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GENES THAT AFFECT MEAT PRODUCTION, FAT DEPOSITION AND CARCASS WEIGHT IN PIGS

A thesis
submitted in fulfilment
of the requirements for the Degree of
Doctor of Philosophy

at
Lincoln University
by
Sajee Kunhareang

Lincoln University
2013
GENES THAT AFFECT MEAT PRODUCTION, FAT DEPOSITION AND CARCASS WEIGHT IN PIGS

by

Sajee Kunhareang

Muscle growth is a critical trait in the pig industry, as increased muscle growth results in increased meat yield. In this context, the aim of this study was to investigate genes that may be involved in muscle growth and carcass traits in pigs.

A total of 474 commercial pigs from New Zealand and Thailand were investigated using polymerase chain reaction-single strand conformational polymorphism (PCR-SSCP) analysis to explore variation in nine candidate genes. These genes included: the myogenic regulatory factor 5 gene (MYF5), the myogenin gene (MYOG), the calpastatin gene (CAST), the calpain 3 gene (CAPN3), the myostatin gene (MSTN), the callipyge gene (CLPG), the leptin gene (LEP), the beta-3 adrenergic receptor gene (ADRB3) and the immunoglobulin heavy alpha chain gene (IGHA). These genes have been reported to influence muscle growth rate, meat production and other production traits in animals.

Sequence analyses revealed genetic variation at all the loci tested, with the exception of MSTN, which was not variable in the region tested in the pigs studied. There were two variants of MYOG, CAPN3, CLPG and ADRB3, and three variants of LEP and IGH, including a new variant, that has not been reported previously. The highest level of variation detected was four variants of MYF5 and CAST.

The variation in each gene was tested for its association with production traits in the pigs studied. The presence of variant A of MYF5 exon 3 was associated with increased weaning-weight and decreased fat depth. Variant C of CAST intron 5 was associated with increased live weight, average daily gain (ADG) and lean growth rate. Variant D of CAST intron 5 was
associated with increased ADG and increased fat depth, while variant B of CAST exon 6 tended (P=0.064) to be associated with increased lean growth rate. The genotype AB of CAST exon 6 tended (P=0.065) to be associated with increased lean growth rate. Variant B of ADRB3 was associated with increased weaning-weight and hot carcass weight. Absence of variant A was associated with increased fat depth, but this finding is weak since only two individual pigs carried this genotype. Variant B of IGH A was associated with decreased fat depth.

Together, these data suggest that genetic variation in MYF5, CAST, ADRB3 and IGH A may be involved in skeletal muscle growth and meat production in pigs. There was no association observed with variation in LEP and some variants of MYOG, CLPG and CAPN3 were at a low frequency, which precluded further analysis. Given that these results could be of benefit to the pig industry, the genes warrant further investigation.

**Keywords:** genetic variation, weaning-weight, carcass traits, pig production, myogenic regulatory factor 5 gene (MYF5), myogenin gene (MYOG), myostatin gene (MSTN), callipyge gene (CLPG), calpastatin gene (CAST), calpain 3 gene (CAPN3), leptin gene (LEP), beta-3 adrenergic receptor gene (ADRB3), immunoglobulin heavy alpha chain gene (IGH A).
Publication/presentations arising from this thesis

Papers:


Notes: Reprints were made with permission from the respective publishers.

Popular press:


Sequences submitted to the NCBI GeneBank:

1. *Sus scrofa* myogenic factor 5 (MYF-5) gene, MYF-5-A allele, exon 1 and partial codons: Accession number EU924175
2. *Sus scrofa* myogenic factor 5 (*MYF-5*) gene, MYF-5-B allele, exon 1 and partial codons: Accession number EU924176

3. *Sus scrofa* myogenic factor 5 (*MYF-5*) gene, MYF-5-C allele, exon 1 and partial codons: Accession number EU924177

4. *Sus scrofa* myogenic factor 5 (*MYF-5*) gene, MYF-5-D allele, exon 1 and partial codons: Accession number EU924178

5. *Sus scrofa* myogenic factor 5 (*MYF-5*) gene, MYF-5-A allele, exon 3 and partial codons: Accession number EU924179

6. *Sus scrofa* myogenic factor 5 (*MYF-5*) gene, MYF-5-A allele, exon 3 and partial codons: Accession number EU924180

7. *Sus scrofa* myogenic factor 5 (*MYF-5*) gene, MYF-5-A allele, exon 3 and partial codons: Accession number EU924175

**Presentations:**


Poster:

Evaluating genetic variation underlying meat production in pigs. *NZBIO 2010: Advancing a Bio-Based Economy*, Sky City Convention Centre, Auckland, New Zealand, 22\textsuperscript{nd}-24\textsuperscript{th} March 2010.

Conferences attended:

1. *Lincoln University Postgraduate Conference 2008*. Lincoln University, Canterbury, New Zealand, 26\textsuperscript{th}-27\textsuperscript{th} August 2008.

2. *Lincoln University Postgraduate Conference 2009*. Lincoln University, Canterbury, New Zealand, 31\textsuperscript{st} August-1\textsuperscript{st} September 2009.

3. *New Zealand Society of Animal Production, the 69\textsuperscript{th} conference*. Lincoln University, 24\textsuperscript{th}-26\textsuperscript{th} June 2009.

4. *ABIC 2009 conference Agricultural Biotechnology for Better Living and a Clean Environment*, Bangkok, Thailand, 22\textsuperscript{nd}-25\textsuperscript{th} September 2009.

5. *NZBIO 2010: Advancing a Bio-Based Economy*, Sky City Convention Centre, Auckland. New Zealand, 22\textsuperscript{nd}-24\textsuperscript{th} March 2010.

6. Queenstown Molecular Biology conference 2010: the 20\textsuperscript{th} Anniversary Meeting, Queenstown. New Zealand, 31\textsuperscript{st} -2\textsuperscript{nd} September 2010.
Acknowledgements

...This thesis is dedicated to the greatest dad and grandmom for their love and inspiration from a peaceful heaven... I do love you.

This thesis could not been completed without the help and the support of many people and I will endeavour to thank everyone. I am sorry if I miss anyone out.

Firstly, I would like to express my respect and most sincere gratitude to my supervisor Professor Jon Hickford. Thanks Jon for your encouragement and lots of constructive advice. Without your assistance I could not have completed this thesis. Thanks also to my co-supervisor, Dr Huitong Zhou, for all of your advice in the laboratory work and also to my associate supervisor, Dr Victoria Metcalf, for your review of my thesis draft. Thanks also to Professor Patrick Morel and Dr. Marinus F. W. te Pas, for all of your comments to complete my final draft for the award of my Doctoral degree.

Thanks to all others members of the Gene-Marker Laboratory (Freeman Fang, Hua Gong, Seung-ok Byun (Mimi), Jin Han, Yang Guo, Andrea Hogan and Vicky Liang) for their help, support and advice, and in particular Dr Grant McKenzie without whose help I would not have been able to collect blood samples. Many thanks to Dr Grant Bennett for your advice and guidance me to get back on track of Ph.D study. Many thanks also to Dr Selwyn Dobbinson, and Piet and Gavin Bloom for all their help collecting samples and the data for analysis, and Dr Chris Frampton and Dr Simon Hodge for helping me with the statistical analyses.

I would like to thank all of the other staffs and postgraduates in the Department of Agricultural Sciences. Thanks also to Caitriona Cameron for your organisation of writing class, which was very useful to improving my writing.

My thanks also to those who provided financial assistance: The Office of International Agriculture, Faculty of Agriculture, Khon Kaen University and Academic and International Affairs, Khon Kaen University. Thank you also to The Lincoln University Gene-Marker Laboratory, which assisted in funding my postgraduate study.

Lastly, I would like to thank my friends (here in Lincoln University and abroad). Thanks also to Assistant Professor Suporn Katawatin, Associate Professor Pornchai Lawilai, Associate Professor Yupa Hanboonsong, Dr Vilailuck Srijongrunson, Dr Montira Watcharasukarn,
Dr Piyaratt Dokkularb, Dr Prapapan Teerawanichpan, Dr Nutsurang Pukkalanun, Mr Wanpuech Parnsen and Mr Jackrit Sathong for their help to motivate me to keep working on my thesis, and my family members, my lovely mom, my sisters (Boonyabhon Kunhareang and Uthaiwan Noi-thong), my gorgeous niece and nephew, for your unconditional love. Thanks for all your support, encouragement and wise counsel. You have all helped me keep life in perspective and for that I am grateful. I love you.
Abbreviations

> greater than
< lower than
% percent
α alpha
β beta
γ gamma
σ sigma
° C degree celsius
µg microgram (s)
µL microlitre (s)
µm micrometre (s)
µM micromolar
ADRB3 beta-3 adrenergic receptor gene
AgNO₃ silver nitrate
ANOVA analysis of variance
Bis N, N’-methylene-bis-acrylamide
bp base pair
CAPN3 calpain 3 gene
CAST calpastatin gene
DNA deoxyribonucleic acid
dH₂O deionised water
dNTP deoxyribonucleoside triphosphates
EDTA ethylenediaminetetraacetate
h hour
IGHA heavy constant region of the IgA gene
kg kilogram
Ltd limited
LEP leptin gene
MAS marker-assisted selection
mg milligram (s)
Mg²⁺ magnesium (ionic)
### Amino acid residue abbreviations

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<tr>
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<td>Cys</td>
<td>cysteine</td>
</tr>
<tr>
<td>D</td>
<td>Asp</td>
<td>aspartic acid</td>
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<tr>
<td>E</td>
<td>Glu</td>
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<tr>
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Chapter 1

Introduction

1.1 Global trends in pork production

Population growth and increases in economic activity throughout the world have raised meat demand by over 75% in the last 20 years (Rosegrant et al., 1995; Gill, 1999; Burke et al., 2008). The global consumption of meat is projected to further increase from approximately 290 million tonnes per annum, to 360 million tonnes by 2020, and to more than 550 million tonnes by 2050 (Figure 1.1) (Rosegrant et al., 1995; Rosegrant et al., 2001; Delgado, 2003; FAO, 2009). While meat consumption is increasing in both developed and developing countries, the increase has been greatest in the developing countries (Rosegrant et al., 2001).

![Figure 1.1 Global demand for meat from 1970 to 2000, and predicted demand between 2010 and 2050](Adapted from Rosegrant et al. (2001) and FAO (2009)).

The commercial pig industry is responding to this global demand by attempting to increase production. Consumer attitudes have also been changing, with an increasing demand for healthy food of a high nutritional quality. To meet these demands, factors affecting pork production and quality are being investigated in many parts of the world.
Pork production can be increased by increasing pig numbers, and by providing a greater quantity and quality of animal feed. However, the cost of feeding pigs remains high (Rauw et al., 2006), so another avenue to improving pork production is to improve pig genetics (Koohmaraie, 2002; te Pas, 2004; Kim et al., 2009).

1.1.1 The international pig industry and pork production

Globalisation and improving global economics have resulted in increased trade in pork, particularly in developing countries (Delgado, 2003; den Hartog, 2004). This has led to an increase in the world production of pork, with in excess of 100 million tonnes of forecasted production globally in 2009 (USDA’s FAS, 2010). China, the European Union and the United States are the largest pig producing countries of the world and they account for 89% of world pig production in 2008 (Table 1.1).

While China is the world’s largest pork producer, all its production is consumed by its domestic market. The main exporters of pork are the United States, the European Union, Canada and Brazil. Of these, the United States is the largest exporter and the countries importing the most pork are Germany, Russia, Mexoco, Japan and South Korea (USDA’s FAS, 2010).

The international trade in pig meat is either in live pigs or processed pork (Kyriazakis & Whittemore, 2006). The largest exporters of live pigs are the Netherlands and Denmark, and they provide live pigs to Germany and Eastern Europe for feeding and slaughter. Japan is the largest destination for processed pork, and this comes from the United State and the European Union (Dransfield et al., 2005).
Table 1.1 World pork production between 2005 and 2008

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<thead>
<tr>
<th>Country</th>
<th>2005</th>
<th>2006</th>
<th>2007</th>
<th>2008</th>
<th>Growth (%)</th>
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<td>World</td>
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<td>96,156</td>
<td>94,700</td>
<td>98,441</td>
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(Data from USDA FAS, 2010)

1 Growth rate of pork production from 2005 to 2008

1.1.2 Requirements of pig meat markets

Pig meat is considered an “essential food and protein source” by some countries, especially since trade in beef and poultry has decreased due to disease-related bans (Morgan & Prakash, 2006). In the last few years, pig meat has become the most consumed meat in the world (Figure 1.2).
Chapter 1: Introduction

Figure 1.2 World meat consumption by meat types in 2010 [Data from USDA’S FAS (2010)].

The increase in pork consumption is changing the global pig industry and production systems are moving from small outdoor-mixed production farming types, to large-unit companies (Edwards, 2005; Honeyman, 2005). As a consequence, the pig industry is now seeking to improve production efficiency and the reproductive characteristics of pigs (Hermesch et al., 2000; Kuhlers et al., 2003; Roehe et al., 2003).

The pork market is changing and is no longer just concerned with low cost production, but is also focusing on niche markets, where customers have a preference for eating high quality pork (Ngapo et al., 2004; Honeyman, 2005; Kyriazakis & Whittemore, 2006). European consumers of pork have become more conscious of consuming lean meat products (Dransfield et al., 2005; Kyriazakis & Whittemore, 2006), while in contrast, US consumers have become more concerned about food safety, pig welfare and the environmental impact of the production system (Ngapo et al., 2004; Dransfield et al., 2005).

Consumer demands also affect what finishing weight is optimal prior to slaughter. Smaller pigs, at 50-80 kg live-weight, are favoured for domestic demand and/or local markets, because they give a superior product for fresh pork consumption. However, live-weights between 90-120 kg are the optimal range for industrial processing (Kyriazakis & Whittemore, 2006), and even heavier weights (160-180 kg) are required to make Parma ham (Virgili et al., 2003). Regardless of the market or ultimate use of pig meat, we need to have a better understanding of the genetic factors that affect muscle growth and development.
Our understanding of the genetics of muscle growth has been improved by recent advances in gene technologies. This has allowed the study of individual genes and their effect on growth metabolism and survival in production systems (te Pas, 2004; Rothschild et al., 2007).

1.2 Genetic improvement to improve pork production

The growth and development of pigs can be influenced by a number of factors such as breed, gender, physiological responses and variation in nutrient supply (Wiseman et al., 2005; te Pas, 2004; Hossner, 2005). In a selection program, the ability to choose superior genetics for growth and meat production is dependent upon pigs having sufficient genetic variability to enable selection and that the trait of interest is heritable (Lo et al., 1992; Robinson et al., 1998).

Heritability indicates the degree to which a character is transmitted from parent to offspring. It is defined as the extent to which individual genetic differences contribute to individual phenotypic differences (Lawrence et al., 2012). Heritability estimates for some commercial traits in pigs are reported in Table 1.2.

Table 1.2 Heritability estimates for some traits in commercial pigs

<table>
<thead>
<tr>
<th>Trait</th>
<th>Heritability (%)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Litter size</td>
<td>10</td>
<td>Taylor &amp; Bogart, 1988</td>
</tr>
<tr>
<td>Birth weight</td>
<td>10</td>
<td>Taylor &amp; Bogart, 1988</td>
</tr>
<tr>
<td>Litter weaning-weight</td>
<td>15</td>
<td>Taylor &amp; Bogart, 1988</td>
</tr>
<tr>
<td>Post-weaning gain</td>
<td>30</td>
<td>Taylor &amp; Bogart, 1988</td>
</tr>
<tr>
<td>Carcass fat thickness</td>
<td>56</td>
<td>Lo et al. (1992)</td>
</tr>
<tr>
<td>Loin eye muscle area</td>
<td>45</td>
<td>Taylor &amp; Bogart, 1988</td>
</tr>
<tr>
<td>Fibre number</td>
<td></td>
<td></td>
</tr>
<tr>
<td>\textit{longissimus} muscle</td>
<td>22</td>
<td>Larzul et al. (1997)</td>
</tr>
<tr>
<td></td>
<td>28-40</td>
<td>Dietl et al. (1993)</td>
</tr>
<tr>
<td></td>
<td>43-48</td>
<td>Staun (1972)</td>
</tr>
<tr>
<td>Fibre size (area or diameter)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>\textit{longissimus} muscle</td>
<td>34</td>
<td>Larzul et al. (1997)</td>
</tr>
<tr>
<td></td>
<td>22-34</td>
<td>Dietl et al. (1993)</td>
</tr>
<tr>
<td></td>
<td>30-50</td>
<td>Staun (1972)</td>
</tr>
</tbody>
</table>

To date, gene-marker tests are commercially available for a limited number of single gene defects in pigs. However, research is progressing into identifying genes controlling
quantitative traits (such as growth-rate, milk production and reproduction; Rothschild et al., 2007), with the objective of using these tests in routine breeding programs. Today, the commercial pig industry is using gene-markers in breeding selection, and alongside the use of traditional performance information to improve production traits.

As a way of improving muscle growth, variation in genes controlling muscle development needs to be identified and how this affects muscle growth quantified. Accordingly, in this thesis, genetic variation in some candidate genes was identified and tested to see if it was associated with pork production traits, and with the aim of identifying candidate genes that could be used as gene-markers to improve pig breeding.

1.3 Study approach

This study assessed genetic variation in some genes that may affect pork production. Candidate gene selection was based on what is known of the genes function and/or if they had been linked to muscle growth metabolism and survival in other animals. The candidate genes selected were MYF5, MYOG, MSTN, CLPG, CAPN3, CAST, LEP, ADRB3 and IGHA.

Variation in these genes was investigated using polymerase chain reaction (PCR) coupled with single-strand conformational polymorphism (SSCP) analysis. PCR-SSCP analysis is based on the relationship between the electrophoretic mobility of a single-stranded DNA molecule and its folded conformations, which in turn reflects its nucleotide sequence. It is an economic, reproducible and effective analytical method for the detection of deletions, insertions, or rearrangement in genes (Hayashi, 1992; Orti et al., 1997; Sunnucks et al., 2000).

Following genotyping, various statistical analyses were used to determine the relationship between variation in the candidate gene and variation in some pork production traits. This established if the genetic variation had the potential to be used as a gene-marker to improve the selection of breeding stock. An overview of the research approach is shown in Figure 1.3.

1.3.1 Research hypotheses

1. It is hypothesised that genes involved in myogenesis, growth, tissue catabolism and anabolism, and animal health will be variable and,

2. That variation in some of these genes may be associated with variation in muscle, growth, and carcass traits in NZ commercial pigs.
Research approach:

Genotypic data:

Identification of genetic variation using PCR-SSCP
- MYF5
- MYOG
- MSTN
- CLPG
- CAST
- CAPN3
- ADRB3
- LEP
- IGH A

Phenotypic data:

Records available
- ID boar/ ID sow
- date to weaning
- date to slaughter
- weaning-weight
- hot carcass weight
- fat depth between the 3rd and 4th rib of the hot carcass

Statistical analysis

Any associations between genetic variation and phenotype

General discussion

Conclusions

Figure 1.3 Overview of the research approach.

1.3.2 Aims of this study

The aims of this study are as follows.

1. To investigate the extent of variation in genes that may be involved in growth and muscle development in pigs
2. To investigate if associations exist between candidate genes and growth and carcass traits in pigs
3. To ascertain the suitability of the genes that positively affects pig production traits for use as gene-markers in selective breeding.
1.4 Outline of chapters

Chapter 1 describes the background of the problem, research approach, hypothesis and aims of the study, followed by a literature review (Chapter 2). The literature review provides information about skeletal muscle development and growth (myogenesis), the regulation of muscle development and the genes involved in the various regulatory networks. It provides a justification for choosing the genes of interest in the candidate gene approach. This is followed by the materials and methods chapter (Chapter 3) and the results (Chapter 4). Chapter 5 is a discussion of the findings and Chapter 6 draws conclusions and describes the implications of the findings.
Chapter 2
Literature review

Meat is one of the most economically important products of the pig industry. To increase meat production, genetic improvement using selection based on heritable meat characteristics is essential (Lawrence et al., 2012). This process has become more efficient with the use of genetic markers that assist in the identification of genes that affect meat growth and production. The development of these gene-markers requires an understanding of the biological processes regulating the differentiation and growth of skeletal muscle.

2.1 The structure of skeletal muscle

Skeletal muscle is one of the main contributors to meat production (Lawrence et al., 2012). Its primary components are muscle fibres, which are surrounded by connective tissue (Figure 2.1). Parallel muscle fibres are organised into a bundle (a fasciculus) and groups of bundles are organised into muscles.

Each fibre is a single multi-nucleated cell with the nuclei found near the surface of the fibre. These muscle cells can contain more than a thousand nuclei that have been derived from the fusion of precursor cells (known as myoblasts) during foetal development (Gregorio & Antin, 2000). Each muscle fibre contains bundles of myofilaments. There are two types of myofilament: the thin myofilaments (actin) and thick myofilaments (myosin). The myofilaments are organised into repeated units called sarcomeres. The myofibril consists of many sarcomeres lined up end-to-end. Each sarcomere can be identified as the area between the Z-disks, which extend across the muscle cell and that attach by transmembrane structures to the extracellular connective tissue (Lawrence et al., 2012).

The structure of skeletal muscle is dynamic and responds to changes in physiology by the remodelling of the structure of an individual fibre. Sarcomeres can be added or removed from the cylindrical myofibril structure, and this leads to changes in the overall mass of muscle tissue (Trotter, 2002). In addition, changes in gene expression alter the metabolic and contractile properties of the myofibrils (Olsen & Williams, 2000), and affect muscle development. To understand muscle development, there is a need to describe the molecular mechanisms that control the pre- and post-natal growth of muscle.
Chapter 2: Literature review

Figure 2.1 Diagram of the structure of skeletal muscle. The basic unit of skeletal muscle is a parallel array of muscle fibres. This muscle fibre contains bundles of myofibrils consisting of actin and myosin myofilaments. The myofilaments are arranged in overlapping structure called sarcomeres and these can change in number in response to changes in physiology.

2.2 Skeletal muscle development and growth

Muscle development and growth is the result of myoblast and/or satellite cell proliferation and muscle fibre formation (te Pas, 2004; Rehfeldt et al., 2002). The potential for muscle growth and meat production in an animal is dependent on both the pre-natal development of muscle

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1 Adapted from http://www.shoppingtrolley.net/skeletal%20muscle.shtml, Downloaded on June the 3rd, 2010.
fibres and the rate of muscle fibre hypertrophy post-natally (Bower et al., 1997; te Pas et al., 1999; Rehfeldt & Kuhn, 2006). Muscle fibres are formed during pre-natal growth from muscle progenitor cells. These muscle fibres become larger during post-natal growth by fusing with muscle satellite cells (Koohmaraie, 2002; Lawrence et al., 2012; Dellavalle et al., 2011).

2.2.1 Myogenesis and pre-natal muscle growth

The formation of muscle fibres during pre-natal growth is called “myogenesis” (Buckingham, 1994). Myogenesis can be split into four phases (Buckingham et al., 2003; Lawrence et al., 2012): i) the formation of fibres in the myotomes of the somites; ii) primary fibre formation during embryo development; iii) secondary fibre formation during late embryogenesis; and iv) fibre formation during late foetal development and after birth.

During the embryonic development of muscle, myoblasts develop from myogenic precursor cells. These precursor cells are derived from the mesoderm, which originates from the limb and interlimb somites (epithelial assemblies that arise from the paraxial mesoderm) (Buckingham et al., 2003; Hossner, 2005). The somites differentiate into the dermomyotome (DM) and the ventrolateral lip (VLL), and in turn these cells give rise to the myotome and the myogenic precursor cells (Figure 2.2) (Cossu et al., 1996; Birchmeier & Brohmann, 2000).

![Figure 2.2 Schematic diagram of skeletal muscle development during embryogenesis](image)

Figure 2.2 Schematic diagram of skeletal muscle development during embryogenesis. The muscle progenitors are a result of differentiation of the embryonic limb and interlimb somites. These processes affect the number of myoblasts, myotubes and myofibres [Adapted from L’Honore et al. (2007) and Buckingham et al. (2003)].
The specification of the mesodermal precursor cells to enter the myogenic lineage requires the up-regulation of the muscle-specific transcriptional activators MyoD and/or MYF5 (Figure 2.3) (Pownall et al., 2002; te Pas, 2004). These MyoD and/or MYF5 positive myogenic cells are able to proliferate a pool of myoblasts (Pownall et al., 2002; Buckingham et al., 2003; Rehfeldt et al., 2011).

Myoblast differentiation is assisted by myogenin (MYOG) and muscle regulatory factor 4 (MRF4) responding to differentiation signals (Buckingham et al., 2003; Rehfeldt et al., 2011). Proliferating myoblasts withdraw from the cell cycle at the first gap phase (G1) to become terminally-differentiated myocytes (Pownall et al., 2002). These subsequently express muscle-cell specific proteins such as the actin and myosin heavy chain proteins, and muscle creatine kinase (Olson, 1992; Hooper & Thuma, 2005). Finally, mononucleated myocytes fuse to form multinucleated myotubes. These mature into muscle fibres (Asakura et al., 2002; te Pas, 2004; Rehfeldt et al., 2011).

Figure 2.3 Schematic diagrams of myogenesis and the regulation of embryogenesis by transcriptional activators. The formation of skeletal muscle involves (i) specification of precursor cells to the myoblast lineage, (ii) proliferation of the myoblasts, (iii) differentiation of the myoblasts and (iv) maturation to produce myofibres. Myogenic precursor cells are induced by MyoD and MYF5, and MYOG and MRF4 expression in the differentiating muscle. During differentiation, embryonic myoblasts differentiate to form secondary fibres that surround the primary fibres [Adapted from Rehfeldt et al. (2000) and Picard et al. (2002)].
The muscle fibres are a combination of differentiated myoblasts. These are developed in two phases: the formation of the primary myofibres and the formation of the secondary myofibres. Primary myofibres are formed during the initial stages of myoblast fusion. These provide a framework for the development of the larger population of smaller secondary myofibres (Rehfeldt et al., 2000; te Pas & Soumillion, 2001; Lawrence et al., 2012). The formation of secondary myofibres occurs through a second wave of differentiation of the foetal myoblasts and this determines the final number of muscle fibres (Rehfeldt et al., 2000; Parker et al., 2003). Heterogeneous populations of myoblasts give rise to muscle fibres (Pownall et al., 2002; te Pas, 2004). The number of muscle fibres that are formed prenatally determines the ultimate size of muscle (Parker et al., 2003; te Pas et al., 2007; Wimmers et al., 2010; Rehfeldt et al., 2011).

Not all myoblast cells differentiate into fibres. Some form satellite cells that are located outside the myofibre cell membranes (Seale et al., 2000). These satellite cells are able to divide and serve as the source of new myonuclei (Tatsumi, 2010) for the post-natal growth, regeneration and repair of muscle (Oksbjerg et al., 2004; Otto et al., 2009; Rehfeldt et al., 2011).

### 2.2.2 Post-natal muscle growth

After birth, increases in skeletal muscle mass are due to an increase in muscle size and by either an increase in muscle fibre cross-sectional area, or an increase in length (Lawrence et al., 2012). This occurs as a consequence of additional undifferentiated satellite cells fusing with the myofibre (Dellavalle et al., 2011; te Pas, 2004; Rehfeldt et al., 2000). Initially, undifferentiated (quiescent) satellite cells are induced to proliferate and differentiate to form mature myoblasts (Rudnicki et al., 2008). Those myoblasts exit the cell cycle to fuse with each other and with surviving fibres to generate new and repaired muscle tissue (Yablonka-Reuveni, 2011). During proliferation, quiescent satellite cells generate two daughter cells (Zammit et al., 2006). One daughter cell remains as a self-renewing stem cell and the second daughter cell is committed to differentiation and fuses with the myofibre (Figure 2.4) (Kuang et al., 2008).
The quiescent satellite cells (SC) and their progeny are created upon activation, proliferation and differentiation by MYF5, MyoD and Fax7 expression. Satellite cells undergo asymmetric division in which they create two daughter cells. One daughter cell returns to a quiescent state and the other daughter cell forms a population of myocytes, prior to fusion with existing muscle fibres [Modified from Bentzinger et al. (2012)].

The quiescent satellite cells can be differentiated based on the expression patterns of transcriptional activators including the paired-homeobox transcription factor Pax7, MyoD and MYF5 (Zammit et al., 2006; Allouh et al., 2008). Experimentally, MYF5 promoter activity has been observed in satellite cells and their proliferating progeny in myogenic cultures from the MYF5nlacZ/+ mice (Beauchamp et al., 2000; Day et al., 2010). It has been found that the MYF5 promoter is active in quiescent satellite cells, but MYF5 protein is not produced until the cells begin to proliferate. Moreover, expression of MYF5 decreases when myoblasts start to differentiate, whereas MyoD expression persists well into the differentiation stage (Day & Yablonka-Reuveni, 2009; Yablonka-Reuveni, 2011).

Figure 2.4 Structure and regeneration of adult skeletal muscle. The quiescent satellite cells (SC) and their progeny are created upon activation, proliferation and differentiation by MYF5, MyoD and Fax7 expression. Satellite cells undergo asymmetric division in which they create two daughter cells. One daughter cell returns to a quiescent state and the other daughter cell forms a population of myocytes, prior to fusion with existing muscle fibres [Modified from Bentzinger et al. (2012)].

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2 Adapted from
Downloaded on April the 27th, 2013.
2.3 The pathways and networks that regulate muscle development

The formation of skeletal muscle can be separated into a sequence of steps. In the beginning, unspecified mesodermal cells are induced to form muscle precursors, which then differentiate into specific skeletal muscle cells. Muscle growth is under the influence of a regulatory network of genes as well as being dependent on the metabolic and nutritional state of the animal. The genes and networks implicated in muscle growth are described below.

2.3.1 The genetic networks controlling myogenesis

During myogenesis, skeletal muscle cells originate from the dermomyotome in the somite (see Figure 2.2) (Pownall et al., 2002). This process is influenced by regulatory transcriptional factors that can be allocated into three stages: induction, specification and differentiation.

Induction of myogenesis is affected by external regulatory factors including the morphogens from the wingless gene (Wnt), sonic hedgehog homolog (Shh) and the bone morphogenetic protein (BMP) factors (Pownall et al., 2002; Rawls et al., 2000). Wnt signalling involves a group of pathway that pass signals from outside of a cell through cell surface receptors to the inside of the cell. Wnt derives from the neural tubes (Birchmeier & Brohmann, 2000; Echelard et al., 1993), while Shh derives from the floor plate and notochord. Together, these act as positive regulatory signals to increase myogenic gene expression. Analysis of Shh “knock-out” mice revealed impaired formation of the dermomyotome, as well as reduced expression of MYF5 in the myotome (Chiang et al., 1996; Straface et al., 2009) and together this suggests that Wnt and Shh are needed for the maturation of dermomyotomal cells. In contrast, bone morphogenetic protein (BMP) from the lateral mesoderm, inhibits expression of the myogenic regulatory genes (Birchmeier & Brohmann, 2000).

Specification is the result of downstream myogenic regulatory factors being expressed by the myogenic progenitor cells (Tajbakhsh et al., 1998). A key regulator of myogenesis is Pax-3 (the paired-type homeobox gene), which is expressed in the epaxial mesoderm and initiates MyoD expression in the somites (Figure 2.5) (Rawls et al., 2000). Pax3 expression in the myotome is a consequence of the combinational effects of the Wnt, Shh, and BMP signalling pathways (Pownall et al., 2002).
Figure 2.5 The myogenic network between the transcriptional regulators (Wnt/BMP/Shh) and the various myogenic factors and the various pathways engaged in the hypaxial mesoderm and the epaxial mesoderm. Six1,4 and Pax3 are regulators of early lineage specification, whereas MYF5 and MyoD commit cells to the myogenic program. Expression of the terminal differentiation genes, required for the fusion of myocytes and the formation of myotubes, are performed by both myogenin (MYOG) and MRF4. Solid lines show direct interaction at the transcriptional level and dashed lines represent genetic interactions [Modified from Pownall et al. (2002) and Heanue et al. (2002)].

In the hypaxial mesoderm, Pax3 expression is a downstream effect of the activity of the sine oculis–related homeobox 1 (Six1) gene and Six4 gene. Six1 and Six4 are considered genetic regulators at the start of a cascade of events that ultimately induce dermomyotomal progenitors to form myogenic cells (Grifone et al., 2007). A lack of Pax3 expression in the hypaxial mesoderm results in the limb and trunk hypaxial muscles not forming (Heanue et al., 2002; Grifone et al., 2007).

The fusion of myocytes and the formation of myotubes is controlled by MYOG and MRF4 (Pownall et al., 2002) and the myocyte enhancer factor 2 (MEF2) (Bertzinger et al., 2012). The MEF2 elements do not affect myogenic cells directly, but they potentiate the function of MRFs through transcriptional cooperation (Molkentin et al., 1995; Potthoff & Olson, 2007).
A negative feedback loop in this system arises from other MEF2 transcriptional targets that associate with, and repress myogenic regulatory factors (Haberland et al., 2007).

### 2.3.2 The genetic network controlling muscle hypertrophy

Muscle growth continues after birth by the enlargement of skeletal muscle fibres (hypertrophy) (Hossner, 2005). Muscle growth and hypertrophy are controlled by regulatory pathways; including the phosphatidyl-inositol-3 kinase (PI3K) and protein kinase (Akt) pathway (Argilés et al., 2012; Gurpur et al., 2009). Akt can delay the caspase-2-mediated death pathway and promote muscle growth by stimulation of cellular metabolism (Kovacheva et al., 2010). Akt can be inactivated by myostatin (MSTN) gene expression (Kovacheva et al., 2010) and MSTN signalling is able to inhibit Akt phosphorylation and down-regulate the PI3K/AKT hypertrophy pathway (Figure 2.6).

The PI3K/AKT pathway controls cell growth by regulating translation processes. It induces phosphorylation, which can promote muscle growth through the direct activation of Notch signalling (Kovacheva et al., 2010). Over-expression of MSTN in muscle leads to a loss of protein mass via inhibition of Akt phosphorylation, whereas under-expression of MSTN results in skeletal muscle hypertrophy (Argilés et al., 2012).

![Figure 2.6 Myostatin signalling in skeletal muscle](#)

Myostatin increases protein degradation and decreases protein synthesis by activation of the PI3K/Akt pathway. This results in activation of atrogenic gene expression and inactivation of protein synthesis. In addition, MSTN activates c-Jun N-terminal kinase (JNK). Activation of JNK inhibits muscle growth by inhibiting muscle cell apoptosis. MSTN also inhibits the myogenic program by induced up-regulation of p21, resulting in a decrease in myoblast proliferation [Modified from Kovacheva et al. (2010)].
The loss of functional MSTN leads to hyperplasia and hypertrophy of skeletal muscle (Thomus et al., 2000). MSTN “knock-out” mice revealed that during myogenesis the signalling for p21 up-regulation was lost. This results in de-regulated (increased) myoblast proliferation, leading to an increase in muscle fibre number (Thomas et al., 2000; McCroskery et al., 2003). An absence of MSTN also causes the satellite cells to re-enter the cell cycle, and proliferate and fuse with existing muscle fibres (Toabin & Celeste, 2005).

![Figure 2.7 A model for the role of myostatin (MSTN) in myogenesis](Modified from Langley et al. (2004) and Thomas et al. (2000)).

A “double muscled” phenotype of cattle (Kambadur et al., 1997) and sheep (Clop et al., 2006) have been reported, which are related to a mutation in MSTN. This effect is reported to contribute to increased growth rates, meat yield and carcass quality in cattle (McPherron & Lee, 1997; Wegner et al., 2000; Bellinge et al., 2005). Other studies have suggested that variation in MSTN could be used for breeding stock selection, since cattle carrying the MSTN variation have increased muscle mass (Esmailizadeh et al., 2008). In sheep, the variability has also been found to result in similar effects to those described in cattle (Dunner et al., 2003; Smith et al., 2000). Thus, variation in MSTN may be beneficial in animal breeding for improved meat production.
Muscle hypertrophy is not only regulated by MSTN. It is also observed in sheep carrying the callipyge (CLPG) mutation (Cockett et al., 1996; Caiment et al., 2010). This point mutation is located in the DLK1-GTL2 imprinted gene cluster (Cockett et al., 1994). The CLPG mutation results in expression of Delta-like 1 homolog (DLK1). DLK1 expression is an effector causing muscle hypertrophy through inhibition of Notch1 (Vuocolo et al., 2007; Conboy & Rando, 2002). In turn, this inhibition enhances the activity of myogenic regulatory factors and results in decreased expression of MEF2. This promotes the formation of muscle fibers and muscle hypertrophy (Waddell et al., 2010; Vuocolo et al. (2007).

2.3.3 Anabolism and catabolism that affects muscle growth

Aside from the genetic networks, muscle growth is affected by the metabolic balance of an animal. Metabolic balance is a reflection of both anabolic pathways (anabolism) and catabolic pathways (catabolism) (Figure 2.8). The balance between the anabolic pathways and catabolic pathways is important for muscle synthesis and degradation, as well as for feed intake regulation and energy expenditure.

![Figure 2.8 The relationship between catabolism and anabolism in cells.](image) Catabolism is a process to prepare energy for the cell (energy-yielding metabolism) and that changes energy sources from one form to another. Some energy is lost in the form of heat. Anabolism involves biosynthetic metabolism to build cell material from energy available from catabolic processes and nutrients. These processes create a metabolic balance and can attribute to accumulation or loss of muscle protein in animals [Modified from Rajan & Mitch, (2008) and Goll et al. (1998)].
2.3.3.1 The balance of muscle synthesis and degradation

Muscle growth is a result of net muscle accumulation and appears to be associated with the activity of specific factors that promote muscle synthesis and reduce muscle degradation (Goll et al., 1998). Muscle growth or loss depends on whether muscle synthesis is occurring more than (hypertrophy) or less than (atrophy) muscle degradation (Rajan & Mitch, 2008; Costelli et al., 2005). The degradation of muscle is the result of myofibrils degrading. This degradation can be induced by the activation of calpain and its associated factors (Smith & Dodd, 2007) (Figure 2.9).

Increased calpain levels are responsible for initiating myofibrillar degradation (Dayton et al., 1975; Goll et al., 1992). This results in a release of myofilaments from the surface of the myofibril (Dargelos et al., 2002; Goll et al., 2008). Moreover, calpains inhibit the Akt pathway of protein synthesis, leading to decreased muscle synthesis (Smith & Dodd, 2007).

The activity of calpains depends on the presence and activity of the inhibitor, calpastatin (Dargelos et al., 2002; Sensky et al., 1999). In skeletal muscle, the calpain-calpastatin system

Figure 2.9 Schematic diagram of the different proteolytic pathways in muscle degradation. Extracellular signals can induce intracellular pathways, and other pathways may also be involved (discontinuous arrows), leading to muscle atrophy [Modified from Costelli et al. (2003)].
is involved in the regulation of striated muscle metabolism (Barnoy et al., 1998; Goll et al., 2003) and other metabolic processes that control protein turnover (Huff-Lonergan et al., 1996; Choi et al., 2006; Goll et al., 2008).

Increasing levels of calpastatin expression are associated with changes in muscle protein synthesis (Goll et al., 2003; Li et al., 2009). Goll et al. (2003) reported that an increasing rate of skeletal muscle growth resulted from a decreased rate of muscle protein degradation. This is due to an increase in calpastatin activity and decreased calpain activity (Goll et al., 2008). Therefore, calpain-calpastatin activity in skeletal muscle is highly related to the rate of muscle protein turnover (Goll et al., 2008).

2.3.3.2 Feed intake and energy expenditure

Feed intake and energy expenditure is responsible for muscle growth (Lawrence et al., 2012; Richards, 2003). These are controlled by physiological signals such as insulin signalling and the hormonal regulation of appetite (Lawrence et al., 2012). One of the most important hormones that regulate appetite is leptin (Friedman & Halaas, 1998).

Leptin is released by adipose tissues in response to food intake and suppresses appetite (Remesar et al., 1997; Friedman, 2000) (Figure 2.10). Leptin synthesis is known to be induced by high circulating insulin, glucose and glucocorticoid levels (Remesar et al., 1997; JÉQuier, 2002). Leptin also induces adipogenesis via signals from the sympathetic nervous system and stimulated adrenergic receptors (ARs). As a result, ARs have been implicated in controlling lipolysis and adipogenesis (Lee et al., 2012). Furthermore, differentiation of myogenic cells into brown fat or muscle cells also appears to be controlled by AR gene expression (Figure 2.10) (Shan et al., 2013; Margareto et al., 2001).
2.3.4 The heritability of various muscle phenotypes

Muscle growth is the result of an increase in muscle fibre number and size. This growth is affected by both environmental and genetic factors. The proportion of phenotypic variation attributed to genetic effects is described as its heritability. In general, if the trait has a high heritability, it can be improved rapidly using selection. Thus, the heritability of a trait is important when conducting a genetic improvement program.

The heritability of some muscle and production traits has been evaluated. These traits typically have a moderate to high heritability in different animal species (between 0.20-0.45, Table 2.1 and Table 2.2). This indicates that these muscle and production traits could be improved using selection. Moreover, selection can be improved by using genetic markers that increase the accuracy of selection and allow for accumulate selections to be made earlier in an animals breeding cycles.
### Table 2.1 Heritability of some muscle traits in meat-producing animals

<table>
<thead>
<tr>
<th>Trait</th>
<th>Species</th>
<th>Heritability</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Loin eye muscle area</td>
<td>Pig</td>
<td>0.45</td>
<td>Taylor &amp; Bogart (1988)</td>
</tr>
<tr>
<td><em>longissimus</em> muscle area</td>
<td></td>
<td>0.28-0.40</td>
<td>Dietl <em>et al.</em> (1993)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.43-0.48</td>
<td>Staun (1972)</td>
</tr>
<tr>
<td>intramuscular fat</td>
<td></td>
<td>0.39</td>
<td>Suzuki <em>et al.</em> (2005)</td>
</tr>
<tr>
<td>Carcass weight</td>
<td>Cattle</td>
<td>0.40</td>
<td>Utrera &amp; Van Vleck (2004)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.36</td>
<td>Robinson <em>et al.</em> (1998)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.40</td>
<td>Fouilloux <em>et al.</em> (1999)</td>
</tr>
<tr>
<td>Back fat thickness</td>
<td></td>
<td>0.29</td>
<td>Wiener <em>et al.</em> (2002)</td>
</tr>
<tr>
<td><em>longissimus</em> muscle area</td>
<td></td>
<td>0.26</td>
<td>Wiener <em>et al.</em> (2002)</td>
</tr>
<tr>
<td>Tenderness</td>
<td></td>
<td>0.40</td>
<td>Dikeman <em>et al.</em> (2005)</td>
</tr>
<tr>
<td>Eye muscle depth</td>
<td>Sheep</td>
<td>0.31</td>
<td>Greeff <em>et al.</em> (2003)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.27</td>
<td>Fogarty <em>et al.</em> (2003)</td>
</tr>
<tr>
<td>Carcass weight</td>
<td>Chicken</td>
<td>0.28</td>
<td>Zerehdaran <em>et al.</em> (2004)</td>
</tr>
</tbody>
</table>

### Table 2.2 Heritability of some production traits

<table>
<thead>
<tr>
<th>Trait</th>
<th>Species</th>
<th>Heritability</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Birth weight</td>
<td>Pig</td>
<td>0.10</td>
<td>Taylor &amp; Bogart, 1988</td>
</tr>
<tr>
<td>Litter weaning weight</td>
<td></td>
<td>0.15</td>
<td>Taylor &amp; Bogart, 1988</td>
</tr>
<tr>
<td>Post weaning gain</td>
<td></td>
<td>0.30</td>
<td>Taylor &amp; Bogart, 1988</td>
</tr>
<tr>
<td>Birth weight</td>
<td>Cattle</td>
<td>0.44</td>
<td>Gregory <em>et al.</em> (1995)</td>
</tr>
<tr>
<td>Pre-weaning weight</td>
<td></td>
<td>0.38</td>
<td></td>
</tr>
<tr>
<td>Post-weaning weight</td>
<td></td>
<td>0.43</td>
<td></td>
</tr>
<tr>
<td>Birth weight</td>
<td>Sheep</td>
<td>0.39</td>
<td>Tosh &amp; Kemp (1994)</td>
</tr>
<tr>
<td>Weaning weight</td>
<td></td>
<td>0.16</td>
<td>Tosh &amp; Kemp (1994)</td>
</tr>
<tr>
<td>Body weight at 16 weeks</td>
<td>Chicken</td>
<td>0.23</td>
<td>Zerehdaran <em>et al.</em> (2004)</td>
</tr>
</tbody>
</table>

Gene-markers are either linked to a single gene that has a major effect on a trait or are linked to a number of genes with additive effects (Jeon *et al.*, 1999). Examples of gene-markers...
associated with meat production traits and that are used for predicting growth and meat production are shown (Table 2.3).

**Table 2.3** Examples of gene-markers used in meat production

<table>
<thead>
<tr>
<th>Marker</th>
<th>Animal</th>
<th>Trait</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>HAL</td>
<td>Pigs</td>
<td>Stress susceptibility, meat quality, meat yield</td>
<td>Fujii <em>et al.</em>, 1991</td>
</tr>
<tr>
<td>MC4R</td>
<td></td>
<td>Dairy gain, feed conversion, lean</td>
<td>Kim <em>et al.</em>, 2000</td>
</tr>
<tr>
<td>IGF2</td>
<td></td>
<td>Lean</td>
<td>Jeon <em>et al.</em>, 1999; Nezer <em>et al.</em>, 1999</td>
</tr>
<tr>
<td>CAST</td>
<td></td>
<td>Meat quality</td>
<td>Ciobanu <em>et al.</em>, 2004; Nonneman <em>et al.</em>, 2011</td>
</tr>
<tr>
<td>MYOG</td>
<td></td>
<td>Meat yield</td>
<td>te Pas <em>et al.</em>, 1999</td>
</tr>
<tr>
<td>MSTN</td>
<td>Cattle</td>
<td>Yield and eating quality</td>
<td>Grobet <em>et al.</em>, 1998</td>
</tr>
<tr>
<td>CLPG</td>
<td>Sheep</td>
<td>Yield and tenderness</td>
<td>Freking <em>et al.</em>, 2002</td>
</tr>
</tbody>
</table>

**Remarks:** CAST (Calpastatin gene), MSTN (Myostatin gene), HAL (Halothane gene), MC4R (melanocortin 4 receptor gene), IGF2 (Insulin-like growth factor 2 gene), MYOG (myogenin gene), CLPG (callipyge gene)

**Inherited muscle diseases, abnormal phenotypes and their underlying genetics**

Muscle growth and phenotypes can be affected by the mutation of single genes. Examples include the inherited muscle diseases: muscular dystrophy, limb-girdle muscular dystrophy, and other abnormal phenotypes, such as “double-muscling”.

Muscular dystrophy is a genetic disease characterised by a weakness of affected skeletal muscle and it can lead to muscle degeneration and death. There are many types of muscular dystrophy that are caused by mutations in single genes. For example: Duchenne muscular dystrophy (DMD) and Becker muscular dystrophy (BMD) are caused by a mutation in the dystrophin gene. The DMD and BMD patients carry mutations that cause premature translation termination and reduced dystrophin protein levels (Kirchmann *et al.*, 2005). In pigs, DMD mutation results in porcine stress syndrome via myofibrillar degeneration and necrosis in both cardiac and skeletal muscle (Nonneman *et al.*, 2011).
Limb-girdle muscular dystrophy is caused by mutation in the calpain 3 gene (CAPN3) (Hauerslev et al., 2012; Ojima et al., 2011; Kramerova et al., 2008). The disease leads to the development of abnormal sarcomeres, impairment of muscle contractile capacity and the death of muscle fibres (Duguez et al., 2006; Richard et al., 1997; Cohen, 2006).

Other abnormal phenotypes include double-muscling caused by mutation of the myostatin gene (MSTN). This mutation causes increased muscling by increasing the number of muscle fibres that form during myogenesis (see Section 2.3.2). While this has been reported in cattle and sheep, no doubled-muscle phenotype has been reported in pigs.

2.4 Genes that may affect muscle growth traits and that require further investigation in pigs

A small number of genes can have a large effect on muscle growth and meat production traits. Variation in key genes has been linked to production traits in pigs elsewhere, but these genes are poorly understood in New Zealand pigs with no information available on their variation, the frequency of genetic variants, or the effect of the genes or variation in them, in New Zealand pig production systems.

Given the lack of knowledge about genetic variation in New Zealand pigs and the importance that some genes appear to have in meat production, candidate genes were selected for study. The genes were selected based on their understood role in myogenesis or growth in either pigs and/or other animal species (Figure 2.11). The chosen genes belonged to four categories: those involved in myogenesis (MYF5 and MYOG); those involved in hypertrophy (MSTN and CLPG); those involved in the balance of anabolism and catabolism (CAST, CAPN3, LEP, ADRB3); and those involved in health/survival (IGHA). The biology of each gene and how it might affect meat production in pigs, is described below.
2.4.1 The myogenic regulatory factor 5 gene (MYF5)

Myogenic regulatory factor 5, (MYF5) is a protein that controls the proliferation of muscle cells and is involved in the regulation of muscle development. Analysis of MYF5 “knock-out” mice revealed that MYF5 (-/-) embryos do not form skeletal muscles and die at birth (Kaul et al., 2000; Rudnicki et al., 1992; Braun et al., 1992; Rudnicki et al., 1993). In farm animals, variation in MYF5 has been linked to changes in growth and carcass traits. Examples include: changes in muscle size, fat depth, pre-weaning weights, weight gain and feed intake in cattle (Robakowska-Hyzorek et al., 2010; Li et al., 2004); increased carcass and muscle weight in chickens (Yin et al., 2011); and changes in the amount of lean meat on a carcass, loin muscle weight, loin muscle area, intramuscular fat and muscle microstructure in pigs (Verner et al., 2007; Klosowska et al. 2004; Cieslak et al. 2002; te Pas et al., 2000; te Pas et al., 1999).

2.4.2 The myogenin gene (MYOG)

MYOG is an important regulatory factor involved in myogenesis. Expression of MYOG induces muscle differentiation, and the timing of expression affects the number of muscle
fibres that form (te Pas et al., 2000). Analysis of MYOG “knock-out” mice revealed that while MYOG (-/-) embryos can form a primary muscle fibre, secondary myogenic does not occur, resulting in a severe deficiency of skeletal muscle at birth. In pigs, variation in MYOG has been associated with increased meat production, muscle weight, muscle fibre growth and muscle fibre number (Rehfeldt et al., 2011; Kim et al., 2009; te Pas et al., 2000; te Pas et al., 1999; Soumillion et al., 1997).

2.4.3 The myostatin gene (MSTN)

Myostatin controls the number of muscle fibres that form during embryonic development (Hathaway et al., 1994). Analysis of MSTN “knock-out” mice revealed that MSTN (-/-) mice had an increase in the number of muscle fibres and an increase in skeletal muscle mass (Stinckens et al., 2008; Wegner et al., 2000; Smith et al., 2000). Moreover, mutation of MSTN increases the severity of limb-girdle muscular dystrophy (Parsons et al., 2006), as well as Duchenne muscular dystrophy in mice (Bogdanovich et al., 2002). Variation in MSTN has been associated with increased muscle weight (Hadjipavlou et al., 2008) and meat yield (Hickford et al., 2009) in sheep and double-muscling in cattle (McPherron et al., 1997). In pigs, variation in MSTN has been reported in the non-coding regions of the gene (Jiang et al., 2002; Stinckens et al., 2008), but no association has been reported with increased muscle growth.

2.4.4 The callipyge gene (CLPG)

CLPG is a type of mutation that results in muscle hypertrophy and growth in sheep (Koohmararie et al., 1995; Freking et al., 2002; Vuocolo et al., 2007). The mutation is reported to cause larger pelvic limbs (Carpenter et al., 1996; Freking et al., 1998) and to increase muscle size by up to 40%, (Koohmararie et al., 1995; Cockett et al., 2001). This mutation has not been reported in pigs.

2.4.5 The calpastatin gene (CAST)

Calpastatin is a specific inhibitor of calpain proteases. The activity of calpastatin has been linked to protein turnover and meat tenderization (Casas et al., 2006; Sensky et al., 2006; Lindhom-Perry et al., 2009). In CAST “knock-out” mice, muscle fibres are degraded by calpain protease and muscle fails to develop (Sato et al., 2011). Variation in CAST is associated with increased birth weight in sheep (Byun et al., 2008a), as well as effecting longevity and fertility in dairy cattle (Garcia et al., 2006). In pigs, variation in CAST is
associated with meat texture development (Ciobanu et al., 2004; Kent et al., 2004), meat shear force (Lindholm-Perry et al., 2009) and meat colour (Lengerken et al., 1994).

2.4.6 The calpain 3 gene (CAPN3)

Calpain 3 is known as a skeletal muscle-specific calpain protease (Goll et al., 2003; Ojima et al., 2007). It has a crucial role in muscle development and affects myofibrillar degradation and organisation (Beckmann & Spencer, 2008; Chung et al., 2007; Goll et al., 2008). Analysis of CAPN3 “knock-out” mice suggests that CAPN3 has a role in sarcomere formation and turnover (Kramerova et al., 2004), with the loss of CAPN3 leading to mice having abnormal sarcomeres, a loss of muscle fibres and reduced contractile capacity (Duguez et al., 2006). In farm animals, variation in CAPN3 is associated with increased birth weight and post-weaning growth in sheep (Chung et al., 2007), meat tenderness in cattle (Barendse et al., 2008), and increased body weight, carcass weight, breast muscle weight and leg muscle weight in chickens (Zhang et al., 2009). In pigs, variation in CAPN3 is association with an increased proportion of fast glycolytic muscle (Jones et al., 1999; Yang et al., 2007), which has been suggested to cause higher carcass weight.

2.4.7 The leptin gene (LEP)

Leptin is a hormone that controls appetite and is linked to body weight, energy balance and thermogenesis (Barb et al., 2001; Mostyn et al., 2006). Analysis of LEP “knock-out” mice revealed that they had increased food intake and body weight (Friedman & Halaas, 1998). It was suggested that a mutation of LEP resulted in a loss of appetite suppression resulting in over-eating and subsequent obesity (MacDougald et al., 1995; Soukas et al., 2000). Mutation of LEP in mice also inhibited muscular atrophy and enhanced muscle cell proliferation (Sáinz et al., 2009). It has been proposed that leptin affects meat production characteristics (Soukas et al., 2000). In farm animals such as cattle, variation in LEP is associated with feed intake variation (Lagonigro et al., 2003), and carcass fat content and fat deposition (Buchanan et al., 2002; Nkrumah et al., 2004). Liefers et al. (2002) found associations with milk performance in dairy cattle.

In pigs, variation in LEP has been linked to low body fat content (Jiang & Gibson, 1999; Urban et al., 2002; Floris et al., 2004; Silveira et al., 2008), litter size and back-fat thickness (Urban et al., 2002; Chen et al., 2004), average daily gain and lean meat content (Urban et al., 2002), and it has been associated with feed intake and growth rate (Kennes et al., 2001).
2.4.8 The beta 3-adrenergic receptor gene (ADRB3)

The beta3-adrenergic receptor (ADRB3) is involved in the regulation of energy metabolism and thermogenesis (Strosberg, 1997). In ADRB3 “knock-out” mice, lipolysis is reduced resulting in increased fat deposition and decreased lean muscle mass (Revelli et al. 1997). In humans, ADRB3 has been reported to be associated with obesity-related characteristics (Marvelle et al. 2008; Kurokawa, 2011). In sheep, variation in ADRB3 is associated with increased birth weight, growth rate, carcass composition, lamb mortality and cold survival (Forrest et al. 2003, 2006). In pigs, variation in ADRB3 is associated with increased loin eye muscle area (Hirose et al., 2009).

2.4.9 The immunoglobulin A gene (IgA)

Immunoglobulin A (IgA) is an antibody molecule found associated with mucosa (Woof & Kerr, 2004). The heavy chain constant region of IgA consists of three homologous domains (CH1, CH2 and CH3) and a hinge region. The Cα gene (Boehm et al., 1999) encodes these domains and region. The hinge region serves an important function by introducing flexibility to the movement of the antigen binding arms (Woof & Kerr, 2004). This allows an optimal fitting of the antigen-recognition sites to antigenic determinants at varying distances and angles on antigens (Furtado et al., 2004).

Variation in the IgA heavy chain gene (IGHA) has been reported in humans (Senior et al., 2000), horses (Wagner et al., 2003), rabbits (Woof & Kerr, 2004), dogs (Peters et al., 2004), sheep (Zhou et al., 2005) and goats (Zhou et al., 2006). In pigs, there is a single copy of the IgA heavy chain constant gene (IGHA) (Navarro et al., 2000a), but it was found to occur in two forms (Navarro et al., 2000b; Brown et al., 1995). One form (IgAa) encodes a form of with a six amino acid hinge, whereas IgAb encodes an IgA with a two amino acid hinge (Brown et al., 1995).

Variation in IGH A in sheep has been linked to nematode resistant and lower faecal egg counts (Lin et al., 2009). In pigs, IgA levels have been linked to infection of muscle tissue by parasite protozoa, Sarcocystis miescheriana. This parasite is widespread in pigs and results in muscle weakness, anorexia and reduced water intake. Mild infections do not cause clinical signs, but they still reduce weight gain and affect meat quality (Dubey et al., 1989). Despite the importance of IGHA, no reports exist describing if variation in IGHA has been linked to disease resistance, survival or other production traits in pigs.
2.5 A candidate gene approach to finding genes associated with pig growth and carcass traits

Muscle growth is likely affected by a small number of genes of moderate to high effect. These genes influence myogenesis and other metabolic pathways that play important roles in the development of muscle and meat production. It is appropriate to study meat production using a candidate gene approach since this trait has a high heritability implying it is under the control of a small number of genes. Furthermore, the nine genes of interest described above are well researched with information available on their functions and biology.

Given the well described biology of the nine candidate genes selected, these genes are worthy of study in NZ pigs using a candidate gene approach to test if they are linked to meat production.
Chapter 3
Materials and methods

Individual genes can affect muscle growth, meat quality and production traits in pigs. In this thesis, genetic variation in the nine candidate genes was examined in pigs and an analysis undertaken to test if it was associated with production traits. Briefly, polymerase chain reaction (PCR) amplification coupled with single strand conformational polymorphism (SSCP) analysis (PCR-SSCP) and DNA sequencing was used to describe variation in the candidate genes. Next, a descriptive analysis was performed to characterise production traits in NZ pigs and Pearson correlation coefficients were calculated to evaluate relationships between the various production and carcass traits. Finally, analysis of variance and General Linear Mixed-effects Models were used to determine the relationship between any candidate gene variation and the various growth and carcass traits.

3.1 Animal samples

Initially, 100 DNA samples from Thai pigs were analysed to develop the methods to screen for genetic variation in the candidate genes. These 100 samples were supplied by a large scale commercial company that produces over 10,000 pigs per annum. While no pedigree or specific data was available, typically this company’s pigs would be derived from cross-bred Large White, Landrace and Duroc pigs.

To explore the genetic variation in the candidate genes in NZ pigs, an investigation of 374 piglets, recruited from a single farm was undertaken. These pigs were produced from crosses of Large White and Landrace sows and terminal meat line boars that were Duroc or Duroc crossed with Landrace or Large White pigs. Artificial insemination (AI) was used for mating. These piglets could be allocated to seven boar groups, six where a specific boar or boars could be identified and a seventh that included all the other piglets that could not be assigned to any specific boar group (see Table 3.1).

All piglets from the mating were marked within a day of birth using ear notching to define a unique identification number. They were weaned at 14 to 28 days. At weaning, the piglets were weighed, with the weight of each individual pig recorded against its ear tag number. After weaning, all piglets were raised together until they reached a set finishing weight. Prior
to the day of slaughter, the pigs were delivered to the abattoir (Bay View Abattoir, Timaru, New Zealand) and weighed upon arrival to give a live weight at slaughter. Before slaughter, the pigs were rested for a minimum of 12 hours.

On the killing chain, blood samples were collected onto FTA™ cards and then dried and pig ears were collected for matching to their pedigree and production data. Hot carcass weight was determined, prior to the carcasses entering the cooler room. Fat depth between the third and fourth rib on the left side of the hot carcass was measured using a Hennessey back-fat probe. This created a data set for each animal as follows:

1) A pedigree file recording boar ID, sow ID, parity, and time to weaning.
2) Production data collected for weaning-weight (kg), live weight at slaughter (kg), hot carcass weight (kg) and fat depth (mm).
3) A calculated average daily gain (ADG) from weaning to slaughter and an average lean growth rate. These calculations were based upon those described in a publication of the National Pork Producers Council (NPPC) (NPPC, 1999).

### 3.2 DNA purification

For each blood sample, a disc of 1.2 mm in diameter was punched from the FTA card, and the genomic DNA on the card was purified using a rapid two-step procedure (Zhou et al., 2006). Briefly, the disc was transferred into 1.2 mL tube and two hundred µL of 20 mM NaOH solution was added before the tube was incubated for 30 min at room temperature. The solution was then discarded and the disc was equilibrated in 200 µL of 1 x TE buffer (10 mM Tris-HCl, 0.1 mM EDTA, pH 8.0) for 5 min. After removal of the TE buffer, the disc was air-dried prior to its use in PCR.

### 3.3 PCR amplification

The specific PCR primers for each candidate genes were designed based on GenBank derived gene sequences. The primers, the target genes and the regions of the genes targeted, the annealing temperature, the GenBank accession number and the gel conditions used in the SSCP analysis are described in Table 3.2.
## Table 3.1 Boar group of the NZ pigs studied

<table>
<thead>
<tr>
<th>Boar group</th>
<th>Boar ID$^1$</th>
<th>Number of sows mated</th>
<th>Sow parity</th>
<th>Number of piglets</th>
<th>Weaning age (days)</th>
<th>Note for boar pedigree</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1010AI</td>
<td>2</td>
<td>1</td>
<td>16</td>
<td>18, 20, 21</td>
<td>1010 AI (may be a semen mixture)</td>
</tr>
<tr>
<td>1</td>
<td>1010AI/2058*</td>
<td>1</td>
<td>6</td>
<td>7</td>
<td>25</td>
<td>1010 AI followed by 2058</td>
</tr>
<tr>
<td>1</td>
<td>2058*</td>
<td>1</td>
<td>1</td>
<td>11</td>
<td>20, 28</td>
<td>-</td>
</tr>
<tr>
<td>2</td>
<td>356AI/2700</td>
<td>1</td>
<td>1</td>
<td>6</td>
<td>17</td>
<td>356 AI followed by boar 2700</td>
</tr>
<tr>
<td>2</td>
<td>356AI/72/79</td>
<td>1</td>
<td>4</td>
<td>10</td>
<td>27</td>
<td>356 AI followed by boar 72 and 79</td>
</tr>
<tr>
<td>2</td>
<td>356AI/01</td>
<td>1</td>
<td>3</td>
<td>10</td>
<td>14</td>
<td>356 AI followed by boar 01</td>
</tr>
<tr>
<td>2</td>
<td>356AI/41</td>
<td>7</td>
<td>3, 5, 7, 8, 9</td>
<td>70</td>
<td>19, 20, 21</td>
<td>356 AI followed by boar 41</td>
</tr>
<tr>
<td>2</td>
<td>356AI/75</td>
<td>2</td>
<td>6, 8</td>
<td>19</td>
<td>20</td>
<td>356 AI followed by boar 75</td>
</tr>
<tr>
<td>2</td>
<td>356AI/80</td>
<td>1</td>
<td>7</td>
<td>12</td>
<td>21</td>
<td>356 AI followed by boar 80</td>
</tr>
<tr>
<td>2</td>
<td>356AI</td>
<td>8</td>
<td>2, 5, 6, 7, 8, 9</td>
<td>101</td>
<td>19, 20, 21</td>
<td>356 AI (may be a semen mixture)</td>
</tr>
<tr>
<td>3</td>
<td>15</td>
<td>1</td>
<td>1</td>
<td>12</td>
<td>21</td>
<td>-</td>
</tr>
<tr>
<td>4</td>
<td>21</td>
<td>1</td>
<td>3</td>
<td>9</td>
<td>25</td>
<td>-</td>
</tr>
<tr>
<td>5</td>
<td>unknown 1</td>
<td>1</td>
<td>1</td>
<td>8</td>
<td>25</td>
<td>No boar ID available</td>
</tr>
<tr>
<td>6</td>
<td>unknown 2</td>
<td>1</td>
<td>1</td>
<td>11</td>
<td>22</td>
<td>No boar ID available</td>
</tr>
<tr>
<td>7</td>
<td>no boar identified</td>
<td>-</td>
<td>-</td>
<td>72</td>
<td>-</td>
<td>Boar ID and sow ID were not available</td>
</tr>
</tbody>
</table>

Total 374

$^1$Artificial insemination was used in all mating and mixed semen samples were sometimes used. Boar group was therefore based on having either a single sire or at least one sire in common

*2058 was a son of 1010
Table 3.2 PCR primers, annealing temperature for amplification and PCR-SSCP conditions

<table>
<thead>
<tr>
<th>Gene</th>
<th>GenBank accession no.</th>
<th>Region targeted</th>
<th>Forward primer sequence (5’ to 3’)</th>
<th>Reverse primer sequence (5’ to 3’)</th>
<th>Expected amplicon size (bp)</th>
<th>Annealing temperature (°C)</th>
<th>Polyacrylamide Concentration (%)</th>
<th>SSCP electrophoresis condition</th>
</tr>
</thead>
<tbody>
<tr>
<td>MYF5</td>
<td>Y17154</td>
<td>Exon 1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>ctgccagttctgctcgcctc</td>
<td>ttaccatgctgctggagc</td>
<td>480</td>
<td>58</td>
<td>11</td>
<td>260V for 18 h at 28°C</td>
</tr>
<tr>
<td></td>
<td>Y17154</td>
<td>Exon 1&lt;sup&gt;b&lt;/sup&gt;</td>
<td>tagatggtctctgactc</td>
<td>cagaggatctccactctg</td>
<td>350</td>
<td>59</td>
<td>11</td>
<td>300V for 18 h at 25°C</td>
</tr>
<tr>
<td></td>
<td>Y17154</td>
<td>Exon 3</td>
<td>tctctgtgaacccctgac</td>
<td>cctctctcggtgaattag</td>
<td>380</td>
<td>58</td>
<td>14</td>
<td>300V for 18 h at 25°C</td>
</tr>
<tr>
<td>MYOG</td>
<td>U14331</td>
<td>Exon 3</td>
<td>ttccacccactgtgctg</td>
<td>Acaacccactctgccagac</td>
<td>300</td>
<td>60</td>
<td>12</td>
<td>250V for 18 h at 25°C</td>
</tr>
<tr>
<td>MSTN</td>
<td>EF490989</td>
<td>5’UT</td>
<td>catatatcttctattttgtgag</td>
<td>gtaaatataaatactgattt</td>
<td>300</td>
<td>62</td>
<td>14</td>
<td>300V for 18 h at 20°C</td>
</tr>
<tr>
<td></td>
<td>EF490989</td>
<td>Exon 1</td>
<td>aacaagggaaagattgtatgg</td>
<td>tgataacactaactggact</td>
<td>425</td>
<td>60</td>
<td>11</td>
<td>350V for 19 h at 25°C</td>
</tr>
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<td>Exon 2</td>
<td>ctgtcttctgaagagttg</td>
<td>gattgttttgtcctagaae</td>
<td>400</td>
<td>60</td>
<td>12</td>
<td>300V for 18 h at 20°C</td>
</tr>
<tr>
<td></td>
<td>EF490989</td>
<td>Exon 3</td>
<td>actctctctatctcttaacc</td>
<td>tgaacccacataaatctgag</td>
<td>420</td>
<td>60</td>
<td>11</td>
<td>280V for 18 h at 25°C</td>
</tr>
<tr>
<td>CLPG</td>
<td>UY682208</td>
<td>Partial sequence</td>
<td>tagctgctacagagagc</td>
<td>Acgaggagcaaggctg</td>
<td>300</td>
<td>64</td>
<td>14</td>
<td>220V for 19 h at 25°C</td>
</tr>
<tr>
<td>CAST</td>
<td>EU137105</td>
<td>Intron 5</td>
<td>aagcaaaaggaacaccacca</td>
<td>Gctartgctgatgtcggg</td>
<td>350</td>
<td>63</td>
<td>11</td>
<td>370V for 18 h at 4°C</td>
</tr>
<tr>
<td></td>
<td>EU137105</td>
<td>Exon 6</td>
<td>ttgtaaactatctgtagg</td>
<td>Tctctatactgtggtttag</td>
<td>210</td>
<td>58</td>
<td>14</td>
<td>370V for 18 h at 4°C</td>
</tr>
<tr>
<td>CAPN3</td>
<td>NM214171</td>
<td>Exon 1</td>
<td>ttgcaattgctcttcctc</td>
<td>Tcactgctgctcctc</td>
<td>350</td>
<td>63</td>
<td>11</td>
<td>370V for 18 h at 4°C</td>
</tr>
<tr>
<td></td>
<td>NM214171</td>
<td>Exon 5</td>
<td>cttcatctatcttaaggg</td>
<td>cagatccctaactgaagg</td>
<td>200</td>
<td>58</td>
<td>14</td>
<td>370V for 18 h at 4°C</td>
</tr>
<tr>
<td></td>
<td>NM214171</td>
<td>Exon 10</td>
<td>cctctctgtgttctatggag</td>
<td>ctgtgcacactccagg</td>
<td>210</td>
<td>58</td>
<td>14</td>
<td>390V for 19 h at 4°C</td>
</tr>
<tr>
<td></td>
<td>NM214171</td>
<td>Exon 16</td>
<td>cccacccactctctcgc</td>
<td>cattcatgcacagtctcag</td>
<td>250</td>
<td>58</td>
<td>14</td>
<td>370 V for 19 h at 4°C</td>
</tr>
<tr>
<td>LEP</td>
<td>U66254</td>
<td>Exon 3</td>
<td>cccctctctctgctgag</td>
<td>ctgtgcacagtctcctg</td>
<td>300</td>
<td>60</td>
<td>12</td>
<td>220V for 19 h at 22°C*</td>
</tr>
<tr>
<td>ADRB3</td>
<td>AF274007</td>
<td>Exon 2</td>
<td>aggtgctcgctgagagactg</td>
<td>tcaaggtttgagagacagag</td>
<td>300</td>
<td>62</td>
<td>12</td>
<td>300V for 18 h at 20°C</td>
</tr>
<tr>
<td>IGH A</td>
<td>U12594</td>
<td>Intron 1-2</td>
<td>cccacccagctccgttaac</td>
<td>attggagccaggacag</td>
<td>250</td>
<td>62</td>
<td>12</td>
<td>220V for 18 h at 25°C</td>
</tr>
</tbody>
</table>

<sup>a</sup> Primers to amplify fragment of *MYF5* exon 1 of Thai pigs

<sup>b</sup> Primers to amplify fragment of *MYF5* exon 1 of NZ pigs

<sup>*</sup> SSCP electrophoresis condition used for PCR-SLCP screening of variation in *LEP*
PCR amplification was performed in 20 µL reactions containing the washed genomic DNA on a 1.2 mm punch of FTA paper, 0.25 µM of each primer (Integrated DNA Technology, Coralville, IA, USA), 150 µM dNTPs (Eppendorf, Hamburg, Germany), 2.5 mM MgCl₂, 0.5U Taq DNA polymerase (Qiagen) and 1x the reaction buffer supplied with the enzyme. Amplification was carried out in an iCycler (Bio-Rad Laboratories, Hercules, CA, USA). The thermal profile consisted of denaturation at 94°C for 2 min, followed by 35 cycles of 94°C for 30s, annealing at an optimal temperature for each pair of primer (Table 3.1) for 30s, and extension at 72°C for 30s, with a final extension step at 72°C for 5 min. The amplicons were visualized by electrophoresis in 1% agarose (Quantum Scientific, Queensland, Australia) gels using 1x TBE buffer (89 mM Tris, 89 mM boric acid, 2 mM Na₂EDTA), containing 200 ng/mL ethidium bromide. A 2 µL aliquot of PCR product was added to 2 µL of loading dye (0.2% bromophenol blue, 0.2% xylene cyanol, 40% (w/v) sucrose) and the gels were run at a constant 10V/cm for 30 min, prior to visualisation by UV transillumination at 254 nm.

3.4 Detection of genetic variation using PCR-SSCP analysis

A PCR-single strand conformational polymorphism (PCR-SSCP) method was used to screen the PCR products of the various candidate genes. SSCP was performed by mixing a 0.7 µL aliquot of each amplicon with 7 µL of loading dye (98% formamide, 10 mM EDTA, 0.025% bromophenol blue, 0.025% xylene cyanol), before heated at 95 °C for 5 min and rapidly cooling on wet ice. The samples were loaded on 16 x 18 cm, acrylamide:bisacrylamide (37.5:1; Bio-Rad) gels. Electrophoresis was performed using Protean II xi cells (Bio-Rad) in 0.5 x TBE buffer. The specific concentration and SSCP electrophoresis conditions are described in Table 3.2. The SSCP gels were silver-stained according to the method of Byun et al. (2009).

3.5 Detection of genetic variation using PCR-SLCP analysis

A PCR-stem-loop conformational polymorphism (PCR-SLCP) method was used to screen the PCR products produced from LEP and that could not be resolved using PCR-SSCP. In brief, PCR-SLCP uses PCR primers with adapters at the 5’ ends which generate amplicons containing inverted terminal repeat sequences. The adapters chosen were 11-mer sequences (5’gagactgactc 3’) attached to the 5’ ends of both the forward and reverse PCR primers (Zhou
et al., 2011). A 0.7 µL aliquot of each amplicon was mixed with 7 µL of loading dye (98% formamide, 10 mM EDTA, 0.025% bromophenol blue, 0.025% xylene cyanol), before being heated at 95 °C for 5 min and rapidly cooled on wet ice. The samples were loaded on 16 x 18 cm, acrylamide:bisacrylamide (37.5:1; Bio-Rad) gels. Electrophoresis was performed using Protean II xi cells (Bio-Rad) in 0.5 x TBE buffer. The specific electrophoresis conditions are similar to those described for PCR-SSCP analysis. The gels were silver-stained according to the method of Byun et al. (2009).

### 3.6 Cloning of amplicons and screening of the clones

To characterise the sequence variation in the PCR fragments, pig DNA samples representative of different PCR-SSCP patterns were amplified using Pwo Super Yield DNA polymerase (Roche Applied Science, Mannheim, Germany), as described above. The amplicons were ligated into the pCR 4 Blunt-TOPO vectors (Invitrogen, Carlsbad, CA). A 2 µL aliquot of the ligation mixture was used to transform competent *Escherichia coli* cells (One Shot INVαF’ Invitrogen), following the manufacturer’s instructions. Ten insert positive colonies for each transformation were picked and incubated overnight in Terrific broth (Invitrogen, Carlsbad, CA) at 37°C, in a shaking rotary incubator at 225 rpm.

Clones were screened using a PCR-SSCP approach (Zhou & Hickford, 2008) and only those clones for which the PCR-SSCP patterns matched those of the corresponding genomic DNA, were selected for sequencing. Plasmid DNA from selected clones was extracted using a QIAprep Spin Miniprep kit (Qiagen) and sequenced in both directions using the M13 forward and reverse primers. To ensure sequences were error free, identical sequences were obtained from at least three clones, before they were subjected to further analysis.

### 3.7 DNA Sequencing and sequence analysis

Sequence alignment, translation and comparisons were performed using DNAMAN (Version 5.2.10). The BLAST algorithm was used to search the NCBI GenBank databases (http://www.ncbi.nlm.gov/) for homologous sequences.

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3 Publication arising from this thesis
3.8 Identification of the extended haplotypes

It is possible to determine the extended haplotypes, as more than one fragment was amplified from MYF5, CAST and CAPN3. These haplotypes were identified by finding pigs homozygous at one region and then typing these at the other region to ascertain the extended haplotypes. Only some pigs could be haplotyped in this way.

3.9 Statistical analyses

Minitab (Version 16, Minitab Statistical Software, Pennsylvania, Minitab Inc., USA) was used to analyse the data. Pearson correlation coefficients were calculated to test the strength of the linear correlations between the various production traits. General Linear Mixed-effects Models were used to calculate and test correlations between the production data. The data was analysed as follows.

1) Pearson’s correlation

Pearson correlation coefficients were calculated to evaluate the strength of the relationship between weaning-weight, live-weight at slaughter, fat depth and hot carcass-weight. A Pearson correlation was performed with a two-tailed significance level of $\alpha=0.05$.

2) Analysis of variance (ANOVA)

ANOVA was used to assess the relationship between boar group, age to weaning, age to slaughter and the production, growth and carcass data. These factors that were associated with variation in the production, growth and carcass data at a threshold at $P<0.05$ were subsequently factored in the General Linear Mixed-effects Model.

3) General Linear Mixed-effects Model (GLMM) analyses were used to assess the effect of presence (or absence) of particular variants in a pig’s genotype. For each trait, a GLMM was performed for each variant of candidate gene observed in the pig studied. In the model that assessed the effect of variant presence/absence on weaning-weight, age to weaning was included in the model as a co-variate. Age to slaughter was included as co-variate in the models testing the variant effect variant presence/absence on ADG and lean growth rate. In the model to assess variation in fat depth, hot carcass-weight was included as co-variate. Boar group was included as a random effect in each model.
GLMM analyses were performed for each genotype observed in the population if they were above 10%. In the model that assessed the effect of genotype on weaning-weight, weaning age was included in the model as a co-variate. Age to slaughter was included as co-variate in the models testing genotype effect on ADG and lean growth rate. In the model to assess variation in fat depth, hot carcass-weight was included as co-variate. Boar group was included as a random effect in each model.

The statistical model used to test the variant and genotype effects was as follows:

\[
Y_{ijkl} = \mu + \tau_i + \beta_j + X_l + \epsilon_{ijkl}
\]

where \( Y_{ijkl} \) is the phenotypic value for each trait
\( \mu \) is population mean value for each trait
\( \tau_i \) is the fixed factor of variant
\( \beta_j \) is the random factor of boar group effect
\( X_l \) is variation in equal to co-variate (s)
\( \epsilon_{ijkl} \) is the random error

To facilitate understanding of the effects of the genotypes on growth and carcase traits, multiple pair-wise comparisons were performed using least significant difference tests to describe trait differences. A P-value was considered statistically significant when \( P < 0.05 \) and trends were noted when \( 0.05 < P < 0.10 \).
Chapter 4

Results

In chapter 3, genetic variation in New Zealand and Thai pigs was investigated. Nine candidate genes were studied and subsequently tested to see if variation in the genes was associated with production traits.

4.1 Nucleotide variation identified by PCR-SSCP and sequencing in New Zealand and Thai pigs

DNA samples were screened for genetic variation in the candidate genes. The genes screened in the NZ pigs were MYF5, MYOG, MSTN, CLPG, CAST, CAPN3, LEP, ADRB3 and IGHA. In the Thai pigs, only four candidate genes (MYF5, CAST, CAPN3 and IGHA) were investigated. Genetic variation was evident at all the loci tested, with the exception of the fragment of MSTN that was analysed since no variation was found in this study.

4.1.1 Detection of variation in MYF5

Two primer sets were used to amplify fragments of MYF5 exon 1 from NZ and Thai pigs. The first set of primers was designed to detect variation of MYF5 in Thai pigs. This resulted in amplicons of the expected size and four PCR-SSCP banding patterns were observed (Figure 4.1-A). The first set of PCR primers worked well, but considering the size of amplicons may be too big for SSCP (expected size of 480 bp). As a result, a second set of primers was subsequently designed to amplify a smaller (expected size of 350 bp) fragments but covered all known SNPs for SSCP analysis, with the aim of 1) having better resolution for the known variants and 2) being able to detect potential new variations. A second primer set was used in NZ pigs. Two banding patterns (350 bp) were identified in the NZ pigs (Figure 4.1-B).

For the exon 3 fragment of MYF5, the amplicons were of the expected size (300 bp) and the result between Thai and NZ pigs were consistent. Four PCR-SSCP patterns were identified in the NZ pigs (Figure 4.1-C), and three in the Thai pigs. These three variants were identical to the SSCP patterns observed for the NZ pigs.

The four sequences obtained for exon 1 were unique and named variants A, B, C and D. They were deposited into GenBank with accession numbers EU924175-EU924178. Variant A has
two nucleotide substitutions at position 1420 and 1435 relative to the previously reported sequence Y17154. It is notable that Y17154 would specify a stop codon at the nucleotide position 1435, but this was not found in either A or B. Variant B had a further nucleotide difference relative to Y17154 at position 1121, a C/G substitution leading to a putative alanine/proline amino acid change in the peptide. Variant C had a nucleotide difference at positions 1288 relative to Y17154, but this would not change the amino acid sequence. Variant D had both of these nucleotide substitutions.

**PCR-SSCP patterns for MYF5**

![Image of PCR-SSCP patterns for MYF5]

**Figure 4.1 PCR-single-strand conformational polymorphism (PCR-SSCP) analysis of pig MYF5 exon 1 and exon 3 fragments.** A) Four unique banding patterns, representing combinations of the variants A, B, C and D in exon 1 were produced from homozygous and heterozygous Thai pigs. B) two unique banding patterns, representing combinations of variants A and B in exon 1 of NZ pigs, and C) four banding patterns, representing the variants A, B, C and D in exon 3 of the NZ pigs.
Table 4.1 Sequence variation detected in the exon 1 region of porcine MYF5

<table>
<thead>
<tr>
<th>Nucleotide change position</th>
<th>Y17154</th>
<th>XM_001924362</th>
<th>Variant A</th>
<th>Variant B</th>
<th>Variant C</th>
<th>Variant D</th>
<th>Notional amino acid change</th>
</tr>
</thead>
<tbody>
<tr>
<td>1121</td>
<td>GCG</td>
<td>GCG</td>
<td>GCG</td>
<td>CCG</td>
<td>GCG</td>
<td>CCG</td>
<td>Alanine/Proline</td>
</tr>
<tr>
<td>1288</td>
<td>CTG</td>
<td>CTG</td>
<td>CTG</td>
<td>CTG</td>
<td>CTC</td>
<td>CTC</td>
<td>Leucine (no change)</td>
</tr>
<tr>
<td>1420</td>
<td>GAA</td>
<td>GAG</td>
<td>GAG</td>
<td>GAG</td>
<td>GAG</td>
<td>GAG</td>
<td>Glutamic acid (no change)</td>
</tr>
<tr>
<td>1435</td>
<td>TAA²</td>
<td>TAC</td>
<td>TAC</td>
<td>TAC</td>
<td>TAC</td>
<td>TAC</td>
<td>Stop/Tyrosine</td>
</tr>
</tbody>
</table>

1 Position relative to GenBank accession number Y17154
2 In-frame stop codon defined in exon 1 of the Y17154 sequence

In exon 3, four sequences were identified. These resulted from a C/T synonymous substitution at position 2931 and the presence of length variation and nucleotide substitutions at the intron boundary (Figure 4.2). These sequences were named variants A, B, C and D. Variation in the exon sequence of variants A, B and C were deposited into the GenBank (accession numbers EU924179-EU924181). All of these sequences were different to the reference MYF5 sequence (Y17154) and in both the exon (at positions 2970, 2973, 2979 and 2982) and in the intronic boundary region.

The variation detected in both the exon 1 and exon 3 regions allowed the determination of extended MYF5 haplotypes. Four extended haplotypes were identified and summarised in Figure 4.3.
**Figure 4.2** Comparison of the porcine MYF5 sequences in intron 2 and exon 3. Nucleotides in exon 3 are indicated in uppercase and those in intron 2 are in lowercase. Dots have been introduced to improve the alignment and “//” indicates that the sequences are discontinuous. Nucleotides identical to the top sequence are represented by dashes and nucleotide positions refer to GenBank sequence Y17154. A published sequence XM001924362 is derived from using the gene prediction method GNOMON.

**Figure 4.3** The four haplotypes of the porcine MYF5 that were found in the pigs studied.

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4 Information for GNOMON linked: [www.sanger.ac.uk/Projects/S_scrofa](http://www.sanger.ac.uk/Projects/S_scrofa). Downloaded on June the 10th, 2010.
4.1.2 Detection of variation in *MYOG*

Two unique PCR-SSCP banding patterns were detected for porcine *MYOG* exon 3. PCR amplicons of the expected sizes (approximately 300 bp) were obtained (Figure 4.4). For variant *B* was found at a low frequency (n= 12) in the piglets studied, so the two variants were not further sequenced and analysed.

**PCR-SSCP for MYOG**

![Image](image.png)

**Figure 4.4** PCR-single-strand conformational polymorphism (PCR-SSCP) analysis of pig *MYOG* exon 3. Two unique banding patterns, representing combinations of the sequences *A* and *B* in exon 3 were produced from homozygous and heterozygous pigs.

4.1.3 Detection of variation in *MSTN*

In NZ commercial pigs, PCR amplicons of the expected sizes 425, 400 and 420 bp were obtained from *MSTN* exons 1, 2 and 3, respectively while a 300 bp fragment was produced from 5’UT region. PCR-SSCP revealed that there was no variation found in the fragments of *MSTN* that were investigated. Therefore, *MSTN* was not further sequenced or analysed.

4.1.4 Detection of variation in *CLPG*

Amplicons of approximately 300 bp were obtained for the pigs studied. These produced two patterns upon PCR-SSCP analysis. One variant was found to be very rare (n=6) in the pigs, so the two variants found were not sequenced or analysed further.
4.1.5 Detection of variation in CAST

PCR amplicons of the expected size (350 bp and 210 bp for intron 5 and exon 6) were obtained from the pig DNA. Four unique PCR-SSCP banding patterns were obtained for CAST intron 5. These were named variants A, B, C and D (Figure 4.5) and these had four unique sequences (Figure 4.6). Two PCR-SSCP patterns were detected in exon 6 and these were named variants A and B. The sequences revealed that variant B was the result of a substitution of A/G at position 76745 and this substitution would cause a serine/asparagine substitution (Figure 4.6).

PCR-SSCP patterns for CAST intron 5

![PCR-SSCP patterns for CAST intron 5](image)

**Figure 4.5** PCR-single-strand conformational polymorphism (PCR-SSCP) analysis of pig CAST intron 5. Four unique banding patterns, representing combinations of the variants A, B, C and D were produced from the NZ pigs.
Figure 4.6 Nucleotide sequences of \textit{CAST} intron 5 (\textit{CAST-in 5A-5D}) and exon 6 (\textit{CAST-ex6A} and \textit{6B}) sequence variants. Nucleotides in the exon are indicated in upper-case and those in the intron are in lower-case. Nucleotides identical to the published porcine sequence (GenBank accession number EU137105) are presented by dashes. Dots have been introduced to improve the alignment. Vertical arrows indicate the putative splice sites. The highlighted variation at position 76745 represents a nucleotide substitution that would result in serine/asparagine amino acid substitution. The green shaded region with underlining represents the primer regions for \textit{CAST} intron 5 and the yellow shaded region is the primer-binding region for \textit{CAST} exon 6.
The variation detected in the intron 5 and exon 6 regions allowed the determination of \textit{CAST} haplotypes. Five extended haplotypes were identified (Figure 4.7).

\begin{figure}
\centering
\includegraphics[width=0.5\textwidth]{haplotypes.png}
\caption{The five haplotypes of the porcine \textit{CAST} that were found in the pigs studied.}
\end{figure}

\subsection*{4.1.6 Detection of variation in \textit{CAPN3}}

PCR-SSCP analysis was conducted to investigate if sequence variation occurred in \textit{CAPN3} exons 1, 5, 10 and 16. Only one PCR-SSCP pattern was detected for the fragments of \textit{CAPN3} exons 5 and 16 that were amplified. These amplicons were not sequenced. The two patterns identified for both exon 1 and exon 10 were named variants \textit{A} and \textit{B}, and both variants in both regions were sequenced.

Two DNA sequences were obtained corresponding to the \textit{CAPN3} exon 1 variants \textit{A} and \textit{B} and four nucleotide substitutions C/T, A/G, G/A and G/T were detected at positions c.18, c.63, c.66 and c.255, respectively. In the exon 10 region, amplicons for variants \textit{A} and \textit{B} had a substitution at position c.1296. An alignment of the exon 1 and exon 10 sequences with a previously reported porcine \textit{CAPN3} sequence (NM214171) is shown in Figure 4.9. The nucleotide substitutions found in the NZ pigs did not change the putative amino acid sequence of \textit{CAPN3} when comparing the \textit{A} and \textit{B} variants (Figure 4.9).

Three insertions of cytosines at position c.45, c.51 and c.65 were also identified in exon 1 (Figure 4.9). These did not match the sequence NM214171. Furthermore, the putative amino acid sequences for both variant \textit{A} and \textit{B} had seven amino acid differences relative to NM214171 because of frameshefts and the insertion of an additional amino acid (Figure 4.10).
PCR-SSCP for *CAPN3*

A) *CAPN3* exon 1

<table>
<thead>
<tr>
<th></th>
<th>AA</th>
<th>AB</th>
<th>AA</th>
<th>AA</th>
<th>AB</th>
<th>AA</th>
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</thead>
</table>

B) *CAPN3* exon 10

<table>
<thead>
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<th></th>
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<th>AA</th>
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<th>AB</th>
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<th>AB</th>
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</table>

Figure 4.8 PCR-single-strand conformational polymorphism (PCR-SSCP) analysis of pig *CAPN3* exon 1 and exon 10. A) Two unique banding patterns, representing combinations of the sequences A and B in exon 1 were produced from the NZ pigs. B) Two banding patterns, representing the sequences A and B in the exon 10, were detected.
Figure 4.9 Nucleotide sequences of \textit{CAPN3} exon 1 and exon 10 sequence variants. Nucleotides identical to the published porcine sequence (GenBank accession number NM214171) are presented by dashes. Dots have been introduced to improve the alignment. The presence of three cytosines at position c.45, c.51 and c.65 in exon 1 are highlighted in blue. The positions are given relative to the coding in \textit{CAPN3} exon 1 of the porcine \textit{CAPN3} sequence (GenBank NM214171).
Chapter 4: Results

Figure 4.10 Amino acid sequences obtained for CAPN3 exon 1 variants A and B compared with porcine sequence NM214171. Dashes indicate amino acids identical to the top sequence. Differences in the predicted amino acid sequences between variants A, B and NM214171 are highlighted in blue. The numbering is given relative to the first codon in exon 1 of the porcine CAPN3 sequence (GenBank NM214171).

The variation detected in the exon 1 and exon 10 regions allowed the determination of CAPN3 haplotypes. Two haplotypes were identified (Figure 4.11).

Figure 4.11 The two haplotypes of the porcine CAPN3 that were found in the pigs studied.

4.1.7 Detection of variation in LEP

PCR-SSCP analysis was conducted to investigate if sequence variation occurred in porcine LEP exon 3. Three PCR-SSCP patterns were identified for porcine LEP and these were named variants A, B and C. However, variants A, B and C of LEP exon 3 could not be resolved under the same SSCP conditions (Figure 4.12). So, they were resolved using PCR-stem-loop conformational polymorphism analysis (PCR-SLCP) (Figure 4.13) instead.
Chapter 4: Results

**PCR-SSCP for LEP**

A) SSCP condition A

```
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<th>AA</th>
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<th>AB</th>
<th>AC</th>
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</table>
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B) SSCP condition B

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Figure 4.12 PCR-single-strand conformational polymorphism (PCR-SSCP) analysis of pig LEP exon 3. Three unique banding patterns, representing combinations of the variants A, B and C were produced from homozygous and heterozygous pigs. Under SSCP condition A), variant C could not be resolved from variant A, while under SSCP condition B), variant B could not resolved from variant A. The electrophoresis conditions were as follows: A) 4 °C, 280 V, 18 h; B) 21 °C, 240 V, 18 h.

**PCR-SLCP for LEP**

```
<table>
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<tr>
<th></th>
<th>AC</th>
<th>AA</th>
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<th>AC</th>
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</thead>
</table>
```

Figure 4.13 PCR-stem-loop conformational polymorphism (PCR-SLCP) analysis of pig LEP exon 3. Three unique banding patterns, representing combinations of the variants A, B and C were produced from homozygous and heterozygous pigs.
Chapter 4: Results

Following sequence analysis, three sequences were obtained corresponding to variant A, B and C. These variations resulted from two nucleotide substitutions at positions 3469 and 3655 relative to GenBank sequence number U66254 (Figure 4.14).

U66254 CATCCCTGGGCTCCATCCTGCTGAGTTTGTCCAAGATG 3480
variant A -------------------------------------------------------
variant B -------------------C---------------------
variant C -------------------------------------------------------

Nucleotides from position 3481 to 3600 not shown

U66254 AGCTGCCCTTTGCCCCAGCGCAGGGCCCTGGAGACCTTGG 3640
variant A ------------------GC---------------------
variant B ------------------GC---------------------
variant C ------------------GC---------------------

U66254 AGAGCCTGGGCGGCGTCCTGGAAGCCTCCCTCTACTCCAC 3680
variant A -------------------------------------------------------
variant B -------------------------------------------------------
variant C --------------A-------------------------

Figure 4.14 The three sequences of porcine LEP exon 3: variant A, B and C are compared with the porcine sequence U66254. A dash indicates nucleotides identical to the top sequence. The highlighted nucleotide represents a substitution at position 3655 that would result in an amino acid change from valine to isoleucine. The nucleotide positions are given relative to a porcine LEP reference sequence (GenBank accession number U66254).

4.1.8 Detection of variation in ADRB3

Sequence analysis showed two unique nucleotide sequences and named variants A and B (Figure 4.15). Variant B was found to contain nucleotide variation at position 2215 relative to variant A, but this did not change the putative amino acid sequence. A difference in variant B was also detected at position 2238 where it contained an insertion of a sixth thymine in a repeating sequencing of five thymine bases relative to variant A (Figure 4.16). The insertion putatively causes a frameshift mutation and generates a stop codon at amino acid 405. The ADRB3 exon 2 sequences are present in Figure 4.17.
Chapter 4: Results

PCR-SSCP for ADRB3

Figure 4.15 PCR-single-strand conformational polymorphism (PCR-SSCP) analysis of pig ADRB3 exon 2. Two unique banding patterns, representing combinations of the variants A and B of exon 2 were produced from homozygous and heterozygous pigs.

<table>
<thead>
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<th>Sequence</th>
<th>Description</th>
<th>Position</th>
</tr>
</thead>
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<tr>
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<td>variant A</td>
<td>2240</td>
</tr>
<tr>
<td>AB252782 AAGTCCCTGACCTTGATCCATTTTCCCCTAATGTCCCTGT</td>
<td>variant B</td>
<td>2280</td>
</tr>
<tr>
<td>AB252782 TCCAACCCTCTGCGCCCTCAGTTTATCTCCATTTTCAGGG</td>
<td>variant A</td>
<td>2320</td>
</tr>
<tr>
<td>AB252782 TTGATCCAGAACCTTTGGAAAGCCTCTGGCCTTGTACAGA</td>
<td>variant B</td>
<td>2399</td>
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</tbody>
</table>

Figure 4.16 Sequence variation detected in ADRB3 exon 2. Variants A and B are compared with the porcine sequence AB252782. A dash indicates nucleotides identical to the top sequence. The highlighted nucleotide represents a nucleotide substitution at position 2338 that would result in a stop codon being produced in exon 2 of AB252782. The positions are given relative to a porcine ADRB3 reference sequence (GenBank accession number AB252782).
Chapter 4: Results

4.1.9 Detection of variation in \textit{IGHA}

PCR-SSCP analysis was conducted to investigate if sequence variation occurred in the porcine \textit{IGHA} intron 1- exon 2 region. Three PCR-SSCP banding patterns were detected (Figure 4.18).

\textbf{PCR-SSCP for \textit{IGHA}}

![PCR-SSCP for \textit{IGHA}]

**Figure 4.18** PCR-single-strand conformational polymorphism (PCR-SSCP) analysis of the pig \textit{IGHA} gene. Three unique banding patterns, representing combinations of the variants \textit{A}, \textit{B} and \textit{C} were revealed in the \textit{IGHA} hinge region.

Sequence analyses revealed the three PCR-SSCP patterns represented three different nucleotide sequences. These sequences were named \textit{A}, \textit{B} and \textit{C}. Variants \textit{A} and \textit{B} were matched to the previously reported porcine \textit{IGHA} sequences (GenBank accession numbers...
U12594 and S71099, respectively). Variant C contained an AG deletion relative to A and B (Figure 4.19). These results have been published (Kunhareang et al., 2009\(^5\)).

\[
\begin{array}{cccccccc}
 & c.302 & c.314 \\
\hline
\text{Variant A} & \text{acttctctctcag} & ^{TTTACCTTCAG\hat{\text{AG}}} & \text{TCATGTCCCCAG} \\
\text{(V)} & L & P & S & D & P & C & P & Q \\
\text{Variant B} & \ldots \ldots \ldots \ldots & \hat{\ldots} \ldots \ldots \ldots & \hat{\ldots} \ldots \ldots \ldots \\
\text{(D)} & P & C & P & Q \\
\text{Variant C} & \ldots \ldots \ldots \ldots & \hat{\ldots} \ldots \ldots \ldots & \hat{\ldots} \ldots \ldots \ldots \\
\text{(D)} & P & C & P & Q \\
\end{array}
\]

\textbf{Figure 4.19 Polymorphism of the porcine IGHA gene.} Nucleotides in the exon are indicated in upper-case and those in the intron are in lower-case. Amino acids are represented in one-letter code and shown in bold. Nucleotides identical to the top sequence are represented by dashes. Nucleotides in the putative exon 2 are shaded. The putative splice-sites are indicated by a vertical arrow. Dots have been introduced to improve alignment. The last nucleotide of exon 1 contributes the first nucleotide of the initial codon of the hinge region and the corresponding amino acids are shown in brackets.

In summary, PCR-SSCP coupled with sequencing analysis identified nineteen sequence variants (Table 4.2). Of these, 15 nucleotide substitutions were detected including variation in exon 1 and exon 3 of MYF5, CAST intron 5, CAST exon 6, CAPN3 exon 1 and exon 10, LEP exon 3 and IGHA intron 1-exon 2. Other variation included deletions in CAST intron 5 and the IGHA intron 1-exon 2 region and an insertion of thymine in ADRB3 exon 2.

\section*{4.2 Sequence variation and genotype frequencies for the candidate genes in the New Zealand and Thai pigs}

The frequencies of the variants of the nine candidate genes investigated in the NZ and Thai pigs are given in Table 4.3. Four of the genes were only studied in the Thai pigs; including MYF5 exons 1 and 3, CAST intron 5 and exon 6, CAPN3 exon 10 and IGHA intron 1-exon 2.

Variation in MYF5 exon 1 in the Thai pigs was found to be greater than in the NZ pigs. While, four variants were identified in the Thai pigs, there were only two variants (A and B)

\(^5\) Publication arising from this thesis
observed in NZ pigs. Variant A was the most common variant with a high frequency in the Thai pigs and the seven of boar groups of the NZ pigs. In the Thai pigs variant C was also present at a high frequency (25%). In exon 3, there were four variants identified in NZ pigs, but only three variants (variants A, B and C) were found in the Thai pigs. Of the three variants identified in the Thai pigs, the frequency of variant C was higher than in all the NZ boar groups.

The frequency of the variants of CAST intron 5 and exon 6 were similar in the NZ and Thai pigs. There were three variants of CAST intron 5 identified in the Thai pigs and four variants detected in the NZ pigs, but variant D identified in the NZ pigs was at a very low frequency (less than 10%), with the exception of a boar group 3 (29%). There were two variants identified in CAST exon 6 and the frequency of these variants was similar in both the NZ and Thai pigs.

Two variants of CAPN3 exon 10 were identified in the NZ and Thai pigs, but variant B had a very low frequency in the NZ pigs.

Variant A and B of IGH A were found in both the NZ and Thai pigs, but variant C was not identified in the Thai pigs studied.

Two variants of MYOG and CLPG were observed in the NZ pigs. However, while variant A was common, variant B was found at a very low frequency. Because of the very low frequency, the variation was deemed unsuitable for further sequencing or statistical testing.

LEP exon 3 variants A and B were the most common variants accounting for 92 to 100% of the population. Two variants of ADRB3 exon 2 were identified, with variant A the most common in all of the boar groups. This variation accounted for 85 to 100% of the population, whereas variant B was present at a low frequency.
Table 4.2 Summary of the nucleotide variations found in the candidate genes in the New Zealand pigs

<table>
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<tr>
<th>Candidate gene and region</th>
<th>Nucleotide variation</th>
<th>Putative amino acid change</th>
<th>GenBank no.</th>
<th>Reference</th>
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<td>MYF5 exon 1</td>
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<td>Leucine/Proline</td>
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1 Position refers to GenBank accession number.
2 Nucleotide position are relative to the first nucleotide of the coding region.

Publication arising from this thesis
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</tr>
</tbody>
</table>

* Boar group 7 included progeny for which no boar could be assigned
4.3 Association of candidate genes with growth and carcass traits in New Zealand pigs

4.3.1 Summary of the production data for the pigs studied

The mean of production traits in NZ pigs was calculated and summarised in Table 4.4.

Table 4.4 Summary of NZ pigs studied

<table>
<thead>
<tr>
<th>Variable</th>
<th>n</th>
<th>Mean</th>
<th>SD</th>
<th>Minimum</th>
<th>Maximum</th>
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</thead>
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<tr>
<td>Age to weaning (day)</td>
<td>374</td>
<td>22</td>
<td>3.79</td>
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<td>31</td>
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<td>Age to slaughter (day)</td>
<td>374</td>
<td>109</td>
<td>6.84</td>
<td>92</td>
<td>127</td>
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<tr>
<td>Weaning weight (kg)</td>
<td>369</td>
<td>6.6</td>
<td>1.72</td>
<td>2.4</td>
<td>12.3</td>
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<td>Live weight (kg)</td>
<td>216</td>
<td>87.2</td>
<td>7.68</td>
<td>65</td>
<td>110</td>
</tr>
<tr>
<td>Carcass weight (kg)</td>
<td>374</td>
<td>65.1</td>
<td>7.04</td>
<td>44.8</td>
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<td>Fat depth (mm)</td>
<td>374</td>
<td>10.4</td>
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<td>ADG (g/day)</td>
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<td>938.7</td>
<td>131.8</td>
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<td>Lean growth rate (g/day)</td>
<td>369</td>
<td>361.6</td>
<td>60.4</td>
<td>227.7</td>
<td>565.8</td>
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</tbody>
</table>

4.3.2 Correlations between pig production, growth and carcass traits

Pearson correlation coefficients were calculated to estimate the direction and strength of the linear relationship between the production, growth and carcass traits. These are tabulated in Table 4.5.

The analyses revealed a positive correlation between weaning-weight and live-weight ($r=0.321$, $P<0.01$), weaning-weight and hot carcass-weight ($r=0.370$, $P<0.01$), weaning-weight and ADG ($r=0.346$, $P<0.01$) and weaning-weight and lean growth rate ($r=0.398$, $P<0.01$). Live-weight and fat depth ($r=0.232$, $P<0.01$) were correlated and a strong, and not unexpected, positive correlation was found between live-weight and hot carcass-weight ($r=0.928$, $P<0.01$), live-weight and ADG ($r=0.812$, $P<0.01$), and live-weight and lean growth rate ($r=0.79$, $P<0.01$). A high correlation was also found between ADG and lean growth ($r=0.942$, $P<0.01$). While a positive correlation was found between lean growth rate and fat depth ($r=0.289$, $P<0.01$), no correlation was found between ADG and fat depth. There was no correlation between weaning-weight and fat depth.
Table 4.5 Pearson correlation coefficients between the production, growth and carcass traits

<table>
<thead>
<tr>
<th></th>
<th>Weaning-weight</th>
<th>Live-weight</th>
<th>Hot carcass-weight</th>
<th>Fat depth</th>
<th>ADG</th>
</tr>
</thead>
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<td>0.321**</td>
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<td></td>
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</tr>
<tr>
<td>Hot carcass-weight</td>
<td>0.370**</td>
<td>0.927**</td>
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</tr>
<tr>
<td>Fat depth</td>
<td>0.094</td>
<td>0.232**</td>
<td>0.338**</td>
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<tr>
<td>ADG</td>
<td>0.346**</td>
<td>0.812**</td>
<td>0.745**</td>
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<tr>
<td>Lean growth rate</td>
<td>0.398**</td>
<td>0.790**</td>
<td>0.905**</td>
<td>0.289**</td>
<td>0.942**</td>
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</tbody>
</table>

** Correlation is significant at the 0.01 level (2-tailed)

### 4.3.3 Association analysis of *MYF5* variation and weaning weight and fat depth in NZ pigs

A general linear model revealed that there was no association between pigs carrying different variants at *MYF5* exon 1 and production traits. There were associations detected between *MYF5* exon 3 variation and changes in weaning-weight and fat depth. The estimated marginal means of the traits for the presence/absence of variants of *MYF5* exon 1 and exon 3 are shown in Table 4.6.

**Association of *MYF5* exon 3 with weaning weight and fat depth**

Analysis of associations between *MYF5* exon 3 and the production traits revealed a significant association with the presence of variant A being associated with increased weaning weight. In addition, the presence of variant A was also associated with decreased fat depth (Table 4.6).

**Genotype analyses**

Genotypes of *MYF5* were analysed for the association of genotypes at exon 1 and exon 3 and various production traits. There was no association between the genotypes AA and AB at exon 1 and the traits (Table 4.7). Similarly, there were no associations between genotypes of exon 3 and variation in the measured traits. Table 4.8 summarises the estimated marginal means for the various genotypes of exon 3.
### Chapter 4: Results

Table 4.6 Association between variation in *MYF5* exon 1 and exon 3 and variation in production, growth and carcass traits in NZ pigs.

<table>
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<tr>
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<th>Status</th>
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<th>Weaning-weight n</th>
<th>Live-weight n</th>
<th>Hot carcass-weight n</th>
<th>Fat depth n</th>
<th>ADG $^3$ n</th>
<th>Lean growth rate $^3$ n</th>
</tr>
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<tr>
<td><strong>MYF5 exon 1</strong></td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>A</td>
<td>Present</td>
<td>369</td>
<td>-</td>
<td>216</td>
<td>374</td>
<td>-</td>
<td>374</td>
<td>214</td>
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<td>Absent</td>
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<td>-</td>
<td>0</td>
<td>0</td>
<td>-</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>B</td>
<td>Present</td>
<td>65</td>
<td>6.6±0.24</td>
<td>87.6±1.68</td>
<td>66</td>
<td>6.6±0.24</td>
<td>936.5±22.1</td>
<td>377.6±8.61</td>
</tr>
<tr>
<td></td>
<td>Absent</td>
<td>304</td>
<td>6.8±0.15</td>
<td>87.8±0.94</td>
<td>308</td>
<td>6.6±0.15</td>
<td>931.7±15.9</td>
<td>379.0±6.52</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(P=0.265)</td>
<td>(P=0.858)</td>
<td>(P=0.259)</td>
<td>(P=0.617)</td>
<td>(P=0.701)</td>
<td>(P=0.829)</td>
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<tr>
<td><strong>MYF5 exon 3</strong></td>
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<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>A</td>
<td>Present</td>
<td>346</td>
<td>6.8±0.16</td>
<td>87.8±0.96</td>
<td>350</td>
<td>65.7±0.71</td>
<td>350</td>
<td>201</td>
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<tr>
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<td>Absent</td>
<td>10</td>
<td>5.7±0.53</td>
<td>87.8±3.69</td>
<td>11</td>
<td>65.3±2.28</td>
<td>11</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(P=0.031)</td>
<td>(P=0.996)</td>
<td>(P=0.825)</td>
<td>(P=0.617)</td>
<td>(P=0.701)</td>
<td>(P=0.829)</td>
</tr>
<tr>
<td>B</td>
<td>Present</td>
<td>25</td>
<td>6.8±0.35</td>
<td>87.5±2.33</td>
<td>25</td>
<td>6.6±0.35</td>
<td>953.0±37.54</td>
<td>384.2±14.15</td>
</tr>
<tr>
<td></td>
<td>Absent</td>
<td>331</td>
<td>6.8±0.16</td>
<td>87.9±0.96</td>
<td>336</td>
<td>65.8±0.72</td>
<td>336</td>
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<tr>
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<td></td>
<td>(P=0.888)</td>
<td>(P=0.857)</td>
<td>(P=0.321)</td>
<td>(P=0.993)</td>
<td>(P=0.742)</td>
<td>(P=0.299)</td>
</tr>
<tr>
<td>C</td>
<td>Present</td>
<td>77</td>
<td>6.7±0.22</td>
<td>88.1±1.39</td>
<td>79</td>
<td>65.9±0.99</td>
<td>79</td>
<td>44</td>
</tr>
<tr>
<td></td>
<td>Absent</td>
<td>279</td>
<td>6.8±0.16</td>
<td>87.8±1.01</td>
<td>282</td>
<td>65.7±0.75</td>
<td>282</td>
<td>162</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(P=0.464)</td>
<td>(P=0.857)</td>
<td>(P=0.753)</td>
<td>(P=0.929)</td>
<td>(P=0.740)</td>
<td>(P=0.966)</td>
</tr>
<tr>
<td>D</td>
<td>Present</td>
<td>34</td>
<td>6.6±0.31</td>
<td>88.1±2.31</td>
<td>35</td>
<td>65.5±1.40</td>
<td>35</td>
<td>14</td>
</tr>
<tr>
<td></td>
<td>Absent</td>
<td>322</td>
<td>6.8±0.16</td>
<td>87.9±0.96</td>
<td>325</td>
<td>65.7±0.72</td>
<td>326</td>
<td>192</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(P=0.489)</td>
<td>(P=0.922)</td>
<td>(P=0.827)</td>
<td>(P=0.493)</td>
<td>(P=0.667)</td>
<td>(P=0.460)</td>
</tr>
</tbody>
</table>

$^1$ Least square mean±standard error and P value derived from GLMM with variant present/absent as fixed effect and boar group as random effect.

$^2$ *P*<0.05 in bold

$^3$ Least square mean±standard error calculated from weaning-weight to slaughter-weight
### Table 4.7 The effect of *MYF5* exon 1 genotypes on variation in production, growth and carcass traits in NZ pigs.

<table>
<thead>
<tr>
<th>Traits</th>
<th>n</th>
<th>AA</th>
<th>n</th>
<th>AB</th>
<th>n</th>
<th>AC</th>
<th>n</th>
<th>AD</th>
<th>n</th>
<th>BC</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weaning weight (kg)</td>
<td>310</td>
<td>6.8±0.15</td>
<td>59</td>
<td>6.5±0.25</td>
<td>0.223</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Live weight (kg)</td>
<td>189</td>
<td>87.8±0.94</td>
<td>27</td>
<td>88.1±1.75</td>
<td>0.848</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hot carcass-weight (kg)</td>
<td>314</td>
<td>65.7±0.70</td>
<td>60</td>
<td>64.9±1.13</td>
<td>0.416</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fat depth (mm)</td>
<td>314</td>
<td>10.1±0.20</td>
<td>60</td>
<td>10.3±0.33</td>
<td>0.479</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ADG&lt;sup&gt;2&lt;/sup&gt; (g/day)</td>
<td>187</td>
<td>930.8±15.94</td>
<td>27</td>
<td>942.1±22.60</td>
<td>0.546</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lean growth rate&lt;sup&gt;2&lt;/sup&gt; (g/day)</td>
<td>313</td>
<td>378.7±6.52</td>
<td>60</td>
<td>379.6±8.74</td>
<td>0.890</td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
</tbody>
</table>

1. Least square mean±standard error and P value derived from GLMM with genotype variation as fixed effect and boar group as random effect.
2. Least square mean±standard error calculated from weaning-weight to slaughter-weight.

### Table 4.8 The effect of *MYF5* exon 3 genotypes on variation in production, growth and carcass traits in NZ pigs.

<table>
<thead>
<tr>
<th>Traits</th>
<th>n</th>
<th>AA</th>
<th>n</th>
<th>AB</th>
<th>n</th>
<th>AC</th>
<th>n</th>
<th>AD</th>
<th>n</th>
<th>BC</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weaning weight (kg)</td>
<td>225</td>
<td>6.9±0.16</td>
<td>22</td>
<td>6.8±0.36</td>
<td>67</td>
<td>6.8±0.22</td>
<td>32</td>
<td>6.7±0.32</td>
<td>3</td>
<td>6.1±0.92</td>
<td>0.213</td>
</tr>
<tr>
<td>Live weight (kg)</td>
<td>139</td>
<td>87.8±1.01</td>
<td>11</td>
<td>86.3±2.49</td>
<td>40</td>
<td>87.9±1.43</td>
<td>13</td>
<td>87.6±2.37</td>
<td>2</td>
<td>93.3±5.62</td>
<td>0.812</td>
</tr>
<tr>
<td>Hot carcass-weight (kg)</td>
<td>228</td>
<td>65.7±0.75</td>
<td>22</td>
<td>63.2±1.62</td>
<td>68</td>
<td>65.9±1.01</td>
<td>12</td>
<td>66.2±1.42</td>
<td>3</td>
<td>71.9±4.12</td>
<td>0.203</td>
</tr>
<tr>
<td>Fat depth (mm)</td>
<td>228</td>
<td>10.2±0.21</td>
<td>22</td>
<td>10.1±0.47</td>
<td>68</td>
<td>9.9±0.30</td>
<td>32</td>
<td>10.1±0.41</td>
<td>3</td>
<td>10.5±1.23</td>
<td>0.175</td>
</tr>
<tr>
<td>ADG&lt;sup&gt;2&lt;/sup&gt; (g/day)</td>
<td>138</td>
<td>928.9±16.32</td>
<td>11</td>
<td>902.9±32.04</td>
<td>39</td>
<td>932.9±21.21</td>
<td>13</td>
<td>939.5±29.78</td>
<td>2</td>
<td>1043.4±66.3</td>
<td>0.568</td>
</tr>
<tr>
<td>Lean growth rate&lt;sup&gt;2&lt;/sup&gt; (g/day)</td>
<td>227</td>
<td>378.2±6.66</td>
<td>22</td>
<td>361.3±11.92</td>
<td>68</td>
<td>378.2±8.40</td>
<td>32</td>
<td>388.8±10.50</td>
<td>5</td>
<td>430.6±27.66</td>
<td>0.174</td>
</tr>
</tbody>
</table>

1. Least square mean±standard error and P value derived from GLMM with genotype variation as fixed effect and boar group as random effect.
2. Least square mean±standard error calculated from weaning-weight to slaughter-weight.
4.3.4 Association analysis of CAST variation and production, growth and carcass traits in NZ pigs

A general linear mixed-effects model revealed that there was no significant difference between pigs carrying different variants of CAST exon 6. There were however associations detected between CAST intron 5 variation and variation in live-weight, fat depth, ADG, and lean growth rate. The estimated marginal means of the traits for the presence/absence of variants of CAST intron 5 and exon 6 are shown in Table 4.9.

Association of CAST intron 5 variation with live-weight

A general linear mixed-effects model revealed that CAST intron 5 variation was associated with variation in some of the traits measured. Specifically, the model suggested that the presence of variant C in CAST intron 5 was associated with increased live-weight, with a live weight of 89.3±1.14 kg compared to 86.8±1.02 kg, when the variant was absent.

Association of CAST intron 5 variation with ADG

An analysis of association between variant C and D, and ADG, revealed a significant association; with the presence of variant C being associated with an ADG of 1005.3±19.85 g/day compared to 967±18.11 g/day when the variant was absent. Similarly, the presence of variant D was associated with increased ADG (1020.7±30.43 g/day present versus 951.6±11.88 g/day absent).

Association of CAST intron 5 variation with fat depth

An association was detected between variant D and increased fat depth, with a fat depth of 11.2±0.45 mm (variant present) compared to 9.9±0.20 mm, when the variant was absent.

Association of CAST intron 5 and exon 6 variation with lean growth rate

Analysis of the association between variant C of CAST intron 5 and lean growth rate also revealed a significant effect. The present of variant C was associated with an increased lean growth rate of 384.2±9.34 g/day compared to 371.9±8.37 g/day when variant was absent.

In addition, the presence of variant B in CAST exon 6 tended to be associated with increased lean growth rate (P=0.064). The presence of variant B in CAST exon 6 was associated with a
higher lean growth rate (383.1±7.22 g/day when present versus 372.8±7.83 g/day when absent).

**Genotype analyses**

There were no significant associations between genotypes of CAST intron 5 (Table 4.10) and exon 6 (Table 4.11) and production traits, but the exon 6 AB genotype of CAST tended to be associated with increased lean growth rate (P=0.065). An analysis of CAST exon 6 showed that the AB genotype had a high lean growth rate, with a lean growth rate of 386.7±7.42 g/day compared to the homozygous genotypes AA and BB (376.8±6.97 g/day and 376.7±8.61 g/day, respectively).
Table 4.9 Association between variation in CAST intron 5 and exon 6 and variation in production, growth and carcass traits in NZ pigs.

<table>
<thead>
<tr>
<th>Region/variant</th>
<th>Status</th>
<th>n</th>
<th>Weaning-weight (kg)</th>
<th>Live-weight (kg)</th>
<th>Hot carcass-weight (kg)</th>
<th>Fat depth (mm)</th>
<th>ADG (g/day)</th>
<th>Lean growth rate (g/day)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CAST intron 5</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A</td>
<td>Present</td>
<td>295</td>
<td>6.8±0.16</td>
<td>174</td>
<td>87.5±0.98</td>
<td>299</td>
<td>65.7±0.72</td>
<td>172</td>
</tr>
<tr>
<td></td>
<td>Absent</td>
<td>71</td>
<td>6.7±0.23</td>
<td>42</td>
<td>88.9±1.40</td>
<td>72</td>
<td>65.0±1.04</td>
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</tr>
<tr>
<td></td>
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<td></td>
<td>(P=0.760)</td>
<td>(P=0.310)</td>
<td>(P=0.409)</td>
<td>(P=0.592)</td>
<td>(P=0.380)</td>
<td>(P=0.140)</td>
</tr>
<tr>
<td>B</td>
<td>Present</td>
<td>147</td>
<td>6.6±0.19</td>
<td>86</td>
<td>87.9±1.13</td>
<td>149</td>
<td>65.2±0.85</td>
<td>85</td>
</tr>
<tr>
<td></td>
<td>Absent</td>
<td>219</td>
<td>6.9±0.16</td>
<td>130</td>
<td>87.7±1.04</td>
<td>222</td>
<td>65.8±0.75</td>
<td>129</td>
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<tr>
<td></td>
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<td>(P=0.124)</td>
<td>(P=0.879)</td>
<td>(P=0.459)</td>
<td>(P=0.569)</td>
<td>(P=0.120)</td>
<td>(P=0.213)</td>
</tr>
<tr>
<td>C</td>
<td>Present</td>
<td>130</td>
<td>6.7±0.16</td>
<td>78</td>
<td>89.3±1.14</td>
<td>133</td>
<td>66.5±0.86</td>
<td>78</td>
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<tr>
<td></td>
<td>Absent</td>
<td>236</td>
<td>6.9±0.19</td>
<td>138</td>
<td>86.8±1.02</td>
<td>238</td>
<td>65.1±0.75</td>
<td>136</td>
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<tr>
<td></td>
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<td>(P=0.117)</td>
<td>(P=0.024)</td>
<td>(P=0.09)</td>
<td>(P=0.732)</td>
<td>(P=0.037)</td>
<td>(P=0.046)</td>
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<td>D</td>
<td>Present</td>
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<td>6.6±0.34</td>
<td>10</td>
<td>91.2±2.54</td>
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<td>64.4±1.53</td>
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<tr>
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<td>Absent</td>
<td>341</td>
<td>6.8±0.16</td>
<td>206</td>
<td>87.6±0.94</td>
<td>346</td>
<td>65.8±0.72</td>
<td>204</td>
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<tr>
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<td>(P=0.640)</td>
<td>(P=0.149)</td>
<td>(P=0.367)</td>
<td>(P=0.007)</td>
<td>(P=0.042)</td>
<td>(P=0.901)</td>
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<tr>
<td>CAST exon 6</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>A</td>
<td>Present</td>
<td>282</td>
<td>6.8±0.16</td>
<td>174</td>
<td>87.5±1.01</td>
<td>286</td>
<td>66.1±0.73</td>
<td>286</td>
</tr>
<tr>
<td></td>
<td>Absent</td>
<td>68</td>
<td>6.7±0.23</td>
<td>41</td>
<td>88.9±1.4</td>
<td>69</td>
<td>64.8±1.05</td>
<td>69</td>
</tr>
<tr>
<td></td>
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<td>(P=0.747)</td>
<td>(P=0.608)</td>
<td>(P=0.230)</td>
<td>(P=0.480)</td>
<td>(P=0.859)</td>
<td>(P=0.161)</td>
</tr>
<tr>
<td>B</td>
<td>Present</td>
<td>224</td>
<td>6.7±0.17</td>
<td>138</td>
<td>88.3±1.02</td>
<td>228</td>
<td>66.1±0.77</td>
<td>228</td>
</tr>
<tr>
<td></td>
<td>Absent</td>
<td>126</td>
<td>6.8±0.19</td>
<td>77</td>
<td>86.9±1.19</td>
<td>127</td>
<td>65.4±0.85</td>
<td>127</td>
</tr>
</tbody>
</table>

1 Least square mean±standard error and P value derived from GLMM with variant variation as fixed effect and boar group as random effect.
2 P<0.05 in bold
3 Least square mean±standard error calculated from weaning-weight to slaughter-weight
### Table 4.10 The effect of CAST intron 5 genotypes and variation in production, growth and carcass traits in NZ pigs.

<table>
<thead>
<tr>
<th>Traits</th>
<th>n</th>
<th>AA</th>
<th>AB</th>
<th>AC</th>
<th>BB</th>
<th>BC</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weaning-weight (kg)</td>
<td>106</td>
<td>6.8±0.20</td>
<td>6.6±0.22</td>
<td>7.0±0.22</td>
<td>6.6±0.38</td>
<td>6.3±0.40</td>
<td>6.8±0.29</td>
</tr>
<tr>
<td>Live-weight (kg)</td>
<td>67</td>
<td>86.1±1.21</td>
<td>86.6±1.3</td>
<td>88.8±1.37</td>
<td>89.7±2.8</td>
<td>84.5±2.70</td>
<td>90.2±1.71</td>
</tr>
<tr>
<td>Hot carcass-weight (kg)</td>
<td>107</td>
<td>65.5±0.89</td>
<td>65.6±0.98</td>
<td>66.8±0.99</td>
<td>63.5±1.70</td>
<td>62.1±1.79</td>
<td>65.8±1.28</td>
</tr>
<tr>
<td>Fat depth (mm)</td>
<td>107</td>
<td>9.8±0.26</td>
<td>10.2±0.28</td>
<td>10.0±0.29</td>
<td>11.1±0.50</td>
<td>9.8±0.53</td>
<td>10.1±0.38</td>
</tr>
<tr>
<td>ADG (g/day)</td>
<td>66</td>
<td>914.0±17.29</td>
<td>929.8±19.78</td>
<td>946.6±20.01</td>
<td>963.7±35.31</td>
<td>918.7±34.23</td>
<td>956.1±24.24</td>
</tr>
<tr>
<td>Lean growth rate (g/day)</td>
<td>107</td>
<td>375.4±7.16</td>
<td>381.7±8.14</td>
<td>384.9±8.11</td>
<td>368.2±12.42</td>
<td>360.9±13.12</td>
<td>384.0±10.01</td>
</tr>
</tbody>
</table>

1. Least square mean±standard error and P value derived from GLMM with genotype variation as fixed effect and boar group as random effect.
2. Least square mean±standard error calculated from weaning-weight to slaughter-weight
Table 4.11 The effect of *CAST* exon 6 genotypes and variation in production, growth and carcass traits in NZ pigs.

<table>
<thead>
<tr>
<th>Traits</th>
<th>n</th>
<th>AA</th>
<th>Mean±SEM</th>
<th>n</th>
<th>AB</th>
<th>Mean±SEM</th>
<th>n</th>
<th>BB</th>
<th>Mean±SEM</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weaning-weight (kg)</td>
<td>125</td>
<td>6.8±0.19</td>
<td>157</td>
<td>6.8±0.18</td>
<td>68</td>
<td>6.7±0.23</td>
<td>0.860</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Live-weight (kg)</td>
<td>77</td>
<td>86.8±1.20</td>
<td>97</td>
<td>88.1±1.13</td>
<td>41</td>
<td>88.9±1.42</td>
<td>0.576</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hot carcass-weight (kg)</td>
<td>126</td>
<td>65.4±0.85</td>
<td>160</td>
<td>66.6±0.83</td>
<td>69</td>
<td>64.9±1.05</td>
<td>0.132</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fat depth (mm)</td>
<td>126</td>
<td>9.9±0.25</td>
<td>160</td>
<td>10.2±0.24</td>
<td>69</td>
<td>10.0±0.31</td>
<td>0.528</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ADG² (g/day)</td>
<td>76</td>
<td>922.3±17.22</td>
<td>96</td>
<td>941.1±17.67</td>
<td>41</td>
<td>941.7±21.33</td>
<td>0.548</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lean growth rate² (g/day)</td>
<td>126</td>
<td>376.1±6.97</td>
<td>159</td>
<td>386.7±7.24</td>
<td>69</td>
<td>376.7±8.61</td>
<td>0.065</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

1 Least square mean±standard error and P value derived from GLMM with genotype variation as fixed effect and boar group as random effect.
2 Least square mean±standard error calculated from weaning-weight to slaughter-weight.
4.3.5 No association detected between LEP variation and production traits, but possible association with hot carcass-weight in NZ pigs

An association analysis was conducted between the variation in LEP exon 3 and variation in the production and carcass traits. A general linear mixed-effects model suggested that the presence (or absence) of variation in LEP was not associated with any of the traits measured. The estimated marginal means are shown in Table 4.12.

The presence of variant C of LEP exon 3 tended (P=0.102) to be associated with increased hot carcass-weight (68.6±1.96 kg when present compared to 65.5±0.71 kg when absent). In addition, C tended (P=0.106) to be associated with increased lean growth rate, with a lean growth rate of 393.4±14.67 g/day when present compared to 372.3±8.01 g/day, when the variant was absent.

Genotype analyses

The data revealed no significant difference between genotypic variation in LEP exon 3 and production traits. The estimated marginal means are shown in Table 4.13.

4.3.6 Association analysis of ADRB3 exon 2 variation and weaning-weight, hot carcass-weight and fat depth in NZ pigs

Association of ADRB3 variation with weaning weight

Analysis of associations between ADRB3 variation and the production traits revealed a significant association between the present variant B of ADRB3 exon 2 and weaning-weight, but not with live-weight, ADG and lean growth rate. As can be seen in Table 4.14, the presence of variant B was associated with a weaning-weight of 7.2±0.25 kg compared to 6.7±0.15 kg, when the variant was absent.

Association of ADRB3 variation with hot carcass-weight

A significant association was detected between variant B and hot carcass-weight (Table 4.14). The presence of variant B was associated with increased hot carcass-weight, with a hot carcass-weight of 67.6±1.14 kg compared to 65.4±0.71 kg, when the variant was absent.
Association of *ADRB3* variation with fat depth

Statistical analysis of the association between variant *A* of *ADRB3* exon 2 and fat depth revealed a significant effect with the presence of variant *A* being associated with a lower fat depth (10.1±0.19 mm) compared to 16.0±1.46 mm when the variant is absent (Table 4.14).

Genotypes analyses

Further analyses of the effect of *ADRB3* genotype on production traits revealed that there was a significant association between *ADRB3* genotype and fat depth (Table 4.15). The presence of the *BB* genotype (n=2) was associated with increased fat depth. There were no associations between genotypic variation and live-weight, ADG and lean growth rate, but variation of genotypes *AB* and *BB* tended to be associated with increased weaning-weight (P=0.061) and hot carcass-weight (P=0.109).

4.3.7 Association analysis of *IGHA* and production, growth and carcass traits in NZ pigs

A general linear mixed model revealed that significant differences were detected between pigs carrying different variants of *IGHA* and the production traits (Table 4.16).

Association of *IGHA* variation with fat depth

Statistical analysis of the association between the presence and absence of variant *B* of *IGHA* and fat depth revealed a significant effect. The presence of variant *B* was associated with a lower fat depth (9.8±0.26 mm) compared to 10.3±0.21 mm when the variant was absent (Table 4.16), but no association was detected with weaning-weight, live-weight, hot carcass-weight, ADG or lean growth rate. In addition, the data revealed no significant relationship between genotypic variation in *IGHA* and the traits (Table 4.17).
Table 4.12 Association between variation in *LEP* exon 3 and variation in production, growth and carcass traits in NZ pigs

<table>
<thead>
<tr>
<th>Region/variant</th>
<th>Status</th>
<th>Weaning-weight (kg)</th>
<th>n</th>
<th>Live-weight (kg)</th>
<th>n</th>
<th>Hot carcass-weight (kg)</th>
<th>n</th>
<th>Fat depth (mm)</th>
<th>n</th>
<th>ADG(^2) (g/day)</th>
<th>n</th>
<th>Lean growth rate(^2) (g/day)</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
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</tr>
<tr>
<td><em>LEP</em> exon 3</td>
<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>A</td>
<td>Present</td>
<td>6.8±0.15</td>
<td>342</td>
<td>87.8±0.95</td>
<td>204</td>
<td>65.7±0.70</td>
<td>347</td>
<td>10.1±0.21</td>
<td>347</td>
<td>946.9±21.20</td>
<td>202</td>
<td>388.3±8.95</td>
<td>346</td>
</tr>
<tr>
<td></td>
<td>Absent</td>
<td>6.4±0.35</td>
<td>24</td>
<td>87.4±2.69</td>
<td>9</td>
<td>64.2±1.57</td>
<td>24</td>
<td>9.8±0.47</td>
<td>9</td>
<td>922.7±36.24</td>
<td>24</td>
<td>377.4±13.01</td>
<td>24</td>
</tr>
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<td></td>
<td></td>
</tr>
<tr>
<td>B</td>
<td>Present</td>
<td>6.6±0.19</td>
<td>148</td>
<td>88.2±1.27</td>
<td>76</td>
<td>65.2±0.87</td>
<td>151</td>
<td>10.1±0.25</td>
<td>151</td>
<td>939.5±25.89</td>
<td>75</td>
<td>384.0±10.19</td>
<td>150</td>
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<tr>
<td></td>
<td>Absent</td>
<td>6.8±0.16</td>
<td>218</td>
<td>87.7±0.98</td>
<td>137</td>
<td>65.8±0.74</td>
<td>220</td>
<td>10.2±0.21</td>
<td>220</td>
<td>930.1±25.74</td>
<td>136</td>
<td>381.7±10.31</td>
<td>220</td>
</tr>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>Present</td>
<td>6.8±0.44</td>
<td>14</td>
<td>89.2±2.45</td>
<td>11</td>
<td>68.6±1.96</td>
<td>14</td>
<td>10.2±0.58</td>
<td>14</td>
<td>947.7±34.21</td>
<td>11</td>
<td>393.4±14.67</td>
<td>14</td>
</tr>
<tr>
<td></td>
<td>Absent</td>
<td>6.8±0.15</td>
<td>352</td>
<td>87.7±0.95</td>
<td>202</td>
<td>65.5±0.71</td>
<td>357</td>
<td>10.1±0.21</td>
<td>357</td>
<td>921.9±21.78</td>
<td>200</td>
<td>372.3±8.01</td>
<td>356</td>
</tr>
<tr>
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<td></td>
</tr>
</tbody>
</table>

1 Least square mean±standard error and P value derived from GLMM with varaint variation as fixed effect and boar group as random effect.
2 Least square mean±standard error calculated from weaning-weight to slaughter-weight
Table 4.13 The effect of *LEP* exon 3 genotypes and variation in production, growth and carcass traits in NZ pigs.

<table>
<thead>
<tr>
<th>Traits</th>
<th>n</th>
<th>AA</th>
<th>Mean±SEM$^1$</th>
<th>n</th>
<th>AB</th>
<th>Mean±SEM</th>
<th>n</th>
<th>AC</th>
<th>Mean±SEM</th>
<th>n</th>
<th>BB</th>
<th>Mean±SEM</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weaning-weight (kg)</td>
<td>204</td>
<td>6.8±0.16</td>
<td></td>
<td>125</td>
<td>6.7±0.21</td>
<td></td>
<td>13</td>
<td>6.9±0.45</td>
<td></td>
<td>23</td>
<td>6.4±0.36</td>
<td></td>
<td>0.633</td>
</tr>
<tr>
<td>Live-weight (kg)</td>
<td>127</td>
<td>87.5±1.01</td>
<td></td>
<td>76</td>
<td>88.3±1.33</td>
<td></td>
<td>10</td>
<td>89.6±2.56</td>
<td></td>
<td>8</td>
<td>87.7±2.85</td>
<td></td>
<td>0.889</td>
</tr>
<tr>
<td>Hot carcass-weight (kg)</td>
<td>207</td>
<td>65.6±0.75</td>
<td></td>
<td>127</td>
<td>65.3±0.91</td>
<td></td>
<td>13</td>
<td>68.6±2.02</td>
<td></td>
<td>23</td>
<td>63.9±1.60</td>
<td></td>
<td>0.535</td>
</tr>
<tr>
<td>Fat depth (mm)</td>
<td>207</td>
<td>10.1±0.21</td>
<td></td>
<td>127</td>
<td>10.1±0.27</td>
<td></td>
<td>13</td>
<td>10.2±0.61</td>
<td></td>
<td>23</td>
<td>9.8±0.48</td>
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<td>0.980</td>
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<tr>
<td>ADG$^2$ (g/day)</td>
<td>126</td>
<td>929.7±16.30</td>
<td></td>
<td>66</td>
<td>938.7±18.97</td>
<td></td>
<td>10</td>
<td>951.2±32.52</td>
<td></td>
<td>8</td>
<td>910.0±35.52</td>
<td></td>
<td>0.775</td>
</tr>
<tr>
<td>Lean growth rate$^2$ (g/day)</td>
<td>206</td>
<td>379.2±6.68</td>
<td></td>
<td>127</td>
<td>376.1±7.56</td>
<td></td>
<td>13</td>
<td>397.8±14.26</td>
<td></td>
<td>23</td>
<td>364.6±11.71</td>
<td></td>
<td>0.377</td>
</tr>
</tbody>
</table>

$^1$ Least square mean±standard error and P value derived from GLMM with genotype variation as fixed effect and boar group as random effect.

$^2$ Least square mean±standard error calculated from weaning-weight to slaughter-weight
### Table 4.14 Association between variation in ADRB3 exon 2 and variation in production, growth and carcass traits in NZ pigs

<table>
<thead>
<tr>
<th>Region/Variant</th>
<th>Status</th>
<th>n</th>
<th>Weaning-weight (kg)</th>
<th>Live-weight (kg)</th>
<th>Hot carcass-weight (kg)</th>
<th>Fat depth (mm)</th>
<th>ADG ^1</th>
<th>Lean growth rate ^3</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>Present</td>
<td>367</td>
<td>6.8±0.15</td>
<td>214</td>
<td>65.6±0.70</td>
<td>372</td>
<td>10.1±0.19</td>
<td>936.3±16.50</td>
</tr>
<tr>
<td></td>
<td>Absent</td>
<td>2</td>
<td>7.5±1.12</td>
<td>2</td>
<td>67.1±5.02</td>
<td>2</td>
<td>16.9±1.46</td>
<td>879.2±68.66</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(P=0.483)</td>
<td>(P=0.693)</td>
<td>(P=0.776)</td>
<td>(P=0.000)</td>
<td>(P=0.400)</td>
<td>(P=0.701)</td>
</tr>
<tr>
<td>B</td>
<td>Present</td>
<td>56</td>
<td>7.2±0.25</td>
<td>40</td>
<td>67.6±1.14</td>
<td>56</td>
<td>9.9±0.34</td>
<td>901.1±38.11</td>
</tr>
<tr>
<td></td>
<td>Absent</td>
<td>313</td>
<td>6.7±0.15</td>
<td>176</td>
<td>65.4±0.71</td>
<td>313</td>
<td>10.1±0.21</td>
<td>914.3±37.11</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(P=0.019) ^2</td>
<td>(P=0.606)</td>
<td>(P=0.035) ^2</td>
<td>(P=0.600)</td>
<td>(P=0.420)</td>
<td>(P=0.111)</td>
</tr>
</tbody>
</table>

1. Least square mean±standard error and P value derived from GLMM with variant variation as fixed effect and boar group as random effect.
2. P<0.05 in bold
3. Least square mean±standard error calculated from weaning-weight to slaughter-weight
4. n=2, a value considered too low to make a safe comparison

---

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Chapter 4: Results

Table 4.15 The effect of ADRB3 exon 2 genotypes and variation in production, growth and carcass traits in NZ pigs.

<table>
<thead>
<tr>
<th>Traits</th>
<th>n</th>
<th>AA Mean±SEM</th>
<th>n</th>
<th>AB Mean±SEM</th>
<th>n²</th>
<th>BB Mean±SEM</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weaning-weight (kg)</td>
<td>313</td>
<td>6.7±0.15</td>
<td>54</td>
<td>7.3±0.25</td>
<td>2</td>
<td>7.6±1.12</td>
<td>0.061</td>
</tr>
<tr>
<td>Live-weight (kg)</td>
<td>176</td>
<td>87.7±0.96</td>
<td>38</td>
<td>88.5±1.52</td>
<td>2</td>
<td>85.7±5.55</td>
<td>0.769</td>
</tr>
<tr>
<td>Hot carcass-weight (kg)</td>
<td>318</td>
<td>65.4±0.71</td>
<td>54</td>
<td>67.5±1.15</td>
<td>2</td>
<td>67.1±5.00</td>
<td>0.109</td>
</tr>
<tr>
<td>Fat depth (mm)</td>
<td>318</td>
<td>10.1±0.19</td>
<td>54</td>
<td>9.7±0.33</td>
<td>2</td>
<td>16.9±1.45</td>
<td>0.000</td>
</tr>
<tr>
<td>ADG³ (g/day)</td>
<td>174</td>
<td>929.7±16.06</td>
<td>38</td>
<td>942.9±20.47</td>
<td>2</td>
<td>885.7±68.01</td>
<td>0.571</td>
</tr>
<tr>
<td>Lean growth rate³ (g/day)</td>
<td>317</td>
<td>377.1±6.55</td>
<td>54</td>
<td>388.2±8.72</td>
<td>2</td>
<td>374.9±34.55</td>
<td>0.279</td>
</tr>
</tbody>
</table>

1 Least square mean±standard error and P value derived from GLMM with genotype variation as fixed effect and boar group as random effect.
2 n=2, a value considered too low to make a safe comparison
3 Least square mean±standard error calculated from weaning-weight to slaughter-weight
### Table 4.16 Association between variation in IGHA and variation in production, growth and carcass traits in NZ pigs

<table>
<thead>
<tr>
<th>Region/variant</th>
<th>Status</th>
<th>n</th>
<th>Weaning-weight (kg)</th>
<th>n</th>
<th>Live-weight (kg)</th>
<th>n</th>
<th>Hot carcass-weight (kg)</th>
<th>n</th>
<th>Fat depth (mm)</th>
<th>n</th>
<th>ADG (g/day)</th>
<th>n</th>
<th>Lean growth rate (g/day)</th>
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<tr>
<td><strong>IGHA</strong></td>
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<td></td>
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<tr>
<td>A</td>
<td>Present</td>
<td>348</td>
<td>6.7±0.16</td>
<td>199</td>
<td>88.1±0.95</td>
<td>352</td>
<td>65.7±0.71</td>
<td>352</td>
<td>10.1±0.21</td>
<td>198</td>
<td>937.2±19.15</td>
<td>351</td>
<td>384.1±7.82</td>
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<tr>
<td></td>
<td>Absent</td>
<td>21</td>
<td>6.9±0.36</td>
<td>17</td>
<td>85.2±1.99</td>
<td>22</td>
<td>64.8±1.59</td>
<td>22</td>
<td>9.6±0.47</td>
<td>16</td>
<td>894.4±26.87</td>
<td>22</td>
<td>371.3±11.8</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>B</td>
<td>Present</td>
<td>117</td>
<td>6.7±0.20</td>
<td>64</td>
<td>86.7±1.25</td>
<td>118</td>
<td>64.9±0.89</td>
<td>118</td>
<td>9.8±0.26</td>
<td>64</td>
<td>911.5±21.54</td>
<td>118</td>
<td>378.8±8.33</td>
</tr>
<tr>
<td></td>
<td>Absent</td>
<td>252</td>
<td>6.8±0.16</td>
<td>152</td>
<td>88.2±0.98</td>
<td>256</td>
<td>65.9±0.73</td>
<td>256</td>
<td>10.3±0.21</td>
<td>150</td>
<td>920.1±19.49</td>
<td>255</td>
<td>376.7±8.97</td>
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<tr>
<td>C</td>
<td>Present</td>
<td>46</td>
<td>6.5±0.27</td>
<td>30</td>
<td>86.8±1.64</td>
<td>48</td>
<td>66.1±1.21</td>
<td>48</td>
<td>10.3±0.35</td>
<td>29</td>
<td>920.5±23.86</td>
<td>48</td>
<td>383.4±10.02</td>
</tr>
<tr>
<td></td>
<td>Absent</td>
<td>323</td>
<td>6.8±0.15</td>
<td>186</td>
<td>87.9±0.95</td>
<td>326</td>
<td>65.6±0.71</td>
<td>326</td>
<td>10.1±0.21</td>
<td>185</td>
<td>911.1±19.56</td>
<td>325</td>
<td>372.1±8.18</td>
</tr>
</tbody>
</table>

1. Least square mean±standard error and P value derived from GLMM with variant variation as fixed effect and boar group as random effect.
2. P<0.05 in bold
3. Least square mean±standard error calculated from weaning-weight to slaughter-weight.
Table 4.17 The effect of IGHA genotypes and variation in production, growth and carcass traits in NZ pigs.

<table>
<thead>
<tr>
<th>Traits</th>
<th>n</th>
<th>AA</th>
<th>n</th>
<th>AB</th>
<th>n</th>
<th>AC</th>
<th>n</th>
<th>BB</th>
<th>n</th>
<th>BC</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weaning-weight (kg)</td>
<td>209</td>
<td>6.8±0.16</td>
<td>104</td>
<td>6.6±0.21</td>
<td>35</td>
<td>6.5±0.30</td>
<td>10</td>
<td>7.7±0.51</td>
<td>10</td>
<td>6.5±0.51</td>
<td>0.106</td>
</tr>
<tr>
<td>Live-weight (kg)</td>
<td>122</td>
<td>88.7±1.03</td>
<td>56</td>
<td>86.9±1.36</td>
<td>21</td>
<td>87.2±1.88</td>
<td>7</td>
<td>83.8±2.99</td>
<td>9</td>
<td>86.4±2.73</td>
<td>0.442</td>
</tr>
<tr>
<td>Hot carcassweight (kg)</td>
<td>211</td>
<td>65.9±0.76</td>
<td>105</td>
<td>65.2±0.95</td>
<td>36</td>
<td>66.3±1.34</td>
<td>10</td>
<td>62.9±2.27</td>
<td>11</td>
<td>66.5±2.23</td>
<td>0.756</td>
</tr>
<tr>
<td>Fat depth (mm)</td>
<td>211</td>
<td>10.2±0.22</td>
<td>105</td>
<td>9.8±0.28</td>
<td>36</td>
<td>10.4±0.39</td>
<td>10</td>
<td>9.2±0.68</td>
<td>11</td>
<td>9.9±0.66</td>
<td>0.413</td>
</tr>
<tr>
<td>ADG² (g/day)</td>
<td>121</td>
<td>939.8±16.41</td>
<td>56</td>
<td>928.4±19.57</td>
<td>21</td>
<td>941.9±25.04</td>
<td>7</td>
<td>863.8±37.10</td>
<td>8</td>
<td>919.5±36.13</td>
<td>0.418</td>
</tr>
<tr>
<td>Lean growth rate² (g/day)</td>
<td>210</td>
<td>708.9±13.47</td>
<td>105</td>
<td>698.1±16.06</td>
<td>36</td>
<td>708.3±20.55</td>
<td>10</td>
<td>656.1±30.45</td>
<td>11</td>
<td>707.7±29.65</td>
<td>0.593</td>
</tr>
</tbody>
</table>

¹ Least square mean±standard error and P value derived from GLMM with genotype variation as fixed effect and boar group as random effect.
² Least square mean±standard error calculated from weaning-weight to slaughter-weight
In this study, variation in nine candidate genes that may affect muscle growth was investigated in NZ pigs and Thai pigs (see chapter 3.1). PCR-SSCP and PCR-SLCP analysis coupled with DNA sequencing analysis were used to identify sequence variation in these genes. Eight (*MYF5, MYOG, CLPG, CAST, CAPN3, LEP, ADRB3* and *IGHA*) out of the nine genes were variable and four of these genes (*MYF5, CAST, CAPN3* and *IGHA*) were variable in both the NZ and Thai pigs. Of these nine candidate genes, the variation in *MYF5, CAST, ADRB3* and *IGHA* were associated with muscle growth and/or carcass traits. The variation and the associations of these genes are discussed below.

**MYF5**

The *MYF5* has 1,534 coding nucleotides. This gene consists of three exons, separated by two introns (te Pas et al. 1999). This study focused on variation in *MYF5* exon 1 (666 nucleotides) and exon 3 (792 nucleotides), that account for 95% of gene. The genetic variations in exon 1 and exon 3 of *MYF5* were compared with *MYF5* GenBank sequence Y17154.

All the *MYF5* variants (variant A, B, C, D) in exon 1 identified in this study possessed a cytosine at position 1435 (Appendix A), resulting in a sequence of TAC which is tyrosine codon whereas Y17154 has a TAA as a stop codon signal. This difference may be response for the result of a protein with 23 amino acids longer in *MYF5* variants A-D. The difference in the nucleotide sequence particularly at position 1435 is not only found in the present study. It was also reported in other species, for example chicken (Saitoh et al., 1993) and cattle (Li et al., 2004). This variation was also found in GenBank sequence NM_001278775, suggesting this is a possible of a sequencing error in the Y17154 sequence.

If genetic expression is presented, the nucleotide substitutions at positions 1121 and 1288 would be the first reported amino acid substitution in *MYF5* in pigs. Liu et al. (2007) did not find variation in this position, but reported variation at position 1205 in exon 1 which expressed differently between different pig populations. The non-detection of this amino acid substitution in previous reports could be the result of those previous studies investigating a limited number of samples and/or less sensitive typing methods (Zhou & Hickford, 2008).
In addition, a substantial difference was observed between NZ and Thai pigs. For exon 1, variant A and B were found in NZ pigs while four variants: A, B, C and D were identified in the Thai pigs. Variant A was found at a high frequency (82-100%) in the NZ pigs compared to variant B (11-18%). Variant A (68%) and variant C (25%) were found frequently in Thai pigs, while variant B (6%) and variant D (1%) were rarely found. It cannot be confirmed what this may reflect but a possible explanation is that it could be a consequence of selection for production, inbreeding or a founder effect.

Nonetheless, the substitution of alanine with proline has a biological consequence for MYF5 function. Although both amino acids have similar charges, proline is an amino acid that can disrupt protein secondary structure (Williamson, 1994; Moriarty & Raleigh, 1999). As a consequence, proline substitution could affect the function of the MYF5 during myogenesis and potentiate muscle growth, and hence affect meat production in pigs.

Robakowska-Hyzorek et al. (2010) reported that variation found in cattle MYF5 exon 1 may affect the NH-terminal transactivation domain of the MYF5. This could influence the binding of MYF5 to other transcriptional factors. This domain has been described with putative binding sites for transcription factors lymphoid enhancer-binding factor 1 (LEF-1) and homeobox protein 5 (Hoxa5) (Merrill et al., 2001). LEF-1 transcription factor is an element of canonical Wnt/β-catenin/ TCF pathway, which is important to induce myogenesis (Ridgeway et al., 2000; Brunelli et al. 2007).

In this study, there were missing thymine bases in the intron 2 such that the length variation was noticed in the intron boundary of MYF5 (Figure 4.2). The length variation found in MYF5 close to the splicing site between intron 2 and exon 3. Genetic variation located at intronic boundary regions can affect mRNA splicing, and consequence to affect the amino acid sequence produced from the transcript (Le Hir et al., 2003; Wang et al., 2007). It results in a splicing error by changing the core donor site (Wang & Burge, 2008) and thus influenced the pre-mRNA secondary structure during skeletal muscle development (Clancy, 2008; Faustino & Cooper, 2003). This could influence the function of MYF5 (Maak et al., 2006) and results in increased muscle growth in pigs.

In addition, Drogemuller and Kempers (2000) reported variation in cattle MYF5 intron 2 at position 1009. The authors suggested that this variation related to the transcription start site of MYF5, which is responsible of MYF5 activation in ventral somitic domains (Ribas et al., 2011; Pownall et al., 2002). Moreover, previous studies have revealed that variation of MYF5
Chapter 5: Discussion

at position 1,948 of intron 2 (GenBank accession No. M95684) (Li et al., 2004) had significant effect on body weight in Korean cattle (Bhuiyan et al., 2009) and growth traits in Qinchuan (Chinese cattle) (Zhang et al., 2007). Other studies also suggest that single nucleotide polymorphism in intron regions had significant associations with carcass traits in cattle (Sherman et al., 2008). These results may suggest that variation in intron region is possibly linked to another variation in the coding regions for the growth traits.

For exon 3, four variants (A, B, C and D) found in NZ pigs and three variants (A, B and C) were found in Thai pigs. The abundance of each variant found varied among the seven boar groups of NZ pigs investigated (Chapter 3.1), with variant A was the most common in all the groups (61-92%). This implies that exon 3 varaint A is in a haplotype with exon 1 variant A, since variant A of both exon 1 and exon 3 is high in the NZ and Thai pigs. This is consistent with the haplotypes analysis (Figure 4.3).

Following the study in genetic variation, the association between genetic variation in MYF5, and variation in muscle growth and carcass traits were explored in 374 NZ pigs. The present study did not find an association between genetic variation in MYF5 exon 1 and muscle growth and carcass traits. However, such association was found between the present of the variant A of exon 3 with increased weaning-weight and decreased fat depth. This finding is consistent with previous reports (Liu et al., 2007; Verner et al., 2007; Cieslak et al., 2002). Liu et al., (2007) and Cieslak et al. (2002) found association in non-coding variation in MYF5 associated with meat and fat deposition. While, other variation in MYF5 exon 2 was associated with increased lean meat content and loin weight (Verner et al., 2007). In addition, associations were found between linked mutations g.580C/T and 613C/T in promoter region and meat production traits (Urbanski et al., 2006); the effect found significant on weight of ham, area of loin eye, and meat content of carcasses. Furthermore, association between variation in MYF5 and productive traits has reported in other meat animal for example cattle (Li et al., 2004).

For pigs, weaning-weight is one of the main indicators of the growth potential of piglets and ultimately reflects their mature body weight potential (Wolter & Ellis, 2001). Mahan & Lepine (1991) and Graham et al. (1981) have suggested that weaning-weight can affect post-weaning growth in the subsequent growing-finishing period, with pigs of less than 5.0 kg at weaning having good growth responses in the nursery, but lower weight gains overall and more time spent reaching market weight than heavier weaned-pigs (Mahan & Lepine, 1991). This suggests that pigs of a heavier weaning-weight may adapt more rapidly to a diet
than lighter weight pigs. The association between variant A of MYF5 exon 3 and weaning-weight and fat depth, may therefore suggest that MYF5 may be a candidate gene for genetic selection for growth of pigs. This is makes it important to further investigate the genetic variation of MYF5 in different pig populations. If variation and its association is representative, variation of MYF5 may be useful as selection tool to improve growth and productive traits in pigs.

**MYOG, MSTN and CLPG**

Other three candidate genes (MYOG, MSTN and CLPG) that are potentially of interest in muscle growth and carcass traits were also studied in the NZ pigs. In this study, the detection of variation in MYOG and CLPG were of low frequency, and variation of MSTN was not found (Table 4.3). Therefore, these three genes were not sequenced or further analysed. Although the variant frequency of these genes detected here was relatively low frequency, it would be worth to say that these three candidate genes should be put aside for the future investigation if improving meat production is of interest.

**CAST**

The CAST contains of thirty-five exons spanning nearly 123 kb (Meyers & Beever, 2008). In this study, variations in CAST in the two regions: CAST intron 5 and CAST exon 6 were studied due to the high variability (Meyers & Beever, 2008; Nonneman et al., 2011).

There were seven nucleotide differences observed in CAST intron 5 (see Table 4). Of these seven variations, three nucleotide variations at positions 76337, 76338, and 76468 have found in the present study. Variation in CAST intron 5 at positions 76337 (variant A) and 76338 (variant D) are located close to the splice site (see Figure 4.6). The variations that located in the intron region can influence splicing error by changing the core donor site (Wang & Burge, 2008) and subsequently influenced the pre-mRNA secondary structure (Le Hir et al., 2003; Greenwood & Kelsoe, 2003). This variation may affect on RNA processing and stability and subsequently affect the amino acid sequence and/or function of CAST.

Furthermore, the substitution of C/T at position 76468 (variant B) creates a 5 thymine base repeat. The repeat of thymine is hotspots for cis-synthymine dimer formation and to cause bending DNA (Wang & Taylor, 1991). Thymine dimer structures have reported to play a role in a number of important protein-mediated processes (Crothers et al., 1990) such as recombination, transcription, and replication (Wada-Kiyama & Kiyama, 1996; Zahn &
Thus, it is possible that the substitution at position 76468 might be related to the distinctive structure and affect gene expression.

In exon 6, the substitution of A/G has affected a putative amino acid changing serine to asparagine (Ciobanu et al., 2004; Meyers & Beever, 2008), and both serine and asparagine are in polar neutral side chains. This substitution could not make much on protein structure of CAST. However, previous study have been reported that the amino acid substitution might predict to affect the physiological and chemical properties of CAST protein including hydrophobicity, amphiphilicity and net charge of protein (Uversky, 2002). Subsequently, it might influence its structure and effect on the activity of Ca\(^{2+}\) channel (Hao et al., 2000), and thus it might regulate calpain activity and afterwards meat tenderness and growth rate.

Kristensen et al. (2002) suggest that the activity of CAST is correlated with muscle growth and the rate of proteolytic changes and the post mortem tenderization of meat. It has also been suggested that the calpain-calpastatin system has a crucial effect on a number of muscle fibres (Goll et al., 2003). The accelerated growth of the skeletal muscles may be due to reduced protein degradation caused either by the reduced activity of calpain or by a significant increase in the activity of CAST (Goll et al., 2008).

Moreover, there is documentation that Ca\(^{2+}\) channel-activity is regulated by CAST domain L (encoded by exon 6), with Ciobanu et al. (2004) describing variation in the domain L that is involved in the activation of Ca\(^{2+}\) channels. The authors also reported an amino acid substitution in this region that has been associated with pork quality, especially tenderness. Others studies have also suggested genetic variation in CAST may affect carcass and meat quality (Kocwin-Podsiadla et al. 2003, Rybarczyk et al. (2012). The results reported by Rybarczyk et al. (2012) show an association between variation in CAST and carcass quality and meat composition in Pietrain crossbred pigs. In a study by Kuryl et al. (2003) a significant association was found between variation in CAST and the meat content of carcasses and backfat thickness in Stamboek pigs, and meat deposition and carcass composition in Landrace pigs, Yorkshire pigs and crossbred pigs (Krzęcio et al., 2008).

In addition, Choi et al (2006) also reported an association between variation in CAST and growth traits and birth weight in Korean crossbred pigs. Other studies reported a relationship between variation in CAST and birth weight and growth rate to weaning in lambs (Byun et al., 2008). Similarly, in the present study, an association between variation in CAST intron 5 and production traits was observed. The pigs carrying variant C had a higher live weight, ADG
and lean growth rate, whereas the presence of variant $D$ resulted in increased ADG. It is possible that variation in $CAST$ could be influencing muscle growth in the pre-weaning growth period and subsequently affect final live weight and carcass weight.

In this study, the mean of weaning-weight showed a positive correlation with live weight and hot carcass weight (Table 4.5). It could therefore be argued that selection for increased weaning-weight might have a similar effect on selection for improved live weight and hot carcass weight in pigs. Furthermore, a genetic correlation of 0.81 and 0.79 were calculated between live weight and ADG and lean growth rate, respectively. Therefore, it should be expected that there is a positive effect or high genetic correlation between the live weight, hot carcass weight, ADG and lean growth rate.

Furthermore, fat depth is an indicator which related to the negative quality of carcass traits and lean deposition in pig is found associated with variant $D$ in $CAST$ intron 5. This finding is consistent with previous studies, which reported association between $CAST$ and backfat thickness (Choi et al., 2006), body fat, and intramuscular fat (Choy et al., 2002). Moreover, variation in fat depth was different depending on growth pattern and the maturity at slaughter age of the pigs (Choy et al., 2002). This implies that variation in $CAST$ could influence fat deposition, but other factors like age to slaughter could also be involved. Since fat deposition has a negative effect on lean deposition, variation detected in $CAST$ intron 5 might also be involved in lean deposition and eventually affect meat production and meat quality in pigs.

The development of muscle has been related to meat yield and meat quality. In this study, the quality of pork was not included, but an association has been reported by Ciobanu et al. (2004). Kristensen et al. (2002) also reported an increase in protein turnover in vivo, which may be a result of variation in $CAST$ levels. Thus, genetic variation in $CAST$ could be potentially used to evaluate meat yield and meat quality.

$CAPN3$

The $CAPN3$ has twenty-four exons and codes 821 amino acids (Duguez et al., 2006). This study focused on variation in exons 1, 5, 10 and 16 of $CAPN3$. The selected regions have found variable in the unique sequence of $CAPN3$ (Goll et al., 2003).

Of these four regions, four variations in $CAPN3$ exon 1 and exon 10 were detected in NZ pigs. These variants have been previously reported (Gandolfi et al., 2011) (Figure 4.9). In addition, three cytosines insertions have observed in this study. The insertion of three
cytosines results in a change of seven putative amino acids (Figure 4.10) from the published sequence (NM 214171). In addition, the predicted amino acid of NM214171 does not resemble the predicted cattle sequence (GenBank accession NM174260) and human (GenBank accession AF127260) (Appendix B). This implies that the sequence NM214171 could be error of sequencing.

However, in this study, variation in CAPN3 in both regions has a low frequency so that statistical analysis was not undertaken. Although the variant frequency for CAPN3 detected here was relatively low frequency, this indicates a potential exists to increase the frequency of these variations if it could have genetic improvement of meat yield in pig.

Nonneman & Koohmaraie (1999) and Chung et al. (2007) suggested that CAPN3 plays a crucial role in muscle development and growth as well as in the regulation of myogenesis (Dargelos et al. (2002). The present study was focused on the exons 1, 5, 10 and 16, containing three unique insertion sequences, for the reason that the genetic variation between these regions may affect the process of regeneration of muscles (Baghdiguian et al., 1999; Goll et al., 2003). Fougerousse et al. (1998) and Kramerova et al. (2004) reported that the locations of the insertions were related to muscle formation process in mice and progressive muscular dystrophies during early human development. Also, other studies reported that expression of CAPN3 is correlated with muscle organisation (Stockholm et al., 2001; Ono et al. 2006; Beckmann & Spencer, 2008; Goll et al., 2008), and a muscle cytoskeleton regulator (Taveau et al. 2003). Genetic variants of exon 1 observed in this study are located in the N-terminal domain, which corresponds to a regulatory propeptide found in the cysteine proteinase.

In addition, several studies show the effect of CAPN3 on birth weight and post-weaning growth in sheep (Chung et al., 2007), and shoulder yield but not leg or loin yield in NZ Romney sheep (Fang et al., 2013). In chickens, variation in CAPN3 has been associated with increased body weight, carcass weight, breast and leg muscle weight (Zhang et al., 2009).

Although no much information reported in pig, this revealed that genetic variation in CAPN3 could also have a potential used for improving body weight and meat yield in pig.

**LEP**

The LEP consists of three exons and two introns (Ramsay et al., 1998). The first exon is a short untranslated sequence, with the coding region is in exons 2 and 3 (Bidwell et al., 1997;
van der Lende et al., 2005). This study focused on variation in LEP exon 3, locate in the region affecting of leptin.

In this study, two nucleotide substitutions were observed in LEP exon 3 so that three variant (variants A, B, C) were detected. One substitution at position 3469 (variant B) has been previously described (Jiang & Ginson, 1999; Kulig et al., 2001; Urban et al., 2002), while variation at position 3655 (variant C) was new finding in this study. This variation causes a putative amino acid changing of valine for isoleucine.

Moreover, at position 3618 and 3619 of variants A, B, C did not have variation. However, there is a reversion of nucleotide from CG to GC (Figure 4.14) comparing to the published porcine sequence U66254. The GC nucleotides will be resulted in GCC alanine coding, whereas the CG of U66254 has a CGC arginine. The GC inversion has also seen in cattle LEP (AJ132764) and sheep LEP (EF534370) (Appendix C).

Association analysis revealed that variants of LEP did not show significance on production traits. This is not in accordance with reported in other studies (Switonski et al., 2003; Kulig et al., 2001; Urban et al., 2002) in which associations between LEP and production traits were shown with weaning-weight, backfat thickness and feeding intake. Kulig et al. (2001) and Urban et al. (2002) reported that variation at position 3469 was associated with increased carcass weight in Duroc pigs, whereas Kennes et al. (2001) described association with decreased feed intake in Landrace pigs.

Although, the present finding could not reveal the association of variation with production traits, the nucleotide substitution at position 3655 may suggest the possible effect of this variation on leptin activity. According to the report of Imagawa et al. (1998), amino acid residues at 106 to 160 were the affecting region of leptin (Imagawa et al., 1998; Grasso et al., 1997). Therefore, the nucleotide substitution at position 3655 results in the change of amino acid valine/isoleucine, which happened to be located at 134 amino acid residues, may affect the expression and/or the activity of leptin.

Robert et al. (1998) reported that southern blot analysis of fat and lean pigs showed a DNA polymorphism related to the lean phenotype. Differences in leptin mRNA levels were associated with subcutaneous fat accumulation in pigs, and a higher leptin level was found in the backfat of fat pigs compared to lean pigs. Observations have also been made in mice (Lalonde et al., 2004), humans (Considine et al. 1995), and pigs (Bidwell et al. 1997), with
Lalonde et al. (2004) suggesting that leptin abolished hyperinsulinemia in obese mice. The effects of leptin on insulin secretion could also have an effect on fat gain and food intake. Reduced leptin levels were related to a reduction in food intake and insulin (Cusin et al. 1995; Saladin et al. 1995; Leroy et al. 1996). The present study found no association with fat depth, but a trend was observed between a leptin substitution at position 3655 (variant C) and hot carcass-weight and increase lean growth rate. Due to the metabolic function of leptin this may imply that the heavier hot carcass-weights and higher lean growth rate, observed in this study result from the lower fat content of the carcasses.

In addition, in this study, a positive correlation was observed between fat depth, hot carcass-weight and lean growth rate (Table 4.5). This is consistent with previous findings that have reported high genetic correlation between serum leptin concentration and the percentage of subcutaneous fat area (Suzuki et al., 2009; Robert et al., 1998), as well as feed conversion ratio (Suzuki et al., 2009). Moreover, Suzuki et al., (2009) have reported high heritabilities for the concentration of serum leptin, backfat thickness, and intramuscular fat content of the loin. If the associations can be confirmed between LEP, carcass weight and lean growth rate, this might prove valuable for improving pig carcasses.

**ADRB3**

The ADRB3 has two exons. It contains a large exon 1 encoding 398 amino acids and a small exon 2 that encodes the nine amino acids (Tanaka et al., 2007). This study focused on variation in ADRB3 exon 2. This region has an important role in signalling efficiency of the coupling of ADRB3 in the carboxyl-terminal residues of the receptor (Smith et al., 2001).

In this study, two variants of ADRB3 were identified in NZ pigs (variants A and B), with variant B caused a frame-shift that resulted in the loss of two amino acids from the predicted polypeptide. These variations reported previously in pigs (Tanaka et al., 2007; Chikuni et al., 2008; Hirose et al., 2009).

Both variant A and B of ADRB3 found to be associated with production traits. The presence of variant A was associated with decreased fat depth, while the presence of B variant was associated with increased weaning-weight and hot carcass weight. Some studies had shown association with increased loin eye muscle area (Hirose et al., 2009), whereas others found no association with productive traits (Cieslak et al. 2009). However, association of production traits and ADRB3 variation has reported in other species, for instance sheep, in which
variation in *ADRB3* has been associated with increased birth weight, growth rate of weaner and carcass fat (Forrest *et al.*, 2003; Horrell *et al.*, 2009).

The premature stop codon in variant *B* of *ADRB3* was found in the carboxyl-terminal region of the putative protein, result in a shorter protein. This region is thought to be essential for receptor-G protein coupling (Strosberg & Gerhardt, 2000) and the carboxyl-tail of *ADRB3* also has important roles in signalling efficiency of *ADRB3* protein (Castro-Fernandez & Conn, 2002; Budd *et al.*, 2003). Likewise, the study in sheep, Forrest *et al.* (2003, 2006, 2007, 2009) identified that variation in the ovine *ADRB3* locus was associated with the cold-related mortality rate in lambs and growth rate. Given association between weaning-weight and hot carcass weight found in pigs and association with growth rate reported in sheep, this gene may be a target for selection to improve growth rate. Thus, increasing the frequency of the variant *B* might be beneficial in the breeding program to accelerate the genetic improvement of meat production traits.

However, in this study, two individual homozygous for *BB* genotype were found, and thus the expected effect of being homozygous could not be assessed. In animals carrying *BB*, an extreme phenotype was observed with much higher fat depth. If this extreme genotype is the result of carrying the *BB* genotype, this gene may be significant mechanism effects on *ADRB3* function. An analysis of more animals is necessary to confirm the association between the *BB* genotype and fat deposition and carcass traits.

**IGHA**

The *IGHA* encodes an amino acid of the hinge region of immunoglobulin A (Senior *et al.*, 2000). The hinge region is flexible and allows some variation in distance between the antigen binding sites and flexibility in how IgA binds antigens (Furtado *et al.*, 2004). This study focused on variation of porcine *IGHA* and to ascertain if the variation could affect muscle growth and carcass traits in pigs.

The present study identified three variants (variants *A, B, C*) of *IGHA* in NZ pigs. Of these, variant *C* was a novel finding of this study, while the other two variants have been described earlier (Brown *et al.*, 1995; Navarro *et al.*, 2000a).

Variants *A* and *B* describe variation in the hinge region of IgA with changes in the predicted polypeptide length as the result of change in a splicing site. The two possible splice-acceptor
sites are ‘AG↓T’ between position c.302-1, c.302-2 and ‘AG↓A’ between position c.314-1, c.314-2 (GenBank accession U12594). In this study, a novel PCR-SSCP method was used, and this may be the reason that variant C was detected. Previously, *IGHA* typing methods was employed and it would not have distinguished variants B and C. This is because the typing method was based on restriction fragment length analysis (Navarro *et al.*, 2000a) that detects the presence of a ‘CTCAG’ sequence at the splice-acceptor site in *IGHA*, which is absent in both variant B and C (Figure 4.18). Likewise, the typing method based on IgA transcriptional analysis (Brown *et al.*, 1995) could not detect the length variation in variant B and C, as they only differ by two nucleotides in the non-coding sequence.

A PCR-SSCP method demonstrated to detect new variants of porcine *IGHA*. This technique provides a reliable, sensitivity and cost-effective method to detect variation in *IGHA* (Hayashi, 1991). Also, with this method, a previously un-described variation in the hinge region of *IGHA* is detectable. Previous authors have suggested that variation in the hinge of IgA may be the result of adaptation to host-parasite interactions and/or positive selection against pathogens (Navarro *et al.*, 2000b). The hinge region has been proposed important since its variation may provide greater flexibility to the IgA molecule (Sumiyama *et al.*, 2002), which may have a consequential impact on pig production.

An association found between the presences of variant B and decreased fat depth. This association has not been previously reported. However, association between presence of variant B and decreased fat depth was only described in one herd of pigs and the mechanism of how *IGHA* would affect fat depth is not clear. If this association can be confirmed a relationship between *IGHA* and fat deposition, this could have valuable consequences for improving pig industry, in term of carcass fat reduction and feed efficiency improvement.

### Factors that may cause bias

This study was conducted as a preliminary study, and thus there are some aspects that have to be considered when drawing a conclusion. These include the biology of the candidate genes.

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7 GenBank accession number predicted by [http://www.fruitfly.org/seq_tools/splice.html](http://www.fruitfly.org/seq_tools/splice.html), Downloaded on June the 10th, 2010
themselves, the sampled animals, the experimental methods, and assumptions in the statistical analysis.

The sampled pigs in this study were not randomly selection and were only from one commercial farm. As a result, there may have bias from the genetic background of the animals on the farm, including boars and sows relatedness, which could result in inbreeding and founder effects causing a loss of genetic diversity. The collected data on productive traits were not directly related to muscle growth and lean deposition. Hence, the results and conclusion cannot directly be interpreted to prove a relationship between the candidate genes and muscle growth and lean deposition.

It is possible that the screening methods did not have detected all the variation in the candidate gene, for example, variant C of LEP required further method development to be resolved. However, the risk of screening method was reduced during the method optimised since many differences PCR-SSCP conditions were tested.

Another potential source of error is because the statistical model only includes information available on sows; farm management effects that could influence the model such as founder effects and the mating systems. No piglet gender data was available, and litter sizes and birth weight were not measured. All these could have affected the genetics or growth of the pigs studied. Consequently, biases due to data quality problems, unmeasured confounding effects and correlations between the various predictor variables may have been present in the data.

The commercial breeds studied have been subjected to intensive selection for commercial traits, such as muscle growth rate and carcass weight. This might explain the significant deviation from the Hardy-Weinberg equilibrium for variants.

It is apparent that the results in this study need to be reproduced in order to minimise the possible error from boar and other related factors. It would be appropriate that in the further investigation, a larger number of samples should be involved. This will be needed to prove that the associations have not occurred by chance.

Although the statistical analysis showed no significant relationship between muscle growth and carcass traits and most of candidate genes, a number of genes show variation with high frequency distribution. Thus, these characteristics attract the further study of these genes as an indication of muscle growth in NZ pigs and each candidate gene is still significant in the biology and metabolic aspects.
Chapter 6
General discussion and future work

The major focus of this thesis was to assess genetic variation of the nine candidate genes that are involved in muscle growth, and to ascertain if this genetic variation is associated with growth, carcass traits and meat production in NZ pigs. If the desired gene could be pointed out it would be the potential biological marker for the meat production in pig industry.

As meat production is controlled by a number of different biological pathways, the studied genes are chosen based on their involved in myogenesis, protein synthesis and degradation, meat quality, energy balance and feed intake as well as health and survival in pigs. Of these nine candidate genes, variations in five (MYF5, CAST, LEP, ADRB3 and IGA) genes were found with high frequencies whereas others (MYOG, CLPG and CAPN3) were less common. Investigation of association in five candidate genes is conceivable that variation in MYF5, CAST, ADRB3 and IGA could affect weaning-weight, hot carcass-weight, ADG, lean growth rate and fat depth. Moreover, this study reports the novel findings of a potential effect of ADRB3 on weaning-weight and hot carcass-weight in pig; and IGA on fat deposition. Hence, the variation of these genes should be further conducted for the benefit of pig industry.

In pig industry, weaning-weight is an important factor, because the process of early stage of muscle formation influences pig’s growth, lean deposition as well as meat production. The present study found a potential effect of variation in MYF5 exon 3 (variant A) and ADRB3 exon 2 (variant B) was associated with increased weaning-weight in NZ pigs. Since these variants could play an important role in the prediction of weaning-weight, it would seem beneficial to promote the selection for pigs carrying variant A in MYF5 exon 3 and variant B in ADRB3 exon 2. These could improve heavier weaning-weight and eventually improve meat production.

The present study also detected that variant A in MYF5 exon 3 was associated with decreased fat depth. This trait is related to backfat thickness which is one important indicator to improve carcass quality in pig industry. In the grading system of pork carcass, the carcass quality is depend on the depth of backfat which consumers continually prefer leaner meat, and pig producers are encouraged to breed leaner breeds and to select for leaner pig (Li et al., 2003; Boys et al., 2007). In addition, the high heritability value substantiates that the backfat
thickness is achievable through genetic selection of breeding stock. Pigs carrying variant A in MYF5 exon 3 could be predicted for the fat depth and hence were selected to improve backfat thickness and the pork carcass quality.

On the other hand, variant D (carrying a missing of thymine base at position 76337, 76338) of the CAST intron 5 was associated with increased fat depth. Since fat depth has a negative correlation with lean deposition, pigs carrying variant D in CAST intron 5 will possess more fat and less lean. Variant D in CAST intron 5 could be used in the prediction of fat depth. However, the pig carrying this variant should not be selected if learner pigs are required.

The live weight and hot carcass-weight are also the indicators related to carcass weight and dressing percentage of the pork yield. The present study find that variations of variant C in CAST intron 5 was associated with increased live weight, ADG and lean growth rate, and variation of variant B in ADRB3 exon 2 was associated with increased hot carcass weight. Since, a significant increase in carcass yield was shown when live weight and carcass weight increase (Fiego et al., 2005), the findings of variant C in CAST intron 5 and variant B in ADRB3 exon 2 could be considered in the breeding scheme of pig production. Selection for pigs carrying variant B in ADRB3 and those carrying variant C in CAST intron 5 should potentiate productivity and economic returns to the pig production industry.

Another finding of genetic variation that associated with pig productive traits that was not reported previously is a potential effect of variation in IGHA on fat deposition in pig. Variant B in IGHA (containing a missing AG at a splicing site) was associated with decreased fat depth in pigs. This has been exposed in their having been varying in the hinge region of IgA. This variant may potentially affect a hinge distance and a flexibility of IgA binding regions. However, the actual mechanism by which IGHA could affect fat deposition is unclear. It is possible that this response is driven by subclinical pathogens. If this is true, it is likely to be inconsistent between farms since pathogen prevalence varies by farm.

Drawing from the present findings suggest that using the genetic variation of MYF5, CAST, ADRB3 and IGHA as a selection tool for improved muscle growth and carcass traits in NZ pigs may be feasible. Gene-marker assisted offers a breeding scheme to take advantage on the genetic potential of the animals by increasing the selection accuracy and reducing the generation interval in the breeding program. However, utilizing these variants may have to consider on the basis of requirement to fit farmers, processors or consumers. Generally, pig selection for growth performance is commonly based on weighting gain. It is mainly based on
the carcass weight with some premium payments for carcass grade. Therefore, heavier carcass weight is an economic benefit to farmers. In contrast, from the meat processor point of view, more meat per unit carcass yield is required, whereas consumers want lean meat. To balance these needs, further work is required to ascertain the effect of the candidate genes on the traits that fit requirement of consumers, processors and farmers.

There are some concerns that need to state here for the future work before utilisation of these identified \textit{MYF5}, \textit{CAST}, \textit{ADRB3} and \textit{IGHA} variations for marker-assisted selection. Validation of the effect of various candidate genes on meat production and quality traits across different pig breeds are still needed. In addition, further investigation is needed to confirm the interaction of \textit{MYF5}, \textit{CAST}, \textit{ADRB3} and \textit{IGHA} with other genes that involved in muscle growth.
In conclusion, the present study provides evidence that there are at least five candidate genes that show genetic variation and promising markers to use for improving meat production traits in NZ pig. *MYF5*, *CAST* and *ADRB3* variant would benefit on weaning-weight and hot carcass-weight. Similarly, there is evidence of effects of the variation in *CAST* and *IGHA* on fat deposition. Although the genotypes for these candidate genes were less balanced within the experimental designs, based on the findings for the production traits it appears that *MYF5*, *ADRB3*, *CAST* and *IGHA* may be suitable for use in marker-assisted selection to improve meat production, fat deposition and carcass weight in pig. However, the need exists for further larger-scale assessments to confirm these findings.
References


Cockett, N. E., Jackson, S. P., Shay, T. L., Nielsen, D., Moor, S. S., Steele, M. R., Barendse, W., Green, R. D., & Georges, M. (1994). Chromosomal location of the


Tanaka, K., Iwaki, Y., Takizawa, T., Murakami, M., Mannen, H., Meada, Y., Kurosawa, Y.,
Dang, V. B., Chum Phith, L., Bouahom, B., Yamamoto, Y., Daing, T. & Namikawa,
Tatsumi, R. (2010). Mechano-biology of skeletal muscle hypertrophy and regeneration:
Possible mechanism of stretch-induced activation of resident myogenic stem cells. J.
activated through autolysis within the active site and lyses sarcomeric and
Company, New York.
Biochemical pathways analysis of microarray results: regulation of myogenesis in
te Pas, M. F. W. (2004). Candidate genes for meat production and meat quality-the MRF
genes. Animal Science Papers and Reports. 22(1)115-118.
te Pas, M. F.W., & Visscher, A. H. (1994). Genetic regulation of meat production by
te Pas, M. F. W., Harders, F. L., Soumillion, A., Born, L., Buist, W., & Meuwissen, T. H. E.
(1999). Genetic variation at the porcine MYF-5 gene locus: Lack of association with
ribonucleic acid expression of the MyoD gene family in muscle tissue at slaughter in
information of the regulation of the determination of skeletal muscle mass in livestock
Myostatin, a negative regulator of muscle growth, functions by inhibiting myoblast
ryanodine receptor, growth hormone, leptin and MYC protooncogene protein and meat
Urbanski, P., & Kuryl, J. (2004). Two new SNPs within exon 1 of the porcine MYOD1
(MYF3) gene and their frequencies in chosen pig breeds and lines. J. Anim. Breed.
Genet.121, 204-208.


### Appendix A

<table>
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<th>Sequence</th>
<th>Variants</th>
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</tr>
<tr>
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<td>B: -----------------------------------------------</td>
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<tr>
<td></td>
<td>C: -----------------------------------------------</td>
</tr>
<tr>
<td></td>
<td>D: -----------------------------------------------</td>
</tr>
<tr>
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<tr>
<td></td>
<td>B: -----------------------------------------------</td>
</tr>
<tr>
<td></td>
<td>C: -----------------------------------------------</td>
</tr>
<tr>
<td></td>
<td>D: -----------------------------------------------</td>
</tr>
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<td>B: -----------------------------------------------</td>
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<tr>
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<td>C: -----------------------------------------------</td>
</tr>
<tr>
<td></td>
<td>D: -----------------------------------------------</td>
</tr>
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<td>B: ------------------------------------------------</td>
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<tr>
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<td>C: ------------------------------------------------</td>
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<td>D: ------------------------------------------------</td>
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<td></td>
<td>C: -------- 491</td>
</tr>
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<td></td>
<td>D: -------- 491</td>
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**Figure 1** Nucleotide sequences of MYF5 exon 1 and sequent variant. Nucleotide identical to the published sequence (GenBank accession number Y17154) are presented by dashes. The yellow shaded region with the underline represents the primer regions for MYF5 of the first set to amplify a 491 bp fragment of MYF5 in Thai pigs and the blue shaded region is the primer-binding region for the second primer to use in NZ pigs.
Appendix B

<table>
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<tr>
<th>Human</th>
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</tr>
<tr>
<td>variantA</td>
<td>--------M-----------------------------M-----G------V---G-K--</td>
</tr>
<tr>
<td>variantB</td>
<td>--------M-----------------------------M----------------------------</td>
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<td>NM_214171</td>
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<table>
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<td>---------------------F---------------------</td>
</tr>
<tr>
<td>variantA</td>
<td>---------------------L---------------------</td>
</tr>
<tr>
<td>variantB</td>
<td>---------------------L---------------------</td>
</tr>
<tr>
<td>NM214171</td>
<td>---------------------L---------------------</td>
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</tbody>
</table>

**Figure 2** Comparison of the amino acid sequences obtained for CAPN3 exon 1 (variant A and B) compared with porcine sequence NM214171, cattle sequence NM174260 and human sequence AF127260. Dots have been introduced to improve alignment and dashes indicate nucleotides identical to the top sequence. Differences in the putative polypeptide sequences are highlighted in red. The positions are given relative to a porcine CAPN3 reference sequence (GenBank accession number NM214171).
### Appendix C

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</tr>
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<tr>
<td>Variant C</td>
<td>-------------------------------</td>
<td></td>
</tr>
<tr>
<td>Cattle</td>
<td>-------------------------------</td>
<td></td>
</tr>
<tr>
<td>Sheep</td>
<td>-------------------------------</td>
<td></td>
</tr>
</tbody>
</table>

**Variant A** and **Variant B** are shown for comparison.

**Variant C** is also shown.

Nucleotides at position 3481 to 3600 not shown.

<table>
<thead>
<tr>
<th>Sequence</th>
<th>Nucleotides</th>
<th>Position</th>
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<tr>
<td>U66254</td>
<td>AGCTGCCCTTGCCCAAGCAGGGCCCTGGAGACCTTGGG</td>
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<td>variant B</td>
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</tr>
<tr>
<td>variant C</td>
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<tr>
<td>Cattle</td>
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<tr>
<td>Sheep</td>
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</table>

**Variant A** and **Variant B** are shown for comparison.

**Variant C** is also shown.

Nucleotides at position 3481 to 3600 not shown.

<table>
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<th>Sequence</th>
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<td>Sheep</td>
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**Variant A** is shown for comparison.

**Variant B** and **Variant C** are not shown.

**Figure 3** Comparison of *LEP* sequences. The three sequences of porcine *LEP* exon 3: variant A, B and C are compared to the porcine sequence U66254, bovine sequence AJ132764 and ovine sequence EF534370. The nucleotides located at position 3469 and 3655 are shaded blue. A dash indicates nucleotides identical to the top sequence. The positions are given relative to a porcine *LEP* reference sequence (GenBank accession number U66254).
Copies of publications arising from this thesis