Thesis structure

This thesis reports the results from three experiments studying residual feed intake (RFI) in cattle by gene expression, expression of RFI-related genes and their relationships with other production traits in beef cattle, effect of HGP treatment on RFI and RFI-related gene expression, and the microRNA expression profile related to RFI. Chapter 1 provides an introduction to the field of study covered by the thesis. Chapter 2 provides background information on the current knowledge of RFI in beef cattle, its relationships with other production traits, genes expression and genomic technologies for genetic improvement used in livestock.

Chapter 3 studied the gene expression levels of eight candidate genes, and their relationship with growth, feed efficiency traits and other economically important production traits in Angus beef cattle. Chapter 4 evaluates the effects of HGP implant on RFI, expression levels of RFI-associated genes, and other production traits. Chapter 5 reports the results of a microRNA profiling experiment using bovine liver by a deep sequencing approach in two pools representing two genetically divergent selection lines for RFI. Chapter 6 is the general discussion which summarises the major findings and important implications of the results of the three experiments.

Each experimental chapter is presented in peer-reviewed journal format. Chapter 3: "Expression of candidate genes for residual feed intake in Angus cattle" was published in the *Animal Genetics*, online DOI: 10.1111/age.12092. Chapter 4: "Effects of hormonal growth implants on feed efficiency and expression of RFI-associated genes in beef cattle" was published in the Journal of *"Animal Production Science"*, on-line 21 May 2013: http://dx.doi.org/10.1071/AN12398. Chapter 5 "Liver microRNAs profiling in cattle divergently selected for residual feed intake with deep sequencing" has been prepared for publication in *BMC Genomics.*

Chapter 1

General Introduction

Profitability of beef production depends on both inputs and outputs. In the past, selection and genetic improvement mainly aimed to improve the output traits, such as liveweight, fertility, carcass and meat quality traits, with little importance placed on reducing input cost. Providing feed to cattle is the single largest variable input cost in beef production. It represents more than 60% of the total input cost. Feed cost for maintenance requirements have been estimated to represent at least 60 to 65% of the total feed requirements for the cow herd with significant variation among individual animals (Montano-Bermudez and Nielsen, 1990; Parnell et al., 1994). Therefore, any improvement in the efficiency of feed utilization will help to reduce the input cost of beef production and, as a consequence, increase profit (Arthur and Herd, 2005; Herd et al., 2003; Moore et al., 2009).

There are also environmental issues associated with cattle production, such as greenhouse gas (GHG) emissions. FAO (2006) states that globally the livestock sector generates more GHG than transport activities, and is responsible for about 18% of total GHG emissions derived from human activities (Steinfeld et al., 2006). Cattle produce methane (CH_4) and nitrous oxide (N_2O), which are GHG with global warming potential 21 times and 310 times greater than carbon dioxide (CO_2; the standard reference gas for GHG), respectively (Arthur, 2009). In cattle, methane is produced through enteric microbial fermentation of hydrolysed dietary carbohydrates. Methane can also be produced, together with N_2O from manure under both pasture and feedlot management conditions. Cattle with better feed efficiency emit less methane and excrete less manure than low feed efficiency cattle (Arthur, 2009; Arthur et al., 2010; Hegarty et al., 2007; Nkrumah et al., 2006). Thus, decreased GHG emissions are additional real benefit from improvement in feed efficiency. Therefore, over the past two decades, there has been greatly increased attention in breeding programs toward improving feed efficiency of beef cattle (Arthur et al., 2010).

The concept of residual feed intake (RFI), or net feed intake (NFI), is one of a number of indices of feed efficiency of cattle. RFI is the difference between an animal's actual feed intake recorded over a test period and its expected feed intake based on its size

and growth rate (Koch et al., 1963). There is significant individual animal variation in feed intake above and below that expected on the basis of animal's size and growth rate. There is strong evidence for a genetic basis of variation in RFI, and it is moderately heritable (Archer et al., 1999; Herd et al., 2003; Herd and Arthur, 2009; Herd et al., 2004). Thus, using bulls and cows genetically-superior for low RFI (higher feed efficiency) will create progeny that require less feed in comparison with the offspring of high-RFI (lower feed efficiency) parents, for the same liveweight and the same level of production, consequently improving profitability (Baker et al., 2006; Herd et al., 2003; Moore et al., 2005).

RFI has been adopted by the major cattle breed societies in Australia as the measure of choice for genetic improvement of feed efficiency in beef cattle. Genetic progress is made by selection based on estimated breeding values (EBV) derived from pedigree and individual-animal RFI measurement. However, the individual-animal measurement of RFI is expensive and inconvenient and both are barriers to genetic improvement. Molecular genetics allows studying the genetic makeup of individuals at the DNA level. Therefore, an alternative would be using DNA markers for genetic variation in RFI and then using such data to estimate breeding values for selection (marker-assisted selection). Marker-assisted selection (MAS) speeds up the rate of genetic progress by increasing selection pressure and reducing generation intervals. It has particular advantage for traits that are expensive or difficult to record (e.g. RFI), or traits that require slaughter of the animal (carcass traits) (Dekkers and Hospital, 2001; Johnston et al., 2012; Meuwissen and Goddard, 1996).

Several quantitative trait loci (QTL) markers and many single nucleotide polymorphisms (SNP) have been found to be associated with RFI in beef cattle (Barendse et al., 2007; Bolormaa et al., 2011; Mujibi et al., 2011; Nkrumah et al., 2007b; Sherman et al., 2008, 2009; Snelling et al., 2011). However, only a small number of these SNPs have been validated across populations and breeds, and most of these markers have failed to maintain association with RFI in these validation studies. So far, these studies have not found a major causative gene affecting RFI.

The completion of the bovine genome sequence and advances in genotyping technology has made it possible to select animals based on genetic markers covering the whole genome (genomic selection) (Meuwissen et al., 2001). Although it is based on the linkage disequilibrium (LD) between markers and causative variants, breeding values can be predicted with high accuracy using genetic markers covering the whole genome.

However, the accuracy of genomic selection depends on how the target population differs from that in which the effect of the markers was estimated, because it based on the LD between the marker and the causative variant. Genomic selection with functional SNP genotypes can be less reliant on LD shared by training and target populations and allows prediction across breeds and populations. Gene expression studies can provide functional information on genes underlying the phenotype and can help identify functional DNA variants.

A global gene expression study using a long-oligo microarray with 24,000 probes was conducted by Chen et al. (2011a) to profile liver tissue of 44 young bulls genetically selected for high and low RFI. This study revealed 161 unique genes expressed differentially between high and low RFI cattle. These genes were involved in seven gene networks affecting cellular growth and proliferation, cell signaling, cellular assembly and organization, protein synthesis, drug metabolism, lipid metabolism and carbohydrate metabolism. The identified gene networks increased understanding of the biological processes underlying variation in RFI and yielded candidate genes for marker assisted selection. A subset of these genes has been validated with quantitative real-time PCR (qPCR) and was found to be associated with RFI in young bulls and steers selected for high and low RFI (Chen et al., 2012b; Chen et al., 2012a; Chen et al., 2011b).

Studies have shown the presence of small (~ 22 nt), noncoding RNA called microRNAs (miRNAs) which can repress thousands of target genes and regulate cellular processes, including cellular proliferation, differentiation and apoptosis (Carmell and Hannon, 2004; Castro et al., 2010; Lu et al., 2005). In the last decade, some miRNAs profiling studies in cattle genomes have been undertaken (Jin et al., 2009; Romao et al., 2012). However, the role of miRNAs in regulating key cellular and physiological pathways that may regulate feed efficiency and RFI are still unclear. In this study, we profiled miRNA abundance in liver tissue of Angus from divergently RFI-selection lines using next generation sequencing (NGS), which is an advanced technology that has significantly promoted transcriptomic studies (Buermans et al., 2010b; Liu et al., 2012).

Scope of this thesis

The overall aim of this thesis was to study the genetic mechanisms underlying variation in RFI in beef cattle by studying of gene expression in beef cattle with quantitative real-time PCR and miRNA profiling by deep sequencing. The objectives of this study were:

1. Study the expression of eight RFI-related genes in bovine liver and their correlation with RFI in a production herd using quantitative real-time PCR.

2. Evaluate the effects of hormonal growth implants on the expression of RFI-associated genes and RFI in beef cattle.

3. Profile miRNA expression in bovine liver and identify differentially expressed miRNA between genetically high and low RFI cattle by deep sequencing.

Chapter 2

Literature review

2.1 Introduction

Traditionally, producers of beef cattle have focused animal breeding on traits related to outputs for making improvements in their production system, such as body weight and carcas measurements. These traits have direct market value and are relatively easy to measure. However, in recent years increased input costs have forced livestock producers to search for new methods to reduce production costs without compromise to levels of production. It is well established that providing feed represents the largest input cost in beef production (Archer et al., 2002; Arthur and Herd, 2008; Arthur et al., 2005; Herd and Bishop, 2000). Approximately 65% of total feed consumed by beef cattle is required to maintain the breeding herd and the remainder of food intake is used for production such as growth and lactation (Arthur et al., 2001a; Basarab et al., 2003). Therefore, a large improvement in profitability could be realized by reducing the amount of feed required per animal in the breeding herd, in another words, by improving individual-animal feed efficiency.

Traditionally, feed efficiency has been measured as the feed-to-gain ratio. This measure of feed efficiency does not separate the feed intake between growth requirements and maintenance requirements. Residual feed intake is the difference between an animal's actual feed intake and its expected feed intake based on its size and growth rate recorded over a test period (Koch et al., 1963). Residual feed intake has been demonstrated as to be a measure of feed efficiency that is independent of growth rates and maturity patterns, and to be an appropriate trait to be used in selection and breeding programs to improve feed efficiency (Arthur et al., 2001b; Crews, 2005; Koch et al., 1963). The main barrier to widespread adoption of RFI in selection programs is the technical difficulties, expense and inconvenience of measuring a large number of individual animals for feed intake over a test period. This makes the development of predictive genetic markers an attractive alternative to direct measurement on large numbers of animals.

This review will focus on measures of feed efficiency for beef cattle, explanation of RFI, and on methods for genetic improvement of feed efficiency. It also includes review of the biological and physiological basis for RFI, the development and application of genetic

markers for RFI, and the genetic technologies applied to evaluate and identify genetic factors that contribute to feed utilization in beef cattle.

2.2 Measures of feed efficiency

The utilization of feed by animals involves a complex of biological processes and interaction with environmental factors. Also, feed intake is highly correlated with body size and outputs traits. Therefore examination of feed intake or production outputs separately from each other usually provides little indication of the efficiency of production. Several measures exist for describing feed efficiency of livestock. Each measure reflects different biological and mathematical aspects of feed efficiency (Archer et al., 1999). The most common measures used for describing feed efficiency in beef cattle are feed conversion ratio (FCR), partial efficiency of growth (PEG), and RFI. In general, the phenotypic and genetic correlations among these measures are high (Arthur and Herd, 2008; Arthur et al., 2001a).

2. 2. 1 Feed conversion ratio

Traditionally, FCR has been the most common measure of feed efficiency, and is the ratio of units of feed required for one unit of gain. Therefore, the lower the numerical value for FCR, the more feed efficient the animal. FCR is moderately heritable (Arthur et al., 2001a; Arthur et al., 2001b; Bishop et al., 1991; Crews, 2005).

FCR may be not the ideal trait for genetic improvement purposes in beef cattle, mainly for two reasons. Firstly, FCR has a significant genetic correlation with mature body weight and daily feed intake (DFI). Selection to reduce post-weaning FCR to improve feed efficiency of growing animals can lead to increase in mature size of breeding females. This will increase the feed intake and the total feed requirements for maintenance of the breeding herd and offset any gain in profitability in the production system (Arthur et al., 2001b; Herd and Bishop, 2000; Schenkel et al., 2004). Secondly, FCR is a gross estimation of feed efficiency and it does not separate the feed intake between growth requirements and maintenance requirements. Thus, the selection for this trait will not necessarily lead to improved maintenance feed requirements of cattle (Carstens and Tedeschi, 2006).

2.2.2 Partial efficiency of growth

Partial efficiency of growth (PEG) is another measure of feed efficiency. It partitions feed intake into requirements for maintenance and requirements for growth. It can be calculated using the formula:

$$ADG / (DMI - DMIm)$$

Where, ADG is average daily gain, DMI is dry matter intake on a daily basis and DMIm is the expected DMI required for maintenance on a daily basis calculated using published population estimates for maintenance (Carstens and Tedeschi, 2006).

There are advantages of PEG over FCR. The genetic and phenotypic correlations between PEG with ADG and weight traits are significantly lower than those for FCR with ADG and weight traits (Arthur et al., 2001a; Lancaster et al., 2005; Nkrumah et al., 2004). Although these lower genetic correlations may be desirable from a genetic prediction viewpoint, PEG does not fully capture individual variation in feed efficiency. As the DMI required for maintenance (DMIm) is obtained using standardized feeding tables based on population estimates for all beef cattle, PEG has an essential disadvantage in not measuring individual-animal variation in maintenance requirements, limiting its use for genetic prediction and genetic improvement of whole herd efficiency (Archer et al., 1999).

2.2.3 Residual feed intake

Residual feed intake (RFI) is sometimes referred to as net feed intake (NFI) or net feed efficiency. It is an approach to defining feed efficiency that separates feed inputs into that required for maintenance and that for growth components. Koch et al. (1963) first to propose this concept in beef cattle and examined a variety of indices for calculating feed efficiency. They recommended that feed intake of an animal could be adjusted for liveweight and weight gain to divide feed eaten into two components. The first component is the feed intake expected for the observed level of production, and the second component is the residual portion. Efficient animals are those animals that consume less feed than expected based on their size and their growth rate, so they will have negative RFI. On the other hand, those animals which consume more feed than expected have positive RFI and are considered inefficient animals (Arthur et al., 2001a; Arthur et al., 2001b; Crews, 2005; Montanholi, 2007).

2.2.3.1 Methodology of measuring RFI

The calculation of RFI requires measurement of each animal's weight, growth and feed intake over a test period and an estimation of its expected feed intake as well. The estimation of expected feed intake can be made using the regression of the data from the actual feed intake test for a group of animals (Arthur et al., 2001a; Kennedy et al., 1993). The phenotypic regression approach has become the standard form of computing RFI for genetic improvement purposes and several studies have used this approach (Archer et al., 1997; Arthur et al., 2001a; Arthur et al., 2001b; Cafe et al., 2010; Crews, 2005).

In general, RFI is calculated by fitting the model:

$$\mathbf{Y}\ i = \boldsymbol{ß0} + \boldsymbol{ß1}\ (ADGi) + \boldsymbol{ß2}\ (MMWTi) + \boldsymbol{ei}$$

Where Y is the daily feed intake of animal i, ***ß0*** is the regression intercept, ***ß1*** is the partial regression coefficient of daily feed intake on ADG, and ***ß2*** is the partial regression coefficient of daily feed intake on metabolic mid-test liveweight (MMWT), and ***ei*** is the residual error term. RFI is equated to the residual error term in the model.

Testing cattle for RFI requires the measurement of actual feed intake and growth over a defined period of time. The optimal duration of the test is around 70 days, after an adjustment period of about 21 days (Archer et al., 1997; Archer and Bergh, 2000). The feed intake by each animal is recorded daily, either manually or with automatic electronic feeders, which dispense and record feed intake. The cattle in the RFI test need to be weighed regularly at least every fortnight (Archer et al., 1997; Wang et al., 2006). Additional measurements, such as ultrasound scanning for subcutaneous fat thickness and area of the eye-muscle *M. longissimus,* can be taken during RFI test as well (Arthur, 2009).

2.2.3.2 Heritability of RFI

There are substantial individual-animal differences in RFI and RFI is moderately heritable and direct selection is effective. Koch *et al.* (1963) reported the first heritability estimates for RFI ($h^2 = 0.28$) in beef cattle. Since that time reported heritability estimates for RFI in beef cattle have ranged from 0.28 to 0.58 (Koch et al., 1963; Archer et al., 1997; Arthur et al., 1997; Arthur et al., 2001a; Arthur et al., 2001b; Herd and Bishop, 2000; Crews et al., 2003; Crowley et al., 2010). There is some variation in estimated heritability

between different age classes of cattle. Arthur et al. (2001a) estimated the heritability of RFI in 15 months-old bulls to be 0.46 and 0.31 in 19 month-old bulls. However, there were very high phenotypic and genetic correlations for RFI between the two ages, 0.93 and 0.82 respectively (Arthur et al., 2001a).

Archer et al. (2002) also reported a high genetic correlation between postweaning RFI and mature cow RFI. These findings suggest that there is likely to be common genetic factors or genes that control RFI through different stages of animal's life. Therefore, genetic selection for lower RFI measured post weaning in young animals has potential benefits for reduction in feed intake in later stages of an animals' life (Archer et al., 2002; Herd and Arthur, 2009).

2.2.3.3 RFI genetic and phenotypic correlations with other production traits

Strong genetic correlations between RFI and feed intake are reported in the literature. These correlations ranged from 0.64 to 0.79 (Arthur et al., 2001a; Herd and Bishop, 2000; Nkrumah et al., 2007a). Phenotypic correlations between RFI and FI were strong as well, ranging from 0.52 to 0.72 (Arthur et al., 1997; Arthur et al., 2001a; Herd and Bishop, 2000; Lancaster et al., 2005; Nkrumah et al., 2007a). Furthermore, high phenotypic correlations (0.65 and 0.67) were estimated between RFI and feed intake in both growing and finishing cattle (Carstens and Tedeschi, 2006). Lower RFI is likely to be associated with reduced total feed intake in the cattle enterprise and lower overall cost of cattle production.

There are moderate to high phenotypic and genetic correlations between RFI and FCR. Reported phenotypic correlations between RFI and FCR ranged from 0.42 to 0.76 (Arthur et al., 1997; Arthur et al., 2001a; Robinson and Oddy, 2004; Lancaster et al., 2005; Baker et al., 2006). Also, Carstens and Tedeschi, (2006) reported strong phenotypic correlations (0.56 and 0.63) for RFI with FCR in both growing and finishing cattle, respectively. Similarly, strong genetic correlations, ranging from 0.66 to 0.85, have been found between RFI and FCR by Herd and Bishop (2000) and by Schenkel et al. (2004).

Most studies report that RFI phenotypically is independent from its component traits (ADG and body weight), since RFI is computed by linear regression model using these traits (Lancaster et al., 2005; Schenkel et al., 2004). Herd and Bishop (2000) have found that RFI genetically was uncorrelated with the body weight of mature cow (r_g = -

0.09). However, Arthur et al., (2001a) found that there is a genetic correlation between RFI and body weight (r_g = 0.32), and a lower genetic correlation between RFI and ADG (r_g = -0.10). These correlations would indicate that selecting for lower RFI to improve efficiency would result in reduction in body weight and ADG (Kennedy et al., 1993), but this has not been observed to accompany RFI-divergent selection in Angus cattle (Herd and Arthur, 2009).

In general, correlations between RFI with body composition traits and carcass traits are weak in cattle tested post-weaning but stronger in older cattle (Herd and Arthur, 2009). Therefore, selection for RFI within a multi-trait index is recommended to achieve improved feed efficiency without risk of unfavourable correlated responses (Arthur et al., 2001a; Carstens and Tedeschi, 2006; Crews, 2005; Richardson et al., 1998).

2.2.3.4 Biological basis of variation in RFI

The biological basis of variation in RFI is yet to be fully understood, and there are many factors that influence variation in RFI and feed utilization in beef cattle (Richardson and Herd, 2004). Herd and Arthur (2009) have reported that there are five major physiological processes that are likely to contribute to variation in RFI, including processes associated with intake of feed, the digestion process, metabolism (anabolism and catabolism), physical activity, and thermoregulation. Studies on Angus steers belonging to divergent-RFI selection lines estimated that 73% of the variation in RFI is explained by heat production from metabolic processes, differences in body composition, and physical activity. The proportions of variation in RFI that these processes explain are protein turnover, tissue metabolism and stress (37%); digestibility (10%); heat increment of feeding and fermentation (9%); physical activity (10%); body composition (5%); and feeding patterns (2%). Approximately 27% of the variation in RFI was due to the variation in other processes such as ion transport (Herd and Arthur, 2009).

2.2.3.5 Physiological indicators for RFI

Several studies have been conducted on physiological traits as indicators for RFI in beef cattle. A comprehensive study looking at a number of physiological parameters through key metabolites in beef steers from weaning through to slaughter was conducted by Richardson et al. (2004), and found phenotypic correlations between RFI and concentrations in plasma of aspartate aminotransferase (r = 0.34); β-hydroxy butyrate (r = 0.55); plasma levels of glucose (r = 0.40); plasma urea (r = 0.26); total blood protein (r =

0.26) ; insulin (r = 0.43) ; blood plasma cortisol (r = - 0.40) ; creatinine (r = - 0.45); and leptin (r = 0.31). Also the concentrations of urea triglycerides, cortisol, and insulin tended to be associated with sire RFI EBV, providing evidence for genetic associations with RFI.

Insulin-like growth factor-1 (IGF-1) concentration in plasma was found to be genetically correlated (r_g = 0.57) with RFI measured at weaning within the limited data (available till 2004) for Australian beef cattle (Moore et al., 2005a). IGF-1 has been used in feed efficiency selection strategies in Australia, United States and Japan (Kahi and Hirooka, 2007; Moore et al., 2005a). In Australia, the IGF-1 information was included in the generation of trial EBVs for RFI in the national genetic improvement system BREEDPLAN (Arthur and Herd, 2005; Herd and Arthur, 2009). However, the re-analysis of Australian data available to 2007 confirmed that RFI measured post-weaning for younger cattle and RFI measured on older feedlot cattle was moderately genetically correlated, but that the concentration of plasma IGF-I was found to be positively genetically correlated with RFI at post-weaning (r_g = 0.17) but correlated in the opposite direction with RFI of feedlot (r_g = –0.22). Plasma IGF-I concentration has ceased to be used for predicting EBV of RFI due to these low and conflicting correlations (Herd and Arthur, 2009; Kelly et al., 2010a; 2010b).

2.2.3.6 Quantitative trait loci (QTL) mapping for RFI

Considerable research has been undertaken and is still underway to determine the genetic basis and to find gene markers for RFI in beef cattle (Arthur and Herd, 2006; Moore et al., 2006; Nkrumah et al., 2007b; Sherman et al., 2008a). A primary genomic scan to identify putative QTL for RFI using multiple-marker interval mapping in half-sib families with a random sire model was conducted by Nkrumah et al. (2007b). The mapping resource included 400 beef cattle steers from 20 Angus, Charolais, or Alberta Hybrid bulls with 100 microsatellites and 355 single nucleotide polymorphism (SNP) covering the 29 bovine autosomes. Eight QTL for RFI were identified on BTA chromosomes 1, 5, 7, 8, 12, 16, 17, and 26. Fine mapping was conducted on BTA 2, 5, 20, and 29, with 423 SNP markers with an average spacing of 1.01 cM (Moore et al., 2006; Sherman, et al., 2008). Using a candidate gene approach, Sherman et al. (2008) analyzed the individual SNP in the genes harbored in the QTL intervals, and to find those genes that were associated with RFI (Sherman et al., 2008). However, no major causative gene/genes affecting RFI were found.

In addition, most of these markers discovered within family-based or population-based experimental designs and they have failed in further validation studies.

2.2.3.7 Genome wide association studies (GWAS) of RFI

With advances in bovine genome sequencing and genome-wide DNA chip technology, Barendse et al. (2007) conducted a GWAS using a commercial bovine SNP chip containing approximately 10,000 SNP, with 189 Australian beef cattle representing animals' genotypes with extreme RFI from a total phenotyped population of 1,472 animals. 161 SNP were found to be significantly associated with RFI. Interestingly, many SNP with flanking sequence contained one or more microRNA motifs and suggests non-protein coding DNA variants may have an important role in unraveling the molecular basis of variation in RFI (Barendse et al., 2007; Moore et al., 2009). Known functions of genes harboring the SNP included involvement in biological processes of energy use such as cell progression, apoptosis, growth, development appetite and body-mass homeostasis, and involvement in DNA-binding proteins.

Development of a commercial 50,000 (50K) SNP chip has provided the opportunity to conduct a more powerful GWAS for economically important traits in beef and dairy cattle (Bolormaa et al., 2011; Cole et al., 2009; Mujibi et al., 2011; Rolf et al., 2011; Snelling et al., 2010). Bolormaa et al., (2011) conducted a GWAS study using genotype data from both 10K and 50K SNP chips and found 75 SNP were significantly associated with RFI. In Angus cattle divergently selected for and against RFI, 111 SNP were found to be associated with RFI with a 10K chip. However, only 27 SNP overlapped with the SNP found in the 50K experiment.

2.2.3.8 Genomic selection for RFI

The revolution in genotyping using high-density SNP chips has resulted in large numbers of individual animals with genome-wide genotype data. This supports the development of genomic selection through the use of genomic predictions or genomic estimated breeding values (GEBVs) developed using the results from GWAS studies. The availability of such genomic information on large numbers of individuals has significantly changed dairy cattle breeding in many countries (Hayes et al., 2009). Recently, a study of genomic selection for RFI in dairy cattle used 625,000 SNP markers and 2,000 heifers measured for growth rate and feed intake in Australia and New Zealand (Pryce et al., 2012). This 625K SNP chip was also used to derive the prediction equations and the

accuracies for GEBV in validation data sets. However, in the beef industry the accuracy of genomic prediction for RFI is low and not enough to capture the benefits of genomic selection (Johnston and Graser, 2010; Johnston et al., 2012). This is likely due to the relatively low number of contemporary animals with genotypes and phenotypes that have been used to develop genomic prediction equations. Improving the accuracy of genomic predictions can also be achieved by incorporated more causative markers by identification of causative DNA variants.

2.3 Gene expression and transcriptomics technologies

When cells use the genetic information to produce the corresponding proteins, the first step is transcription of gene sequence into an intermediate molecule known as messenger RNA (mRNA). Although all cells in an organism contain the same genetic information, not all genes are actively transcribed in all cells at all times, and there are certain regions in DNA (i.e., regulatory elements) that help coordinate which gene is transcribed and at what time, in another words, regulate the gene expression (Saba et al., 2008). The entirety of all mRNAs found in a cell, tissue, or organism at a given time is called the "transcriptome". Genome-wide transcriptional profiles can contribute to a better understanding of the molecular architecture of complex traits (Cheung and Spielman, 2002; Saba et al., 2008; Visscher and Goddard, 2011). Theoretically, gene expression can explain more phenotypic variation than the DNA sequences alone as it can capture both genetic and some environmental-caused variation (Edwards et al., 2001; Piano et al., 2004).

There are many techniques to evaluate gene expression. Historically, Northern blot analysis and ribonuclease protection assay techniques were employed for determining gene expression levels. These techniques have been limited to the gene-by-gene approach. Recently, high-throughput technologies such as microarrays and next generation sequencing (NGS) have become essential tools for large scale studies for transcriptional profiles and identification of the molecular networks associated with complex traits (Urmila et al., 2012).

2.3.1 Microarray technology

DNA microarrays comprising hundreds or thousands of DNA fragments arrayed on small glass slides which can be used to probe the entire transcriptome to give a global picture of gene expression behavior (Stoughton, 2005). In DNA microarrays, thousands of single strands of DNA or gene fragments (called probes) are attached into a solid substrate

(Schena et al., 1995). The complementary strands (known as the target) interact specifically with the immobilized probes forming a highly stable duplex structure in a lower energy state. In typical microarray detection procedures, the RNAs are initially reverse transcribed into cDNA and fluorescently labeled with fluorescence molecules (Shalon et al., 1996). These targets are then purified, mixed together and simultaneously hybridized to the same microarray. Following the hybridization, the microarrays are washed, dried, and scanned for detection of fluorescence on the DNA probes. The intensity in each spot is then related to the expression level of that gene.

There are many array technologies including commercially-available genome expression arrays such as Affymetrix and Agilent, or custom long-oligo arrays. (Cristancho and Lazar, 2010; Lim et al., 2012; Pantoja et al., 2008). The Affymetrix Bovine Genome Array can be used to study gene expression of over 23,000 bovine transcripts. The bovine oligonucleotide array has over 24,000 probes with 60 bases in length (Prokesch et al., 2009; Urmila et al., 2012). Microarray experiments are capable of generating long lists of genes with differential expression; often followed by validation of specific results of interest with quantitative real-time PCR (MacHugh et al., 2009; Sarmento et al., 2008).

2.3.2 Quantitative real-time PCR (qPCR)

The polymerase chain reaction (PCR) is one of the most powerful technologies in molecular biology (Saiki et al., 1985) and specific genomic sequence can be amplified with sequence specific primers using PCR (Stolovitzky and Cecchi, 1996). Theoretically, the number of target molecules doubles every amplification cycle and a typical PCR program has 25-50 cycles, dependent on the initial template concentration. In real-time PCR, the amount of DNA is measured after each cycle via fluorescent dyes that yield increasing fluorescent signal in direct proportion to the number of amplicons.

Quantitative real-time PCR (qPCR) has dramatically changed the field of measuring gene expression. The most common method to analyse qPCR data is C_t (threshold cycle) which is the cycle number at which the fluorescent signal of the reaction crosses the threshold. The threshold of the real-time PCR is the level of signal that reflects a statistically significant increase over the baseline signal. Often a dilution series of known template concentrations can be used to establish a standard curve for estimating the initial template. C_t methods are widely used as the gold standard in diagnosis and genetics for

DNA or cDNA quantification. qPCR is sensitive and accurate technique for quantifying differential gene expression and it is often used to validate results from microarray analysis or other gene expression platforms (Lim et al., 2012; Morey et al., 2006).

2.3.3 Next -generation sequencing (NGS)

For more than two decades, sequencing methods have used the chain termination technique developed by Frederick Sanger in 1977 (Sanger et al., 1977; Metzker, 2005; Hutchison, 2007). Next-generation sequencing (NGS) employs massively parallel sequencing by laying millions of DNA fragments on a single chip and sequencing all fragments in parallel. DNA fragments are used directly to build DNA fragment libraries that are subsequently used as sequencing templates without the cloning process. Both ends of each fragment of the DNA fragment libraries are linked with specific adaptor oligonucleotides by ligation.

Many gene expression studies have been carried out by deep sequencing (RNA-seq) instead of microarrays. Importantly, RNA-seq is able to identify and quantify rare transcripts without prior knowledge of a particular gene and provides additional information such as alternative splicing and sequence variation in transcripts (Wold and Myers, 2008; Auer and Doerge, 2010; Dames et al., 2010; Metzker, 2010; Wang et al., 2009). Furthermore, NGS has the following advantages over microarrays: since it has i) greater sensitivity without any background issues as found in microarrays, ii) no errors due to non-match of single base variation in probe sequences of microarrays, iii) better method to find information about isomers, and iv) better suited for small RNAs (including miRNA and other non-coding RNAs) profiling studies, particularly for elucidating novel miRNA. Consequently, NGS has become the gold standard in current transcriptomic research and has gained in importance in the fields where in-depth analysis of RNA and high accuracy are considered critical (Morozova and Marra, 2008; Motameny et al., 2010; Liu et al., 2012).

The prominent commercial sequencing platforms include the Roche /454 system, Illumina / HiSeq, and AB SOLiD System (Liu et al., 2012). These NGS technologies consist of various strategies for template preparation, sequencing and imaging, and genome alignment and assembly methods (Metzker, 2010; Liu et al., 2012).

Currently, the Illumina /Solexa Genome Analyzer dominates the NGS market due to its features of largest output and lowest reagent cost (Liu et al., 2012). This platform implements sequencing by synthesis, a method in which the synthesis of the complementary template is used to determine the sequence. It can analyse different RNA samples in parallel by incorporating both bridge amplification and dye terminated nucleotides to the process, as illustrated in Figure 2.1 (b). Once the primer is hybridized, the nucleotides which are labelled by four colour fluorescent dyes are added and all the remaining free nucleotides are removed by a washing step in each sequencing cycle (Figure 2.1 a). At the end of each cycle, the terminator and dye group are cleaved off and a new sequencing cycle is started. In the present study, the Illumina Genome Analyzer IIx was used for sequencing microRNAs (miRNA) in liver tissue of cattle from genetically divergent lines of high and low RFI.

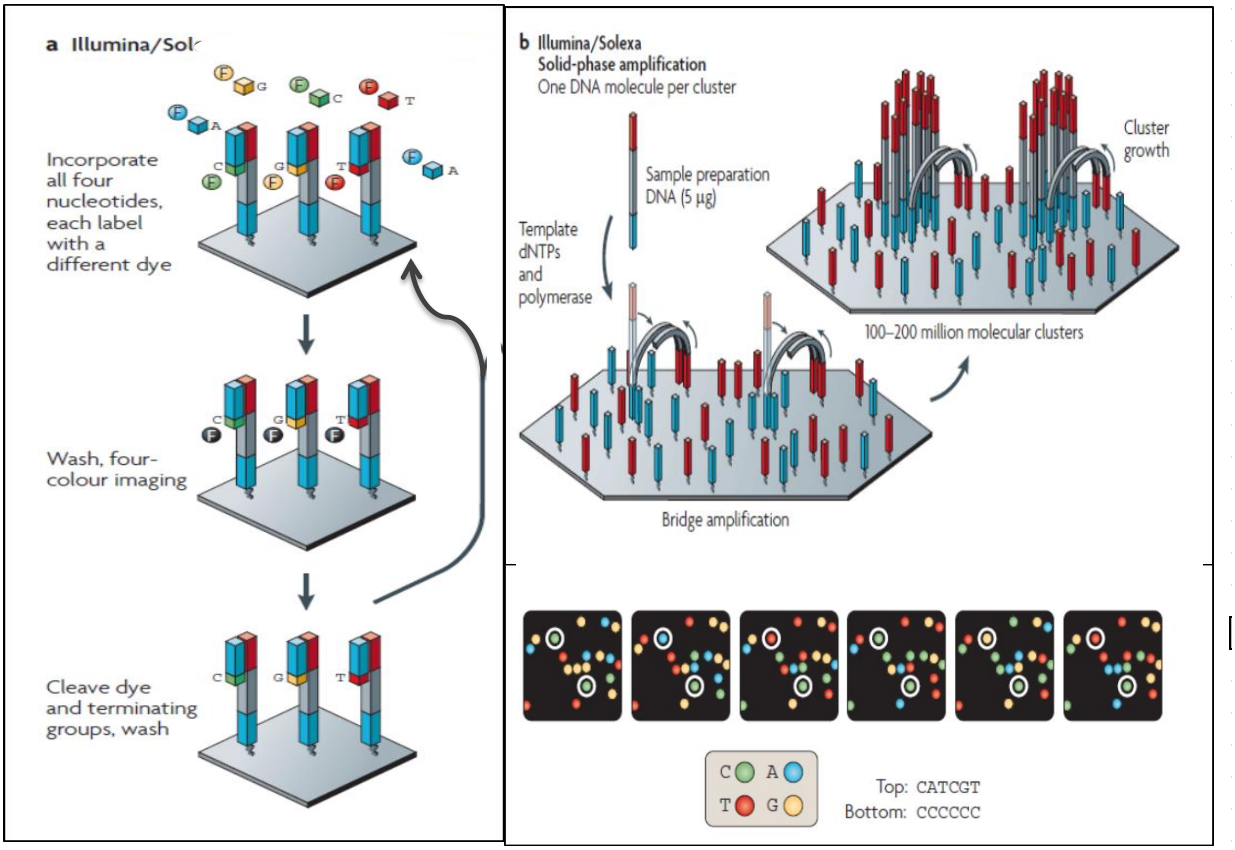

Figure 2.1 Template immobilization strategy and four-colour nucleotides termination method of Illumina/Solexa (Adapted from Metzker, 2010).

2.4 MicroRNA and its function

MicroRNAs (miRNA) are non-coding, single strand, short, approximately 22 nucleotides long, RNA molecules that regulate gene expression in a sequence-specific manner (He and Hannon, 2004; Hutvagner, 2005). MicroRNAs target mRNA through binding at the 3' un-translated region (3'UTR) to translational repression or mRNA degradation (Carmell and Hannon, 2004; Yu et al., 2007; Castro et al., 2010). A single mature miRNA can target hundreds of distinct mRNAs for decreased translation. MicroRNAs have been shown to participate in the regulation of almost every cellular process investigated so far. For instance, they were reported to guide developmental decisions including cell fate, cell cycle progression, muscle development, growth and differentiation (Anderson et al., 2006; Carthew, 2006; Garzon et al., 2006; Nakajima et al., 2006; Callis et al., 2007; Filipowicz et al., 2008; McDaneld et al., 2009a; Wei et al., 2009; Xie et al., 2010; Dávalos et al., 2011; Romao et al., 2012).

2.4.1 Transcription and biogenesis of microRNA

MicroRNAs are individually encoded by nuclear genes. Some are located in the non-protein coding regions of the genome, whereas others occur in the introns and inter genic regions of protein coding genes of the genome. As illustrated in Figure 2.2, complex processes are required for the development and function of miRNA (Chen, 2005). Mature functional miRNA (~ 22 nucleotides) are produced from primary miRNA (pri-miRNA) transcripts. First, the long pri-miRNAs are processed in the nucleus into stem-loop precursors RNA (pre-miRNA) by the RNase III endonuclease Drosha. The pre-miRNAs are then actively transported into the cytoplasm by Exportin 5 and further processed into small RNA double stranded (duplexes) of ~ 22 nt, by the Dicer RNase III enzyme. The functional strand of the miRNA-duplex, which is called mature miRNA, is then loaded into a multiprotein complex known as the "RNA-induced silencing complex" (RISC) (Hutvagner, 2005; Lai, 2005), while the other strand, which is called miRNA*, is degraded in most cases (Buermans et al., 2010a).

Selection of the strands is likely to depend on the relative thermodynamic stability of the ends of the miRNA-duplex. However, in some cases, both strands have been detected at similar expression levels (Buermans et al., 2010b). The sequence of the miRNA loaded into the complex targets the RISC to specific binding sites in the 3' UTR region of target mRNA transcripts, resulting in either degradation of the miRNA: mRNA complex into a cytoplasmic vesicle termed as a processing body, or translational repression. In either case, the association of RISC with mRNA results in a decrease in the level of the targeted gene product (Bushati and Cohen, 2007; Chen, 2005; Lai, 2005; Qi et al., 2008). However, it has been reported (Buchan and Parker, 2007; Vasudevan et al., 2007) that miRNA can also activate the gene expression under certain conditions, for instance during cell cycle arrest.

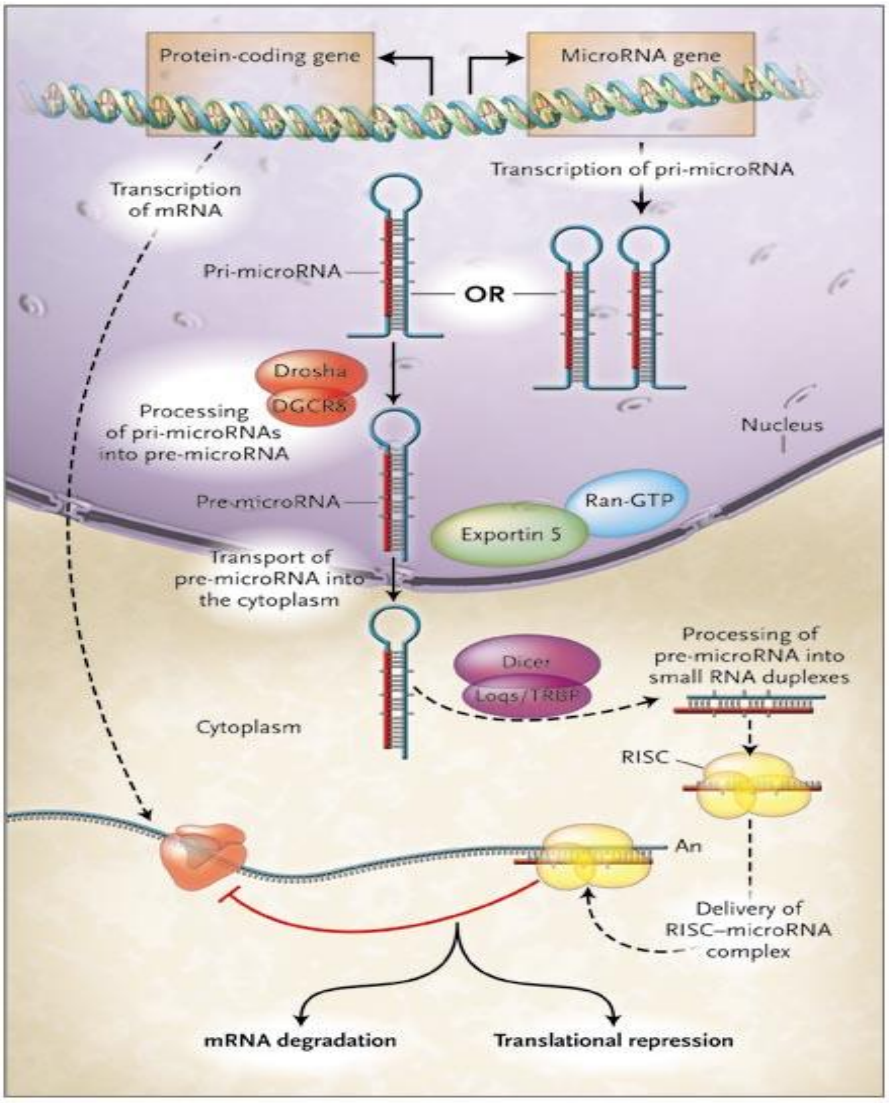

Figure 2.2 Biogenesis of MicroRNA and MicroRNA-Mediated Gene Regulation in Animal Cells (figure reproduced from Chen (2005)).

2.4.2 Profiling microRNA studies in livestock animals

Numerous profiling studies have been carried out in characterizing miRNA encoded in livestock species genomes. It has been demonstrated that there is a wide range of species-specific and tissue-specific miRNA (Liu et al., 2009). These small RNA play important roles in essential physiological processes in livestock, including muscle and organ development (Cho et al., 2010; McDaneld et al., 2009a), adipose tissue differentiation (Jin et al., 2009, 2010), and embryo development (Darnell et al., 2006,

2007; Glazov et al., 2008; Hicks et al., 2010). It has been postulated that many microRNAs may be linked to phenotypic differences or quantitative trait variation of livestock such as feed efficiency (McDaneld, 2009).

MicroRNAs have been evaluated in the skeletal muscle of sheep, pigs, and cattle. Muscle-specific miRNA (miR-1, miR-133, and miR-206) were reported to have roles in skeletal muscle development through regulation of the different stages of myogenesis (Brennecke et al., 2005; Chen, 2005; Nakajima et al., 2006). For instance, in heavily muscled Belgian Texel sheep, miR-1 and miR-206 have been found to regulate myostatin gene expression by decreasing translation of the myostatin protein resulting in an increase in the muscle mass of these sheep (Clop et al., 2006). Recently, McDaneld et al. (2012) utilized NGS to profile porcine skeletal muscle. From that study, a list of target genes for microRNA expressed in porcine skeletal muscle has been identified and reported to be associated with signaling pathways that impact skeletal muscle growth and function, including calcium ion binding, actin structure, and the insulin signaling pathway.

MicroRNAs have been found to have important roles in the embryonic development of livestock species. In chickens, a large and diverse group of miRNAs was found to be expressed differently in different stages of chicken embryonic development (Darnell et al., 2006, 2007; Glazov et al., 2008). Different miRNA expression patterns during ovarian folliculogenesis and early embryogenesis in bovine have been reported (Tripurani et al., 2010).

In Holstein cattle, Huang et al. (2011) reported that testis and ovary have different microRNA expression profiles. Approximately 30.5% of the identified bovine miRNA were found to be expressed differentially between testicular and ovarian tissues. The putative target genes of these miRNAs were found to be involved in pathways associated with reproductive physiology and suggest these diverse miRNAs have potential regulatory roles in the development of the reproductive organs in Holstein cattle.

The role of miRNA in beef cattle has been evaluated in various tissues including ovarian, oocyte, testicular and adipose tissues (Huang et al., 2011; Miles et al., 2009; 2012). Gu et al. (2007) identified 54 adipose tissue-specific miRNAs in bovine adipose tissue compared with miRNAs from mammary gland. Jin et al., (2010) studied miRNA expression levels in subcutaneous adipose tissue and 86 differentially-expressed miRNAs between high and low back-fat thickness cattle were reported. Bovine miR-378 was found to be the most differently expressed miRNA associated with back-fat thickness. Romao et al. (2012) have evaluated the differences in miRNAs expression between bovine

subcutaneous fat tissue and visceral fat depots (perirenal fat) and the effect of diet on miRNA expression patterns with an Agilent miRNA microarray. It was demonstrated that the profiles of miRNAs were different among fat depots and the expression profiles were affected by diet. Recently, Wang et al. (2013) reported 30 differentially-expressed miRNAs between intramuscular adipose and subcutaneous adipose of adult beef cattle.

The effects of miRNA in bovine muscle type specification and maintenance have been studied by Muroya et al. (2013). Differentially-expressed miRNAs between semitendinosus and masseter muscles were determined. Bovine miR-196a and miR-885 were expressed exclusively in semitendinosus muscle. Recently, Sun et al. (2013) studied miRNA expression patterns of bovine muscles at fetal and adult stages using deep sequencing NGS. Bovine miRNA-206, miRNA-1, and miRNA-133 were found to have role in muscles development and were highly expressed in muscle-related tissues and organs.

2.5 Conclusions

Feed intake and feed efficiency are important factors that affect overall profitability of beef production. Residual feed intake (RFI) is a measure of feed efficiency and has been adopted in Australia for genetic improvement.

Molecular genetics facilitates the study of the genetic make-up of individuals at the DNA level. Many QTL and SNP markers have been found to be associated with RFI in several studies and all so far have failed validation in other than their discovery population. No major causative genes affecting RFI were found.

An alternative approach to discover the causative genes underlying RFI is to gain a better understanding of the molecular architecture of RFI by gene expression via genome-wide transcriptional profiling. Genetic analyses of variation in gene expression have provided valuable models for studying the genetic architecture of complex traits (Cheung and Spielman, 2002). A long-oligo microarray experiment has identified 161 differentially expressed genes in cattle from two genetically divergent selection lines for RFI (Chen et al., 2011a). Further studies are required to evaluate these genes in other cattle herds and to determine the correlation of expression levels of these genes with RFI and other production traits.

One of the major classes of small RNA is microRNA which can regulate a number of cellular and genetic functions. Their role in regulating key cellular and physiological pathways that may impact the variation of RFI in beef cattle remains to be determined. It is necessary to study the role of miRNAs in growth and feed utilization in beef cattle.

The current study will: 1) Evaluate the expression level, in liver, of eight RFI-related genes in a production herd and their correlation with RFI; 2) Evaluate the effect of hormonal growth promotants (HGP) on the expression of RFI-associated genes and RFI in beef cattle in the feedlot; 3) Perform the first miRNA expression profile in bovine liver and to elucidate the role of miRNAs in regulation of RFI in cattle.

Chapter 3

Expression of candidate genes for residual feed intake in Angus cattle

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Chapter 4

Hormonal growth implants affect feed efficiency and expression of residual feed intake-associated genes in beef cattle

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Chapter 5

Liver microRNAs profiling with deep sequencing in cattle divergently selected for residual feed intake

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Chapter 6

General Discussion

6.1 introduction

The advance of genomics in past two decades has increased our understanding of the scientific basis of livestock biology and provided novel methods for improving the genetic gain in animal breeding. These new developments have the potential to achieve increases of feed efficiency and sustainable productivity and lessen environmental impact. Feed efficiency measured by RFI is highly related to the basic metabolic processes, and genetic association between energy requirement for maintenance of body weight and RFI has been reported in cattle (Herd and Bishop 2000). Many physiological processes may contribute to variation of RFI, such as feed intake, digestion of feed, metabolism, physical activity, and thermoregulation (Herd & Arthur 2009). Studies on Angus steers following divergent selection for RFI estimated that heat production from metabolic processes, body composition, and physical activity explained about 73% of the variation in RFI.

The genomic basis to variation in these physiological processes yet not very clear. Early genomic studies have shown many hundred genes to be associated with differences in RFI. A first attempt to elucidate the genetic basis of variation in RFI using global gene expression was conducted by Chen and colleges (2011a) using liver biopsies from Angus bulls from divergent selection lines of high and low RFI. They have identified 161 genes expressed different between high and low RFI cattle. These genes were involved in seven gene networks affecting cellular growth and proliferation, cellular assembly and organization, cell signaling, drug metabolism, protein synthesis, lipid metabolism and carbohydrate metabolism.

In follow up work using animals from the same selection lines, Chen et al. (2011b) and Chen et al. (2012) used real time PCR to measure gene expression on a subset of the 14 most promising candidate genes identified in the microarray study. These genes were selected based on the magnitude of the expression difference between high and low RFI animals and because they are located within reported QTL regions for RFI. Eight of the 14 genes were significantly associated with RFI-EBV and the expression levels showed high accuracy in discriminating animals into high and low RFI groups via linear discriminant analysis.

This thesis was aimed to understand the genetic mechanisms involved in RFI in beef cattle and demonstrated that the level of gene expression of eight candidate genes was correlated with RFI, which is highly correlated with FCR and DFI. Expression levels of these genes can be used in combination to build prediction equations for RFI, which can explain one third of the phenotype variation. This thesis, further evaluated these gene expression changes caused by HGP implants which promote growth and improve FCR. Finally, extensive hepatic microRNA profiling with divergently-selected RFI lines was carried out and identified novel bovine microRNAs and differentially expressed microRNA between the high and low RFI animals by deep sequencing. This general discussion will consider the current results and future directions of such studies, particularly how they will be affected by a rapid development of next generation sequencing.

6.2 RFI-related genes expression with qPCR

RFI-related genes (*AHSG*, *GHR*, *GSTM1*, *INHBA*, *PCDH19*, *SERPINI2*, *S100A10*, and *SOD3)* were confirmed associated with RFI phenotype in steers of Angus production herd. Also, the expression levels of these genes were used in combination to build prediction equations for RFI, which can explain about 32.2% of the phenotypic variation. The proportion of the variation explained by these genes is consistent with that reported in young bulls and 250-day feed lot steers (Chen et al., 2012a; 2012b). However, this prediction equation was based on small number of samples. There are some challenges in practical implementation in beef genetic improvement. These include target tissue biopsy procedures, standardizing the gene expression measurements across labs, and high cost of assays.

6. 3 RFI-related gene expression was not affected by HGP

Our results demonstrated that HGP treatment did not affect RFI nor expression levels of the RFI-associated genes. These results indicate that the metabolic pathways affected by HGP, which result in improved FCR, are different from the metabolic pathways involved in RFI. There is evidence that HGP implants improve growth rates through their action in effectively increasing protein synthesis and reducing protein degradation (Dunshea et al., 2005; Hunter et al., 2001a; 2001b; Hunter, 2010). It has been hypothesized that protein turnover may be one of the regulators of RFI in cattle (Herd et al., 2004; Richardson and Herd, 2004; Herd and Arthur, 2009). The lack of effect of HGP treatment on RFI suggests that RFI in cattle is regulated by other mechanisms not impacted

by the HGP treatment such as mechanisms can be found the functions of xenobiotic pathway as have been reported previously (Chen et al., 2011a).

6.4 Profiling miRNAs in bovine liver

Liver miRNAs could play an important role in regulation of the physiological processes and pathways underlying variation in RFI in beef cattle. Forty two bovine miRNAs were identified as being differentially expressed between the high and low RFI line cattle. Bovine *miR-143* was the most abundant miRNA and was down-regulated in low RFI (high feed efficiency) cattle. Hepatic *miR-143* has been found to be contributing to obesity-associated insulin resistance in mice by impairing insulin-stimulated AKT activation and glucose homeostasis, and the knockout *miR-143* mice did not develop obesity-associated insulin resistance (Jordan et al., 2011). Interestingly, *bta-miR-143* has a target site in the 3'UTR of *CD4* which expressed higher in low RFI line cattle (Chen et al., 2011a). These findings indicate that *bta-miR-143* plays a key role in feed efficiency of beef cattle.

Among differentially expressed isomiRs miRNA (mature-star miRNA), *bta-mir-122-3p* was found down-regulated in low RFI cattle have a targeted *COL3A1* that was up-regulated in low RFI cattle (Chen et al., 2011a). *MiR-122* is well known as liver-specific miRNA in human and mouse, and it was the first miRNA linked to cholesterol and lipid metabolic control. Suppression of *mir-122* by antisense reduced plasma cholesterol levels by 25–30% in mice. It also reduced expression of genes involved in lipid synthesis in liver and decreased hepatic cholesterol and fatty acids (Esau et al., 2006; Jin et al., 2009; McDaneld, 2009; Rottiers and Näär, 2012). These findings indicate that *bta-miR-122* plays a key regulator of cholesterol and fatty-acid metabolism that might affect feed efficiency in cattle as well.

Our results showed that *bta-miR-19b, bta-miR-101, bta-miR-106b,* and *bta-miR-142-3p* were down-regulated in low RFI cattle. It has been reported that their expression levels are highly influenced by the changes in the energy density of the diet (mainly lipid levels in the diet) in steers (Romao et al., 2012). Furthermore, we found that *bta-miR-19b* was down-regulate in low RFI cattle and it targets five genes, *EDNRB, IGFBP3, POSTN, DHRS3,* and *EDNRB,* which were all up-regulated in low RFI cattle (Chen et al., 2011a). Together these results suggest that these miRNAs, particular *bta-miR-19b,* might be key regulators of specific functions or pathways in feed utilization in bovine.

The current studies used pooled total RNA for identification of differentially expressed microRNA between high and low RFI animals. The lack of biological replication has limited the statistical power of the experiment. With the dramatic reduction of sequencing cost, future studies will be able to generate microRNA profiling for large numbers of animals with genome sequence information. This will enable not only listing of miRNAs correlated to RFI; it also will have the potential to identify the DNA variants in the genome which regulate miRNA expression.

6.5 Points for consideration in future research

- **Gene expression can explain a good portion of RFI phenotype variance.** As the eight RFI-associated genes (*AHSG, GHR, GSTM1, INHBA, PCDH19, SERPINI2, S100A10,* and *SOD3*) have explain a reasonable proportion of the variance in RFI, and they can be used to build prediction equations for RFI. It is likely because it can capture both the genetic and some environmental variance. However, the exact nature of the relationship between expression and RFI is still unclear and future research need to be done to address following issues:

1) **Samples' size:** While this work validated and extended previous discovery of gene expression related to RFI, the number of animals used in this work was quite small and no validation of the prediction equations obtained in this study with independent samples was carried out. How well these predictions will hold in other populations need to be investigated with a larger number of independent animals.

2) **Samples' tissue:** Liver samples are not practical for routine usage, although gene expression data can potentially be used for phenotypic prediction and can capture a large proportion of the phenotypic variance. Therefore, investigation of expression of these genes in other more practical tissues such as blood should be considered. As blood (WBCs) can easily be taken from the living animal, it has been selected as ideal tissue for biomarker screening. Recently Silva et al. (2013) used RT-PCR technique to measure the expression of BPV2 in the blood to diagnose healthy and papillomatosis-affected cattle.

- **Using microRNAs as a biomarker:** Profiling bovine liver microRNAs from divergent-RFI selection-lines increased our understanding of the role of miRNAs in

bovine gene expression and feed efficiency of beef cattle. Liver microRNAs could play an important role in regulation of the physiological processes and pathways underlying variation in RFI in beef cattle. Differentially expressed miRNAs between the high and low RFI line cattle regulate the gene expression of key genes involved in glucose homeostasis, obesity-associated insulin resistance mechanisms, and cholesterol and fatty-acid metabolism that might affect feed efficiency in cattle. Further research is required to study and validate differentially expressed miRNAs and addressing issues such as:

- **Validation in other tissues:** Detected differentially expressed miRNAs need to be validated in other tissues such as muscles and blood (WBC). Predicted miRNAs targets should be functionally annotated. Besides, the potential RNA-sequencing to identify causative mutations by combine genomic sequencing is also need to be considered in future studies.

6.6 Conclusion

This thesis demonstrated that expression of RFI-associated genes (*AHSG, GHR, GSTM1, INHBA, PCDH19, SERPINI2, S100A10*, and *SOD3*) can explain a reasonable proportion of the variance in RFI, and they can be used in combination to build prediction equations for RFI. In the cattle studied, RFI was highly correlated with FCR and DFI, and uncorrelated with ADG and weight, intramuscular fat, eye-muscle area, subcutaneous $12/13^{th}$ rib fat depth, P8-rump fat depth and hot-carcass weight. These are typical of results from many previous RFI tests with many cattle breeds. Our findings confirmed the differential expression of these genes and described their correlation with RFI. These results can be used to inform genomic studies on other cattle breeds.

Hormonal growth promotants did give the expected improvement in FCR and ADG, but did not affect RFI or expression levels of the RFI-associated genes. This implies that the metabolic pathways affected by HGP which resulted in improved FCR are different from the metabolic pathways involved in RFI.

Profiling of miRNAs in liver by deep sequencing has improved the miRNA annotation in the bovine genome. Differentially expressed miRNAs between the cattle from divergent RFI selection lines increased our understanding of the role of miRNAs in bovine gene expression and feed efficiency of beef cattle.

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