The objective of this study was to examine the transcriptional regulation of key genes and pathways controlling *M. longissimus thoracis et lumborum* growth during feed restriction and compensatory growth in Aberdeen Angus steers with a view to elucidating key gene and pathways regulating this growth phenomenon.

During the differential feeding period 40 pathways were identified as statistically significantly different between restricted and unrestricted steers, with four key pathways of interest chosen for further discussion. These pathways include PPAR signalling, adipocytokine signalling, insulin signalling and metabolism of lipids and lipoproteins. These pathways, relating to lipid metabolism, support the findings for differences in plasma leptin concentrations and ultrasonically scanned fat depths between L-H and H-H steers (chapter 5).

During the realimentation period, 19 pathways were identified as statistically significantly different between previously restricted and unrestricted steers, with four key pathways of interest chosen for further discussion. These pathways include: TGF-β signalling, MAPK signalling, FOXM1 transcription factor network and M phase. The TGF-β signalling pathway generally is a negative regulator of growth and TGF-βR1. *TGF-βR1*, a key gene in this pathway, was down-regulated in animals exhibiting compensatory growth during the feed realimentation period.

The results obtained from this study offer a new and exciting insight into key regulatory genes and pathways controlling compensatory growth in skeletal muscle of

cattle which following appropriate validation may be incorporated into genomically assisted selection strategies for beef cattle.

7.3.2 Chapter discussion

7.3.2.1 L-H v H-H steers at the end of the differential feeding period

The timing of the first biopsy marked the end of 97 days of feed restriction for the L-H steers. By this time restricted steers had lower live weights, lower ultrasonically scanned muscle and fat depths and smaller carcass size proportional to bodyweight. In addition, restricted steers had lower plasma concentrations of IGF-1, insulin, leptin, glucose and BHB, as discussed in chapter 5.

Five key pathways of interest were selected for further discussion with many of these pathways containing genes involved in lipid metabolism. Firstly, the PPAR signalling pathway is an important route during adipocyte tissue development, differentiation and activation of lipogenesis (Cánovas *et al*., 2010). Adiponectin C1Q and collagen domain (*ADIPOQ*) is regulated by this pathway and was down-regulated in L-H compared to H-H steers. The protein, ADIPOQ, is mainly secreted from adipose tissue in mammals; however, recent findings have reported its expression in muscle tissue (Krause *et al*., 2008). *In vitro, ADIPOQ* stimulates fatty acid oxidation and glucose uptake in muscle cells (Yamauchi *et al*., 2002). In the current study, restricted steers had lower concentrations of plasma glucose and ultrasonically scanned subcutaneous fat (chapter 5) at the end of the differential feeding period compared to animals offered a high energy diet, thereby highlighting concordance between biochemical and genetic findings. Whether ADIPOQ expression levels are originating from muscle cells, adipocytes, or both, warrants further investigation before detailed conclusions can be drawn, as whole muscle tissue was used in this study, and it is

expected that small quantities of adipocytes would be included in the cellular milleu of harvested tissue. A second differentially expressed gene of particular interest targeted by the PPAR signalling pathway is angiopoietin-like 4 (ANGPTL4), also referred to as the fasting induced adipose factor. ANGPTL4 plays a key role in maintaining metabolic homeostasis and inhibiting fat accumulation (Backhed *et al*., 2007; Mamedova *et al*., 2010). This gene was up-regulated in L-H compared to H-H steers supporting the findings of many studies (Kersten, 2000; Yoon *et al*., 2000; Dutton and Trayhurn, 2008; Kersten *et al*., 2009; Kim *et al*., 2010) which stated that caloric restriction increased ANGPTL4 plasma concentrations and mRNA expression in skeletal muscle. As a well characterised marker of caloric restriction, this result indicates that the L-H animals were adequately restricted in dietary intake.

Additional pathways associated with lipid metabolism (i.e. adipocytokine signalling, insulin signalling pathway and metabolism of lipids and lipoproteins) were identified as having differentially expressed genes. Although adipose tissue is often thought of as a major storage depot for triglycerides; adipose functions as an active endocrine tissue which secretes hormones, called adipocytokines. Plasma concentrations of leptin (chapter 5) and leptin (LEP) gene expression were down-regulated in L-H compared to H-H steers supporting the findings of Geary *et al*. (2003) where plasma leptin concentrations were positively correlated to *M. longissimus* fat thickness. Furthermore, Delavaud *et al*. (2002) reported that circulating leptin is positively regulated by feed energy intake. Thus results of the current study suggest that decreased plasma leptin was a direct result of a down-regulation of *LEP* gene expression in response to reduced energy intake and fat tissue accumulation.

Studies to date have examined the transcriptional response to nutritional restriction in skeletal muscle of rats, pigs, fish, human (Sreekumar *et al*., 2002; da Costa *et al*., 2004; Johansen and Overturf, 2006; Tsintzas *et al*., 2006; Rescan *et al*., 2007) and

cattle (Byrne *et al*., 2005; Lehnert *et al*., 2006; Connor *et al*., 2009). The degree of feed restriction applied in the current study, did not alter genes relating to protein catabolism or muscle fibre type redistribution as previously described in Byrne *et al*. (2005) and Lehnert *et al.* (2006). This suggests that although animals were restricted in energy intake, and were always in a positive energy balance, the length and degree of restriction applied in this study did not produce adverse or potentially harmful effects.

7.3.2.2 L-H v H-H steers during the realimentation period

Although both groups of steers were offered the same diet during the realimentation period, animals that were previously feed restricted prior to feed realimentation had higher live weight gains, as expected and discussed in chapter 5. The molecular mechanisms controlling this compensatory growth phenomenon are unknown, as previous attempts by other researchers to fully characterise the transcriptome and proteome during this realimentation period have been unsuccessful (Lametsch *et al*., 2006; Lehnert *et al*., 2006). Lehnert *et al*. (2006) examined transcriptional regulation in bovine muscle tissue during a compensatory growth period using microarray technology. The authors reported that just one gene, relating to muscle fibre type, was differentially expressed between treatments. Evidently, 84 days following feed realimentation was too late to examine differences in gene expression as animals had entered a normal growth trajectory (Lehnert *et al*., 2006). Additionally, Lametsch *et al*. (2006) examined the proteomic profile of muscle from pigs at the end of the realimentation period and reported that just 7 proteins were significantly altered with functions relating to glycolysis and lipogenesis. Again, in the Lametsch *et al.* (2006) study, muscle sampling was late into the realimentation period and it was hypothesised that key proteins regulating compensatory growth had returned to baseline levels (Lametsch *et al*., 2006). Therefore, in the current study, 32 days post realimentation was

chosen for muscle sampling to examine compensatory growth when previously restricted steers had greater average daily gains compared to animals on a continual plane of nutrition. Additionally, RNA*seq* was employed as it provided a highly sensitive approach to examine global transcriptional gene expression. As a result, numerous differential gene expression changes were identified with potential roles in regulating compensatory growth in bovine. Four pathways of interest were chosen for further discussion and these offer novel insight into potential and promising molecular mechanisms regulating compensatory growth in the bovine.

The role of the TGF-β signalling pathway is to translocate an activated SMAD transcription factor to the nucleus of the cell. By doing so, a potential number of target genes may respond to the SMAD transcription factor (Massague, 2000). In general, TGF-β signalling has a negative effect on cell growth and controls a diverse range of processes including cell proliferation, differentiation and apoptosis. TGF-β does not interact with type 1 receptors directly; however, they bind TGF-βR2 as a result of its high affinity for type 2 receptors (Massague, 1998; Shi and Massague, 2003). This binding facilitates the phosphorylation and activation of TGF-βR1 which results in down stream signalling of the SMAD proteins. In the current study, genes in the TGF-β signalling pathway exhibited differential expression. As TGF-βR1, a key member in the TGF-β signalling pathway was down-regulated it is hypothesised that the signalling effects of this pathway are reduced thereby promoting cell growth and proliferation in previously restricted steers experiencing compensatory growth.

The signalling effects of the TGF-β pathway are not restricted to the SMAD proteins. In fact, in human prostate stromal cells, TGF-β1, a member of the TGF signalling pathway, although not identified as differentially expressed in this study, is known to inhibit expression of FOXM1 (Untergasser *et al.*, 2005). FOXM1, a transcription factor encoded by the *FOXM1* gene, stimulates cell proliferation and

promotes cell cycle progression. *FOXM1* was up-regulated in steers experiencing compensatory growth; however, whether the stimulatory effects of TGF-β1 on FOXM1 are also evident in skeletal muscle is unknown and warrants further investigation. In addition, FOXM1 regulates genes that control G1/S-transition, S-phase progression, G2/M-transiton and M-phase progression. Interestingly, genes relating to the M-phase pathway (*CDC20*, *CDCA8*) were up-regulated in L-H compared to H-H steers. The M-phase pathway is the last stage in the cell cycle. A key component of the anaphase promoting complex, which is FOXM1 dependent, is CDC20 (Wang *et al*., 2002; Davis *et al*., 2010). Gene expression of *CDC20* was found to be up-regulated in muscle of L-H steers. These data suggest that during compensatory growth, genes regulating the cell cycle were up-regulated to meet the demands of accelerated muscle growth and development.

Extracellular signals are transmitted to their intracellular targets through the interaction of proteins (Seger and Krebs, 1995). Cells up-regulate or down-regulate gene expression as a consequence of these actions, leading to altered metabolism, differentiation or apoptosis (Hwang and Rhee, 1999). The MAPK signalling pathways are frequently used as a paradigm for receptor-mediated protein kinase cascades (Hwang and Rhee, 1999). One gene associated with this pathway, PPM1A, is a protein phosphatase, the mRNA levels of which were down-regulated in L-H compared to H-H steers. Research (Lin *et al*., 2006) has shown that PPM1A functions to dephosphorylate and inhibit MAPK and TGF-β signalling. Based on the observations of Lin *et al*. (2006), it would be hypothesised that PPM1A would be up-regulated in L-H steers promoting muscle growth and therefore its down-regulation in the current study warrants further consideration. Additionally, PPM1A has also been known to positively regulate insulin sensitivity through direct activation of P13K (Yoshizaki *et al*., 2004). Alternatively, knockout of PPM1A led to decreased insulin-stimulated GLUT4 translocation

(Yoshizaki *et al*., 2004). During the realimentation period in the current study, plasma concentrations of glucose and insulin rose sharply with glucose levels higher in L-H compared to H-H animals (chapter 5). It may be hypothesised that lower expression levels of PPM1A in L-H animals during the realimentation period resulted in higher peripheral concentrations of glucose as glucose uptake was reduced due to a decrease in GLUT4 translocation. However, the biological reasoning behind this and particularly the down-regulation of PPM1A in the current study may become clearer with further research in this area when the full functionality of this protein phosphatase becomes more apparent.

7.3.3 Chapter conclusion

To the authors' knowledge, this study is the first to effectively characterise the bovine transcriptome during a compensatory growth model. Through the harvesting of muscle tissue just 32 d post feed realimentation at the peak of compensatory growth, and the subsequent use of RNA*seq* technology key genes and pathways regulating this growth phenomenon were identified, which again, until now had not been previously identified during accelerated muscle growth. TGF-βR1, a key receptor in the TGF-β signalling pathway was down-regulated in previously restricted animals during compensatory growth. It is hypothesised that the signalling effects of the TGF-β pathway are reduced thereby promoting accelerated cell growth and proliferation in muscle tissue of animals experiencing compensatory growth. Overall, during the realimentation period, 65 differentially expressed genes were annotated to 19 over-represented pathways using InnateDB software (Lynn *et al*., 2008). These data indicate that transcriptional mechanisms regulating compensatory growth in the bovine are not limited to many differential gene expression changes in a few pathways but fewer discreet changes in many pathways. Although, further investigation of mechanisms regulating

compensatory growth is required, this work offers revealing and novel insight into the transcriptional regulation of *M. longissimus thoracis et lumborum* during feed realimentation following feed restriction in AA steers. Earlier muscle sampling time points, possibly just weeks or even days following the start of feed realimentation and across a range of other metabolically important tissues, will additionally serve to further elucidate the transcriptional mechanisms regulating compensatory growth in the bovine.

Chapter 8

General Discussion

8.1 Introduction

The focus of this thesis was to investigate how skeletal muscle growth in beef cattle is controlled at a molecular level with a view to better understanding these key mechanisms. The approach taken in this thesis was to combine key physiological and molecular analyses regulating the growth and development of *M. longissimus thoracis et lumborum* 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 set up to undergo compensatory growth. The focus of this thesis is primarily on *M. longissimus thoracis et lumborum* due to its primary economic important in cattle production.

In order to elucidate these key genes and proteins regulating and controlling muscle growth in beef cattle a number of areas needed to be examined, which involved two studies being undertaken with the following five objectives:

1. To examine the effect of sire breed and sire EPD_{cwt} on the mRNA expression of genes of the somatotropic axis in *M. longissimus thoracis et lumborum* in AA and BB cattle using qRT-PCR (chapter 3).

2. To examine the effect of sire breed and sire EPD_{cwt} on the expression of proteins in *M. longissimus thoracis et lumborum* in AA and BB cattle using 2D gel electrophoresis and mass spectrometry (chapter 4).

3. To study the response of crossbred steers, AA and BB, to differential feeding treatments with a view to examining the potential of these two genotypes to exhibit compensatory growth following feed realimentation (chapter 5).

4. To examine the effect of compensatory growth on meat quality and sensory analysis in *M. longissimus thoracis et lumborum* from AA and BB steers (chapter 6).

5. To examine the transcriptome for key regulatory pathways controlling *M. longissimus thoracis et lumborum* growth during feed restriction and compensatory growth in AA steers using RNA*seq* analysis (chapter 7).

8.2 Discussion

This thesis first examined potential gaps in the knowledge with regard to regulatory systems controlling growth potential and muscle growth in cattle. From a review of the literature it was concluded that animals of higher growth potential grew at faster and higher growth rates (Keane and Diskin, 2007; Campion *et al.*, 2009; Clarke *et al.*, 2009; Keane *et al.*, 2011); however, the reasons behind and the regulatory systems controlling this higher growth rate are poorly understood, with a dearth of published information on this area (Bernard *et al.*, 2009). Additionally, mechanisms regulating compensatory growth were insufficiently characterised in bovine muscle although efforts to characterise the molecular mechanisms regulating compensatory growth at a transcriptome and proteome level were undertaken previously (Lametsch *et al.*, 2006; Lehnert *et al.*, 2006).

Chapters 3 and 4 of this thesis focused on potential differences in gene expression and protein abundance in *M. longissimus thoracis et lumborum* between progeny of sires varying in their growth potential. Chapter 3 employed a candidate gene approach examining potential differences in expression of genes between breeds and growth potentials in the somatotropic axis while chapter 4 focused on the differential protein abundance across genotype and growth potential. It was evident from both of

these chapters that growth potential is under molecular control in skeletal muscle, which supports findings of a previously published report in a similar area (Bernard *et al.*, 2009). Following proteomic analysis, proteins involved in the glycolytic pathway were up-regulated in animals of high compared low growth potential. The somatotropic axis and glucose metabolism are unavoidably linked with GH synthesis influencing glucose metabolism (Renaville *et al.*, 2002). Furthermore, following a fasting period in cattle, feed intake is associated with a decline in plasma GH concentrations (Hornick *et al.*, 1998).

A common thread running between chapters 5 and 7 was both the plasma concentrations of leptin and its gene expression reduced at the end of the differential feeding period in animals offered a restricted feed intake compared to animals on a continual plane of nutrition. Leptin is involved in the hypothalamic control of body energy homeostasis, an indicator of body fat reserves and regulator of appetite and energy expenditure (Delavaud *et al.*, 2002); however, knowledge on its full functionality is continually growing. During the differential feeding period when feed intakes were decreased in L-H steers, subcutaneous fat depth was reduced and therefore the decrease in peripheral concentrations of leptin supports the findings of Geary *et al*. (2003) and Delavaud *et al*. (2002) who found that plasma leptin concentrations were positively correlated to subcutaneous fat thickness and feed energy intake.

It was evident from the ultrasonically scanned fat measurements, taken at the end of the differential feeding period, that animals on a restricted feed intake had lower subcutaneous fat accumulation (chapter 5) as previously mentioned. In agreement, genes in pathways relating to lipid metabolism, identified from RNA*seq* analysis, were differential expressed between treatments (chapter 7). Furthermore, fat tissue anabolism returned fat depths to similar depths as observed in the steers on a continual plane of

nutrition just 32 d post feed realimentation (chapter 5), which resulted in similar expression profiles for genes and pathways involved in lipid metabolism.

In the compensatory growth model (chapter 5), ultrasonically scanned *M. longissimus thoracis et lumborum* in previously restricted animals had returned to its inherent size by day 32 of the realimentation period with no difference also observed in muscle depth or *M. longissimus thoracis et lumborum* area at slaughter between treatments. Full recovery happened quickly in this muscle tissue making it an ideal and promising tissue to assess compensatory growth in bovine muscle. Thirty-two days post feed realimentation, when previously restricted animals were offered a higher energy intake, a second biopsy was taken from all AA steers. RNA*seq* technology, a highly sensitive approach to examine gene expression changes, effectively allowed the inspection of differential gene expression change between animals that exhibited compensatory growth and control animals. Interestingly, RNA*seq* analysis, carried out on mRNA extracted from the muscle tissue harvested during the realimentation period, found that the TGF-β and M-phase pathways were activated in that muscle at the same time that accelerated growth was occurring in the *M. longissimus thoracis et lumborum* making these pathways of immense interest when assessing increased growth in cattle. Furthermore, these pathways are potential targets to assess compensatory growth in all muscles in the bovine. Additionally, the TGF-β signalling pathway is a key target of interest for future differential gene expression studies between animals of high and low growth potential with the purpose of identifying a biomarker of high growth potential.

Tenderness and flavour are important factors determining eating quality as indicated by consumer research (Becker *et al*., 1998: Moloney *et al*., 2001). Chapter 6 aimed to assess the effect of a compensatory growth feeding regime on meat quality characteristics in AA and BB genotypes. An interesting result observed was the effect of compensatory growth on meat quality (chapter 6) especially on such critical factors

as meat tenderness and flavour. Many previous studies to date found no difference or even an improved quality (Sinclair *et al.*, 2001; Kristensen *et al.*, 2004; Hansen *et al.*, 2006; Therkildsen *et al.*, 2008; Stolzenbach *et al.*, 2009; Therkildsen *et al.*, 2011) from this type of feeding regime, so therefore a reduction in tenderness and overall flavour was interesting. Many of the effects of restricted nutrition that were evident in the steers (lower metabolite and blood hormone concentrations, ultrasonically scanned muscle and fat depths etc.) were transient, with levels returning to baseline or to similar levels to that of the control animals with five weeks (chapter 5). However, although animals were restricted for a relatively short period of time during their lifetime, residual effects of this restriction event (or the accelerated growth during feed realimentation) had an effect on meat quality characteristics at slaughter. Although these differences in meat quality attributes were statistically significant, the differences were small and unlikely to negatively impact eating quality (chapter 6). To assess this, future work could evaluate these small differences and whether they are deemed noticeable by the consumer, rather than a trained sensory team.

Additionally, meat quality attributes as affected by breed were also assessed (chapter 6). Peroxiredoxin (PRDX6) is an antioxidant enzyme and has been suggested as a potential marker for meat tenderness of beef (Jia *et al.*, 2009; Picard *et al.*, 2011). Jia *et al.* (2009) reported that PRDX6 was more abundant in tender meat, both in muscle from living animals and in the meat sampled post slaughter. In the study outlined in chapter 4 of this thesis, protein abundance of PRDX6 was increased in AA compared to BB which was consistent with the results of tenderness assessments, examined using both a mechanical and sensory approach, showing increased values for AA compared to BB steers (chapter 6). Intramuscular fat in the Angus breed has been shown to favourably influence tenderness of the meat and this was also shown in both shear force and sensory assessments in chapter 6 when intramuscular fat was used as a covariate in

the statistical analysis. Although intramuscular fat attributed highly to tenderness and juiciness, attributes such as beef flavour and overall flavour were still found to be greater in meat from AA compared to BB, irrespective of fat concentrations. Recent findings from Hocquette *et al.* (2011) suggested that intramuscular fat in bulls, between the ranges of ~ 10 and 25 mg/g, showed a close relationship with flavour; however, when intramuscular fat concentrations were greater than this no relationship was observed. Perhaps in the current study, as intramuscular fat concentrations were high, the relationship was in a 'plateau' phase (Hocquette *et al.*, 2011) and thus the variables were independent of each other. Possibly differences in fibre type distribution or myoglobin concentrations as a result of the BB animals being heterozygous for the myostatin mutation, negatively affected flavour (Clinquart *et al.*, 1998).

RNA*seq* is classed as the 'next-generation' in sequencing and many researchers have opted to use this sequencing technique as it offers a highly sensitive approach to transcriptome sequencing. This study utilised this most up to date technology and carefully chosen bioinformatic tools to carefully identify differentially gene expression in muscle tissue. Paired-end sequencing, compared to single read or fluorescence based sequencing approaches, offers users an increased confidence in results obtained. Additionally, a strict bioinformatic approach was carried out to address potential PCR and gene length bias and therefore confidence in identified genes and pathways was ensured.

8.3 Main conclusions

In conclusion, the main findings of this thesis are:

1. Plasma concentrations of IGF-1 and insulin throughout the lifetime of the animal, from 7 months of age to slaughter, were not affected by either breed or genetic merit for carcass weight (*H* or *L*) in AA or BB steers (chapter 3).
2. An increase in gene expression for *IGF-1* and a reduction in transcript levels of *IGFBP3* in *M. longissimus thoracis et lumborum* may play a regulatory role in increased muscle growth potential in steers during the finishing period (chapter 3).
3. Proteins relating to the glycolytic pathway, ENO1, GPI and PKM2, were increased in AA*H* compared to AA*L* steers, which offers revealing insights into the molecular mechanisms regulating muscle growth rate potential in AA steers (chapter 4). The finding support previously published data in this area (Bernard *et al.*, 2009) which aids in the understanding of genetic influences regulating muscle growth.
4. Proteins related to fibre type, such as MYL1 and MYLPF, were increased in BB steers, while proteins involved in the glycolytic and citric acid cycle were in greater abundance in muscle of AA animals (chapter 4). These data provide evidence for different metabolic processes taking place in muscle of crossbred AA and BB steers, supporting research findings in porcine studies (Murgiano *et al.*, 2010; Sieczkowska *et al.*, 2010).
5. In the compensatory growth study (chapter 5), there was no difference in DMI during feed realimentation; however, live weight gain was increased in previously restricted animals compared to animals on a continual plane of nutrition. The animals which underwent nutritional restriction exhibited greater feed efficiency when offered *ad libitum* access to feed.

6. Following a simple economic analysis, the compensatory growth model yielded on average a €35 premier per head over the continual feeding regime (chapter 5).
7. *M. longissimus thoracis et lumborum* has the ability to recover to its inherent size quickly during feed realimentation following a period of restricted feed intake (chapter 5). The fact that *M. longissimus thoracis et lumborum* is of high economic importance, and its ability to recover following restricted feed intake, made it an excellent tissue for further investigation into the molecular control of compensatory growth in bovine (chapter 7).
8. A compensatory growth feeding regime resulted in an increased shear force, and reduced tenderness and overall flavour. Irrespective of intramuscular fat, there was still a trend for overall flavour to be lower in animals that experienced compensatory growth compared to animals on a continual plane of nutrition (chapter 6).
9. Meat from AA had a reduced shear value and greater sensory flavour characteristics compared to BB steers. Intramuscular fat, which affects flavour and juiciness of the meat was greater in AA. When the sensory data were adjusted for intramuscular fat overall flavour was still greater in meat from AA compared to BB steers (chapter 6).
10. During the differential feeding period, restricted steers had lower peripheral concentrations of leptin. At a transcription level, the *LEP* gene was also identified as down-regulated in restricted animals. These data support much of the research in the area of fat reverses and energy intake, as reviewed by Wylie *et al*. (2011)
11. During feed realimentation, the TGF-β signalling pathway was identified as having genes with differential expression in animals exhibiting compensatory

growth compared to control steers. This finding has offered revealing insight into the molecular control of compensatory growth in bovine muscle (chapter 7).

12. Overall, the transcriptional control of compensatory growth in *M. longissimus thoracis et lumborum* is not limited to changes in expression of many genes in a few pathways but fewer discreet changes in many pathways.

8.4 Future work and implications

There are a number of possible future directions for further investigation arising from the results obtained from the studies conducted in this thesis:

1. It is evident from this work that EPD_{cwt} is under molecular control in cattle. Although chapters 3 and 4 offer enlightening insight into some genes and pathways, this regulation of muscle growth potential is still elusive. RNA*seq* technology, applied in chapter 7 of this thesis, offered new and revealing insights into the regulation of increased muscle growth. Therefore a similar approach whereby the entire transcriptome of animals divergent for growth rate potential are characterised, would offer revealing insight into all mechanism regulating growth in *M. longissimus thoracis et lumborum*.
2. As mentioned in chapter 3, the *IGFBP3* and *IGF-1* genes are potential candidates for future investigation of molecular markers for muscle growth. Future studies could focus on sequencing the entire *IGF-1* and *IGFBP3* genes and regulatory regions in large numbers of animals divergent in growth performance for SNP discovery and subsequent association studies.
3. Clearly, compensatory growth of muscle tissue is under molecular control in cattle and therefore it would be of great interest to also assess changes arising from feed restriction and transcriptional regulation in other metabolically

important tissues such as liver harvested using a biopsy technique to avoid any confounding gene expression changes relating to slaughter i.e. stress relating to transport, fasting, strange environment and mixing with unfamiliar cohorts.

4. In the compensatory growth model, rumen weight both full and empty was larger at slaughter in animals that exhibited compensatory growth compared to animals on a continual plane of nutrition suggesting a greater capacity for recovery. However, a larger empty rumen would consume more energy and reduce feed efficiency and therefore the biological reasoning behind this warrants further investigation to assess the implications of this observation.
5. There was no difference in absolute DMI across treatments; however, animals of high genetic merit for growth potential and animals offered a restricted ration during the differential feeding period exhibited higher live weight gains compared to animals of low genetic merit for growth potential and animals on a continual plane of nutrition, respectively (chapters 3 and 5). Research (Zhou *et al.*, 2009) has shown that changes in microbial populations may influence feed efficiency and therefore a future study could be designed to incorporate the collection of both rumen fluid and solids for the assessment of differences in both quantity and species of microbial populations across treatments to address this question in a compensatory growth model.
6. Additionally, gastrointestinal tissue such as duodenum, jejunum, and ileum could be examined to access differences in expression of genes relating to nutrient absorption during the differential feeding period and feed realimentation from restricted and control animals as well between animals of high and low genetic merit for carcass weight.
7. Although a compensatory growth feeding regime can save money in feed costs, as evident in chapter 5, from chapter 6 it is apparent that this type of feeding

regime may negatively impact both sensory and flavour characteristics. Although, at this stage the reasoning behind this is unclear and further investigatory work is required before a compensatory growth feeding regime is encouraged for fear that meat quality may be affected and beef consumption reduced. As mentioned in section 8.2, assessing these differences in meat quality on consumers, rather than a trained sensory panel as observed in this thesis, may truly identify differences, if any, in meat quality characteristics.

8. Key pathways regulating compensatory growth in *M. longissimus thoracis et lumborum*, such as the TGF-β signalling pathway were identified. This is novel and insightful information which had never been previously known and therefore these genes and pathways warrant further research across varying bovine breeds and tissues.

This work has offered insight into key pathways regulating growth in cattle such as the somatotropic axis, glycolytic pathway and TGF-β signalling pathways. Although introduction of biomarkers into any environment is a difficult task to achieve, and findings from this thesis are at a very early stage of investigation, following future investigatory work as outlined above, such information could be incorporated into future breeding programs, as potential biomarkers for muscle growth, or used along side production traits in assessing AI bulls around the country. Breeding programs and information databases incorporating genetic merit for growth are already in existence in Ireland (section 1.4). These programs include both live and carcass information regarding the sire itself and information regarding its progeny with a reliability score assigned to that animal. When choosing and assessing biomarkers for growth for inclusion in breeding programs, care needs to be taken to choose animals not only with the largest carcass weights and highest growth rates but also animals which are the most

efficient. Choosing biomarkers to assess in selection criteria for large, fast growing animals for breeding studies is potentially harmful as large animals may have larger maintenance requirements and therefore selectively breeding low efficiency animals may persist. In this thesis, both models looking at genetic merit for increased growth as well as a response to dietary feed allowance focused on improving growth in terms of increasing muscle growth and carcass weight but also examined feed efficiency. Therefore, not only did this thesis offer revealing insight into key genes and proteins regulating growth, it presented potential markers for assessing efficiency in steers, such as animals of high and low residual feed intake (RFI).

Chapter 9

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Chapter 10

Appendix

Table A.1 List of sensory terms[1] with definitions derived for sensory profiling of beef samples

Term and scale[2]	Definition
Tenderness (1-8)	Easiness with which the meat is divided into fine particles (ranging from very tough to very tender)
Juiciness (1-8)	Amount of juice released from the meat during mastication (ranging from very dry to very juicy)
Beef (1-8)	Amount of cooked beef flavour (ranging from little beef flavour to a lot of beef flavour)
Abnormal (1-8)	Amount of abnormal flavour (ranging from little abnormal to very abnormal)
On cutting (1-100)	
Ease of cutting	Ease with which sample is cut through by knife
Cleanness of cut	Appearance of sample on cutting with knife (jagged fibres to very clean cutting)
Initial eating (1-100)	
Toughness	Amount of resistance to teeth on initial chewing
Juiciness	Amount of moisture in the sample on initial chewing
Sponginess	Amount of springiness in the sample, bounce back to bite
Crunchy	Amount of perceived crispness in the sample on initial chewing
On eating (1-100)	
Toughness	Toughness on eating
Moisture	The perceived moisture content in the sample during eating
Pulpy	Pulpiness in the sample on eating
Chewiness	The total perceived effort required to prepare the sample to a state ready for swallowing
Gristle	Amount of gristle in the sample
Fibres	Amount of perceived fibres in the sample on eating
Greasiness	Amount of perceived oil or fatty matter in the sample on eating
Dissoluble	Degree to which it melts or disintegrates in mouth
Residue (1-100)	
Greasy	Amount of greasy coating in the mouth
Swallow	Degree to which the residue is easy to swallow
Particles	Fine particles in residue
Pulpy	Pulpiness in the residue
Mouthfeel	Sensation in the mouth after chewing (dry or wet)
Flavour (1-100)	
Greasy	The taste associated with fresh fat
Bloody	The taste associated with raw undercooked meat
Livery	The taste associated with liver flavour
Metallic	Tangy metal taste
Bitter	The taste on the tongue associated with caffeine/quinine
Sweet	The taste on the tongue associated with sugars
Rancid	The taste associated with “off” oil and fat
Fishy	The taste associated with fresh fish
Acidic	The taste associated with acids
Cardboard	The taste associated with smell of damp cardboard
Vegetable	Flavour of green vegetables and grass
Dairy	The taste associated with milk products
Overall	Preference rating for the sample

[1]Warner *et al*. (2008); Eight point scale or one hundred point scale.

Table A.2 Individual flowcell information relating to reads per lane aligning to the bovine genome

Flowcell	Lane	Sample ID	Feeding treatment	Time point	Direction	Raw reads	Aligned		Failed to align	
1	2	553RT1	RES	T1	Left	33251052	19311977	(58.08%)	9299795	(27.97%)
					Right	33152759	18766924	(56.61%)	9848453	(29.71%)
1	4	23HT1	CON	T1	Left	34472465	14837739	(43.04%)	16519875	(47.92%)
					Right	34253480	13848629	(40.43%)	17537037	(51.20%)
1	7	30HT2	CON	T2	Left	36206791	20141309	(55.63%)	11694719	(32.30%)
					Right	36125384	19617376	(54.30%)	12316535	(34.09%)
2	1	553RT2	RES	T2	Left	30570978	13219866	(43.24%)	15397092	(50.37%)
					Right	30430813	15696491	(44.61%)	12127620	(39.85%)
2	2	30HT1	CON	T1	Left	30541405	17254687	(57.60%)	10225017	(33.48%)
					Right	30397183	17526308	(57.60%)	9097225	(29.33%)
2	3	465RT1	RES	T1	Left	27944600	13326501	(47.69%)	12749119	(45.62%)
					Right	27810958	27810958	(51.65%)	10605465	(38.13%)
2	4	414HT2	CON	T2	Left	32157077	15326129	(47.66%)	14622949	(45.47%)
					Right	32003199	16410540	(51.28%)	12274302	(38.35%)
2	6	587RT2	RES	T2	Left	33507209	11614085	(34.66%)	19924386	(59.46%)
					Right	33350519	11257807	(33.76%)	19748364	(59.21%)
2	7	468HT1	CON	T1	Left	38017734	18273944	(48.07%)	16631118	(43.75%)
					Right	37626210	21544539	(57.27%)	11157729	(29.66%)
2	8	926RT1	RES	T1	Left	30646248	17881385	(58.35%)	8593096	(28.04%)
					Right	30489637	18317376	(60.08%)	7314151	(23.99%)
3	1	23HT2	CON	T2	Left	30336084	13084666	(43.13%)	14814783	(48.84%)
					Right	30239170	12982531	(42.93%)	14811870	(48.98%)
3	2	921RT2	RES	T2	Left	36565578	1.6E+08	(43.73%)	17177979	(46.98%)
					Right	36449125	15798284	(43.34%)	17294619	(47.45%)
3	3	414HT1	CON	T1	Left	37321183	22039250	(59.05%)	9549763	(25.59%)

					Right	37198408	21788077	(58.57%)	9747608	(26.20%)
3	4	587RT1	RES	T1	Left	38287817	22779727	(59.50%)	9774334	(25.53%)
					Right	38164056	22562220	(59.10%)	9962943	(26.11%)
3	6	584HT2	CON	T2	Left	34420285	17492886	(50.82%)	13054082	(37.93%)
					Right	34305151	17332214	(50.52%)	13162062	(38.37%)
3	7	976RT2	RES	T2	Left	29167189	18128566	(62.15%)	7108090	(24.37%)
					Right	29073554	17950304	(61.74%)	7254690	(24.95%)
3	8	521HT1	CON	T1	Left	35312124	20616842	(58.37%)	10009481	(28.35%)
					Right	35187614	26276430	(57.62%)	10339098	(29.38%)
4	1	921RT1	RES	T1	Left	39400798	20211102	(51.30%)	15191911	(38.50%)
					Right	39321575	19950576	(50.74%)	15360955	(39.06%)
4	2	521HT2	CON	T2	Left	32412306	19513385	(60.20%)	7455460	(23.00%)
					Right	32352403	19331603	(59.75%)	7668908	(23.70%)
4	3	465RT2	RES	T2	Left	34471150	17551615	(50.92%)	13324830	(38.66%)
					Right	34400196	17256958	(50.17%)	13636961	(39.69%)
4	4	584HT1	CON	T1	Left	26623369	12950913	(48.64%)	10950095	(41.13%)
					Right	26567654	12805454	(48.20%)	11098714	(41.78%)
4	6	976RT1	RES	T1	Left	33724753	21590698	(64.02%)	8010793	(23.75%)
					Right	33657044	21349076	(63.43%)	8256466	(24.53%)
4	7	468HT2	CON	T2	Left	33484756	16571197	(49.49%)	13321183	(39.78%)
					Right	33410853	16330858	(48.88%)	13575157	(40.63%)
4	8	926RT2	RES	T2	Left	29652762	18684824	(63.01%)	6846096	(23.07%)
					Right	29585770	18488188	(62.49%)	7054396	(23.84%)
					Average	**33209342**	**20479312**	**(52.57%)**	**11947861**	**(36.00%)**

Table A.3 All significantly over-enriched pathways generated from InnateDB between L-H and H-H steers at the end of the differential feeding period (d 97)

Pathway Name	Pathway Id	Pathway uploaded gene count	Genes in InnateDB for this entity	Genes Ratio	Pathway *P*-value	Pathway *P*-value (corrected)	Gene Symbols	Genes (Symbol|IDBG-ID|Ensembl|Entrez|Fold Change|*P*-Value)
PPAR signaling pathway	566	6	70	9%	5.76E-07	3.11E-05	ADIPOQ ; ANGPTL4 ; FABP4 ; PCK1 ; PCK2 ; PLIN1 ;	ADIPOQ|IDBG-69167|ENSG00000181092|9370|-2.6|0.00002 ; ANGPTL4|IDBG-24662|ENSG00000167772|51129|1.8|0.01591 ; FABP4|IDBG-26715|ENSG00000170323|2167|-3.1|<1.0E-5 ; PCK1|IDBG-82362|ENSG00000124253|5105|-2.4|0.00214 ; PCK2|IDBG-3497|ENSG00000100889|5106|-4|<1.0E-5 ; PLIN1|IDBG-29477|ENSG00000166819|5346|-2|0.00343 ;
GTP + Oxaloacetic acid = GDP + Phosphoenol-pyruvic acid + CO2 (Citrate cycle)	9570	2	2	100%	1.99E-05	0.000536626	PCK1 ; PCK2 ;	PCK1|IDBG-82362|ENSG00000124253|5105|-2.4|0.00214 ; PCK2|IDBG-3497|ENSG00000100889|5106|-4|<1.0E-5 ;
Amino Acid conjugation	1578	2	3	67%	5.95E-05	0.000642123	ACSM1 ; GLYAT ;	ACSM1|IDBG-18511|ENSG00000166743|116285|-3.9|<1.0E-5 ; GLYAT|IDBG-47902|ENSG00000149124|10249|-26.8|0.09522| ;
Conjugation of benzoate with glycine	1781	2	3	67%	5.95E-05	0.000642123	ACSM1 ; GLYAT ;	ACSM1|IDBG-18511|ENSG00000166743|116285|-3.9|<1.0E-5 ; GLYAT|IDBG-47902|ENSG00000149124|10249|-26.8|0.09522| ;
Conjugation of carboxylic acids	1266	2	3	67%	5.95E-05	0.000642123	ACSM1 ; GLYAT ;	ACSM1|IDBG-18511|ENSG00000166743|116285|-3.9|<1.0E-5 ; GLYAT|IDBG-47902|ENSG00000149124|10249|-26.8|0.09522| ;
Adipocytokine signaling pathway	590	4	68	6%	0.0002319	0.002087102	ADIPOQ ; LEP ; PCK1 ; PCK2 ;	ADIPOQ|IDBG-69167|ENSG00000181092|9370|-2.6|0.00002 ; LEP|IDBG-39587|ENSG00000174697|3952|-4.7|<1.0E-5 ; PCK1|IDBG-82362|ENSG00000124253|5105|-2.4|0.00214 ; PCK2|IDBG-3497|ENSG00000100889|5106|-4|<1.0E-5 ;
Biological oxidations	3694	4	87	5%	0.0005965	0.004601652	ACSM1 ; CYP2B6 ; CYP4F2 ; GLYAT ;	ACSM1|IDBG-18511|ENSG00000166743|116285|-3.9|<1.0E-5 ; CYP2B6|IDBG-52438|ENSG00000197408|1555|-6.1|<1.0E-5 ; CYP4F2|IDBG-35066|ENSG00000186115|8529|3.6|<1.0E-5 ; GLYAT|IDBG-47902|ENSG00000149124|10249|-26.8|0.09522| ;
Hormone-sensitive lipase (HSL)-mediated triacylglycerol hydrolysis	1754	2	12	17%	0.001275	0.008606354	FABP4 ; PLIN1 ;	FABP4|IDBG-26715|ENSG00000170323|2167|-3.1|<1.0E-5 ; PLIN1|IDBG-29477|ENSG00000166819|5346|-2|0.00343 ;
Fatty Acyl-CoA Biosynthesis	1307	2	16	13%	0.0022921	0.013752393	ELOVL6 ; FASN ;	ELOVL6|IDBG-34174|ENSG00000170522|79071|-2.8|0.03419 ; FASN|IDBG-73235|ENSG00000169710|2194|-2|0.0021 ;
Insulin signalling pathway	531	4	135	3%	0.0030427	0.016430576	FASN ; PCK1 ; PCK2 ; SOCS2 ;	FASN|IDBG-73235|ENSG00000169710|2194|-2|0.0021 ; PCK1|IDBG-82362|ENSG00000124253|5105|-2.4|0.00214 ; PCK2|IDBG-3497|ENSG00000100889|5106|-4|<1.0E-5 ; SOCS2|IDBG-51299|ENSG00000120833|8835|1.5|0.09522| ;
Gluconeogenesis	1370	2	20	10%	0.0035882	0.017614887	PCK1 ; PCK2 ;	PCK1|IDBG-82362|ENSG00000124253|5105|-2.4|0.00214 ; PCK2|IDBG-3497|ENSG00000100889|5106|-4|<1.0E-5 ;

Proximal tubule bicarbonate reclamation	10353	2	22	9%	0.0043379	0.019520518	PCK1 ; PCK2 ;	PCK1|IDBG-82362|ENSG00000124253|5105|-2.4|0.00214 ; PCK2|IDBG-3497|ENSG00000100889|5106|-4|<1.0E-5 ;
Citrate cycle (Citrate cycle)	10239	2	23	9%	0.0047376	0.019679275	PCK1 ; PCK2 ;	PCK1|IDBG-82362|ENSG00000124253|5105|-2.4|0.00214 ; PCK2|IDBG-3497|ENSG00000100889|5106|-4|<1.0E-5 ;
Triglyceride Biosynthesis	1811	2	26	8%	0.0060344	0.023275721	ELOVL6 ; FASN ;	ELOVL6|IDBG-34174|ENSG00000170522|79071|-2.8|0.03419 ; FASN|IDBG-73235|ENSG00000169710|2194|-2|0.0021 ;
Citrate cycle (TCA cycle)	464	2	30	7%	0.0079861	0.028750027	PCK1 ; PCK2 ;	PCK1|IDBG-82362|ENSG00000124253|5105|-2.4|0.00214 ; PCK2|IDBG-3497|ENSG00000100889|5106|-4|<1.0E-5 ;
Phase II conjugation	1394	2	32	6%	0.0090547	0.030559775	ACSM1 ; GLYAT ;	ACSM1|IDBG-18511|ENSG00000166743|116285|-3.9|<1.0E-5 ; GLYAT|IDBG-47902|ENSG00000149124|10249|-26.8|0.09522| ;
Glucose metabolism	1341	2	33	6%	0.0096118	0.030531494	PCK1 ; PCK2 ;	PCK1|IDBG-82362|ENSG00000124253|5105|-2.4|0.00214 ; PCK2|IDBG-3497|ENSG00000100889|5106|-4|<1.0E-5 ;
Neuroactive ligand-receptor interaction	416	5	316	2%	0.0130049	0.03901479	CHRNA6 ; CRHR2 ; GHRH ; LEP ; PRSS1 ;	CHRNA6|IDBG-20385|ENSG00000147434|8973|4.5|0.08194| ; CRHR2|IDBG-11186|ENSG00000106113|1395|-2.1|0.01235 ; GHRH|IDBG-74024|ENSG00000118702|2691|28.3|0.00002 ; LEP|IDBG-39587|ENSG00000174697|3952|-4.7|<1.0E-5 ; PRSS1|IDBG-45037|ENSG00000204983|5644,5645|-1.7|0.05464| ;
Pyruvate metabolism	450	2	40	5%	0.0139218	0.039567212	PCK1 ; PCK2 ;	PCK1|IDBG-82362|ENSG00000124253|5105|-2.4|0.00214 ; PCK2|IDBG-3497|ENSG00000100889|5106|-4|<1.0E-5 ;
Lipid digestion, mobilization, and transport	1651	2	41	5%	0.0145946	0.039405383	FABP4 ; PLIN1 ;	FABP4|IDBG-26715|ENSG00000170323|2167|-3.1|<1.0E-5 ; PLIN1|IDBG-29477|ENSG00000166819|5346|-2|0.00343 ;
Cytochrome P450 - arranged by substrate type	3441	2	45	4%	0.0174231	0.044802259	CYP2B6 ; CYP4F2 ;	CYP2B6|IDBG-52438|ENSG00000197408|1555|-6.1|<1.0E-5 ; CYP4F2|IDBG-35066|ENSG00000186115|8529|3.6|<1.0E-5 ;
Class B/2 (Secretin family receptors)	4776	2	46	4%	0.0181639	0.04458416	CRHR2 ; GHRH ;	CRHR2|IDBG-11186|ENSG00000106113|1395|-2.1|0.01235 ; GHRH|IDBG-74024|ENSG00000118702|2691|28.3|0.00002 ;
Metabolism of lipids and lipoproteins	8971	4	231	2%	0.0195852	0.04598255	ELOVL6 ; FABP4 ; FASN ; PLIN1 ;	ELOVL6|IDBG-34174|ENSG00000170522|79071|-2.8|0.03419 ; FABP4|IDBG-26715|ENSG00000170323|2167|-3.1|<1.0E-5 ; FASN|IDBG-73235|ENSG00000169710|2194|-2|0.0021 ; PLIN1|IDBG-29477|ENSG00000166819|5346|-2|0.00343 ;
Type II diabetes mellitus	577	2	48	4%	0.0196851	0.044291421	ADIPOQ ; SOCS2 ;	ADIPOQ|IDBG-69167|ENSG00000181092|9370|-2.6|0.00002 ; SOCS2|IDBG-51299|ENSG00000120833|8835|1.5|0.09522| ;
Phase 1 - Functionalization of compounds	3606	2	56	4%	0.0262783	0.056761167	CYP2B6 ; CYP4F2 ;	CYP2B6|IDBG-52438|ENSG00000197408|1555|-6.1|<1.0E-5 ; CYP4F2|IDBG-35066|ENSG00000186115|8529|3.6|<1.0E-5 ;
Arachidonic acid metabolism	418	2	57	4%	0.0271577	0.056404373	CYP2B6 ; CYP4F2 ;	CYP2B6|IDBG-52438|ENSG00000197408|1555|-6.1|<1.0E-5 ; CYP4F2|IDBG-35066|ENSG00000186115|8529|3.6|<1.0E-5 ;
NOD-like receptor signaling pathway	8112	2	61	3%	0.0307925	0.061584936	CASP5 ; NOD2 ;	CASP5|IDBG-69591|ENSG00000137757|838|-2.1|0.02913 ; NOD2|IDBG-30654|ENSG00000167207|64127|-1.8|0.09471| ;
Jak-STAT signaling pathway	568	3	155	2%	0.0322982	0.062289303	CNTF ; CNTF ; LEP ; SOCS2 ;	CNTF|IDBG-409278|ENSG00000242689|1270|3|0.0001 ; LEP|IDBG-39587|ENSG00000174697|3952|-4.7|<1.0E-5 ; SOCS2|IDBG-51299|ENSG00000120833|8835|1.5|0.09522| ;
Tryptophan degradation (Tryptophan degradation)	10017	2	63	3%	0.0326787	0.060849979	CYP2B6 ; IDO1 ;	CYP2B6|IDBG-52438|ENSG00000197408|1555|-6.1|<1.0E-5 ; IDO1|IDBG-18750|ENSG00000131203|3620|-1.7|0.07553| ;
Glycolysis / Gluconeogenesis	414	2	64	3%	0.0336386	0.060549489	PCK1 ; PCK2 ;	PCK1|IDBG-82362|ENSG00000124253|5105|-2.4|0.00214 ; PCK2|IDBG-3497|ENSG00000100889|5106|-4|<1.0E-5 ;
Metabolism of xenobiotics by cytochrome P450	599	2	69	3%	0.0386016	0.067241562	CYP2B6 ; MGST1 ;	CYP2B6|IDBG-52438|ENSG00000197408|1555|-6.1|<1.0E-5 ; MGST1|IDBG-21764|ENSG00000008394|4257|-1.8|0.04879 ;

Drug metabolism - cytochrome P450	2806	2	70	3%	0.0396262	0.066869138	CYP2B6 ; MGST1 ;	CYP2B6|IDBG-52438|ENSG00000197408|1555|-6.1|<1.0E-5 ; MGST1|IDBG-21764|ENSG00000008394|4257|-1.8|0.04879 ;
Cell junction organization	6970	2	75	3%	0.0449023	0.073476467	CDH11 ; LAMA3 ;	CDH11|IDBG-34793|ENSG00000140937|1009|-3.8|0.09522| ; LAMA3|IDBG-1631|ENSG00000053747|3909|-1.9|0.02913 ;
Integration of energy metabolism	1360	2	77	3%	0.0470824	0.074777909	FASN ; LEP ;	FASN|IDBG-73235|ENSG00000169710|2194|-2|0.0021 ; LEP|IDBG-39587|ENSG00000174697|3952|-4.7|<1.0E-5 ;
Protein digestion and absorption	10383	2	79	3%	0.049301	0.076064437	PRSS1 ; XPNPEP2 ;	PRSS1|IDBG-45037|ENSG00000204983|5644,5645|-1.7|0.05464| ; XPNPEP2|IDBG-85631|ENSG00000122121|7512|-2.1|0.01722 ;
Fatty acid, triacylglycerol, and ketone body metabolism	9255	2	80	3%	0.0504245	0.075636801	ELOVL6 ; FASN ;	ELOVL6|IDBG-34174|ENSG00000170522|79071|-2.8|0.03419 ; FASN|IDBG-73235|ENSG00000169710|2194|-2|0.0021 ;
Mammalian Wnt signaling pathway (Mammalian Wnt signaling pathway Diagram)	9534	2	81	2%	0.0515574	0.075245888	DKK2 ; SFRP4 ;	DKK2|IDBG-33265|ENSG00000155011|27123|-1.7|0.08135| ; SFRP4|IDBG-12815|ENSG00000106483|6424|-1.8|0.0326 ;
Metabolism of carbohydrates	1815	2	83	2%	0.0538506	0.076524541	PCK1 ; PCK2 ;	PCK1|IDBG-82362|ENSG00000124253|5105|-2.4|0.00214 ; PCK2|IDBG-3497|ENSG00000100889|5106|-4|<1.0E-5 ;
Canonical Wnt signaling pathway (Canonical Wnt signaling pathway Diagram)	9882	2	88	2%	0.0597398	0.080648762	DKK2 ; SFRP4 ;	DKK2|IDBG-33265|ENSG00000155011|27123|-1.7|0.08135| ; SFRP4|IDBG-12815|ENSG00000106483|6424|-1.8|0.0326 ;
Canonical Wnt signaling pathway (Mammalian Wnt signaling pathway Diagram)	10351	2	88	2%	0.0597398	0.080648762	DKK2 ; SFRP4 ;	DKK2|IDBG-33265|ENSG00000155011|27123|-1.7|0.08135| ; SFRP4|IDBG-12815|ENSG00000106483|6424|-1.8|0.0326 ;

Table A.4 All significantly over-enriched GO terms identified between L-H and H-H steers at the end of the differential feeding period (d 97)

GO ID	Term
GOID: GO:0044421	extracellular region part
GOID: GO:0006641	triglyceride metabolic process
GOID: GO:0006638	neutral lipid metabolic process
GOID: GO:0006639	acylglycerol metabolic process
GOID: GO:0019216	regulation of lipid metabolic process
GOID: GO:0005578	proteinaceous extracellular matrix
GOID: GO:0006662	glycerol ether metabolic process
GOID: GO:0018904	organic ether metabolic process
GOID: GO:0032502	developmental process
GOID: GO:0005576	extracellular region
GOID: GO:0031012	extracellular matrix
GOID: GO:0030154	cell differentiation
GOID: GO:0004611	phosphoenolpyruvate carboxykinase activity
GOID: GO:0004613	phosphoenolpyruvate carboxykinase (GTP) activity
GOID: GO:0006629	lipid metabolic process
GOID: GO:0048878	chemical homeostasis
GOID: GO:0048869	cellular developmental process
GOID: GO:0042221	response to chemical stimulus
GOID: GO:0042180	cellular ketone metabolic process
GOID: GO:0006094	gluconeogenesis
GOID: GO:0032720	negative regulation of tumor necrosis factor production
GOID: GO:0048585	negative regulation of response to stimulus
GOID: GO:0010646	regulation of cell communication
GOID: GO:0043408	regulation of MAPKKK cascade
GOID: GO:0019319	hexose biosynthetic process
GOID: GO:0032501	multicellular organismal process
GOID: GO:0050873	brown fat cell differentiation
GOID: GO:0046486	glycerolipid metabolic process
GOID: GO:0009968	negative regulation of signal transduction
GOID: GO:0046890	regulation of lipid biosynthetic process
GOID: GO:0051046	regulation of secretion
GOID: GO:0060341	regulation of cellular localization
GOID: GO:0042592	homeostatic process
GOID: GO:0010033	response to organic substance
GOID: GO:0000036	acyl carrier activity
GOID: GO:0019752	carboxylic acid metabolic process
GOID: GO:0043436	oxoacid metabolic process
GOID: GO:0044283	small molecule biosynthetic process
GOID: GO:0046364	monosaccharide biosynthetic process
GOID: GO:0006082	organic acid metabolic process
GOID: GO:0023057	negative regulation of signaling
GOID: GO:0010648	negative regulation of cell communication
GOID: GO:0044255	cellular lipid metabolic process
GOID: GO:0007275	multicellular organismal development
GOID: GO:0051259	protein oligomerization
GOID: GO:0050810	regulation of steroid biosynthetic process
GOID: GO:0006637	acyl-CoA metabolic process
GOID: GO:0035383	thioester metabolic process

GOID: GO:0048731	system development
GOID: GO:0048513	organ development
GOID: GO:0043392	negative regulation of DNA binding
GOID: GO:0042981	regulation of apoptosis
GOID: GO:0001890	placenta development
GOID: GO:0044421	extracellular region part
GOID: GO:0006641	triglyceride metabolic process
GOID: GO:0006638	neutral lipid metabolic process
GOID: GO:0006639	acylglycerol metabolic process
GOID: GO:0019216	regulation of lipid metabolic process
GOID: GO:0005578	proteinaceous extracellular matrix
GOID: GO:0006662	glycerol ether metabolic process
GOID: GO:0018904	organic ether metabolic process

Table A.5 All significantly over-enriched pathways between L-H and H-H steers during the realimentation period (d 131) when L-H animals were exhibiting compensatory growth.

Pathway Name	Pathway Id	Pathway uploaded gene count	Genes in InnateDB for this entity	Gene Ratio	Pathway *P*-value	Pathway *P*-value (corrected)	Gene Symbols	Genes (Symbol|IDBG-ID|Ensembl|Entrez|Fold Change|*P*-Value)
							COMP ;	COMP|IDBG-38816|ENSG00000105664|1311|2.5|0.05655| ;
							PPP2CB ;	PPP2CB|IDBG-15988|ENSG00000104695|5516|2.2|0.05655| ;
TGF-beta signaling							PPP2R1B ;	PPP2R1B|IDBG-70936|ENSG00000137713|5519|-2|0.05955| ;
pathway	465	4	84	5%	3.10E-05	0.000743	TGFBR1 ;	TGFBR1|IDBG-78488|ENSG00000106799|7046|-2.9|0.08293| ;
							FOS ;	FOS|IDBG-12957|ENSG00000170345|2353|-1.9|0.0054 ;
Chagas disease							PPP2CB ;	PPP2CB|IDBG-15988|ENSG00000104695|5516|2.2|0.05655| ;
(American							PPP2R1B ;	PPP2R1B|IDBG-70936|ENSG00000137713|5519|-2|0.05955| ;
trypanosomiasis)	10366	4	103	4%	6.91E-05	0.000829	TGFBR1 ;	TGFBR1|IDBG-78488|ENSG00000106799|7046|-2.9|0.08293| ;
							FOS ;	FOS|IDBG-12957|ENSG00000170345|2353|-1.9|0.0054 ;
					0.0004031		PPP2CB ;	PPP2CB|IDBG-15988|ENSG00000104695|5516|2.2|0.05655| ;
IL6	3910	3	66	5%	03	0.003225	PPP2R1B ;	PPP2R1B|IDBG-70936|ENSG00000137713|5519|-2|0.05955| ;
							CDC20 ;	CDC20|IDBG-97410|ENSG00000117399|991|1.8|0.08823| ;
					0.0017412		PPP2CB ;	PPP2CB|IDBG-15988|ENSG00000104695|5516|2.2|0.05655| ;
Oocyte meiosis	8113	3	109	3%	65	0.010448	PPP2R1B ;	PPP2R1B|IDBG-70936|ENSG00000137713|5519|-2|0.05955| ;
							MYH3 ;	MYH3|IDBG-30560|ENSG00000109063|4621|2.8|<1.0E-5 ;
					0.0030090		PPP2CB ;	PPP2CB|IDBG-15988|ENSG00000104695|5516|2.2|0.05655| ;
Tight junction	522	3	132	2%	53	0.014443	PPP2R1B ;	PPP2R1B|IDBG-70936|ENSG00000137713|5519|-2|0.05955| ;
							IFIT1 ;	IFIT1|IDBG-81960|ENSG00000185745|3434|-3.3|0.01127 ;
					0.0031401		PPP2CB ;	PPP2CB|IDBG-15988|ENSG00000104695|5516|2.2|0.05655| ;
Hepatitis C	10404	3	134	2%	84	0.012561	PPP2R1B ;	PPP2R1B|IDBG-70936|ENSG00000137713|5519|-2|0.05955| ;
FOXM1 transcription					0.0033987		FOS ;	FOS|IDBG-12957|ENSG00000170345|2353|-1.9|0.0054 ;
factor network	9456	2	39	5%	84	0.011653	FOXM1 ;	FOXM1|IDBG-12457|ENSG00000111206|2305|2.7|0.00136 ;
TGF-beta receptor					0.0057556		PPP2CB ;	PPP2CB|IDBG-15988|ENSG00000104695|5516|2.2|0.05655| ;
signaling	9443	2	51	4%	41	0.017267	TGFBR1 ;	TGFBR1|IDBG-78488|ENSG00000106799|7046|-2.9|0.08293| ;
					0.0084126		FOS ;	FOS|IDBG-12957|ENSG00000170345|2353|-1.9|0.0054 ;
Colorectal cancer	442	2	62	3%	97	0.022434	TGFBR1 ;	TGFBR1|IDBG-78488|ENSG00000106799|7046|-2.9|0.08293| ;
					0.0103404		PPP2CB ;	PPP2CB|IDBG-15988|ENSG00000104695|5516|2.2|0.05655| ;
Long-term depression	555	2	69	3%	62	0.024817	PPP2R1B ;	PPP2R1B|IDBG-70936|ENSG00000137713|5519|-2|0.05955| ;
MRNA surveillance					0.0130793		PPP2CB ;	PPP2CB|IDBG-15988|ENSG00000104695|5516|2.2|0.05655| ;
pathway	10376	2	78	3%	65	0.028537	PPP2R1B ;	PPP2R1B|IDBG-70936|ENSG00000137713|5519|-2|0.05955| ;
					0.0134013		CDC20 ;	CDC20|IDBG-97410|ENSG00000117399|991|1.8|0.08823| ;
Mitotic Prometaphase	1932	2	79	3%	22	0.026803	CDCA8 ;	CDCA8|IDBG-96468|ENSG00000134690|55143|2.3|0.02546 ;
					0.0147236		CDC20 ;	CDC20|IDBG-97410|ENSG00000117399|991|1.8|0.08823| ;
M Phase	1554	2	83	2%	91	0.027182	CDCA8 ;	CDCA8|IDBG-96468|ENSG00000134690|55143|2.3|0.02546 ;
MAPK signaling					0.0210583		FOS ;	FOS|IDBG-12957|ENSG00000170345|2353|-1.9|0.0054 ;
pathway	487	3	268	1%	99	0.0361	PPM1A ;	PPM1A|IDBG-8210|ENSG00000100614|5494|-1.7|0.09419| ;

							TGFBR1 ;	TGFBR1|IDBG-78488|ENSG00000106799|7046|-2.9|0.08293| ;
Osteoclast					0.0331359		FOS ;	FOS|IDBG-12957|ENSG00000170345|2353|-1.9|0.0054 ;
differentiation	10367	2	128	2%	09	0.053017	TGFBR1 ;	TGFBR1|IDBG-78488|ENSG00000106799|7046|-2.9|0.08293| ;
Wnt signaling					0.0442551		PPP2CB ;	PPP2CB|IDBG-15988|ENSG00000104695|5516|2.2|0.05655| ;
pathway	445	2	150	1%	53	0.066383	PPP2R1B ;	PPP2R1B|IDBG-70936|ENSG00000137713|5519|-2|0.05955| ;
					0.0453275		FOS ;	FOS|IDBG-12957|ENSG00000170345|2353|-1.9|0.0054 ;
TGFBR	3930	2	152	1%	9	0.063992	TGFBR1 ;	TGFBR1|IDBG-78488|ENSG00000106799|7046|-2.9|0.08293| ;
Mitotic M-M/G1					0.0480514		CDC20 ;	CDC20|IDBG-97410|ENSG00000117399|991|1.8|0.08823| ;
phases	9071	2	157	1%	65	0.064069	CDCA8 ;	CDCA8|IDBG-96468|ENSG00000134690|55143|2.3|0.02546 ;
					0.0595307		CDC20 ;	CDC20|IDBG-97410|ENSG00000117399|991|1.8|0.08823| ;
DNA Replication	1964	2	177	1%	82	0.075197	CDCA8 ;	CDCA8|IDBG-96468|ENSG00000134690|55143|2.3|0.02546 ;