Identification of functional genes for feed efficiency traits via transcriptome analyses to enhance the genomic prediction accuracy in beef cattle

by

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Abstract

Residual feed intake (RFI), a measure of feed efficiency, and its component traits including average daily gain (ADG), dry matter intake (DMI) and metabolic weight (MWT) are traits of great economic importance to the beef industry. The genetic improvement of these traits can improve the industry's profitability as well as reduce the environmental footprints of beef production, thus leading to more sustainable beef production. However, these traits are difficult and expensive to measure on individual animals, making them good candidates for genomic prediction, a method that predicts animal's genetic potential based on DNA markers. However, the accuracies of genomic prediction on these traits are general low in beef cattle and it is believed that incorporation of information on genetic mechanisms controlling these traits will improve the accuracy of genomic prediction. Therefore, in the current project we aimed to use RNAseq technology to identify candidate functional genes associated with RFI and its component traits in beef cattle. We further investigated the potential of improving genomic prediction accuracy of RFI and its component traits by utilizing the functional gene information.

In the first study we analyzed whole liver transcriptome RNAseq data between six (n = 6) high and six (n = 6) low-RFI steer groups from three beef cattle breeds including Angus, Charolais and Kinsella Composite (KC) raised together. Similar analyses were performed in the second study between the steer groups with divergent component trait phenotypes from the three breeds. In total we identified 253, 252, 375 and 206 differentially expressed (DE) genes associated with RFI, ADG, DMI and MWT, respectively. For each trait the majority (82 – 88%) of the DE genes were breed specific. Functional enrichment analyses revealed that the identified DE genes are mainly involved in metabolism of lipids, carbohydrates, amino acids, molecular transport, cellular movement and cell-to-cell signaling.

In the third study we explored differential micro RNA (miRNA) expression between six (n = 6) high and six (n = 6) low-RFI steers in the three beef breeds considered in studies I and II. Likewise, in the fourth study we investigated the association of miRNA expression with ADG, DMI and MWT in the three beef breeds. We identified 39 DE miRNAs associated with RFI, 36 DE miRNAs associated with ADG and 46 miRNAs were identified as associated with both DMI and MWT. Consistent with the DE genes findings in the first and second studies, DE miRNAs were also majorly breed specific. Additionally, DE miRNAs were predicted to target 55 – 76% of the identified DE genes which are involved in key molecular and cellular functions such as metabolism of lipids, carbohydrates, protein and amino acids as well as cell proliferation, and cell death and survival.

To explore the possibility of improving genomic prediction accuracy through integration of transcriptomic information in the fifth study, we compiled a functional single nucleotide polymorphisms (SNP) panel from a total of 3,735 DE and miRNA target genes from this project and from literature (coding and miRNA precursor genes), and compared it to a commercial 50K SNP panel and a randomly selected (Random) SNP panel. Genomic prediction accuracies for RFI and its component traits were estimated for 7,372 beef animals from six beef breed populations including Angus, Charolais, KC, Elora, PG1 and TX. Results from this study indicated that generally the functional panel did not significantly yield higher genomic prediction accuracies than the other two panels. However, it had slightly higher accuracy for all the four traits for within Charolais evaluation.

In conclusion, results from this PhD thesis project contribute to the understanding of genetic architectures of feed efficiency and its component traits. The results also demonstrated the potential to enhance genomic prediction accuracy through integration of functional information.

However, further research on the utilization of functional information is required to enhance genomic prediction accuracy of feed efficiency and its related traits in beef cattle.

Preface

This thesis contains original PhD research work by Robert Mukiibi, and it is part of a collaborative research project between Dr. Changxi Li of the Department of Agricultural, Food and Nutritional Science, Faculty of Agricultural, Life and Environmental Sciences, University of Alberta, Canada, and Dr. Sinéad Waters of the Animal and Bioscience Research Department, Teagasc, Ireland, under the UAlberta-Teagasc Walsh Fellowship program. I drafted the whole thesis and finalized the thesis with inputs from Dr. Changxi Li, Dr. Sinéad M. Waters, and Dr. Paul Stothard.

Chapter two of this thesis has been published as Mukiibi Robert, Michael Vinsky, Kate A. Keogh, Carolyn Fitzsimmons, Paul Stothard, Sinéad M. Waters, and Changxi Li. "Transcriptome analyses reveal reduced hepatic lipid synthesis and accumulation in more feed efficient beef cattle." *Scientific reports* 8.1 (2018): 7303. In this study, I performed all the data analyses, interpreted the results, and drafted the manuscript. Changxi Li conceived, designed, and supervised the study. Kate Keogh contributed to functional enrichment analyses. Sinéad Waters, Paul Stothard, Michael Vinsky and Carolyn Fitzsimmons contributed to the design of the study and acquisition of data. All authors read, commented and approved the final manuscript.

Chapter three has been accepted for publication in the Journal of Animal Science as Robert Mukiibi, Michael Vinsky, Kate Keogh, Carolyn Fitzsimmons, Paul Stothard, Sinéad M. Waters, and Changxi Li.” Liver transcriptome profiling of beef steers with divergent growth rate, feed intake or metabolic body weight phenotypes.” In this study, I performed all the data analyses, interpreted the results, and drafted the manuscript. Changxi Li conceived, designed, and supervised the study. Kate Keogh contributed to functional enrichment analyses. Sinéad Waters, Paul

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For Chapter six, the manuscript is under preparation for publication in a peer-reviewed scientific journal as Robert Mukiibi, Yining Wang, Michael Vinsky, Paul Stothard, Sinéad M. Waters and Changxi Li. "Evaluation of genomic prediction accuracy of feed efficiency and the related traits with integration of differentially expressed gene SNPs" In this study I compiled all

candidate genes, performed genomic prediction analyses, interpreted the results, and drafted the manuscript. Yining Wang performed imputation and contributed to designing the functional panel. Changxi Li conceived, designed, and supervised the study. Paul Stothard, Sinéad M. Waters. Michael Vinsky contributed to the design of the study and SNP data acquisition.

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Chapter 1. General introduction

1.1 Beef production

Beef is a major protein and other nutrients source for human consumption whose demand continues to increase due to the growing human population size and the improving economic status of people (Gerber *et al.* 2013; Li 2017; Smith *et al.* 2018). Beef also contributes largely to the world economies, for example, in Canada the beef industry accounts for up to 16% of total farm cash receipts (years 2014-2018), as well as provides an average of 228,000 jobs to Canadians with an annual contribution of $18 billion to the gross domestic product (GDP), (CCA 2018). In the USA, beef is the fourth largest agricultural export contributing up to $7.3 billion to the annual (for 2017) export revenues (USDA 2017). With the projected increase of the global population to 9.6 billion by the year 2050 (Gerber *et al.* 2013), the increase demand for beef is likely to impart more pressure on already limited production resources including land, water, fertilizers, labor and feed materials (Gerber *et al.* 2013). Additionally, cattle contribute up 65% of the total livestock greenhouse gas emissions, of which beef and dairy cattle account for 41% and 20% respectively (Gerber *et al.* 2013). This environmental impact is expected to cause public concerns on increasing beef production unless stainable production strategies are implemented.

1.2 Feed efficiency in beef cattle

For sustainable beef production, improving feed efficiency of the beef animals is among the practical strategies that have been proposed (Hocquette & Chatellier 2011). Feed efficiency can generally be defined as the ability of the beef cattle animal to convert consumed feed (nutrients) into salable product/beef (Carstens & Tedeschi 2006). Feed efficiency is of great interest to the beef

production industry mainly because feed and feeding related costs represent the largest variable production cost for the industry ranging between 55 to 75% of the total variable costs (Ahola & Hill 2012; Brandebourg *et al.* 2013). The high costs of feeds or feed materials can majorly be attributed to numerous factors including the increased cost of feed production inputs such as fertilizers, land, labor, water, and thus feed processing costs (Archer *et al.* 1999). The cost of feed is further exacerbated by the competition with human food or bioenergy production from the same materials that are used to produce animal feeds. In beef cattle, feed efficiency is measured by several traits including partial efficiency of gain (Kellner 1909), feed conversion ratio (Brody 1945), Kleiber ratio or growth/metabolic weight (Kleiber 1947), residual feed intake (Koch *et al.* 1963), residual body gain (Crowley *et al.* 2010) and residual intake and body weight gain (Berry & Crowley 2012).

1.3 Residual feed intake

Of the feed efficiency measures, residual feed intake (RFI), which is defined as the difference between the actual feed intake of the animal and expected intake of the animal based on its body weight and growth rate (Koch *et al.* 1963), has gained popularity as a measure of feed efficiency in beef cattle due to its phenotypic independency from body weight and growth rate of the animal (Kennedy *et al.* 1993; Archer *et al.* 1999). Numerous studies in beef cattle have revealed RFI as having moderate heritability estimates (0.16 – 0.68), (Herd & Bishop 2000; Arthur *et al.* 2001; Schenkel *et al.* 2004; Nkrumah *et al.* 2006; Mao *et al.* 2013; Pryce *et al.* 2014). This has enabled the beef industry to improve feed efficiency through genetic selection for RFI. Moreover, studies have also reported positive

genetic correlation estimates between RFI and methane emission (Nkrumah *et al.* 2006; Hegarty *et al.* 2007), hence revealing possibilities of reducing the carbon footprint impact of beef cattle to the environment through selection of more feed efficient animals.

1.4 Physiological background of residual feed intake

RFI is a complex trait whose phenotypic variability is affected by multiple body organs, and hence numerous physiological process. Some of the main physiological mechanisms influencing RFI include feeding patterns, digestibility, heat increment of fermentation, activity and body composition which have been reported to account for 2%, 10%, 9%, 10% and 5% of the RFI variability in beef cattle respectively (Herd *et al.* 2004; Richardson & Herd 2004). In addition, protein turnover, tissue metabolism and stress together were predicted to account for around 37% of the RFI variability, whereas other processes such as ion or molecular transport accounted for 27% of RFI variation (Herd *et al.* 2004; Richardson & Herd 2004).

1.5 Molecular genetic background of RFI

With the advancement of functional genomics and molecular genomic tools such as identification of molecular markers (including microsatellites and single nucleotide polymorphisms or SNPs) in the bovine genome, efforts have been made to genetically characterize RFI and its component traits in beef cattle. The initial attempts to characterize the genomic basis of feed efficiency and the component traits was performed through linkage and association analysis with a few markers distributed across the bovine genome. Multiple QTLs were located on chromosomes for RFI, dry matter intake (DMI) and average daily gain (ADG) (Barendse *et al.* 2007; Nkrumah *et al.* 2007; Sherman *et al.* 2009). Additionally, SNP markers within candidate genes believed to have effects on RFI or its component traits have been evaluated for associations with these traits (Sherman *et al.* 2008; Karisa *et al.* 2013; Alexandre *et al.* 2014). These studies

revealed associations between feed efficiency or/and its component traits with SNP markers in candidate genes such as *GHR*, *NR1I3*, *IGF2*, *NPY* and *UCP2* (Sherman *et al.* 2008; Karisa *et al.* 2013; Alexandre *et al.* 2014). With the development of denser commercial SNP panels spanning the entire genome, several studies have performed genome-wide association studies with the aim of identifying genomic regions or SNPs associated with RFI, or/and its component traits in different beef cattle populations/breeds (Abo-Ismail *et al.* 2010; Bolormaa *et al.* 2011; Mujibi *et al.* 2011b; Rolf *et al.* 2012; Abo-Ismail *et al.* 2014; de Oliveira *et al.* 2014; Saatchi *et al.* 2014; Santana *et al.* 2014; Olivieri *et al.* 2016; Santana *et al.* 2016; Seabury *et al.* 2017). In general, RFI and its component traits are controlled by many QTLs that are distributed on all the 29 autosomal bovine chromosomes, except MWT for which no QTL has been identified yet on chromosome 29 (Hu *et al.* 2018). The estimated additive effects of the significant SNP markers or genomic windows associated to these traits are generally small, and the studies considering multiple breeds have indicated that the identified QTLs are largely breed specific (Saatchi *et al.* 2014). Some QTLs have been reported to have pleotropic effects of controlling RFI and its component traits (Nkrumah *et al.* 2007; Seabury *et al.* 2017). Moreover, genes close or within the regions associated with RFI have been identified to be generally involved in multiple biological functions such as energy generation and use, protein metabolism, lipid metabolism, molecule transport, the immune response, cellular secretion, cellular activity, and growth (Abo-Ismail *et al.* 2010; Mujibi *et al.* 2011a; de Oliveira *et al.* 2014; Olivieri *et al.* 2016; Santana *et al.* 2016).

In addition to genome-based QTL linkage mapping and DNA marker association or genome-wide association studies (GWAS), transcriptome analyses have also been performed to characterize feed efficiency and the related traits. Differential gene expression in the tissue from several organs such as the liver (Chen *et al.* 2011; Alexandre *et al.* 2015; Paradis *et al.* 2015;

Tizioto *et al.* 2015; Weber *et al.* 2016), adipose tissue (Weber *et al.* 2016), spleen, skeletal muscle (Tizioto *et al.* 2016; Weber *et al.* 2016), pituitary gland (Weber *et al.* 2016), rumen epithelia (Kern *et al.* 2016; Kong *et al.* 2016; Reynolds *et al.* 2017), jejunum (Lindholm-Perry *et al.* 2016; Foote *et al.* 2017; Reynolds *et al.* 2017), duodenum (Lindholm-Perry *et al.* 2016; Weber *et al.* 2016) and ileum (Lindholm-Perry *et al.* 2016) have been investigated in relation to feed efficiency (especially RFI) and its component traits in beef cattle. Indeed, consistent with the results of GWAS, multiple genes associated with feed efficiency and the related traits have been identified through these whole transcriptome studies on all the 29 autosomal chromosomes. Some of the major biological functions identified in these studies as associated with include lipid metabolism, amino acid metabolism, carbohydrate metabolism, drug or xenobiotic metabolism, transport of molecules and immune response and inflammation (Chen *et al.* 2011; Alexandre *et al.* 2015; Tizioto *et al.* 2015; Weber *et al.* 2016; Foote *et al.* 2017; Reynolds *et al.* 2017).

Furthermore, post translational gene expression regulation processes play key functions in modulating different cellular processes to maintain homeostasis and optimal or normal cell function (Lackner & Bähler 2008; Lu & Clark 2012). In this regard studies on the differential expression of microRNAs (miRNAs) and potential regulatory activities on their target genes have been studied in beef cattle in the skeletal muscle (De Oliveira *et al.* 2018) and liver (Al-Husseini *et al.* 2016; De Oliveira *et al.* 2018). These studies have identified multiple miRNAs associated with feed efficiency and some of the miRNAs have been predicted to target differentially expressed genes in the same tissues (Al-Husseini et al. 2016; De Oliveira et al. 2018). Also, more recently some proteomic studies have been conducted to investigate the molecular controls of RFI at the proteome level in the liver tissue (Baldassini *et al.* 2018; Fonseca *et al.* 2019). Identified differentially abundant proteins are majorly involved in energy metabolism, xenobiotic

metabolism, vitamin metabolism, amino acid metabolism, mitochondrial function, oxygen transport, blood flow, ion transport, and cell survival, microbial metabolism, biosynthesis of fatty acids, and antigen processing and presentation (Baldassini *et al.* 2018; Fonseca *et al.* 2019). Despite all the reviewed studies, we still do not have a clear picture of the molecular architecture of RFI and its component traits as the concordance of the identified genes across studies is low even for the same tissue type. This could generally be attributed to the differences in the breed/population, sex type and age of the animals used in the different studies, and in the environmental conditions under which the experimental animals in the different experiments were tested.

1.6 Genomic selection for feed efficiency

Traditional genetic selection for feed efficiency requires accurate measurement of feed intake for each of the selection candidate or their relatives which is currently an expensive and time-consuming process (Chen *et al.* 2013; Pryce *et al.* 2014). This rendered genomic selection a greatly desirable method of genomic evaluation and selection for feed efficiency. Genomic selection involved estimation of genetic merit of selection candidates based only on their genome-wide marker genotypes after the SNP markers' effects are estimated based on the reference animals that have both marker genotype and phenotypic information (Meuwissen *et al.* 2001; Meuwissen *et al.* 2013). The accuracy of genomic prediction or genetic merit estimation is of great interest as it is a key factor affecting the response to genomic selection (Goddard 2009; Goddard & Hayes 2009). Genomic prediction accuracy is affected by several factors including the size of the reference population, genetic relationship between the selection candidates and the reference population, heritability of the trait, number of SNP markers in the evaluation panel, genetic architecture of the trait and the statistical method used for the genetic evaluation (Goddard 2009;

Goddard & Hayes 2009; Zhang *et al.* 2019). In beef cattle, achieving a reasonably greater accuracy of genomic predictions is a challenge due to the difficulty to establish large reference populations with good genetic representation of crossbred commercial animals from the industry, which is characterized by less usage of artificial insemination and high rate of crossbreeding (Stock & Reents 2013). Consequently, multiple strategies have been evaluated with the objective of improving genomic prediction accuracy for feed efficiency or the related traits. For example, Mujibi et al. (2011) evaluated effect of BayesB and random regression BLUP (RR-BLUP) methods of genomic evaluation, together with SNP density and structure of the reference population on genomic prediction accuracy of RFI, DMI and ADG in a crossbred population (Mujibi *et al.* 2011c). In general, they obtained higher genomic prediction accuracy with RR-BLUP than the BayesB method. Also, they obtained higher genomic prediction accuracy when they allowed sire overlap between the reference and validation populations than when no sire overlap was allowed. Interestingly, higher accuracies were also obtained using a subset of 200 SNP with larger SNP effects than using the commercial 50K SNP genotypes (37,959 SNPs). However, they hypothesized that SNP effects of the top 200 SNPs for each trait could have been limited to the studied population. In another study using two purebred (Angus and Charolais) populations, Chen et al. (2013) evaluated the impact of the reference population construction strategies, and statistical methods (GBLUP and BayesB) for RFI. On average, allowing sire overlap between the validation and training population resulted in higher accuracies than when no sire overlap was allowed. Within breed evaluations had higher accuracies than across breed genomic prediction. In a study by Khansefid et al. (2014), residual feed intake records and SNPs of high density (HD) chip of 842 Holstein heifers and 2,009 beef cattle from Australia, and 2,763 Canadian beef cattle were combined as a reference population in an attempt to improve the

genomic prediction accuracy for RFI (Khansefid *et al.* 2014). The results showed that the multi-breed reference population increased the accuracy of genomic prediction slightly by an average of 5% through using a larger multibreed population (Khansefid *et al.* 2014).

From the above highlighted studies, it is evident that there is potential for improving genomic prediction accuracies for feed efficiency and the related traits in beef cattle through incorporation of functional information into genomic prediction as proposed by Snelling et al. (2013). This is mainly because of reduced reliance on linkage disequilibrium (LD) for gene or regulatory SNP markers to capture the effects of QTLs (Snelling *et al.* 2013). It is believed that functionally enriched SNP panels would also result in a higher genomic prediction accuracy for crossbred animals as prediction would be based on the functional SNP effects expected to be relatively stable across breeds or breeds, rather than LD that is usually unstable across population or usually broken down through crossing(Snelling *et al.* 2013). Transcriptome studies provide an important source of such biological information as they have the potential to identify transcribed genes associated with the traits (Snelling *et al.* 2013). However, differentially expressed genes and enriched molecular processes in key tissues related to RFI and its component traits in beef cattle remain largely unknown, especially when difference breeds are considered, and utilization of gene SNPs or functional SNPs has not been investigated in beef cattle regarding genomic prediction accuracy of feed efficiency and its related traits.

1.7 Research objectives

In this thesis project we performed five studies whose specific objectives were to:

(i) Identify genes associated with feed efficiency in beef cattle and biological functions through liver global transcriptome analyses in three beef breed populations (Angus, Charolais and Kinsella Composite).

(ii) Identify genes associated with average daily gain or dry matter intake or metabolic weight in beef cattle and biological functions through liver global transcriptome analyses in three beef breed populations (Angus, Charolais and Kinsella Composite).

(iii) Identify microRNAs associated with feed efficiency in beef cattle and their potential target genes through liver differential microRNA expression analyses in three beef breed populations (Angus, Charolais and Kinsella Composite).

(iv) Identify microRNAs associated with genes associated to average daily gain or dry matter intake or metabolic weight in beef cattle and their potential target genes through liver differential microRNA expression analyses in three beef breed populations (Angus, Charolais and Kinsella Composite).

(v) Investigate the potential of improving genomic prediction accuracy for feed efficiency and the component traits in beef cattle through integrating SNPs of DE genes identified by transcriptome analyses from our current project as well as from literature into a functional SNP panel for genomic selection.

1.8 References

Abo-Ismail M., Squires E., Swanson K., Lu D., Wang Z., Mah J., Plastow G., Moore S. & Miller S. (2010) Fine mapping QTL and candidate genes discovery for residual feed intake on Chromosomes 5, 15, 16, and 19 in beef cattle. In: Proceedings of the 9th World Congress on Genetics Applied to Livestock Production: 1-6 August 2010; Leipzig.

Abo-Ismail M.K., Vander Voort G., Squires J.J., Swanson K.C., Mandell I.B., Liao X., Stothard P., Moore S., Plastow G. & Miller S.P. (2014) Single nucleotide polymorphisms for feed efficiency and performance in crossbred beef cattle. BMC genetics 15, 14.

Ahola J.K. & Hill R.A. (2012) Input Factors Affecting Profitability: a Changing Paradigm and a Challenging Time. In: Feed efficiency in the beef industry (ed. by Hill RA), p. 10. Wiley-Blackwell, Ames, Iowa.

Al-Husseini W., Chen Y., Gondro C., Herd R.M., Gibson J.P. & Arthur P.F. (2016) Characterization and profiling of liver microRNAs by RNA-sequencing in cattle divergently selected for residual feed intake. Asian-Australasian journal of animal sciences 29, 1371.

Alexandre P.A., Gomes R.C., Santana M.H., Silva S.L., Leme P.R., Mudadu M.A., Regitano L.C., Meirelles F.V., Ferraz J.B. & Fukumasu H. (2014) Bovine NR1I3 gene polymorphisms and its association with feed efficiency traits in Nellore cattle. Meta gene 2, 206-17.

Alexandre P.A., Kogelman L.J., Santana M.H., Passarelli D., Pulz L.H., Fantinato-Neto P., Silva P.L., Leme P.R., Strefezzi R.F. & Coutinho L.L. (2015) Liver transcriptomic networks reveal main biological processes associated with feed efficiency in beef cattle. BMC genomics 16, 1073.

Archer J., Richardson E., Herd R. & Arthur P. (1999) Potential for selection to improve efficiency of feed use in beef cattle: a review. Australian Journal of Agricultural Research 50, 147-62.

Arthur P., Renand G. & Krauss D. (2001) Genetic and phenotypic relationships among different measures of growth and feed efficiency in young Charolais bulls. Livestock production science 68, 131-9.

Baldassini W., Bonilha S., Branco R., Vieira J., Padilha P. & Lanna D. (2018) Proteomic investigation of liver from beef cattle (Bos indicus) divergently ranked on residual feed intake. Molecular biology reports 45, 2765-73.

Barendse W., Reverter A., Bunch R.J., Harrison B.E., Barris W. & Thomas M.B. (2007) A validated whole-genome association study of efficient food conversion in cattle. Genetics 176, 1893-905.

Berry D. & Crowley J. (2012) Residual intake and body weight gain: a new measure of efficiency in growing cattle. Journal of animal science 90, 109-15.

Bolormaa S., Hayes B., Savin K., Hawken R., Barendse W., Arthur P., Herd R. & Goddard M. (2011) Genome-wide association studies for feedlot and growth traits in cattle. Journal of animal science 89, 1684-97.

Brandebourg T., Wolfe D. & Foradori C. (2013) US beef industry: a sustainable success story, challenges and priorities. J. Fisheries Livest. Prod 1.

Brody S. (1945) Bioenergetics and growth with special reference to the energetic efficiency complex in domestic animals. Reinhold Publ., New York 403.

Carstens G.E. & Tedeschi L.O. (2006) Defining feed efficiency in beef cattle. In: Proceedings of Beef Improvement Federation 38th Annual Research Symposium and Annual Meeting, Choctaw, Mississippi, pp. 12-21.

CCA (2018) Canadian Beef Industry Statitistics. URL http://www.cattle.ca/cca-resources/industry-stats/.

Chen L., Schenkel F., Vinsky M., Crews D. & Li C. (2013) Accuracy of predicting genomic breeding values for residual feed intake in Angus and Charolais beef cattle. Journal of animal science 91, 4669-78.

Chen Y., Gondro C., Quinn K., Herd R., Parnell P. & Vanselow B. (2011) Global gene expression profiling reveals genes expressed differentially in cattle with high and low residual feed intake. Animal genetics 42, 475-90.

Crowley J., McGee M., Kenny D., Crews Jr D., Evans R. & Berry D. (2010) Phenotypic and genetic parameters for different measures of feed efficiency in different breeds of Irish performance-tested beef bulls. Journal of animal science 88, 885-94.

de Oliveira P.S., Cesar A.S., do Nascimento M.L., Chaves A.S., Tizioto P.C., Tullio R.R., Lanna D.P., Rosa A.N., Sonstegard T.S. & Mourao G.B. (2014) Identification of genomic regions associated with feed efficiency in Nelore cattle. BMC genetics 15, 100.

De Oliveira P.S., Coutinho L.L., Tizioto P.C., Cesar A.S., de Oliveira G.B., Diniz W.J.d.S., De Lima A.O., Reecy J.M., Mourão G.B. & Zerlotini A. (2018) An integrative transcriptome analysis indicates regulatory mRNA-miRNA networks for residual feed intake in Nelore cattle. Scientific reports 8, 17072.

Fonseca L.D., Eler J.P., Pereira M.A., Rosa A.F., Alexandre P.A., Moncau C.T., Salvato F., Rosa-Fernandes L., Palmisano G. & Ferraz J.B. (2019) Liver proteomics unravel the metabolic pathways related to Feed Efficiency in beef cattle. Scientific reports 9, 5364.

Foote A., Keel B., Zarek C. & Lindholm-Perry A. (2017) Beef steers with average dry matter intake and divergent average daily gain have altered gene expression in the jejunum. Journal of animal science 95, 4430-9.

Gerber P.J., Steinfeld H., Henderson B., Mottet A., Opio C., Dijkman J., Falcucci A. & Tempio G. (2013) Tackling climate change through livestock: a global assessment of emissions and mitigation opportunities. Food and Agriculture Organization of the United Nations (FAO).

Goddard M. (2009) Genomic selection: prediction of accuracy and maximisation of long term response. Genetica 136, 245-57.

Goddard M.E. & Hayes B.J. (2009) Mapping genes for complex traits in domestic animals and their use in breeding programmes. Nature Reviews Genetics 10, 381-91.

Hegarty R., Goopy J., Herd R. & McCorkell B. (2007) Cattle selected for lower residual feed intake have reduced daily methane production. Journal of animal science 85, 1479-86.

Herd R. & Bishop S. (2000) Genetic variation in residual feed intake and its association with other production traits in British Hereford cattle. Livestock production science 63, 111-9.

Herd R., Oddy V. & Richardson E. (2004) Biological basis for variation in residual feed intake in beef cattle. 1. Review of potential mechanisms. Australian Journal of Experimental Agriculture 44, 423-30.

Hocquette J.-F. & Chatellier V. (2011) Prospects for the European beef sector over the next 30 years. Animal Frontiers 1, 20-8.

Hu Z.-L., Park C.A. & Reecy J.M. (2018) Building a livestock genetic and genomic information knowledgebase through integrative developments of Animal QTLdb and CorrDB. Nucleic acids research 47, D701-D10.

Karisa B., Thomson J., Wang Z., Stothard P., Moore S. & Plastow G. (2013) Candidate genes and single nucleotide polymorphisms associated with variation in residual feed intake in beef cattle. Journal of animal science 91, 3502-13.

Kellner O.J. (1909) The Scientific Feeding of Animals. Macmillan.

Kennedy B., Van der Werf J. & Meuwissen T. (1993) Genetic and statistical properties of residual feed intake. Journal of animal science 71, 3239-50.

Kern R.J., Lindholm-Perry A.K., Freetly H.C., Snelling W.M., Kern J.W., Keele J.W., Miles J.R., Foote A.P., Oliver W.T. & Kuehn L.A. (2016) Transcriptome differences in the rumen of beef steers with variation in feed intake and gain. Gene 586, 12-26.

Khansefid M., Pryce J., Bolormaa S., Miller S., Wang Z., Li C. & Goddard M. (2014) Estimation of genomic breeding values for residual feed intake in a multibreed cattle population. Journal of animal science 92, 3270-83.

Kleiber M. (1947) Body size and metabolic rate. Physiological reviews 27, 511-41.

Koch R.M., Swiger L.A., Chambers D. & Gregory K.E. (1963) Efficiency of feed use in beef cattle. Journal of animal science 22, 486-94.

Kong R.S., Liang G., Chen Y. & Stothard P. (2016) Transcriptome profiling of the rumen epithelium of beef cattle differing in residual feed intake. BMC genomics 17, 592.

Lackner D.H. & Bähler J. (2008) Translational control of gene expression: from transcripts to transcriptomes. International review of cell and molecular biology 271, 199-251.

Li C. (2017) The role of beef in human nutrition and health. Dikeman E, editorEnsuring safety and quality in the production of beef 2, 329-38.

Lindholm-Perry A., Butler A., Kern R., Hill R., Kuehn L., Wells J., Oliver W., Hales K., Foote A. & Freetly H. (2016) Differential gene expression in the duodenum, jejunum and ileum among crossbred beef steers with divergent gain and feed intake phenotypes. Animal genetics 47, 408-27.

Lu J. & Clark A.G. (2012) Impact of microRNA regulation on variation in human gene expression. Genome research 22, 1243-54.

Mao F., Chen L., Vinsky M., Okine E., Wang Z., Basarab J., Crews Jr D. & Li C. (2013) Phenotypic and genetic relationships of feed efficiency with growth performance,

ultrasound, and carcass merit traits in Angus and Charolais steers. Journal of animal science 91, 2067-76.

Meuwissen T., Hayes B. & Goddard M. (2001) Prediction of total genetic value using genome-wide dense marker maps. Genetics 157, 1819.

Meuwissen T., Hayes B. & Goddard M. (2013) Accelerating improvement of livestock with genomic selection. Annu. Rev. Anim. Biosci. 1, 221-37.

Mujibi F., Nkrumah J., Durunna O., Grant J., Mah J., Wang Z., Basarab J., Plastow G., Crews D. & Moore S. (2011a) Associations of marker panel scores with feed intake and efficiency traits in beef cattle using preselected single nucleotide polymorphisms. Journal of animal science 89, 3362-71.

Mujibi F., Nkrumah J., Durunna O., Grant J., Mah J., Wang Z., Basarab J., Plastow G., Crews Jr D. & Moore S. (2011b) Associations of marker panel scores with feed intake and efficiency traits in beef cattle using preselected single nucleotide polymorphisms. Journal of animal science 89, 3362-71.

Mujibi F., Nkrumah J., Durunna O., Stothard P., Mah J., Wang Z., Basarab J., Plastow G., Crews D. & Moore S. (2011c) Accuracy of genomic breeding values for residual feed intake in crossbred beef cattle. Journal of animal science 89, 3353-61.

Nkrumah J., Okine E., Mathison G., Schmid K., Li C., Basarab J., Price M., Wang Z. & Moore S. (2006) Relationships of feedlot feed efficiency, performance, and feeding behavior with metabolic rate, methane production, and energy partitioning in beef cattle. Journal of animal science 84, 145-53.

Nkrumah J., Sherman E., Li C., Marques E., Crews D., Bartusiak R., Murdoch B., Wang Z., Basarab J. & Moore S. (2007) Primary genome scan to identify putative quantitative trait

loci for feedlot growth rate, feed intake, and feed efficiency of beef cattle. Journal of animal science 85, 3170-81.

Olivieri B.F., Mercadante M.E.Z., Cyrillo J.N.d.S.G., Branco R.H., Bonilha S.F.M., de Albuquerque L.G., de Oliveira Silva R.M. & Baldi F. (2016) Genomic regions associated with feed efficiency indicator traits in an experimental Nellore cattle population. PloS one 11, e0164390.

Paradis F., Yue S., Grant J., Stothard P., Basarab J. & Fitzsimmons C. (2015) Transcriptomic analysis by RNA sequencing reveals that hepatic interferon-induced genes may be associated with feed efficiency in beef heifers. Journal of animal science 93, 3331-41.

Pryce J., Wales W.d., De Haas Y., Veerkamp R. & Hayes B. (2014) Genomic selection for feed efficiency in dairy cattle. Animal 8, 1-10.

Reynolds J., Foote A., Freetly H., Oliver W. & Lindholm-Perry A. (2017) Relationships between inflammation-and immunity-related transcript abundance in the rumen and jejunum of beef steers with divergent average daily gain. Animal genetics 48, 447-9.

Richardson E. & Herd R. (2004) Biological basis for variation in residual feed intake in beef cattle. 2. Synthesis of results following divergent selection. Australian Journal of Experimental Agriculture 44, 431-40.

Rolf M., Taylor J., Schnabel R., McKay S., McClure M., Northcutt S., Kerley M. & Weaber R. (2012) Genome-wide association analysis for feed efficiency in Angus cattle. Animal genetics 43, 367-74.

Saatchi M., Beever J.E., Decker J.E., Faulkner D.B., Freetly H.C., Hansen S.L., Yampara-Iquise H., Johnson K.A., Kachman S.D. & Kerley M.S. (2014) QTLs associated with dry matter

intake, metabolic mid-test weight, growth and feed efficiency have little overlap across 4 beef cattle studies. BMC genomics 15, 1004.

Santana M., Freua M., Do D., Ventura R., Kadarmideen H. & Ferraz J. (2016) Systems genetics and genome-wide association approaches for analysis of feed intake, feed efficiency, and performance in beef cattle. Genet. Mol. Res 15, 1-10.

Santana M.H., Utsunomiya Y.T., Neves H.H., Gomes R.C., Garcia J.F., Fukumasu H., Silva S.L., Junior G.A.O., Alexandre P.A. & Leme P.R. (2014) Genome-wide association analysis of feed intake and residual feed intake in Nellore cattle. BMC genetics 15, 21.

Schenkel F., Miller S. & Wilton J. (2004) Genetic parameters and breed differences for feed efficiency, growth, and body composition traits of young beef bulls. Canadian Journal of Animal Science 84, 177-85.

Seabury C.M., Oldeschulte D.L., Saatchi M., Beever J.E., Decker J.E., Halley Y.A., Bhattarai E.K., Molaei M., Freetly H.C. & Hansen S.L. (2017) Genome-wide association study for feed efficiency and growth traits in US beef cattle. BMC genomics 18, 386.

Sherman E., Nkrumah J., Li C., Bartusiak R., Murdoch B. & Moore S. (2009) Fine mapping quantitative trait loci for feed intake and feed efficiency in beef cattle. Journal of animal science 87, 37-45.

Sherman E., Nkrumah J., Murdoch B., Li C., Wang Z., Fu A. & Moore S. (2008) Polymorphisms and haplotypes in the bovine neuropeptide Y, growth hormone receptor, ghrelin, insulin-like growth factor 2, and uncoupling proteins 2 and 3 genes and their associations with measures of growth, performance, feed efficiency, and carcass merit in beef cattle. Journal of animal science 86, 1-16.

Smith S.B., Gotoh T. & Greenwood P.L. (2018) Current situation and future prospects for global beef production: overview of special issue. Asian-Australasian journal of animal sciences 31, 927.

Snelling W., Cushman R., Keele J., Maltecca C., Thomas M., Fortes M. & Reverter A. (2013) BREEDING AND GENETICS SYMPOSIUM: Networks and pathways to guide genomic selection–. Journal of animal science 91, 537-52.

Stock K. & Reents R. (2013) Genomic selection: status in different species and challenges for breeding. Reproduction in Domestic Animals 48, 2-10.

Tizioto P.C., Coutinho L.L., Decker J.E., Schnabel R.D., Rosa K.O., Oliveira P.S., Souza M.M., Mourão G.B., Tullio R.R. & Chaves A.S. (2015) Global liver gene expression differences in Nelore steers with divergent residual feed intake phenotypes. BMC genomics 16, 242.

Tizioto P.C., Coutinho L.L., Oliveira P.S., Cesar A.S., Diniz W.J., Lima A.O., Rocha M.I., Decker J.E., Schnabel R.D. & Mourão G.B. (2016) Gene expression differences in Longissimus muscle of Nelore steers genetically divergent for residual feed intake. Scientific reports 6.

USDA (2017) Top U.S. Agricultural Exports in 2017. URL https://www.fas.usda.gov/data/top-us-agricultural-exports-2017.

Weber K.L., Welly B.T., Van Eenennaam A.L., Young A.E., Porto-Neto L.R., Reverter A. & Rincon G. (2016) Identification of gene networks for residual feed intake in angus cattle using genomic prediction and RNA-seq. PloS one 11, e0152274.

Zhang H., Yin L., Wang M., Yuan X. & Liu X. (2019) Factors affecting the accuracy of genomic selection for agricultural economic traits in maize, cattle, and pig populations. Frontiers in genetics 10, 189.

Chapter 2. Transcriptome analyses reveal reduced hepatic lipid synthesis and accumulation in more feed efficient beef cattle

2.1 Abstract

The genetic mechanisms controlling residual feed intake (RFI) in beef cattle are still largely unknown. Here we performed whole transcriptome analyses to identify differentially expressed (DE) genes and their functional roles in liver tissues between six extreme high and six extremes low RFI steers from each of the three beef breed populations including Angus, Charolais, and Kinsella Composite (KC). On average, next generation sequencing yielded 34 million single-end reads per sample, of which 87% were uniquely mapped to the bovine reference genome. At false discovery rate (FDR) < 0.05 and fold change (FC) > 2, 72, 41, and 175 DE genes were identified in Angus, Charolais, and KC, respectively. Most of the DE genes were breed-specific, while five genes including *TP53INP1*, *LURAP1L*, *SCD*, *LPIN1,* and *ENSBTAG00000047029* were common across the three breeds, with *TP53INP1*, *LURAP1L*, *SCD*, and *LPIN1* being downregulated in low RFI steers of all three breeds. The DE genes are mainly involved in lipid, amino acid and carbohydrate metabolism, energy production, molecular transport, small molecule biochemistry, cellular development, and cell death and survival. Furthermore, our differential gene expression results suggest reduced hepatic lipid synthesis and accumulation processes in more feed efficient beef cattle of all three studied breeds.

2.2 Introduction

An animal's ability to convert consumed feed into saleable meat is of central importance to the meat production industry because feed and feeding related costs are the single largest variable expense in animal production (Shalev & Pasternak 1989; Ramsey *et al.* 2005; Van

Heugten 2010; Ahola & Hill 2012; Brandebourg *et al.* 2013). As the global demand for meat products continues to increase due to population growth, and improved economic prosperity in the developed and developing world, provision of feed for meat animal production will become a potential burden on global resources including land, water, fertilizers, and labor (Archer *et al.* 1999; Naylor *et al.* 2005; Salter 2017). In addition, environmental footprints including greenhouse gas emission associated with meat animal production have become a public concern (Gerber *et al.* 2013). Of meat production animals, beef cattle are the largest animals and a major contributor to environmental footprints (Gerber *et al.* 2013). Studies have shown that more feed efficient beef cattle not only consume less feed for the same amount of meat produced, but also have a reduced methane emission (Nkrumah *et al.* 2006; Hegarty *et al.* 2007; Fitzsimons *et al.* 2013). Therefore, decreasing production inputs through improving feed efficiency and reducing environmental footprints will be a vital step in improving the sustainability of the beef production industry.

Feed efficiency is a complex trait that can be measured using a variety of methods (Archer *et al.* 1999). Residual feed intake (RFI) is one of the measurements of feed efficiency and is defined as the difference between actual and expected feed or dry matter intake required for maintenance and growth (Koch *et al.* 1963). RFI has become a more preferred measure of feed efficiency in beef cattle due to its phenotypic independence from production traits (Archer *et al.* 1999; Mao *et al.* 2013) and moderate heritability (Nkrumah *et al.* 2007a; Mao *et al.* 2013), which allow a reasonable response to genetic selection for more efficient animals without compromising their growth rate and mature weight.

It has been proposed that RFI is controlled by several physical, physiological and metabolic processes such as feed intake, digestion, body composition, tissue metabolism, activity and thermoregulation (Herd *et al.* 2004; Richardson & Herd 2004; Herd & Arthur 2009). With the

advancement of DNA markers and genotyping technologies, multiple candidate chromosomal regions or quantitative trait loci (QTL) that contribute to the variation of RFI in beef cattle have been identified through DNA markers, based linkage and association studies (Barendse *et al.* 2007; Nkrumah *et al.* 2007b; Sherman *et al.* 2009; Abo-Ismail *et al.* 2014; de Oliveira *et al.* 2014; Saatchi *et al.* 2014). However, the significant QTL regions and DNA variants vary largely across studies. To further identify genes associated with RFI, whole transcriptome profiling studies between beef cattle with divergent RFI phenotypes have also been performed for several tissues such as liver (Chen *et al.* 2011; Alexandre *et al.* 2015; Paradis *et al.* 2015; Tizioto *et al.* 2015; Khansefid *et al.* 2017), skeletal muscle (Tizioto *et al.* 2016; Weber *et al.* 2016; Khansefid *et al.* 2017), adipose (Weber *et al.* 2016), pituitary (Weber *et al.* 2016), rumen (Kong *et al.* 2016) and duodenum (Weber *et al.* 2016). However, only a small proportion of the reported differentially expressed genes were shared across these studies. This discrepancy of DE genes identified across studies could be attributed to the differences in breed types, sex type, tissue, and age of the animals used in the studies, as well as the differences in management and environmental conditions under which animals were raised and tested. These confounding factors hinder our understanding on genetic mechanisms that regulate RFI. Therefore, to better elucidate genetic influence on feed efficiency in beef cattle, we calculated RFI on steers from three distinctive beef breeds including Angus, Charolais, and Kinsella Composite (KC) of similar age that were born, raised, and managed under the same environmental conditions, and then identified DE genes and molecular functions/processes associated with RFI within and across the breeds using whole transcriptome RNAseq analyses of liver tissues of high and low RFI phenotype steers from each breed population.

2.3 Materials and Methods

2.3.1 Animal populations and management

All animals used in this study were managed according to the guidelines established by the Canadian Council of Animal Care (Olfert *et al.* 1993) and the experiment procedures were approved by the University of Alberta Animal Care and Use Committee (AUP00000777). Beef steers from three beef cattle herds including purebred Angus, purebred Charolais, and Kinsella Composite (KC) were used in this study. The three beef cattle herds were located and managed alike at the Roy Berg Kinsella Ranch, University of Alberta, Canada. These cattle herd populations were described previously (Nkrumah *et al.* 2007b; Mao *et al.* 2013). Briefly, the purebred Angus and Charolais cows were bred by artificial insemination (AI) and natural service bulls with their pedigree information maintained by the Canadian Angus or Charolais Associations, respectively. The KC herd was produced from crosses between Angus, Charolais, or Alberta Hybrid bulls and the University of Alberta's hybrid dam line that was generated by crossing composite cattle lines of multiple beef breeds as described by (Goonewardene *et al.* 2003).The animals used in this study were born between April to May of 2014 and were weaned at approximately six months of age. They were then fed a background diet composed of 80% barley silage, 17% barley grain, and 3% rumensin pellet supplement, and then a transition diet with gradually decreasing barley silage and increasing barley grain proportions for 3 weeks prior to the finishing diet of 75% barley grain, 20% barley silage, and 5% rumensin pellet supplement (as fed basis).

2.3.2 Growsafe feedlot test and residual feed intake calculation

In 2015, 50 Angus, 48 Charolais, and 158 KC steers were measured for individual feed intake between April to August using the GrowSafe system® (GrowSafe Systems Ltd., Airdrie, Alberta, Canada), and were fed a finishing diet during the feed intake test. Details of individual

animals' daily feed intake data collection using the GrowSafe automated system was described previously by (Mao *et al.* 2013). Briefly, daily dry matter intake (DMI) of each steer was calculated as the average of daily feed intakes over the test period (70 to 73 days), standardized to 12 MJ ME per kg dry matter based on the energy content of the diet. Initial body weight and average daily gain (ADG) for each animal were obtained from a linear regression of serial body weight (BW) measurements that were recorded on two consecutive days at the beginning, at approximately 14 day intervals during the feedlot test, and on two consecutive days at the end of test. Metabolic body weight (MWT) was calculated as midpoint $BW^{0.75}$, where midpoint BW was computed as the sum of initial BW of the animal and the product of its ADG multiplied by half the number of days under the feedlot test. For each breed, the expected DMI for each animal was predicted using the regression intercept and regression coefficients of ADG and MWT on actual standardized daily DMI, and RFI was computed as the difference between the actual standardized daily DMI and the expected DMI as proposed by Koch et al (1963).

2.3.3 Liver tissue collection

Animals with extreme RFI phenotype values were slaughtered at Agriculture and Agri-Food Canada (AAFC) Lacombe Research Centre (Lacombe, AB) between July and September of 2015. Steers were targeted for slaughter at an average backfat thickness of 8 mm between the 12th and 13th ribs as measured by ultrasound using an Aloka 500V diagnostic realtime ultrasound machine with a 17cm 3.5Mhz linear array transducer (Overseas Monitor Corporation Ltd., Richmond BC), which resulted in an average slaughter age of 494 ± 3, 518 ± 4, and 457 ± 4 days for Angus, Charolais, and KC, respectively. The liver sample of each animal was collected immediately after slaughter and the tissue was dissected from approximately the same location on the right lobe with the fibrous capsule removed. Samples were separately bagged and labeled, and

were immediately flash frozen in liquid nitrogen, transported on dry ice, and stored at -80ºC until RNA extraction.

2.3.4 RNA isolation and purification

From the frozen liver samples, a total of 36 samples (12 from each breed) consisting of six samples from animals with the highest and six animals with the lowest RFI phenotypes from each of the three breeds were selected for total RNA extraction and consequently differential gene expression analyses. The frozen liver tissue of each steer was pulverised into fine powder using liquid nitrogen with a pre-chilled mortar and pestle on dry ice. Total RNA was then extracted from 10 mg of the pulverised tissue using a Qiagen RNeasy Plus Universal Kit (Qiagen, Toronto, ON, Canada) and further purified using a Zymo RNA Clean & Concentrator (Zymo, Irvine, CA, USA). RNA was quantified using a NanoDrop 2000 Spectrophotometer (Thermo Scientific, Wilmington, DE, USA) and was deemed acceptable if its absorbance (A260/280) was between 1.8 and 2.0. RNA integrity was confirmed using a TapeStation-Agilent instrument (Agilent Technologies, Mississauga, ON, Canada), and the RNA integrity number (RIN) values for all samples were higher than eight.

2.3.5 cDNA library preparation and sequencing

Preparation of cDNA library and sequencing for each of the 36 animal samples were performed at the Clinical Genomics Centre (Toronto, ON, Canada), where mRNA was purified and enriched from 1 µg of each of the total RNA samples and then fragmented. Thereafter, the first strand of the cDNA was synthesized using SuperScript II Reverse Transcriptase enzyme (Thermo Fisher Scientific, San Jose, CA, USA) and the second strand was synthesized using the DNA Polymerase I and RNase H enzymes (Illumina, San Diego, CA, USA). The cDNA libraries were validated using gel electrophoresis to confirm that the fragment size was 150bp (on average)

and concentration was on average 25ng/µl per sample. Unique oligonucleotide adapters were added to the cDNA of each sample to allow for multiplexing. Of the prepared sample cDNA libraries, 27 (all Angus, all KC and 3 Charolais samples) were single end sequenced (100bp) under the high output run mode of the Illumina Hiseq 2500 System on eight flow cell lanes, while the other 9 Charolais samples were sequenced under the rapid run mode of the same sequencing equipment. High quality single end reads of 101bp with an average Phred score of 36 and 37 for high output run mode and rapid run mode, respectively, were obtained with an average of 31 and 46 million reads per sample for high output run mode and rapid run mode, respectively. All sequence data generated for this study has been submitted to the Gene Expression Omnibus repository under the accession number GSE107477.

2.3.6 RNAseq data analyses

Raw single-end sequence reads for each sample were assessed for sequencing quality using FastQC (Version 0.11.5) with default parameters (Andrews 2010). Reads of each sample were independently aligned and mapped to the bovine genome UMD3.1 using the TopHat (version 2.1.1) RNAseq mapper with default single end read alignment parameters (Kim *et al.* 2013). Reads that were uniquely aligned to each gene annotated in the GTF Bovine gene annotation file (ftp://ftp.ensembl.org/pub/release-89/gtf/bos_taurus/Bos_taurus.UMD3.1.89.gtf.gz) were counted using HTSeq-count with default parameters (Anders *et al.* 2015) which generated the read count tables that were used for downstream differential gene expression statistical analyses.

2.3.7 Differential gene expression statistical analysis

Gene read count tables from HTSeq-count, the annotation file downloaded from Ensembl Biomart (http://www.ensembl.org/biomart/martview/9153354bb2bef3f0fe8126460f4804ae), and sample information file were used for differential gene expression statistical analyses using edgeR

(Robinson *et al.* 2010). Genes within each breed with less than one count per million (CPM) of mapped reads in at least six samples (half of the analyzed samples) were removed from further analyses as proposed by Anders and colleagues (Anders *et al.* 2013). For the retained genes, their counts were normalized using the trimmed mean M values (TMM) method to account for the variation in library sequencing depths between samples (Anders *et al.* 2013). The TMM normalization method implemented in edgeR was proposed by Robinson & Oshlack (Robinson & Oshlack 2010), and it assumes that the majority of the sequenced genes in the libraries are not differentially expressed. With one sample considered as a reference, a TMM factor was calculated for each sample as a weighted mean of log ratios of gene-wise log fold changes and absolute expression level after exclusion of genes with the highest (30%) log-fold change ratios and highest (5%) absolute expression. The TMM value for each sample was expected to be equal or close to one, if not, correction factors were calculated and applied to the original library sizes to calculate new effective library sizes. Normalized read counts were then analyzed with a generalized linear model for each of the breed populations with an assumption of a negative binomial distribution of gene counts to identify differentially expressed genes, as implemented in egdeR. The statistical models used for analyses are as described below:

Model.1 $\log (CPM)_{ijkl} = \mu + RFI_i + SIRE_j + e_{ijkl}$

Model.2 $\log (CPM)_{ijmkl} = \mu + RFI_i + SIRE_j + SEQ_m + e_{ijmkl}$

Model 1 was used for Angus and KC steer gene expression analyses, where $\log (CPM)_{ijkl}$ was the log transformed read counts per million of mapped reads for the gene l in sample k from i^{th} RFI group (high or low) and j^{th} SIRE group, and e_{ijkl} as the random error term. Model 2 was used for Charolais steer gene expression analyses, where $\log (CPM)_{ijmkl}$ was the log transformed counts per

million of gene l in sample k from the i^{th} RFI group, j^{th} SIRE group, and m^{th} SEQ, and e_{ijmkl} was the random error term. The term μ was the population mean and RFI, SIRE and SEQ were treated as fixed effects in the models. For each model, the RFI group consisted of 6 steers with high RFI values in the high-RFI group and 6 steers with low RFI values in the low-RFI group. The SIRE effect of Angus, Charolais, and KC steers included 6, 5, and 9 sires, respectively. For Charolais, SEQ was included as an additional fixed effect to account for differences due to the sequencing modes (i.e. high output run mode or rapid run mode) (Model.2). Differentially expressed (DE) genes were identified using a likelihood ratio test of each gene expression level between the two RFI groups with the high-RFI group (or less feed efficient group) used as the reference group. The analysis was performed for each gene; therefore, Benjamin-Hochberg method was used to control the false discovery rate (FDR) due to multiple testing (Benjamini & Hochberg 1995). A threshold FDR of 0.05 and fold change (FC) of greater than two (> 2) were used as the cut off to indicate significant differential gene expression.

2.3.8 Functional enrichment analysis

To understand the biological functionality of the DE genes identified, functional analyses for the DE genes within each breed were performed using Ingenuity Pathway Analysis software (IPA) (Redwood City, CA; www.qiagen.com/ingenuity). Ensembl bovine gene IDs and log_2-fold change (logFC) of the DE genes were used as identity (ID) and expression level (Observation 1), respectively, in IPA. To increase the number of mapped genes, Ensembl IDs for the unmapped genes were extracted and replaced with their closest human orthologue gene Ensembl IDs. Thereafter a combined list of bovine Ensembl for the mapped and human ortholog Ensembl IDs for unmapped genes was used for IPA biological function analysis. Molecular and cellular functions or biological functions were considered significantly enriched if the p-value for the

overlap comparison test between the input gene list and the IPA Knowledge base database for a given biological function was less than 0.05. Activation or deactivation level of a specific enriched metabolic process within a biological function was defined by the Z-score (Krämer *et al.* 2013) that was calculated from the expression levels of the overlapping DE genes, where a negative or a positive score indicated deactivation or activation, respectively.

2.4 Results

2.4.1 Difference of RFI and other performance traits between high and low-RFI groups

The averages and the t-test P-values for RFI and other performance traits are presented in Table 2.1. The animals used in this study had raw RFI values ranging from 1.55 to -1.096, 1.82 to -1.38, and 1.99 to -1.63 kg/day of dry matter intake for Angus, Charolais, and KC, respectively. The average RFI values of the low and high RFI steer groups were significantly different ($P \leq$ 1.69E-07) for all the three breed populations (Table 2.1). Of the RFI component traits, only DMI was significantly different between the two RFI groups for all the three populations, with low RFI or more feed efficient animals consuming significantly ($P \leq 0.01$) less feed than their counterparts in the high RFI group for all the three breed populations. All the averages of growth and carcass traits as well as slaughter ages were not significantly different between the high and low RFI groups for all the studied breeds ($P > 0.01$).

2.4.2 Sequencing and alignment quality assessment

The Illumina sequencing yielded an average of 32,059,334 (SD = 2,575,908), 42,028,676 (SD = 8,852,805), and 30,259,896 (SD = 5,977,827) raw single-end sequence reads from the 12 cDNA libraries of Angus, Charolais, and KC samples, respectively. On average, the rapid run output mode produced more reads per sample (46,335,115 (SD = 5,355,272)) than the high output sequencing mode (30,931,809 (SD = 4,435,107)). The reads had an average length of 101bp and

an average Phred quality score of 36.2 ± 0.07. All reads were free of any sequencing adaptors and no read was flagged as having poor quality. On average 87% of the total sequences per sample were uniquely aligned and mapped to annotated genes in the bovine reference genome. The number of raw sequence reads, sequencing quality assessment, and alignment summary results for each sample are provided in the Supplementary Data S1 file.

2.4.3 Differential gene expression analysis

After filtering out non-expressed genes, 11,823, 11,942 and 11,819 genes were found to have sufficient expression for further analyses (> 1 CPM for at least half of the samples) in the liver tissues of Angus, Charolais, and KC, respectively. The majority (96.1%) of the expressed genes were common to all the three breeds as shown in Figure 2.1a, hence showing a great similarity between the breeds in terms of genes expressed in the liver tissue. Of the expressed genes, 72 (46 downregulated and 26 downregulated in low-RFI steers), 41 (19 downregulated and 22 upregulated in low-RFI steers), and 175 (108 downregulated and 67 upregulated in low-RFI steers) DE genes were identified in Angus, Charolais, and KC, respectively at the significance threshold of FDR <0.05 and FC >2. A subset of the most significantly differentially expressed genes (by FDR values) from each breed is shown in Table 2.2, whereas the full lists of all differentially expressed genes for each breed are provided in the Supplementary Data S2, Supplementary Data S3, and Supplementary Data S4 for Angus, Charolais, and KC, respectively. When we compared DE genes across breeds, the majority of them (68.1% for Angus, 63.4% for Charolais, and 84.6% for KC) were breed specific, with only a few genes being shared between breeds (8 to 20 DE genes) or across the breeds (5) as shown in Figure 2.1b. The five common DE genes across all the three breeds included *TP53INP1*, *LURAP1L*, *SCD*, *LPIN1, and ENSBTAG00000047029* (paralogous to *RPS23*) (Figure 2.2). Four of these genes (*TP53INP1,*

LURAP1L, SCD and LPIN1) were downregulated in all low RFI steers across the three breeds, whereas *ENSBTAG00000047029* was upregulated in low RFI steers of Angus and Charolais but downregulated in KC low RFI steers as illustrated in Figure 2.2. Between two breeds, Angus and KC shared the most unique DE genes (n = 15), of which the majority (n = 13) had the same expression direction in low RFI animals of the two breeds, and only two genes had a different expression direction in the efficient animals of the two breeds. Angus and Charolais shared the fewest (n = 3) DE genes (Figure 2.1b), of which *GNAZ* and *DLK1* were both downregulated in Angus but upregulated in Charolais low RFI animals (Supplementary Data S2 and Supplementary Data S3).

2.4.4 IPA Functional Enrichment Analysis

From the DE genes identified, 70, 37 and 169 were successfully mapped to the IPA knowledge base database for Angus, Charolais, and KC respectively. Subsequently, 27 significantly enriched biological functions (P-value < 0.05) were detected for Angus and KC, and 23 functions for Charolais. All significant biological functions and their enrichment P-values for each breed are provided in the Supplementary Data S5. The majority (n = 23 or 85.2%) of the identified biological functions were common across the three-studied breeds (Figure 2.1c). The most significantly enriched biological functions included lipid metabolism, amino acid metabolism, carbohydrate metabolism, energy production, molecular transport, small molecule biochemistry, cellular development, and cell death and survival. Table 2.3 shows the DE genes involved in the top five most significantly enriched biological functions for each of the studied breeds. A full list of all biological functions identified is provided together with the list of DE genes for each breed in the Supplementary Data S2, Supplementary Data S3 and Supplementary Data S4 for Angus, Charolais and KC respectively.

Of the five shared DE genes identified in this study across the three breeds, *LPIN1* and *SCD* were involved in lipid metabolism, small molecule biochemistry, carbohydrate metabolism and energy production. *LURAP1L* was involved in small molecule biochemistry, and *TP531NP1* was involved in carbohydrate metabolism and molecular transport. Within the lipid metabolism function, further analyses of regulatory gene networks revealed several enriched fat or lipid related metabolic processes as presented in Figure 2.3, Figure 2.4, and Figure 2.5 for Angus, Charolais, and KC, respectively. Lipid synthesis was predicted to be downregulated in the liver tissues of more feed efficient animals (low-RFI steers) across all the three breeds (Figure 2.3, Figure 2.4, and Figure 2.5). Lipid accumulation was also predicted to be downregulated in KC and Charolais feed efficient steers. Additionally, downregulation of accumulation of triglycerides was predicted in Angus and KC for low-RFI steers. These results indicate that more feed efficient beef cattle have reduced hepatic lipid synthesis and accumulation. However, oxidation of fatty acids was relatively upregulated in KC and Charolais while downregulated in Angus.

2.5 Discussion

The liver is a relatively small organ (1-2% of body mass) although metabolically it is a very active and important organ sharing 18-26% of the total body oxygen for its metabolic activities (Reynolds 1992). The liver is a central physiological and metabolic organ of ruminant animals. It is responsible for modulation and distribution of nutrients to peripheral tissues and organs for maintenance and production purposes such as muscle deposition in beef cattle or milk production in dairy cattle (Seal & Reynolds 1993). The liver is also involved in important metabolic and physiological functions relating to glucose, lipid, protein, mineral and vitamin metabolism as well as immune function, steroid hormone catabolism and detoxification of ammonia and endotoxins (Drackley *et al.* 2005; Donkin 2012). Therefore, transcriptome

differences in the liver tissues between efficient and inefficient animals offer a great potential to shed some light on genes and biological functions that are involved in determining RFI in beef cattle. In the current study we employed RNAseq to explore whole transcriptome expression differences between individuals with divergent RFI phenotypes in three beef cattle breed populations. Angus and Charolais are two distinct beef breeds with Angus being a British breed characterized by its moderate frame and early age fattening, whereas Charolais is a continental European breed with a larger frame, and later maturity and fattening (Briggs & Briggs 1980). KC is a composite herd composed of animals bred through crossing of multiple breeds as reported by Nkrumah et al. (2007). Breed composition analyses showed that the 12 KC steers used in this study had an average of 22.3% Angus and 6.7% Charolais influence along with multiple other beef breeds, indicating that KC is genetically distinct from the two pure breeds included in this study.

Our results showed that the majority of the identified DE genes related to RFI were breed or breed population specific although 96.1% of expressed genes in liver were common across the three breeds. This could be an indication that causal genes and causals mutations contributing to RFI variation in beef cattle are likely breed specific. This concurs with a low level of overlapped QTL regions of RFI across multiple breeds as reported by Saatchi and colleagues in multi-breed QTL analysis study (Saatchi *et al.* 2014), as well as concurs with a greater discrepancy of QTL regions reported in different studies (Barendse *et al.* 2007; Nkrumah *et al.* 2007b; Abo-Ismail *et al.* 2014; de Oliveira *et al.* 2014). Furthermore, with respect to previous liver tissue whole transcriptomic studies in beef cattle, only 31 of the 253 DE genes identified in the current study have been previously reported in the liver tissue of beef cattle with divergent RFI phenotypes (Chen *et al.* 2011; Alexandre *et al.* 2015; Tizioto *et al.* 2015; Weber *et al.* 2016), as listed in the supplementary excel files S2, S3, and S4. It is interesting to note that of the five genes differentially

expressed across all three cattle populations in our study, two genes including *Stearoyl Co-A desaturase (SCD) and Lipin 1* (*LPIN1*) code for key enzymes involved in lipid metabolism. Tumor protein p53 inducible protein 1 (*TP53INP1*) gene codes for a stress inducible protein (SIP) that is involved in regulation of cell death (apoptosis) and cell cycle arrest influenced by cell stressors (Tomasini *et al.* 2003). *Leucine rich adaptor protein 1 like* (*LURAP1L*) codes for an adaptor protein reported to be involved in regulation of cell motility and migration (Cheng *et al.* 2017), while *ENSBTAG00000047029* codes for an uncharacterized protein and its sequence is paralogous to ribosomal protein S23 (*RPS23*) that encodes a protein that is a component of the 40S subunit of the ribosomes (protein synthesis organelles) (Kitaoka *et al.* 1994), suggesting that these genes play key roles in altering RFI across the studied beef breeds.

Although the DE genes we identified were mainly breed specific, the enriched biological functions were greatly similar across the breeds, indicating that genes influencing RFI in beef cattle are involved in the same biological functions underlying the trait across different breeds even though the specific genes underlying RFI are different between breeds. Some of the major biological functions identified in our study included lipid metabolism, molecular transport, small molecule biochemistry, energy production, amino acid metabolism, carbohydrate metabolism, cell development, and cell death and survival. Our results showed that lipid metabolism was the most significantly enriched biological function in Angus and Charolais, and the fourth most enriched function in KC, indicating the significant biological importance of lipid metabolism in regulating RFI in beef animals. Lipid metabolism has also been previously identified as an important biological function in relation to beef cattle RFI in other hepatic transcriptome studies (Chen *et al.* 2011; Alexandre *et al.* 2015; Weber *et al.* 2016).

Regarding lipid metabolism, our results showed that lipid synthesis (including triacylglycerol synthesis) was predicted to be downregulated in the liver tissues of low-RFI animals from all the three beef breeds (Figure 2.3, Figure 2.4 and Figure 2.5). Similarly, downregulation of genes involved in lipogenesis and steroidogenesis in both liver and fat tissue of low-RFI Yorkshire pigs has been reported by Lkhagvadorj and colleagues (Lkhagvadorj *et al.* 2010). In a liver transcriptomic study of Nellore steers, downregulation of *fatty acid synthase* (*FASN*) was reported in steers with low residual intake and body weight gain (low-RIG) (Alexandre *et al.* 2015), implying possible reduced fatty acid synthesis in the liver tissue of those animals. In a more recent study in Angus cattle, predicted downregulation of lipid synthesis was reported in the adipose tissue of low-RFI steers (Weber *et al.* 2016). These observations suggest that feed efficient animals (not only cattle) direct consumed energy/nutrients away from lipid synthesis and probably towards protein or lean muscle synthesis. Notably, *SCD* and *LIPN1* genes identified as differentially expressed across all the three studied cattle breeds are involved in lipid synthesis. *SCD* codes for Stearoyl Co-A desaturase enzyme, a rate limiting enzyme in the biosynthesis of monounsaturated fatty acids, predominantly oleic and palmitoleic acid (Ntambi & Miyazaki 2004). The synthesized fatty acids are then used as substrates for biosynthesis of other lipids such as phospholipids, triglycerides and cholesterol esters. Therefore, differential expression of this gene between feed efficient and inefficient animals may contribute to the genetic linkage/correlations between feed efficiency and carcass fatty acid composition that have been reported in beef cattle (Inoue *et al.* 2011; Zhang *et al.* 2017). Differential expression of the *SCD* gene between RFI divergent beef animals has been reported in pituitary, muscle, adipose and duodenum tissues where it was also downregulated in low-RFI Angus steers (Weber *et al.* 2016). *LIPN1* encodes for *Lipin-1* a phosphatidate phosphatase (PAP) enzyme, and a member of the Lipin

protein family, which are mainly involved in triacylglycerol (TAG) synthesis in the glycerol phosphate pathway where they dephosphorylate phosphatidic acid to diacylglycerol (Csaki *et al.* 2013). Diacylglycerol is then converted to triacylglycerol by diacylglycerol transferase (*DGAT*). Triacylglycerol is a major and vital form of energy storage in adipose tissues and source of fatty acids for oxidation in both cardiac and skeletal muscles (Csaki *et al.* 2013). We acknowledge the fact that in ruminants such as cattle, lipogenesis or lipid synthesis predominantly occurs in the adipose tissue and a limited capacity of lipogenesis occurs in the liver (Roh *et al.* 2006). This limited lipogenesis in the liver does however generate new fatty acids that are either esterified into triglycerides for storage in adipose tissue, oxidized in the liver or exported to other parts of the body as lipoproteins where they are used as a source of energy and structurally as membrane building components. Additionally, downregulation of accumulation and storage of lipids (such as triglycerides) was predicted in the low-RFI animals in all three studied breed populations. This could be another metabolic advantage of feed efficient animals have over inefficient animals. It is worth mentioning that species with limited hepatic lipogenesis like cattle also have limited potential to secrete triglycerides from the liver as compared to those species that use the liver as the major tissue for lipogenesis (Pullen *et al.* 1990). Therefore, increased hepatic lipid synthesis and accumulation predicted in the high-RFI animals could consequently lead to increased fat accumulation in the hepatic cells of inefficient animals. Increased accumulation of fat in the liver cells may lead to the development of fatty liver (Drackley *et al.* 2005). Fatty liver then impairs the liver tissue's optimal functionality of gluconeogenesis, β-oxidation, endotoxin and metabolic waste detoxification, exposing the animals to a number of metabolic stressors (Drackley *et al.* 2005). Interestingly, our results showed predicted upregulation of lipid secretion, transport and efflux from the hepatic cells of low-RFI steers which could be another mechanism of minimizing

fat accumulation in those cells. In this regard, reduced liver fat synthesis and accumulation might be an adaptive metabolic or physiological advantage for feed efficient animals to maintain an optimal functioning liver tissue as compared to the inefficient animals. Although we did not perform histological evaluation of the liver tissues of the animals studied in the current study, an independent study on Nellore steers by Alexandre et al. (2015) through histopathological evaluation observed different liver tissue health status between the less feed efficient or high-RIG animals as compared to high efficient animals or low-RIG (Alexandre *et al.* 2015). In that study, they reported increased periportal liver lesions in the less feed efficient compared to high feed efficient animals, which they hypothesized was because of increased hepatic lipid biosynthesis and elevated bacterial infection in the less feed efficient animals (Alexandre *et al.* 2015), hence revealing that hepatic tissue health could influence observed differences in feed efficiency in beef cattle.

Although phenotype records of the fat related traits (FUFAT, AFAT and marbling score) in our study did not show significant difference between the high and low RFI steer groups, low fat accumulation or deposition in more feed efficient beef animals in different body parts has been reported by a number of studies. For example, Trejo et al. (2010) and Nascimento et al. (2016) reported significantly lower internal fat content in more feed efficient beef cattle carcasses as compared to inefficient animals (Trejo 2010; Nascimento *et al.* 2016). Richardson and colleagues also reported lower carcass and internal fat in low-RFI Angus steers than high-RFI steers (Richardson *et al.* 2001). In our previous studies, it was observed that more feed efficient beef cattle tended to have less backfat and slightly less marbling (Nkrumah *et al.* 2007a; Mao *et al.* 2013). In a more recent transcriptomic study, higher specific gravity of carcasses from feed efficient Angus steers was observed in comparison to the inefficient steers, indicating lower fat

and higher lean content in the carcasses of more feed efficient animals (Weber *et al.* 2016). In the same study, transcriptome analysis results predicted reduced fat synthesis and accumulation in the adipose tissue of the animals with low-RFI or more feed efficient animals (Weber *et al.* 2016). Therefore, our results and the previous reports showing fat synthesis and accumulation differences between feed efficient and inefficient animals could be a result of metabolic prioritization of nutrients, especially energy. The efficient animals probably spend less energy on lipid synthesis and accumulation/deposition, which metabolically require more energy than lean tissue or protein deposition (McDonald 2002; Robinson & Oddy 2004), thus indicating that energy required to deposit fat may play a major role in determining feed efficiency in growing steers.

The liver modulates body nitrogen through several amino acid and other nitrogen compound metabolic processes, such as protein synthesis (Eisemann *et al.* 1989; Keiding & Sørensen 2007), protein and amino acid catabolism and ureagenesis (Reynolds 1992; Seal & Reynolds 1993). Indeed, our data demonstrates that amino acid metabolism was the most significantly enriched biological function in the crossbred animals with 22 DE genes involved (shown in Table 3), though only three DE genes (*SLC7A5*, *ANXA2* and *ABCC4*) and two DE genes (*GATM* and *EDNRA*) were identified as involved in amino acid metabolism in Charolais and Angus, respectively. The genes identified in KC are involved in several amino acid metabolic processes such as catabolism of amino acids (*AASS, ARG1, ASL, GOT1, HAL, SDS* and *TAT*), amino acid transport (*ARG1, IGF1, SLC16A10, SLC22A7, SLC25A15* and *SLC7A2*) and the urea cycle (*ARG1* and *ASL*). Even though we could not obtain activation/deactivation prediction scores for the identified processes from IPA because of low DE gene numbers, the majority of the DE genes identified in these processes were downregulated in low-RFI steers. For example, of the seven genes involved in amino acid catabolism, six (*ARG1, ASL, GOT1, HAL, SDS* and *TAT*) were

downregulated in low-RFI animals, and this suggests reduced protein and amino acid breakdown in the feed efficient animals. *Argininosuccinate lyase* (*ASL*) and *arginase* (*ARG1*) are key enzymes in ureagenesis, where *Argininosuccinate lyase* catalyzes conversion of argininosuccinate to arginine, and arginase catalyzes conversion of arginine to urea and ornithine (Morris Jr 2002). Hence, downregulation of these genes could be an indication of reduced amino acid catabolism and/or reduced synthesis of urea in the liver. Lower levels of blood urea concentration have been reported in low-RFI steers as compared to high-RFI beef cattle by Richardson et al. (2004) and Fitzsimons et al. (2013), suggesting that amino acid metabolism also plays a considerable role in regulating RFI of beef cattle (Richardson *et al.* 2004; Fitzsimons *et al.* 2013).

Carbohydrate metabolism was another interesting enriched biological function in our study with 13 DE genes involved in Angus (genes shown in Table 2.3), 10 in Charolais, and 31 in the crossbred KC population (genes of both populations shown in Supplementary Data S3 and Supplementary Data S4). Association between RFI variation and carbohydrate metabolism has been previously reported in a liver whole transcriptome study between efficient and inefficient Angus steers (Khansefid *et al.* 2017). More interestingly, some of the DE genes we identified are involved in gluconeogenesis, and these included *ADIPORA, GATM and SCD* for Angus, *NROB2* and *SCD* for Charolais, and *DUSP1, FGF21, GNMT, NROB2, PPARGC1A, SCD, SDS* and *TAT* for KC. Carbohydrates are a very important nutrient to an animal as they provide more than half of the total energy needed by an animal for maintenance, growth and production (muscle deposition in beef cattle) (Nafikov & Beitz 2007). Furthermore, glucose is the main source of metabolic energy in the body, however, in ruminants most of the carbohydrates (cellulose and starch) are fermented by rumen microbes into volatile fatty acids (VFAs) which are absorbed into the blood stream and transported to the liver (Nafikov & Beitz 2007), where VFAs are utilized for

biosynthesis of several organic molecules including carbohydrates. Therefore, differential expression of genes involved in carbohydrate metabolism between inefficient and efficient animals may reflect the difference in catabolic or anabolic efficiency difference in carbohydrate synthesis and utilization by these animals.

2.6 Conclusions

We investigated differential gene expression through RNAseq analyses in the liver tissues of steers with divergent feed efficiency phenotypes from two beef pure breeds and a composite breed population that were born, raised and managed under the same environments, and with a similar age. We identified a total of 253 unique genes associated with RFI in the three Canadian beef cattle breeds, of which five DE genes were shared across all three breeds. The study showed a great similarity in the biological functions associated with RFI across the three breeds, with lipid metabolism, amino acid metabolism, carbohydrate metabolism, molecular transport, energy production, small molecule biochemistry, cell death and survival, and cellular development being the major functions we identified. Our results further suggest reduced hepatic lipid synthesis and fat accumulation in more feed efficient beef cattle across all the studied breeds, which may be an indication of energy prioritization away from lipid deposition and towards lean growth or maintaining better health or function of liver tissue. However, most of DE genes identified in this study were breed specific, which indicates that most causative genetic mutations contributing to RFI variation are likely not the same across beef breeds or expressed differently in different breeds. Further studies including blood tissue whole metabolome profiling, liver lipid biosynthesis and accumulation evaluation, and transcriptome analyses from multiple tissues at various developmental stages would help generate a better understanding of the genetic influence and

would contribute to identification of causative mutations for RFI in beef cattle, especially when different beef breeds are examined.

2.7 References

Abo-Ismail M.K., Vander Voort G., Squires J.J., Swanson K.C., Mandell I.B., Liao X., Stothard P., Moore S., Plastow G. & Miller S.P. (2014) Single nucleotide polymorphisms for feed efficiency and performance in crossbred beef cattle. BMC genetics 15, 14.

Ahola J.K. & Hill R.A. (2012) Input Factors Affecting Profitability: a Changing Paradigm and a Challenging Time. In: Feed efficiency in the beef industry (ed. by Hill RA), p. 10. Wiley-Blackwell, Ames, Iowa.

Alexandre P.A., Kogelman L.J., Santana M.H., Passarelli D., Pulz L.H., Fantinato-Neto P., Silva P.L., Leme P.R., Strefezzi R.F. & Coutinho L.L. (2015) Liver transcriptomic networks reveal main biological processes associated with feed efficiency in beef cattle. BMC genomics 16, 1073.

Anders S., McCarthy D.J., Chen Y., Okoniewski M., Smyth G.K., Huber W. & Robinson M.D. (2013) Count-based differential expression analysis of RNA sequencing data using R and Bioconductor. Nature protocols 8, 1765-86.

Anders S., Pyl P.T. & Huber W. (2015) HTSeq–a Python framework to work with high-throughput sequencing data. Bioinformatics, btu638.

Andrews S. (2010) FastQC: a quality control tool for high throughput sequence data.

Archer J., Richardson E., Herd R. & Arthur P. (1999) Potential for selection to improve efficiency of feed use in beef cattle: a review. Crop and Pasture Science 50, 147-62.

Barendse W., Reverter A., Bunch R.J., Harrison B.E., Barris W. & Thomas M.B. (2007) A validated whole-genome association study of efficient food conversion in cattle. Genetics 176, 1893-905.

Benjamini Y. & Hochberg Y. (1995) Controlling the false discovery rate: a practical and powerful approach to multiple testing. Journal of the royal statistical society. Series B (Methodological), 289-300.

Brandebourg T., Wolfe D. & Foradori C. (2013) US beef industry: a sustainable success story, challenges and priorities. J. Fisheries Livest. Prod 1.

Briggs M.H. & Briggs M.D. (1980) Modern breeds of livestock. Macmillan, New York.

Chen Y., Gondro C., Quinn K., Herd R., Parnell P. & Vanselow B. (2011) Global gene expression profiling reveals genes expressed differentially in cattle with high and low residual feed intake. Animal genetics 42, 475-90.

Cheng X.-N., Shao M., Li J.-T., Wang Y.-F., Qi J., Xu Z.-G. & Shi D.-L. (2017) Leucine repeat adaptor protein 1 interacts with Dishevelled to regulate gastrulation cell movements in zebrafish. Nature Communications 8, 1353.

Csaki L.S., Dwyer J.R., Fong L.G., Tontonoz P., Young S.G. & Reue K. (2013) Lipins, lipinopathies, and the modulation of cellular lipid storage and signaling. Progress in lipid research 52, 305-16.

de Oliveira P.S., Cesar A.S., do Nascimento M.L., Chaves A.S., Tizioto P.C., Tullio R.R., Lanna D.P., Rosa A.N., Sonstegard T.S. & Mourao G.B. (2014) Identification of genomic regions associated with feed efficiency in Nelore cattle. BMC genetics 15, 100.

Donkin S.S. (2012) The role of liver metabolism during transition on postpartum health and performance. In: 2012 Florida Ruminant Nutrition Symposium Proceedings. 23rd Meeting, pp. 97-106.

Drackley J.K., Dann H.M., Douglas N., Guretzky N.A.J., Litherland N.B., Underwood J.P. & Loor J.J. (2005) Physiological and pathological adaptations in dairy cows that may increase susceptibility to periparturient diseases and disorders. Italian Journal of Animal Science 4, 323-44.

Eisemann J., Hammond A. & Rumsey T. (1989) Tissue protein synthesis and nucleic acid concentrations in steers treated with somatotropin. British Journal of Nutrition 62, 657-71.

Fitzsimons C., Kenny D., Deighton M., Fahey A. & McGee M. (2013) Methane emissions, body composition, and rumen fermentation traits of beef heifers differing in residual feed intake. Journal of animal science 91, 5789-800.

Gerber P.J., Steinfeld H., Henderson B., Mottet A., Opio C., Dijkman J., Falcucci A. & Tempio G. (2013) Tackling climate change through livestock: a global assessment of emissions and mitigation opportunities. Food and Agriculture Organization of the United Nations (FAO).

Goonewardene L., Wang Z., Price M., Yang R.-C., Berg R. & Makarechian M. (2003) Effect of udder type and calving assistance on weaning traits of beef and dairy× beef calves. Livestock production science 81, 47-56.

Hegarty R., Goopy J., Herd R. & McCorkell B. (2007) Cattle selected for lower residual feed intake have reduced daily methane production. Journal of animal science 85, 1479-86.

Herd R. & Arthur P. (2009) Physiological basis for residual feed intake. Journal of animal science 87, E64-E71.

Herd R., Oddy V. & Richardson E. (2004) Biological basis for variation in residual feed intake in

beef cattle. 1. Review of potential mechanisms. Australian Journal of Experimental Agriculture 44, 423-30.

Inoue K., Kobayashi M., Shoji N. & Kato K. (2011) Genetic parameters for fatty acid composition and feed efficiency traits in Japanese Black cattle. Animal 5, 987-94.

Keiding S. & Sørensen M. (2007) Textbook of Hepatology: From Basic Science to Clinical Practice.

Khansefid M., Millen C., Chen Y., Pryce J., Chamberlain A., Vander Jagt C., Gondro C. & Goddard M. (2017) Gene expression analysis of blood, liver, and muscle in cattle divergently selected for high and low residual feed intake. Journal of animal science 95, 4764-75.

Kim D., Pertea G., Trapnell C., Pimentel H., Kelley R. & Salzberg S.L. (2013) TopHat2: accurate alignment of transcriptomes in the presence of insertions, deletions and gene fusions. Genome biology 14, R36.

Kitaoka Y., Olvera J. & Wool I.G. (1994) The primary structure of rat ribosomal protein S23. Biochemical and Biophysical Research Communications 202, 314-20.

Koch R.M., Swiger L.A., Chambers D. & Gregory K.E. (1963) Efficiency of feed use in beef cattle. Journal of animal science 22, 486-94.

Kong R.S., Liang G., Chen Y. & Stothard P. (2016) Transcriptome profiling of the rumen epithelium of beef cattle differing in residual feed intake. BMC genomics 17, 592.

Krämer A., Green J., Pollard Jr J. & Tugendreich S. (2013) Causal analysis approaches in ingenuity pathway analysis. Bioinformatics 30, 523-30.

Lkhagvadorj S., Qu L., Cai W., Couture O.P., Barb C.R., Hausman G.J., Nettleton D., Anderson L.L., Dekkers J.C. & Tuggle C.K. (2010) Gene expression profiling of the short-term

adaptive response to acute caloric restriction in liver and adipose tissues of pigs differing in feed efficiency. American Journal of Physiology-Regulatory, Integrative and Comparative Physiology 298, R494-R507.

Mao F., Chen L., Vinsky M., Okine E., Wang Z., Basarab J., Crews D. & Li C. (2013) Phenotypic and genetic relationships of feed efficiency with growth performance, ultrasound, and carcass merit traits in Angus and Charolais steers. Journal of animal science 91, 2067-76.

McDonald P. (2002) Animal nutrition. Pearson education.

Morris Jr S.M. (2002) Regulation of enzymes of the urea cycle and arginine metabolism. Annual review of nutrition 22, 87-105.

Nafikov R.A. & Beitz D.C. (2007) Carbohydrate and lipid metabolism in farm animals. The Journal of nutrition 137, 702-5.

Nascimento M., Souza A., Chaves A., Cesar A., Tullio R., Medeiros S., Mourão G., Rosa A., Feijó G. & Alencar M. (2016) Feed efficiency indexes and their relationships with carcass, non-carcass and meat quality traits in Nellore steers. Meat science 116, 78-85.

Naylor R., Steinfeld H., Falcon W., Galloway J., Smil V., Bradford E., Alder J. & Mooney H. (2005) Losing the links between livestock and land. Science 310, 1621-2.

Nkrumah J., Basarab J., Wang Z., Li C., Price M., Okine E., Crews D. & Moore S. (2007a) Genetic and phenotypic relationships of feed intake and measures of efficiency with growth and carcass merit of beef cattle. Journal of animal science 85, 2711-20.

Nkrumah J., Okine E., Mathison G., Schmid K., Li C., Basarab J., Price M., Wang Z. & Moore S. (2006) Relationships of feedlot feed efficiency, performance, and feeding behavior with metabolic rate, methane production, and energy partitioning in beef cattle. Journal of animal science 84, 145-53.

Nkrumah J., Sherman E., Li C., Marques E., Crews D., Bartusiak R., Murdoch B., Wang Z., Basarab J. & Moore S. (2007b) Primary genome scan to identify putative quantitative trait loci for feedlot growth rate, feed intake, and feed efficiency of beef cattle. Journal of animal science 85, 3170-81.

Ntambi J.M. & Miyazaki M. (2004) Regulation of stearoyl-CoA desaturases and role in metabolism. Progress in lipid research 43, 91-104.

Olfert E.D., Cross B.M. & McWilliam A.A. (1993) Guide to the care and use of experimental animals. Canadian Council on Animal Care Ottawa.

Paradis F., Yue S., Grant J., Stothard P., Basarab J. & Fitzsimmons C. (2015) Transcriptomic analysis by RNA sequencing reveals that hepatic interferon-induced genes may be associated with feed efficiency in beef heifers. Journal of animal science 93, 3331-41.

Pullen D., Liesman J. & Emery R. (1990) A species comparison of liver slice synthesis and secretion of triacylglycerol from nonesterified fatty acids in media. Journal of animal science 68, 1395-9.

Ramsey R., Doye D., Ward C., McGrann J., Falconer L. & Bevers S. (2005) Factors affecting beef cow-herd costs, production, and profits. Journal of Agricultural and Applied Economics 37, 91-9.

Reynolds C.K. (1992) Metabolism of Nitrogenous Compounds by Ruminant Liver1. The Journal of nutrition 122, 850.

Richardson E. & Herd R. (2004) Biological basis for variation in residual feed intake in beef cattle. 2. Synthesis of results following divergent selection. Animal Production Science 44, 431-40.

Richardson E., Herd R., Archer J. & Arthur P. (2004) Metabolic differences in Angus steers divergently selected for residual feed intake. Australian Journal of Experimental Agriculture 44, 441-52.

Richardson E., Herd R., Oddy V., Thompson J., Archer J. & Arthur P. (2001) Body composition and implications for heat production of Angus steer progeny of parents selected for and against residual feed intake. Australian Journal of Experimental Agriculture 41, 1065-72.

Robinson D. & Oddy V. (2004) Genetic parameters for feed efficiency, fatness, muscle area and feeding behaviour of feedlot finished beef cattle. Livestock production science 90, 255-70.

Robinson M.D., McCarthy D.J. & Smyth G.K. (2010) edgeR: a Bioconductor package for differential expression analysis of digital gene expression data. Bioinformatics 26, 139-40.

Robinson M.D. & Oshlack A. (2010) A scaling normalization method for differential expression analysis of RNA-seq data. Genome biology 11, R25.

Roh S.G., Hishikawa D., Hong Y.H. & Sasaki S. (2006) Control of adipogenesis in ruminants. Animal Science Journal 77, 472-7.

Saatchi M., Beever J.E., Decker J.E., Faulkner D.B., Freetly H.C., Hansen S.L., Yampara-Iquise H., Johnson K.A., Kachman S.D. & Kerley M.S. (2014) QTLs associated with dry matter intake, metabolic mid-test weight, growth and feed efficiency have little overlap across 4 beef cattle studies. BMC genomics 15, 1004.

Salter A.M. (2017) Improving the sustainability of global meat and milk production. Proceedings of the Nutrition Society 76, 22-7.

Seal C. & Reynolds C. (1993) Nutritional implications of gastrointestinal and liver metabolism in ruminants. Nutrition Research Reviews 6, 185-208.

Shalev B. & Pasternak H. (1989) Meat production efficiencies of turkey, chicken and duck broilers. World's Poultry Science Journal 45, 109-14.

Sherman E., Nkrumah J., Li C., Bartusiak R., Murdoch B. & Moore S. (2009) Fine mapping quantitative trait loci for feed intake and feed efficiency in beef cattle. Journal of animal science 87, 37-45.

Tizioto P.C., Coutinho L.L., Decker J.E., Schnabel R.D., Rosa K.O., Oliveira P.S., Souza M.M., Mourão G.B., Tullio R.R. & Chaves A.S. (2015) Global liver gene expression differences in Nelore steers with divergent residual feed intake phenotypes. BMC genomics 16, 242.

Tizioto P.C., Coutinho L.L., Oliveira P.S., Cesar A.S., Diniz W.J., Lima A.O., Rocha M.I., Decker J.E., Schnabel R.D. & Mourão G.B. (2016) Gene expression differences in Longissimus muscle of Nelore steers genetically divergent for residual feed intake. Scientific reports 6.

Tomasini R., Samir A.A., Carrier A., Isnardon D., Cecchinelli B., Soddu S., Malissen B., Dagorn J.-C., Iovanna J.L. & Dusetti N.J. (2003) TP53INP1s and homeodomain-interacting protein kinase-2 (HIPK2) are partners in regulating p53 activity. Journal of Biological Chemistry 278, 37722-9.

Trejo C.O. (2010) Residual feed intake, breed of sire and dam, and individual sire affect performance and carcass characteristics and rates of back fat and intramuscular fat of feedlot steers. University of Illinois at Urbana-Champaign.

Van Heugten E. (2010) Growing-finishing swine nutrient recommendations and feeding management. National Swine Nutrition Guide (ed. DJ, Meisinger), 80-95.

Weber K.L., Welly B.T., Van Eenennaam A.L., Young A.E., Porto-Neto L.R., Reverter A. & Rincon G. (2016) Identification of gene networks for residual feed intake in angus cattle using genomic prediction and RNA-seq. PloS one 11, e0152274.

Zhang F., Ekine-Dzivenu C., Vinsky M., Basarab J., Aalhus J., Dugan M. & Li C. (2017) Phenotypic and genetic relationships of residual feed intake measures and their component traits with fatty acid composition in subcutaneous adipose of beef cattle. Journal of animal science.

2.8 Figures and Tables

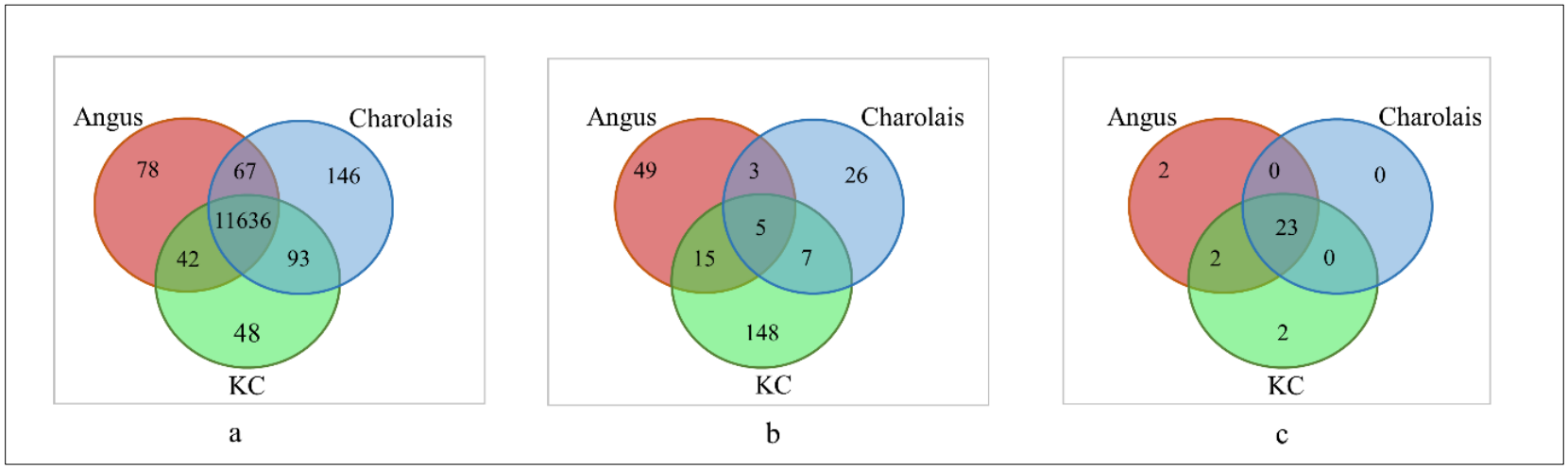


Figure 2.1. Venn diagrams showing: (a) overlap of expressed genes (> 1CPM in ≥ 6 samples) in the three studied breed populations; (b) overlap of differentially expressed genes (DE genes) in the three studied breed populations; (c) overlap of biological functions identified.

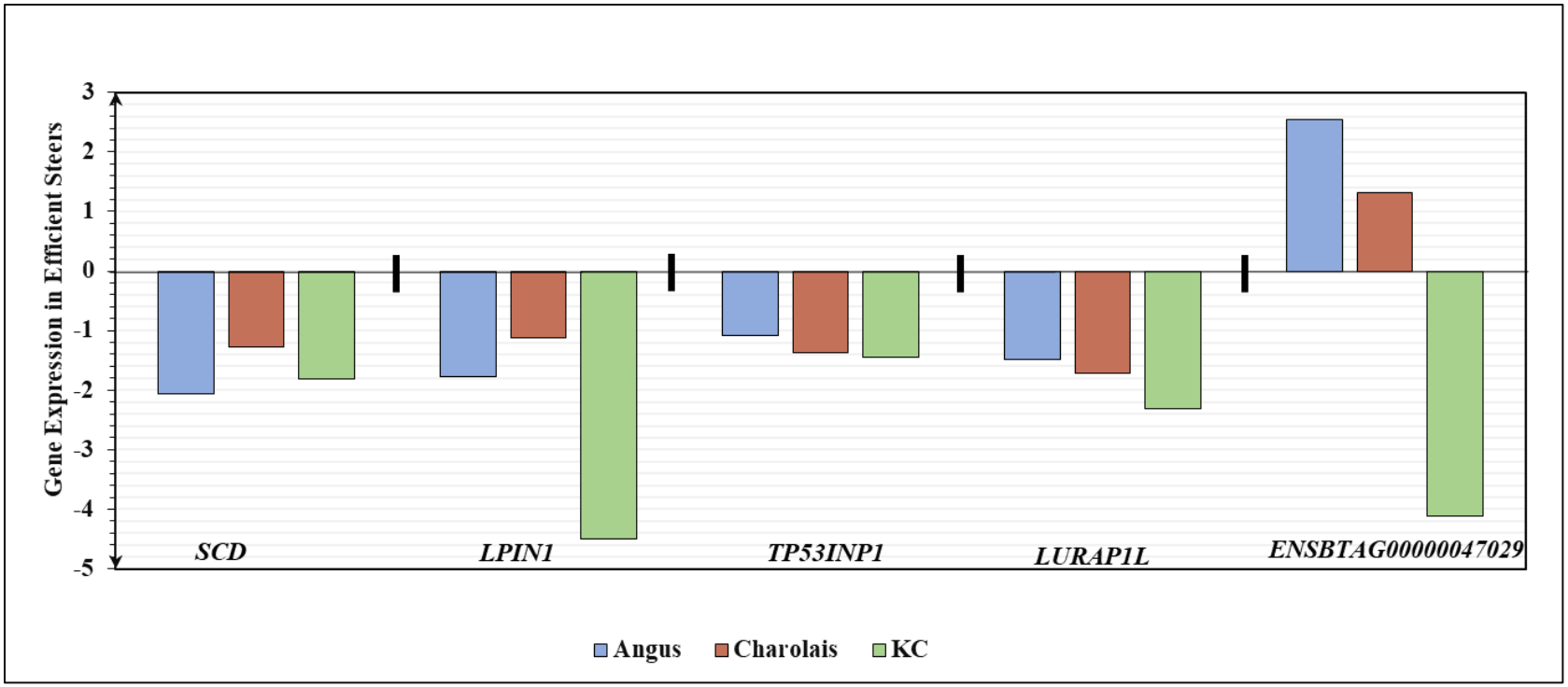


Figure 2.2. Expression profile (log_2(Fold-change)) in low-RFI steers of the five differentially expressed (DE) genes common across all three breeds.

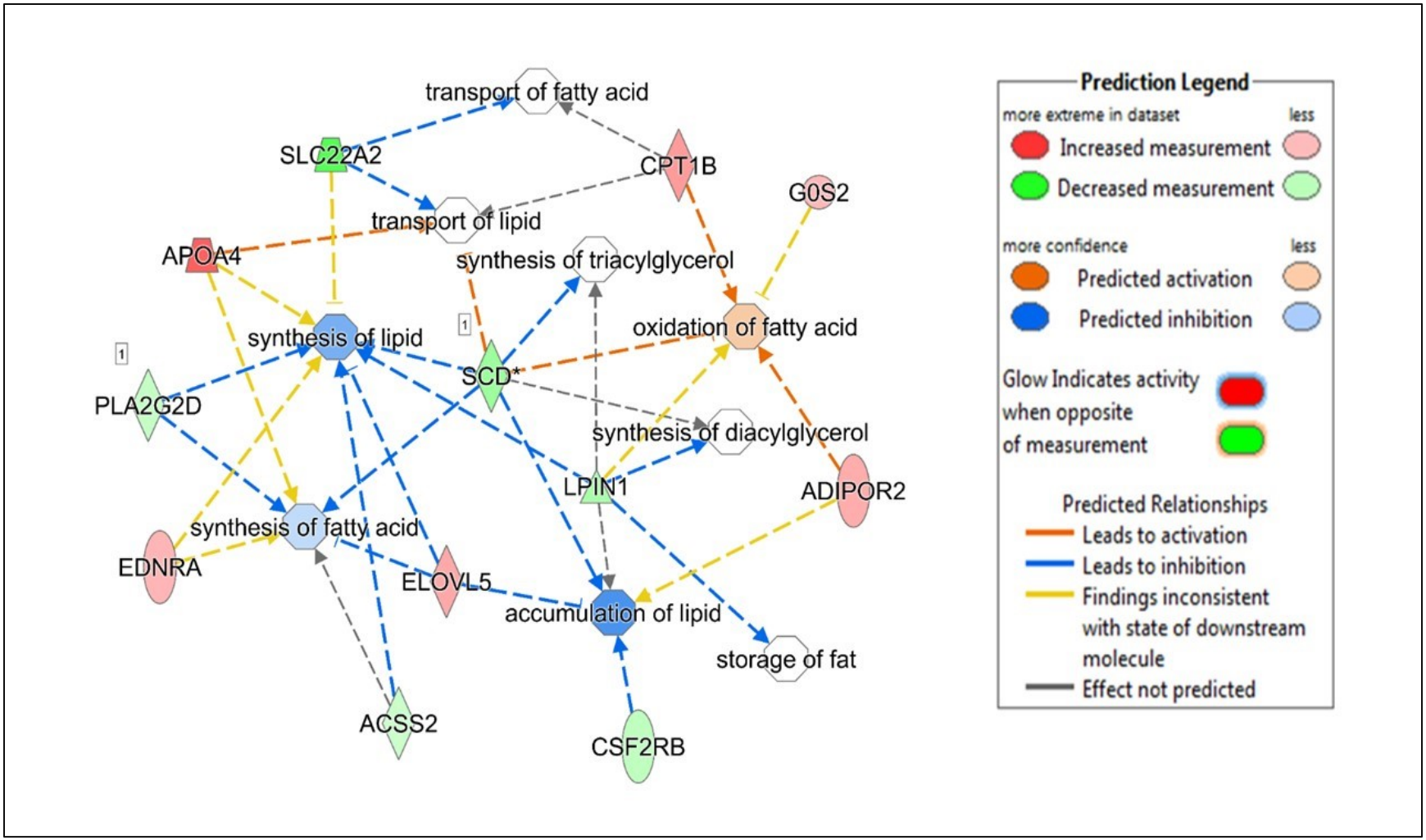


Figure 2.3. Metabolic process regulatory gene network showing differentially expressed (DE) genes involved in the different lipid metabolic processes and their predicted activation or deactivation levels in Angus low-RFI steers.

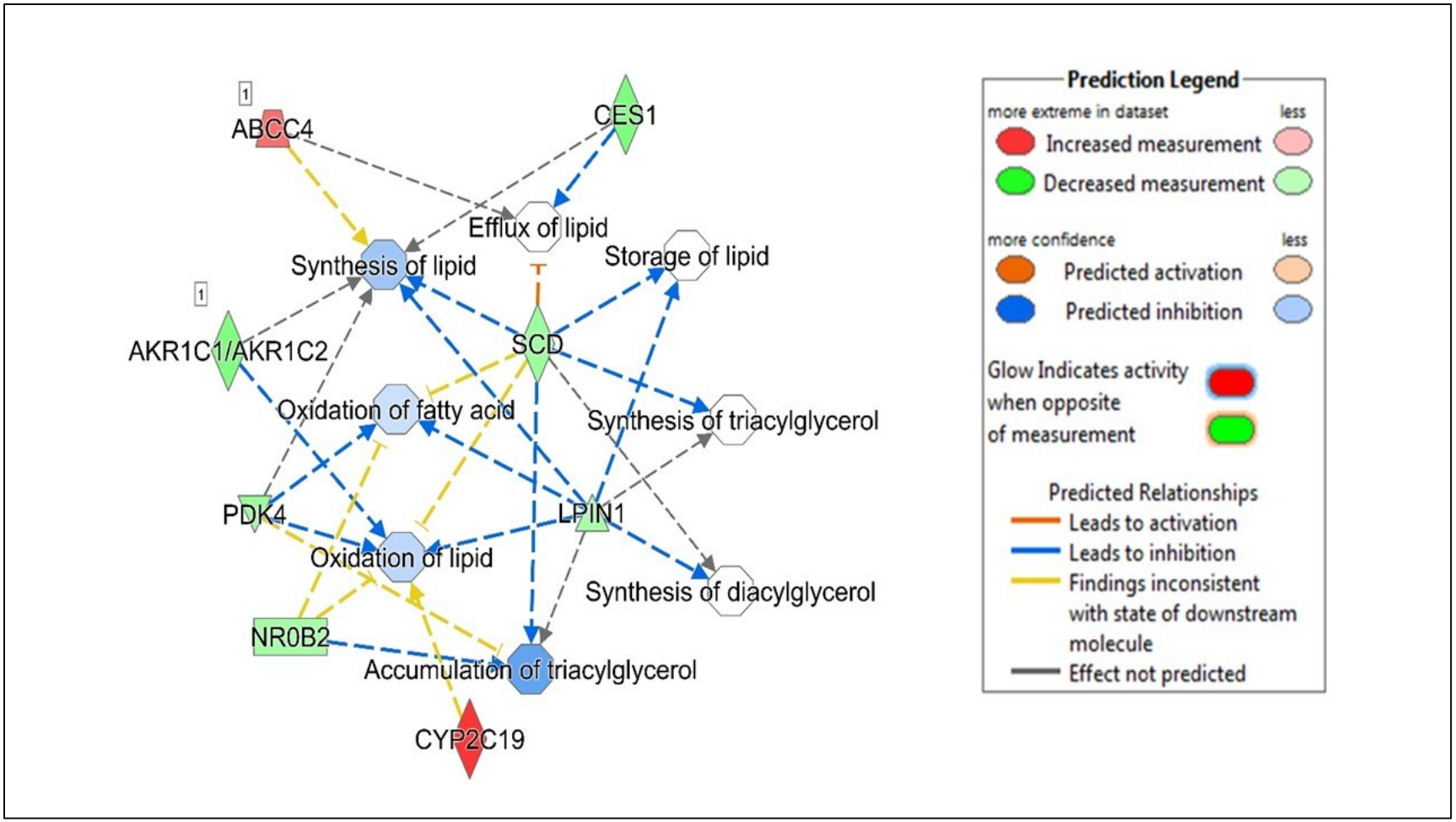


Figure 2.4. Metabolic process regulatory gene network showing differentially expressed (DE) genes involved in the different lipid metabolic processes and their predicted activation or deactivation levels in Charolais low-RFI steers.

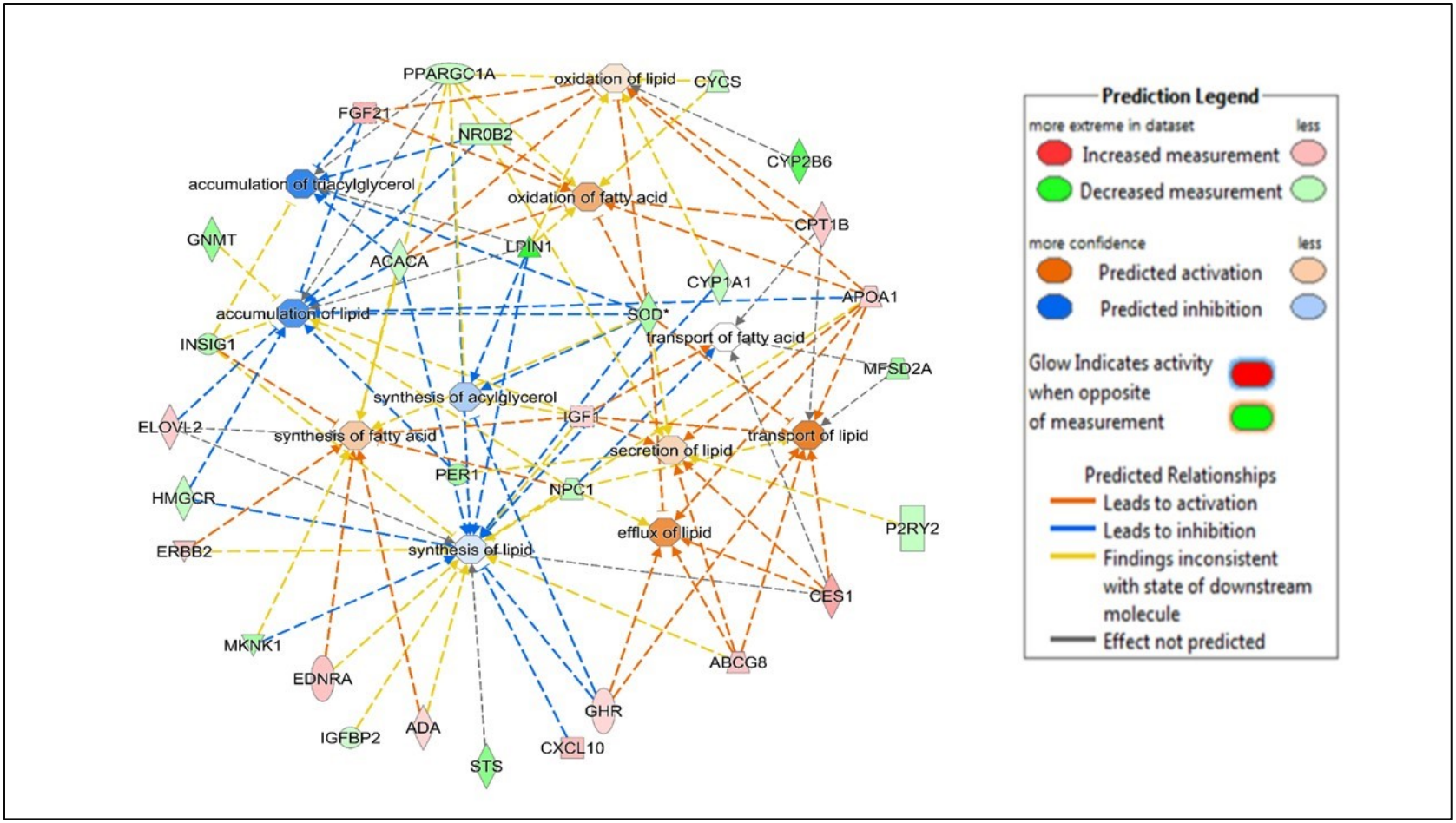


Figure 2.5. Metabolic process regulatory gene network showing differentially expressed (DE) genes involved in the different lipid metabolic processes and their predicted activation or deactivation levels in KC low-RFI steers.

Table 2.1. Differences of RFI and other performance traits between groups of high (n = 6) and low RFI steers (n = 6) of the three breeds (Angus, Charolais and Kinsella Composite).

	Angus			**Charolais**			**Kinsella Composite (KC)**		
Trait	**L_RFI±SE**	**H_RFI±SE**	**P-value**	**L_RFI±SE**	**H_RFI±SE**	**P-value**	**L_RFI±SE**	**H_RFI±SE**	**P-value**
RFI/kg/day	-0.84±0.07	1.29±0.10	9.24E-09*	-1.10±0.0.08	1.15±0.16	1.69E-07*	-1.29±0.11	1.52±0.12	1.18E-08*
DMI/kg/day	11.46±0.51	13.31±0.43	0.01*	10.11±0.16	12.32±0.16	2.21E-06*	9.21±0.36	12.74±0.36	3.95E-05*
ADG/kg/day	1.88±0.11	1.74±0.12	0.38	1.64±0.04	1.67±0.08	0.78	1.48±0.10	1.63±0.07	0.26
MWT/kg	115.58±5.41	115.63±2.75	0.99	120.73±1.50	119.74±1.79	0.68	99.7±2.70	104.67±2.77	0.23
FUREA/cm2	84.41±1.56	80.34±3.08	0.27	93.80±2.27	91.99±3.25	0.66	70.28±2.90	74.22±1.52	0.26
FUFAT/mm	9.23±1.24	9.57±0.68	0.73	7.08±0.85	5.67±0.63	0.21	8.67±0.55	8.98±0.45	0.67
HCW/lb	763.23±44.26	753.47±22.00	0.85	855.17±23.18	843±9.89	0.64	656.67±21.52	697.33±24.54	0.24
AFAT/mm	10.67±1.09	12.17±1.40	0.42	8.33±1.11	6.67±0.49	0.20	11.67±1.18	10±0.51	0.22
CREA/cm2	75.83±2.34	74.33±4.45	0.77	95.3±4.44	94±3.12	0.81	69.67±2.54	76.33±2.23	0.08
LMY/%	56.43±1.18	55.2±1.76	0.57	60.88±1.09	61.94±0.62	0.42	55.79±0.87	57.81±0.56	0.08
Marbling score	393.33±23.47	438.33±17.78	0.16	370±36.79	398.33±14.24	0.49	378.33±20.56	378.33±20.56	1.00
Slaughter age/day	488.9±5.2	500.3±4.4	0.12	517.3±6.6	522.0±5.0	0.58	445.2±3.4	464.0±7.1	0.04

"*" indicates significant difference (P-value≤ 0.01). RFI – residual feed intake, DMI – daily dry matter intake, ADG–average daily gain, MWT– metabolic body weight, FUREA - final ultrasound ribeye area at the end of feedlot test ; FUFAT - final ultrasound backfat at the end of feedlot test; HCW - hot carcass weight; AFAT - carcass average backfat; REA = carcass ribeye area; LMY - lean meat yield; Marbling score (100–399 = trace marbling or less, 400–499 = slight marbling, 500–799 = small to moderate marbling, and 800–1199 = slightly abundant or more marbling). L_RFI±SE- mean RFI values for the low RFI group ± standard error (SE); H_RFI±SE- mean RFI values for the high RFI group ± standard error (SE).

Table 2.2. Twenty-nine most significantly (by FDR value) differentially expressed genes in Angus, Charolais, and KC.

Angus			Charolais			Kinsella Composite (KC)		
Gene	**logFC**	**FDR**	**Gene**	**logFC**	**FDR**	**Gene**	**logFC**	**FDR**
RPL12	3.05	6.72E-20	*PRAP1*	-2.93	9.4E-22	*SERPINI2*	5.52	1.92E-47
Sectm1b	2.67	1.33E-19	*CYP2C19*	3.146	1.9E-21	*FKBP5*	-4.66	2.2E-25
CDHR5	-3.17	9.81E-19	*SLC13A2*	1.987	2.6E-08	*LPIN1*	-4.49	4.9E-23
PRSS2	-4.73	4.79E-18	*REC8*	-1.416	1.9E-05	*CYP2B6*	-3.49	9.67E-15
HLA-DQA1	2.99	2.55E-17	*CES1*	-1.694	3.3E-05	*CES1*	2.42	3.04E-14
APOA4	2.36	8.67E-14	*GPX3*	1.412	2.6E-04	*PRAP1*	-4.00	1.83E-12
HLA-DQA2	-2.83	3.55E-13	*LURAP1L*	-1.726	2.6E-04	*NAV2*	2.29	8.5E-11
ECEL1	-2.50	5.51E-11	*AK4*	1.225	2.6E-04	*AK4*	2.26	3.38E-10
DOPEY2	2.39	3.29E-10	*LAMB3*	-1.462	2.6E-04	*AKR1B10*	-3.98	4.73E-10
LOC690507	-2.77	5.79E-10	*TP53INP1*	-1.367	5.6E-04	*COL27A1*	1.94	1.17E-08
SLC22A2	-3.59	1.06E-09	*SLC7A5*	-1.71	07E-04	*SLC16A6*	-2.46	3.59E-08
GIMAP4	1.93	3.46E-09	*TMEM176B*	1.195	1.35E-03	*STS*	-2.41	4.52E-08
SCD	-2.07	1.40E-08	*HLA-DQB1*	-2.194	2.6E-03	*ALAS1*	-2.23	9.65E-08
HLA-B	-1.97	3.55E-07	*TNC*	1.233	2.71E-03	*GLCE*	-2.19	1.3E-07
HOPX	1.71	4.97E-07	*CXCL2*	1.474	3.37E-03	*GNMT*	-2.23	4.85E-07
UGT2B7	1.64	1.14E-06	*NR0B2*	-1.18	4.12E-03	*SDS*	-2.14	7.61E-07
HLA-B	-1.79	1.44E-06	*THEM4*	-1.239	9.12E-03	*ARG1*	-1.99	3.05E-06
CCDC80	-1.95	1.47E-06	*PDK4*	-1.33	0.0184	*ABHD2*	1.67	4.49E-06
CABYR	1.66	3.91E-06	*GPNMB*	1.155	0.0192	*NMNAT2*	-3.02	5.49E-06
UGT2B17	-1.78	4.86E-06	*LPIN1*	-1.118	0.0192	*PER1*	-2.03	7.37E-06
LPIN1	-1.77	5.94E-06	*SERPINA3*	-1.225	0.0192	*GLS2*	-1.95	7.65E-06
SLCO4A1	-1.87	5.94E-06	*TBATA*	1.02	0.0195	*WFDC2*	-2.02	8.16E-06
ASCL1	-1.71	6.26E-06	*RND1*	1.021	0.02	*MKNK1*	-1.90	1.13E-05
IFI6	-1.79	9.22E-06	*INMT*	1.104	0.0291	*OAT*	-2.01	1.51E-05
RXRG	1.42	1.06E-04	*ANXA2*	1.065	0.0309	*MFSD2A*	-2.05	1.64E-05
FKBP5	-1.53	2.14E-04	*SCD*	-1.275	0.0356	*MYCL*	2.03	1.88E-05
ALAS1	-1.54	2.47E-04	*SLC4A4*	-1.063	0.0389	*ERBB2*	1.51	4.23E-05
TSKU	-1.54	9.20E-04	*KLHL13*	-1.184	0.0429	*HLA-B*	-1.75	4.36E-05
LURAP1L	-1.49	1.26E-03	*SPNS2*	1.028	0.0466	*ASB9*	-2.80	4.80E-05

FDR = False discovery rate; logFC = log2 (Fold-Change in low RFI steers in comparison with high RFI steers).

Table 2.3. Five topmost significantly enriched biological functions within each breed and the DE genes involved within each specific function.

	Biological Function	**No. of genes**	**Genes involved in the biological function**
(Angus) *1*	Lipid metabolism	21	*ELOVL5, GATM, HP, LPIN1, ADIPOR2, CSF2RB, SLC22A2, CCDC80, ZBTB16, ACSS2, EDNRA, CPT1B, RXRG, APOA4, UGT2B17, SCD, FKBP5, G0S2, MARCO, PLA2G2D, DLK1*
2	Molecular transport	20	*ADIPOR2, APOA4, CCDC80, CPT1B, CSF2RB, DLK1, EDNRA, ELOVL5, G0S2, GATM, HP, LPIN1, MARCO, PLA2G2D, RXRG, SCD, SLC22A2, TP53INP1, UGT2B17, ZBTB16*
3	Small molecular biochemistry	23	*ACSS2, ADIPOR2, APOA4, CCDC80, CPT1B, CSF2RB, DLK1, EDNRA, ELOVL5, FKBP5, G0S2, GATM, HP, LPIN1, LURAP1L, MARCO, PLA2G2D, RXRG, SCD, SLC22A2, TP53INP1, UGT2B17, ZBTB16*
4	Carbohydrate metabolism	13	*SCD, CCDC80, UGT2B17, LPIN1, CSF2RB, PLA2G2D, GNAZ, TP53INP1, EDNRA, GATM, ADIPOR2, ELOVL5, APOA4*
5	Energy production	6	*SCD, CCDC80, LPIN1, G0S2, ADIPOR2, CPT1B*
(Charolais) *1*	Lipid metabolism	14	*ABCC4, AKR1C1/AKR1C2, ANXA2, CES1, CYP2C19, DLK1, LPIN1, NR0B2, PDK4, SCD, SLC4A4, SPNS2, THEM4, TNC*
2	Molecular transport	17	*ABCC4, AKR1C1/AKR1C2, ANXA2, CES1, CXCL2, DLK1, LPIN1, NR0B2, PDK4, SCD, SIRPA, SLC13A2, SLC4A4, SLC7A5, SPNS2, TNC, TP53INP1*
3	Small molecule biochemistry	21	*ABCC4, AK4, AKR1C1/AKR1C2, ANXA2, CES1, CYP2C19, DLK1, GPX3, LPIN1, LURAP1L, MIOX, NR0B2, PDK4, SCD, SLC13A2, SLC4A4, SLC7A5, SPNS2, THEM4, TNC, TP53INP1*
4	Energy production	6	*AKR1C1/AKR1C2, CYP2C19, LPIN1, NR0B2, PDK4, SCD*
5	Cellular development	15	*ANXA2, CXCL2, DLK1, GNAZ, GPNMB, LAMB3, LPIN1, NR0B2, PDK4, RND1, SCD, SIRPA, SLC7A5, TNC, TP53INP1*

*(**KC**)1*	Amino acid metabolism	22	*AASS, ACMSD, ARG1, ASL, ERBB2, GCH1, GCLC, GHR, GLS2, GNMT, GOT1, HAL, IGF1, IGFBP2, OAT, RXRG, SDS, SLC16A10, SLC22A7, SLC25A15, SLC7A2, TAT*
2	Small Molecule biochemistry	64	*AASS, ABCG8, ACACA, ACMSD, ADA, AK4, AKR1B10, APOA1, ARG1, ASL, ASPG, ATP2A2, BAG3, CDKN1A, CES1, CPT1B, CXCL10, CYCS, CYP1A1, CYP2B6, DUSP1, EDNRA, ELOVL2, ERBB2, ERBB3, FGF21,GATA4, GCH1, GCLC, GHR, GLS2, GNMT, GOT1, HAL, HMGCR, IGF1, IGFBP2, INSIG1, LPIN1, MFSD2A, MKNK1, NMNAT2, NPC1, NR0B2, OAS1, OAT, OGDH, P2RY2, PER1, PNP, PPARGC1A, RBP5, RHOJ, RXRG, SCD, SDS, SLC16A10, SLC22A7, SLC25A15, SLC7A2, SQLE, STS, TAT, ZBTB16*
3	Cell death and survival	64	*ACACA, ADA, APMAP, APOA1, ARG1, ATP2A2, BAG3, BTG2, CCND1, CDKN1A, CES1, CXCL2, CXCL10, CYCS, CYP2B6, DDIT4, DUSP1, EDNRA, ERBB2, ERBB3, FGF21, FKBP5, GADD45B, GATA4, GCH1, GCLC, GHR, GLS2, GNL3, GNMT, HEYL, HLA-B, HLA-F, HMGCR, IGF1, IGFBP2, INSIG1, IRAK3, ITGA7, KYAT1, LRIG1, MANF, MKNK1, MOB3B, NMNAT2, NPC1, NR0B2, OAS1, OGDH, PER1, PIGR, PNP, PPARGC1A, PRAP1, RHOJ, RRS1, SCD, SERPINA3, TOP1, TP53INP1, TRIB2, UHRF1, USP2, ZBTB16*
4	Lipid metabolism	43	*ABCG8, ACACA, ADA, AKR1B10, APOA1, ASPG, ATP2A2, BAG3, CDKN1A, CES1, CPT1B, CXCL10, CYCS, CYP1A1, CYP2B6, DUSP1, EDNRA, ELOVL2, ERBB2, FGF21, GATA4, GHR, GNMT, GOT1, HMGCR, IGF1, IGFBP2, INSIG1, LPIN1, MFSD2A, MKNK1, NPC1, NR0B2, OGDH, P2RY2, PER1, PPARGC1A, RBP5, RXRG, SCD, SQLE, STS, ZBTB16*
5	Molecular Transport	45	*ABCG8, ACACA, ADA, APOA1, ARG1, ATP2A2, BAG3, CDKN1A, CES1, CPT1B, CXCL10, CXCL2, CYP1A1, DUSP1, EDNRA, ELOVL2, ERBB2, ERBB3, FGF21, GATA4, GHR, GNMT, HMGCR, HOOK1, IGF1, INSIG1, LPIN1, MFSD2A, NPC1, NR0B2, P2RY2, PER1, PIGR, PNP, PPARGC1A, RHOJ, RXRG, SCD, SLC16A10, SLC16A6, SLC22A7, SLC25A15, SLC38A7, SLC7A2, ZBTB16*

Chapter 3. Liver transcriptome profiling of beef steers with divergent feed intake, metabolic weight or growth rate phenotypes

3.1 Abstract

Average daily (ADG) and dry matter intake (DMI) are key determinants of the beef production profitability. These traits together with metabolic weight (MWT) are combined as component traits to calculate residual feed intake (RFI), a common measure of feed efficiency in beef cattle. Recently, there have been significant efforts towards molecular genetic characterization of RFI through transcriptomic studies with different breeds and tissues. However, molecular mechanisms of RFI component traits remain largely unexplored. Therefore, in the current study we investigated the hepatic transcriptomic profiles and their association with ADG, DMI and MWT in Angus, Charolais, and Kinsella Composite (KC) populations through global RNAseq analyses. In each population and for each trait, 12 steers with extreme phenotypes (n = 6 low and n = 6 high) were analyzed for differential gene expression. On average, 11854, 11900 and 11792 genes were expressed in the liver tissue of Angus, Charolais and KC steers, respectively. We identified 123, 102 and 78 differentially expressed (DE) genes between high and low-ADG animals of Angus, Charolais and KC populations, respectively. For DMI, 108, 180 and 156 DE genes were identified between high and low-DMI from Angus, Charolais and KC populations, respectively, while for MWT, 80, 82 and 84 genes were differentially expressed between high and low-MWT animals in Angus, Charolais and KC populations, respectively. The identified DE genes were largely breed-specific (81.7% for ADG, 82.7% for DMI, and 83% for MWT). However, the DE genes were largely involved in the same biological functions across the breeds. Among the most enriched biological functions included metabolism of lipid, carbohydrates, amino acids, vitamins and minerals, small molecule biochemistry, cellular movement, cell morphology and cell

to cell signaling and interaction. Notably, we identified multiple DE genes that were involved in cholesterol biosynthesis, and immune response pathways for the three studied traits. Together, our findings provide insight into the genetic mechanisms and candidate genes that influence feed intake, growth, and metabolic weight of beef cattle.

3.2 Introduction

Animal growth rate and feed intake are very important traits to the beef industry as they both directly affect the productivity, and thus profitability of the industry. To finish beef cattle for meat production, feedlot operators maintain their animals in the feedlot and incur costs such as labor, management, veterinary, feed and feeding related costs, with the latter accounting for over 65% of the total production costs (Ahola & Hill 2012). It is therefore of great interest to beef producers to raise faster growing animals with minimal or reduced daily feed consumption to optimize productivity of production systems profits (Hill & Ahola 2012). It has been reported that growth rate measured as average daily gain (ADG) and feed intake measured as daily dry mater intake (DMI) are moderately to highly heritable traits, with estimated heritability of 0.35 to 0.59 (Schenkel *et al.* 2004; Nkrumah *et al.* 2007; Mao *et al.* 2013), hence the potential to breed and select for more efficient animals. Additionally, ADG and DMI together with metabolic weight (MWT) are key component traits used in the calculation of animal feed efficiency termed as residual feed intake (RFI) in beef cattle (Koch *et al.* 1963). Furthermore, ADG and DMI are also important traits that can be included in beef cattle genetic selection and breeding programs to improve the efficiency of beef production.

In recent years, several transcriptome studies on different tissues including the liver tissue have been performed to identify molecular mechanisms of feed efficiency traits including RFI in different beef cattle breeds or populations (Chen *et al.* 2011; Al-Husseini *et al.* 2014; Alexandre

et al. 2015; Paradis *et al.* 2015; Kong *et al.* 2016; Tizioto *et al.* 2016; Weber *et al.* 2016; Khansefid *et al.* 2017; Mukiibi *et al.* 2018). However, only a few studies have focused on identifying such mechanisms for ADG and DMI (Foote *et al.* 2017; Lindholm-Perry *et al.* 2017; Reynolds *et al.* 2017; Zarek *et al.* 2017). ADG, DMI and MWT are complex traits whose molecular architecture involve multiple organs, and the liver is a major physiological and metabolic organ involved in nutrient metabolism and homeostasis (Van den Berghe 1991), immune response (Racanelli & Rehermann 2006), and growth regulation through its endocrine function (Baruch 2000). Given this metabolic vitality, it is eminent that genes expressed in liver probably directly or indirectly influence animal growth rate, feed intake and metabolic weight of the animal. Therefore, in the current study we analyzed RNAseq data of liver tissues of selected beef steers of extreme phenotypes, with the aim to identify differentially expressed genes and metabolic or biological functions that underlie ADG, DMI and MWT phenotypic differences in Canadian beef populations including Angus, Charolais, and Kinsella Composite.

3.3 Materials and Methods

3.3.1 Animal populations and management

Populations and management of the animals used in this study have been described extensively in our previous study by Mukiibi et al. (2018) (Mukiibi *et al.* 2018). Briefly, the animals were managed under the Canadian Council of Animal Care (CCAC) guidelines on the care and use of farm animals in research teaching and testing (CCAC 2009), and the experimental procedures were approved by the University of Alberta Animal Care and Use Committee (AUP00000777). Beef steers from three beef cattle herds including purebred Angus, Charolais, as well as Kinsella Composite (KC) were used in this study. All animals were born, raised and managed similarly at the Roy Berg Kinsella Ranch, University of Alberta, Canada. The purebred

Angus and Charolais cows were bred by artificial insemination (AI) followed by natural service bulls and their pedigree information was maintained by the Canadian Angus or Charolais Association, respectively. The KC herd descended from crosses between Angus, Charolais, or Alberta Hybrid bulls and the University of Alberta's hybrid dam line that was generated by crossing composite cattle lines of multiple beef breeds as described by Goonewardene et al. (2003). Commercial crossbred bulls have also been used in the KC herd since 2012. The animals used in this study were born during the months of April and May in 2014 and were castrated right after birth. The steer calves remained with their dams over the summer and grazed mixed tame grass pasture, then weaned at approximately six months of age. They were transitioned to backgrounding diet composed of 80% barley silage, 17% barley grain, and 3% rumensin pellet supplement, and then fed set-up diets with gradually decreasing barley silage and increasing barley grain proportions for 3 weeks prior to introducing them to the finishing diet of 75% barley grain, 20% barley silage, and 5% rumensin pellet supplement (as fed basis).

3.3.2 GrowSafe feedlot test, phenotype measurement and calculations

In 2015, 50 Angus, 48 Charolais and 158 KC steers were measured for individual feed intake between April and August using the GrowSafe system® (GrowSafe Systems Ltd., Airdrie, Alberta, Canada). During this test period, animals were fed a finishing diet as described above. The process of measuring the individual animals' daily feed intake using the GrowSafe automated system has been described previously by Mao et al. (2013) (Mao *et al.* 2013). Briefly, DMI of each steer was calculated as the average of daily feed intakes over the test period (70 to 73 days), standardized to 12 MJ ME per kg dry matter based on the energy content of the diet. Initial body weight and ADG for each animal were obtained from a linear regression of serial body weight (BW) measurements that were recorded on two consecutive days at the beginning, at

approximately 14-day intervals during the feedlot test, and on two consecutive days at the end of test. MWT was calculated as midpoint $BW^{0.75}$, where midpoint BW was computed as the sum of initial BW of the animal and the product of its ADG multiplied by half the number of days under the feedlot test.

3.3.3 Liver tissue collection

At the end of the feedlot test all animals from each of the three breeds were slaughtered at Agriculture and Agri-Food Canada (AAFC) Lacombe Research and Development Centre (Lacombe, AB) between July and September of 2015. Steers were rendered fit for slaughter at a backfat thickness of ≥ 8 mm as predicted from a final ultrasound backfat measurement that was performed between the 12th and 13th ribs at the end of the GrowSafe feedlot test using an Aloka 500 diagnostic Realtime ultrasound machine with a 17cm 3.5Mhz linear array transducer (Overseas Monitor Corporation Ltd., Richmond BC). The three steer groups had average slaughter ages of 494 ± 3, 518 ± 4, and 457 ± 4 days for Angus, Charolais, and KC respectively. The liver sample of each animal was collected immediately after slaughter and the tissue was dissected from approximately the same location on the right lobe with the fibrous capsule removed. Samples were separately bagged, labeled and were immediately flash frozen in liquid nitrogen. Subsequently the liver samples were transported to the laboratory on dry ice, and then stored at -80ºC until RNA extraction.

3.3.4 RNA isolation and purification

From the frozen liver samples, a total of 60 samples (20 from each breed) were selected for total RNA extraction based on their residual feed intake values (i.e. 10 steers with high and 10 with low RFI values). The frozen liver tissue of each steer was pulverized into fine powder in liquid nitrogen with a pre-chilled mortar and pestle on dry ice. Total RNA was then extracted from

10 mg of the pulverized tissue using a Qiagen RNeasy Plus Universal Kit (Qiagen, Toronto, ON, Canada) and further purified using a Zymo RNA Clean & Concentrator (Zymo, Irvine, CA, USA). RNA was quantified using a NanoDrop 2000 Spectrophotometer (Thermo Scientific, Wilmington, DE, USA) and was deemed acceptable if its absorbance (A260/280) was between 1.8 and 2.0. RNA integrity was confirmed using a TapeStation-Agilent instrument (Agilent Technologies, Mississauga, ON, Canada), and the RNA integrity number (RIN) values for all samples were higher than 8.

3.3.5 cDNA library preparation and sequencing

Preparation of cDNA libraries and subsequent next generation sequencing of each of the 60 libraries were performed at the Clinical Genomics Centre (Toronto, ON, Canada) using the Illumina TruSeq® RNA Sample Prep Kit v2 (Illumina, San Diego, CA, USA), where mRNA was purified and enriched from 1 µg of each of the total RNA samples using oligo-dT attached magnetic beads, and then fragmented through elevated heating to produce mRNA fragments of length 120-200bp and a median of 150pb. Thereafter, the first strand of the cDNA was synthesized using SuperScript II Reverse Transcriptase enzyme (Thermo Fisher Scientific, San Jose, CA, USA) and the second strand was synthesized using the DNA Polymerase I and RNase H enzymes (Illumina, San Diego, CA, USA). The cDNA libraries were validated using gel electrophoresis to confirm that the fragment size was 150bp (on average) and concentration was on average 25ng/µl per sample. Unique oligonucleotide adapters were added to the cDNA of each sample to allow for multiplexing. Of the prepared cDNA sample libraries, 48 (all Angus, all KC and 8 Charolais) samples were single end sequenced (100bp) under the high output run mode of the Illumina Hiseq 2500 System on eight flow cell lanes. The other 12 Charolais samples were sequenced under the rapid run mode of the same sequencing equipment. All sequence and phenotype data used in this

study have been submitted to the Gene Expression Omnibus repository under the accession number GSE107477.

3.3.6 RNAseq data bioinformatic analyses

Raw sequence data for each sample was assessed for sequencing quality using FASTQC software (Version 0.11.5) with default parameters (Andrews 2010). Tophat2 (version 2.1.1) RNAseq mapper was used to align and map the reads to the bovine reference genome UMD3.1 using default single end read alignment parameters (Kim *et al.* 2013). Reads that were uniquely aligned to each gene annotated in the bovine gene transfer format (GTF) file (ftp://ftp.ensembl.org/pub/release-89/gtf/bos_taurus/Bos_taurus.UMD3.1.89.gtf.gz) were counted using HTSeq-count package (Anders *et al.* 2015) with default parameters to generate read count tables that were further used for differential gene expression statistical analyses.

3.3.7 Differential gene expression analyses

The 20 animal samples from each of the three cattle populations were sorted independently by ADG, DMI or MWT. The 12 samples within each breed with extreme phenotypes (n= 6 high and n= 6 low) for each trait were then analyzed for differential gene expression. The gene count tables generated by HTSeq-count, the gene annotation file downloaded from Ensembl Biomart (http://uswest.ensembl.org/biomart/martview/beba0f867162345fad64c14ad5232f2c), and the sample information file were used for differential gene expression statistical analysis using the R Bioconductor package edgeR (Robinson *et al.* 2010). To increase statistical power of the analyses, genes within each breed that had less than one count per million (CPM) of mapped reads in at least six samples (half of the analyzed samples) were filtered out from the analyses as proposed by Anders et al. (2013) (Anders *et al.* 2013). For the genes retained after filtration, their counts were normalized using the trimmed mean M values (TMM) method (Robinson & Oshlack 2010), to

account for the technical variations between samples due to RNA extraction, cDNA library construction and differences library sequencing depths of genes (Robinson & Oshlack 2010). To test for differential gene expression between high and low-phenotype groups for each trait within a breed, normalized counts were modeled using a generalized linear model likelihood ratio test under assumption of a negative binomial distribution with the trait group as a fixed effect. For Charolais, sequencing run mode, either rapid or high output, was also included in the model to account for the difference in the sequencing modes. Genes were considered significantly differentially expressed between the trait groups at a threshold of Benjamin-Hochberg's false discovery rate (FDR) of 0.05 and fold change (FC) of greater than 1.5 (> 1.5).

3.3.8 Functional enrichment analysis

Functional enrichment analysis of the DE genes for each trait within a breed was performed using Ingenuity Pathway Analysis software (IPA) (Redwood City, CA; www.qiagen.com/ingenuity), with Ensembl bovine gene IDs and log_2-fold change (log_2FC) of the DE genes as input data. The core analysis in IPA was performed on the mapped genes to identify significantly enriched biological functions, canonical pathways and upstream regulators. Molecular and cellular functions (biological functions), canonical pathways, and upstream regulators were considered significantly enriched if the overlap comparison test (Fisher's exact test) between the input DE gene list and the IPA Knowledge base database for that given biological function had a p value less than 0.05. Activation or deactivation of a specific enriched metabolic process, pathway or gene expression regulator was defined by the Z-score (Krämer *et al.* 2013) that was calculated based on the log_2-fold changes of the overlapping DE genes involved in a process or canonical pathway, where a negative or a positive score indicated deactivation or activation of a process, respectively.

3.4 Results

3.4.1 Phenotypic differences between animal groups

For ADG, the steer groups of high-ADG and low-ADG within all the three studied breeds were significantly different from each other at $P < 0.0042$ with Bonferroni correction of 12 multiple tests at $\alpha < 0.05$, Table S1 in Supplementary Data S6. There were no significant differences between the groups for all the other phenotypic traits except for final ultrasound ribeye area at the end of feedlot test for the KC steers. For DMI, steers from low-DMI group significantly consumed less feed per day as compared to those from the high-DMI group within each breed ($P < 0.0042$), Table S2 in Supplementary Data S6. As expected, low-DMI steers had significantly lower RFI than high-DMI animals in Charolais and KC ($P < 0.0042$), and for Angus Low-DMI steers also had lower RFI than their high-DMI counterparts although the difference did not reach the significance level of $P < 0.0042$. When the steer groups in each breed were compared for the other production phenotypes, no significant difference was observed between high and low-DMI animals except for MWT, for which, low-DMI steers showed lower MWT than the high-DMI steers and the difference reached the significance level of $P < 0.0042$ at the KC population. For metabolic weight, our results showed that animals in the high-MWT group within each of the studied population had significantly ($P < 0.0042$) higher metabolic weight than those in the low-MWT group (Table S3 in Supplementary Data S6). It is observed that animals with high metabolic weights on average also had significantly ($P < 0.0042$) higher hot carcass weights (HCW) than those with lower MWT. For Angus, animals with low-MWT ate significantly less feed per day as compared to the high-MWT animals. All the other phenotypes were not significantly different between the MWT groups across the three breeds.

3.4.2 Sequencing and alignment quality assessment

On average next generation transcriptome sequencing (NGS) generated more than 32, 40 and 29 million raw single end sequence reads for the Angus, Charolais and KC cDNA libraries, respectively (Table 3.1). FASTQC sequence data quality assessment results showed that the sequence reads were of high quality with the reads having average length of 101bp and average Phred quality score of more than 36. For alignment to the bovine reference genome, we obtained high unique alignment of approximately 87% reads per sample (Table 3.1).

3.4.3 Differential gene expression

Of the 24,616 annotated bovine genes, 11,849, 11,923 and 11,809 were found to be of sufficient expression level (i.e. Counts per million or CPM in at least six samples > 1) for differential liver gene expression analyses in ADG divergent steers from Angus, Charolais and KC populations, respectively. For Angus, 123 DE genes were identified between the ADG divergent steers, of which 74 genes were unregulated and 49 genes downregulated in fast growing (high-ADG group) animals. For Charolais, we identified 102 DE genes for ADG with 39 and 63 DE genes that were up and downregulated respectively in high-ADG steers. For KC, 78 genes showed significant DE between high and low-ADG steers, with 23 and 55 of these genes respectively up and down regulated in high-ADG steers. Based on FDR, the forty topmost significantly differentially expressed genes which code for characterized proteins for each breed are presented in Table 3.2. The full list of all DE genes identified as associated with ADG for each breed are provided in the Supplementary Data S7. Most of the DE genes (81.7%) were breed specific, however, a sizable number of DE genes were shared at least between two breeds (Figure 3.1a). Five DE genes including *SLC17A9*, *CXCL3*, *IFI27*, *JSP.1* and *ENSBTAG00000003492* were shared among three breeds, with *SLC17A9, CXCL3* and *IFI27* showing consistent direction of

expression in fast growing steers across the three studied populations as presented in Figure S1 in the Supplementary Data S8.

For DMI, 11871, 11961 and 11793 genes were expressed sufficiently for differential gene expression in Angus, Charolais and KC steers, respectively. For Angus, we identified 108 DE genes, with 57 genes up and 51 genes downregulated in low-DMI steers. Among the Charolais steers, 180 genes (120 upregulated and 60 downregulated in low-DMI animals) were differentially expressed. For KC, 156 genes (107 upregulated and 49 downregulated in low-DMI steers) were differentially expressed. The forty most significant protein coding DE genes by FDR are presented in Table 3.3, and all identified DE genes associated with DMI for each breed are provided in the Supplementary Data S9. Also for DMI, most (82.7%) of the identified DE genes were breed specific, with only four DE genes including *IFI27, ENSBTAG00000003492, ENSBTAG00000024700, ENSBTAG00000047029* common among the three studied breeds, and a considerable number of DE genes (17-24 DE genes) were uniquely shared between breed pairs as shown in Figure 2.1b. However, none of the common DE genes showed consistent expression direction across the three breeds (Figure S2 in Supplementary Data S8).

For MWT, 11843, 11908 and 11774 genes were adequately expressed and hence were considered for analysis for the Angus, Charolais and KC steers, respectively. Of these expressed genes, 80 (34 upregulated and 46 downregulated in low-MWT steers), 82 (21 upregulated and 61 downregulated in low-MWT steers), 84 (40 upregulated and 44 downregulated in low-MWT steers) genes were differentially expressed in Angus, Charolais and KC steers respectively. The forty most significant DE genes (coding for characterized proteins) by FDR are presented in Table 4 and all identified DE genes associated with MWT in each of the studied breeds are provided in the Supplementary Data S10. Comparison of the identified DE genes across breeds showed a

similar trend as for ADG and DMI with most of the DE genes (83%) being breed specific and only five DE genes including *MT1E*, *CTGF*, *PRAP1*, *TMEM45A* and *CYP2B6* were identified as common across the three breeds (Figure 3.1c). Two of these shared genes (i.e. *MT1E* and *CTGF*) showed consistent expression across the three populations with *MT1E* being downregulated and *CTGF* upregulated in low-MWT animals as shown in Figure S3 in Supplementary Data S8.

3.4.4 Gene expression across traits within breed

DE genes identified to be associate with ADG, DMI and MWT were compared with the DE genes for RFI reported by Mukiibi et al. (2018) within each studied population as shown in Figure S4 in Supplementary Data S8. Within each breed, the DE genes were largely trait specific with only four (*HP, ENSBTAG00000047029, SERPINA3 and IFI27*), one (*ENSBTAG00000048094*) and two (*ENSBTAG00000022590* and *ENSBTAG00000003492*) DE genes shared across four traits in Angus, Charolais and KC, respectively. However, there was some considerable number of genes shared between pairs of the traits. For example, 46 genes were common between ADG and MWT, 31 genes shared between ADG and DMI, and 39 genes shared between ADG and RFI in Angus, Charolais and KC steers respectively.

3.4.5 Functional Enrichment Analyses

For ADG, a total of 120, 102 and 78 DE genes were mapped to the IPA knowledgebase database for Angus, Charolais and KC, respectively. These mapped DE genes were significantly ($P < 0.05$) involved in 20 molecular and cellular functions for Angus, 27 for Charolais and 28 for KC. Of all the identified molecular and cellular functions, 18 (58.1%) were common to all the three breeds as shown in Figure 3.1d. The most significantly enriched functions included cellular movement, lipid metabolism, small molecule biochemistry, vitamin and mineral metabolism, cell-to-cell signaling and interaction, molecular transport, amino acid metabolism and carbohydrate

metabolism (Figure S5, Figure S6 and Figure S7 in the Supplementary Data S8). It is worth noting that lipid metabolism and small molecule biochemistry functions were among the top five enriched biological functions across the three breeds.

Within lipid metabolism, several metabolic processes related to lipid accumulation, lipid synthesis, lipid oxidation and lipid transport were identified as enriched by the differentially expressed genes as shown in Figure 3.2, Figure 3.3 and Figure 3.4 for Angus, Charolais and KC respectively. Synthesis of lipid (including steroids, fatty acids and acylglycerol) was predicted to be downregulated in the liver tissue of high-ADG animals from Charolais and KC steers (Figure 3.3 and Figure 3.4). Accumulation of lipid was predicted to be downregulated in Charolais (Figure 3.3), while upregulated in Angus and KC high-ADG steers, as shown in Figure 3.2 and Figure 3.4 respectively. Transport of lipid and fatty acid oxidation were predicted as upregulated in both Charolais and KC high-ADG steers. Some of the key DE genes associated with lipid metabolism identified in the current study include *CYP7A1, IGF1, SAA1, HMGCR* and *NROB2* for Angus, *SCD, FASCN, APOA1*, APOA*4, SAA1, PDK4* and *HMOX1* for Charolais, and *SCD, LPIN1, FGF21, CYP7*A1 and *CES1* for KC. Lists of all DE genes involved in each of the five topmost enriched functions within each breed are provided in Table S4 in the Supplementary Data S6.

Other than being the among the topmost enriched molecular and cellular functions for KC, amino acid and carbohydrate metabolism were also enriched for both Angus and Charolais with important enriched underlying processes. In relation to amino acid metabolism, some of the enriched metabolic processes included transport of amino acids, synthesis of amino acids and catabolism of amino acids. Top amino acid metabolism related processes for each breed and the DE genes involved in these processes, activation/deactivation score and overlap test p-values are presented in Table S5 in Supplementary Data S6. For carbohydrate metabolism biological

function, glucose uptake, and carbohydrate synthesis (gluconeogenesis), carbohydrate oxidation and transport were among the enriched metabolic processes as shown in Table S6 in Supplementary Data S6.

IPA also revealed several interesting enriched activated or deactivated pathways for the identified differentially expressed genes in relation to growth rate in the three studied breed populations, with the topmost enriched pathways for each breed shown in Table 3.5. In Angus, superpathway of cholesterol biosynthesis was the most significantly (P = 1.35E-05) enriched pathway involving four of the DE genes (*SQLE, HMGCR, HMGCS1* and *CYP51A1*) and was predicted to be inactivated in high-ADG steers with a Z-score of -2.00 (Table 3.5). LXR/RXR and PXR/RXR activation pathway was the most significant pathway involving seven (*IL1R2, SCD, RXRG, APOA1, APOA4, FASN and SAA1*) and five (*GSTM1, SCD, CYP7A1, IGFBP1* and *ALAS1*) DE genes for Charolais and KC, respectively (Table 3.5). Additionally, IPA identified several upstream gene expression regulators and their predicted activation or deactivation level in the liver tissue of high-ADG animals across the three studied breeds. *SREBF1* is a transcription factor that was predicted as the most significant (P = 9.41E-11) expression regulator in Angus and was shown to regulate expression of 14 (*AK4, CYP51A1, CYP7A1, GPNMB, GPX3, HMGCR, HMGCS1, IFI30, IL1R2, NR0B2, OAT, SERPINA3, SERPINE1 and SQLE*) of the identified DE genes in this breed (Table S7A in Supplementary Data S6). For Charolais, the *P450 oxidoreductase* (*POR*) enzyme was the most significant (P = 1.86E-12) regulator, regulating expression of 13 DE genes (*ACTG1, APOA4, CSAD, CYP2B6, ELOVL2, GADD45B, HMOX1, NOCT, PDK4, SCD, SDS, SERPINA3 and SQLE*) (Table S7A in Supplementary Data S6). For KC, interferon beta (IFN-β) was the most significant (P = 5.63E-14) upstream regulator, predicted to regulate 12 DE genes (*DUSP1, HLA-B, IFI44, IFI6, ISG15, MX1, MX2, MYC, OAS1, RSAD2, SLC16A6 and USP18)*

and to be inactivated in high-ADG animals with a Z-score of -3.08 (Table S7A in Supplementary Data S6).

For DMI, 107, 177 and 155 DE genes were mapped to the IPA database for Angus, Charolais and KC, respectively, and we identified 27, 22 and 25 significantly enriched biological functions for Angus, Charolais and KC respectively, with 18 of them (60%) common to all breeds (Figure 3.1e). The top enriched functions associated with DMI included lipid metabolism, molecular transport, small molecule biochemistry, cell death and survival, carbohydrate metabolism, vitamin and mineral metabolism, cellular movement, cellular function and maintenance, cell-to-cell signaling and interaction and cellular development (Figure S8, Figure S9 and Figure S10 in the Supplementary Data S8). The genes involved in the top enriched molecular and cellular functions associated with DMI in each breed are provided in Table S4 in the Supplementary Data S6. Lipid metabolism was among the top enriched molecular and cellular functions in Angus and Charolais. For KC, all the top enriched functions we identified were related functionality of cells, with cellular function and maintenance being the most significantly enriched function. Within lipid metabolism for Angus, 30 DE genes were involved in several lipid related metabolic processes including concentration and accumulation of multiple lipids (cholesterol, phospholipids, triacylglycerol and acylglycerol), and catabolism of lipid as shown in Figure 3.5. Accumulation of lipid and concentration of lipids such as cholesterols and triacyl glycerides were predicted to be downregulated in low-DMI steers, whereas metabolism of membrane lipid derivative and quantity of polyunsaturated fatty acids were predicted to be upregulated. Some key DE genes involved in the metabolism of lipids in Angus include *SCD, ARNTL, LIPN1, APOA4* and *ABHD6*. For Charolais, 47 DE genes were identified as involved in different lipid metabolism processes. Some of the processes including uptake of lipid, accumulation of lipid and uptake of

cholesterol were predicted to be downregulated in the liver tissue of low feed intake animals as shown in Figure 3.6. However, other processes such as synthesis of lipid, synthesis of cholesterol, transport of lipid, and fatty acid metabolism were predicted to be upregulated in the same animals as shown in Figure 3.6. Some of the major DE genes related to lipid metabolism identified in Charolais included *ABCA1, ABCG5, ABCG8, CYP7A1, NROB2, NPC1, CES1, SAA1, IL1B* and *SULT1E1*. For DMI in KC, 51 DE genes were identified as involved in cellular function and maintenance, and these genes are mainly involved in a number of immune related functions such as proliferation of T lymphocytes, T cell development, phagocytosis of cells and T cell homeostasis which were predicted to be upregulated in liver tissue of low-DMI animals as shown in Figure 3.7.

Pyrimidine ribonucleotides interconversion was identified as the most significantly (P = 1.20E-03) enriched pathway in Angus with three (*NUDT5*, *CMPK2* and *AK8*) DE genes involved in this pathway (Table 3.5). For Charolais, LPS/IL-1 mediated inhibition of RXR function was the most significant (P = 2.63E-10) pathway, with 15 of the DE genes (*IL1R2, ABCG8, GSTM1, ABCG5, SULT1E1, JUN, SULT1C4, NR0B2, CYP7A1, SLC27A6, IL1B, ALDH3B1, HMGCS1, ABCA1, CYP2C19*) involved in this pathway and it was predicted to be relatively activated (Z-score = 0.33) in low feed intake steers (Table 3.5). For KC, we identified interferon signaling pathway as the most significant (P = 1.00E-10) pathway for this breed involving eight of the identified DE genes (all upregulated) as shown in Figure S11 in the Supplementary Data S8, and was predicted to be activated (Z-score = 2.83) in low-DMI animals (Table 3.5). All the top enriched canonical pathways associated with DMI for each breed are presented in Table 3.5. Besides canonical pathways, IPA also predicted the top gene expression regulators and their activation/deactivation state as associated with feed intake for each of the breeds. Interferon alpha

cytokine group was predicted as the most significant gene expression regulator in Angus and KC. It was predicted to be inactivated (Z-score = -0.63) in Angus and activated in KC (Z-score = 3.86). For Charolais, *FGF19* growth factor was the most significant (P = 2.30E-16) regulator involved in the regulation of 15 DE genes. Top five enriched upstream gene expression regulators and their predicted activation or deactivation state in low-DMI steers from the three studied populations are presented in Table S7B in Supplementary Data S6.

For MWT, 80, 81 and 83 DE genes from Angus, Charolais and KC, respectively were mapped to the IPA database. These genes significantly enriched 24, 24 and 19 molecular and cellular functions for Angus, Charolais and KC respectively, with 17 of the enriched functions in common (63%) across breeds (Figure 3.1f). The major functions that were identified as associated with MWT included lipid metabolism, amino acid metabolism, small molecule biochemistry, vitamin and mineral metabolism, molecular transport, cell morphology, cellular movement, cell-to-cell signaling and interaction, cell death and survival and drug metabolism. The genes involved in these major molecular and cellular functions associated with MWT in each breed are provided in Table S4 in the Supplementary Data S6. As for ADG and DMI, lipid metabolism and small molecule metabolism were among the top functions for both Angus and Charolais. Topmost (by P-value) enriched processes within amino acid metabolism and lipid metabolism for Angus, lipid metabolism and cellular movement for Charolais, and cell death and survival, and cellular movement for KC are presented in Table S8 in Supplementary Data S6. IPA additionally identified several significantly enriched canonical pathways associated with metabolic weight in each of the breeds, the top enriched canonical pathways are presented in Table 3.5. LPS/IL-1 mediated inhibition of RXR function was the most significant (P = 1.38E-05) pathway for Angus and was predicted to be inactivated (Z-score = -2.00) in low metabolic weight steers. For Charolais,

neuroinflammation signaling pathway was identified as the most significant (P = 1.45E-04) pathway and predicted to be activated (Z-score = 1.89) in low-MWT steers (Table 3.5). For KC, antigen presentation pathway was the most significant (P = 3.39E-04) involving three of the identified DE genes (*HLA-B, HLA-DQB1* and *HLA-DQA2*) (Table 3.5). For the upstream gene expression regulation factors, the ligand dependent nuclear receptor *RORA* was identified as the most significant (P = 5.29E-07) expression regulator in Angus and was shown to affect expression of eight of the DE genes including *CCL24, CYP2B6, HMGCR, IGF1, ITPR1, SLC13A2, SULT1E1, SULT2A1.* Albeit, cytokines *IFNG* and *IL6* were identified as the most significant gene expression regulators in Charolais and KC steers respectively. *IFNG* was predicted to be activated (Z-score = 1.22), whereas *IL6* was predicted to be inactivated (Z-score = -1.27) in low-MWT steers of the respective breeds. The top enriched gene expression regulators associated with MWT for each breed are presented in Table S7 C in Supplementary Data S6.

3.5 Discussion

The liver is a central metabolic organ that provides and distributes energy and other essential nutrients to the muscles, brain, adipose tissue and other peripheral organs in the animal's body (Jeremy *et al.* 2002; Rui 2011), thus implying its critical importance in energy and other nutrient assimilation and distribution. The liver is also an important immunological organ involved in both innate and adaptive immunologic systems of the animal (Parker & Picut 2005; Racanelli & Rehermann 2006), which are vital for not only animal's health but also influence many other systems including animal growth and development.

In the current study we employed RNAseq analyses of liver tissue from 60 steers of three Canadian beef cattle populations (Angus, Charolais and KC) to study gene expression difference between the high and low phenotype steer groups for three feed efficiency related traits ADG, DMI

and MWT. To maximize the phenotypic difference of the animals for the particular trait under investigation, we sorted the 20 steers of each of the three breeds and selected the 6 highest and 6 lowest steers for differential gene expression gene analyses. Each trait under investigation showed significant differences between the high and low extreme phenotype groups (Table S1, S2 and S3 in Supplementary Data S6). It is worth noting that with the aim of minimising environmental differences between the studied animals, they were raised on the same experimental farm and were managed similarly. In addition, the two extreme groups of each population did not differ significantly in their age when the liver samples were collected. (Table S1, S2 and S3 in Supplementary Data S6). Although the two extreme groups of the target trait also exhibited significant differences in a few of other production traits due to their biological correlations, the strongest divergence for ADG, DMI or MWT provided suitable contrast of animal groups for differential gene expression analysis for each of the trait under investigation.

Our results showed a great diversity in terms of differentially expressed genes between breeds for the same trait (Figure 3.1, a-c). For example, of the 252, 375 and 206 DE genes associated with ADG, DMI and MWT, only 1% to 2% of them were shared between all three breeds, while, 81.7% to 83% were breed specific, and 3% to 8% uniquely common between two breeds for a trait. This diversity of differential gene expression implies that probably these traits are largely controlled by different genes in the different breeds. A similar trend of predominantly breed specific differential gene expression profiles across breeds in the same animal populations (including animals used in the current study) has been previously observed and reported for residual feed intake by Mukiibi et al. (2018) (Mukiibi *et al.* 2018). However, DE gene comparison within a breed showed a relatively moderate number of gene overlap (12 – 29%) between MWT and ADG or DMI. This could be an indication of shared genetic mechanisms underlying these

traits, which supports the moderate to high genetic correlations between MWT and ADG or DMI reported in beef cattle (Crowley *et al.* 2010; Mao *et al.* 2013).

3.5.1 Association of lipid metabolism with growth rate, feed intake and metabolic weight

Although the DE genes we identified were largely breed specific for each of the trait, the significant enriched molecular and cellular functions are mostly common with a 58% to 63% overlap between breeds for the three traits (i.e. ADG, DMI and MWT). Our results showed lipid metabolism as an important metabolic function that were strongly associated with growth rate, feed intake, and maintenance weight in at least two of the studied populations. Lipid metabolism has also been reported to be associated with feed efficiency and component traits such as RFI (Chen *et al.* 2011; Tizioto *et al.* 2015; Weber *et al.* 2016; Mukiibi *et al.* 2018), residual intake and gain (Alexandre *et al.* 2015) and growth rate (Foote *et al.* 2017) in beef cattle.

For the growth rate, lipid metabolism was significantly associated with enriched molecular and cellular function across all the cattle populations studied, indicating that hepatic lipid metabolic processes play important roles in regulating body weight gain in beef steers irrespective of the breed. Accumulation of lipid, concentration of cholesterol, synthesis of cholesterol, metabolism of membrane lipid derivative and concentration of lipid were among the enriched processes across the three breeds. Notably, lipid synthesis as a metabolic process was enriched for Charolais and KC, where it was predicted to be downregulated in the fast-growing animals (Figure 3.3 and Figure 3.4). Additionally, in Angus, 14 lipogenic genes (*AKR1C3, IL1R2, SOCS3, NR0B2, F2R, IGF1, ELOVL5, CYP7A1, ABHD6, HMGCR, PGAP1, SQLE, CYP51A1* and *HMGCS1*) were identified as associated with ADG, of which eight genes (*IGF1, ELOVL5, CYP7A1, ABHD6, HMGCR, PGAP1, SQLE, CYP51A1* and *HMGCS1*) were downregulated in the liver tissue of high ADG animals (Figure 3.2). The predicted down regulation of lipid synthesis genes in the fast-

growing animals might be an indication that these animals divert their energy from lipid synthesis away from lipid metabolism and towards muscle deposition, hence resulting in faster body weight gain. However, this speculation should be considered with caution as the liver's contribution to total endogenous lipogeneses in ruminants is minimal as compared to the adipose tissue (Roh *et al.* 2006). Therefore, further transcriptome studies considering the muscle and adipose tissues should be pursued. Despite lipid synthesis not being enriched as a process within lipid metabolism in KC, synthesis of different lipid species including cholesterol ester (*CYP7A1, SCD*), diacylglycerol (*LPIN1, SCD*), long chain fatty acid (*GSTM4, SCD*), phospholipid (*CES1, CYP7A1, LPIN1, MFSD2A, PIP5K1A*) and triacylglycerol (*CYP7A1, LPIN1, SCD*) were identified in this population (Figure 3.3).

For DMI, lipid metabolism was also among the top five significantly enriched cellular and molecular functions for both Angus and Charolais (Figure 3.4 and Figure 3.5, respectively). Seven DE genes involved in lipid metabolism (*CYP7A1, SAA1, CCDC80, LPIN1, GNMT, HP and PIP5K1A)* in both Angus and Charolais were identified. Although accumulation of lipid was predicted as downregulated in the liver tissue of low-DMI animals of both breeds, our results showed difference between the steer groups in terms of predicted concentration of different lipids. For example, cholesterol and fatty acids concentration were predicted to be downregulated in low-DMI Angus animals (Figure 3.4) but upregulated in Charolais animals with similar phenotypes (Figure 3.5). Indeed, bovine breeds differ in their hepatic lipid concentrations as reported by O'Kelly (1974) (O'Kelly 1974). Therefore the predicted difference in hepatic lipid concentrations between Angus and Charolais could potentially be due to the genetic distinctiveness between the two breeds as Angus is a breed of moderate frame and earlier maturing and fattening, whereas Charolais is characterized by a larger frame and later maturity to fattening (Briggs & Briggs 1980).

Similarly, our results revealed a strong association between lipid metabolism with metabolic weight within Angus and Charolais steers. Steroid metabolism, synthesis of lipid, metabolism of terpenoid and secretion of testosterone were among the commonly shared enriched processes between the two breeds. Lipid synthesis in Angus (involving *ABHD6, CYR61, ETNPPL*, *HMGCR, HMGCS1, IGF1, IGFBP2, IL1R2, PIP5K1A* and *SULT1E1*) and Charolais (involving *FADS1, HMOX1, IL1B, LPIN1, PDK4, PLTP, PTGS1, SOAT2, SULT1E1, TBXAS1* and *THRSP*) was predicted to be upregulated (activated) in low metabolic weight animals. However, as highlighted above, the liver is not a major lipogenic organ in the ruminant animal's body. Differential expression of key lipogenic genes including steroidogenic genes and the predicted upregulation of lipid synthesis in low-MWT steers as compared to high-MWT could be an indication that these animals not only differ in the amount of metabolically active tissue but also differ in terms of metabolic activity level in those same tissues.

It is essential to highlight that herbivorous animals such as cattle that almost entirely depend on endogenous cholesterol biosynthesis, with the liver contributing substantially to this biosynthesis (Bell 1981). Cholesterol is utilized for synthesis of steroid hormones, bile acids, vitamin D, and cholesteryl esters which are a major lipid transport mechanism in ruminants (Bell 1981). In the current study, several DE genes involved in numerous cholesterol or steroid metabolism processes (e.g. synthesis of cholesterol, sterol metabolism and accumulation of sterol) , canonical pathways (e.g. superpathway of cholesterol biosynthesis, LXR/RXR activation and mevalonate pathway I) and upstream gene expression regulator (*SREBF1*) have been identified. The superpathway of cholesterol biosynthesis was identified among the top significantly enriched pathways for ADG in Angus where it was predicted to be downregulated in high-ADG steers, and for DMI in Charolais where it was predicted to be downregulated in low-DMI steers. Four of the

DE genes identified as associated with ADG in Angus (*SQLE, HMGCR, HMGCS1* and *CYP51A1)* are involved in the cholesterol biosynthesis pathway and were all downregulated in high-ADG animals. For DMI in Charolais, five DE genes including *SQLE, PMVK, IDI1, HMGCR* and *HMGCS1* are involved in the cholesterol biosynthesis pathway, and they were all downregulated in low-DMI steers. Interestingly, *SQLE, PMVK, IDI1, HMGCS1, HMGCR, PMVK, SQLE,* and *IDI1* are key enzymes catalyzing important steps in cholesterol biosynthesis (Brown & Sharpe 2015). For example; *HMGCS1* codes for the 3-hydroxy-3-methylglutaryl-CoA synthase 1 that characterizes the condensation of acetoacetyl-CoA and acetyl-CoA to 3-hydroxy-3-methylglutaryl-CoA, an initial reaction in cholesterol biosynthesis (Brown & Sharpe 2015). *HMGCR* encodes for 3-hydroxy-3-methylglutaryl-CoA reductase, an enzyme that characterizes the reduction of 3-hydroxy-3-methylglutaryl-CoA to mevalonic acid, a rate-limiting step in cholesterol synthesis (Brown & Sharpe 2015). *SQLE* codes for squalene monooxygenase, which is an enzyme that oxidises the first oxygenation step in cholesterol/sterol biosynthesis and is considered a rate-limiting enzyme in this process (Brown & Sharpe 2015). Liver X receptors (LXR) and retinoid X receptors (RXR), which are heterodimer nuclear receptors that regulate cholesterol metabolism through regulation of cholestrogenic enzymes, and carriers (Sharpe & Brown 2013; Hong & Tontonoz 2014) was identified to be associated with ADG, DMI and MWT. Additionally, *SREBF1* codes for sterol regulatory element-binding protein 1, which is a key (together with *SREBF2*) expression regulator of genes involved in cholesterol biosynthesis (Hua *et al.* 1995). The association of cholesterol biosynthesis with growth and feed intake is an interesting revelation from our results as they imply that selection of fast-growing animals or low feed intake could result in the production of beef with low cholesterol content, a dietary health concern of many beef consumers. Consistent with our findings, association of cholesterol

metabolism with feed efficiency was reported by Karisa et al. (2014) (Karisa *et al.* 2014), and lower blood cholesterol content has been observed in more efficient beef animals as compared to inefficient animals (Alexandre *et al.* 2015; Bourgon *et al.* 2017). Also, downregulation of *HMGCR* and *SQLE* in the liver tissue of feed efficient animals has been previously reported in crossbred steers (Mukiibi *et al.* 2018).

3.5.2 Association of amino acid and carbohydrate metabolism with growth, feed intake and maintenance weight

The liver is also an important regulator of nitrogenous compounds including amino acids, through transamination, anabolic, and catabolic processes (Reynolds 1992). Amino acids released from the liver are necessary for protein synthesis in different tissues including muscle, leading to tissue deposition and overall growth (Huntington & Eisemann 1988). Additionally, in ruminants the liver is the main tissue of glucose that is a major source of energy for the different tissues (Nafikov & Beitz 2007). The liver synthesizes glucose through gluconeogenesis, a process that utilizes amino acids, volatile fatty acids and glycerol as precursors (Nafikov & Beitz 2007), hence making it the principle organ in carbohydrate and energy metabolism in ruminant animals. For ADG, amino acid metabolism was among the top enriched cellular and molecular functions in Charolais and KC animals, with carbohydrate metabolism was one of the top enriched functions in KC. With respect to amino acid metabolism in Charolais, processes such as synthesis of amino acids, metabolism of serine family amino acids and other amino acid metabolic related processes were strongly enriched, whereas catabolism of amino acids, synthesis of L-proline, metabolism of essential amino acids and others were enriched for KC with DE genes *ARG1* and *AASS* being involved in most of these processes. *ARG1* and *AASS* code for critical enzymes in amino acid metabolism, *ARG1* codes for arginase enzyme which catalyzes conversion of arginine to urea and

orthenine in the urea cycle (Morris Jr 2002), whereas *AASS* codes for alpha-aminoadipate-semialdehyde synthase, a bifunctional enzyme that catalyzes a twostep conversion of lysine to alpha-aminoadipic semialdehyde in the lysine degradation pathway(Sacksteder *et al.* 2000). For Angus, amino acid metabolism was not among the most significant functions associated with ADG, however, it was significantly enriched by mainly amino acid transport DE genes including *SLC16A10*, *SLC1A2*, *SLC25A15* and *SLC3A1* as presented in Table S5 of the Supplementary Data S6. Additionally, amino acid metabolism also showed strong association with MWT in Angus steers with numerous enriched amino acid metabolic processes such as uptake of cystine (Table S5 in Supplementary Data S6). Carbohydrate metabolism was among the most significantly enriched functions in Angus and enriched in Charolais and KC as well, with respect to ADG. Important processes including oxidation of carbohydrate, synthesis of carbohydrate, glycogenolysis, intake of glucose and gluconeogenesis were identified as associated to growth rate in the studied animals (Table S6 in Supplementary Data S6). In agreement with our results, Foote et al (2017) have reported carbohydrate and amino acid metabolism association with beef cattle growth and feed intake in the jejunum tissue (Foote *et al.* 2017). Studying the rumen epithelial tissue transcriptome of crossbred steers with divergent feed intake and growth phenotypes, Kern et al (2016) reported carbohydrate metabolism associated with feed intake and gain (Kern *et al.* 2016). Besides feed intake and body weight gain, hepatic carbohydrate and amino acid metabolism have also been identified to be associated with feed efficiency in beef cattle (Chen *et al.* 2011; Mukiibi *et al.* 2018).

3.5.3 Association of immunological functions with growth, feed intake and maintenance weight

In the current study, we identified immune function related genes that were differentially expressed in the liver tissue of animals with divergent growth rate or feed intake or metabolic weight phenotypes. For example, acute phase response signaling was among the top enriched canonical pathway for Angus steers with divergent growth rate phenotypes involving *SOCS3, HP, SAA1, SOCS2, SERPINA3, LBP* and *SERPINE1*, and was predicted as upregulated in the fast-growing animals. Within the composite breed KC, steers with divergent dry mater intake had a large number of differentially expressed immune related genes, and they were involved in multiple immune function processes such as engulfment by macrophages, T cell homeostasis, T cell development and differentiation of T lymphocytes, which were predicted to be upregulated in the liver tissue of low feed intake KC. Additionally, interferon signaling pathway (involving *OAS1, IFI6, PSMB8, STAT1, TAP1, IRF1, IFITM1* and *ISG15*), Th1 pathway (involving *NFIL3, CD3E, HLA-B, CD274, HLA-DQB1, HLA-DQA2, STAT1, CD3D* and *IRF1*) and PKCθ signaling in T lymphocytes (involving *CACNA1I, RAC2, CACNG1, CD3E, HLA-B, HLA-DQB1, CD3D* and *LCP2*) were among the top enriched pathways associated with feed intake in KC and also predicted to be activated in KC steers with lower feed intake (Table 3.5). Since the liver is a major organ to process absorbed materials from the gastrointestinal tract including microbes and toxins, it plays an important role in defending the body against invading pathogens through phagocytosis by the Kupfer cells or killing the infected cells through lysis and inducing apoptosis by natural killer cells and natural killer T cells (Nakamoto & Kanai 2014). Previously, associations of feed efficiency or its component traits with immune related functions have been reported in beef cattle through transcriptome studies. For example, Kern et al (2016) reported association of immune response

related genes expression in the rumen epithelial tissue with feed intake and body weight gain phenotypic differences (Kern *et al.* 2016). With respect to hepatic transcriptome studies, associations of feed efficiency and hepatic immune response in beef cattle have been reported by Alexandre et al (2015) and Paradis et al (2015) (Alexandre *et al.* 2015; Paradis *et al.* 2015). Paradis et al (2015) identified five immune genes (*HBB, MX1, ISG5*, *HERC6* and *IF44*) associated with feed efficiency in crossbred heifers. Similarly, in our study *MX1*, *ISG5*, *HERC6* and *IF44* were also identified as associated either with ADG, DMI or MWT in KC steers, whereas for Charolais steers, *MX1*, *ISG5* and *IF44* were either associated with DMI or MWT. These and several other genes we identified in this study are regulated by α and β interferon signaling as shown by canonical pathways and upstream regulator results and are hence involved in innate immune function (Stetson & Medzhitov 2006; Boxx & Cheng 2016). These reported DE genes related to immune functions indicate possible immunological adaptations to the feedlot challenges by some of the animals, which probably have implications on animal's feed intake and growth.

3.6 Conclusions

We identified a total of 252, 375 and 206 protein coding genes associated with growth rate, feed intake, and maintenance weight of beef cattle, respectively, through hepatic transcriptome sequence data analyses. The majority of the identified DE genes for the traits were breed specific. However, most of the enriched biological functions are common across the three breeds. Functional enrichment showed that the identified DE genes were involved in multiple cellular and molecular functions that mainly include metabolism of lipid, carbohydrates, amino acids, vitamins and minerals, small molecule biochemistry, cellular movement, cell morphology and cell to cell signaling and interaction. Our functional results further revealed strong associations of both cholesterol biosynthesis and immune related functions with growth, feed intake and metabolic

weight through identification of pathways and upstream gene expression regulators involved in these processes or functions. The DE genes and major biological functions associated with growth, feed intake, and metabolic weight advance our understanding of genetic mechanisms that regulate feed intake, growth, and feed efficiency in beef cattle respective to various breeds/breed populations, which will also help design strategies of genetic and genomic selection and breeding to improve the traits.

3.7 References

Ahola J.K. & Hill R.A. (2012) Input Factors Affecting Profitability: a Changing Paradigm and a Challenging Time. In: Feed efficiency in the beef industry (ed. by Hill RA), p. 10. Wiley-Blackwell, Ames, Iowa.

Al-Husseini W., Gondro C., Quinn K., Herd R., Gibson J. & Chen Y. (2014) Expression of candidate genes for residual feed intake in Angus cattle. Animal genetics 45, 12-9.

Alexandre P.A., Kogelman L.J., Santana M.H., Passarelli D., Pulz L.H., Fantinato-Neto P., Silva P.L., Leme P.R., Strefezzi R.F. & Coutinho L.L. (2015) Liver transcriptomic networks reveal main biological processes associated with feed efficiency in beef cattle. BMC genomics 16, 1073.

Anders S., McCarthy D.J., Chen Y., Okoniewski M., Smyth G.K., Huber W. & Robinson M.D. (2013) Count-based differential expression analysis of RNA sequencing data using R and Bioconductor. Nature protocols 8, 1765-86.

Anders S., Pyl P.T. & Huber W. (2015) HTSeq–a Python framework to work with high-throughput sequencing data. Bioinformatics, btu638.

Andrews S. (2010) FastQC: a quality control tool for high throughput sequence data.

Baruch Y. (2000) The liver: a large endocrine gland. Journal of hepatology 32, 505-7.

Bell A. (1981) Lipid metabolism in liver and selected tissues and in the whole body of ruminant animals. In: Lipid metabolism in ruminant animals (pp. 363-410. Elsevier.

Bourgon S., de Amorim M.D., Miller S. & Montanholi Y. (2017) Associations of blood parameters with age, feed efficiency and sampling routine in young beef bulls. Livestock Science 195, 27-37.

Boxx G.M. & Cheng G. (2016) The roles of type I interferon in bacterial infection. Cell host & microbe 19, 760-9.

Briggs M.H. & Briggs M.D. (1980) Modern breeds of livestock. Macmillan, New York.

Brown A.J. & Sharpe L.J. (2015) Cholesterol Synthesis. In: Biochemistry of lipids, lipoproteins and membranes (eds. by Ridgway N & McLeod R), pp. 327-58. Elsevier, Amsterdam, Netherlands.

CCAC (2009) Guidelines on the care and use of farm animals in research, teaching and testing. Canadian Council on Animal Care Ottawa, ON, Canada.

Chen Y., Gondro C., Quinn K., Herd R., Parnell P. & Vanselow B. (2011) Global gene expression profiling reveals genes expressed differentially in cattle with high and low residual feed intake. Animal genetics 42, 475-90.

Crowley J., McGee M., Kenny D., Crews Jr D., Evans R. & Berry D. (2010) Phenotypic and genetic parameters for different measures of feed efficiency in different breeds of Irish performance-tested beef bulls. Journal of animal science 88, 885-94.

Foote A., Keel B., Zarek C. & Lindholm-Perry A. (2017) Beef steers with average dry matter intake and divergent average daily gain have altered gene expression in the jejunum. Journal of animal science 95, 4430-9.

Goonewardene L., Wang Z., Price M., Yang R.-C., Berg R. & Makarechian M. (2003) Effect of udder type and calving assistance on weaning traits of beef and dairy× beef calves. Livestock production science 81, 47-56.

Hill R.A. & Ahola J.K. (2012) Feed Efficiency Interactions with Other Traits: Growth and Product Quality. In: Feed efficiency in the beef industry (ed. by Hill RA), p. 148. Wiley-Blackwell, Ames, Iowa.

Hong C. & Tontonoz P. (2014) Liver X receptors in lipid metabolism: opportunities for drug discovery. Nature reviews Drug discovery 13, 433.

Hua X., Wu J., Goldstein J.L., Brown M.S. & Hobbs H.H. (1995) Structure of the human gene encoding sterol regulatory element binding protein-1 (SREBF1) and localization of SREBF1 and SREBF2 to chromosomes 17p11. 2 and 22q13. Genomics 25, 667-73.

Huntington G.B. & Eisemann J.H. (1988) Regulation of nutrient supply by gut and liver tissues. Journal of animal science 66, 35-48.

Jeremy M.B., John L.T. & Lubert S. (2002) Each Organ Has a Unique Metabolic Profile. In: Biochemistry (ed. by Moran S), p. 1259. W. H. Freeman and Company, New York.

Karisa B., Moore S. & Plastow G. (2014) Analysis of biological networks and biological pathways associated with residual feed intake in beef cattle. Animal Science Journal 85, 374-87.

Kern R.J., Lindholm-Perry A.K., Freetly H.C., Snelling W.M., Kern J.W., Keele J.W., Miles J.R., Foote A.P., Oliver W.T. & Kuehn L.A. (2016) Transcriptome differences in the rumen of beef steers with variation in feed intake and gain. Gene 586, 12-26.

Khansefid M., Millen C., Chen Y., Pryce J., Chamberlain A., Vander Jagt C., Gondro C. & Goddard M. (2017) Gene expression analysis of blood, liver, and muscle in cattle

divergently selected for high and low residual feed intake. Journal of animal science 95, 4764-75.

Kim D., Pertea G., Trapnell C., Pimentel H., Kelley R. & Salzberg S.L. (2013) TopHat2: accurate alignment of transcriptomes in the presence of insertions, deletions and gene fusions. Genome biology 14, R36.

Koch R.M., Swiger L.A., Chambers D. & Gregory K.E. (1963) Efficiency of feed use in beef cattle. Journal of animal science 22, 486-94.

Kong R.S., Liang G., Chen Y. & Stothard P. (2016) Transcriptome profiling of the rumen epithelium of beef cattle differing in residual feed intake. BMC genomics 17, 592.

Krämer A., Green J., Pollard Jr J. & Tugendreich S. (2013) Causal analysis approaches in ingenuity pathway analysis. Bioinformatics 30, 523-30.

Lindholm-Perry A., Cunningham H., Kuehn L., Vallet J., Keele J., Foote A., Cammack K. & Freetly H. (2017) Relationships between the genes expressed in the mesenteric adipose tissue of beef cattle and feed intake and gain. Animal genetics 48, 386-94.

Mao F., Chen L., Vinsky M., Okine E., Wang Z., Basarab J., Crews D. & Li C. (2013) Phenotypic and genetic relationships of feed efficiency with growth performance, ultrasound, and carcass merit traits in Angus and Charolais steers. Journal of animal science 91, 2067-76.

Morris Jr S.M. (2002) Regulation of enzymes of the urea cycle and arginine metabolism. Annual review of nutrition 22, 87-105.

Mukiibi R., Vinsky M., Keogh K.A., Fitzsimmons C., Stothard P., Waters S.M. & Li C. (2018) Transcriptome analyses reveal reduced hepatic lipid synthesis and accumulation in more feed efficient beef cattle. Scientific reports 8, 7303.

Nafikov R.A. & Beitz D.C. (2007) Carbohydrate and lipid metabolism in farm animals. The Journal of nutrition 137, 702-5.

Nakamoto N. & Kanai T. (2014) Role of toll-like receptors in immune activation and tolerance in the liver. Frontiers in immunology 5, 221.

Nkrumah J., Basarab J., Wang Z., Li C., Price M., Okine E., Crews D. & Moore S. (2007) Genetic and phenotypic relationships of feed intake and measures of efficiency with growth and carcass merit of beef cattle. Journal of animal science 85, 2711-20.

O'Kelly J. (1974) The concentrations of lipids in the plasma, liver and bile of genetically different types of cattle. Comparative Biochemistry and Physiology Part B: Comparative Biochemistry 49, 491-500.

Paradis F., Yue S., Grant J., Stothard P., Basarab J. & Fitzsimmons C. (2015) Transcriptomic analysis by RNA sequencing reveals that hepatic interferon-induced genes may be associated with feed efficiency in beef heifers. Journal of animal science 93, 3331-41.

Parker G.A. & Picut C.A. (2005) Liver immunobiology. Toxicologic Pathology 33, 52-62.

Racanelli V. & Rehermann B. (2006) The liver as an immunological organ. Hepatology 43, S54-S62.

Reynolds C.K. (1992) Metabolism of nitrogenous compounds by ruminant liver. The Journal of nutrition 122, 850-4.

Reynolds J., Foote A., Freetly H., Oliver W. & Lindholm-Perry A. (2017) Relationships between inflammation-and immunity-related transcript abundance in the rumen and jejunum of beef steers with divergent average daily gain. Animal genetics 48, 447-9.

Robinson M.D., McCarthy D.J. & Smyth G.K. (2010) edgeR: a Bioconductor package for differential expression analysis of digital gene expression data. Bioinformatics 26, 139-40.

Robinson M.D. & Oshlack A. (2010) A scaling normalization method for differential expression analysis of RNA-seq data. Genome biology 11, R25.

Roh S.G., Hishikawa D., Hong Y.H. & Sasaki S. (2006) Control of adipogenesis in ruminants. Animal Science Journal 77, 472-7.

Rui L. (2011) Energy metabolism in the liver. Comprehensive physiology 4, 177-97.

Sacksteder K.A., Biery B.J., Morrell J.C., Goodman B.K., Geisbrecht B.V., Cox R.P., Gould S.J. & Geraghty M.T. (2000) Identification of the α-aminoadipic semialdehyde synthase gene, which is defective in familial hyperlysinemia. The American Journal of Human Genetics 66, 1736-43.

Schenkel F., Miller S. & Wilton J. (2004) Genetic parameters and breed differences for feed efficiency, growth, and body composition traits of young beef bulls. Canadian Journal of Animal Science 84, 177-86.

Sharpe L.J. & Brown A.J. (2013) Controlling cholesterol synthesis beyond 3-hydroxy-3-methylglutaryl-CoA reductase (HMGCR). Journal of Biological Chemistry, jbc. R113. 479808.

Stetson D.B. & Medzhitov R. (2006) Type I interferons in host defense. Immunity 25, 373-81.

Tizioto P.C., Coutinho L.L., Decker J.E., Schnabel R.D., Rosa K.O., Oliveira P.S., Souza M.M., Mourão G.B., Tullio R.R. & Chaves A.S. (2015) Global liver gene expression differences in Nelore steers with divergent residual feed intake phenotypes. BMC genomics 16, 242.

Tizioto P.C., Coutinho L.L., Oliveira P.S., Cesar A.S., Diniz W.J., Lima A.O., Rocha M.I., Decker J.E., Schnabel R.D. & Mourão G.B. (2016) Gene expression differences in Longissimus muscle of Nelore steers genetically divergent for residual feed intake. Scientific reports 6.

Van den Berghe G. (1991) The role of the liver in metabolic homeostasis: implications for inborn errors of metabolism. In: Journal of inherited metabolic disease (pp. 407-20. Springer.

Weber K.L., Welly B.T., Van Eenennaam A.L., Young A.E., Porto-Neto L.R., Reverter A. & Rincon G. (2016) Identification of gene networks for residual feed intake in angus cattle using genomic prediction and RNA-seq. PloS one 11, e0152274.

Zarek C., Lindholm-Perry A., Kuehn L. & Freetly H. (2017) Differential expression of genes related to gain and intake in the liver of beef cattle. BMC research notes 10, 1.

3.8 Figures and Tables

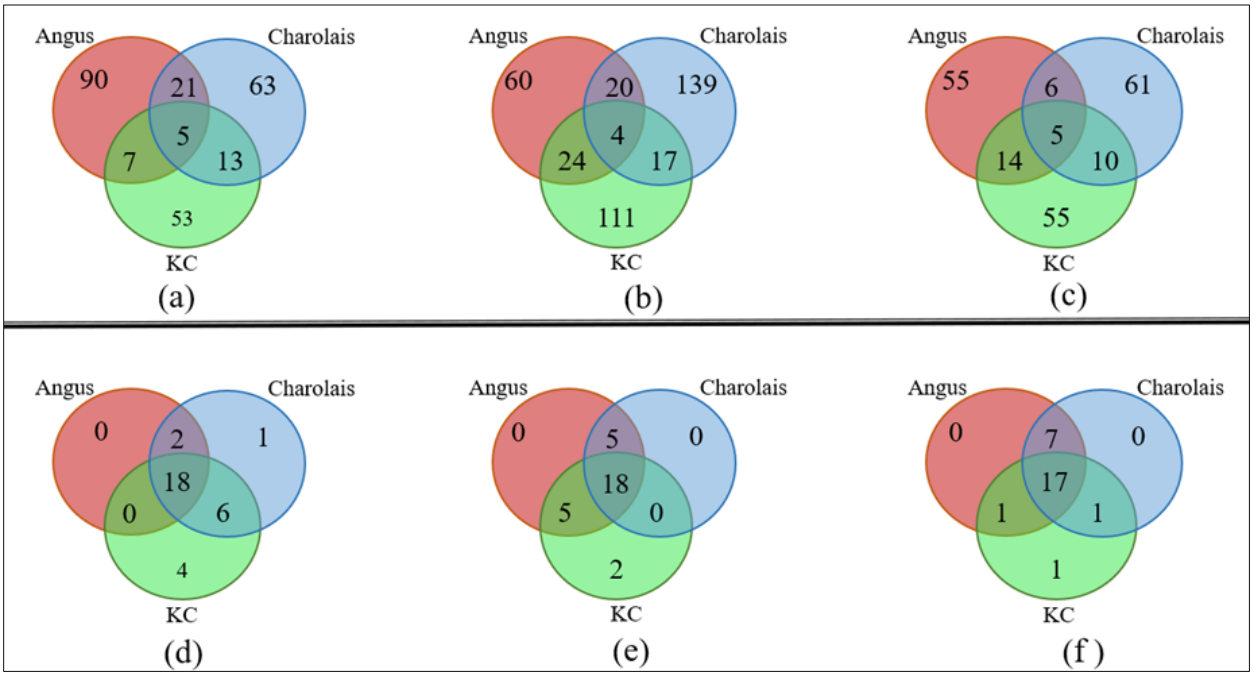


Figure 3.1. Venn diagrams showing differentially expressed genes overlap among Angus, Charolais, and Kinsella Composite (KC) for (a) Average daily gain (ADG), (b) Average daily dry matter intake (DMI) and (c) metabolic weight (MWT). Venn diagrams showing significant enriched biological functions overlap among breeds for (d) ADG, (e) DMI and (f) MWT.

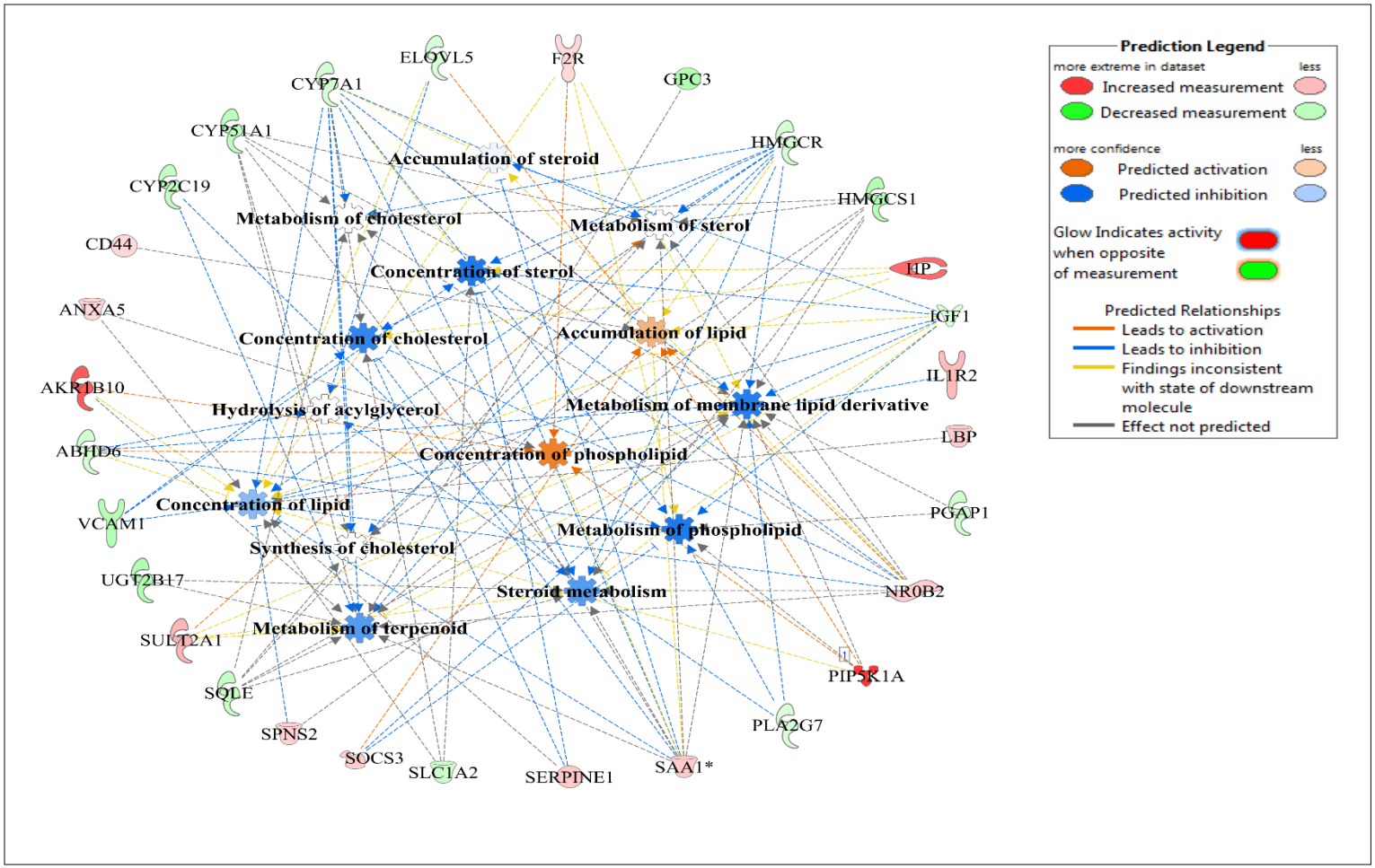


Figure 3.2. Lipid metabolism gene and molecular processes interaction network within lipid metabolism function as associated to average daily gain (ADG) in Angus steers.

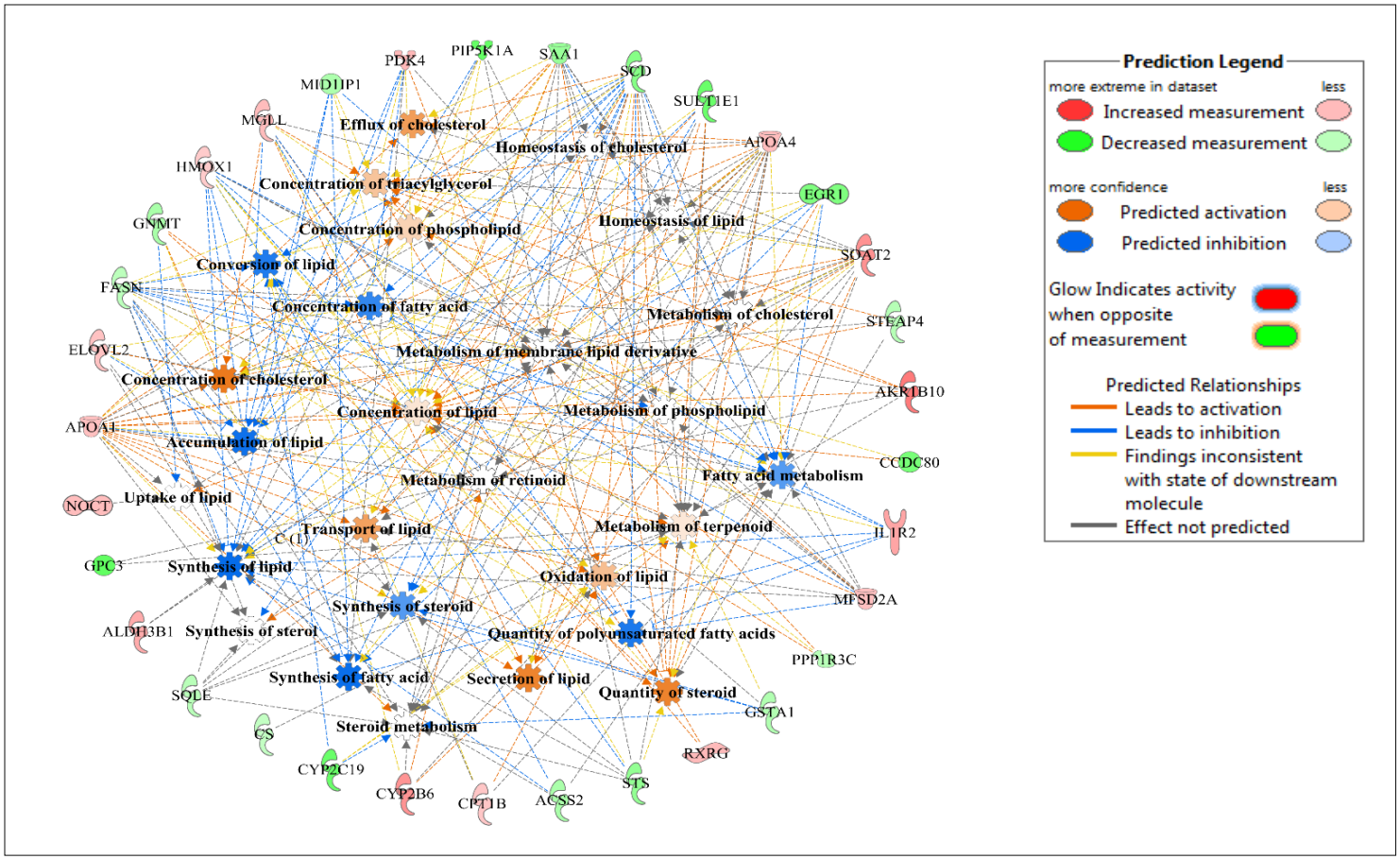


Figure 3.3. Lipid metabolism gene and molecular processes interaction network within lipid metabolism function as associated to average daily gain (ADG) in Charolais steers.

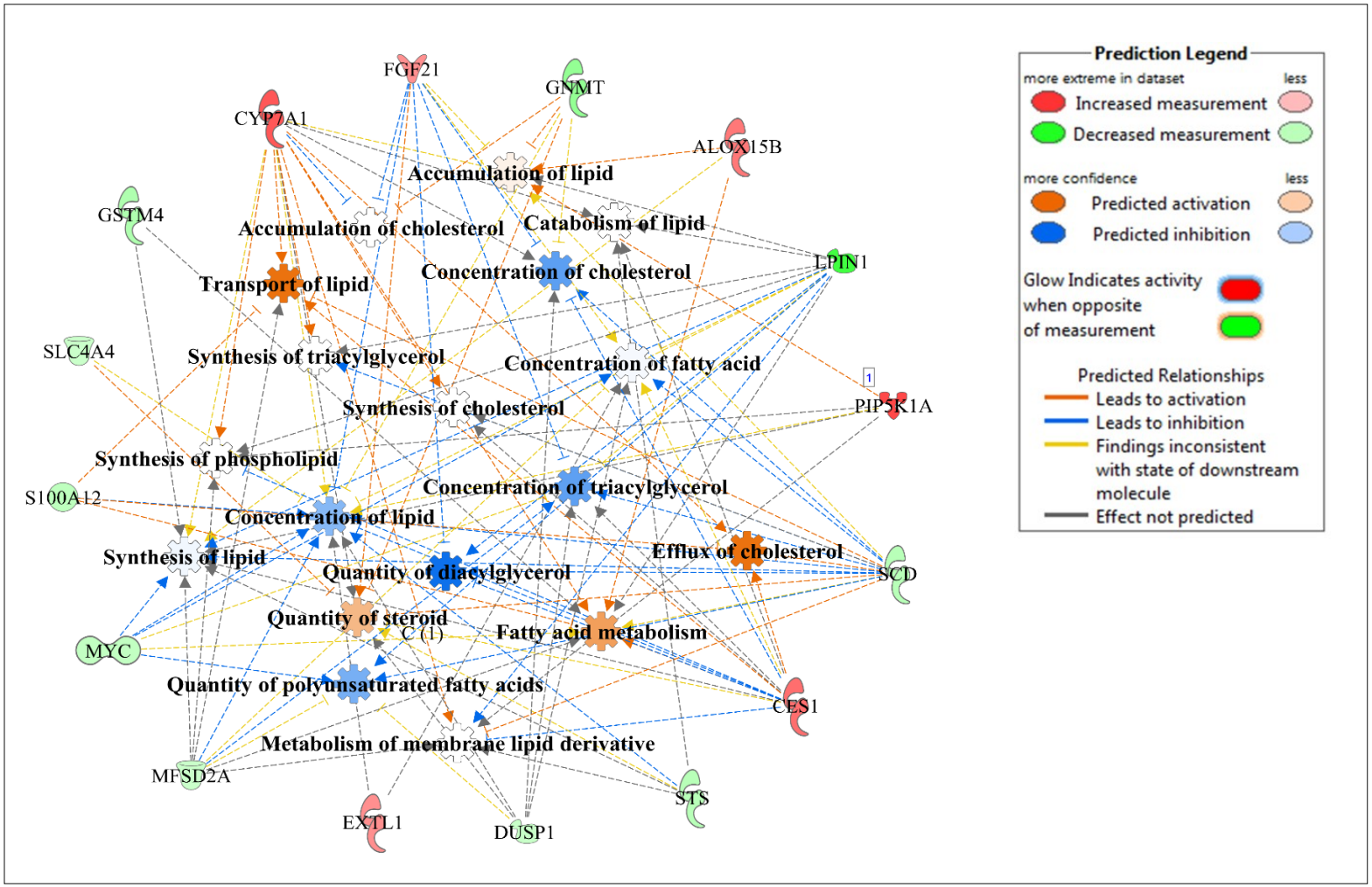


Figure 3.4. Lipid metabolism gene and molecular processes interaction network within lipid metabolism function as associated to average daily gain (ADG) in Charolais steers.

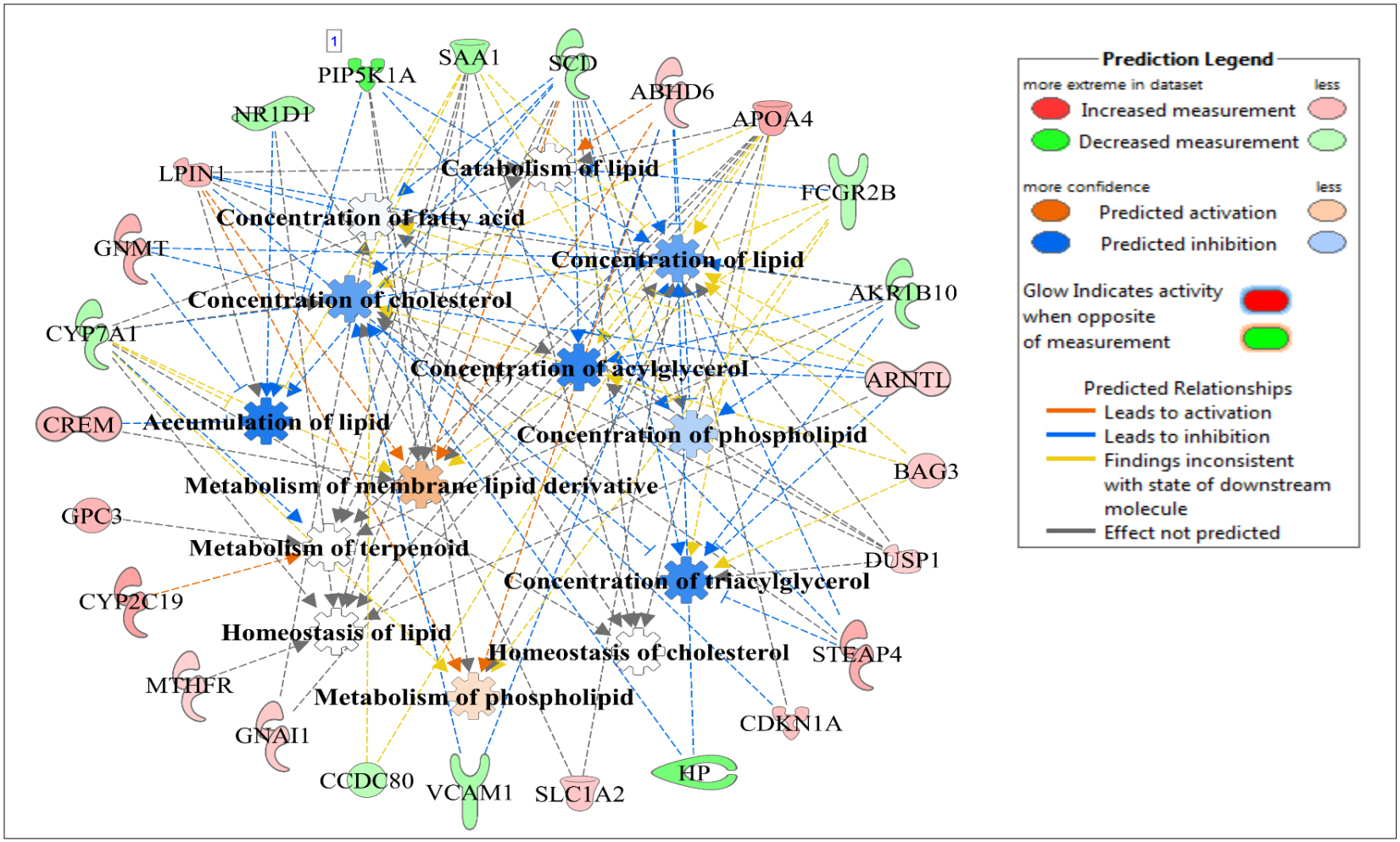


Figure 3.5. Lipid metabolism gene and molecular processes interaction network within lipid metabolism function as associated to average daily dry matter intake (DMI) in Angus steers.

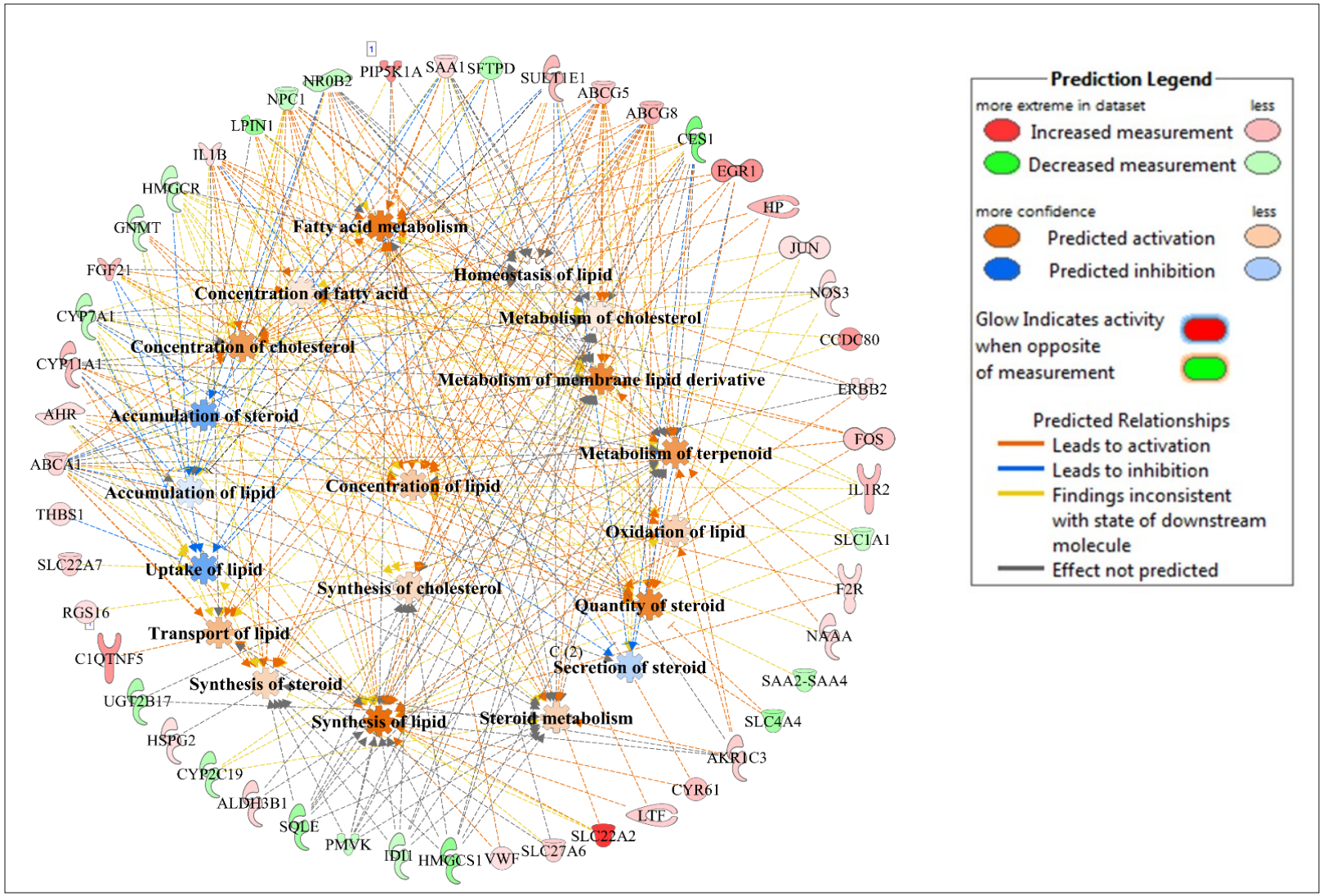


Figure 3.6. Lipid metabolism gene and molecular processes interaction network within lipid metabolism function as associated to average daily dry matter intake (DMI) in Charolais steers.

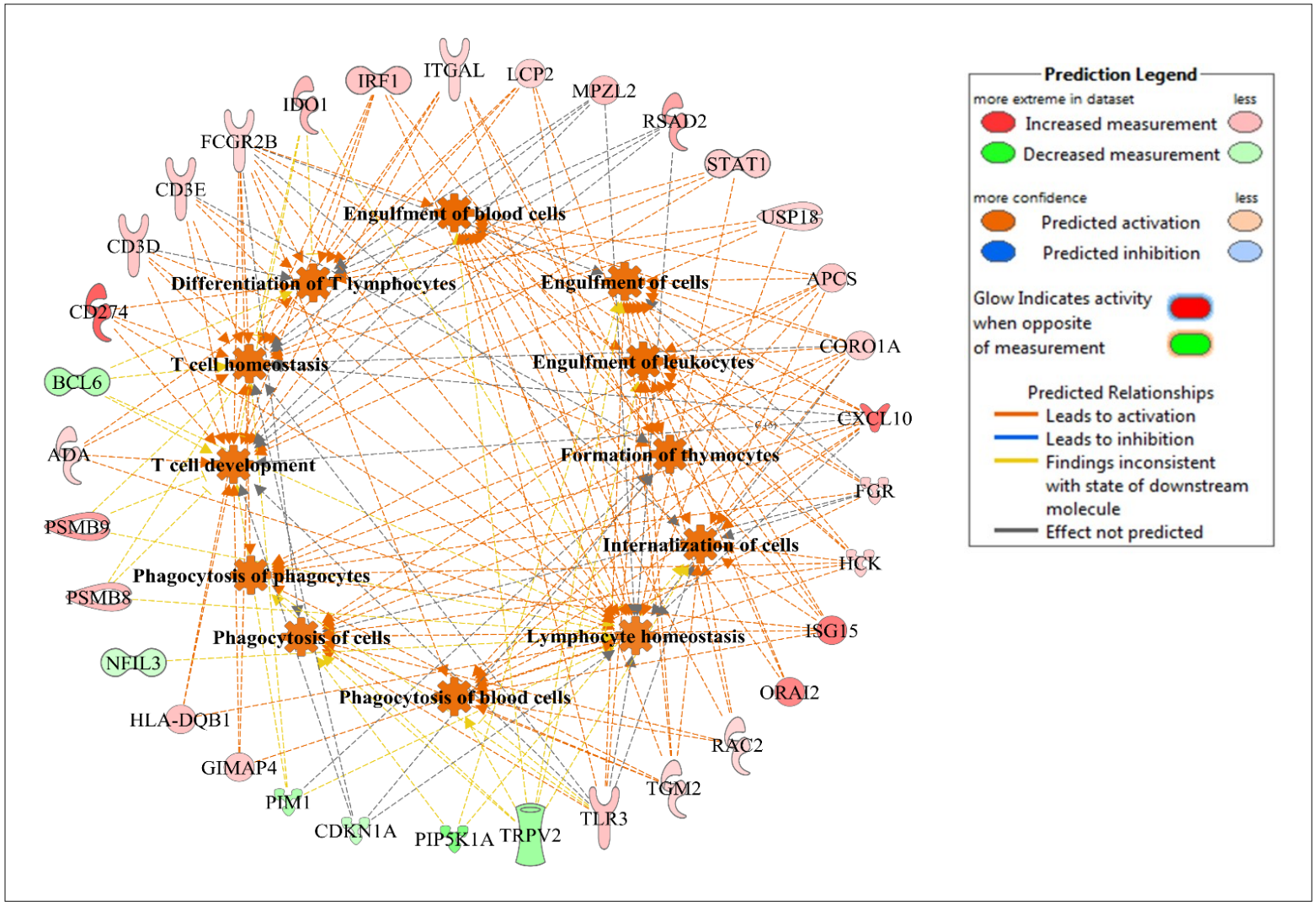


Figure 3.7. Cellular function and maintenance gene and molecular processes interaction network within lipid metabolism function as associated to average daily dry matter intake (DMI) in Kinsella Composite (KC) steers.

Table 3.1. Averages of the sequencing quality and alignment assessment parameters for Angus, Charolais, and Kinsella Composite (KC) animals.

	Angus (SD)	**Charolais (SD)**	**KC (SD)**
Total number of reads	32,419,572 (2527134)	40,796,790 (8826642)	29,571,035 (5,730,204)
Uniquely aligned reads	28,388,072 (2394131)	35,584,367 (8,224,313)	25680361 (4,964,214)
Average Phred Score	35.6 (0.23)	35.6 (0.60)	37.8 (0.18)
Uniquely aligned reads (%)	87.5 (1.12)	87.0 (2.01)	86.9 (0.84)

SD = standard deviation

Table 3.2. Top 40 significantly (by FDR) differentially expressed genes of characterized proteins between high and low-ADG steers from Angus, Charolais and Kinsella Composite populations.

Angus			**Charolais**			**Kinsella Composite**		
Gene	**log_2FC**	**FDR**	**Gene**	**Log_2FC**	**FDR**	**Gene**	**log_2FC**	**FDR**
TMEM45A	-3.87	4.69E-76	*TMEM45A*	-1.81	2.08E-19	*IFI27*	-2.69	9.87E-39
SERPINA3	3.32	1.74E-55	*HOPX*	-1.63	5.59E-16	*LPIN1*	-2.54	1.48E-34
GPX3	2.94	1.75E-53	*AKR1B15*	1.59	1.66E-14	*IFI6*	-1.67	5.27E-15
AKR1B15	2.68	2.33E-38	*TNC*	-1.73	2.20E-13	*SERPINA3*	1.56	1.01E-14
GPNMB	1.85	3.79E-21	*HLA-DQB1*	-1.86	6.68E-13	*ISG15*	-1.59	8.76E-14
HP	2.43	8.42E-18	*GPC3*	-1.64	3.17E-12	*HBB*	-1.49	8.54E-13
S100A2	1.58	1.09E-15	*KEL*	1.53	1.19E-11	*HERC6*	-1.44	2.45E-12
SERPINA3	1.95	7.74E-15	*DDO*	-1.90	2.07E-10	*GNMT*	-1.42	2.57E-12
HOPX	1.43	1.32E-14	*GPX3*	-2.20	2.72E-10	*GADD45G*	-1.35	6.14E-11
IFI6	-1.47	1.96E-13	*SERPINA3*	2.45	3.14E-10	*SLC5A8*	1.75	9.76E-10
UGT2B7	-1.35	4.93E-13	*AC108941.2*	-1.55	3.09E-09	*CES1*	1.24	5.26E-09
IFI27	-1.42	2.42E-12	*IGLV2-18*	-1.60	3.17E-08	*SERPINI2*	1.29	3.15E-08
GPC3	-1.37	1.83E-11	*CYP2B6*	1.29	4.48E-08	*CYP7A1*	1.44	4.51E-08
HMGCS1	-1.30	2.50E-10	*SLC25A45*	1.80	1.42E-07	*TSKU*	-1.24	1.43E-07
SECTM1	1.19	4.95E-10	*SERPINA3*	-1.61	1.97E-07	*IFIT1*	-1.38	1.43E-07
SULT2A1	1.18	7.65E-10	*IGLV2-18*	-1.44	3.27E-07	*UHRF1*	-1.60	2.15E-07
SPIDR	1.20	7.83E-10	*SCD*	-1.26	3.28E-07	*HLA-DQA1*	-1.29	2.72E-06
IGHG1	1.17	2.32E-08	*S100A10*	-1.14	4.06E-07	*NOCT*	-1.10	3.39E-06
ECEL1	1.10	5.27E-08	*STS*	-1.12	7.69E-07	*WFDC2*	-1.03	4.29E-06
JAKMIP2	1.44	9.89E-08	*UHRF1*	-1.47	1.48E-06	*ZNF385B*	1.19	4.29E-06
CYP51A1	-1.12	1.57E-07	*CCDC80*	-1.41	4.63E-06	*MX2*	-1.26	6.20E-06

AIF1L	1.02	2.62E-07	*SLC13A2*	-1.21	5.92E-06	*RSAD2*	-1.01	6.91E-06
AKR1C1	1.22	2.91E-07	*EGR1*	-1.33	6.60E-06	*GSTM2*	-1.07	9.32E-06
CCL24	1.35	4.38E-07	*CRYAB*	-1.04	7.08E-06	*C12orf45*	-1.01	1.15E-05
DLK1	-1.28	4.54E-07	*HIST1H2BI*	-0.99	7.11E-06	*IGFBP1*	0.97	1.50E-05
VCAM1	-1.28	2.21E-06	*GNMT*	-0.90	3.75E-05	*EXTL1*	0.96	2.94E-05
MT1G	1.07	6.21E-06	*EPCAM*	-1.05	8.16E-05	*ALAS1*	-1.00	3.46E-05
SQLE	-0.99	1.05E-05	*IFI27*	-0.90	9.13E-05	*STS*	-0.95	4.15E-05
IL1R2	1.09	2.69E-05	*ACSS2*	-0.88	1.24E-04	*PLEKHG6*	1.11	1.36E-04
SERPINE1	0.88	4.03E-05	*SLC17A9*	0.92	1.31E-04	*LURAP1L*	-1.02	1.65E-04
SLC13A2	0.91	5.28E-05	*SCD*	-1.29	1.37E-04	*PRAP1*	-0.93	1.92E-04
SERPINI2	-1.21	5.40E-05	*THNSL2*	0.91	2.19E-04	*SCD*	-0.95	2.95E-04
TNFRSF10A	-1.21	7.39E-05	*RCL1*	-0.88	2.30E-04	*FKBP5*	-0.89	5.62E-04
RAP1GAP	0.96	7.48E-05	*MID1IP1*	-0.85	2.42E-04	*TAT*	-1.02	6.56E-04
OXER1	-0.84	8.35E-05	*MBOAT2*	-1.24	2.42E-04	*FGF21*	0.95	6.85E-04
SLC1A2	-0.88	1.25E-04	*SOAT2*	1.30	3.09E-04	*ACE2*	-0.84	7.03E-04
MAMDC2	-1.08	1.25E-04	*FOXA3*	-0.84	3.13E-04	*MX1*	-0.87	7.98E-04
DENND2A	0.82	1.50E-04	*MAMDC2*	-1.09	4.79E-04	*IFI44L*	-0.83	8.80E-04
ROS1	-1.09	1.83E-04	*REC8*	-0.84	7.97E-04	*WFS1*	0.81	1.24E-03
CLBA1	1.15	2.55E-04	*ISG15*	-0.78	9.07E-04	*ALOX15B*	1.15	1.64E-03

log_2FC = log_2(Fold Change of a gene in high ADG animals with reference to low-ADG animals) and [2]FDR = False discovery rate adjusted P-Value.

Table 3.3. Top 40 significantly (by FDR) differentially expressed genes of characterized proteins between high and low-DMI steers from Angus, Charolais and Kinsella Composite populations.

Angus			**Charolais**			**Kinsella Composite**		
Gene	log_2FC	FDR	Gene	log_2FC	FDR	Gene	log_2FC	FDR
IFIT1	-2.49	1.38E-34	*SLC22A2*	4.47	1.92E-47	*IFI27*	3.13	9.17E-50
GPX3	-2.38	1.42E-30	*REC8*	-2.61	1.22E-37	*CXCL9*	2.82	2.83E-44
GPNMB	-2.23	4.16E-27	*EGR1*	2.38	3.46E-31	*GBP3*	2.99	3.15E-41
HBB	2.97	3.93E-26	*IGLC1*	2.48	3.46E-31	*IFI6*	2.24	1.09E-27
SERPINA3	-1.90	1.24E-20	*IGHG1*	2.31	3.95E-28	*CYP2B6*	-2.17	1.62E-27
ISG15	-1.83	1.04E-17	*SERPINA3*	2.47	3.92E-26	*HERC6*	2.07	1.07E-25
SFRP2	1.72	9.15E-17	*CCDC80*	2.25	1.56E-23	*IFIT1*	2.35	3.23E-25
HERC6	-1.44	1.18E-11	*SFRP1*	-1.79	1.00E-18	*ISG15*	2.03	3.28E-23
DDO	-1.63	3.99E-10	*GPX3*	2.08	6.20E-17	*CXCL10*	2.22	1.06E-22
FKBP5	1.37	7.10E-10	*HLA-DQB1*	-2.11	5.26E-16	*TMEM45A*	-2.84	1.63E-22
RSAD2	-1.35	1.01E-09	*CLDN15*	1.61	4.46E-15	*MX2*	2.07	4.80E-20
SDS	1.36	1.82E-09	*ABCG8*	1.54	2.98E-14	*AK4*	1.79	2.11E-19
APOA4	1.29	6.27E-09	*CES1*	-1.52	2.20E-12	*CD274*	2.44	7.13E-16
MBOAT2	-1.28	7.41E-09	*S100A10*	1.41	1.49E-11	*SERPINA3*	-1.60	2.60E-15
CDHR5	-1.25	7.65E-09	*CYP11A1*	1.41	2.03E-11	*AKR1B15*	-1.75	7.10E-14

MX1	-1.26	1.44E-08	*NNAT*	1.63	8.45E-11	*OAS1*	1.44	1.49E-12
IL20RA	-1.32	4.02E-08	*FGF21*	1.43	6.40E-10	*GBP7*	1.71	2.55E-11
SLC2A5	1.28	1.40E-07	*AC108941.2*	1.50	8.82E-10	*RSAD2*	1.36	2.55E-11
STEAP4	1.19	2.94E-07	*CYR61*	1.32	1.15E-09	*MKI67*	1.45	4.35E-11
GNMT	1.10	1.18E-06	*PRAP1*	1.26	1.73E-09	*PSMB9*	1.46	7.64E-11
MYOM1	1.21	2.78E-06	*CUX2*	1.41	2.73E-09	*KYAT1*	-1.21	1.03E-08
GPC3	1.06	7.85E-06	*CARNS1*	1.27	3.43E-09	*IFI44L*	1.15	1.01E-07
SERPINA3	-1.31	1.98E-05	*TNC*	1.32	4.57E-09	*RTP4*	1.17	1.01E-07
HP	-1.52	2.27E-05	*SLC7A2*	-1.26	6.17E-09	*ATP6V1C2*	-1.66	2.12E-07
LPIN1	1.03	2.27E-05	*HMGCS1*	-1.26	2.55E-08	*RBP5*	-1.12	3.20E-07
SECTM1	1.02	2.28E-05	*IL1R2*	1.48	3.82E-08	*GBP3*	1.31	5.23E-07
SCD	-0.99	3.23E-05	*LPIN1*	-1.23	4.91E-08	*HAPLN3*	-1.14	9.08E-07
NR1D1	-0.96	3.32E-05	*CDH17*	1.13	1.82E-07	*CTGF*	1.49	1.29E-06
CREM	0.96	3.57E-05	*HP*	1.61	3.99E-07	*PSMB8*	1.04	2.77E-06
PYROXD2	0.98	4.35E-05	*IGLV2-18*	1.29	6.43E-07	*TAP1*	1.02	4.52E-06
RTP4	-0.93	8.43E-05	*ABCG5*	1.16	6.55E-07	*FOXS1*	1.45	7.19E-06
SCD	-0.95	9.57E-05	*SLC4A4*	-1.08	9.88E-07	*PIM1*	-1.00	8.79E-06
RNF125	0.96	1.20E-04	*IFI27*	-1.13	1.19E-06	*WFS1*	-0.99	9.48E-06
IFI44L	-0.91	2.21E-04	*CLDN4*	1.47	2.00E-06	*IFIT2*	1.41	1.53E-05
NOCT	0.92	2.59E-04	*FOS*	1.21	2.91E-06	*NLRC5*	1.05	2.76E-05
CYP7A1	-0.88	3.43E-04	*HOOK1*	-1.04	2.91E-06	*UBA7*	0.97	2.77E-05
AKR1B15	-0.94	4.51E-04	*SQLE*	-1.09	3.37E-06	*RAB20*	-0.96	3.20E-05
DDIT4	0.91	4.80E-04	*STRIP2*	-1.03	3.92E-06	*CITED4*	0.96	3.20E-05
SPTB	-0.83	9.02E-04	*IGLC1*	1.15	4.94E-06	*RRM2*	1.36	3.20E-05
CKAP4	-0.83	9.42E-04	*DLK1*	1.19	5.02E-06	*IL20RA*	1.13	3.33E-05

$\log_2$FC = $\log_2$(Fold Change of a gene in low-DMI animals with reference to high-DMI animals) and FDR = False discovery rate adjusted P-Value.

Table 3.4. Top 40 significantly (by FDR) differentially expressed genes of characterized proteins between high and low-DMI steers from Angus, Charolais and Kinsella Composite populations.

Angus			**Charolais**			**Kinsella Composite**		
Gene	**logFC**	**FDR**	**Gene**	**logFC**	**FDR**	**Gene**	**logFC**	**FDR**
GPX3	-2.55	5.66E-37	*SERPINA3*	4.94	1.74E-99	*TMEM45A*	-5.23	2.12E-119
IFI27	2.50	1.14E-35	*CYP2B6*	-1.57	2.13E-17	*HOPX*	-1.76	1.53E-17
SERPINA3	-2.45	9.25E-32	*HLA-DQB1*	-1.83	1.04E-13	*SERPINA3*	-1.73	1.04E-16
IFI6	1.98	5.95E-22	*IGLC1*	-1.44	5.06E-13	*IFI27*	1.64	3.16E-15
SERPINA3	-2.51	4.61E-21	*TMEM45A*	-1.25	2.49E-11	*IFI6*	1.54	5.51E-14
GPNMB	-1.57	1.91E-14	*ECEL1*	1.17	6.28E-10	*GPX3*	-1.36	3.81E-11
SERPINI2	1.73	5.62E-13	*AC108941.2*	1.16	6.19E-08	*CYP2B6*	-1.36	1.02E-10
HBB	1.99	5.62E-13	*KEL*	1.20	6.19E-08	*FBLN2*	-1.36	6.92E-09
AKR1B15	-1.58	1.39E-12	*FBLN2*	1.17	6.19E-08	*WFS1*	-1.21	1.34E-08
AC108941.2	1.37	1.84E-11	*HOPX*	-1.02	2.74E-07	*CTGF*	1.53	5.43E-08

SDS	1.37	5.82E-11	*FGF21*	-1.12	7.30E-07	*HBB*	1.32	1.72E-07
AIF1L	-1.27	6.18E-10	*S100A10*	0.97	2.04E-06	*CXCL9*	1.29	5.13E-07
TMEM45A	1.26	9.39E-09	*PLA2G2D*	1.41	2.12E-06	*UGT2B7*	-1.08	7.53E-07
SULT2A1	-1.16	5.72E-08	*PLTP*	0.92	7.78E-06	*CYP3A7-CYP3A51P*	-1.19	7.53E-07
CKAP4	-1.10	2.23E-07	*IL1B*	1.11	8.46E-06	*ATP5MGL*	-1.47	9.00E-07
RAP1GAP	-1.22	1.55E-06	*KCTD12*	1.02	1.93E-05	*PIM1*	-1.06	1.74E-06
NUF2	1.44	1.55E-06	*CSF2RB*	0.93	4.06E-05	*IGHG1*	1.06	2.99E-06
TNC	1.08	1.55E-06	*SFRP1*	-0.87	4.19E-05	*ZNF385B*	-1.21	4.85E-06
CDHR5	-1.00	5.21E-06	*MARCO*	0.85	6.56E-05	*IGLC1*	1.39	9.42E-06
SLC13A2	-1.11	6.80E-06	*PRAP1*	-1.00	8.72E-05	*IGLC1*	1.00	2.61E-05
ROS1	1.32	9.40E-06	*FAM47E*	-1.01	1.05E-04	*HLA-DQB1*	0.97	3.59E-05
HP	-1.57	1.81E-05	*HLA-DQB1*	1.05	1.25E-04	*CES1*	0.94	4.26E-05
AK4	0.92	6.05E-05	*CSF1R*	0.83	1.84E-04	*SERPINI2*	1.08	4.26E-05
DENND2A	-0.89	1.12E-04	*UCP2*	0.80	3.12E-04	*ASIP*	1.00	9.88E-05
SFRP2	0.89	1.16E-04	*ADGRE1*	0.79	3.31E-04	*UGT2B7*	0.91	1.57E-04
SDCBP2	1.20	2.19E-04	*PTN*	0.91	3.31E-04	*ACE2*	0.88	1.79E-04
CYP2B6	0.88	4.01E-04	*IGHA1*	-0.89	5.98E-04	*HP*	-1.34	1.89E-04
PLCD4	-0.89	4.20E-04	*SLC13A2*	1.02	1.05E-03	*AKR1C1*	1.01	2.44E-04
FAM13A	0.99	4.81E-04	*PDK4*	0.79	1.15E-03	*REEP5*	-0.86	3.59E-04
GNMT	0.82	6.79E-04	*PTGS1*	0.75	1.23E-03	*TGM2*	0.85	4.61E-04
CFH	0.95	7.04E-04	*FADS1*	0.91	1.25E-03	*CDH11*	-0.99	5.44E-04
CYR61	0.94	1.04E-03	*SLC7A5*	1.00	1.46E-03	*PRAP1*	0.85	5.49E-04
ABHD6	0.85	1.44E-03	*SOAT2*	0.86	1.94E-03	*DDO*	0.99	6.82E-04
HMGCR	-0.83	1.44E-03	*PPP1R3C*	-0.74	2.00E-03	*CARNS1*	0.88	1.00E-03
GPC3	0.81	1.76E-03	*IGHG1*	-0.71	2.79E-03	*SLCO4A1*	-0.95	1.46E-03
BICC1	-0.96	2.27E-03	*LPIN1*	0.72	2.96E-03	*RAB20*	-0.81	1.62E-03
IGFBP2	-0.80	2.49E-03	*IGLV2-18*	-0.95	2.96E-03	*RFLNA*	-0.83	1.74E-03
ISG15	0.76	3.08E-03	*RASL10A*	0.99	3.33E-03	*SDS*	-0.81	1.91E-03
PRAP1	0.79	3.30E-03	*TMEM176B*	0.71	4.09E-03	*ECEL1*	0.88	2.09E-03
DLK1	0.97	3.49E-03	*SMPDL3B*	0.81	4.75E-03	*CLDN15*	0.78	2.09E-03

$\log_2$FC = $\log_2$(Fold Change of a gene in low-DMI animals with reference to high-DMI animals) and FDR = False discovery rate adjusted P-Value.

Table 3.5. Top enriched canonical pathways associated with growth rate, feed intake and metabolic weight in Angus, Charolais and Kinsella Composite (KC) animals.

Trait_Breed	Ingenuity Canonical Pathways	P-value	Ratio	Z-score	Molecules
ADG_ Angus	Superpathway of Cholesterol Biosynthesis	1.35E-05	0.14	-2.00	*SQLE, HMGCR, HMGCS1, CYP51A1*
	Nicotine Degradation II	2.40E-05	0.08	-2.24	*UGT2B17, FMO2, INMT, CYP51A1, CYP2C19*
	LPS/IL-1 Mediated Inhibition of RXR Function	2.40E-05	0.04	2.24	*IL1R2, NR0B2, FMO2, CYP7A1, LBP, HMGCS1, SULT2A1, CYP2C19*
	Acute Phase Response Signaling	4.17E-05	0.04	0.82	*SOCS3, HP, SAA1, SOCS2, SERPINA3, LBP, SERPINE1*
	LXR/RXR Activation	4.37E-05	0.05	-1.00	*IL1R2, SAA1, CYP7A1, LBP, HMGCR, CYP51A1*
ADG_Charolais	LXR/RXR Activation	1.23E-06	0.06	0.45	*IL1R2, SCD, RXRG, APOA1, APOA4, FASN, SAA1*
	LPS/IL-1 Mediated Inhibition of RXR Function	6.61E-05	0.03	0.00	*IL1R2, SULT1E1, CPT1B, ALDH3B1, GSTA1, CYP2B6, CYP2C19*
	PXR/RXR Activation	2.09E-04	0.06		*SCD, GSTA1, CYP2B6, CYP2C19*
	FXR/RXR Activation	2.63E-04	0.04		*APOA1, APOA4, FASN, SAA1, FOXA3*
	Glycine Betaine Degradation	8.71E-04	0.20		*SDS, SHMT2*
ADG_KC	PXR/RXR Activation	3.39E-06	0.08		*GSTM1, SCD, CYP7A1, IGFBP1, ALAS1*
	Interferon Signaling	7.76E-06	0.11	-2.00	*OAS1, MX1, IFI6, ISG15*
	LPS/IL-1 Mediated Inhibition of RXR Function	1.38E-04	0.03		*GSTM1, IL36G, GSTM4, CYP7A1, ALAS1, SOD3*
	2-amino-3-carboxymuconate Semialdehyde Degradation to Glutaryl-CoA	3.55E-03	1.00		*ACMSD*
	4-hydroxybenzoate Biosynthesis	3.55E-03	1.00		*TAT*
DMI_ Angus	Pyrimidine Ribonucleotides Interconversion	1.20E-03	0.07		*NUDT5, CMPK2, AK8*
	Pyrimidine Ribonucleotides De Novo Biosynthesis	1.38E-03	0.07		*NUDT5, CMPK2, AK8*
	LXR/RXR Activation	2.69E-03	0.03		*SCD, APOA4, SAA1, CYP7A1*
	Activation of IRF by Cytosolic Pattern Recognition Receptors	3.39E-03	0.05		*DHX58, IFIT2, ISG15*
	GADD45 Signaling	3.63E-03	0.11		*GADD45B, CDKN1A*

DMI_Charolais	LPS/IL-1 Mediated Inhibition of RXR Function	2.63E-10	0.07	0.33	*IL1R2, ABCG8, GSTM1, ABCG5, SULT1E1, JUN, SULT1C4, NR0B2, CYP7A1, SLC27A6, IL1B, ALDH3B1, HMGCS1, ABCA1, CYP2C19*
	Superpathway of Cholesterol Biosynthesis	2.34E-06	0.18	-2.24	*SQLE, PMVK, IDI1, HMGCR, HMGCS1*
	Mevalonate Pathway I	2.45E-06	0.31	-2.00	*PMVK, IDI1, HMGCR, HMGCS1*
	LXR/RXR Activation	5.13E-06	0.07	-1.34	*IL1R2, ABCG8, ABCG5, SAA1, CYP7A1, IL1B, HMGCR, ABCA1*
	Superpathway of Geranylgeranyl diphosphate Biosynthesis I (via Mevalonate)	7.94E-06	0.24	-2.00	*PMVK, IDI1, HMGCR, HMGCS1*
DMI_KC	Interferon Signaling	1.00E-10	0.22	2.83	*OAS1, IFI6, PSMB8, STAT1, TAP1, IRF1, IFITM1, ISG15*
	Antigen Presentation Pathway	6.31E-09	0.18		*PSMB9, NLRC5, HLA-B, HLA-DQB1, PSMB8, HLA-DQA2, TAP1*
	Th1 Pathway	3.80E-07	0.07	1.41	*NFIL3, CD3E, HLA-B, CD274, HLA-DQB1, HLA-DQA2, STAT1, CD3D, IRF1*
	Th1 and Th2 Activation Pathway	5.25E-06	0.05		*NFIL3, CD3E, HLA-B, CD274, HLA-DQB1, HLA-DQA2, STAT1, CD3D, IRF1*
	PKCθ Signaling in T Lymphocytes	1.82E-05	0.05	2.45	*CACNA1I, RAC2, CACNG1, CD3E, HLA-B, HLA-DQB1, CD3D, LCP2*
MWT_Angus	LPS/IL-1 Mediated Inhibition of RXR Function	1.38E-05	0.03	-2.00	*IL1R2, SULT1E1, CYP2B6, LBP, HMGCS1, SULT2A1, CYP2C19*
	Melatonin Degradation I	8.13E-05	0.06	1.00	*SULT1E1, CYP2B6, SULT2A1, CYP2C19*
	Superpathway of Melatonin Degradation	1.10E-04	0.06	1.00	*SULT1E1, CYP2B6, SULT2A1, CYP2C19*
	IGF-1 Signaling	6.46E-04	0.04		*CTGF, IGF1, CYR61, IGFBP2*
	Mevalonate Pathway I	9.33E-04	0.15		*HMGCR, HMGCS1*
MWT_Charolais	Neuroinflammation Signaling Pathway	1.45E-04	0.02	1.89	*HMOX1, VCAM1, HLA-B, IL1B, HLA-DQB1, CSF1R, GRIN3A*
	Prostanoid Biosynthesis	4.68E-04	0.22		*PTGS1, TBXAS1*
	Granulocyte Adhesion and Diapedesis	5.37E-04	0.03		*VCAM1, SELL, IL1B, MMP11, SDC3*
	Graft-versus-Host Disease Signaling	7.24E-04	0.06		*HLA-B, IL1B, HLA-DQB1*
	Altered T Cell and B Cell Signaling in Rheumatoid Arthritis	4.47E-03	0.03		*HLA-B, IL1B, HLA-DQB1*
MWT_KC	Antigen Presentation Pathway	3.39E-04	0.08		*HLA-B, HLA-DQB1, HLA-DQA2*

	Pathogenesis of Multiple Sclerosis	4.47E-04	0.22		*CXCL10, CXCL9*
	Nicotine Degradation III	1.07E-03	0.05		*UGT2B17, CYP2B6, CYP2C19*
	Th1 Pathway	1.41E-03	0.03		*SOCS3, HLA-B, HLA-DQB1, HLA-DQA2*
	Melatonin Degradation I	1.62E-03	0.05		*UGT2B17, CYP2B6, CYP2C19*

ADG = Average daily gain; DMI = Average daily dry matter intake; MWT = metabolic weight.

Chapter 4. Bovine hepatic miRNAome profiling and differential miRNA expression analyses between beef steers with divergent feed efficiency phenotypes

4.1 Abstract

Micro RNAs (miRNAs) are a group of small (~22nt) RNAs that negatively regulate their target genes and have been widely recognized as principle regulators or modulators of a broad range of biological processes of living organisms including cattle. Since miRNAs regulate multiple biological processes, it is indicative that miRNAs are involved in differential regulation of genes involved in economically important traits in the beef industry, such as feed efficiency. Multiple studies have identified genes and biological functions associated with feed efficiency traits such as residual feed intake (RFI). However, limited studies have been performed to identify miRNAs associated with RFI in beef cattle. The objective of the current study was to apply RNAseq to profile the liver miRNAome of 60 beef steers and identify differentially expressed (DE) miRNAs between high and low-RFI animals from Angus, Charolais and Kinsella Composite (KC) populations. We identified 588 miRNAs as expressed in the liver tissue of the studied animals, 90% of which were expressed in animals from all three populations. Ten previously identified (known) miRNAs including *bta-miR-192, bta-miR-143, bta-miR-148a, bta-miR-26a, bta-miR-30a-5p, bta-miR-22-3p, bta-miR-27b, bta-let-7f, bta-miR-27a-3p* and *bta-miR-101* were identified as exceptionally highly expressed in the liver tissue of all steers, accounting for over 78% of the aligned reads. Additionally, we identified 241 novel bovine miRNAs, of which the majority were breed specific. We performed differential miRNA expression analysis between low and high-RFI steers from Angus (high (n = 6), low (n = 6)), Charolais (high (n = 6), low (n = 6)) and KC (high (n = 6), low (n = 6)) populations. At a threshold of fold-change ≥ 1.5 and P-value < 0.05, we

identified 12 (7 up- and 5 downregulated in low-RFI animals), 18 (12 up- and 6 downregulated in low-RFI animals) and 12 (8 up- and 4 downregulated in low-RFI animals) DE miRNAs for Angus, Charolais and KC steers, respectively. The majority of the DE miRNAs were breed specific, with only *bta-miR-449a* being differentially expressed in all the three breeds. The DE miRNAs were predicted to target up to 76% of previously identified genes associated with RFI in the same populations. Our results provide insights into the bovine hepatic miRNAome and their potential roles in molecular regulation of RFI in beef cattle.

4.2 Introduction

Genetic selection and the breeding of more feed efficient beef animals is of great interest to beef producers, since increased efficiency would potentially reduce the cost of beef production with respect to feed and feeding related costs, which contribute up to 75% of the total variable production costs (Ahola & Hill 2012). Additionally, studies have shown that breeding for more feed efficient animals can significantly reduce methane emission from beef cattle (Nkrumah *et al.* 2006; Hegarty *et al.* 2007), which would consequently lower the carbon footprint of these animals. Understanding genetic control of complex traits such as feed efficiency can help enhance the rate of genetic improvement of such traits via more effective genetic or genomic selection (Fang *et al.* 2017). In this regard, multiple genome wide (Barendse *et al.* 2007; Nkrumah *et al.* 2007; Abo-Ismail *et al.* 2014; de Oliveira *et al.* 2014; Saatchi *et al.* 2014) and global transcriptomic (Chen *et al.* 2011; Alexandre *et al.* 2015; Paradis *et al.* 2015; Tizioto *et al.* 2015; Kong *et al.* 2016; Tizioto *et al.* 2016; Weber *et al.* 2016; Khansefid *et al.* 2017; Mukiibi *et al.* 2018) studies have endeavored to identify genes and biological functions associated with feed efficiency in beef cattle.

MicroRNAs are a group of small RNAs with an average length of about 22 nucleotides, resulting from the enzymatic cleavage of longer RNA molecules (mainly of intergenic and intronic

origin) by DROSHA (nucleic) and DICER (cytoplasmic) RNase endonucleases (Gregory & Shiekhattar 2005; Moutinho & Esteller 2017; Bartel 2018). MicroRNAs combine with Argonaute proteins to form a miRNA-induced silencing complex (miRISC) (O'Brien *et al.* 2018), which under the guidance of the miRNA, bind to the seed region of the 3' untranslated regions (UTRs) of the target mRNA protein coding gene (O'Brien *et al.* 2018). This process leads to gene expression repression by promoting mRNA decay or repressing mRNA translation into proteins (Creighton *et al.* 2009; O'Brien *et al.* 2018). MicroRNAs have also been demonstrated to be directly or indirectly involved in the epigenetic regulation of gene expression (Moutinho & Esteller 2017). In mammalian cells, miRNAs target and regulate expression of up to 60% of the transcribed genes (Friedman *et al.* 2009), hence, they are involved in multiple biological functions including cell proliferation, cell cycle, cell development, apoptosis, metabolism of amino acids, metabolism of lipids, metabolism of carbohydrates and metabolism of minerals and vitamins (Murakami & Kawada 2017). In liver tissue, miRNAs have been implicated in regulating hepatic cell proliferation, hepatic metabolism of nutrients (including lipids, carbohydrates, vitamins and minerals, and proteins and amino acids), energy metabolism and detoxification (Chen & Verfaillie 2014).

Liver transcriptomic studies have demonstrated the potential for the hepatic tissue's involvement in the molecular control of feed efficiency through identification of differentially expressed genes between efficient and inefficient animals (Chen *et al.* 2011; Alexandre *et al.* 2015; Paradis *et al.* 2015; Tizioto *et al.* 2015; Weber *et al.* 2016; Khansefid *et al.* 2017; Mukiibi *et al.* 2018). For example, in our most recent study, we identified multiple differentially expressed genes associated with feed efficiency, some of which are involved in key hepatic functions such as lipid metabolism, energy production, amino acid metabolism and carbohydrate metabolism, in beef

cattle (Mukiibi *et al.* 2018). However, only a few studies have sought to identify possible miRNA regulation of genes involved in the molecular control of feed efficiency in beef cattle (Al-Husseini *et al.* 2016; De Oliveira *et al*. 2018; Carvalho *et al.* 2019). Therefore, in the current study we aimed to profile the hepatic miRNAome of beef steers from three beef breed populations, including Angus, Charolais and Kinsella Composite, and to identify miRNAs associated with feed efficiency through RNAseq differential expression analyses.

4.3 Materials and Methods

4.3.1 Animal populations and management

The management practices and population descriptions of the experimental animals used in the current study have been presented in our recent study (Mukiibi *et al.* 2018). In summary, the animals were raised and managed following the Canadian Council of Animal Care (CCAC) guidelines on the care and use of farm animals in research teaching and testing (CCAC 2009), and all the experimental procedures applied to the animals were approved by the University of Alberta Animal Care and Use Committee (AUP00000777). We used a total of 256 beef steers from two purebred populations including Angus and Charolais, and crossbred beef steers from the Kinsella Composite population. The steers were born, raised and managed similarly at the Roy Berg Kinsella Ranch, University of Alberta, Canada. All the purebred Angus and Charolais cows were serviced through artificial insemination, followed by natural service by purebred Angus and Charolais bulls whose pedigree records were maintained by the Canadian Angus or Charolais Association, respectively. KC animals were produced through crossing Angus, Charolais, or Alberta Hybrid bulls with the University of Alberta's hybrid dam line. The crossbreeding design used to generate the University of Alberta's hybrid dam line from composite cattle lines of multiple beef breeds has been previously described by Goonewardene *et al*. (2003). Additionally, since

2012, commercial crossbred bulls have been added to the KC herd for natural service. The animals used in the current study were born between the months of April and May of 2014 and were castrated immediately after birth. The steer calves were maintained with their dams on pasture and weaned at an average age of six months. The weaned animals were transitioned to a backgrounding diet composed of 80% barley silage, 17% barley grain, and 3% rumensin pellet supplement, and thereafter fed set-up diets with gradually decreasing barley silage and increasing barley grain proportions for 3 weeks. Subsequently, they were introduced to the finishing diet of 75% barley grain, 20% barley silage, and 5% rumensin pellet supplement (as fed basis).

4.3.2 GrowSafe feedlot test, phenotype measurement and calculations

Between the months of April and August in 2015, 50 Angus, 48 Charolais and 158 KC steers were measured for individual feed intake using the GrowSafe system® (GrowSafe Systems Ltd., Airdrie, Alberta, Canada). A detailed account of the process for measuring each individual animal's daily feed intake using the GrowSafe automated system has been provided by Mao *et al.,* (2013). Briefly, the animals were tested for feed intake for a period ranging from 70 to 73 days, during which animals were fed on a finishing diet. Average daily intake of each animal was calculated as the average of the daily feed intake records over the test period, standardized to 12 MJ ME per kg dry matter, based on the energy content of the diet. Initial body weight (BW) and average daily gain (ADG) for each animal were obtained from a linear regression between serial body weight measurements and time (days), and they were recorded on two consecutive days at the start, at approximately 14-day intervals during the feedlot test, and on two consecutive days prior to the end of test. Metabolic mid-weight (MWT) was calculated as midpoint $BW^{0.75}$, where midpoint BW was computed as the sum of the initial BW of the animal and the product of its ADG, multiplied by half the number of days under the feedlot test. RFI was calculated as the residual

from a linear regression model where dry matter intake was regressed on ADG and MWT as proposed by Koch et al. (1963).

4.3.3 Liver tissue collection

Tissue collection and processing procedures have been previously described in our recent study (Mukiibi *et al.* 2018). Briefly, all the animals used in the current study were slaughtered at the Agriculture and Agri-Food Canada (AAFC) Lacombe Research Centre (Lacombe, AB) between July and September of 2015. Animals were considered ready for slaughter at an average back-fat thickness of 8 mm between the 12^{th} and 13^{th} ribs, which was measured using an Aloka 500 diagnostic real time ultrasound machine with a 17 cm 3.5Mhz linear array transducer. The animals were on average slaughtered at the age of 494 ± 3, 518 ± 4, and 457 ± 4 days for Angus, Charolais and KC respectively. The liver of each animal was collected immediately after slaughter and dissected at relatively the same location on the right lobe and the fibrous capsule was removed from the sliced liver tissue samples. The samples were there after bagged separately in plastic re-closable bags, labelled, flash frozen in liquid nitrogen and transported on dry ice to the laboratory where they were stored at -80^{o}C until total RNA extraction.

4.3.4 Total RNA extraction

Total RNA extraction was performed on 20 samples (10 with positive and 10 with negative RFI phenotype values) from each breed. Each of the selected liver tissue samples were pulverized into a fine powder using liquid nitrogen and a pre-chilled mortar and pestle on dry ice. Total RNA containing small RNAs was then extracted using the Qiagen RNeasy Plus Universal Mini Kit (Qiagen, Toronto, ON), according to the manufacturer's instructions. A NanoDrop 2000 Spectrophotometer (Thermo Scientific, Wilmington, DE, USA) was used to quantify the RNA. We obtained total RNA with an average concertation of 1851.8ng/µl per sample, and with

absorbance ratios (A260/280) ranging between 1.8 and 2.0. RNA integrity was confirmed using a TapeStation-Agilent instrument (Agilent Technologies Canada, Mississauga, ON). RNA integrity number (RIN) values for all samples were higher than 8 which deemed them to be high quality and suitable for cDNA library preparation and downstream transcriptomic profiling.

4.3.5 Construction of cDNA libraries and sequencing

In total 60 cDNA libraries were prepared and sequenced at the Clinical Genomics Centre (Toronto, ON, Canada). The libraries were prepared using the Illumina Truseq Small RNA Library Prep Kit (Illumina, San Diego, CA, USA) from 1 μg of each of total RNA. Initially, an RNA 3' adapter was ligated to the 3' end of the RNAs in the total RNA samples using a T4 RNA Ligase 2 enzyme, thereafter, an RNA 5' adapter was added to the 5' end of the 3' adaptor-ligated-RNAs using a T4 RNA Ligase. The RNA 3' and RNA 5' adapters were designed to specifically target miRNAs and other small RNAs resulting from similar biogenic processing. The 5' and 3' adapter ligated RNA was then reverse transcribed using the SuperScript II Reverse Transcriptase (Thermo Fisher Scientific, San Jose, CA, USA) and the RNA RT primer to generate single stranded cDNA. The cDNA was then PCR amplified with a universal RNA PCR primer, and a second RNA PCR primer containing a six-nucleotide indexing sequence to allow multiplexed sequencing of multiple samples on the same flow cell lane. The cDNA libraries were purified via gel electrophoresis using a 6% PAGE Gel, and the 160bp and 145bp cDNA bands were excised for subsequent sequencing. Four sequencing pools of 15 miRNA libraries were constructed by pooling an average of 2nM cDNA from each library. The pooled cDNA libraries were sequenced on two flow cells using the Illumina Hiseq 2500 sequencing platform under Rapid run mode, with expected read length of 50bp (1x50bp single read (SR)). After sequencing, the raw sequence data were demultiplexed into

individual FASTQ files for each sample using the Illumina bcl2fastq-v2.17.1.14 conversion software (Illumina).

4.3.6 Bioinformatic sequence data processing and miRNA expression profiling

Raw sequence reads were firstly assessed for sequencing quality using FASTQC version 0.11.7(Andrews 2010). The reads were evaluated for quality based on numerous parameters such as; average read length, adaptor content, per sequence GC content and per base sequence quality scores. Thereafter, the Illumina 3' adaptor sequence (TGGAATTCTCGGGTGCCAAGG) was clipped off all the raw read sequences using cutadapt version 1.16 (Martin 2011). Reads of lengths shorter than 15bp, and longer than 28bp were removed as short and long reads, respectively. The retained reads were filtered for other bovine short RNA species including ribosomal RNAs (rRNAs), transfer RNAs (tRNAs), small nuclear RNAs (snRNAs) and small nucleolar RNAs (snoRNAs) downloaded from https://rnacentral.org/. The final processed sequence reads were re-evaluated for quality using FASTQC. 0.11.7 (Andrews 2010).

To profile both novel and known miRNA expression in the samples from the cleaned sequence data, the miRDeep2 package (version 2.0.0.8) modules (Friedländer *et al.* 2011) were used together with the UMD3.1 bovine genome from Ensembl version 93, and the known bovine mature miRNA sequences and their precursor sequences from the miRBase database (release 22) (Griffiths-Jones *et al.* 2007). The mapper module (mapper.pl) with default parameters was used to collapse reads of the sequences into clusters, and then it employed the bowtie-1.1.1 short sequence aligner (Langmead *et al.* 2009) to align the collapsed reads to the indexed UMD3.1 reference genome. Using default parameters, and input files including the reference genome, collapsed reads versus reference genome alignment, known bovine (and human) mature miRNAs and their precursors sequences (including the hairpin structures), and *Bos taurus* (bta) as the species of

interest, the miRDeep2 module (miRDeep2.pl) was used to quantify bovine miRNAs. Using known mature miRNAs and their precursor sequences as input files, miRDeep2 enacted the quantifier module to quantify all known expressed miRNAs in our sequence data, hence producing read counts for each individual sample.

Subsequently, miRDeep2 predicted possible novel miRNAs and their respective precursors based on their read alignment to the bovine reference genome. Genomic regions stacked with aligned reads were excised as potential precursors and evaluated by the RNAfold tool (Markham & Zuker 2008) within ViennaRNA-1.8.4 for their potential to form stable secondary structures (hairpins), their ability to be partitioned into mature, loop and star strand, and their base pairing in the mature miRNA region. Overall, the RNAfold P-value, the miRDeep2 score and the probability that the miRNA candidate was a true positive, were estimated and produced as output together with novel mature miRNA consensus sequences and their respective precursor sequences, for each novel miRNA.

4.3.7 Differential miRNA expression analysis

Initially, counts for each mature miRNA coming from more than one precursor were averaged. Thereafter, all miRNAs that had less than 10 total read counts across the studied samples were filtered out. Then miRNA expression variation patterns between 20 samples in each breed were visualized through principle component analysis of the read counts from the mirDeep2 module using the DESeq2 Bioconductor package (Love *et al.* 2014) and ggplot2 R packages. Twelve samples including six samples with extreme high and six samples with extreme low-RFI phenotypes that showed regular miRNA expression (as compared to all other samples in the same breed) were considered for differential miRNA expression using the egdeR package in R (Robinson *et al.* 2010). To increase the statistical power of the analyses, miRNAs within samples

from each of the breeds that had less than one count per million (CPM) in at least six samples (half of the analyzed samples) were filtered out from the analyses, as proposed by Anders *et al.* (2013). For the retained miRNAs, their counts were normalized using the TMM method (Robinson and Oshlack, 2010). To test for differential miRNA expression between high and low-RFI steer groups from each breed, the normalized counts were modeled using a generalized linear model under a binomial distribution with the high-RFI group as a reference. MicroRNAs were deemed differentially expressed at a P-value less than 0.05, and fold change (FC) greater than 1.5. We performed differential miRNA expression for all expressed known mature miRNAs and the top 25 expressed novel miRNAs in each breed.

4.3.8 Validation of differentially expressed miRNAs

Six differentially DE miRNAs with relatively high expression per sample were selected for validation of the small RNAseq results. These included *bta-miR-2415-3p*, *bta-miR-133a* and *bta-miR-2419-5p* for Charolais, and *bta-miR-424-5p*, *bta-miR-223* and *bta-miR-155* for KC. *bta-miR-192* and *bta-miR-93* were selected as endogenous controls for Charolais, whereas *bta-miR-2284x* and *bta-let-7b* were selected as reference miRNA genes for KC, based on their expression abundance and stability across samples (average M values of 0.18 (KC) and 0.22 (Charolais)), determined using geNorm in the GenEx Software v.5.2.7.44 (2010) Relative expression of the selected miRNAs were obtained through stem-loop RT- TaqMan qPCR(Chen *et al.* 2005) using the high quality total RNA (minimum RIN value of 8, and average concentration of 1870ng/µl) that was also used for small RNA sequencing. Reverse-transcription (RT) stem-loop primers and TaqMan qPCR assays (containing the probe and forward and reverse primers) were purchased from Thermo Fisher Scientific (https://www.thermofisher.com). RT primer IDs and TaqMan qPCR assay IDs for each validated internal control are provided in Supplementary Data S11. Serial

dilutions of pooled cDNA samples were used to determine amplification efficiencies using the equation $E = -1 + 10^{(-1/slope)}$. The slope was calculated by plotting the linear curve of cycle threshold (C_T) values against the log dilutions (Pfaffl, 2001). Primers had PCR efficiencies of between 89 and 110%.

Initially, the reverse transcription reactions for each sample including the no template controls were performed using the TaqMan® MicroRNA Reverse Transcription Kit. Each sample reaction contained 5 µl of total RNA (2ng/µl), 1µl of MultiScribe Reverse Transcriptase enzyme, 3µl of stem-loop RT primer, 0.15µl of dNTP mix, 1.5µl of 10x RT buffer, 0.19µl of RNase inhibitor and 4.16µl of nuclease free water. The 15µl reactions were incubated in an Eppendorf 5331 Mastercycler Gradient v2.30.31 thermocycler for 30 minutes at 16ºC, 30 minutes at 42ºC and 5 minutes at 85ºC.Thereafter, real-time quantitative PCR (qPCR) was performed using the TaqMan® Fast Advanced Master Mix Protocol. The 20µl qPCR reaction contained 10µl of TaqMan Fast Advanced Master Mix, 1µl of TaqMan MicroRNA Assay, 1.33µl of RT reaction product (cDNA) and 7.67µl of nuclease free water. All qPCR reactions were performed in triplicate on a MicroAmp® Fast Optical 96-Well Reaction Plate in the Applied Biosystems™ 7500 Fast Real-Time PCR System v2.0.1 (Applied Biosystems, Foster City, California, USA). The reactions were incubated for 2 minutes at 50ºC, for 20 seconds at 95ºC, and followed subsequently by 40 PCR cycles of 3 seconds at 95ºC for denaturation and 30 seconds at 60ºC for annealing and extension. Threshold cycle values from the Real-Time PCR thermocycler were then imported into GenEx Software v.5.2.7.44 (2010) (MultiD Analyses AB, Göteborg, Sweden). The C_T values were adjusted to account for inter-plate variation using the inter-plate calibrator sample included on the plates and to account for amplification efficiencies. The replicates were averaged, and the resulting C_T values were normalized to the reference genes and Log_2 relative quantities were calculated to

the highest-p C_T value. The relative quantities were then analyzed for differential miRNA expression between high and low-RFI steers using a two tailed t-test.

4.3.9 miRNA Target genes prediction

Target genes for highly expressed known miRNAs and most expressed novel microRNAs across the three populations, , and the differentially expressed known and novel miRNAs from each breed were predicted using three TargetScan 7.0 Perl scripts (Agarwal *et al.* 2015) downloaded from http://www.targetscan.org/cgi-bin/targetscan/data_download.vert72.cgi. TargetScan and its associated packages were used to predict mammalian miRNA target genes based on a scoring model that includes the following attributes; 3'-UTR target-site abundance, predicted seed-pairing stability, identity of the nucleotide at position 1 of the sRNA, identity of the nucleotide at the 8th position of the sRNA, identity of the nucleotide at the 8th position of the target site, local AU content near the target site, supplementary pairing at the miRNA 3' end, predicted structural accessibility, minimum distance of the site from the stop codon or polyadenylation site, probability of target site conservation, ORF length, 3'-UTR length, number of offset-6mer sites in the 3' UTR, and the number of 8mer sites in the ORF (Agarwal *et al.* 2015).

Firstly, we predicted the conserved and non-conserved target sites using targetscan_70.pl by providing all the known gene transcripts UTR sequence alignments and the miRNA family information as inputs. The miRNA family information file included the miRNA family IDs, the seed sequence (from 2nd position nucleotide to 8th position nucleotide 5' of miRNA sequences) and the NCBI IDs of 8 species (cow, sheep, domestic goat, horse, human, mouse, rat and pig). Thereafter, we used targetscan_70_BL_bins.pl and targetscan_70_BL_PCT.pl to calculate the branch length and the conservation probability of the conserved target sites. Finally, in

combination with RNAplfold-2.4.11, targetscan_70_context_scores.pl was used to calculate context++ scores for the miRNA target genes based mainly on the 14 attributes mentioned above.

4.3.10 Functional Enrichment, and target-miRNA interaction networks analysis

Target genes for the top expressed known and novel miRNAs with the 99th context++ score percentile rank were considered for functional enrichment analysis using ingenuity pathway analysis (IPA). Additionally, for each breed target genes for the DE miRNAs with context++ percentile rank higher than 50 and those which were identified as DE genes in our previous mRNA study (Mukiibi *et al.* 2018) were retained for DE mRNA - DE miRNA (Target-miRNA) interaction network analyses for each breed using Cytoscape 3.7.1 (Shannon *et al.* 2003). Functional enrichment analyses were also performed on the DE targets to identify the major biological functions that are potentially differentially modulated by the identified DE miRNAs.

4.4 Results

4.4.1 miRNA sequence data and alignment quality

On average Illumina next generation sequencing yielded over 9M high quality raw reads per sample for Angus and Charolais, and over 11M for KC samples (Table 4.1). After 3' adaptor clipping, 36.91% of the reads were removed as long reads (>28bp), 8.29% were removed as very short reads (< 15bp) (Table S2 in the Supplementary Data S11). Additionally, on average 0.25%, 0.14%, 0.03% and 0.03% of the reads were removed as they aligned to bovine rRNAs, tRNAs, snRNAs and snoRNAs, respectively (Table S3 in the Supplementary Data S11). An average of 5.5M reads were retained for miRNA profiling analysis by mirDeep2 (Table 4.1). The retained reads were of high quality as depicted by high average Phred scores in Figure 4.1a, and majority of the reads ranged between 20 and 24bp in length as shown Figure 4.1b. Of the retained reads,

74.8 ± 0.4% mapped to the UMD3.1 bovine reference genome, with KC samples having the highest mapping rate of 77 ± 0.3% (Table 4.1).

4.4.2 Known miRNA expression and novel miRNA identification

We identified 541, 552 and 576 expressed known miRNAs in Angus, Charolais and KC samples, respectively. Of all the known miRNAs, 90% were common to all the three breeds as shown in Figure 4.2a. Among the most expressed miRNAs, *bta-miR-192* was the most abundant miRNA in all the three breeds with an average of 867,342, 1,060,828 and 1,272,798 aligned reads per sample from Angus, Charolais and KC populations, respectively. Ten miRNAs showed predominantly high expression including *bta-miR-192, bta-miR-143, bta-miR-148a, bta-miR-26a, bta-miR-30a-5p, bta-miR-22-3p, bta-miR-27b, bta-let-7f, bta-miR-27a-3p* and *bta-miR-101* and they accounted for an average of 78.4%, 78.3% and 77.9% percent of the total aligned sequence reads in Angus, Charolais and KC animals, respectively. The top 20 expressed miRNAs across studied samples from each of the breeds are presented in Table 4.2, while all the expressed miRNAs identified, and their average read counts in each breed are presented in Supplementary Data S12. At a mirDeep2 score ≥ 4, an estimated probability that the predicted miRNA candidate is a true positive ≥ 70 ± 4%, and a significant Randfold p-value that the miRNA precursor sequence could be folded into a thermodynamically stable hairpin, we identified 126 (from 129 precursors), 101 (from 103 precursors) and 125 (from 125 precursors) novel miRNAs in Angus, Charolais and KC samples, respectively. The identified miRNAs were largely breed specific, with only 31 of them being common to all the three breeds, (Figure 4.2b). The novel miRNA *bta-miR-AB-10* was the most expressed of all the identified novel miRNAs across the three breeds with a total of 37,061, 57,018 and 64,372 read aligned to it in Angus, Charolais and KC samples, respectively (Table 4.3). The hairpin structure of the precursor and the read alignment distribution

(i.e. alignment to the mature, star and loop sequences) across the three breeds are presented in Figure S2 in Supplementary Data S11. The top 20 expressed novel miRNAs and their miRDeep2 prediction scores are presented in Table 4.3, whereas all the identified novel miRNAs miRDeep2 prediction scores from all the three breeds are provided in the Supplementary Data S12.

4.4.3 miRNA differential expression

Differential miRNA expression analysis was performed between low- and high-RFI steer groups from the profiled animals from three studied populations. The average RFI phenotypic values between the two steer groups (high and low-RFI) were all significant at P-value < 0.0042 after Bonferroni Correction for multiple comparisons (Table S2 in the Supplementary Data S11). For other traits, low-RFI animals consumed significantly less feed per day than their high-RFI counterparts in Charolais and KC as expected. For Angus, low-RFI animals on average also consumed less feed as compared to the high-RFI animals but the difference did not reach to the significant level (i.e. P-value > 0.0042, Bonferroni Correction). The average phenotypic values of other traits including animal slaughter age were not significantly different between the high and low RFI groups (Table S2 in the Supplementary Data S11).

At a fold change of 1.5 and a P-value < 0.05, we identified 12 DE miRNAs in the liver tissue of Angus including (two novel miRNAs), of which five were downregulated and seven upregulated in low-RFI animals. In Charolais, we identified 18 DE miRNAs including (two novel miRNAs), of which six were downregulated and 12 upregulated in low-RFI Charolais steers. In KC, 12 DE miRNAs including (two novel miRNAs) were identified, of which 5 and 7 were downregulated and upregulated, respectively, in low-RFI steers (Table 4.4). Of the identified DE miRNAs, only *bta-miR-449a* was common to all the three breeds where it was upregulated in the low-RFI steers of the three population (Figure 4.3). The novel miRNA *bta-miR-AB-2* was

differentially expressed in both Angus and Charolais steers, however it was downregulated and upregulated in low-RFI Angus and Charolais steers, respectively.

4.4.4 TaqMan qPCR miRNA differential expression validation

Of the six selected miRNAs for validating RNAseq results using TaqMan qPCR, *bta-miR-133a* was significantly differentially expressed between high and low-RFI Charolais steers at P-value = 0.003 (< 0.05). *Bta-miR-223* and *bta-miR-424-5p* for KC had suggestive P-values of 0.054 and 0.086, respectively. Despite the qPCR expression profiles of most of the selected miRNAs not being significantly different between high and low-RFI steers as revealed by RNAseq, the methods showed similar trends of the miRNAs in the low-RFI animals as shown in Figure 4.4. All qPCR t-test and fold-changes between high and low-RFI animals are presented in Table S4 in Supplementary Data S11. Additionally, there was a 0.81 Pearson correlation between the qPCR and RNAseq $\log_2$-fold changes of the six miRNAs as shown Figure S2 of Supplementary Data S11.

4.4.5 Target gene prediction and functional enrichment analyses for the most abundant known and novel miRNAs

We performed target gene prediction for 16 known miRNAs that were highly expressed in the liver tissue of the animals from the three breeds (Table 4.2). At a threshold of the 99th context++ score percentile, we identified 1094 target genes. Of these target genes, 1053 mapped to the IPA ingenuity database and they are mainly involved in cell morphology, cellular assembly and organization, cell death and survival, cellular function and maintenance, and cellular development biological functions. All metabolic and cellular functions significantly enriched by the identified targets are presented in Figure S3 of the Supplementary Data S11. Within the cell death and survival category, the target genes are primarily involved in necrosis and apoptosis. For cellular

function and maintenance, the targets are mainly involved in maintaining cellular homeostasis. For cellular development, the target genes are involved in different cell type proliferation and development processes such as immune cell development and hepatic cell proliferation. Also, IPA identified sirtuin signaling, mitochondrial dysfunction, oxidative phosphorylation, LXR/RXR activation and acute phase response signaling as the most enriched pathways among the target genes.

For the novel miRNAs, the overlap among the top expressed miRNAs across the breeds was low, hence, we performed target gene prediction for the 20-top expressed of each breed (Table 4.3.). At a threshold of the 99th context++ score percentile, we identified 1584, 1973 and 1755 target genes for Angus, Charolais and KC. For Angus, of the identified targets, 1520 genes mapped to the IPA database and they were mainly involved in cellular development, cellular growth and proliferation, protein synthesis, cell cycle, and cell morphology. All metabolic and cellular functions significantly enriched by these targets are presented in Figure S4 of the Supplementary Data S11. Key IPA canonical pathways enriched by these target genes included oxidative phosphorylation, mitochondrial dysfunction, molybdenum cofactor biosynthesis, spermine biosynthesis, and estrogen-mediated S-phase entry pathways. For Charolais, 1898 targets mapped to the IPA database and were mainly involved in cellular movement, RNA post-transcriptional modification, cell cycle, cellular growth and proliferation and cell death and survival. All metabolic and cellular functions significantly enriched by these identified targets are presented in Figure S5 of the Supplementary Data S11. Mitochondrial dysfunction, oxidative phosphorylation, sirtuin signaling, assembly of RNA polymerase II complex and estrogen receptor signaling pathways were identified as the major enriched IPA canonical pathways. For KC, 1358 genes mapped to the IPA database and are mainly involved in RNA post-transcription modification,

RNA damage and repair, protein synthesis, cell morphology, and cell cycle biological functions. All significantly enriched metabolic and cellular identified targets are presented in Figure S6 of the Supplementary Data S11. In addition to oxidative phosphorylation, we identified four cholesterol biosynthesis related pathways including cholesterol biosynthesis I, cholesterol biosynthesis II (via 24, 25-dihydrolanpsterol), cholesterol biosynthesis III (via Desmosterol) and zymosterol biosynthesis, as the major enriched pathways by the target genes. All targets identified for the 16 most highly expressed miRNAs and the 20 highly expressed novel miRNAs and their Targetscan scoring parameter scores are provided in Supplementary Data S13.

4.4.6 DE miRNAs and DE target gene prediction

For the differentially expressed known and novel miRNAs (Table 4.4), we identified 44, 31 and 118 target DE genes that we previously reported as differentially expressed between high and low-RFI in the liver tissue of Angus, Charolais and KC, respectively, at a context++ score percentile > 50. Five of the identified targets included *SCD, LIPN1, LURAP1L, SERPINA3* and *TP53INP1,* and were common to all the three breeds (Figure 4.5). *DLK1* and *GNAZ* were common between Angus and Charolais. *AK4* and *NROB2* were shared between Charolais and KC. The Other 7 target genes included *FKBP5, SLCO4A1, ENDRA, TLE1, ZBTB16, TSKU* and *TTC39C,* and were common between Angus and KC, Figure 4.5. TargetScan results for the identified target DE genes for the DE miRNAs for the three breeds are provided in the Supplementary Data S14.

4.4.7 DE miRNAs and DE target genes interaction networks

The miRNA bta-miR-449a which was identified as the common DE miRNA in the three populations (upregulated in low-RFI steers in all the three breed), was predicted to target 16, 11 and 35 DE gene in Angus, Charolais and KC steers respectively, with three targets genes common to the three populations (*SERPINA3, TP53INP1* and *LPIN1*). For Angus, 8 DE genes were

identified as the major target genes for the DE miRNAs including *FKBP5* and *RAB30,* with each was targeted by six miRNAs. The other six major target genes including *COL1A1, ELOVL5, SCD, TLE1, TP53INP1* and *TTC39C* were predicted to be regulated by five DE miRNAs each, as shown in Figure 4.6. Of these targets, *FKBP5*, *COL1A1*, *SCD*, *TLE1* and *TP53INP1* were downregulated in low-RFI animals, whereas *RAB30*, *ELOVL5* and *TTC39C* were upregulated in the same animals. Additionally, the miRNAs and DE target gene interaction networks show *bta-miR-2285n, bta-miR-2285u* and *bta-miR-449a* (all upregulated) as the major miRNAs targeting 23, 19 and 16 DE genes, respectively (Figure 4.6). Of these target genes, 4 including *CHL1*, *TENM4 (*upregulated in low-RFI animals*)*, *LPIN1* and *ASCL1 (downregulated in low-RFI animals*) are co-regulated by the three miRNAs, whereas 10 including *SCD, FKBP5, MBNL3, GNAZ, TLE1 (*downregulated*)* and *EDNRA, ELOVL5, CPT1B, HOPX* and *DOPEY2* (upregulated) are predicted to be regulated by *bta-miR-2285* and *bta-miR-2285u*. Other major miRNAs including *bta-miR-AB-2, bta-miR-AB-47*, and *bta-miR-424-3p*, are predicted to regulate 14, 13 and 12 DE genes respectively.

For Charolais, the major targeted genes included *SIRPA* (predicted to be targeted by 10 DE miRNAs), *ABCC4* (predicted to be targeted by 10 DE miRNAs), *DLK1* and *TP53INP1* (each predicted to be targeted by eight DE miRNAs), *SCD*, *SLC7A5* and *THEM4* (each predicted to be targeted by seven DE miRNAs) and *AK4* (predicted to be regulated by six DE miRNAs) (Figure 4.7). *SLC7A5, TP53INP1, SCD and THEM4* were downregulated, whereas *AK4, SIRPA, DLK1 and ABCC4* were upregulated in low-RFI animals. Furthermore, *bta-miR-2285ai-5p* (14 targets), *bta-miR-7859* (12 targets), *bta-miR-2284ac* (11 targets), *bta-miR-AB-2* (11 targets), *bta-miR-449a* (10 targets) were predicted as the major regulators among the identified DE miRNAs and were all upregulated in liver tissue of low-RFI steers as shown in Figure 4.7. *SIRPA (*upregulated in low-RFI steers*)* is predicted to be co-regulated by all the five major DE miRNAs. The majority of DE

genes predicted to be co-regulated by *bta-mir-2284ac* and *bta-mir-2285ai-5p* were downregulated in low-RFI animals, and included *LURAP1L, SLC7A5, TP53INP1, PDK4, SCD, KLHL13* and *SLC4A4 (*downregulated in the low-RFI animals), and *ANXA2, SIRPA, ABCC4* and *CYP2C19* (upregulated in the liver tissue of Charolais steers of low-RFI).

For KC, the main predicted target DE genes included *FKBP5* (targeted by 9 DE miRNAs), *TP53INP1* (targeted by 8 DE miRNAs), *PPARGC1A* (targeted by 7 DE miRNAs), and *EDNRA*, *GCH1*, *IGF1* and *SCD* which were targeted by 6 DE miRNAs each. *FKBP5, SCD, TP53INP1, GCH1* and *PPARGC1A* were downregulated in the liver tissue of low-RFI animals, and *IGF1* and *EDNRA* were upregulated in the liver tissue of the same animals. Among the DE miRNAs, *bta-miR-424-5p* (targeting 61 DE genes), *bta-miR-2411-3p* (targeting 40 DE genes), bta-miR-223 (targeting 36 DE genes) and *bta-miR-449a* (targeting 35 DE genes) were identified as major regulators as shown in Figure 4.8. Two DE targets *CXCL10* (downregulated in low-RFI animals*)* and *TP53INP1* (downregulated in low-RFI animals) were predicted to be regulated by all these four major DE miRNAs. However, there were varying numbers of target gene co-regulated by two or three of these DE miRNAs. For example, *bta-miR-424-5p* (upregulated) and *bta-miR-2411-3p* (downregulated), were predicted to uniquely target 11 of the identified DE targets including *NMNAT2, USP2* and *SLC25A15* that were downregulated in low-RFI animals, and *GHR, CCND1, TTC39C, ELOVL2, IRAK3, IL20RA, MPZL2, NYNRIN* which were upregulated in the same animals. Also, *bta-miR-424-5p* and *bta-miR-223* and were predicted to co-regulate eight DE genes including *PER1, SLC7A2, RCL1*, *CYP1A1, PPARGC1A* and *PNP* (downregulated in low-RFI animals) and**,** *GCLC* and *MYCL* (upregulated in low-RFI animals). Other main DE miRNAs included *bta-miR-AB-63* (targeting 28 DE genes), *bta-miR-363* (targeting 26 DE genes), *bta-miR-*

155 (targeting 23 DE genes), *bta-miR-1246* (targeting 20 DE genes) and *bta-miR-2483-5p* (targeting 19 DE genes) as shown in Figure 4.8.

4.4.8 Functional enrichment analyses of the DE target genes

We performed IPA analysis to further characterize the biological importance of the identified DE targets. For Angus, the target genes are mainly involved in lipid metabolism, molecular transport, small molecule biochemistry, energy production and carbohydrate metabolism (Table 4.5). For Charolais, lipid metabolism, molecular transport, small molecule biochemistry, cellular movement, and cell-to-cell signaling, and interaction were identified as the main biological functions involving the identified DE targets (Table 4.5). For KC, the target DE genes are mainly involved in cell death and survival, amino acid metabolism, small molecule biochemistry, lipid metabolism, and vitamin and mineral metabolism (Table 4.5).

4.5 Discussion

4.5.1 RNAseq miRNA abundancy profiling

RNA sequencing offers greater resolution to profile miRNAs even at a low expression level in the cells (Motameny *et al.* 2010) and allows for parallel profiling of the abundance of known miRNAs, and the identification of novel miRNAs (Pritchard *et al.* 2012). Additionally, with the profiled miRNA sequences, the prediction of potential target genes for both known and novel miRNAs is possible (Motameny *et al.* 2010). In the current study we employed deep sequencing of small RNAs to profile miRNA expression in the liver tissue of 60 beef steers from three distinct beef breed populations. We obtained high quality sequence reads as revealed by our sequencing quality results with average Phred quality score of 36.7 across the samples of the three breeds. Additional sequence data quality control processing (including removal of other small miRNAs) resulted in read sequences with an average length of 22bp, and the majority of the reads ranging

between 20 and 24bp across the samples from the three breeds as shown in Figure 4.1a, hence, providing high quality and reliable reads for downstream alignment and abundancy profiling of miRNAs whose natural average length is 22 nucleotides (Friedländer *et al.* 2011).

4.5.2 Known miRNAs expression and functionality

With a high average mapping rate of 75%, we identified, 541, 551 and 575 known mature miRNAs expressed in the liver tissues of Angus, Charolais and KC steers, respectively. Interestingly, 90% of these miRNAs were expressed in the three populations, indicating a high similarity among the breed in term of hepatic miRNA expression. Similar expression patterns were also observed with the expression of protein coding genes in the same populations, where over 96% of the expressed genes were common among the liver tissues of the three beef breeds (Mukiibi *et al.* 2018).

Of the expressed miRNAs, 10 miRNAs including *bta-miR-192, bta-miR-143, bta-miR-148a, bta-miR-26a, bta-miR-30a-5p, bta-miR-22-3p, bta-miR-27b, bta-let-7f, bta-miR-27a-3p* and *bta-miR-101* showed extremely high expression in the liver tissue of the profiled animals across the three breeds accounting for 78.2% of the average aligned read counts in each breed. Interestingly, *bta-miR-101, bta-miR-143, bta-miR-30a-5p, bta-let-7f, bta-miR-192* and *bta-miR-148a* were previously reported among the 10 top-most expressed miRNAs in the liver tissue of Australian Angus steers (Al-Husseini *et al.* 2016) and Chinese Holstein dairy cows(Sun *et al.* 2019), indicating their stable high expression across a wide range of cattle breeds, despite the genetic distinctiveness of these animals. Additionally, Sun *et al.* (2019) reported high expression of these miRNAs in multiple studied tissues, with *bta-miR-143* and *bta-miR-27b* particularly showing high levels of expression in all the tissues from both beef and dairy animals. This implies the potential vitality of these miRNAs to modulate the biological processes of different tissues in

the body. The miRNA *bta-miR-192,* which was the most expressed miRNA across the three populations belongs to the *miR-192/215* family, whose homologous members have been implicated in several biological functions and disease disorders in different species. For example, *miR-192* in mice, has been reported to regulate genes involved in glucose metabolism, cell adhesion and migration, tumorigenesis and tumor progression, protein SUMOylation, epigenetic regulation and epithelial-mesenchymal transition of the hepatic cells through the *HNF4–miR-194*/ *miR-192* signaling pathway (Morimoto *et al.* 2017). In sheep, *miR-192* has been reported to be involved in regulating the growth and development of the skeletal muscle (Zhao *et al.* 2016).

Additionally, we identified the target genes of the highly expressed miRNAs across the three breeds. Functional enrichment analysis revealed that the candidate target genes were involved in some key biological processes including maintaining cellular homeostasis, proliferation of liver cells and apoptosis of cells. Consistent with our results, some miRNAs the majorly expressed miRNAs have been identified as important modulators of liver cellular metabolic homeostasis, liver cell proliferation and development, liver cell death and regeneration in different species (Chen & Verfaillie 2014). For example, *miR-143* which was the second most abundant miRNA in our studied samples was reported to be involved in glucose and insulin metabolism in mice (Jordan *et al.* 2011). *MiR-148* and *miR-26a* are involved in the regulation of mice hepatocyte proliferation (Zhou *et al.* 2012; Gailhouste *et al.* 2013), a key process in liver tissue regeneration. In the human and mouse liver tissue, *miR-148a* (Takagi *et al.* 2008) and miR-27 (Bates *et al.* 2010), respectively, have been identified as regulators liver detoxification. Based on our results and the conserved nature of miRNA-mRNA interaction across mammalian species (Friedman *et al.* 2009), we speculate that these highly expressed miRNAs in the bovine liver might play similar functions as those highlighted in other species, however molecular studies are needed

to validate the precise functions of these miRNAs in cattle given the physiological differences between the species.

We also identified several novel miRNAs, some of which showed substantial expression levels in the bovine liver tissues where they might be involved in regulating different metabolic or growth and development of the liver tissue. Indeed, our functional enrichment results showed the most highly expressed novel miRNA target genes are involved in key biological functions that relate to the normal liver functionality such as cell cycle, cellular growth and proliferation, cell death and survival and protein synthesis. Functional enrichment results for both highly expressed known and novel miRNAs suggest a strong connection between these miRNAs and liver tissue growth and development. These observations are plausible, since the liver is in constant self-regeneration or regrowth to recover hepatic tissue lost due to assault by pathogens, toxins, and exogenous antigens (Tao *et al.* 2017). Liver regeneration is a complex and highly regulated process that includes the initiation phase, the cell proliferation phase, and the regeneration termination phase, all of which are modulated by miRNAs (Chen *et al.* 2015; Yi *et al.* 2016).

4.5.3 Differentially expressed miRNAs between efficient and inefficient animals

The liver is a central metabolic organ serving major biological functions in the mammalian body including nutrients (lipids, carbohydrates, proteins/amino acid, and vitamins and minerals) metabolism, xenobiotics and toxin metabolism, pathogen processing and growth regulation (Häussinger 1996; Parker & Picut 2005). MicroRNAs are known to modulate all these functions (Chen & Verfaillie 2014), therefore, differential hepatic miRNA expression between efficient and inefficient animals can potentially contribute to the molecular variability in feed efficiency in beef cattle. To investigate the possible associations between liver miRNA expression and feed efficiency variability in beef cattle, we performed differential miRNA expression analysis between

steer groups of RFI phenotypes that were significantly different (high and low-RFI groups) from Angus, Charolais and KC populations. Indeed, we identified 39 differentially expressed known and novel miRNA between the steer groups in all the three studied populations. Most of the identified miRNAs were breed specific, which was consistent with our mRNA differential expression study (Mukiibi *et al.* 2018) that considered to the great extent the same animals from the three breeds, where most of the DE genes were breed specific as well. However, *bta-miR-449a* was differentially expressed in all the three breeds, with consistent upregulation in the liver tissue of low-RFI steers in all the breeds.

Within each breed, the majority (i.e. 58.3% for Angus, 66.7% for Charolais and 66.7% for KC) of the differentially expressed miRNAs were upregulated in low-RFI animals, and hence suggests a general expectation of reduced expression of their target genes. Compared with the previous studies that have investigated the association of miRNA expression with feed efficiency (Al-Husseini *et al.* 2016; De Oliveira *et al.* 2018), *bta-miR-424-5p* was the only miRNA that was common between the DE miRNAs identified in our study and those reported by Al-Hussein *et al.* (2015)**.** However, in our study, *bta-miR-424-5p* was upregulated in the liver tissue of low-RFI animals (KC), whereas Al-Hussein *et al.* (2015) reported it to be downregulated in the liver tissue of low-RFI Angus bulls. This difference could be due to the genetic differences between the animals or the diverse sequencing library construction methods used in the two studies. Al-Hussein *et al.* (2015) sequenced two cDNA libraries of pooled RNA from efficient and inefficient animals, whereas in our study we independently sequenced the cDNA libraries of the studied animals.

We performed, qPCR validation of the RNAseq differential miRNA expression using six of the identified differentially expressed miRNAs. Of these, *bta-miR-133a* showed significant differential expression, whereas, *bta-miR-424-5p* and *bta-miR-223* showed suggestive differential

expression between high and low-RFI animals. However, qPCR results of all the six miRNAs showed a similar trend of expression (in low-RFI animals) as revealed by RNAseq in the same animals. Additionally, the miRNA expression profiles from the two methods show a high correlation of 0.81. These results provide great confidence about the reliability of both the technical miRNA profiling by RNA sequencing and the consequent bioinformatic processing of the sequence data.

To investigate the biological importance of the DE miRNAs within each breed, we predicted target genes that were among the differentially expressed DE genes previously reported in the same animal populations (Mukiibi *et al.* 2018). Of the DE genes, 61%, 75.6% and 67.4% were predicted as potential targets of the DE miRNAs in Angus, Charolais and KC steers, respectively. These target genes are mainly involved in lipid metabolism, molecular transport, small molecule biochemistry, energy production, carbohydrate metabolism, cellular movement, cell-to-cell signaling and interaction, cell death and survival, amino acid metabolism and vitamin and mineral metabolism, implying that the identified DE miRNAs influence feed efficiency through differential modulation of the different processes underlying these functions in the liver. From the DE miRNA – DE mRNA interaction networks in in Figure 4.4, Figure 4.5 and Figure 4.6, it can be observed that miRNAs were predicted to target multiple DE genes, and single genes were predicted to be targets for multiple miRNAs. This complex form of miRNAs-mRNAs interaction emanates from the fact that a single miRNA using its seed region, can bind to multiple sites in the 3'-UTRs of different genes (mRNAs), and also one target can have multiple binding sites for several miRNAs (Creighton *et al.* 2009; Hashimoto *et al.* 2013), hence allowing miRNAs to modulate multiple biological processes even though they are small in numbers as compared to the mRNAs that they regulate.

To a large extent, we observed contrasting expression between DE genes and DE miRNAs in the liver tissues of low-RFI animals, for example, in Angus, of the identified DE target genes for the upregulated miRNAs *bta-miR-2285n*, *bta-miR-2285u*, *bta-miR-449a* and *bta-miR-47*, 52%, 63%, 75% and 61.5% were downregulated. For Charolais, of the predicted DE targets of upregulated (in low-RFI animals) miRNAs *bta-miR-2285ai-5p*, *bta-miR-7859*, *bta-miR-2284ac* and *bta-miR-449a*, 57%, 50%, 45%, 72% and 36%, respectively, were downregulated. In KC, of the predicted DE targets of upregulated (in low-RFI animals) major DE miRNAs *bta-miR-424-5p*, *bta-miR-223*, *bta-miR-449a* and *bta-miR-363*, 62%, 67%, 66% and 54%, respectively, were downregulated. However, we also observed a significant number of upregulated predicted target genes despite being targeted by multiple upregulated miRNAs. These observations could be attributed to the different mechanisms of miRNA gene regulation in the mammalian cells including augmenting mRNA degradation through deadenylation and translation (proteins) repression when they bind to the 3' UTRs of their targets (Stroynowska-Czerwinska *et al.* 2014). Therefore, at the transcriptome level we can only detect miRNA's regulation activity if its mode of action on genes is through degradation of the mRNA. However, if the mode of action is via repression of mRNA translation into protein, the regulation affected can only be detected at the proteome level (through proteomics) since mRNA copies will not be changed by the increased expression of the miRNAs (Creighton *et al.* 2009; Saito & Sætrom 2010).

4.6 Conclusions

In the current study we employed RNAseq to performed hepatic miRNAome profiling of beef steers from Angus, Charolais and KC populations. We identified a total of 588 expressed known bovine miRNAs of which 90% were expressed in the liver tissue of the animals from the three populations. Of these miRNAs, *bta-miR-192, bta-miR-143, bta-miR-148a, bta-miR-26a, bta-*

miR-30a-5p, bta-miR-22-3p, bta-miR-27b, bta-let-7f, bta-miR-27a-3p and *bta-miR-101* were identified as the most expressed miRNAs in all three breeds. We also identified 241 novel bovine miRNAs expressed in the liver tissue, 69% identified as expression in only one of the three breeds, whereas, 13% were identified as expressed in all the three populations. Differential miRNA expression analyses identified 39 miRNAs as associated with feed efficiency including five novel miRNAs (*bta-miR-AB-2, bta-miR-AB-47, bta-miR-AB-15, bta-miR-AB-63* and *bta-miR-AB-225*). The majority of the DE miRNAs were breed specific, and *bta-miR-449a* was differentially express in all three populations. The identified DE miRNAs were predicted to target mainly the genes that have been previously identified as differentially expressed in the liver tissues of feed efficient and inefficient animals from the same populations. Our results provide insight into the hepatic miRNAome expression profile of beef cattle and potential molecular regulatory mechanism of feed efficiency in beef cattle.

4.7 References

Abo-Ismail M.K., Vander Voort G., Squires J.J., Swanson K.C., Mandell I.B., Liao X., Stothard P., Moore S., Plastow G. & Miller S.P. (2014) Single nucleotide polymorphisms for feed efficiency and performance in crossbred beef cattle. *BMC genetics* 15, 14.

Agarwal V., Bell G.W., Nam J.-W. & Bartel D.P. (2015) Predicting effective microRNA target sites in mammalian mRNAs. *elife* 4, e05005.

Ahola J.K. & Hill R.A. (2012) Input Factors Affecting Profitability: a Changing Paradigm and a Challenging Time. In: *Feed efficiency in the beef industry* (ed. by Hill RA), p. 10. Wiley-Blackwell, Ames, Iowa.

Al-Husseini W., Chen Y., Gondro C., Herd R.M., Gibson J.P. & Arthur P.F. (2016) Characterization and profiling of liver microRNAs by RNA-sequencing in cattle

divergently selected for residual feed intake. *Asian-Australasian journal of animal sciences* 29, 1371.

Alexandre P.A., Kogelman L.J., Santana M.H., Passarelli D., Pulz L.H., Fantinato-Neto P., Silva P.L., Leme P.R., Strefezzi R.F. & Coutinho L.L. (2015) Liver transcriptomic networks reveal main biological processes associated with feed efficiency in beef cattle. *BMC genomics* 16, 1073.

Andrews S. (2010) FastQC: a quality control tool for high throughput sequence data.

Barendse W., Reverter A., Bunch R.J., Harrison B.E., Barris W. & Thomas M.B. (2007) A validated whole-genome association study of efficient food conversion in cattle. *Genetics* 176, 1893-905.

Bartel D.P. (2018) Metazoan MicroRNAs. *Cell* 173, 20-51.

Bates D.J., Li N., Liang R., Sarojini H., An J., Masternak M.M., Bartke A. & Wang E. (2010) MicroRNA regulation in Ames dwarf mouse liver may contribute to delayed aging. *Aging cell* 9, 1-18.

Carvalho E.B., Gionbelli M.P., Rodrigues R.T., Bonilha S.F., Newbold C.J., Guimarães S.E., Silva W., Verardo L.L., Silva F.F. & Detmann E. (2019) Differentially expressed mRNAs, proteins and miRNAs associated to energy metabolism in skeletal muscle of beef cattle identified for low and high residual feed intake. *BMC genomics* 20, 501.

Chen C., Ridzon D.A., Broomer A.J., Zhou Z., Lee D.H., Nguyen J.T., Barbisin M., Xu N.L., Mahuvakar V.R. & Andersen M.R. (2005) Real-time quantification of microRNAs by stem–loop RT–PCR. *Nucleic acids research* 33, e179-e.

Chen X., Zhao Y., Wang F., Bei Y., Xiao J. & Yang C. (2015) MicroRNAs in liver regeneration. *Cellular Physiology and Biochemistry* 37, 615-28.

Chen Y., Gondro C., Quinn K., Herd R., Parnell P. & Vanselow B. (2011) Global gene expression profiling reveals genes expressed differentially in cattle with high and low residual feed intake. *Animal genetics* 42, 475-90.

Chen Y. & Verfaillie C.M. (2014) Micro RNA s: the fine modulators of liver development and function. *Liver International* 34, 976-90.

Creighton C.J., Reid J.G. & Gunaratne P.H. (2009) Expression profiling of microRNAs by deep sequencing. *Briefings in bioinformatics* 10, 490-7.

de Oliveira P.S., Cesar A.S., do Nascimento M.L., Chaves A.S., Tizioto P.C., Tullio R.R., Lanna D.P., Rosa A.N., Sonstegard T.S. & Mourao G.B. (2014) Identification of genomic regions associated with feed efficiency in Nelore cattle. *BMC genetics* 15, 100.

De Oliveira P.S., Coutinho L.L., Tizioto P.C., Cesar A.S., de Oliveira G.B., Diniz W.J.d.S., De Lima A.O., Reecy J.M., Mourão G.B. & Zerlotini A. (2018) An integrative transcriptome analysis indicates regulatory mRNA-miRNA networks for residual feed intake in Nelore cattle. *Scientific reports* 8, 17072.

Fang L., Sahana G., Ma P., Su G., Yu Y., Zhang S., Lund M.S. & Sørensen P. (2017) Use of biological priors enhances understanding of genetic architecture and genomic prediction of complex traits within and between dairy cattle breeds. *BMC genomics* 18, 604.

Friedländer M.R., Mackowiak S.D., Li N., Chen W. & Rajewsky N. (2011) miRDeep2 accurately identifies known and hundreds of novel microRNA genes in seven animal clades. *Nucleic acids research* 40, 37-52.

Friedman R.C., Farh K.K.-H., Burge C.B. & Bartel D.P. (2009) Most mammalian mRNAs are conserved targets of microRNAs. *Genome research* 19, 92-105.

Gailhouste L., Gomez-Santos L., Hagiwara K., Hatada I., Kitagawa N., Kawaharada K., Thirion M., Kosaka N., Takahashi R.u. & Shibata T. (2013) miR-148a plays a pivotal role in the liver by promoting the hepatospecific phenotype and suppressing the invasiveness of transformed cells. *Hepatology* 58, 1153-65.

Gregory R.I. & Shiekhattar R. (2005) MicroRNA biogenesis and cancer. *Cancer research* 65, 3509-12.

Griffiths-Jones S., Saini H.K., van Dongen S. & Enright A.J. (2007) miRBase: tools for microRNA genomics. *Nucleic acids research* 36, D154-D8.

Hashimoto Y., Akiyama Y. & Yuasa Y. (2013) Multiple-to-multiple relationships between microRNAs and target genes in gastric cancer. *PloS one* 8, e62589.

Häussinger D. (1996) Physiological functions of the liver. In: *Comprehensive human physiology* (pp. 1369-91. Springer.

Hegarty R., Goopy J., Herd R. & McCorkell B. (2007) Cattle selected for lower residual feed intake have reduced daily methane production. *Journal of animal science* 85, 1479-86.

Jordan S.D., Krüger M., Willmes D.M., Redemann N., Wunderlich F.T., Brönneke H.S., Merkwirth C., Kashkar H., Olkkonen V.M. & Böttger T. (2011) Obesity-induced overexpression of miRNA-143 inhibits insulin-stimulated AKT activation and impairs glucose metabolism. *Nature cell biology* 13, 434.

Khansefid M., Millen C., Chen Y., Pryce J., Chamberlain A., Vander Jagt C., Gondro C. & Goddard M. (2017) Gene expression analysis of blood, liver, and muscle in cattle divergently selected for high and low residual feed intake. *Journal of animal science* 95, 4764-75.

Kong R.S., Liang G., Chen Y. & Stothard P. (2016) Transcriptome profiling of the rumen epithelium of beef cattle differing in residual feed intake. *BMC genomics* 17, 592.

Langmead B., Trapnell C., Pop M. & Salzberg S.L. (2009) Ultrafast and memory-efficient alignment of short DNA sequences to the human genome. *Genome biology* 10, R25.

Love M.I., Huber W. & Anders S. (2014) Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. *Genome biology* 15, 550.

Markham N.R. & Zuker M. (2008) UNAFold. In: *Bioinformatics* (pp. 3-31. Springer.

Martin M. (2011) Cutadapt removes adapter sequences from high-throughput sequencing reads. *EMBnet. journal* 17, 10-2.

Morimoto A., Kannari M., Tsuchida Y., Sasaki S., Saito C., Matsuta T., Maeda T., Akiyama M., Nakamura T. & Sakaguchi M. (2017) An HNF4α–microRNA-194/192 signaling axis maintains hepatic cell function. *Journal of Biological Chemistry* 292, 10574-85.

Motameny S., Wolters S., Nürnberg P. & Schumacher B. (2010) Next generation sequencing of miRNAs–strategies, resources and methods. *Genes* 1, 70-84.

Moutinho C. & Esteller M. (2017) MicroRNAs and epigenetics. In: *Advances in cancer research* (pp. 189-220. Elsevier.

Mukiibi R., Vinsky M., Keogh K.A., Fitzsimmons C., Stothard P., Waters S.M. & Li C. (2018) Transcriptome analyses reveal reduced hepatic lipid synthesis and accumulation in more feed efficient beef cattle. *Scientific reports* 8, 7303.

Murakami Y. & Kawada N. (2017) MicroRNAs in hepatic pathophysiology. *Hepatology Research* 47, 60-9.

Nkrumah J., Okine E., Mathison G., Schmid K., Li C., Basarab J., Price M., Wang Z. & Moore S. (2006) Relationships of feedlot feed efficiency, performance, and feeding behavior with

metabolic rate, methane production, and energy partitioning in beef cattle. *Journal of animal science* 84, 145-53.

Nkrumah J., Sherman E., Li C., Marques E., Crews D., Bartusiak R., Murdoch B., Wang Z., Basarab J. & Moore S. (2007) Primary genome scan to identify putative quantitative trait loci for feedlot growth rate, feed intake, and feed efficiency of beef cattle. *Journal of animal science* 85, 3170-81.

O'Brien J., Hayder H., Zayed Y. & Peng C. (2018) Overview of microrna biogenesis, mechanisms of actions, and circulation. *Frontiers in endocrinology* 9, 402.

Paradis F., Yue S., Grant J., Stothard P., Basarab J. & Fitzsimmons C. (2015) Transcriptomic analysis by RNA sequencing reveals that hepatic interferon-induced genes may be associated with feed efficiency in beef heifers. *Journal of animal science* 93, 3331-41.

Parker G.A. & Picut C.A. (2005) Liver immunobiology. *Toxicologic Pathology* 33, 52-62.

Pritchard C.C., Cheng H.H. & Tewari M. (2012) MicroRNA profiling: approaches and considerations. *Nature Reviews Genetics* 13, 358.

Robinson M.D., McCarthy D.J. & Smyth G.K. (2010) edgeR: a Bioconductor package for differential expression analysis of digital gene expression data. *Bioinformatics* 26, 139-40.

Robinson M.D. & Oshlack A. (2010) A scaling normalization method for differential expression analysis of RNA-seq data. *Genome biology* 11, R25.

Saatchi M., Beever J.E., Decker J.E., Faulkner D.B., Freetly H.C., Hansen S.L., Yampara-Iquise H., Johnson K.A., Kachman S.D. & Kerley M.S. (2014) QTLs associated with dry matter intake, metabolic mid-test weight, growth and feed efficiency have little overlap across 4 beef cattle studies. *BMC genomics* 15, 1004.

Saito T. & Sætrom P. (2010) MicroRNAs–targeting and target prediction. *New biotechnology* 27, 243-9.

Shannon P., Markiel A., Ozier O., Baliga N.S., Wang J.T., Ramage D., Amin N., Schwikowski B. & Ideker T. (2003) Cytoscape: a software environment for integrated models of biomolecular interaction networks. *Genome research* 13, 2498-504.

Stroynowska-Czerwinska A., Fiszer A. & Krzyzosiak W.J. (2014) The panorama of miRNA-mediated mechanisms in mammalian cells. *Cellular and Molecular Life Sciences* 71, 2253-70.

Sun H.-Z., Chen Y. & Guan L., Luo (2019) MicroRNA expression profiles across blood and different tissues in cattle. *Scientific data* 6.

Takagi S., Nakajima M., Mohri T. & Yokoi T. (2008) Post-transcriptional regulation of human pregnane X receptor by micro-RNA affects the expression of cytochrome P450 3A4. *Journal of Biological Chemistry* 283, 9674-80.

Tao Y., Wang M., Chen E. & Tang H. (2017) Liver regeneration: analysis of the main relevant signaling molecules. *Mediators of inflammation* 2017.

Tizioto P.C., Coutinho L.L., Decker J.E., Schnabel R.D., Rosa K.O., Oliveira P.S., Souza M.M., Mourão G.B., Tullio R.R. & Chaves A.S. (2015) Global liver gene expression differences in Nelore steers with divergent residual feed intake phenotypes. *BMC genomics* 16, 242.

Tizioto P.C., Coutinho L.L., Oliveira P.S., Cesar A.S., Diniz W.J., Lima A.O., Rocha M.I., Decker J.E., Schnabel R.D. & Mourão G.B. (2016) Gene expression differences in Longissimus muscle of Nelore steers genetically divergent for residual feed intake. *Scientific reports* 6.

Weber K.L., Welly B.T., Van Eenennaam A.L., Young A.E., Porto-Neto L.R., Reverter A. & Rincon G. (2016) Identification of gene networks for residual feed intake in angus cattle using genomic prediction and RNA-seq. *PloS one* 11, e0152274.

Yi P.-S., Zhang M. & Xu M.-Q. (2016) Role of microRNA in liver regeneration. *Hepatobiliary & Pancreatic Diseases International* 15, 141-6.

Zhao Q., Kang Y., Wang H.-Y., Guan W.-J., Li X.-C., Jiang L., He X.-H., Pu Y.-B., Han J.-L. & Ma Y.-H. (2016) Expression profiling and functional characterization of miR-192 throughout sheep skeletal muscle development. *Scientific reports* 6, 30281.

Zhou J., Ju W., Wang D., Wu L., Zhu X., Guo Z. & He X. (2012) Down-regulation of microRNA-26a promotes mouse hepatocyte proliferation during liver regeneration. *PloS one* 7, e33577.

4.8 Figures and Tables

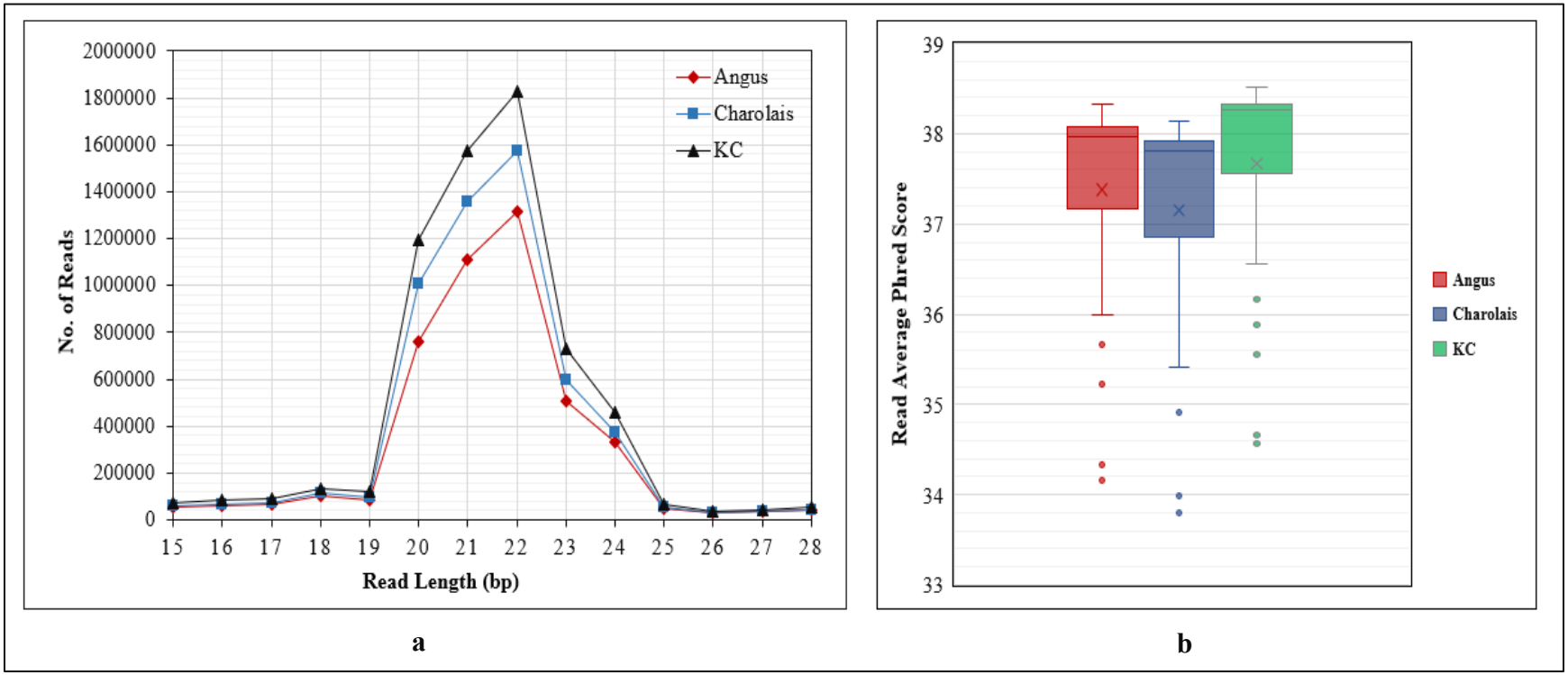


Figure 4.1. (a) Line plot showing read lengths distribution in the final cleaned sequence data after quality control involving; 3' Illumina sequencing adaptor clipping, removing very long reads (>28bp) and short reads (< 18bp) and removing reads that mapped other small RNA species (rRNAs, snRNAs, tRNAs and snoRNAs) for Angus, Charolais and KC samples**; (b)** Box plots showing average Phred quality score of the retained reads**.**

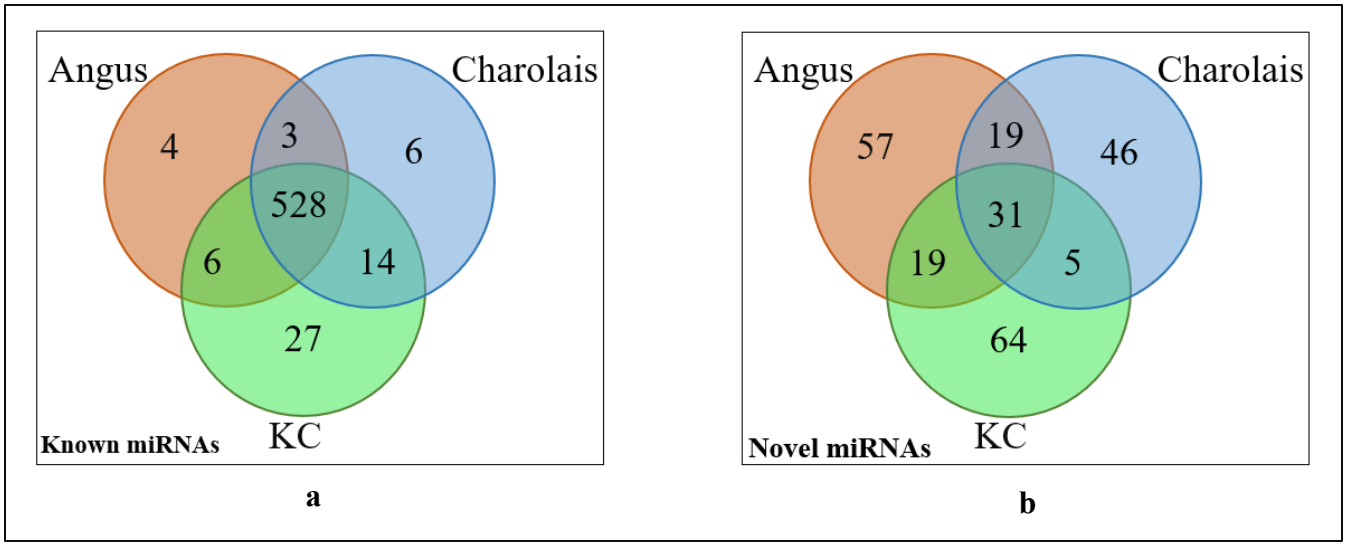


Figure 4.2.(a) Venn diagram showing overlap of expressed known miRNAs in the liver tissue of steers from the three studied breeds (Angus, Charolais and KC); (b) Venn diagram showing overlap of Novel miRNAs identified between the three studied breeds (Angus, Charolais and KC).

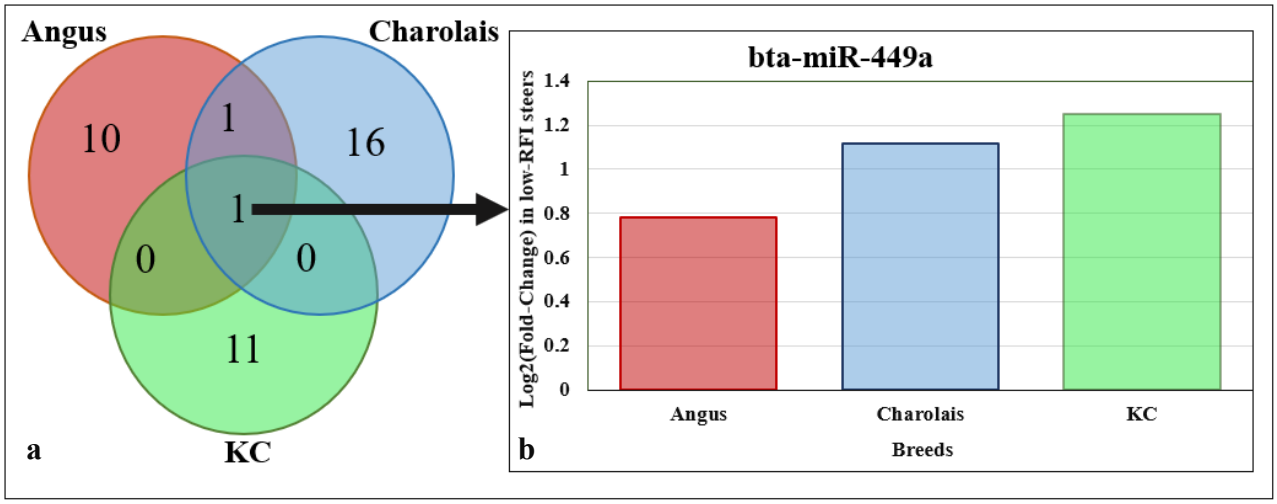


Figure 4.3.(a)Venn diagram showing differentially expressed miRNA overlap between the studied populations (Angus, Charolais and KC); (b) Bar plot showing expression of bta-miR-449a in low-RFI steers of the three breeds.

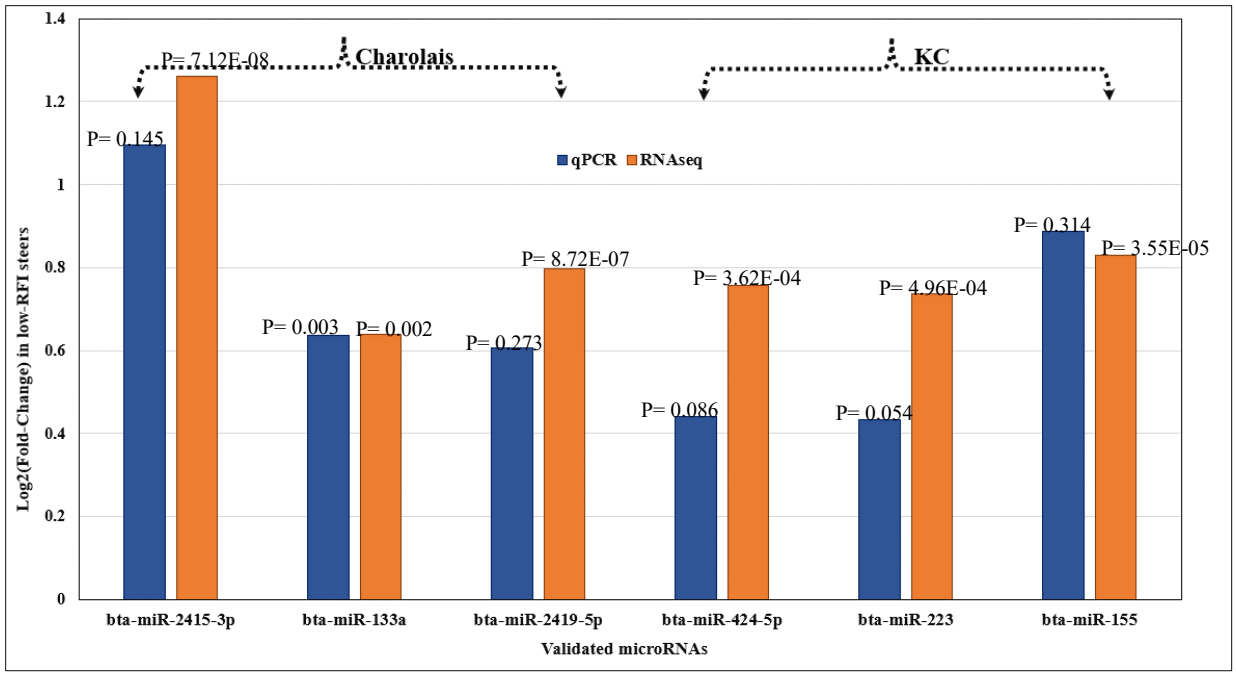


Figure 4.4. Comparison of the expression (in log_2(Fold-Change)) of six differentially expressed miRNAs in low-RFI animals as estimated using qPCR and RNAseq methods, P= P-value of expression difference test between high and low-RFI animals.

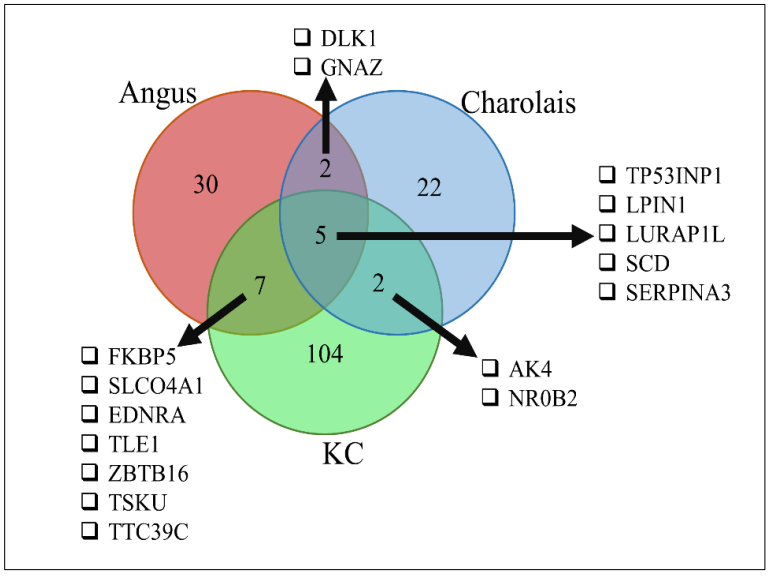
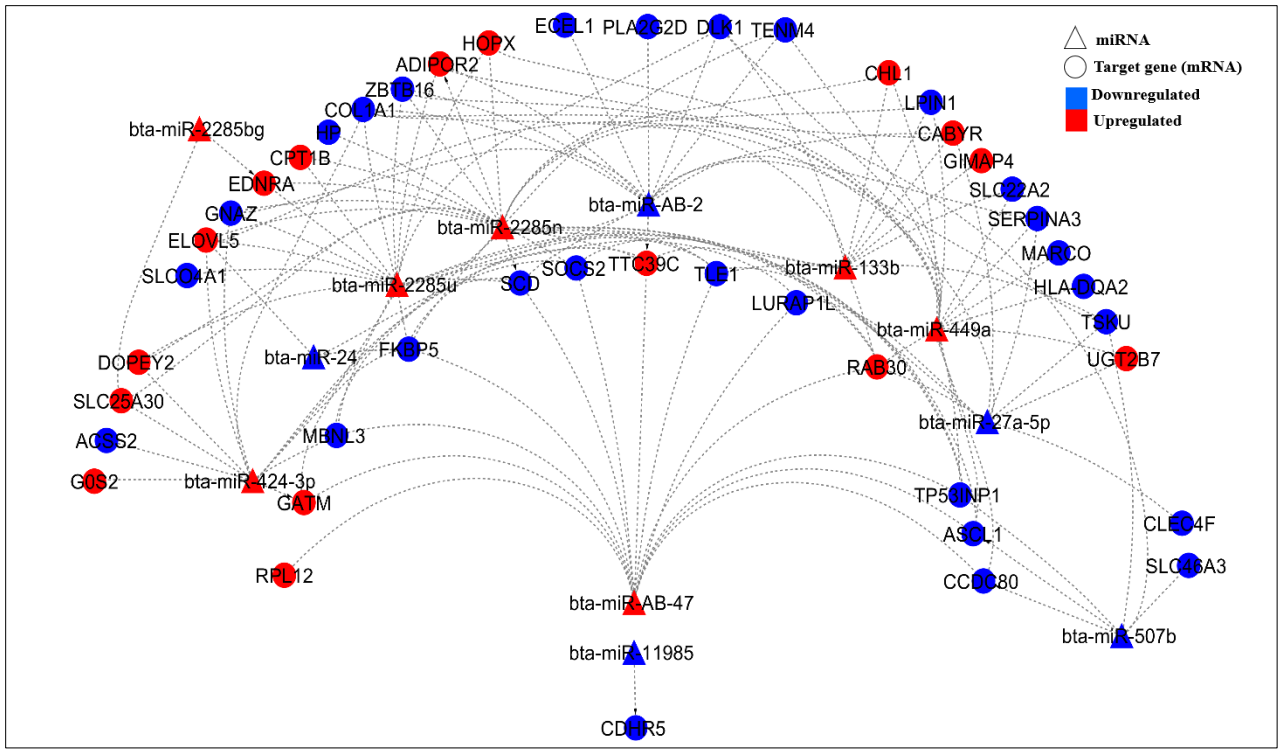
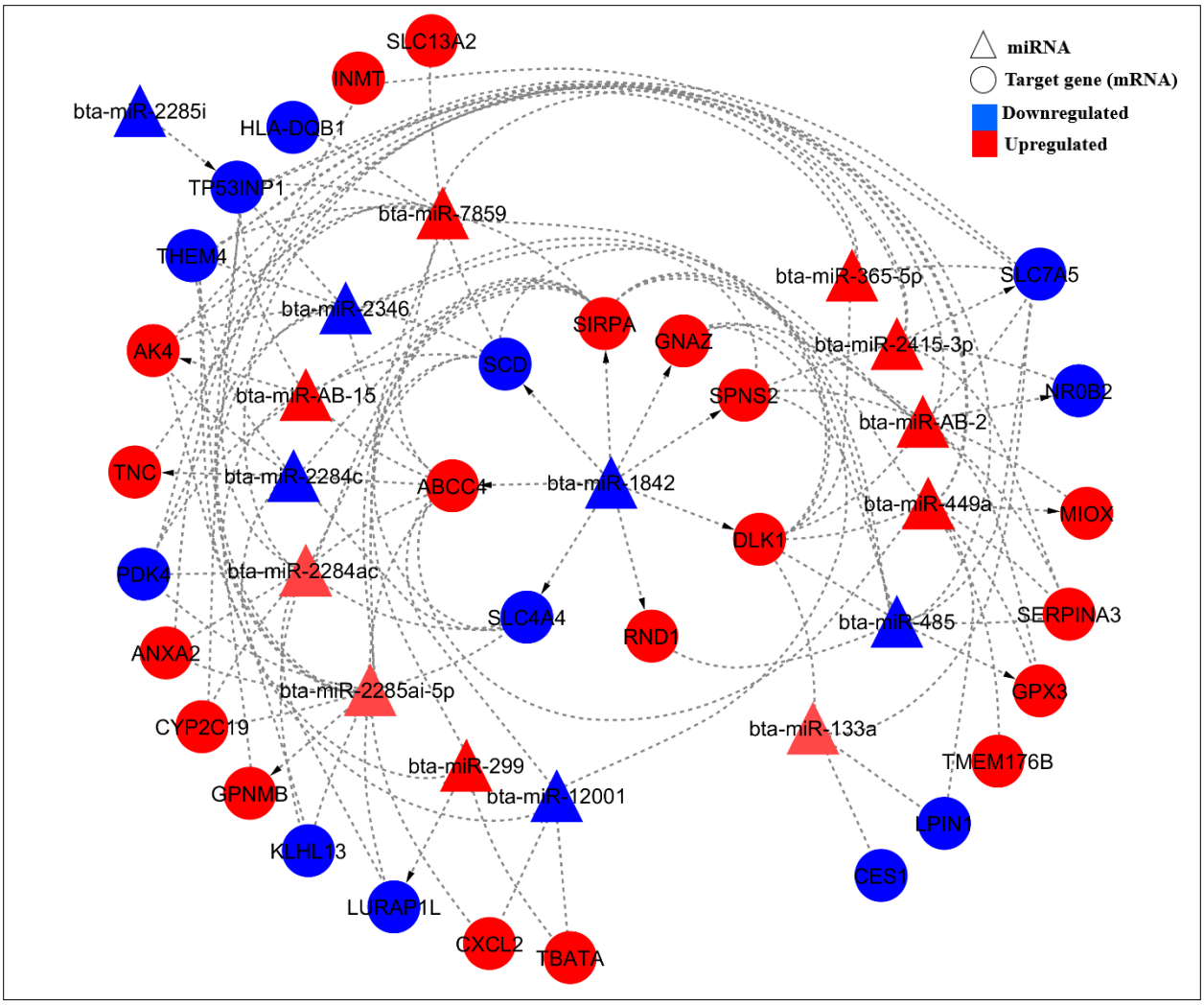


Figure 4.5. Venn diagram showing differentially expressed target genes for the DE miRNAs in the liver tissue of Angus, Charolais and KC steers.



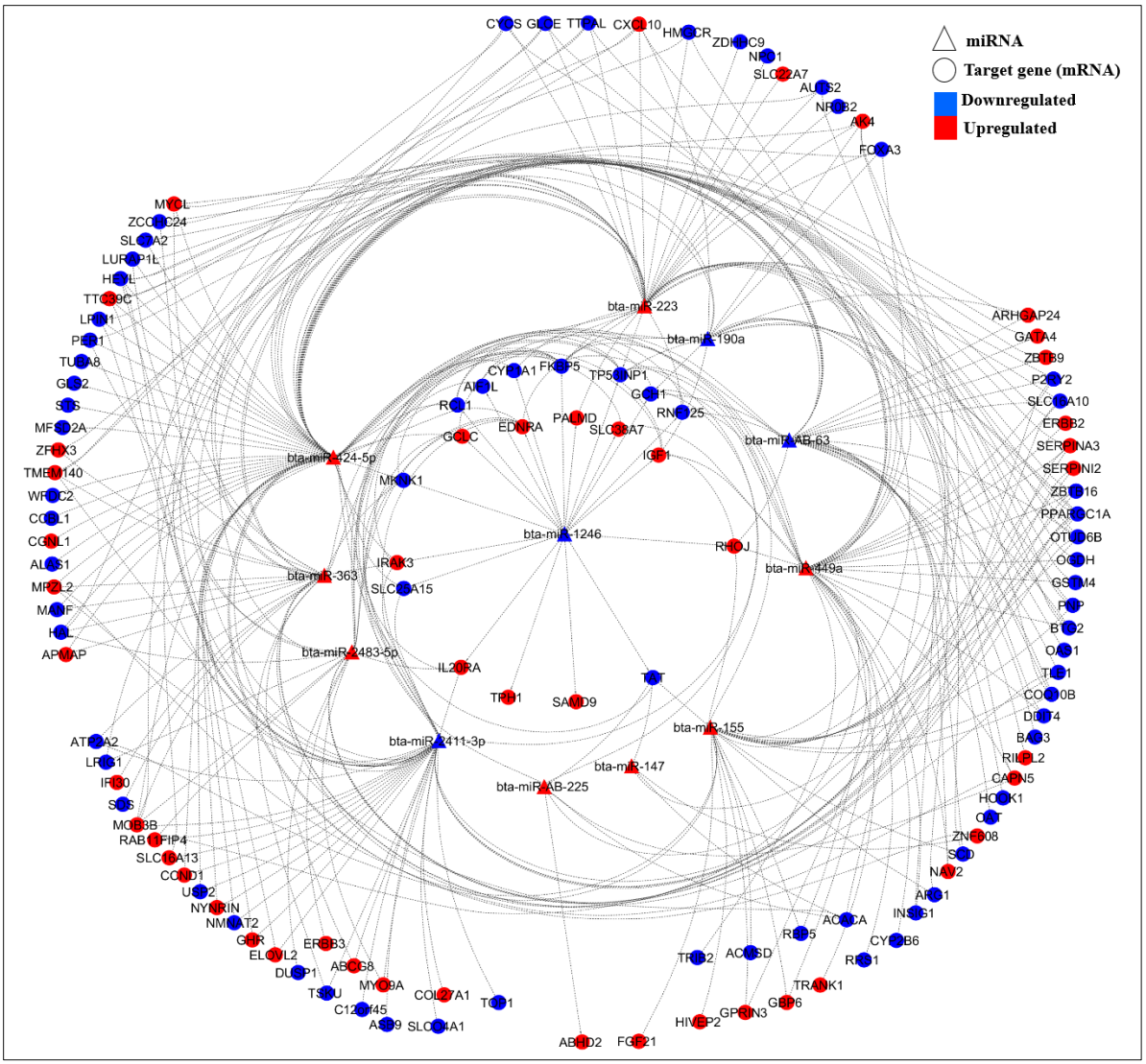
Red and blue indicate increased and reduced expression respectively of the gene or micro RNA in the liver tissue of low-RFI Angus steers relative to high-RFI Angus animals.

Figure 4.6. DE target genes and DE miRNA interaction network and regulation of both DE miRNAs and DE targets in the liver tissue of low-RFI Angus steers.



Red and blue indicate increased and reduced expression respectively of the gene or micro RNA in the liver tissue of low-RFI Charolais steers relative to high-RFI Charolais animals.

Figure 4.7. DE target genes and DE miRNA interaction network and regulation of both DE miRNAs and DE targets in the liver tissue of low-RFI Charolais steers.



Red and blue indicate increased and reduced expression respectively of the gene or micro RNA in the liver tissue of low-RFI Angus steers relative to high-RFI Angus animals.

Figure 4.8. DE target genes and DE miRNA interaction network and regulation of both DE miRNAs and DE targets in the liver tissue of low-RFI KC steers.

Table 4.1. miRNA sequence data quality and expression.

	Angus	**Charolais**	**Kinsella Composite**
Before quality control			
Average no. of reads	9,450,928	9,620,729	11,230,561
Read length/bp	51	51	51
Average quality score	37.30	37.00	35.93
After quality control			
Average no. of reads	4,553,319	5,497,788	6,483,795
Average lengths	22bp	21bp	22bp
Mapping/alignment rate	74.8%	72.5%	77.1%

Table 4.2. Twenty highly expressed miRNAs (by aligned read counts) from each of the three populations (Angus, Charolais and KC) studied.

	Angus		Charolais		KC	
	Expressed Known miRNAs	**Average count/sample**	**Expressed Known miRNAs**	**Average count/sample**	**Expressed Known miRNAs**	**Average count/sample**
1	***bta-miR-192***	867342	***bta-miR-192***	1060828	***bta-miR-192***	1272798
2	***bta-miR-143***	613476	***bta-miR-143***	778165	***bta-miR-143***	961386
3	***bta-miR-148a***	479349	***bta-miR-148a***	535207	***bta-miR-148a***	656136
4	***bta-miR-26a***	177987	***bta-miR-26a***	225551	***bta-miR-26a***	273009
5	***bta-miR-30a-5p***	163172	***bta-miR-30a-5p***	180528	***bta-miR-30a-5p***	225973
6	***bta-miR-22-3p***	145620	***bta-miR-22-3p***	156016	***bta-miR-22-3p***	183337
7	***bta-miR-27b***	110066	***bta-miR-27b***	120648	***bta-miR-27b***	154701
8	***bta-let-7f***	108663	***bta-let-7f***	117980	***bta-let-7f***	126847
9	***bta-miR-27a-3p***	70791	***bta-miR-27a-3p***	77866	***bta-miR-27a-3p***	96651
10	***bta-miR-101***	65614	***bta-miR-101***	74408	***bta-miR-101***	90249
11	***bta-miR-126-5p***	56376	***bta-miR-21-5p***	66516	***bta-miR-126-5p***	84323
12	***bta-miR-21-5p***	52293	***bta-miR-126-5p***	66357	***bta-miR-21-5p***	73380
13	***bta-miR-92a***	44493	***bta-miR-191***	51924	***bta-miR-191***	69342
14	***bta-miR-191***	42488	***bta-miR-215***	49126	***bta-miR-92a***	60130
15	***bta-let-7a-5p***	38480	***bta-miR-92a***	47179	*bta-miR-100*	53849
16	***bta-miR-215***	35905	***bta-let-7a-5p***	43199	***bta-let-7a-5p***	50790
17	*bta-miR-486*	30488	*bta-miR-122*	37749	***bta-miR-215***	46908
18	*bta-miR-30e-5p*	30000	*bta-miR-181a*	36215	*bta-miR-122*	45063
19	*bta-miR-100*	29097	*bta-miR-26b*	33505	*bta-miR-486*	42147
20	*bta-miR-181a*	28292	*bta-miR-30e-5p*	32822	*bta-miR-26b*	41433

"**Bolded miRNAs**" = 16 highly expressed miRNAs across the three breeds.

Table 4.3. Top 20 expressed novel miRNAs identified in the liver tissue of Angus, Charolais and KC steers.

Angus				
Provisional ID	**miRDeep2 score**	**Estimated probability that the miRNA candidate is a true positive**	**Total read count**	**Mature miRNA consensus sequence**
bta-miR-AB-10	18895.5	83±5%	37061	aaagcugaaugaacuuuuuggc
bta-miR-AB-9	4.9	77±4%	35497	agagaugaagcacuggagc
bta-miR-AB-122	5.5	83±4%	9964	ugggcugcagugcgcuaugcc
bta-miR-AB-83	3438.3	83±5%	6743	aaaaccugaaugaacuuuu
bta-miR-AB-93	1927.8	83±5%	3784	aaagaaguuuguuuggguuuu
bta-miR-AB-59	5.1	83±4%	3766	caaaaaguuuguuuggguuuu
bta-miR-AB-65	1854.7	83±5%	3641	aaaaagguuuguuuggguuuu
bta-miR-AB-27	1789.4	83±5%	3501	aaaaaaguuuguuuggauuuu
bta-miR-AB-95	5.2	83±4%	3466	aaaaaaguuuguguggguuuu
bta-miR-AB-52	1663.5	83±5%	3254	aaaaaaguuuguuuggguuuu
bta-miR-AB-29	1435.9	83±5%	2816	acucgaacgaauuuuuggcc
bta-miR-AB-3	4.8	77±4%	2725	guccaguuuucccaggaa
bta-miR-AB-2	6.2	84±5%	1536	gggggccggcggcggcggcggc
bta-miR-AB-54	4.6	77±4%	1210	gaaaaaguuuguuuggguuu
bta-miR-AB-67	4.3	77±4%	1116	aaaaaaguuuguuugggauu
bta-miR-AB-28	4.8	77±4%	1051	caaaaaguucguccagauuuu
bta-miR-AB-12	4.9	77±4%	1041	aucccacuucugacacca
bta-miR-AB-23	502	83±5%	985	acaaccugaaugaacuuuuuga
bta-miR-AB-19	5.1	83±4%	976	ucaaguagcucacagucuag
bta-miR-AB-63	467.3	83±5%	915	ggaauaccggguacuguaggcu
Charolais				
Provisional ID	**miRDeep2 score**	**Estimated probability that the miRNA candidate is a true positive**	**Total read count**	**Mature miRNA consensus sequence**
bta-miR-AB-10	29069.9	77±6%	57018	aaagcugaaugaacuuuuuggc
bta-miR-AB-9	4.9	70±4%	54090	agagaugaagcacuggagc
bta-miR-AB-3	4.8	70±4%	3570	guccaguuuucccaggaa

bta-miR-AB-29	1680.6	77±6%	3296	acucgaacgaauuuuuggcc
bta-miR-AB-19	5.1	78±5%	1897	ucaaguagcucacagucuag
bta-miR-AB-2	6.2	78±5%	1291	gggggccggcggcggcggcggc
bta-miR-AB-12	4.9	70±4%	1173	aucccacuucugacacca
bta-miR-AB-23	569.8	77±6%	1118	acaaccugaaugaacuuuuuga
bta-miR-AB-148	561.9	77±6%	1101	uuguccgacucuuagcgg
bta-miR-AB-28	4.8	70±4%	1046	caaaaaguucguccagauuuu
bta-miR-AB-137	416.7	77±6%	818	aaaucugaacaagcuuuuuggc
bta-miR-AB-156	406.5	77±6%	796	aaaaaguucguuuggguuuuu
bta-miR-AB-7	401.1	77±6%	785	aaaacugaaugaacauuuuggc
bta-miR-AB-48	333.4	77±6%	653	cgaaaaguucguuuggguuuu
bta-miR-AB-47	251.3	77±6%	491	aaaaguucguuucgguuuuucc
bta-miR-AB-145	4.3	70±4%	381	ucuuggagcucaccgucuag
bta-miR-AB-168	4.7	70±4%	375	cugaccuaugaauugaag
bta-miR-AB-158	190.1	77±6%	372	aaaaaguuccuuuggguuuuc
bta-miR-AB-34	174.6	77±6%	341	ucuagaagcucacagucuag
bta-miR-AB-146	171.8	77±6%	335	uucucagguuggacaguccuga
KC				
Provisional ID	**miRDeep2 score**	**Estimated probability that the miRNA candidate is a true positive**	**Total read count**	**Mature miRNA consensus sequence**
bta-miR-AB-10	32819.1	80±5%	64372	aaagcugaaugaacuuuuuggc
bta-miR-AB-225	3118.6	80±5%	6111	cucucgagucgcgacguguaucuc
bta-miR-AB-65	2566.5	80±5%	5037	aaaaagguuuguuuuggguuuu
bta-miR-AB-27	2410.2	80±5%	4719	aaaaaaguuuguuuuggauuuu
bta-miR-AB-52	2256.4	80±5%	4417	aaaaaaguuuguuuuggguuuuu
bta-miR-AB-29	2249.6	80±5%	4412	acucgaacgaauuuuuggcc
bta-miR-AB-63	1079.1	80±5%	2115	ggaauaccggguacuguaggcu
bta-miR-AB-23	750.3	80±5%	1472	acaaccugaaugaacuuuuuga
bta-miR-AB-18	406.1	80±5%	790	aaacccugaaggaacuuuu
bta-miR-AB-7	376.6	80±5%	737	aaaacugaaugaacauuuuggc

bta-miR-AB-198	289.8	80±5%	567	aaaaucugaacaaacuuuu
bta-miR-AB-187	236.7	80±5%	463	aaaguucguucaggguuuuuc
bta-miR-AB-13	174.9	80±5%	335	cgggugggaagaggcggg
bta-miR-AB-58	159.8	80±5%	310	caccuagugcauggucuugggc
bta-miR-AB-57	158.4	80±5%	302	aaaaaaguuuguuugguuu
bta-miR-AB-219	136.8	80±5%	267	uucauaggaaggugucauuca
bta-miR-AB-205	135.1	80±5%	269	aaaacccgaacaaacuuuu
bta-miR-AB-11	130.8	80±5%	255	uccaggauaugugcguguaacuc
bta-miR-AB-241	102.3	80±5%	203	uguucaguggcuaaguuc
bta-miR-AB-62	98.7	80±5%	184	uuggccagaaaguucguuuggau

Table 4.4. Differentially expressed known micro RNAs between high and low-RFI animals within each breed (Angus, Charolais and KC), with a differential expression threshold of P-value < 0.05 and Fold-change of 1.5.

Angus	MicroRNA	logFC	P-value
Known	*bta-miR-11985*	-1.377	2.02E-04
	bta-miR-2285bg	1.185	3.85E-03
	bta-miR-2285n	1.082	4.30E-03
	bta-miR-2285u	0.836	7.75E-03
	bta-miR-424-3p	0.959	8.38E-03
	bta-miR-27a-5p	-0.978	0.016
	bta-miR-24	-0.967	0.016
	bta-miR-507b	-0.900	0.019
	bta-miR-449a	0.782	0.023
	bta-miR-133b	0.663	0.033
Novel	*bta-miR-AB-2*	-0.833	1.35E-04
	bta-miR-AB-47	0.617	0.014
Charolais			
Known	*bta-miR-2415-3p*	1.261	7.12E-08
	bta-miR-2419-5p	0.797	8.72E-07
	bta-miR-449a	1.117	0.002
	bta-miR-2285i	-0.966	0.002
	bta-miR-133a	0.640	0.002
	bta-miR-2346	-1.330	0.003
	bta-miR-1842	-1.026	0.013
	bta-miR-2284ac	0.656	0.014
	bta-miR-2285ai-5p	0.645	0.015
	bta-miR-12001	-0.729	0.019
	bta-miR-299	0.772	0.024
	bta-miR-2284c	-1.104	0.029
	bta-miR-365-5p	0.747	0.043
	bta-miR-485	-0.872	0.049

	bta-miR-7859	0.916	0.049
	bta-miR-6521	0.696	0.0496
Novel	*bta-miR-AB-2*	0.770	1.00E-03
	bta-miR-AB-15	0.735	0.0158
KC			
Known	*bta-miR-190a*	-3.133	1.47E-18
	bta-miR-449a	1.252	7.61E-06
	bta-miR-155	0.829	3.55E-05
	bta-miR-424-5p	0.756	3.62E-04
	bta-miR-223	0.737	4.96E-04
	bta-miR-1246	-0.683	1.27E-03
	bta-miR-363	0.866	3.25E-03
	bta-miR-147	0.801	5.69E-03
	bta-miR-2411-3p	-0.677	0.025
	bta-miR-2483-5p	0.877	0.039
Novel	*bta-miR-AB-63*	-0.897	3.18E-04
	bta-miR-AB-225	1.227	0.008

logFC = $\log_2$(Fold-change), and the sign on the of shows the direction of miRNA expression in low-RFI steers relative to high-RFI animals.

Table 4.5. Top five molecular and cellular (biological) functions enriched by DE target genes for Angus, Charolais and KC populations.

	Biological function	***No. of DE targets***	***Targets involved in the biological function***
Angus	Lipid metabolism	18	*ACSS2, ADIPOR2, CCDC80, CPT1B, DLK1, EDNRA, ELOVL5, FKBP5, G0S2, GATM, HP, LPIN1, MARCO, PLA2G2D, SCD, SLC22A2, UGT2B7, ZBTB16*
	Molecular transport	16	*ADIPOR2, CCDC80, CPT1B, DLK1, EDNRA, ELOVL5, G0S2, GATM, HP, LPIN1, MARCO, PLA2G2D, SCD, SLC22A2, TP53INP1, ZBTB16*
	Small molecule biochemistry	21	*ACSS2, ADIPOR2, CCDC80, CPT1B, DLK1, EDNRA, ELOVL5, FKBP5, G0S2, GATM, HP, LPIN1, LURAP1L, MARCO, PLA2G2D, SCD, SLC22A2, SLCO4A1, TP53INP1, UGT2B7, ZBTB16*
	Energy production	7	*ACSS2, ADIPOR2, CCDC80, CPT1B, G0S2, LPIN1, SCD*
	Carbohydrate metabolism	9	*ADIPOR2, CCDC80, ELOVL5, GATM, GNAZ, LPIN1, PLA2G2D, SCD, TP53INP1*
Charolais	Lipid metabolism	12	*ABCC4, CES1, CYP2C19, DLK1, LPIN1, NR0B2, PDK4, SCD, SLC4A4, SPNS2, THEM4, TNC*
	Molecular transport	16	*ABCC4, ANXA2, CES1, CXCL2, DLK1, LPIN1, NR0B2, PDK4, SCD, SIRPA, SLC13A2, SLC4A4, SLC7A5, SPNS2, TNC, TP53INP1*

	Small molecule biochemistry	19	*ABCC4, ANXA2, CES1, CYP2C19, DLK1, GPX3, LPIN1, MIOX, NR0B2, PDK4, SCD, SLC13A2, SLC4A4, SLC7A5, SPNS2, THEM4, TNC, TP53INP1*
	Cellular movement	13	*ABCC4, ANXA2, CES1, CXCL2, GNAZ, GPNMB, PDK4, SERPINA3, SIRPA, SLC7A5, SPNS2, TNC, TP53INP1*
	Cell-to-cell signalling and interaction	16	*ABCC4, ANXA2, CES1, CXCL2, CYP2C19, DLK1, GNAZ, GPNMB, GPX3, HLA-DQB1, PDK4, RND1, SIRPA, SLC4A4, SLC7A5, TNC*
KC	Cell death and survival	48	*ACACA, APMAP, ARG1, ATP2A2, BAG3, BTG2, CCND1, CXCL10, CYCS, DDIT4, DUSP1, EDNRA, ERBB2, ERBB3, FGF21, FKBP5, GATA4, GCH1, GCLC, GHR, GLS2, HEYL, HMGCR, IGF1, INSIG1, IRAK3, KYAT1, LRIG1, MANF, MFSD2A, MKNK1, MOB3B, NMNAT2, NPC1, NR0B2, OAS1, PER1, PNP, PPARGC1A, RHOJ, RRS1, SCD, SERPINA3, TOP1, TP53INP1, TRIB2, USP2, ZBTB16*
	Amino acid metabolism	15	*ACMSD, ARG1, GCH1, GCLC, GLS2, HAL, IGF1, KYAT1, OAT, SDS, SLC16A10, SLC22A7, SLC25A15, SLC7A2, TAT*
	Small molecule biochemistry	57	*ABCG8, ACACA, ACMSD, AK4, ARG1, ATP2A2, BAG3, CXCL10, CYCS, CYP1A1, CYP2B6, DUSP1, EDNRA, ELOVL2, ERBB2, ERBB3, FGF21, FOXA3, GATA4, GCH1, GCLC, GHR, GLS2, GSTM4, HAL, HMGCR, IGF1, INSIG1, KYAT1, LPIN1, MFSD2A, MKNK1, NMNAT2, NPC1, NR0B2, OAS1, OAT, OGDH, P2RY2, PER1, PNP, PPARGC1A, RHOJ, SCD, SDS, SLC16A10, SLC22A7, SLC25A15, SLC7A2, SLCO4A1, STS, TAT, TP53INP1, TPH1, USP2, ZBTB16*
	Lipid metabolism	32	*ABCG8, ACACA, ATP2A2, BAG3, CXCL10, CYCS, CYP1A1, CYP2B6, DUSP1, EDNRA, ELOVL2, ERBB2, FGF21, GATA4, GHR, GSTM4,*

			HMGCR, IGF1, INSIG1, LPIN1, MFSD2A, MKNK1, NPC1, NR0B2, OGDH, P2RY2, PER1, PPARGC1A, RBP5, SCD, STS, ZBTB16
	Vitamin and mineral metabolism	16	*ABCG8, ACACA, ACMSD, CXCL10, CYP1A1, CYP2B6, GCLC, HMGCR, IGF1, INSIG1, NPC1, NR0B2, PPARGC1A, RBP5, SCD, STS*

No. of DE targets = Number of differentially expressed targets.

Chapter 5. Differential hepatic miRNA expression between beef steers with divergent feed efficiency component trait phenotypes

5.1 Abstract

MicroRNAs (miRNAs) are major post transcription gene expression regulators involved in modulating multiple cellular and molecular processes. In the current study we explored associations of liver miRNA expression with average daily growth (ADG), dry matter intake (DMI), and metabolic weight (MWT) of beef steers from Angus, Charolais and Kinsella Composite (KC) populations. Beef steers including 50, 48 and 158, Angus, Charolais and KC steers, respectively, were tested for individual feed intake, growth rate and their metabolic weight consequently calculated, After slaughter, a total liver tissues of 60 animals from Angus (n=20), Charolais (n=20) and KC (n=20) were used in the current study. MicroRNA expression profiles of the 60 beef were obtained through high throughput sequencing of the cDNA library of each animal. Within each breed population, animals were independently sorted for each of the three traits accordingly. Six animals (n = 6) with extreme high and six animals (n = 6) with extreme low phenotype values for the respective trait were selected for differential miRNA analysis. For ADG, we identified 11(5 downregulated and 6 upregulated), 12 (8 downregulated and 4 upregulated) and 15 (8 downregulated and 7 upregulated) differentially expressed (DE) microRNA for Angus, Charolais and KC steers, respectively. For DMI, 9 (3 downregulated and 6 upregulated), 21 (8 downregulated and 13 upregulated) and 19 (9 downregulated and 10 upregulated) DE-miRNAs were identified for Angus, Charolais and KC animals respectively. For MWT, we identified 13 (3 downregulated and 10 upregulated), 19 (8 downregulated and 11 upregulated), and 18 (8 downregulated and 10 upregulated) DE-miRNAs for Angus, Charolais and KC steers respectively.

The differentially identified miRNAs for each trait were mainly specific each population (89% to 98%). Target gene prediction for the DE-miRNAs across the three populations revealed that up to 71%, 75% and 65% of the DE genes for ADG, DMI and MWT, respectively, are potential targets of the DE-miRNAs identified in this study. The predicted target genes are involved multiple biological processes including lipid metabolism, molecule transport, amino acid metabolism, cell death and survival, cellular movement, cellular function and maintenance, and small molecule biochemistry. Our findings demonstrate potential involvement of miRNAs in modulating growth rate, feed intake and metabolic weight of beef cattle.

5.2 Introduction

The liver plays key important metabolic functions in the body such as carbohydrate metabolism, amino acid metabolism, protein synthesis, lipid metabolism, bile synthesis, toxin biotransformation, microbial processing and xenobiotic metabolism (Häussinger 1996). Therefore, variability in metabolic activities of the liver tissues in the body is most likely to result into measurable difference in key production traits such feed efficiency and the related traits including average daily growth (ADG), dry matter intake (DMI), and metabolic weight (MWT) of the animal. Indeed, a sizable gene expression of some genes in the liver has been shown to be associated with RFI (Chen *et al.* 2011; Alexandre *et al.* 2015; Paradis *et al.* 2015; Tizioto *et al.* 2015; Mukiibi *et al.* 2018), DMI (Mukiibi *et al.* 2019b), ADG (Mukiibi *et al.* 2019b), and MWT(Mukiibi *et al.* 2019b) in beef cattle. The potential post transcription regulation of the genes associated with RFI through microRNAs (miRNAs) has been studied (Al-Husseini *et al.* 2016; De Oliveira *et al.* 2018; Mukiibi *et al.* 2019a), however no comprehensive study on the regulation of DMI or ADG or MWT by miRNAs has been done. MicroRNAs are small (~22 nucleotides) noncoding RNAs that play principle regulators of cellular metabolism and homeostasis by

targeting mRNA possessing their binding cites in the 3' untranslated regions (UTRs) (Hartig *et al.* 2015). The miRNAs exert their modulation action through promoting mRNA degradation (as a result of deadenylation) and repression of translation, both of which result into reduced protein product (Fabian *et al.* 2010; Stroynowska-Czerwinska *et al.* 2014). In the current study we investigated the associations of the liver miRNA expression with ADG, DMI and MWT in beef steers from Angus, Charolais and KC populations through RNAseq analyses and predicted the target genes of DE- miRNA for the feed efficiency related traits.

5.3 Materials and Methods

5.3.1 Animal populations and management

The management handling and raising of the animals used in this study have been described in our previous studies on the same animals (Mukiibi *et al.* 2018; Mukiibi *et al.* 2019a; Mukiibi *et al.* 2019b). Briefly, the Canadian Council of Animal Care (CCAC) guidelines on the care and use of farm animals in research teaching and testing (CCAC 2009) were followed to manage the experimental animals until tissue collection, and all the experimental protocols followed throughout the experiment were approved by the University of Alberta Animal Care and Use Committee (AUP00000777). A total of 256 steers from three populations in purebred Angus, purebred Charolais and Kinsella Composite were initially used in the current study. All the animals were born and raised under the same conditions at the University of Alberta's Roy Berg Kinsella Research Ranch, Alberta, and Canada. The purebred Angus and Charolais animals were born by purebred cows that serviced through artificial insemination followed by natural service by purebred Angus or Charolais bulls respectively. The cross or composite breed animals were born by University of Alberta's hybrid dam line (Goonewardene *et al.* 2003), were either serviced by Angus or Charolais or University of Alberta hybrid bulls. The calves were born between April and

May of 2014 and castrated into steer calves after at birth. The steer calves were maintained with their dams on pasture until weaning at an average age of six months. A backgrounding diet composed 80% barley silage, 17% barley grain and 3% rumensin pellet supplement was feed to the weaned calves as a transition diet. Subsequently, animals were fed on set-up diets that had decreasing barley silage and increasing barley grain proportions for three weeks, and then introduced to a finishing diet that was composed of 75% barley grain, 20% barley silage and 5% rumensin pellet supplement.

5.3.2 Feed intake and growth and body weight measurement

Measurement and calculation of the individual phenotypes of the experimental animals used in the current experiment have been also described in our recent studies on the same animals (Mukiibi *et al.* 2018; Mukiibi *et al.* 2019a; Mukiibi *et al.* 2019b). The GrowSafe Systems® (GrowSafe Systems Ltd., Airdrie, Alberta, Canada) equipment was used to measure individual feed intake of each of the 50 purebred Angus, 48 purebred Charolais and 158 KC steers for a feedlot test period ranging between 70 to 73 days. Daily dry matter intake (DMI) of each animal was computed as the average of the feed intake records through the test days, standardized to 12 MJ ME per kg dry matter. Body weights (BW) were also measured initially for two consecutive days, followed by 14-day interval points during the test and then for two consecutive days at the end of the test. Average daily gain (ADG) and the initial body weight of each animal were computed through linear regression of the serial body weights on time (days). Metabolic mid-weight (MWT) of each animal was calculated as midpoint $BW^{0.75}$ where midpoint BW was calculated as the sum of the initial BW and the product of ADG and half the test days of the animal in the feedlot.

5.3.3 Liver tissue sample collection

Tissue collection, processing and storage have been described in our previous studies (Mukiibi *et al.* 2018; Mukiibi *et al.* 2019a; Mukiibi *et al.* 2019b). All were slaughtered at the Agriculture and Agri-Food Canada (AAFC) Lacombe Research Centre (Lacombe, AB) during the months of July and September in 2015, at an average age of 494 ± 3, 518 ± 4, and 457 ± 4 days for Angus, Charolais and KC steers respectively. For each slaughtered animal the liver was immediately extracted, and liver tissue sample dissections used in the current samples were cut from the right lobe of the organ with the fibrous capsule removed. The sample dissections were immediately bagged in plastic re-closable bags, labelled, snap frozen in liquid nitrogen, and then kept on dry ice during transportation to the laboratory where they were kept under -80^{o}C until RNA extraction.

5.3.4 Total RNA extraction

Extraction of total RNA used in the current study has been described in our previous study (Mukiibi *et al.* 2019a) as they are the same animal samples in both studies. Briefly, each of the three populations, total RNA was extracted from 20 samples (10 with high and 10 low RFI phenotypes). For each sample, 10 mg of the liver tissue was pulverised to fine powder under liquid nitrogen using a prechilled mortar and pestle. Total RNA was then extracted from pulverised tissue using the Qiagen RNeasy Plus Universal Mini Kit (Qiagen, Toronto, ON), following the manufacturers guidelines. The concentration of the extracted total RNA for each sample was measured using a NanoDrop 2000 Spectrophotometer (Thermo Scientific, Wilmington, DE, USA). On average, the total RNA concentration was 1851.8ng/µl per sample, with A260/280 absorbance ratios ranging between 1.8 and 2.0. The integrity or quality of the RNA samples was assessed using a TapeStation-Agilent instrument (Agilent Technologies Canada, Mississauga, ON), which

showed that RNA integrity number values of all our samples were greater than 8, hence deemed of high quality for cDNA library construction and sequencing consequently.

5.3.5 Construction of cDNA libraries and sequencing

Sixty cDNA libraries were prepared from high quality total RNA, enriched for miRNAs and subsequently sequenced at the Clinical Genomics Centre (Toronto, ON, Canada) as described in our recent study (Mukiibi *et al.* 2019a). The Illumina Truseq Small RNA Library Prep Kit (Illumina, San Diego, CA, USA) was used with a start concentration of 1 µg of each of total RNA sample. Firstly, an RNA 3' adapter was ligated to the 3' ends of the RNA using *T4 RNA Ligase 2* enzyme, and subsequently an RNA 5' adaptor was added to the 5' end of the 3' adapter-ligated-RNA using the *T4 RNA Ligase* enzyme. The RNA 3'and 5' adapters are designed specially target miRNAs and other small RNAs that are products DROSHA and DICER RNA cleavage processes. The 3'-5'-adapter-ligated-RNA was reverse transcribed to single stranded cDNA using the SuperScript II Reverse Transcriptase enzyme (Thermo Fisher Scientific, San Jose, CA, USA) and an RNA RT primer, and then PCR amplified and indexed for multiplex sequencing. The cDNA constructs were then purified and enriched for miRNAs through gel electrophoresis. Four cDNA sequencing pools of 15 samples each were constructed by using an average 2nM of cDNA from each sample. Sequencing was performed by the Illumina Hiseq 2500 sequencer, on two flow cells, under rapid run mode and expected length of 50bp single end read. After sequencing, the Illumina bcl2fastq-v2.17.1.14 conversion software (Illumina) was used to demultiplex raw sequence data into individual FASTQ file for each sample.

5.3.6 Bioinformatic sequence data processing and miRNA quantification

Raw sequence data quality assessment, processing and miRNA quantification performed for the current study has been previously extensively described (Mukiibi *et al.* 2019a). FASTQ

version 0.11.7 (Andrews 2010) software was used to assess the sequencing quality of the raw reads, considering multiple quality parameters including read lengths, adapter content, per sequence CG content and per base sequence quality score. The Illumina 3' prime end primer TGGAATTCTCGGGTGCCAAGG was trimmed from all the raw reads using cutadapt version 1.16 software (Martin 2011), and shorter (read length < 15bp) and longer reads (read length > 28bp) removed eliminated. Reads with read length between 15bp and 28bp were further screen for presence of other bovine short RNA species including ribosomal RNAs (rRNAs), transfer RNAs (tRNAs), small nuclear RNAs (snRNAs) and Small nucleolar RNAs (snoRNAs) that were downloaded from https://rnacentral.org/. Identification of novel bovine miRNAs and quantification of both known and novel miRNAs were performed using miRDeep2 package modules version 2.0.08 (Friedländer *et al.* 2011), the UMD3.1 reference genome (downloaded from Ensembl genome browser release 93), and mature and precursor sequences of known bovine miRNAs downloaded from miRBase database release 22 (Griffiths-Jones *et al.* 2007).

5.3.7 Differential miRNA expression analyses

Within each breed, expressed known miRNAs plus the 25 most expressed novel miRNAs as previously reported by (Mukiibi *et al.* 2019a) were analyzed for differential miRNA expression. To avoid expression profile bias during differential expression analyses, miRNA expression profiles across the 20 samples from each breed were initially evaluated by principle component analysis (PCA) using DESeq2 (Love *et al.* 2014). Consequently, 17 samples were considered suitable for differential miRNA expression analyses for Angus and Charolais populations, whereas 20 samples were all suitable for KC. Animals within each breed were independently ranked for ADG or DMI or MWT phenotypes. For each trait within the breed, 12 animals with extreme phenotypes (i.e. n = 6 low and n = 6 high) were selected for differential miRNA expression analysis

using dgeR package (Robinson *et al.* 2010) in R. Firstly, in each analysis miRNAs that had less than one count per million (CPM) in at least six samples were filtered out from the analysis. The counts of the remaining miRNAs were then normalized using the trimmed mean of M values (TMM) method (Robinson & Oshlack 2010). Thereafter, using the trait group as a fixed effect, the normalized counts were modeled using a generalized linear model likelihood ratio test assuming a negative binomial distribution of the counts. Low-ADG, high-DMI and high-MWT were considered as reference groups for ADG, DMI and MWT analyses respectively within each group. Micro-RNAs were identified as differentially expressed at a likelihood ratio test P-value lower than 0.05 and fold change (FC) of greater than 1.5.

5.3.8 Validation of the differentially expressed miRNAs

Six miRNAs that were differentially expressed between high and low-ADG steers were selected for DE validation through TaqMan quantitative polymerase chain reaction (TaqMan qPCR). These DE-miRNAs included *bta-miR-6123* and *bta-miR-2415-3p* for Charolais, and *bta-miR-486, bta-miR-7, bta-miR-424-3p* and *bta-miR-424-5p* for KC. Additionally based on the their expression level (counts) and uniformity across samples four miRNAs were selected as endogenous controls including, *bta-mir-192* and *bta-mir-2284x* for Charolais, and *bta-mir-2284x* and *bta-let-7b* for KC. Stem-loop RT primer of each DE-miRNA and endogenous controls, and qPCR primers were all purchased from Thermo Fisher Scientific (Waltham, Massachusetts, USA). Similar pProcedure of miRNA qPCR validation followed in this study were similar to that provided in our recent publication on residual feed intake (Mukiibi *et al.* 2019a). Briefly, part of the total RNA used in the small RNA sequencing was used for the validation process. Firstly, for each miRNA, 10ng of RNA was reverse transcribed using TaqMan® MicroRNA Reverse Transcription Kit (Thermo Fisher Scientific, Waltham, Massachusetts, USA) and a stem-loop RT

primer on an Eppendorf 5331 Mastercycler Gradient v2.30.31 thermocycler. Thereafter, for each miRNA qPCR was performed on the reverse transcription products using the TaqMan qPCR primers and TaqMan® Fast Advanced Master Mix following the manufacturer's instructions. The Applied Biosystems™ 7500 Fast Real-Time PCR System v2.0.1 (Applied Biosystems, Foster City, California, USA) was used to run qPCR, which produced C_T values that were normalized to the reference miRNAs. Relative quantities based on the C_T values average were computed and then t-tests between the relative quantities of high and low-ADG were performed. P-values less than 0.05 indicated significant differential expression of the miRNA.

5.3.9 Target gene prediction, miRNA-target interaction and functional enrichment analyses

Target gene prediction for the differentially expressed miRNAs was performed using TargetScan Perl scripts version 7.0 (Agarwal *et al.* 2015). Targets of context++ score percentile higher than the 50^{th} percentile and differentially expressed as reported by (Mukiibi *et al.* 2019b) were identified as targets for the DE-miRNAs, and subsequently interaction between these targets and DE-miRNAs were constructed in Cytoscape 3.7.1 (Shannon *et al.* 2003). Additionally, functional enrichment analysis of the identified DE-target genes was performed through core analyses in the ingenuity pathway analysis (IPA) software to identify the major biological functions modulated by DE-miRNAs, through which they regulate ADG, DMI and MWT of beef cattle.

5.4 Results

5.4.1 Sequencing qualities and miRNA profiling

Sequence data quality and miRDeep2 miRNA profiling results have been previously reported in our previous study on residual feed intake (Mukiibi *et al.* 2019a). Briefly, on average

NGS platform produced 10 million (M) reads per sample, of which 5.5M high quality (average phred-score = 37.96) reads were retained for known miRNA quantification and identification of novel ones after the quality control analyses and filtrations. On average we identified 556 expressed known miRNAs and a total of 241 novel miRNAs.

5.4.2 Phenotypic differences

With respect to ADG, the animals with high and low-ADG in each breed on average significantly (P-value < 0.0042 after Bonferroni Correction for multiple comparisons) differed (i.e. high-ADG animals grew faster than the low-ADG animals) as shown in Table 5.1. In general, the animals in the high and low-ADG groups did not differ in terms of other recorded phenotypes as shown in Table 5.1. Also, for DMI, the animal groups (low- and high-DMI) significantly (P <0.0042) differed with high-DMI animals on average consuming more feed per day than their counterparts in the low-DMI group in each of the three breeds (Table 5.2). It is noteworthy that across the three breeds, animals with low-DMI phenotypes also had significantly (P<0.0042) lower RFI than those in the high-DMI group as expected. Additionally, the MWT contrasting groups (high and low-MWT) in Charolais and KC were on average significantly (P <0.0042) different, with the animals in the high-MWT group having higher metabolic weight than those in the low-MWT group across the three populations (Table 5.3). However, for Angus, the contrasting MWT steer groups were only suggestively (P = 0.02) different from each other

5.4.3 Differential miRNA expression

5.4.3.1 ADG

For Angus, we identified 11 DE-miRNAs, of which five were downregulated and six upregulated in the high-ADG animals. In Charolais, 12 DE-miRNAs were identified, seven of these were downregulated and five upregulated in the high-ADG steers. For KC, we identified 15 DE-miRNAs of which eight were downregulated and seven upregulated in high-ADG steers. The majority of the DE-miRNAs were breed specific (Figure 1a), with no DE-miRNA found to be DE across all the three breeds. However, *bta-miR-2411-3p* was common between Angus and KC, it was downregulated and upregulated in high-ADG Angus and KC steers respectively. Additionally, *bta-miR-2284c* was common between Charolais and KC, where it was downregulated and upregulated in low-ADG Charolais and KC steers respectively. All differentially expressed DE-miRNAs in Angus, Charolais and KC, P-values and $\log_2$Fold changes are presented in Table 5.4.

5.4.3.2 DMI

For Angus animals, nine miRNAs were differentially expressed between high and low-DMI animals, two (*bta-miR-96* and *bta-miR-200a*) of these miRNAs were downregulated in low-DMI animals, whereas seven were miRNAs upregulated in the same animals. For Charolais, 21 DE-miRNAs were identified, of which 8 and 13 miRNAs were downregulated and upregulated respectively in the low-DMI animals. For KC, we identified 17 miRNAs as differentially expressed, of these, 9 were downregulated and 8 upregulated in the low-DMI KC animals. The DE-miRNAs were largely breed specific, with none of them common across three breeds, Figure 1b. One miRNA (*bta-miR-424-3p*) was common between Angus and KC and was upregulated in the low-DMI animals of the two breeds. The identified DE-miRNAs from each of the three breeds and presented in Table 5.5.

5.4.3.3 MWT

We identified 13 differentially expressed miRNAs between high and low-MWT Angus animals that included 3 and 10 miRNAs that were downregulated and upregulated respectively in low-MWT animals. For the Charolais animals, 21 DE-miRNAs were identified, 8 of these miRNAs were downregulated, whereas 13 were upregulated in the low-MWT animals. In KC, 18 miRNAs were differentially expressed, of which 10 and 8 were upregulated and downregulated respectively in the low-MWT animals. As for ADG and DMI, also the DE-miRNA identified were mainly breed specific, Figure 5.1c, however, one miRNA (*bta-miR-10164-3p*) was common to all the three breeds, it was upregulated in low-MWT animals from Angus and KC populations, downregulated in Charolais animals with similar phenotype. Two miRNAs (*bta-miR-1246* and *bta-miR-2411-3p*) were common to Angus and KC. All the differentially expressed miRNAs between high and low-MWT within each breed are presented in Table 5.6.

Moreover, comparison of the DE-miRNAs of the three traits together with those of RFI reported by (Mukiibi *et al.* 2019a) within each breed showed that most of the DE-miRNAs were associated to a single trait, however, some DE-miRNAs were associated with more than one trait. For example, within Angus (Figure 5.2a), *bta-miR-11985* was associated with ADG, MWT and RFI, and *bta-miR-2285bg* and *bta-miR-424-3p* was associated to both DMI and RFI. Within Charolais (Figure 5.2b), *bta-miR-2415-3p* and *bta-miR-2284c* were associated with ADG, DMI and RFI, whereas, *bta-miR-2419-5p, bta-miR-AB-2, bta-miR-2285ai-5p, bta-miR-2284ac, bta-miR-2285i, bta-miR-299* and *bta-miR-2346* were all associated with DMI and RFI. Within KC (Figure 5.2c), bta-miR-190a and bta-miR-2411-3p were identified as associated to all the four traits, and, *bta-miR-147*, *bta-miR-155* and *bta-miR-363* were associated with DMI and RFI.

5.4.4 qPCR Validation

We performed qPCR validation for six selected DE-miRNAs. For the Charolais animals, of the two validated miRNAs *bta-miR-6123* showed significant ($P < 0.05$) differential expression between high and low-ADG animals as shown in Figure 5.3. For KC, of the four miRNAs considered for validation, *bta-miR-486* showed significantly differentially expressed between the trait groups. The other four miRNA did not show significant expression ($P > 0.05$). However, in general there was a high correlation (0.84) between expression profiles from RNAseq and qPCR as shown in Figure 5.4.

5.4.5 DE-miRNAs – DE-targets genes interactions and functional enrichment

5.4.5.1 Average daily gain

For ADG, 68, 65 and 55 DE-genes were identified as potential targets for DE-miRNAs identified in Angus, Charolais and KC animals respectively. For Angus as shown in Figure 5.5, genes that are targeted by the most DE-miRNAs included *CD44*, *MBNL3*, *FAM13A* and *HMGCS1* that were targeted by six DE-miRNAs each, and *HMGCR, SERPINE1, PLA2G7, AIF1L, IL20RA*, *SLC25A15, SOCS2* and *AK4* which were predicted to be targeted by five DE-miRNAs each. The novel DE-miRNA *bta-miR-AB-47* (upregulated in high-ADG animals) was predicted to target the most DE-genes (i.e. targeting 30 genes). Of these targets, 17 and 13 DE-genes were downregulated and upregulated in low-ADG Angus animals. *bta-miR-2411-3p* was predicted to target 24 DE-genes, and then *bta-miR-12001* and *bta-miR-34c* were predicted to target 21 DE-genes each. The DE-miRNAs *bta-miR-363* and *bta-miR-487a* were predicted to target 18 DE-genes each. Additionally, the six upregulated (in high-ADG animals) DE-miRNAs identified in the current study were predicted to target 59 of the DE-genes, and 30 of these predicted targets were identified as downregulated in high-ADG animals, whereas 29 were downregulated in the same animals. The

identified 68 targets are mainly involved in cellular movement, cell-to-cell signaling and interaction, cellular development, cellular function and maintenance and cellular growth and proliferation.

For Charolais, *SOCS2* was identified as the most targeted gene (targeted by nine DE-miRNAs), followed by *MBOAT2* that was targeted by seven DE-miRNAs, and *FKBP5, CCDC80* and *FAM13A* that were predicted to be targeted by six DE-miRNAs each as shown in Figure 5.6. On the other hand, *bta-miR-2284c* was identified to target the most DE-genes (27 genes), *bta-miR-2285cp* was predicted to target 21 DE-genes, and *bta-miR-2415-3p* and *bta-miR-AB-47* were predicted to target 20 DE-genes each. The four upregulated (in the liver tissue of high-ADG animals) DE-miRNAs including *bta-miR-6123*, *bta-miR-655*, *bta-miR-95* and *bta-miR-376b* were predicted to target 31 DE-genes, and 18 of these genes were identified as downregulated in the liver tissue of Charolais animals with high-ADG phenotype. Functional analysis revealed that the identified 65 targets are majorly involved in lipid metabolism, molecular transport, small molecular biochemistry, amino acid metabolism and protein synthesis.

For KC, the top targeted DE-genes included *FKBP5* and *FOSL2,* which were predicted to be targeted by 14 and 11 DE-miRNAs respectively as shown in Figure 5.7. DE-genes *SLC4A4* and *TAT* were predicted to be targeted by eight miRNAs each, whereas *CYP7A1, SCD* and *ARRDC3* were predicted to be targets of six DE-miRNAs each. As shown in Figure 5.7, some DE-miRNAs were predicted to target more DE-genes than the others. For example, *bta-miR-2285aj-5p* (upregulated in high-ADG animals) was predicted to target 24 DE-genes. Of the 24 targets, 20 were identified as downregulated in high-ADG KC animals, and four upregulated in the animals with the same phenotypes in the same population. Three DE-miRNAs including *bta-miR-424-5p, bta-miR-6523a* and *bta-miR-7* were predicted to target 19 DE-genes each, and *bta-miR-AB-228*

was predicted to target 16 genes. The seven upregulated DE-miRNAs were predicted to target 43 DE-genes, of which 30 were downregulated in high-ADG animals. Additionally, the 55 DE-genes identified as targets are mainly involved in amino acid metabolism, small molecule biochemistry, lipid metabolism, molecular transport and cell death and survival. All DE target genes predicted for ADG DE miRNAs in Angus, Charolais and KC are provided in the Supplementary Data S15.

5.4.5.2 Dry matter intake

For DMI, 65, 135 and 105 DE-genes were identified as potential targets for DE-miRNAs identified in Angus, Charolais and KC animals respectively. As illustrated in the DE-miRNA-DE target interaction of Figure 5.8 for Angus**.** *ZFAND5, FKBP5, SCD* and *PTGER3* were the most targeted genes with each predicted to be targeted by five DE-miRNAs. The DE-miRNA bta-miR-2285u was predicted to target the highest number of DE-genes (30 genes), followed by bta-miR-200a which was predicted to regulate 29 genes. *bta-miR-455-5p* and *bta-miR-424-3p* were also predicted to target a relatively high number of DE-genes, with each predicted to target, 19 and 17 DE-genes respectively. Of the 30 genes predicted to be targets of bta-miR-2285u, 11 and 19 were identified as downregulated and upregulated respectively in low-DMI animals. The seven DE-miRNAs identified as upregulated in low-DMI animals were predicted to target 56 DE-genes of which 26 and 30 were identified as downregulated and upregulated respectively in the liver tissue of Angus animals with the same phenotype. Functional analysis revealed that the 65 DE-genes (targets) are mainly involved in carbohydrate metabolism, amino acid metabolism, small molecule biochemistry, lipid metabolism and molecule transport.

DE-target genes and DE-miRNAs interactions for the Charolais are illustrated in Figure 5.9. The top targeted genes included *TTPAL* was predicted to be the most targeted gene (i.e. targeted by 14 DE-miRNAs), *STRIP2* and *FKBP5* were both predicted by 13 DE-miRNAs, and

SLC16A10 and *PAQR3* which were each predicted to be targeted by 12 DE-miRNAs. Among the miRNAs that were predicted to target the most DE-genes included *bta-miR-495* (predicted to target 57 DE-genes), *bta-miR-2284c* (predicted to target 55 DE-genes), *bta-miR-2285ai-5p* (predicted to target 53 DE-genes), *bta-miR-10167-3p* (predicted to target 48 DE-genes), *bta-miR-2285n* (predicted to target 46 DE-genes), and *bta-miR-2284ac* and *bta-miR-AB-47* both targeted by 42 DE-miRNAs. Of the 57 DE-genes predicted to be targeted by *bta-miR-495*, 28 and 29 were downregulated and upregulated respectively in the liver of low-DMI Charolais animals. Additionally, the 13 DE-miRNAs identified as upregulated in low-DMI animals were predicted to target 45 downregulated and 83 upregulated DE-genes in low-DMI animals. The IPA results showed that the major molecular and cellular functions enriched by the identified 135 targets included cellular movement, lipid metabolism, molecular transport, small molecule biochemistry, and cell-to-cell signaling and interaction.

For KC, as illustrated in Figure 5.10, the most targeted DE-genes included, *RRM2*, predicted to be targeted by seven DE-miRNAs, *MPZL2, GPRIN3, SGK1, AK4, PDE9A* and *HEBP2* all of which are predicted to be regulated by six DE-miRNAs each. On the other side, the DE-miRNA predicted to target the most DE-genes included *bta-miR-424-5p* and *bta-miR-4286,* both of which were predicted to target 32 DE-genes. *bta-miR-10164-3p* and *bta-miR-490* were both predicted to target 27 DE-genes and *bta-miR-2411-3p* predicted to target 24 genes. Twelve of the predicted targets of *bta-miR-424-5p,* which was upregulated in low-DMI animals, were downregulated in the same animals, whereas 20 of these genes were upregulated in the same animals. Of the 72 DE-genes predicted to be targeted by at least one of the upregulated DE-miRNAs in low-DMI animals in the current study, 26 and 46 DE-genes were downregulated and upregulated respectively in the same animals. Moreover, the 105 identified targets are majorly

involved in cellular function and maintenance, cellular development, cellular growth and proliferation, cell death and survival and cell morphology. All DE target genes predicted for DMI DE miRNAs in Angus, Charolais and KC are provided in the Supplementary Data S16.

5.4.5.3 Metabolic weight

We identified 52, 50 and 52 DE-genes as potential targets for the miRNAs that were differentially expressed between high and low-MWT animals from Angus, Charolais and KC populations respectively. For Angus, the DE-genes that were predicted to be targeted by the most DE-miRNAs included *FAM13A* and *IGF1* each of which were predicted to be targeted by seven miRNAs, and, *AIF1L, YR61, HMGCR, HMGCS1, DLK1* and *CKAP4* that were each predicted to be targeted by six DE-miRNAs as shown in Figure 5.11 The DE-miRNAs predicted to target the most DE-genes included *bta-miR-677* (predicted to target 27 genes), *bta-miR-449a* (predicted to target 17 genes), *bta-miR-10164-3p* (predicted to target 16 genes), *bta-miR-2285u* (predicted to target 16 genes) and *bta-miR-362-5p* (predicted to target 13 genes). In total the 10 upregulated miRNAs in low-MWT animals were predicted to target 49 DE-genes including 22 and 27 genes that were respectively downregulated and upregulated in the liver tissue of low-MWT steers. The 52 identified targets are mainly involved in amino acid metabolism, small molecule biochemistry, DNA replication, recombination and repair, cellular movement and cellular development.

For Charolais, some of the genes predicted to be targeted by the most DE-miRNAs included *FADS1* and *COL8A1* which were both predicted to be targeted by eight DE-miRNAs, and *KCTD12 and GAS2* that were predicted to be targeted by seven miRNAs each as shown in Figure 5.12. For the miRNAs, *bta-miR-7, bta-miR-11991* and *bta-miR-2285as* were identified as the miRNAs that targeted the most DE-genes, with each targeting 18, 14 and 13 DE-genes respectively. Forty-four genes were identified as targeted by 11 DE-miRNAs (upregulated in liver

tissue of low-MWT animals), and seven of these were downregulated and 37 upregulated in low-MWT animals. The major molecular and cellular functions enriched by the 50 targets included cellular movement, cell-to-cell signaling and interaction, cellular development, cellular growth and proliferation and cell death and survival.

For KC, *PHLDA1* (targeted by 13 DE-miRNAs), *REEP5* (targeted by 10 DE-miRNAs), *MYCL* (targeted by 9 DE-miRNAs), *SGK1* (targeted by 8 DE-miRNAs), *IFRD1* (targeted by 8 DE-miRNAs) and *CDH11* (targeted by 7 DE-miRNAs) were identified as the most targeted genes as shown in Figure 5.13. The miRNAs predicted to target the genes included *bta-miR-7,* which was predicted to target 20 DE-genes. *bta-miR-2285bc* and *bta-miR-2285bt* were predicted to target 17 DE-genes each, and *bta-miR-7859* and *bta-miR-AB-185* were predicted to target 15 DE-genes. Of the 44 DE-genes predicted to be targeted by the 11 upregulated DE-miRNAs (in low-MWT animals), 27 were downregulated and 17 upregulated in low-MWT KC animals. Cellular movement, cell morphology, cell-to-cell signaling, and interaction, molecular transport and cellular function and maintenance were identified as the major molecular and cellular functions enriched by the 52 targets. All DE target genes predicted for MWT DE miRNAs in Angus, Charolais and KC are provided in the Supplementary Data S17.

5.5 Discussion

MicroRNAs are principle post transcription gene regulators in cells, which have been implicated in modulation of several important biological processes (Stroynowska-Czerwinska *et al.* 2014). The liver is a major metabolic organ in the body whose differential gene expression has been previously associated to feed intake or growth rate and metabolic weight. It is plausible to hypothesize that these genes are at least partially differentially modulated by miRNAs. The differential gene modulation consequently results into antagonistic metabolic activity in tissues,

and hence leads to divergence in observable phenotypes between animals. Therefore, in the current study we investigated the liver tissue differential miRNA expression between beef steers with divergent ADG or DMI or MWT phenotypes from Angus, Charolais and KC populations through RNAseq profiling.

In general, the animal groups considered for differential miRNAs expression for the respective trait within the three populations on average had the most significant contrasting phenotypes, offering confidence for the differential expression analyses for the trait under investigation although other traits also showed significant differences to some extent between the trait groups due to their correlation (Table 5.1, 5.2, 5.3). With the data, we identified 11, 12 and 16 miRNAs as associated with ADG in Angus, Charolais and KC populations respectively. Regarding DMI, 9, 21 and 17 DE-miRNAs were identified for Angus, Charolais and KC populations respectively. For MWT, we identified 10, 19 and 18 DE-miRNAs for Angus, Charolais and KC animals respectively. With population comparison of the DE-miRNAs among the three traits and RFI reported previously (Mukiibi *et al.* 2019a), the results showed some overlap of the DE-miRNA between the traits, implying that these are pleotropic miRNAs modulating multiple traits. Additionally, *bta-miR-486*, identified as associated to ADG and MWT in KC and Charolais respectively, was also reported to be differentially expressed in the Longissimus muscle of high and low-RFI in Nellore cattle (De Oliveira *et al.* 2018). Of the six validated DE-miRNAs identified by RNAseq, two were found to show significant differential expression level between high and low-ADG steers using qPCR. However, both the RNAseq and qPCR methods showed a high similarity in terms of expression direction for all the six miRNAs with a Pearson's correlation of 0.84. The inability of qPCR to detect significant differences revealed by RNAseq could be due to the detection/profiling resolution differences between the two methods. Indeed, RNAseq offers

a way higher profiling resolution than qPCR, especially for those miRNAs with generally low expression in the samples (Motameny *et al.* 2010).

Target-prediction results revealed that the identified DE-miRNAs targeted 55% to 75% of the previously identified DE-genes by Mukiibi et al. (2019b). We observed that many genes were predicted to be targeted by more than one DE-miRNA, and some of the DE-miRNAs were predicted to target multiple genes. This observation demonstrates the target-miRNA regulatory interaction, as target 3' UTRs have been reported to have binding sites for multiple miRNAs, and a single miRNA through its seed region can modulate several targets that possess binding sites for that miRNA(Creighton *et al.* 2009; Lu & Clark 2012; Pritchard *et al.* 2012; Hashimoto *et al.* 2013; Hartig *et al.* 2015). These characteristics enable miRNAs to regulate large numbers of genes despite their small number. Also, some miRNAs show cooperative activity to effectively co-regulate a gene they target (Hashimoto *et al.* 2013). Our results also showed that large number of the target genes predicted to be regulated by at least one of the upregulated DE-miRNAs, had low (downregulated) expression in the liver tissue of animals in the same phenotype group. Nevertheless, some target genes maintained high expression in the liver tissue despite being predicted to be targeted by upregulated DE-miRNAs in the liver tissue of animals with the same phenotype. These observations could be attributed to the major modes of actions through which mammalian miRNAs modulate expression of their targets, i.e. promoting mRNA deadenylation consequently resulting into increased mRNA degradation, and mRNA translation repression (Fabian *et al.* 2010; Stroynowska-Czerwinska *et al.* 2014). The ultimate effect of the two modes of action is a reduced final protein product of the targeted mRNA or gene (Baek *et al.* 2008; Lu & Clark 2012). The first mode of action (mRNA degradation) results in decreased concentration of the target mRNA in the cytoplasm, and the difference in the level of mRNA concentration or

expression can be detected through transcriptomic studies like our current study (Creighton *et al.* 2009). However, for the second mode of action (mRNA translation repression) the modulation/regulation effect can only be studied or detected through transcriptomic studies as the concentration of mRNA in the cell would not be antagonized by the modulating miRNA (Creighton *et al.* 2009). It is also interesting to note that some target genes that were predicted to targeted by only downregulated DE-miRNAs, also had low expression levels. Downregulation of such targets could be by other pre-translational gene expression mechanisms including epigenetic control of transcription and regulation of transcription by transcription factors (Lackner & Bähler 2008). We recommend further molecular experimental validation of these regulatory interactions as bioinformatic target-miRNA prediction might yield multiple false positives (Stroynowska-Czerwinska *et al.* 2014).

In general, the major biological functions enriched by DE target genes for each of the traits within each population were largely similar to those identified for all the DE-genes (Mukiibi *et al.* 2019b), which was expected since large portions of the DE-genes were predicted as targets of the DE-miRNAs. This implies that probably the identified DE-miRNAs modulate the associated traits through differential modulation of functions such as lipid metabolism, molecule transport, amino acid metabolism, cell death and survival, cellular movement, cellular function and maintenance and small molecule biochemistry. Additionally, some other functions such as cellular growth and proliferation were also identified among the most enriched cellular and molecular functions by some of the identified DE-target gene sets. Cellular growth and proliferation are important processes of the liver that contribute to constant liver tissue regeneration to replace damaged tissue and maintain the tissue at optimal metabolic state (Song *et al.* 2010).

One of the most important discovery is that the differentially expressed miRNAs were majorly breed specific, and only MWT had a DE-miRNA (*bta-miR-10164-3p*) that was common to all the three breeds. This finds concur with the results of RNA transcriptome analyses for the same traits in the same breed populations, where the DE genes were also largely breed-specific (Mukiibi et al. 2019b), indicating that the feed efficiency and related traits are likely regulated by different sets of genes across the different beef breeds.

5.6 Conclusions

We identified 26, 46 and 46 miRNAs associated with ADG, and, DMI and MWT respectively. Most of these identified DE-miRNAs for each trait were majorly breed specific. Target gene prediction showed that majority (55% to 75%) of the previously identified DE-genes of the respective traits within the populations were targeted by the DE-miRNAs. Additionally, those target genes that were predicted to be targeted by at least one upregulated DE-miRNA generally showed contrasting expression profile, however, a substantial number of the DE-genes did not show contrasting expression as compared to their predicted regulators. These findings could be related to the different regulatory mechanisms through which miRNAs regulate their targets, such as augmentation of mRNA degradation and transcription repression. RNAseq can only detect miRNA differential gene modulation through augmentation of target mRNA degradation. Transcription repression could be the mode of regulation for the targets whose expression remained high despite being predicted to be targeted by upregulated DE-miRNAs and can only be confirmed through proteomic studies. Our results provide further insights about the molecular regulation of growth rate, feed intake and metabolic weight in beef cattle.

5.7 References

Agarwal V., Bell G.W., Nam J.-W. & Bartel D.P. (2015) Predicting effective microRNA target sites in mammalian mRNAs. elife 4, e05005.

Al-Husseini W., Chen Y., Gondro C., Herd R.M., Gibson J.P. & Arthur P.F. (2016) Characterization and profiling of liver microRNAs by RNA-sequencing in cattle divergently selected for residual feed intake. Asian-Australasian journal of animal sciences 29, 1371.

Alexandre P.A., Kogelman L.J., Santana M.H., Passarelli D., Pulz L.H., Fantinato-Neto P., Silva P.L., Leme P.R., Strefezzi R.F. & Coutinho L.L. (2015) Liver transcriptomic networks reveal main biological processes associated with feed efficiency in beef cattle. BMC genomics 16, 1073.

Andrews S. (2010) FastQC: a quality control tool for high throughput sequence data.

Baek D., Villén J., Shin C., Camargo F.D., Gygi S.P. & Bartel D.P. (2008) The impact of microRNAs on protein output. Nature 455, 64.

Chen Y., Gondro C., Quinn K., Herd R., Parnell P. & Vanselow B. (2011) Global gene expression profiling reveals genes expressed differentially in cattle with high and low residual feed intake. Animal genetics 42, 475-90.

Creighton C.J., Reid J.G. & Gunaratne P.H. (2009) Expression profiling of microRNAs by deep sequencing. Briefings in bioinformatics 10, 490-7.

De Oliveira P.S., Coutinho L.L., Tizioto P.C., Cesar A.S., de Oliveira G.B., Diniz W.J.d.S., De Lima A.O., Reecy J.M., Mourão G.B. & Zerlotini A. (2018) An integrative transcriptome analysis indicates regulatory mRNA-miRNA networks for residual feed intake in Nelore cattle. Scientific reports 8, 17072.

Fabian M.R., Sonenberg N. & Filipowicz W. (2010) Regulation of mRNA translation and stability by microRNAs. Annual review of biochemistry 79, 351-79.

Friedländer M.R., Mackowiak S.D., Li N., Chen W. & Rajewsky N. (2011) miRDeep2 accurately identifies known and hundreds of novel microRNA genes in seven animal clades. Nucleic acids research 40, 37-52.

Goonewardene L., Wang Z., Price M., Yang R.-C., Berg R. & Makarechian M. (2003) Effect of udder type and calving assistance on weaning traits of beef and dairy× beef calves. Livestock production science 81, 47-56.

Griffiths-Jones S., Saini H.K., van Dongen S. & Enright A.J. (2007) miRBase: tools for microRNA genomics. Nucleic acids research 36, D154-D8.

Hartig S.M., Hamilton M.P., Bader D.A. & McGuire S.E. (2015) The miRNA interactome in metabolic homeostasis. Trends in Endocrinology & Metabolism 26, 733-45.

Hashimoto Y., Akiyama Y. & Yuasa Y. (2013) Multiple-to-multiple relationships between microRNAs and target genes in gastric cancer. PloS one 8, e62589.

Häussinger D. (1996) Physiological functions of the liver. In: Comprehensive human physiology (pp. 1369-91. Springer.

Lackner D.H. & Bähler J. (2008) Translational control of gene expression: from transcripts to transcriptomes. International review of cell and molecular biology 271, 199-251.

Love M.I., Huber W. & Anders S. (2014) Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. Genome biology 15, 550.

Lu J. & Clark A.G. (2012) Impact of microRNA regulation on variation in human gene expression. Genome research 22, 1243-54.

Martin M. (2011) Cutadapt removes adapter sequences from high-throughput sequencing reads. EMBnet. journal 17, 10-2.

Motameny S., Wolters S., Nürnberg P. & Schumacher B. (2010) Next generation sequencing of miRNAs–strategies, resources and methods. Genes 1, 70-84.

Mukiibi R., Johnston D., Vinsky M., Fitzsimmons C., Stothard P., Waters S.M. & Li C. (2019a) Bovine hepatic miRNAome profiling and differential miRNA expression analyses between beef steers with divergent feed efficiency phenotypes.

Mukiibi R., Vinsky M., Keogh K., Fitzsimmons C., Stothard P., Waters S.M. & Li C. (2019b) Liver transcriptome profiling of beef steers with divergent growth rate, feed intake or metabolic body weight phenotypes. Journal of animal science.

Mukiibi R., Vinsky M., Keogh K.A., Fitzsimmons C., Stothard P., Waters S.M. & Li C. (2018) Transcriptome analyses reveal reduced hepatic lipid synthesis and accumulation in more feed efficient beef cattle. Scientific reports 8, 7303.

Paradis F., Yue S., Grant J., Stothard P., Basarab J. & Fitzsimmons C. (2015) Transcriptomic analysis by RNA sequencing reveals that hepatic interferon-induced genes may be associated with feed efficiency in beef heifers. Journal of animal science 93, 3331-41.

Pritchard C.C., Cheng H.H. & Tewari M. (2012) MicroRNA profiling: approaches and considerations. Nature Reviews Genetics 13, 358.

Robinson M.D., McCarthy D.J. & Smyth G.K. (2010) edgeR: a Bioconductor package for differential expression analysis of digital gene expression data. Bioinformatics 26, 139-40.

Robinson M.D. & Oshlack A. (2010) A scaling normalization method for differential expression analysis of RNA-seq data. Genome biology 11, R25.

Shannon P., Markiel A., Ozier O., Baliga N.S., Wang J.T., Ramage D., Amin N., Schwikowski B. & Ideker T. (2003) Cytoscape: a software environment for integrated models of biomolecular interaction networks. Genome research 13, 2498-504.

Song G., Sharma A.D., Roll G.R., Ng R., Lee A.Y., Blelloch R.H., Frandsen N.M. & Willenbring H. (2010) MicroRNAs control hepatocyte proliferation during liver regeneration. Hepatology 51, 1735-43.

Stroynowska-Czerwinska A., Fiszer A. & Krzyzosiak W.J. (2014) The panorama of miRNA-mediated mechanisms in mammalian cells. Cellular and Molecular Life Sciences 71, 2253-70.

Tizioto P.C., Coutinho L.L., Decker J.E., Schnabel R.D., Rosa K.O., Oliveira P.S., Souza M.M., Mourão G.B., Tullio R.R. & Chaves A.S. (2015) Global liver gene expression differences in Nelore steers with divergent residual feed intake phenotypes. BMC genomics 16, 242.

5.8 Figures and Tables

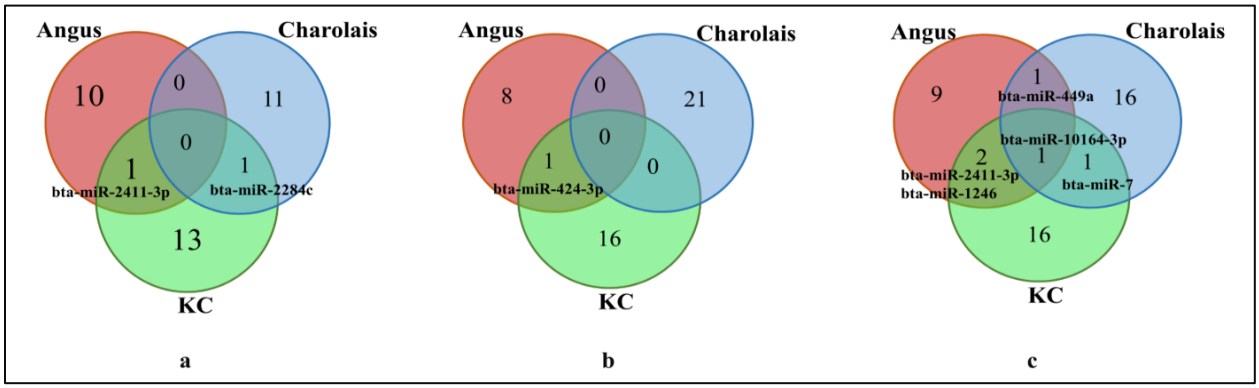


Figure 5.1. Venn diagrams showing between breed DE-miRNA comparisons for: (a) ADG; (b) DMI and (c) MWT.

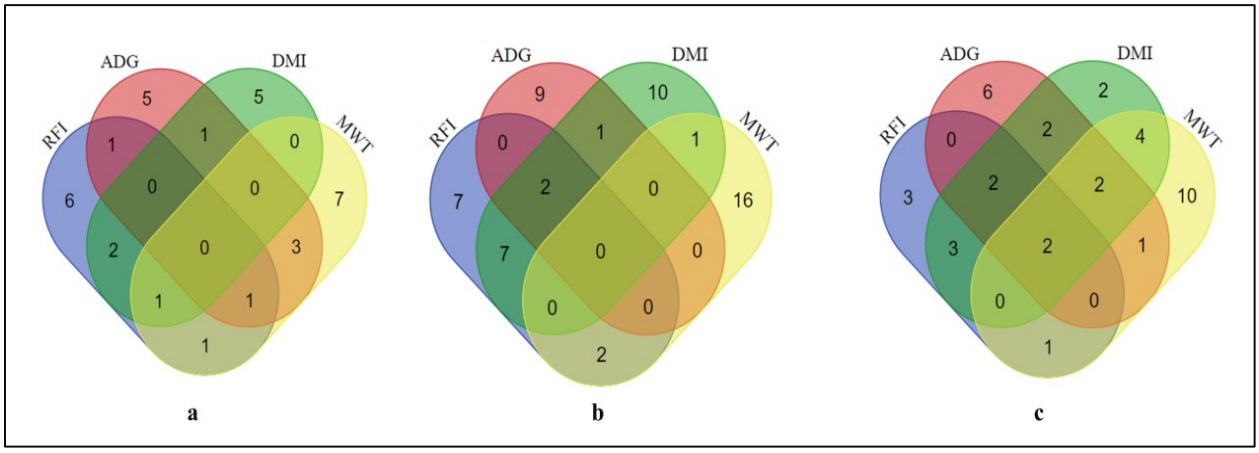


Figure 5.2. Venn diagrams showing between traits DE-miRNA comparisons for: (a) Angus, (b) Charolais and (c) KC.

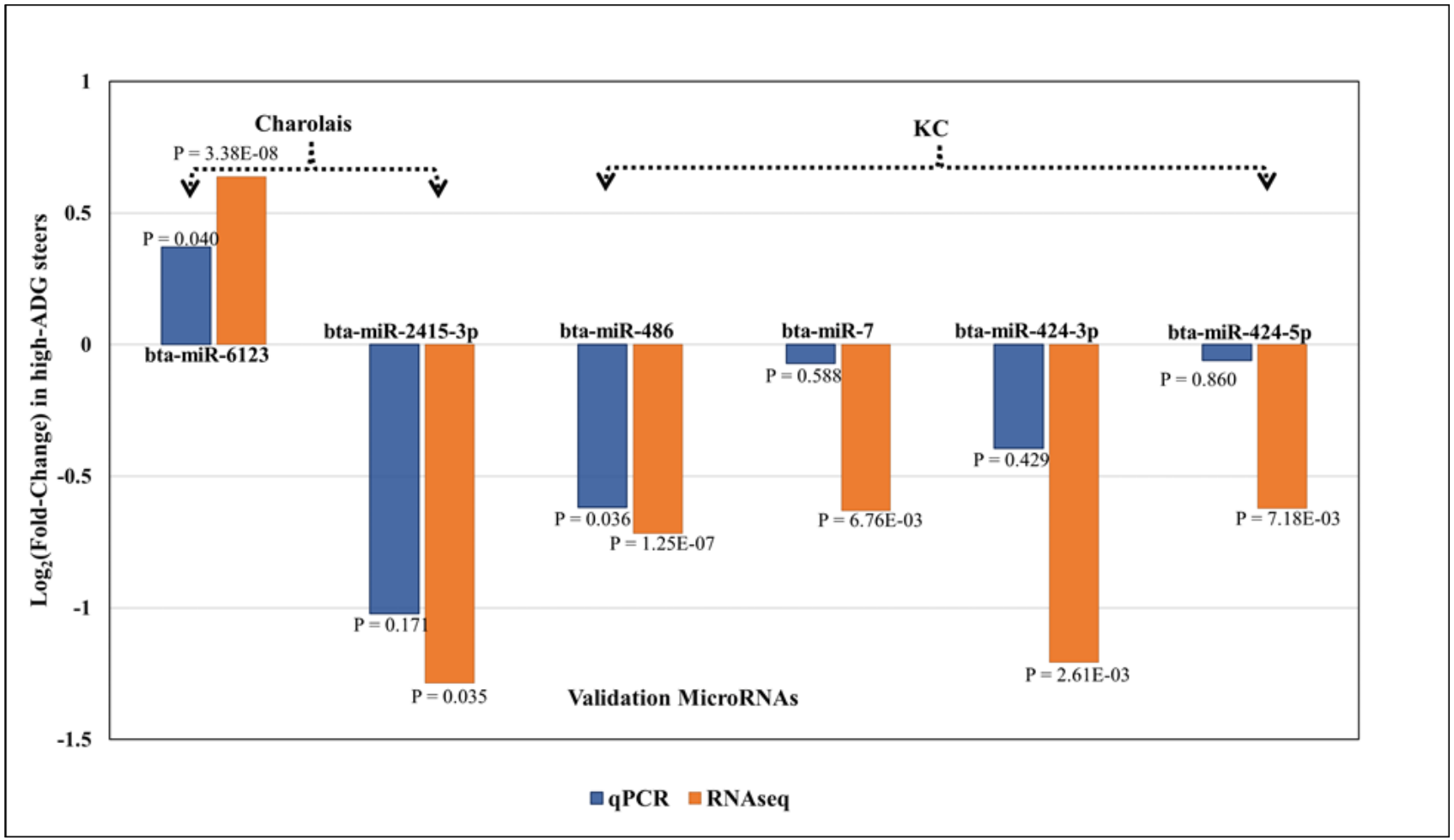


Figure 5.3. Bar plot showing expression profile of the six validation DE-miRNAs by qPCR and RNAseq in the liver tissue of high-ADG steers. (P = P-value)

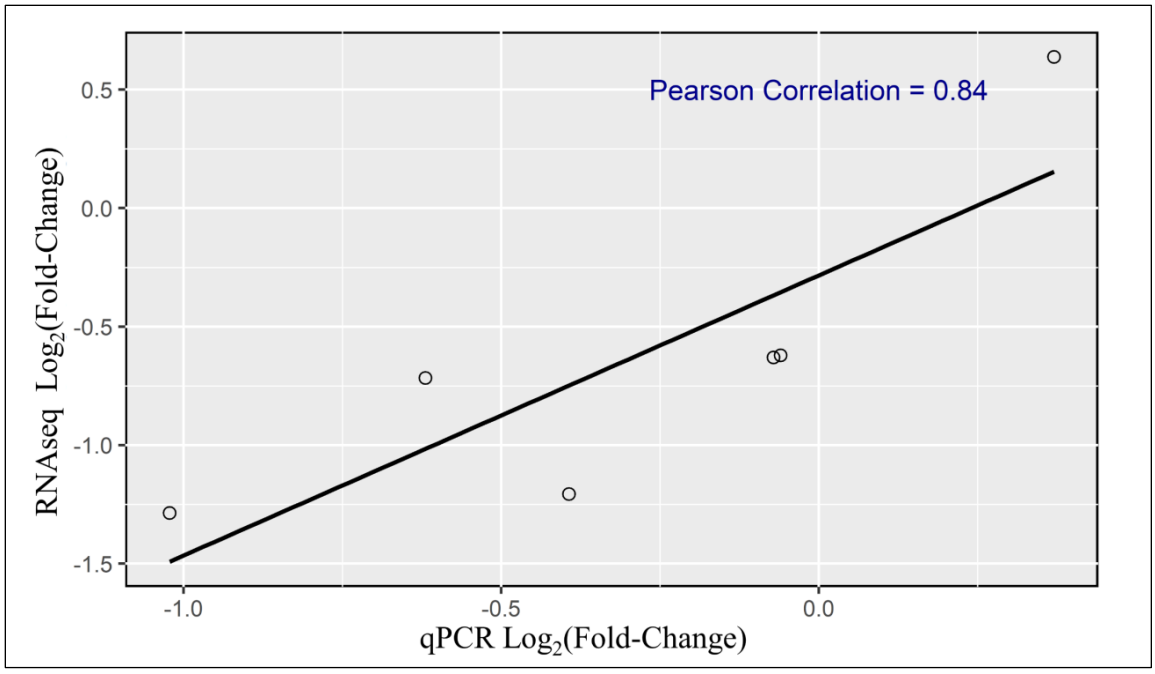
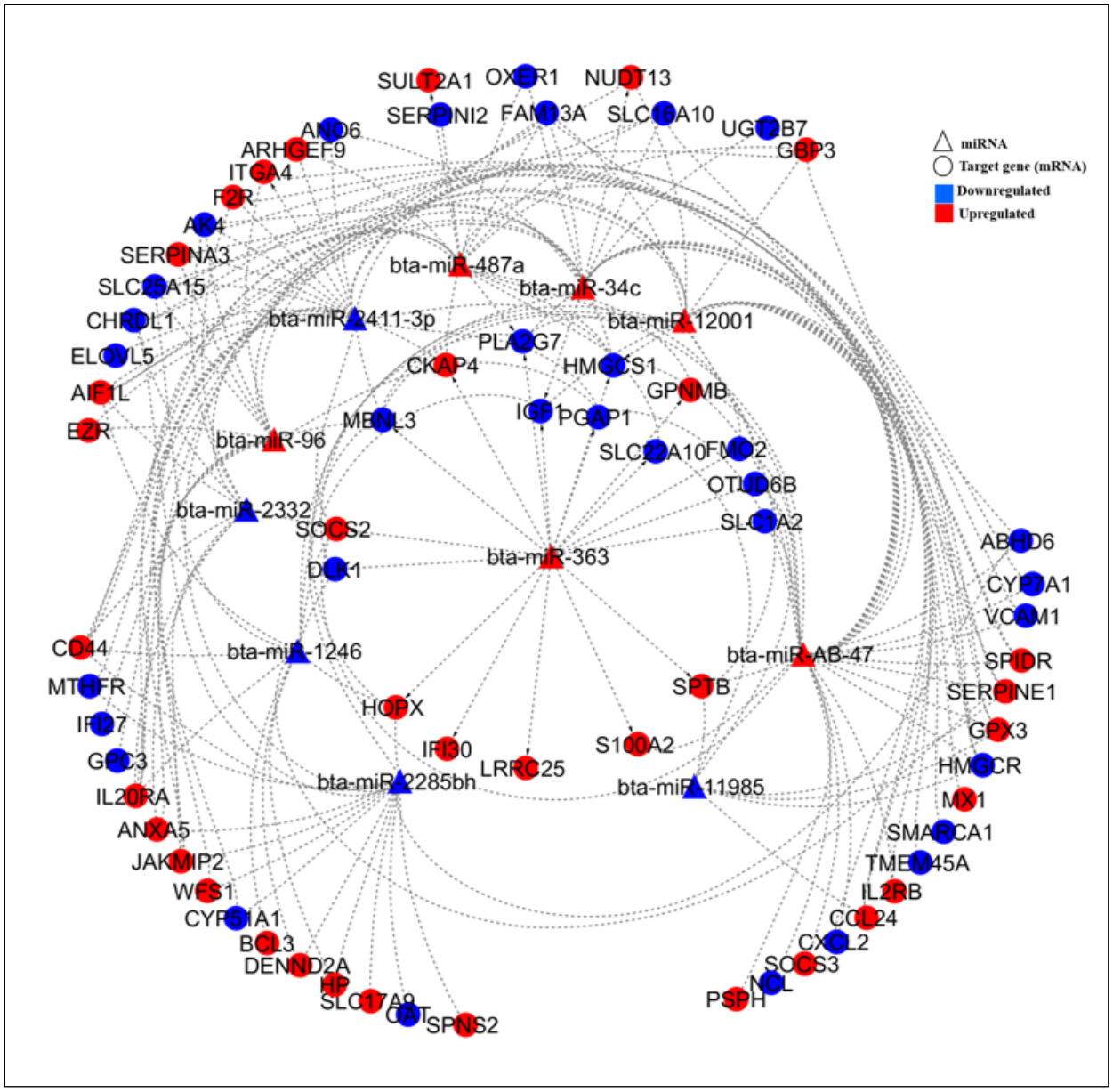
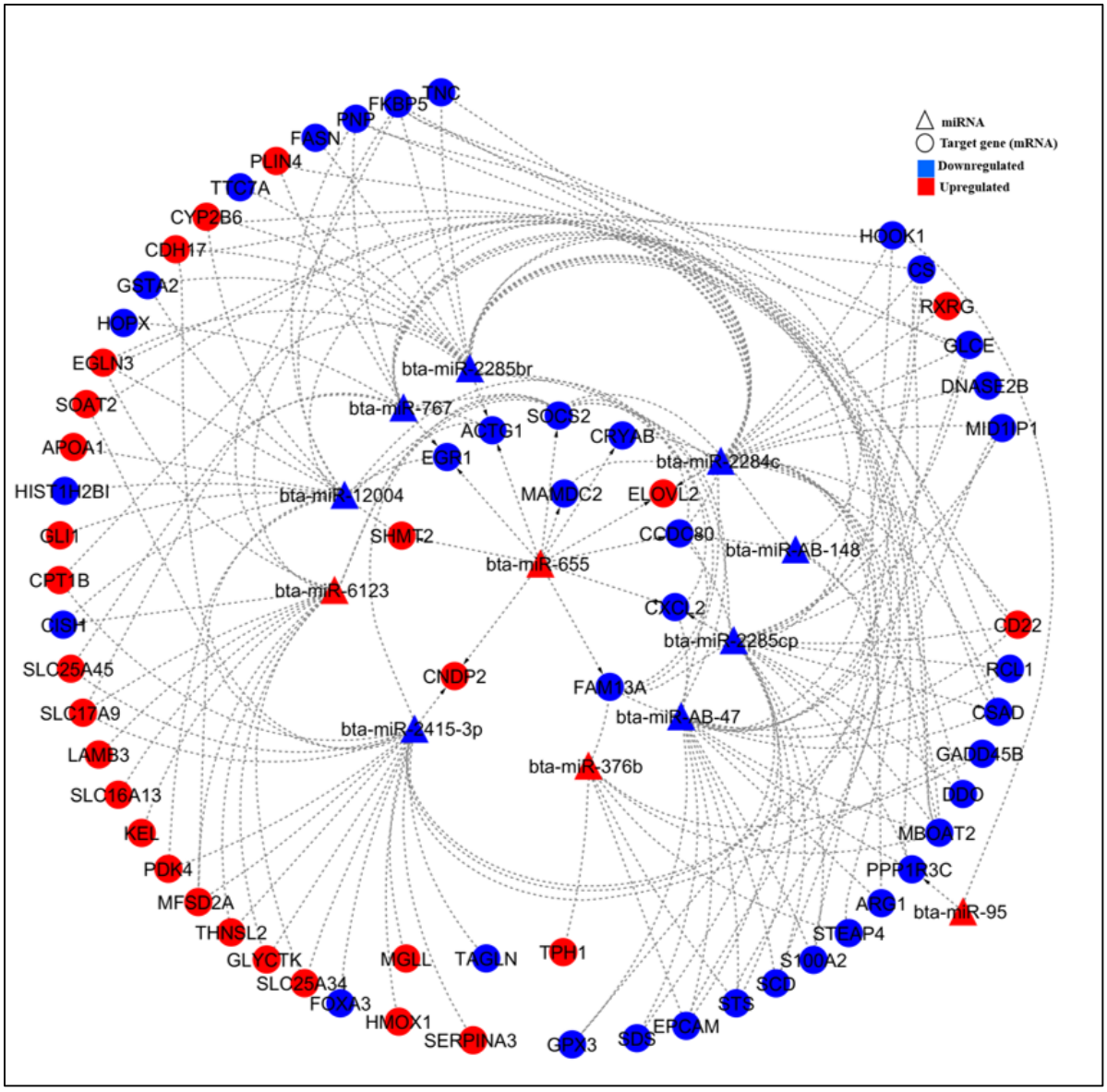


Figure 5.4. Correlation plot showing the correlation between Log_2(Fold-Change) for RNAseq and qPCR for the six validation DE-miRNAs by qPCR and RNAseq in the liver tissue of high-ADG steers.



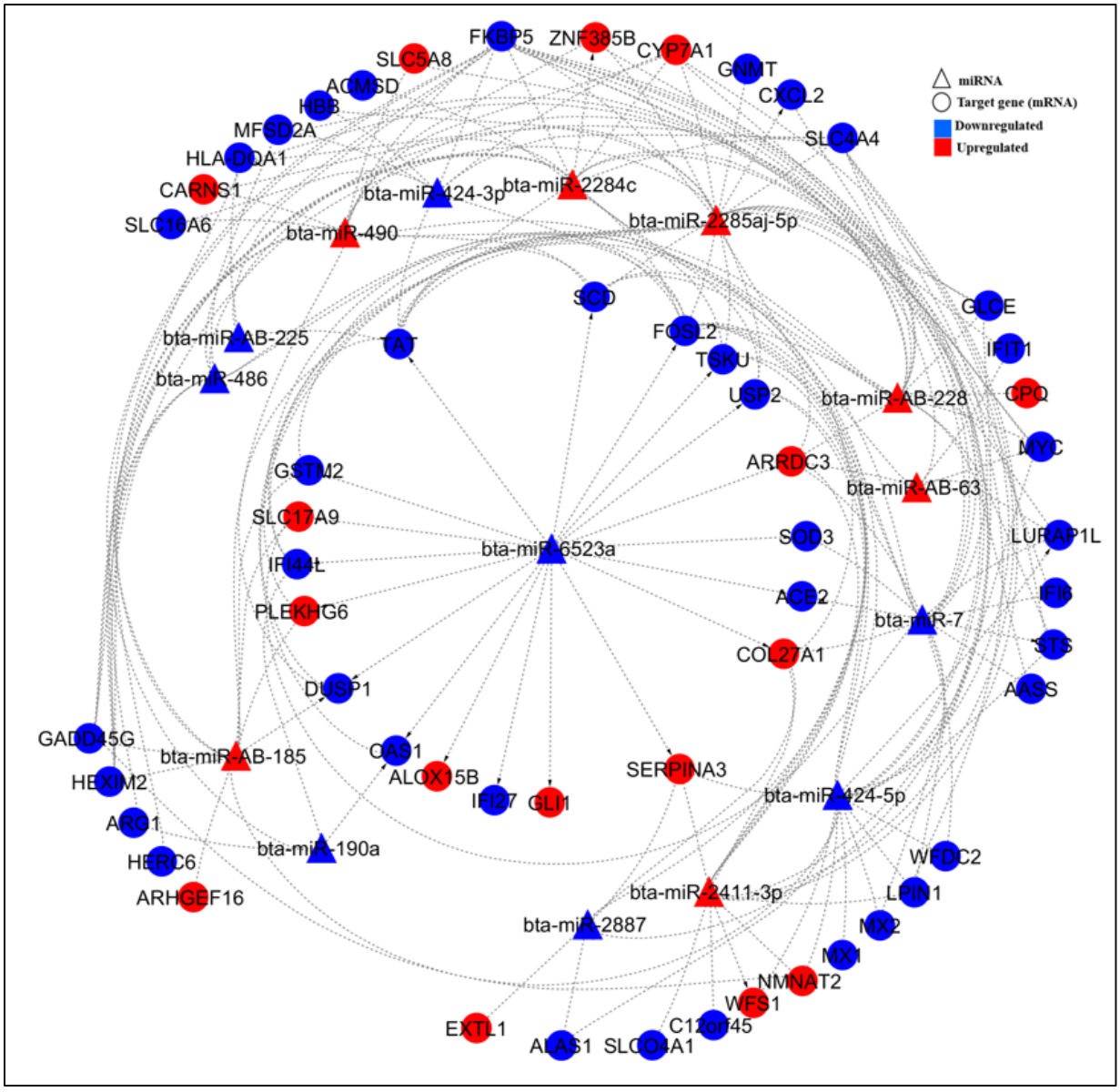
Red and blue indicate increased and reduced expression respectively of the gene or micro RNA in the liver tissue of high-ADG Angus steers relative to low-ADG Angus animals.

Figure 5.5. DE-miRNA-DE-genes predicted interaction network for average daily growth (ADG) for Angus steers.



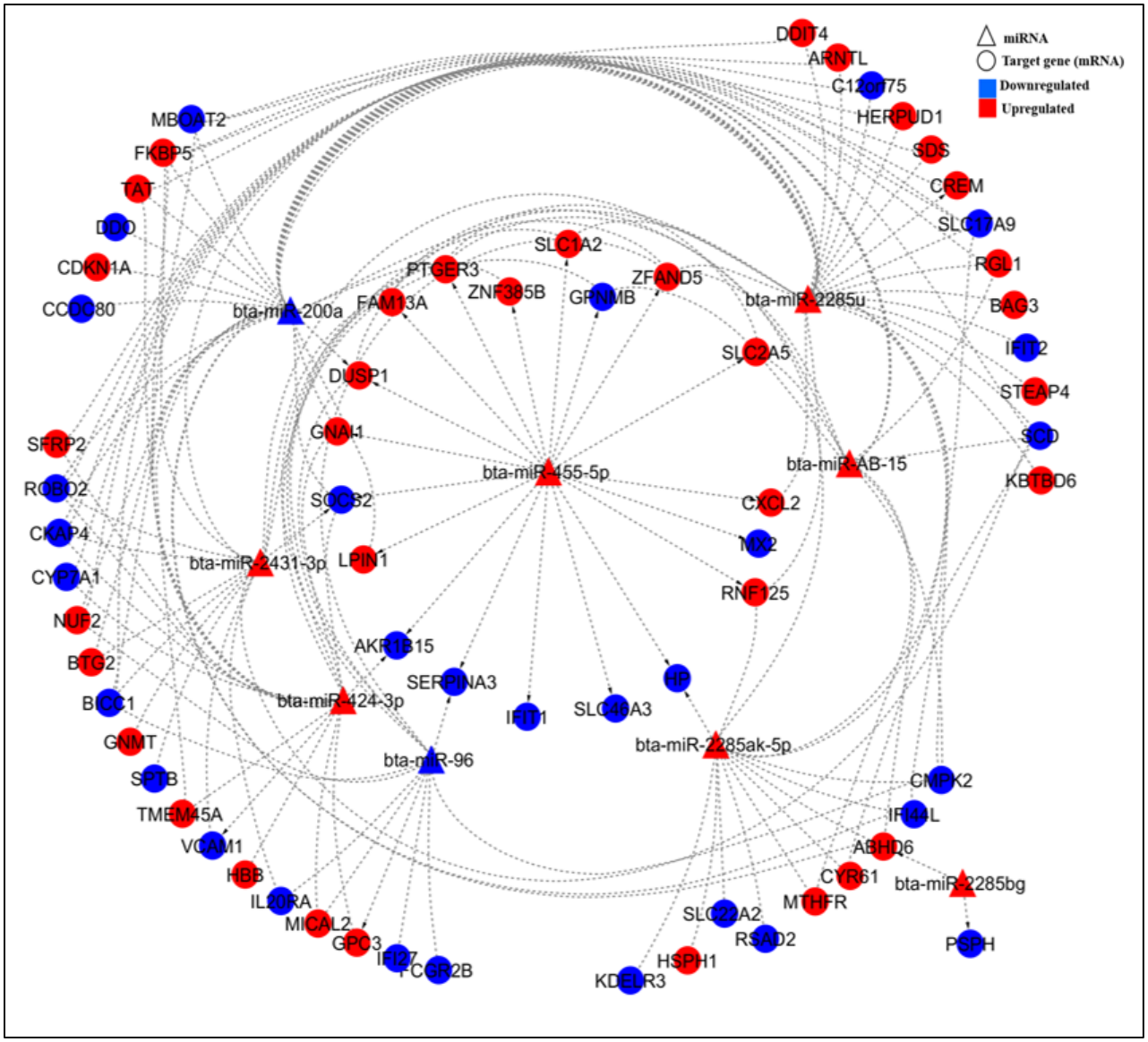
Red and blue indicate increased and reduced expression respectively of the gene or micro RNA in the liver tissue of high-ADG Charolais steers relative to low-ADG Charolais animals.

Figure 5.6. DE-miRNA-DE-genes predicted interaction network for average daily growth (ADG) for Charolais steers.



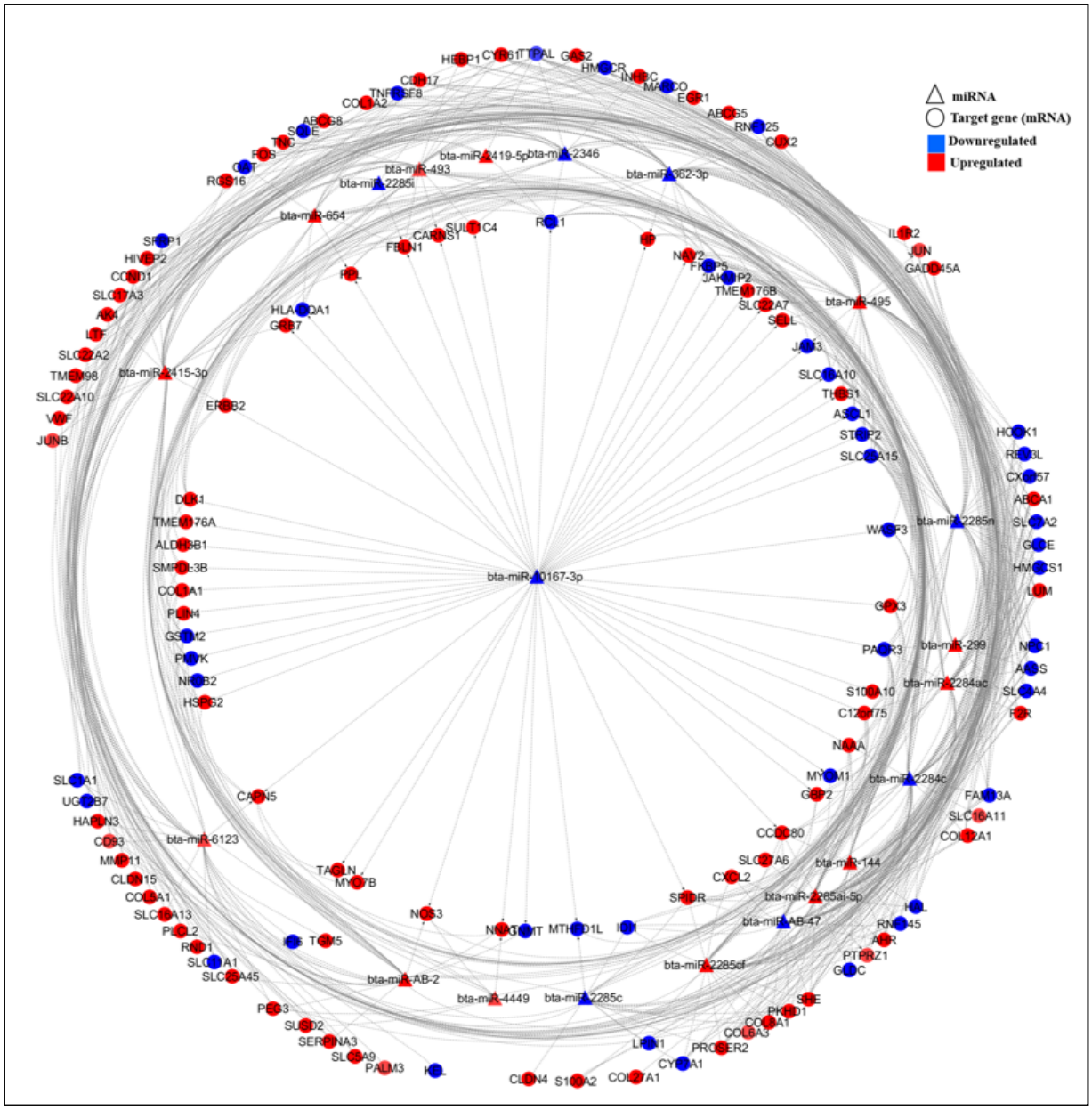
Red and blue indicate increased and reduced expression respectively of the gene or micro RNA in the liver tissue of high-ADG Kinsella Composite steers relative to low-ADG Kinsella Composite animals.

Figure 5.7. DE-miRNA-DE-genes predicted interaction network for average daily growth (ADG) for Kinsella Composite steers.



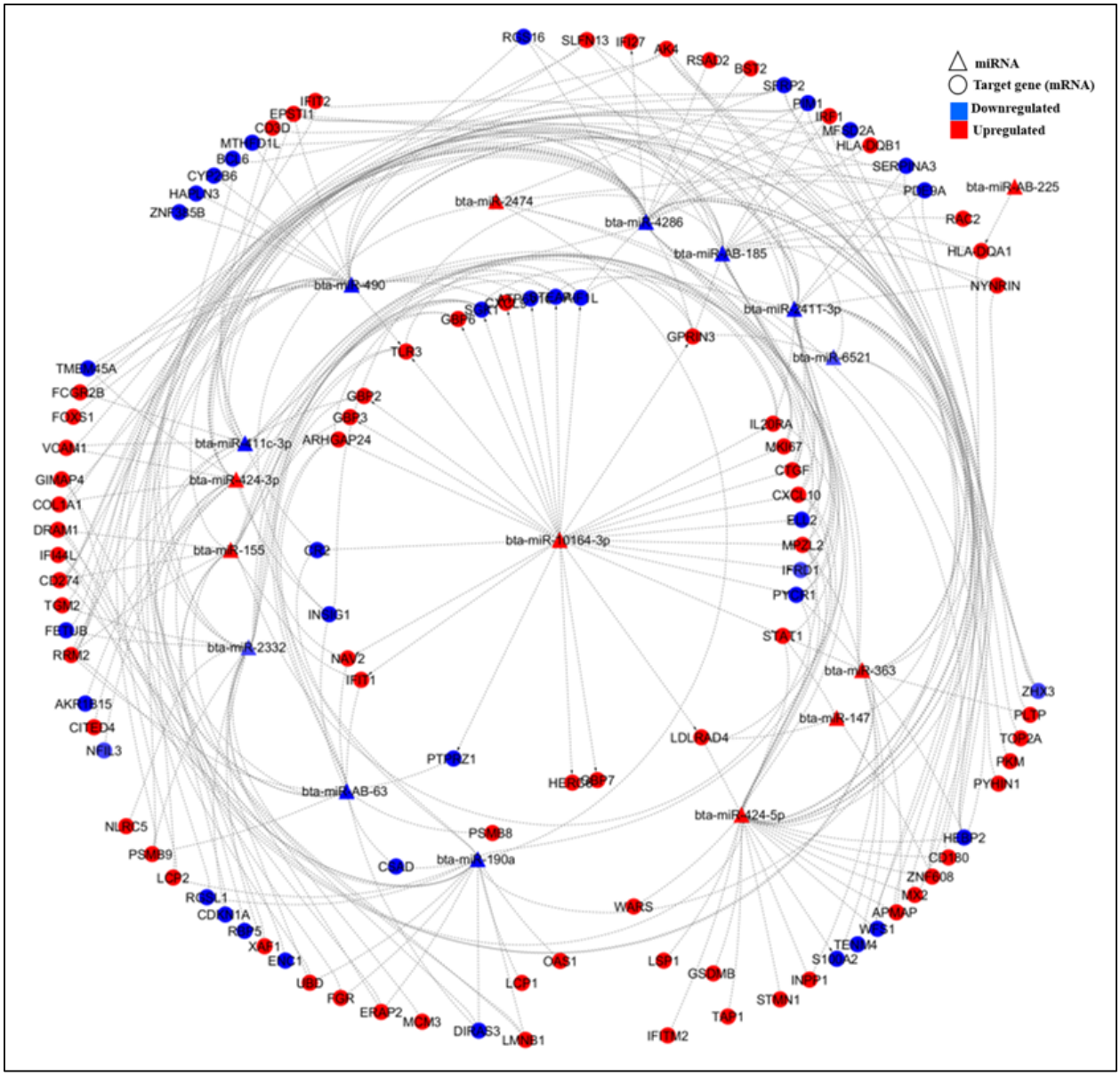
Red and blue indicate increased and reduced expression respectively of the gene or micro RNA in the liver tissue low-DMI Angus steers relative to high-DMI Angus animals.

Figure 5.8. DE-miRNA-DE-genes predicted interaction network for dry matter intake (DMI) for Angus steers.



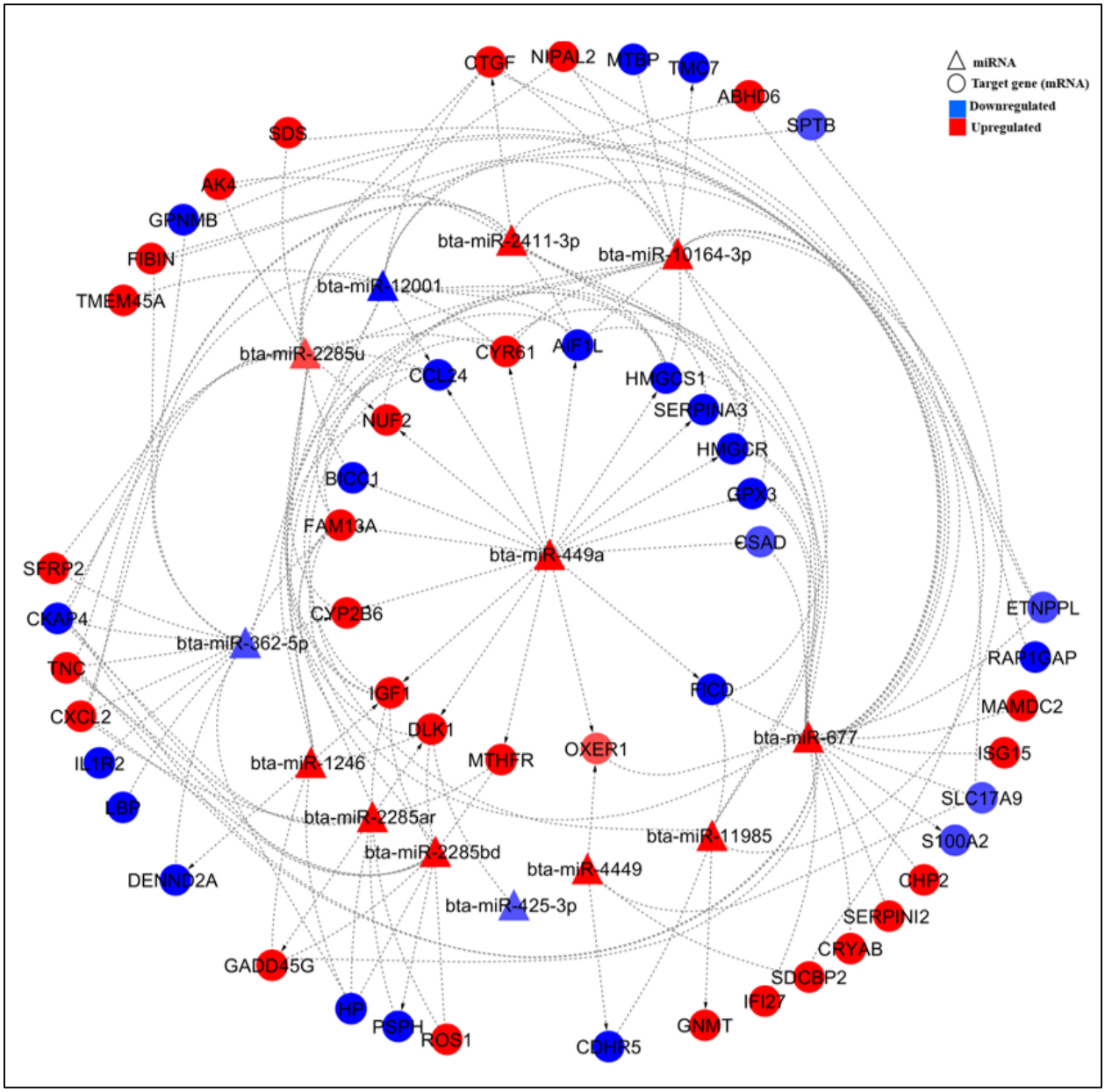
Red and blue indicate increased and reduced expression respectively of the gene or micro RNA in the liver tissue low-DMI Charolais steers relative to high-DMI animals.

Figure 5.9. DE-miRNA-DE-genes predicted interaction network for dry matter intake (DMI) for Charolais steers.



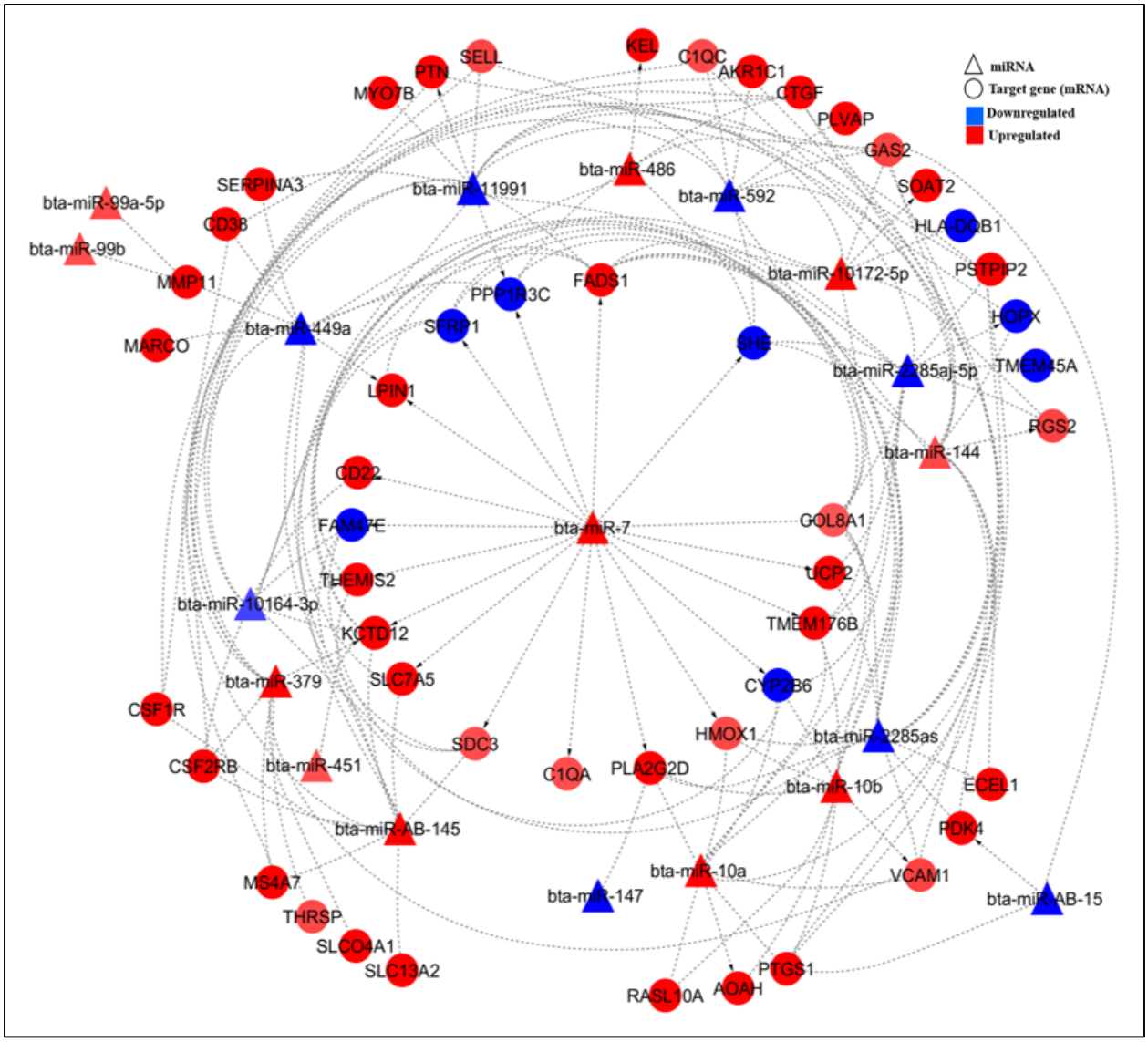
Red and blue indicate increased and reduced expression respectively of the gene or micro RNA in the liver tissue low-DMI Kinsella Composite steers relative to high-DMI Kinsella Composite animals.

Figure 5.10. DE-miRNA-DE-genes predicted interaction network for dry matter intake (DMI) for Kinsella Composite steers.



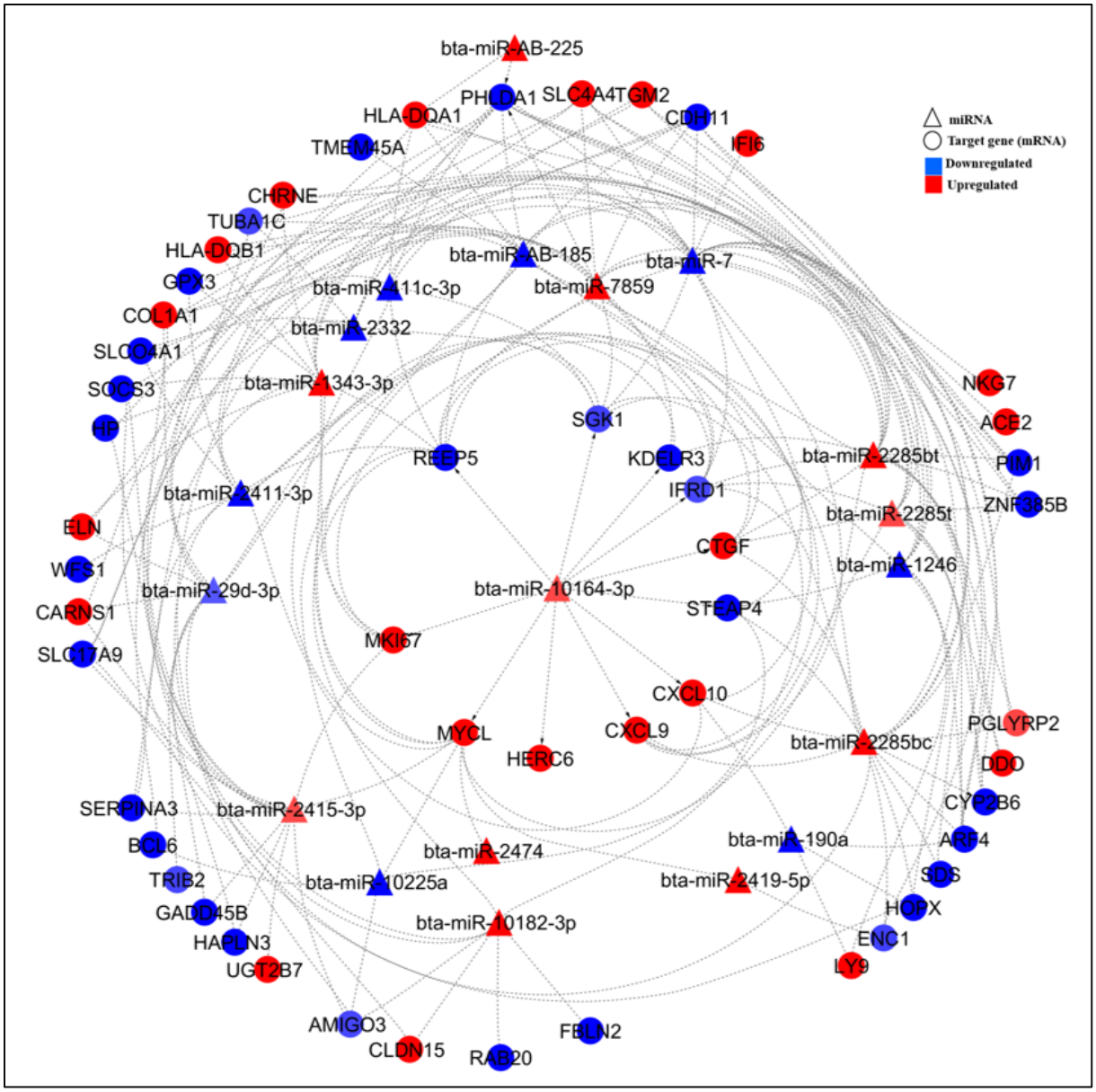
Red and blue indicate increased and reduced expression respectively of the gene or micro RNA in the liver tissue low-MWT Angus steers relative to high-MWT Angus animals.

Figure 5.11. DE-miRNA-DE-genes predicted interaction network for metabolic weight (MWT) in Angus steers.



Red and blue indicate increased and reduced expression respectively of the gene or micro RNA in the liver tissue low-MWT Charolais steers relative to high-MWT Charolais animals.

Figure 5.12. DE-miRNA-DE-genes predicted interaction network for metabolic weight (MWT) in Charolais steers.



Red and blue indicate increased and reduced expression respectively of the gene or micro RNA in the liver tissue low-MWT Kinsella Composite steers relative to high-MWT Kinsella Composite animals.

Figure 5.13. DE-miRNA-DE-genes predicted interaction network for metabolic weight (MWT) in KC steers.

Table 5.1. Phenotypic differences between steers with divergent average daily gain (ADG) phenotypes, tested by two sample t-tests.

	Angus			Charolais			KC		
Trait	**L_ADG±SE**	**H_ADG±SE**	**P-value**	**L_ADG±SE**	**H_ADG±SE**	**P-value**	**L_ADG±SE**	**H_ADG±SE**	**P-value**
ADG/kg/day	1.54±0.02	1.99±0.1	3.98E-03	1.48±0.03	1.92±0.05	3.15E-05	1.23±0.04	1.88±0.09	**4.80E-05**
RFI/kg/day	0.63±0.35	-0.19±0.42	0.17	0.36±0.39	0.02±0.34	0.53	-0.65±0.3	-0.06±0.56	0.33
DMI/kg/day	12.2±0.34	12.84±0.41	0.25	11.43±0.43	11.3±0.42	0.84	9.57±0.31	11.12±0.78	0.1
MWT/kg	115±2.33	122.52±3.06	0.08	121.86±2.21	116.67±2.14	0.12	98.45±1.66	103.53±3.02	0.17
FUREA/cm2	83.08±2.89	81.69±2.34	0.72	95.46±4.95	89.64±3.01	0.34	65.34±1.62	74.45±1.49	0.002
FUFAT/mm	10.57±0.93	10.02±0.8	0.66	5.9±0.63	5.96±0.43	0.93	8.28±0.94	8.83±0.32	0.59
HCW/lb	752.95±21.68	810.7±27.48	0.13	860.83±20.69	822.67±14.94	0.17	657.67±20.83	703.33±26.7	0.21
AFAT/mm	12±1.13	11.83±1.22	0.92	96±3.27	86.83±4.3	0.12	11.17±1.78	9.83±0.95	0.52
CREA/cm2	73.17±2.87	72.33±3.18	0.85	6.17±0.65	7.33±0.49	0.19	66.83±3.33	77.17±2.98	0.04
LMY/%	55.08±1.17	54.32±1.45	0.69	62.48±0.89	60.27±0.8	0.1	55.55±1.36	58.02±0.94	0.17
Marbling score	448.33±24.95	391.67±21.97	0.12	381.67±19.39	371.67±28.68	0.78	372.5±9.64	380±18.08	0.72
Slaughter age/days	491.67±7.52	500.5±2.54	0.29	525.33±1.33	504.5±9.05	0.05	470.83±8.35	448.5±6.14	0.06

Bolded = significant differences (Bonferroni correction of P-value < 0.0042).

Table 5.2. Phenotypic differences between steers with divergent average dry matter intake (DMI) phenotypes, tested by two sample t-tests.

	Angus			Charolais			Kinsella Composite		
Trait	**L_DMI±SE**	**H_DMI±SE**	**P-value**	**L_DMI±SE**	**H_DMI±SE**	**P-value**	**L_DMI±SE**	**H_DMI±SE**	**P-value**
DMI/kg/day	10.88±0.21	13.27±0.26	**2.87E-05**	10.01±0.08	12.4±0.15	**7.06E-08**	8.83±0.16	12.8±0.32	**6.47E-07**
RFI/kg/day	-0.7±0.03	0.57±0.45	0.02	-1.01±0.11	1.1±0.18	**1.90E-06**	-1.18±0.14	1.4±0.17	**3.77E-07**
ADG/kg/day	1.72±0.03	1.91±0.13	0.2	1.65±0.08	1.6±0.06	0.63	1.39±0.11	1.61±0.08	0.14
MWT/kg	110.29±2.8	120.11±2.17	0.01	117.98±1.72	122.98±2.21	0.1	96.28±1.43	106.11±1.88	**1.94E-03**
FUREA/cm2	84.24±1.3	82.11±3.02	0.53	97.28±3.83	93.23±1.96	0.37	68.78±3.26	74±1.64	0.74
FUFAT/mm	9.06±1.01	9.65±0.85	0.72	5.58±0.58	6.46±0.66	0.34	9.39±0.83	9.07±0.46	0.18
HCW/lb	721.87±22.91	792.03±15.36	0.03	834.83±14.71	863.5±17.24	0.23	624.33±7.85	701.17±22.54	**1.16E-06**
AFAT/mm	11.17±1.47	12±1.46	0.7	96.33±3.74	93.83±1.3	0.54	13.17±1.62	9.67±0.49	0.06
CREA/cm2	76.17±2.75	74.17±3.99	0.69	6.5±0.76	7±0.58	0.61	65.83±2.89	75.83±2.51	0.03
LMY/%	56.66±1.55	54.81±1.67	0.43	62.62±0.86	61.42±0.72	0.31	54.35±1.09	57.89±0.53	0.02
Marbling	423.33±28.47	410±20.97	0.71	386.67±36.75	405±26.17	0.69	373.33±21.09	361.67±12.5	0.64
Slaughter age	505.83±5.77	495.17±4.08	0.16	518.33±6.85	528±1.21	0.2	461.17±5.45	446.67±2.83	0.04

Bolded = significant differences (Bonferroni correction of P-value < 0.0042).

Table 5.3. Phenotypic differences between steers with divergent metabolic weight (MWT) phenotypes, tested by two sample t-tests.

	Angus			Charolais			Kinsella Composite		
Trait	**L_MWT±SE**	**H_MWT±SE**	**P-value**	**L_MWT±SE**	**H_MWT±SE**	**P-value**	**L_MWT±SE**	**H_MWT±SE**	**P-value**
MWT/kg	110.1±3.05	121.8±3.73	0.04	114.57±0.83	125.56±1.08	**1.05E-05**	94.24±0.72	107.93±1.47	**7.97E-06**
RFI/kg/day	0.61±0.42	-0.37±0.41	0.12	0.24±0.36	-0.11±0.44	0.55	-0.07±0.51	0.57±0.53	0.41
DMI/kg/day	12±0.52	12.43±0.57	0.59	11.22±0.38	11.42±0.47	0.75	9.75±0.54	12.22±0.62	**2.93E-08**
ADG/kg/day	1.63±0.05	1.9±0.12	0.07	1.8±0.07	1.63±0.06	0.1	1.56±0.13	1.64±0.07	0.57
FUREA/cm2	83.4±2.97	81.18±2.11	0.55	86.81±3.67	96.33±1	0.03	8.26±0.36	9.16±0.39	0.12
FUFAT/mm	9.26±0.34	10.64±1	0.22	5.97±0.43	7.01±0.85	0.3	68.52±2.35	73.94±1.54	0.08
HCW/lb	720.32±25.85	804.4±33.02	0.07	803.33±6.97	898.17±14.31	**1.40E-04**	641.17±17.59	727.67±16.62	**5.07E-03**
AFAT/mm	11.5±1.2	12.17±1.49	0.74	88.17±3.74	96.5±3.92	0.15	10.67±0.71	8.83±0.6	0.08
CREA/cm2	75±2.83	72.67±3.62	0.62	7.33±0.61	8.17±1.14	0.53	69±3.51	78.5±1.63	0.03
LMY/%	56.21±0.98	54.23±1.83	0.36	60.78±0.77	60.71±1.04	0.96	56.54±0.59	58.69±0.56	0.02
Marbling	426.67±14.53	376.67±14.98	0.04	353.33±17.44	363.33±26.28	0.76	372.5±22.05	375±6.71	0.92
Slaughter age	486.17±4.43	505.17±4.77	0.02	503±8.22	525±4.42	0.04	460.17±11.3	448.67±3.4	0.35

Bolded = significant differences (Bonferroni correction of P-value < 0.0042).

Table 5.4. Differentially expressed known micro RNAs between high and low-ADG animals, with a differential expression threshold of P-value < 0.05 and Fold-change > 1.5.

Angus	**MicroRNA**	**Log_2FC**	**P-value**
1	*bta-miR-11985*	-1.633	1.97E-05
2	*bta-miR-12001*	0.903	1.84E-03
3	*bta-miR-2411-3p*	-0.902	3.13E-03
4	*bta-miR-1246*	-0.622	3.69E-03
5	*bta-miR-2332*	-0.724	4.49E-03
6	*bta-miR-487a*	1.155	8.31E-03
7	*bta-miR-96*	0.691	9.89E-03
8	*bta-miR-34c*	1.165	0.012
9	*bta-miR-363*	0.801	0.013
10	*bta-miR-AB-47*	0.621	0.014
11	*bta-miR-2285bh*	-0.962	0.044
Charolais	**MicroRNA**	**Log_2FC**	**P-value**
1	*bta-miR-2415-3p*	-1.287	7.38E-08
2	*bta-miR-AB-148*	-0.826	6.49E-04
3	*Bta-miR-AB-47*	-0.901	1.32E-03
4	*bta-miR-767*	-1.217	3.80E-03
5	*bta-miR-376b*	0.866	0.019
6	*bta-miR-2284c*	-1.012	0.021
7	*bta-miR-95*	0.752	0.025
8	*bta-miR-2285br*	-0.82	0.03
9	*bta-miR-2285cp*	-0.996	0.035
10	*bta-miR-6123*	0.638	0.035
11	*bta-miR-12004*	-0.798	0.041
12	*bta-miR-655*	0.639	0.041
KC	**MicroRNA**	**Log_2FC**	**P-value**
1	*bta-miR-AB-225*	2.287	1.85E-09
2	*bta-miR-486*	-0.717	1.25E-07
3	*bta-miR-6523a*	-1.497	1.86E-04
4	*bta-miR-424-3p*	-1.207	2.61E-03
5	*bta-miR-2887*	-1.183	2.69E-03
6	*bta-miR-AB-10*	-0.614	3.09E-03
7	*bta-miR-7*	-0.631	6.76E-03
8	*bta-miR-424-5p*	-0.621	7.18E-03
9	*bta-miR-190a*	-1.026	0.012
10	*bta-miR-2284c*	1.108	0.016
11	*bta-miR-AB-63*	0.719	0.020

12	*bta-miR-2285aj-5p*	0.659	0.025
13	*bta-miR-490*	0.627	0.03
14	*bta-miR-2411-3p*	0.679	0.035
15	*bta-miR-AB-185*	0.912	0.042

logFC = log_2(Fold-change) and he sign of logFC shows the direction of miRNA expression in high-ADG steers relative to low-ADG animals.

Table 5.5. Differentially expressed known micro RNAs between high and low-DMI animals, with a differential expression threshold of P-value < 0.05 and Fold-change > 1.5.

Angus	**MicroRNA**	**Log_2FC**	**P-value**
1	*bta-miR-455-5p*	0.61	1.22E-04
2	*bta-miR-96*	-1.025	1.78E-04
3	*bta-miR-2285bg*	1.415	1.04E-03
4	*bta-miR-200a*	-0.608	1.40E-03
5	*bta-miR-424-3p*	1.196	1.53E-03
6	*bta-miR-AB-15*	0.859	6.37E-03
7	*bta-miR-2285u*	0.727	0.019
8	*bta-miR-2431-3p*	1.001	0.023
9	*bta-miR-2285ak-5p*	0.964	0.036
Charolais	**MicroRNA**	**Log_2FC**	**P-value**
1	*bta-miR-2415-3p*	1.194	2.27E-07
2	*bta-miR-2419-5p*	0.788	5.78E-07
3	*bta-miR-AB-2*	0.866	6.24E-05
4	*bta-miR-2284c*	-1.731	2.57E-04
5	*bta-miR-2346*	-1.483	4.75E-04
6	*bta-miR-144*	0.754	5.37E-04
7	*bta-miR-2285i*	-0.98	8.48E-04
8	*bta-miR-10167-3p*	-1.098	1.71E-03
9	*bta-miR-654*	0.937	1.83E-03
10	*bta-miR-495*	0.666	4.88E-03
11	*bta-miR-AB-47*	-0.719	5.08E-03
12	*bta-miR-493*	0.616	7.87E-03
13	*bta-miR-299*	0.863	9.91E-03
14	*bta-miR-2285n*	-0.966	0.014
15	*bta-miR-2285ai-5p*	0.65	0.017
16	*bta-miR-2285c*	-1.022	0.018
17	*bta-miR-2284ac*	0.635	0.021

18	*bta-miR-362-3p*	-0.94	0.022
19	*bta-miR-6123*	0.63	0.027
20	*bta-miR-4449*	0.628	0.028
21	*bta-miR-2285cf*	0.664	0.033
KC	**MicroRNA**	**Log$_2$FC**	**P-value**
1	*bta-miR-190a*	-1.868	2.05E-10
2	*bta-miR-155*	0.883	2.30E-05
3	*bta-miR-424-5p*	0.951	3.01E-05
4	*bta-miR-AB-63*	-0.9521	2.17E-04
5	*bta-miR-490*	-1.046	4.66E-04
6	*bta-miR-363*	1.009	1.01E-03
7	*bta-miR-4286*	-1.304	1.80E-03
8	*bta-miR-10164-3p*	0.832	2.22E-03
9	*bta-miR-AB-225*	1.43194	2.27E-03
10	*bta-miR-2411-3p*	-0.938	3.39E-03
11	*bta-miR-AB-185*	-1.134	8.37E-03
12	*bta-miR-411c-3p*	-0.829	0.015
13	*bta-miR-2474*	0.953	0.015
14	*bta-miR-2332*	-0.612	0.025
15	*bta-miR-147*	0.681	0.027
16	*bta-miR-6521*	-0.604	0.042
17	*bta-miR-424-3p*	0.74	0.048

logFC = $\log_2$(Fold-change) and he sign of logFC shows the direction of miRNA expression in low-DMI steers relative to high-DMI animals.

Table 5.6. Differentially expressed known micro RNAs (miRNAs) between high and low-MWT animals, with a differential expression threshold of P-value < 0.05 and Fold-change >1.5.

Angus	**MicroRNA**	**log$_2$FC**	**P-value**
1	*bta-miR-1246*	0.959	2.22E-06
2	*bta-miR-4449*	0.967	1.13E-04
3	*bta-miR-2285ar*	0.945	1.72E-04
4	*bta-miR-449a*	1.233	2.23E-04
5	*bta-miR-11985*	1.217	4.98E-04
6	*bta-miR-2285bd*	0.894	2.37E-03
7	*bta-miR-12001*	-0.825	4.67E-03
8	*bta-miR-2411-3p*	0.759	8.10E-03
9	*bta-miR-10164-3p*	0.69	9.02E-03
10	*bta-miR-425-3p*	-0.616	9.68E-03

11	*bta-miR-677*	0.93	0.017
12	*bta-miR-2285u*	0.668	0.034
13	*bta-miR-362-5p*	-0.662	0.034
Charolais	**MicroRNA**	**log_2FC**	**P-value**
1	*bta-miR-10b*	2.19	1.01E-19
2	*bta-miR-99a-5p*	0.646	1.71E-04
3	*bta-miR-451*	0.616	2.16E-04
4	*bta-miR-10a*	0.827	3.03E-04
5	*bta-miR-379*	0.819	8.54E-04
6	*bta-miR-99b*	0.627	1.40E-03
7	*bta-miR-486*	0.709	2.47E-03
8	*bta-miR-7*	0.722	4.45E-03
9	*bta-miR-10172-5p*	0.756	5.72E-03
10	*bta-miR-449a*	-1.002	8.57E-03
11	*bta-miR-AB-15*	-0.7661	8.84E-03
12	*bta-miR-144*	0.645	9.11E-03
13	*bta-miR-AB-145*	0.7143	0.010
14	*bta-miR-11991*	-0.792	0.016
15	*bta-miR-147*	-0.772	0.016
16	*bta-miR-2285aj-5p*	-0.661	0.035
17	*bta-miR-2285as*	-0.964	0.038
18	*bta-miR-592*	-0.705	0.039
19	*bta-miR-10164-3p*	-0.639	0.039
KC	**MicroRNA**	**log_2FC**	**P-value**
1	*bta-miR-190a*	-2.102	7.47E-09
2	*bta-miR-10225a*	-0.748	5.10E-06
3	*bta-miR-2419-5p*	0.814	8.33E-06
4	*bta-miR-7*	-0.839	8.18E-05
5	*bta-miR-1246*	-0.752	3.16E-04
6	*bta-miR-2285t*	0.646	3.83E-04
7	*bta-miR-2332*	-0.832	1.71E-03
8	*bta-miR-2474*	1.063	3.79E-03
9	*bta-miR-1343-3p*	0.711	4.19E-03
10	*bta-miR-2415-3p*	0.626	9.59E-03
11	*bta-miR-2411-3p*	-0.75	0.015
12	*bta-miR-2285bt*	0.791	0.017
13	*bta-miR-10164-3p*	0.639	0.02
14	*bta-miR-29d-3p*	-0.587	0.021
15	*bta-miR-7859*	0.856	0.03

16	*bta-miR-10182-3p*	1.022	0.032
17	*bta-miR-411c-3p*	-0.799	0.032
18	*bta-miR-2285bc*	0.845	0.039

logFC = $\log_2$(Fold-change) and he sign of logFC shows the direction of miRNA expression in low-MWT steers relative to high-MWT animals.

Chapter 6. Evaluation of genomic prediction accuracy of feed efficiency and the related traits with integration of differentially expressed gene SNPs

6.1 Abstract

Due to the costs and time required to individually measuring feed intake phenotypes for traditional genetic evaluation and selection, genomic selection has been implemented as a method of choice for genetic evaluation for feed efficiency and its related traits. Improving genomic prediction accuracy is among the principle ways of increasing the rate of genetic improvement in the breeding programs. In the current study we investigated the potential of improving genomic prediction accuracy for residual feed intake (RFI), dry matter intake (DMI), average daily gain (ADG), and metabolic weight (MWT) of beef cattle through utilization of a functionally enriched SNP panel. We used a total of 7,372 beef animals from six populations including Angus (n = 1148), Charolais (n = 700), Kinsella Composite or KC (n = 1477), PG1 (n = 1868), Elora (n = 729) and TX (n = 1450). We employed genomic best linear unbiased prediction (GBLUP) for genomic prediction with three SNP panels including the Illumina 50K Bovine BeadChip SNP chip (50K panel), functional gene SNP panel (Functional panel), and randomly selected SNP panel (Random panel). The Functional gene SNP panel was mined from candidate genes associated with RFI or DMI or ADG or MWT identified through transcriptomic differential gene analyses. We conducted GBLUP within each breed and across combined breeds (pooled data from all the 6 breeds). All the three SNP panels captured considerably large additive genetic variances of the traits. In general, the 50K and the Random panels captured similar amount of additive variance while the Functional panel accounted for slightly less amount of additive variance except for RFI within the Charolais, within Elora, and within TX populations, for which the Functional panel led to greater estimates of genomic heritability. The Functional panel also yielded a slightly greater accuracy for ADG,

DMI, MWT, and RFI for within Charolais genomic prediction, but generally had a slightly lower genomic prediction than the 50K and the Random Panel in other populations or genomic prediction scenarios. However, the genomic prediction accuracies of the three SNP panels were similar for all the traits under all the genomic prediction scenarios (within-breed or across breeds) when the standard errors were considered, indicating that the Functional panel did not lead to tangible improvement in genomic prediction for the feed efficiency and the related traits in the beef cattle populations investigated. The results also suggest the need to employ multiple omics tools to identify all and refine genes associated with feed efficiency and the related traits across different breeds to enable establishing a more comprehensive functional SNP panel for genomic prediction for the traits.

6.2 Introduction

Feed and feeding related costs are major beef production costs for the beef industry as they account for up to 75 percent of the total production costs (Ahola & Hill 2012). Therefore, improvement of feed efficiency can significantly improve the production returns for the producers. Feed efficiency can be defined as residual feed intake (RFI), which is the difference between the actual feed intake of the animal and the expected feed intake of the animal based on its growth rate and body weight (Koch *et al.* 1963). Additionally, improving beef cattle's feed efficiency could also reduce the carbon footprint of beef animals to the environment since a number of studies have reported positive genetic correlations between feed efficiency measured as residual feed intake and methane emission, implying that genetic selection for low RFI animals can consequently produce animals with reduced methane emission (Nkrumah *et al.* 2006; Hegarty *et al.* 2007).

Traditional genetic evaluation and selection for feed efficiency requires measurement of individual animals feed intake, which is still an expensive process (Chen *et al.* 2013; Khansefid *et al.* 2014) and/or recording acurate pedigree information, hence making genomic selection a method of choice for selective breeding and improvement of feed efficiency. Genomic selection involves utilization of a reference population of animals with phenotype and genome wide SNP genotype information to predict genetic merit of selection candidates with only genotypic information (Meuwissen *et al.* 2001). The accuracy of the prediction of the genomic merit of the candidates is one of the major factors that affect the progress rate of genetic improvement (Georges *et al.* 2018). The accuracy of genomic prediction is influenced by a number of factors including DNA marker density, informativeness of the SNP panel, heritability of the trait, size of the reference population, genetic relationship between the reference and selection candidates, and the statistical model used for genomic evaluation (Goddard & Hayes 2009; Meuwissen *et al.* 2013; Snelling *et al.* 2013; Zhang *et al.* 2019).

Currently, industrial genomic evaluation in beef cattle for the various economic traits is largely based on genotype data from the commercially available medium density panels such as the Illumina 50K Bovine BeadChip SNP chip (Matukumalli *et al.* 2009), that relies on linkage disequilibrium (LD) between the SNPs and the quantitative loci (QTLs) or genes to capture the additive genetic variance accounted for by these QTLs or genes (Snelling *et al.* 2013). This LD dependency likely contributes to a relatively lower genomic prediction accuracy, due to LD break down especially for the across breed genomic predictions (Goddard 2009; Snelling *et al.* 2013). With respect to feed efficiency and the related traits in beef cattle, different attempts including different strategies of constructing the training and validation populations, increasing the reference population size, and increasing SNP densities have been investigated as approaches to improve

genomic prediction accuracy (Mujibi *et al.* 2011; Chen *et al.* 2013; Khansefid *et al.* 2014; Lu *et al.* 2016). However, the genomic prediction accuracy still remains low. Therefore, inclusion of biological or functional information or causative DNA variants into the SNP panels or in the statistical models provides an opportunity to improve genomic prediction as the causative DNA markers does not involve the LD issues (Snelling *et al.* 2013; Fang *et al.* 2017). Additionally, identification of functional genes and markers associated with important traits and their utilization in genomic prediction are expected to yield higher genomic prediction accuracies for outbred or crossbred industrial animals whose representation in the reference populations are generally low (Snelling *et al.* 2013; Edwards *et al.* 2016; Fang *et al.* 2017).

Functional genomics provides several tools including genome wide association studies, transcriptomics studies and epigenomic studies to identify functional genomic regions or genes associated with complex traits, and these regions/genes can be integrated into genomic evaluations to augment prediction accuracy for such traits (Snelling *et al.* 2013; Edwards *et al.* 2016; Spindel *et al.* 2016; Fang *et al.* 2017; Lozano *et al.* 2017; Gebreyesus *et al.* 2019). Currently transcriptomic studies through RNAseq analyses for both protein coding and non-coding RNAs has been identified as a viable tool to identify functional genes that contribute to variability of economically important traits such as feed efficiency and the related traits, and these genes can be used to enhance genomic prediction accuracy of those associated traits (Snelling *et al.* 2013; Lozano *et al.* 2017). Therefore, in the current study we utilized the differentially expressed (DE) genes that were identified in previous studies as associated with feed efficiency and its related traits and assembled a functional SNP panel in which SNPs are located within or close to the DE genes, with the aim of improving genomic prediction of these traits in beef cattle.

6.3 Materials and Methods

6.3.1 Animal populations

In the current study the genotype and phenotype data beef animals were collected through various research projects across Canada. In total we used 7,372 animals (steers and heifers) from six breed populations including two pure breeds, Angus (1148 animals) and Charolais (700 animals), and four cross breeds, KC (1477 animals), Elora (729 animals), PG1(1868 animals) and TX (1450 animals). The Angus, Charolais and Kinsella Composite herds are maintained at the University of Alberta's Roy Berg Kinsella Research Ranch. Angus and Charolais cows are bred with artificial insemination, followed by purebred Angus and Charolais bulls respectively, whose pedigree information is kept by the Canadian Angus and Charolais breed associations respectively. The KC population descended from the crossings between Angus, Charolais, or Alberta Hybrid bulls and the University of Alberta's hybrid dam line that was generated through crossing of multiple composite cattle lines as described by (Goonewardene *et al.* 2003). Elora were a crossbreed population from the University of Guelph's Elora Beef Research Center, and was mainly composed of Angus, Simmental and other breeds (Schenkel *et al.* 2005). PG1 population was composed of crossbred animals from the Phenomics Gap Project and the animals were raised at the Agriculture and Agri-Food Canada Lacombe Research and Development Centre (Lu *et al.* 2016). The beef cattle of TX population were terminal crossbred animals from multiple commercial herds with Angus, Charolais, Hereford, Simmental, Limousin, and Gelbvieh being the major breeds used in the beef production.

6.3.2 Phenotypic data collection and calculations

Since the animals were from different research projects, the phenotypic records were collected at different time points. Data were collected between 2004 and 2014 for the Angus and

Charolais animals, between 2002 and 2014 for KC, between 1998 and 2006 for Elora, and between 2008 and 2011 for both PG1 and TX animals. Individual feed intake of each animal was measured at a finishing stage using GrowSafe Systems® (GrowSafe Systems Ltd., Airdrie, Alberta, Canada) located at the respective research centers. Animals were tested for feed intake for period ranging between 76 and 112 days. Average dry mater intake (DMI) of each animal was calculated as the average of the feed intakes measured through the test period. DMI was standardized to 12 MJ ME per Kg of dry matter for steers and 10 MJ ME per Kg of dry matter for heifers based on the energy content of the diets. Initial body weight and average daily gain (ADG) for each animal were obtained from a linear regression of serial body weight (BW) measurements that were recorded on two consecutive days at the commencement, at approximately 14-day intervals during the feedlot test, and on two consecutive days at the end of test or on the day at the commencement, at approximately 14-day intervals during the feedlot test, and on the day at the end of test depending on the day of test. Metabolic weight (MWT) was calculated as midpoint $BW^{0.75}$, where midpoint BW was calculated as the sum of initial BW of the animal and the product of its ADG multiplied by half the number of days under the feed intake measurement test. To estimate the expected feed intake of the animals based on their body weight and growth, actual DMI was regressed on MWT and ADG, and RFI was calculated as the difference between the actual DMI, and the expected DMI as shown in equation 1 below.

$$RFI = DMI - (\beta_0 + \beta_1 ADG + \beta_2 MWT) \qquad [1]$$

Where RFI is the residual feed intake, DMI is the actual dry matter intake, β_0 is the regression intercept, β_1 and β_2 are the regression coefficients of average daily gain (ADG) and metabolic weight (MWT) on DMI respectively. The R^2 of the regression model ranged between 0.50 and 0.78 (Mao et al. (2013).

6.3.3 Genotyping, phenotype adjustment and imputation

All the animals with phenotypes were genotyped on either version 2 or 3 of the Illumina BovineSNP50 DNA Genotyping BeadChip (Illumina, San Diego, CA, USA). Raw genotypes of all the animals were merged and consolidated resulting in 54,609 SNPs retained across all the animals. Quality control assessments were performed on the SNP genotypes as follows: SNPs with minor allele frequency less than 0.05, SNPs with missing genotype call rate > 0.05, SNPs that deviated from Hardy-Weinberg equilibrium (HWE) by $P < 0.001$. After all the quality control, 33,321 SNPs were retained. These SNP genotypes were used to estimate the genetic structure of the studied animals through principle component analysis (PCA) using PLINK version 1.9 (Purcell *et al.* 2007). Additionally, using the same SNPs, breed compositions of the animals were estimated using ADMIXTURE (Alexander & Lange 2011), with K (assumed ancestral populations) of six ($K = 6$). Consequently, phenotypic records were adjusted for animal birth year, sex type, contemporary group of feedlot test location and pen, breed composition of the six ancestral breed composition and the test age of the animal.

6.3.4 Genotype imputation for full genome

Imputation processes from 50K genotypes of 7,372 animals to whole genome variants used in the current study has been extensively reported in our previous studies (Wang *et al.* 2019; Zhang *et al.* 2019). Briefly, Imputation was performed using Fimpute 2.2 software (Sargolzaei *et al.* 2011). Firstly, 7,372 animals were imputed from 30,155 SNPs of Illumina 50K bovine BeadChip to 428,895 SNP marker genotypes on the Axion ^{TM} Genome-Wide BOS 1 Bovine Array high density (HD) panel from Affymetrix (Affymetrix, Inc., Santa Clara), using 4,059 animals genotyped on the HD panel as the reference. Thereafter, using a reference population of 1,570

animals with full genome sequence genotypes for the 1,000 Bull Genomes Project (Daetwyler *et al.* 2014), animal genotypes were imputed from 428,895 SNP genotypes to 38,318,974 (38M) whole genome sequence variants (SNPs and INDELs). Imputation accuracy for the genotypes was evaluated using a total of 240 animal samples that had both 50K and whole genome genotype information. The 240 animals were randomly assigned to 5 groups (N=48 for each group). Each group of animals was then used as a validation group in turn where their whole genome sequence variants were masked, and the rest of individuals were merged into the whole genome sequence reference population to impute WGS genotypes for all animals in the validation group. The accuracy of imputation was then calculated as the average proportion of whole genome sequence variant genotypes of the animals in the validation group that were correctly imputed assuming that the real genotypes of WGS variants genotypes had no errors (Wang *et al.* 2019). The DNA variants with imputation accuracy of less than 95%, minor allele frequency less than 0.005, significantly deviated from HWE by $P < 0.00001$ were removed from the 38M variants leaving 7,853,211 (7.8M) variants for further analyses.

6.3.5 Functional and random SNP panel designing

A total of 3735 autosomal candidate genes, including 3,642 protein coding genes and 93 miRNA precursors identified as associated with feed efficiency traits were compiled from our own transcriptomic studies (Mukiibi *et al.* 2017; Mukiibi *et al.* 2018a; Mukiibi *et al.* 2018b; Mukiibi *et al.* 2019a, b; Mukiibi *et al.* 2019c), and other published literature (Chen *et al.* 2011; Paradis *et al.* 2015; Kern *et al.* 2016; Kong *et al.* 2016; Lindholm-Perry *et al.* 2016; Weber *et al.* 2016; Foote *et al.* 2017; Keel *et al.* 2018). Protein coding genes included genes directly identified as differentially expressed between divergent phenotypes of feed efficiency or related traits, and differentially expressed miRNAs' targets of 99th percentile context score that were not among the identified

differentially expressed genes. The chromosome-wise distribution of the candidate genes is illustrated in Figure 6.1. From the 7.8M whole genome variants, a total of 432,170 SNP markers were extracted for the 3,735 autosomal candidate genes, within a window of 1,000bp from the transcription start and end sites of the coding genes to include SNPs in proximal gene regulatory regions. All the 3,735 autosomal candidate genes and their chromosomal positions are provided in the Supplementary Data S18. To maintain the SNP number close to that from 50K panel, linkage disequilibrium pruning was performed on the 411,591 SNPs using a threshold of SNP independent-pairwise squared correlation of 0.2, within a 50 SNP window, and a stepwise shift of 5 SNPs within a window in PLINK. After LD pruning, 33,147 SNPs were retained as the functional panel. Additionally, for comparison 33,147 SNPs were randomly selected from the rest of 7.8M variants that excluded the SNPs in the functional panel to form what we termed a random panel in this study.

6.3.6 Genomic prediction and accuracy calculations

Genomic prediction was performed through genomic best linear unbiased prediction (GBLUP) method using GCTA software (Yang *et al.* 2011) for the three SNP panels (Functional, Random and 50K). The linear mixed model shown as equation 2 below was used for evaluation, where y is the vector of adjusted phenotypes (RFI, ADG, DMI or MWT), μ is the overall phenotypic mean, g is the vector of the random breeding values of the animals, Z is the incidence matrix linking the random breeding values in g to the phenotypic records in y.

$$y = 1\mu + Zg + e \text{ , } g \sim N(0, G\sigma_g^2), e \sim N(0, I\sigma_e^2) \qquad [2]$$

The expanded mixed model equations (MME) used in the genomic evaluation was as equation 3.

$$\begin{bmatrix} \hat{\mu} \\ \hat{g} \end{bmatrix} = \begin{bmatrix} 1'_n 1 & 1'_n Z \\ Z'1_n & Z'Z + G^{-1} \end{bmatrix}^{-1} \begin{bmatrix} 1'_n y \\ Z'y \end{bmatrix} \qquad [3]$$

G^{-1} was the inverse of the inverse of the genomic relationship matrix (GRM) calculated using equation 4, where A_{jk} was the genomic relationship between animals j and k, N is the total number of SNPs, x_{ij} was the number of copies of the reference allele of the SNP i for the animal j, x_{ik} was the number of copies of the reference allele of the SNP i for the animal k, and p_i was the allelic frequency of the reference allele of the i^{th} SNP marker (Yang *et al.* 2011).

$$A_{jk} = \frac{1}{N}\sum_{i=1}^{N}\frac{(x_{ij} - 2p_i)(x_{ik} - 2p_i)}{2p_i(1-p_i)} \qquad [4]$$

Genomic prediction was performed within each of the six breeds (i.e. using animals within breed as the training population) and pooled data (i.e. using all animals in the study as the training population).Within each breed, animals were randomly divided into five cross-validation groups according to sire families. In each genomic prediction analysis for each breed, phenotypes of animals in the cross-validation group were masked and their genomic breeding values (GEBV) were consequently estimated using other animals within the breed or all animals combined as the training population. The accuracy of genomic prediction was then calculated as the ratio of the Pearson's correlation between the adjusted phenotypes and the GEBVs of the validation individuals, divided by the square root of the genomic heritability (Meuwissen *et al.* 2013) of the respective trait, as shown in equation 5 for each validation group. Thereafter, the genomic prediction accuracy and its standard error were obtained from the five cross validation accuracies.

$$\boldsymbol{GEBV\ Accuracy} = \frac{Cor(P_{adj}, GEBV)}{\sqrt{h^2}} \qquad [5]$$

The heritability estimates were estimated from the 7.8M single nucleotide variants for each trait (RFI = 0.25, ADG = 0.26, DMI = 0.39, and MWT = 0.52), (Zhang *et al.* 2019a) and they were mantained constant for all the evaluation scenarios.

6.4 Results

6.4.1 Genomic heritability estimates

Genetic relationships based on 50K panel genotypes among the six populations used in the current study are illustrated by a PCA plot in Figure 6.2. Genomic heritability estimates from GCTA --reml run for RFI, DMI, ADG and MWT for within and pooled data analyses for the three SNP panels are provided in Table 6.1. Generally, MWT had the highest heritability estimates across the tested scenarios ranging from 0.21 ± 0.091 to 0.61 ± 0.049, whereas RFI generally had the lowest heritability estimates (0.15 ± 0.078 to 0.46 ± 0.066). ADG and DMI had heritability estimates ranging from 0.19 ± 0.061 to 0.45 ± 0.102 and 0.2 ± 0.04 to 0.58 ± 0.065 respectively. Additionally, heritability estimates under within-breed analyses were generally higher than pooled data evaluations. In general, the 50K and the Random panels captured a similar amount of additive variance while the Functional panel accounted for slightly less amount of additive variance except for RFI within the Charolais, within Elora, and within TX populations, for which the Functional panel led to greater estimates of genomic heritability. However, when the standard errors of the heritability estimates are considered, all the SNP panels accounted for a similar amount of additive genetic variance for the traits investigated.

6.4.2 Genomic prediction accuracy

Genomic prediction accuracies for the four considered traits for within breed and using pooled data as reference evaluations are summarized in Table 6.2. For RFI, the highest accuracy (0.58 ± 0.06) was observed under within Angus prediction using 50K panel, whereas lowest

accuracy (0.20 ± 0.05) was observed using the pooled data (all animals as reference) with the Functional panel in the same population. For ADG, 0.44 ± 0.06 was the highest accuracy we obtained, which was for the pooled data evaluation for Elora with the Random panel, and 0.2 ± 0.06 was the lowest observed accuracy which was under within TX using the same Random panel. For DMI, 0.55 ± 0.06 was observed as the highest accuracy, and was obtained when we utilized the pooled data as reference for Angus genomic evaluation with the 50K panel, and 0.23 ± 0.05 was the lowest which was observed under pooled data evaluation for Elora using the Functional panel. We observed, 0.68 ± 0.03 as the highest accuracy for MWT and this was under the pooled data evaluation for PG1 using the Random panel, whereas 0.21 ± 0.04 was the lowest accuracy for this trait and was observed under within Elora analysis with the Functional panel.

Using the 50K and the Random panel, the genomic prediction accuracy for ADG, DMI and MWT were generally higher using the pooled data as reference than within breed evaluation across the six breeds, with the highest (increased by 0.16 points) observed in KC for MWT. However, for the Functional panel using pooled data yielded relatively lower genomic prediction than within breed evaluations for ADG, DMI and MWT for Charolais, Elora, PG1 and TX animals, but not for Angus and KC animals. Interestingly, for RFI, within breed evaluations generally resulted into higher genomic accuracy than using the pooled data for the three panels. Compared with the other two SNP panels in this study, a slightly greater genomic prediction accuracy for ADG (0.42 ± 0.10), DMI (0.37 ± 0.09), MWT (0.36 ± 0.08), and RFI (0.44 ± 0.05) for within Charolais genomic prediction was obtained when the Functional SNP panel was used (Table 6.2 and Figure 6.3). However, on average the Functional panel had a slightly lower genomic prediction than the 50K and the Random Panel under most of the genomic prediction scenarios considered in the current study. Nevertheless, the genomic prediction accuracies of the three SNP panels were similar for

all the traits under all the genomic prediction scenarios (within-breed or across breeds) when the standard errors were considered, indicating that the Functional panel did not lead to tangible improvement in genomic prediction for the feed efficiency and the related traits in the beef cattle populations investigated.

6.5 Discussion

In the current study we investigated the potential usage of functional information obtained through transcriptomic analyses to enhance genomic prediction accuracy in beef cattle. We compared three SNP panels including a commercially available 50K SNP panel, a Functional panel of SNPs from candidate genes identified through transcriptome analyses, and a Random SNP panel composed of randomly selected SNPs from the whole genome DNA variants. The genomic prediction analyses for feed efficiency (RFI) and its component traits were performed within each of the six breed populations and with pooled genotype and phenotype data of all the six breed populations as the training population.

Our results showed that in general the 50K panel had relatively better genomic prediction accuracy for RFI, ADG and DMI across populations we studied than the Functional and the Random panels for within breed and pooled data evaluations. These results imply that even though the Functional panel SNPs were mined from genes identified to be associated with the traits under study, some genetic variance remains uncounted for by these SNPs. However, it should also be noted that in some scenarios, the Functional panel performed slightly better than both the 50K and the Random panels, for example within Charolais evaluation for all the four traits, the Functional panel had higher genomic prediction accuracy than the 50K and the Random panel by points ranging between 0.4% to 6.1%. Additionally, for within Elora and PG1 evaluation, the Functional panel had slightly higher accuracy than the 50K and the Random panels for both RFI and DMI.

However, these results are not consistent across the evaluated breed populations to show the potential of improving genomic prediction accuracy through utilization of this functionally enriched SNP panel. For traits that are majorly controlled by a few genomic regions or genes with larger effects across different populations, such as milk fatty acids, it is possible to improve their genomic accuracy through utilization a single genomic tool like GWAS to identify the genomic regions associated with those traits (Gebreyesus *et al.* 2019). However, for feed efficiency and its component traits, genome-wide association studies have identified multiple genomic regions associated with feed efficiency or the component traits but have not found genomic regions or genes with larger effects on the traits (Abo-Ismail *et al.* 2014; de Oliveira *et al.* 2014; Saatchi *et al.* 2014; Seabury *et al.* 2017; Zhang *et al.* 2019a). As a new gene expression technique, global transcriptome studies via RNAseq have identified a number of genes associated to feed efficiency or its components (Chen *et al.* 2011; Alexandre *et al.* 2015; Paradis *et al.* 2015; Tizioto *et al.* 2015; Kong *et al.* 2016; Tizioto *et al.* 2016; Weber *et al.* 2016; Khansefid *et al.* 2017; Mukiibi *et al.* 2018b; Mukiibi *et al.* 2019a, b; Mukiibi *et al.* 2019c). The identification of these multiple genes associated with the feed efficiency or its component traits implies that these are more complex traits that are controlled by many genes that are involved in various metabolic networks. Although it was expected that the Functional SNP panel would lead to greater genomic prediction accuracy for the traits as the SNPs in the panel are from the DE genes associated with the traits. However, the Functional SNP panel did not result in better genomic prediction accuracies for most of the traits under most of the genomic prediction scenarios in comparison to the 50K and Random panels. This is likely due to the reason that the Functional panel is just part of all the genes or genomic regions controlling feed efficiency or its component traits. Moreover, the DE genes were compiled from studies of various beef breeds and were identified via gene expression analyses of

limited tissues at a snapshot of animal development stage, which might not be able to capture a good set of functional genes and hence causative DNA variants for the traits. Furthermore, multi-populations/breeds GWAS (Saatchi et al. 2014) and transcriptomic studies (Mukiibi et al. 2018b; Mukiibi et al. 2019a, b; Mukiibi et al. 2019c), have shown that the genes that regulated feed efficiency and its component traits are largely population or breed specific. Therefore, to optimise utilization of functional gene information from the genome for a complex trait to enhance genomic prediction, one would require employing multiple omics tools including transcriptomics of multiple tissues (such as muscles, adipose tissue and digestive tract) at key development stages, genome-wide association studies, proteomics, metagenomics, metabolomics and epigenomics to identify key genes and metabolic gene networks associated with the traits across different beef breeds. Indeed, combination of function information from multiple functional genomic tools improves genomic prediction accuracy in a crop species as reported by (Lozano et al. 2017).

Genomic feature BLUP (GFBLUP) is a promising and recently proposed method of integrating genomic functional information into genomic prediction, by allowing fitting of multiple genomic random effects of SNPs from different functional genomic regions. This method has previously yielded reasonable enhancement of genomic prediction accuracy than GBLUP (Edwards *et al.* 2016; Fang *et al.* 2017; Gebreyesus *et al.* 2019). However, its application to very complex traits like feed efficiency (with many genomic regions of small effects) is likely to be limited by their intensive computational demands to fit the vast number of genomic features into the model (Fang *et al.* 2017). Therefore, attempts could further be made to develop less computationally intensive strategies for feed efficiency and the related traits genomic evaluation using GFBLUP or other genomic prediction methods, especially when many SNPs from several genomic regions are to be modeled.

6.6 Conclusions

In this study we explored the possibility of enhancing the accuracy of estimating the genomic merit of beef animals for feed efficiency and its component traits through utilization of transcriptomic functional gene information. Our findings showed that on average the commercial 50K SNP panel performed slightly better than the Functional SNP panel for within breed evaluations, as well as for the pooled data evaluations. However, in some considered scenarios the Functional SNP panel had slightly higher accuracy than both the 50K and Random SNP panels. The lack of consistencye in the improvement of genomic prediction accuracy across the populations and across the traits by the Functional SNP panel could be dues to partial discovery of the genomic regions or genes affecting these traits in beef cattle. We therefore recommend further studies involving multiple omics tools in different breed populations to identify key genomic functional regions/gene variants regulating the traits across different beef cattle breeds. We also believe that utilization of genomic feature BLUP or other statistical methods to integrate functional information of key or causative DNA variants into genomic prediction has the potential to yield higher genomic prediction accuracies for feed efficiency and the related traits.

6.7 References

Abo-Ismail M.K., Vander Voort G., Squires J.J., Swanson K.C., Mandell I.B., Liao X., Stothard P., Moore S., Plastow G. & Miller S.P. (2014) Single nucleotide polymorphisms for feed efficiency and performance in crossbred beef cattle. BMC genetics 15, 14.

Ahola J.K. & Hill R.A. (2012) Input Factors Affecting Profitability: a Changing Paradigm and a Challenging Time. In: Feed efficiency in the beef industry (ed. by Hill RA), p. 10. Wiley-Blackwell, Ames, Iowa.

Alexander D.H. & Lange K. (2011) Enhancements to the ADMIXTURE algorithm for individual ancestry estimation. BMC bioinformatics 12, 246.

Alexandre P.A., Kogelman L.J., Santana M.H., Passarelli D., Pulz L.H., Fantinato-Neto P., Silva P.L., Leme P.R., Strefezzi R.F. & Coutinho L.L. (2015) Liver transcriptomic networks reveal main biological processes associated with feed efficiency in beef cattle. BMC genomics 16, 1073.

Chen L., Schenkel F., Vinsky M., Crews D. & Li C. (2013) Accuracy of predicting genomic breeding values for residual feed intake in Angus and Charolais beef cattle. Journal of animal science 91, 4669-78.

Chen Y., Gondro C., Quinn K., Herd R., Parnell P. & Vanselow B. (2011) Global gene expression profiling reveals genes expressed differentially in cattle with high and low residual feed intake. Animal genetics 42, 475-90.

Daetwyler H.D., Capitan A., Pausch H., Stothard P., Van Binsbergen R., Brøndum R.F., Liao X., Djari A., Rodriguez S.C. & Grohs C. (2014) Whole-genome sequencing of 234 bulls facilitates mapping of monogenic and complex traits in cattle. Nature genetics 46, 858.

de Oliveira P.S., Cesar A.S., do Nascimento M.L., Chaves A.S., Tizioto P.C., Tullio R.R., Lanna D.P., Rosa A.N., Sonstegard T.S. & Mourao G.B. (2014) Identification of genomic regions associated with feed efficiency in Nelore cattle. BMC genetics 15, 100.

Edwards S.M., Sørensen I.F., Sarup P., Mackay T.F. & Sørensen P. (2016) Genomic prediction for quantitative traits is improved by mapping variants to gene ontology categories in Drosophila melanogaster. Genetics 203, 1871-83.

Fang L., Sahana G., Ma P., Su G., Yu Y., Zhang S., Lund M.S. & Sørensen P. (2017) Use of biological priors enhances understanding of genetic architecture and genomic prediction of complex traits within and between dairy cattle breeds. BMC genomics 18, 604.

Foote A., Keel B., Zarek C. & Lindholm-Perry A. (2017) Beef steers with average dry matter intake and divergent average daily gain have altered gene expression in the jejunum. Journal of animal science 95, 4430-9.

Gebreyesus G., Bovenhuis H., Lund M.S., Poulsen N.A., Sun D. & Buitenhuis B. (2019) Reliability of genomic prediction for milk fatty acid composition by using a multi-population reference and incorporating GWAS results. Genetics Selection Evolution 51, 16.

Georges M., Charlier C. & Hayes B. (2018) Harnessing genomic information for livestock improvement. Nature Reviews Genetics, 1.

Goddard M. (2009) Genomic selection: prediction of accuracy and maximisation of long term response. Genetica 136, 245-57.

Goddard M.E. & Hayes B.J. (2009) Mapping genes for complex traits in domestic animals and their use in breeding programmes. Nature Reviews Genetics 10, 381-91.

Goonewardene L., Wang Z., Price M., Yang R.-C., Berg R. & Makarechian M. (2003) Effect of udder type and calving assistance on weaning traits of beef and dairy× beef calves. Livestock production science 81, 47-56.

Hegarty R., Goopy J., Herd R. & McCorkell B. (2007) Cattle selected for lower residual feed intake have reduced daily methane production. Journal of animal science 85, 1479-86.

Herd R. & Arthur P. (2009) Physiological basis for residual feed intake. Journal of animal science 87, E64-E71.

Keel B.N., Zarek C.M., Keele J.W., Kuehn L.A., Snelling W.M., Oliver W.T., Freetly H.C. & Lindholm-Perry A.K. (2018) RNA-Seq Meta-analysis identifies genes in skeletal muscle associated with gain and intake across a multi-season study of crossbred beef steers. BMC genomics 19, 430.

Kern R.J., Lindholm-Perry A.K., Freetly H.C., Snelling W.M., Kern J.W., Keele J.W., Miles J.R., Foote A.P., Oliver W.T. & Kuehn L.A. (2016) Transcriptome differences in the rumen of beef steers with variation in feed intake and gain. Gene 586, 12-26.

Khansefid M., Millen C., Chen Y., Pryce J., Chamberlain A., Vander Jagt C., Gondro C. & Goddard M. (2017) Gene expression analysis of blood, liver, and muscle in cattle divergently selected for high and low residual feed intake. Journal of animal science 95, 4764-75.

Khansefid M., Pryce J., Bolormaa S., Miller S., Wang Z., Li C. & Goddard M. (2014) Estimation of genomic breeding values for residual feed intake in a multibreed cattle population. Journal of animal science 92, 3270-83.

Koch R.M., Swiger L.A., Chambers D. & Gregory K.E. (1963) Efficiency of feed use in beef cattle. Journal of animal science 22, 486-94.

Kong R.S., Liang G., Chen Y. & Stothard P. (2016) Transcriptome profiling of the rumen epithelium of beef cattle differing in residual feed intake. BMC genomics 17, 592.

Lindholm-Perry A.K., Kern R.J., Keel B.N., Snelling W.M., Kuehn L.A. & Freetly H.C. (2016) Profile of the spleen transcriptome in beef steers with variation in gain and feed intake. Frontiers in genetics 7, 127.

Lozano R., Del Carpio D.P., Kayondo S.I., Amuge T., Adebo O.A., Ferguson M. & Jannink J.-L. (2017) Leveraging Transcriptomics Data for Genomic Prediction Models in Cassava. bioRxiv, 208181.

Lu D., Akanno E., Crowley J., Schenkel F., Li H., De Pauw M., Moore S., Wang Z., Li C. & Stothard P. (2016) Accuracy of genomic predictions for feed efficiency traits of beef cattle using 50K and imputed HD genotypes. Journal of animal science 94, 1342-53.

Mao F., Chen L., Vinsky M., Okine E., Wang Z., Basarab J., Crews Jr D. & Li C. (2013) Phenotypic and genetic relationships of feed efficiency with growth performance, ultrasound, and carcass merit traits in Angus and Charolais steers. Journal of animal science 91, 2067-76.

Matukumalli L.K., Lawley C.T., Schnabel R.D., Taylor J.F., Allan M.F., Heaton M.P., O'Connell J., Moore S.S., Smith T.P. & Sonstegard T.S. (2009) Development and characterization of a high density SNP genotyping assay for cattle. PloS one 4, e5350.

Meuwissen T., Hayes B. & Goddard M. (2001) Prediction of total genetic value using genome-wide dense marker maps. Genetics 157, 1819.

Meuwissen T., Hayes B. & Goddard M. (2013) Accelerating improvement of livestock with genomic selection. Annu. Rev. Anim. Biosci. 1, 221-37.

Mujibi F., Nkrumah J., Durunna O., Stothard P., Mah J., Wang Z., Basarab J., Plastow G., Crews D. & Moore S. (2011) Accuracy of genomic breeding values for residual feed intake in crossbred beef cattle. Journal of animal science 89, 3353-61.

Mukiibi R., Johnston D., Vinsky M., Fitzsimmons C., Stothard P., Waters S.M. & Li C. (2019a) Bovine hepatic miRNAome profiling and differential miRNA expression analyses between beef steers with divergent feed efficiency phenotypes.

Mukiibi R., Johnston D., Vinsky M., Fitzsimmons C., Stothard P., Waters S.M. & Li C. (2019b) Differential hepatic micro RNA expression between beef steers with divergent growth rate or feed intake phenotypes.

Mukiibi R., Vinsky M., Keogh K., Fitzsimmons C., Stothard P., Waters S.M. & Li C. (2017) Transcriptome analysis of skeletal muscle tissue in Canadian beef cattle with divergent residual feed intake phenotypes. In: 8th Annual Livestock Gentec Conference, Edmonton, AB, Canada.

Mukiibi R., Vinsky M., Keogh K., Fitzsimmons C., Stothard P., Waters S.M. & Li C. (2019c) Liver transcriptome profiling of beef steers with divergent growth rate, feed intake or metabolic body weight phenotypes. Journal of animal science.

Mukiibi R., Vinsky M., Keogh K., Fitzsimmons C., Waters S.M., Stothard P. & Li C. (2018a) Skeletal muscle transcriptional profiling of Canadian crossbred beef cattle divergent for feed efficiency traits. Journal of animal science 96, 175-6.

Mukiibi R., Vinsky M., Keogh K.A., Fitzsimmons C., Stothard P., Waters S.M. & Li C. (2018b) Transcriptome analyses reveal reduced hepatic lipid synthesis and accumulation in more feed efficient beef cattle. Scientific reports 8, 7303.

Nkrumah J., Okine E., Mathison G., Schmid K., Li C., Basarab J., Price M., Wang Z. & Moore S. (2006) Relationships of feedlot feed efficiency, performance, and feeding behavior with metabolic rate, methane production, and energy partitioning in beef cattle. Journal of animal science 84, 145-53.

Paradis F., Yue S., Grant J., Stothard P., Basarab J. & Fitzsimmons C. (2015) Transcriptomic analysis by RNA sequencing reveals that hepatic interferon-induced genes may be associated with feed efficiency in beef heifers. Journal of animal science 93, 3331-41.

Purcell S., Neale B., Todd-Brown K., Thomas L., Ferreira M.A., Bender D., Maller J., Sklar P., De Bakker P.I. & Daly M.J. (2007) PLINK: a tool set for whole-genome association and population-based linkage analyses. The American Journal of Human Genetics 81, 559-75.

Saatchi M., Beever J.E., Decker J.E., Faulkner D.B., Freetly H.C., Hansen S.L., Yampara-Iquise H., Johnson K.A., Kachman S.D. & Kerley M.S. (2014) QTLs associated with dry matter intake, metabolic mid-test weight, growth and feed efficiency have little overlap across 4 beef cattle studies. BMC genomics 15, 1004.

Sargolzaei M., Chesnais J. & Schenkel F. (2011) FImpute-An efficient imputation algorithm for dairy cattle populations. J Dairy Sci 94, 421.

Schenkel F., Miller S., Ye X., Moore S., Nkrumah J., Li C., Yu J., Mandell I., Wilton J. & Williams J. (2005) Association of single nucleotide polymorphisms in the leptin gene with carcass and meat quality traits of beef cattle. Journal of animal science 83, 2009-20.

Seabury C.M., Oldeschulte D.L., Saatchi M., Beever J.E., Decker J.E., Halley Y.A., Bhattarai E.K., Molaei M., Freetly H.C. & Hansen S.L. (2017) Genome-wide association study for feed efficiency and growth traits in US beef cattle. BMC genomics 18, 386.

Snelling W., Cushman R., Keele J., Maltecca C., Thomas M., Fortes M. & Reverter A. (2013) BREEDING AND GENETICS SYMPOSIUM: Networks and pathways to guide genomic selection–. Journal of animal science 91, 537-52.

Spindel J., Begum H., Akdemir D., Collard B., Redoña E., Jannink J. & McCouch S. (2016) Genome-wide prediction models that incorporate de novo GWAS are a powerful new tool for tropical rice improvement. Heredity 116, 395.

Tizioto P.C., Coutinho L.L., Decker J.E., Schnabel R.D., Rosa K.O., Oliveira P.S., Souza M.M., Mourão G.B., Tullio R.R. & Chaves A.S. (2015) Global liver gene expression differences in Nelore steers with divergent residual feed intake phenotypes. BMC genomics 16, 242.

Tizioto P.C., Coutinho L.L., Oliveira P.S., Cesar A.S., Diniz W.J., Lima A.O., Rocha M.I., Decker J.E., Schnabel R.D. & Mourão G.B. (2016) Gene expression differences in Longissimus muscle of Nelore steers genetically divergent for residual feed intake. Scientific reports 6.

Wang Y., Zhang F., Mukiibi R., Chen L., Vinsky M., Plastow G., Basarab J., Stothard P. & Li C. (2019) Genetic architecture of quantitative traits in beef cattle revealed by a genome wide association study of imputed whole genome sequence variants: II: carcass merit traits. BMC genomics.

Weber K.L., Welly B.T., Van Eenennaam A.L., Young A.E., Porto-Neto L.R., Reverter A. & Rincon G. (2016) Identification of gene networks for residual feed intake in angus cattle using genomic prediction and RNA-seq. PloS one 11, e0152274.

Yang J., Lee S.H., Goddard M.E. & Visscher P.M. (2011) GCTA: a tool for genome-wide complex trait analysis. The American Journal of Human Genetics 88, 76-82.

Zhang F., Wang Y., Mukiibi R., Chen L., Vinsky M., Plastow G., Basarab J., Stothard P. & Li C. (2019a) Genetic architecture of quantitative traits in beef cattle revealed by a genome wide association study of imputed whole genome sequence variants: I: feed efficiency and component traits. BMC genomics.

Zhang H., Yin L., Wang M., Yuan X. & Liu X. (2019b) Factors affecting the accuracy of genomic selection for agricultural economic traits in maize, cattle, and pig populations. Frontiers in genetics 10, 189.

6.8 Figures and Tables

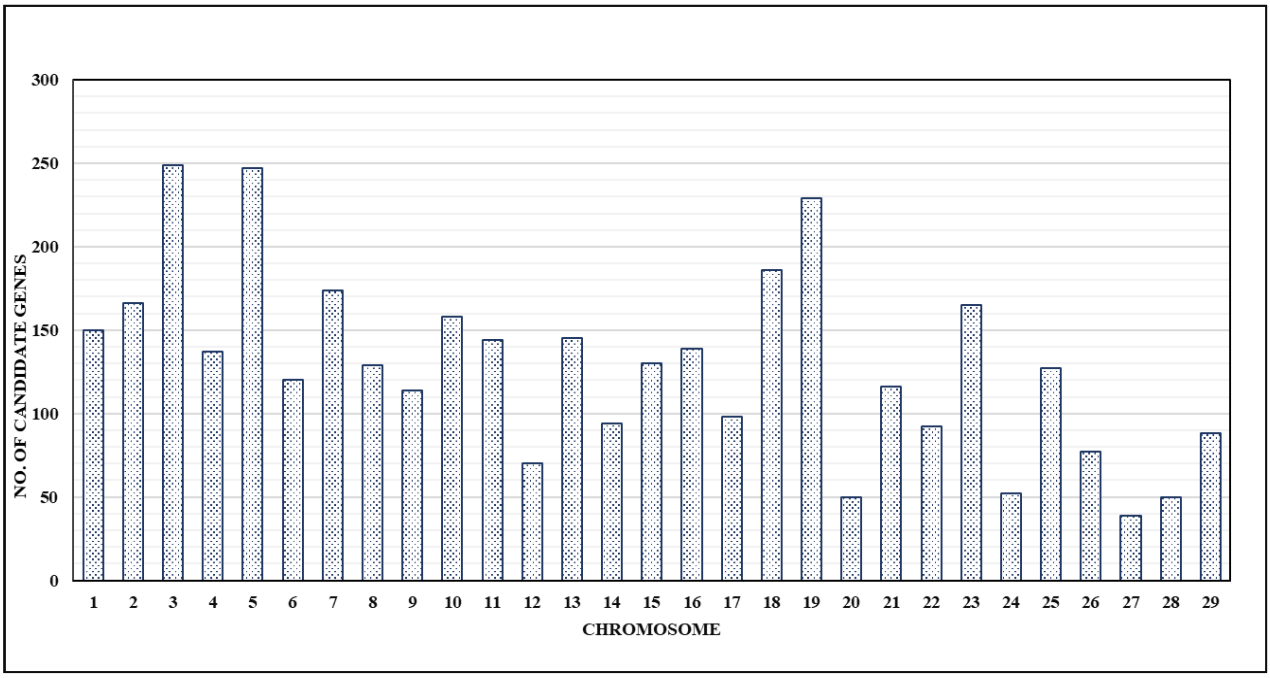


Figure 6.1. Bar chart showing the genomic distribution of the 3,735 candidate genes (coding and miRNAs) from which a Functional panel was compiled.

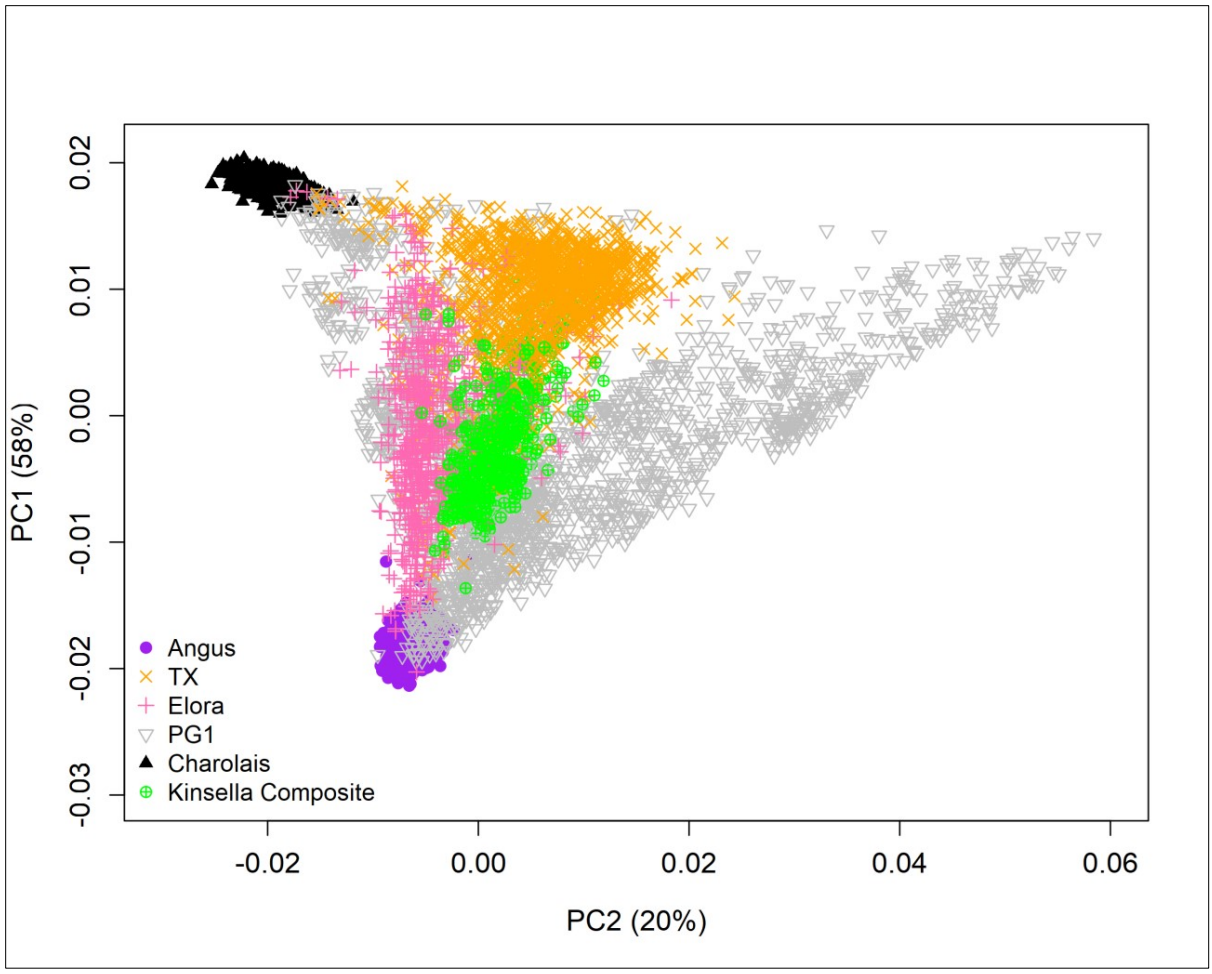


Figure 6.2. Genetic relationships between the beef cattle populations used in the current study as revealed through Principle Component Analysis, PC1 = 1st Principle component (accounting for 58% of the variability) and PC2 = 2nd Principle component (accounting for 20% of variability).

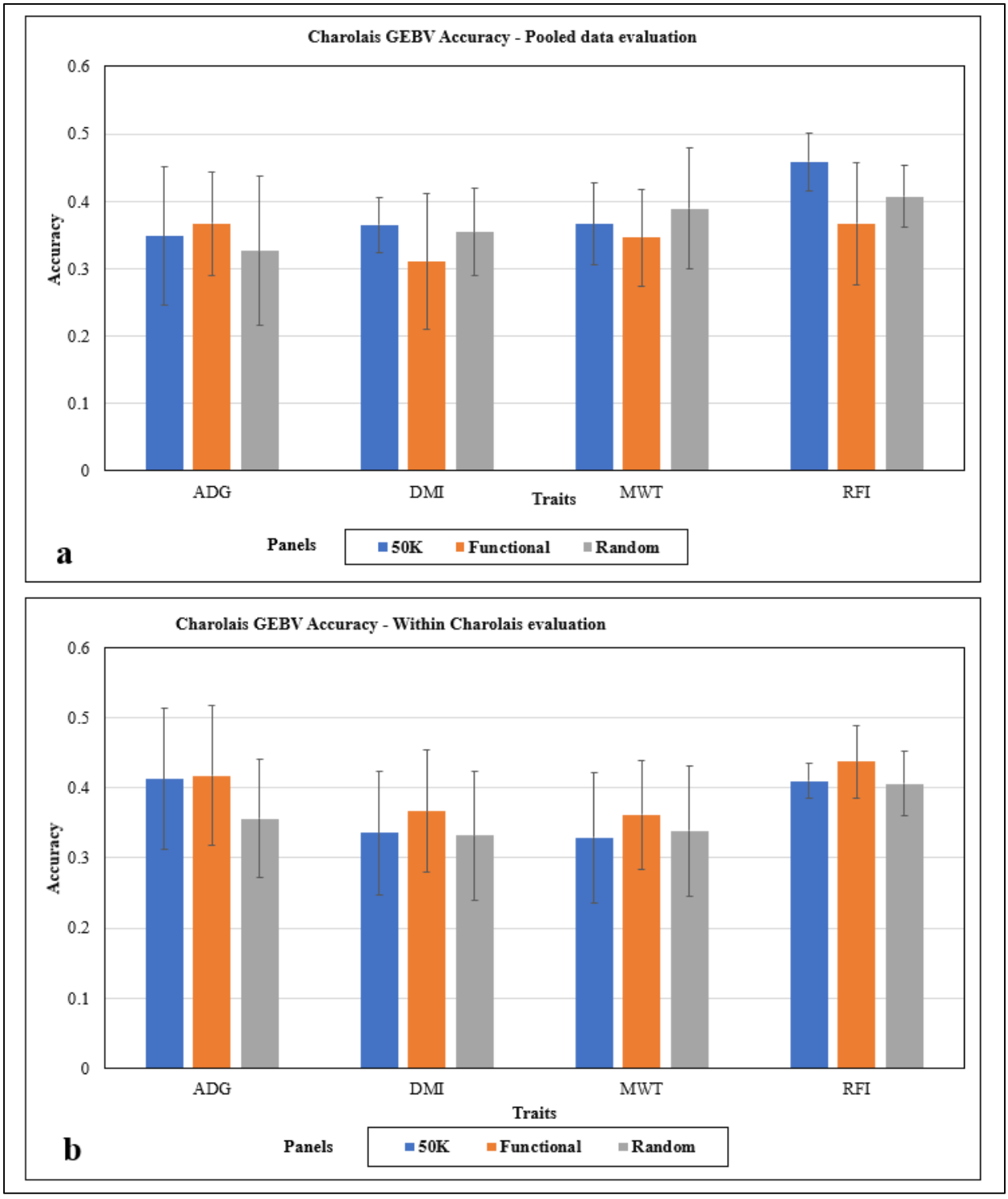


Figure 6.3. Bar chart showing genomic prediction accuracy results for Charolais animals for, a) using pooled data as reference/training population, b) within Charolais (using Charolais as reference/training population).

Table 6.1. Genomic heritability estimated (± Standard error) for feed efficiency and the related traits estimated using the three SNP panels (50K, Functional and Random).

			Panels		
Breed	**Within/Pooled data**	**Trait**	**50K**	**Functional**	**Random**
Angus	**Pooled data**	ADG	0.23±0.019	0.21±0.019	0.23±0.020
		DMI	0.32±0.020	0.30±0.020	0.33±0.020
		MWT	0.44±0.020	0.40±0.020	0.45±0.020
		RFI	0.22±0.019	0.20±0.018	0.21±0.019
	Within Angus	ADG	0.26±0.062	0.24±0.061	0.25±0.062
		DMI	0.57±0.063	0.52±0.067	0.58±0.065
		MWT	0.56±0.061	0.56±0.065	0.57±0.064
		RFI	0.46±0.066	0.40±0.067	0.46±0.067
Charolais	**Pooled data**	ADG	0.22±0.019	0.21±0.019	0.23±0.019
		DMI	0.33±0.020	0.31±0.020	0.34±0.020
		MWT	0.44±0.019	0.41±0.020	0.46±0.020
		RFI	0.22±0.018	0.20±0.018	0.22±0.019
	Within Charolais	ADG	0.39±0.090	0.45±0.102	0.44±0.097
		DMI	0.37±0.092	0.44±0.105	0.38±0.097
		MWT	0.48±0.092	0.56±0.105	0.53±0.099
		RFI	0.31±0.088	0.34±0.099	0.30±0.092
KC	**Pooled data**	ADG	0.22±0.019	0.21±0.019	0.23±0.020
		DMI	0.33±0.020	0.31±0.020	0.34±0.020
		MWT	0.45±0.020	0.41±0.020	0.46±0.020
		RFI	0.23±0.019	0.20±0.018	0.22±0.019
	Within KC	ADG	0.25±0.057	0.23±0.058	0.26±0.060
		DMI	0.34±0.061	0.34±0.064	0.34±0.063
		MWT	0.44±0.062	0.42±0.064	0.46±0.063
		RFI	0.24±0.056	0.25±0.058	0.25±0.057
Elora	**Pooled data**	ADG	0.22±0.019	0.21±0.019	0.23±0.019
		DMI	0.33±0.020	0.31±0.020	0.35±0.020
		MWT	0.45±0.019	0.41±0.020	0.46±0.020
		RFI	0.23±0.019	0.21±0.018	0.23±0.019
	Within Elora	ADG	0.34±0.094	0.36±0.102	0.37±0.099
		DMI	0.20±0.081	0.22±0.09	0.20±0.084
		MWT	0.21±0.091	0.22±0.098	0.22±0.095
		RFI	0.15±0.076	0.18±0.087	0.15±0.078
PG1	**Pooled data**	ADG	0.23±0.019	0.22±0.020	0.23±0.020
		DMI	0.33±0.020	0.30±0.020	0.34±0.020
		MWT	0.44±0.020	0.40±0.020	0.45±0.020

		RFI	0.22±0.019	0.20±0.018	0.22±0.019
	Within PG1	ADG	0.25±0.050	0.22±0.049	0.26±0.052
		DMI	0.46±0.054	0.43±0.055	0.45±0.055
		MWT	0.61±0.049	0.60±0.051	0.61±0.050
		RFI	0.26±0.051	0.28±0.054	0.26±0.052
TX	**Pooled data**	ADG	0.23±0.019	0.21±0.019	0.24±0.020
		DMI	0.33±0.020	0.31±0.020	0.34±0.020
		MWT	0.44±0.020	0.40±0.020	0.45±0.020
		RFI	0.23±0.019	0.20±0.018	0.22±0.019
	Within TX	ADG	0.19±0.061	0.23±0.068	0.20±0.064
		DMI	0.40±0.064	0.44±0.07	0.42±0.066
		MWT	0.56±0.061	0.57±0.068	0.59±0.064
		RFI	0.26±0.059	0.25±0.061	0.25±0.060

Within = using animals of the respective breed as training population for evaluation, Pooled data = using data of all the six breeds pooled together as a reference for evaluation. ADG = average daily gain, DMI= dry matter intake, and MWT= metabolic weight and RFI = residual feed efficiency.

Table 6.2. Genomic prediction accuracy (± Standard error) for feed efficiency and the related traits estimated using the three SNP panels (50K, Functional and Random).

			Panels		
Breed	**Within/Pooled data**	**Trait**	**50K**	**Functional**	**Random**
Angus	**Pooled data**	ADG	0.42±0.05	0.35±0.04	0.38±0.03
		DMI	0.55±0.06	0.42±0.04	0.54±0.05
		MWT	0.41±0.02	0.38±0.06	0.48±0.05
		RFI	0.51±0.11	0.32±0.06	0.44±0.08
	Within Angus	ADG	0.38±0.07	0.27±0.06	0.30±0.05
		DMI	0.50±0.07	0.38±0.07	0.47±0.09
		MWT	0.35±0.08	0.29±0.09	0.33±0.10
		RFI	0.58±0.06	0.42±0.08	0.52±0.07
Charolais	**Pooled data**	ADG	0.35±0.10	0.37±0.08	0.33±0.11
		DMI	0.36±0.04	0.31±0.10	0.35±0.06
		MWT	0.37±0.06	0.35±0.07	0.39±0.09
		RFI	0.46±0.04	0.37±0.09	0.41±0.05
	Within Charolais	ADG	0.41±0.10	0.42±0.10	0.36±0.08
		DMI	0.34±0.09	0.37±0.09	0.33±0.09
		MWT	0.33±0.09	0.36±0.08	0.34±0.09

		RFI	0.41±0.03	0.44±0.05	0.41±0.05
KC	**Pooled data**	ADG	0.31±0.07	0.31±0.06	0.35±0.08
		DMI	0.36±0.05	0.34±0.04	0.40±0.07
		MWT	0.40±0.04	0.35±0.01	0.50±0.06
		RFI	0.35±0.04	0.33±0.06	0.37±0.05
	Within KC	ADG	0.24±0.07	0.20±0.05	0.21±0.04
		DMI	0.29±0.02	0.26±0.01	0.29±0.03
		MWT	0.32±0.03	0.28±0.02	0.33±0.02
		RFI	0.37±0.03	0.35±0.02	0.36±0.02
Elora	**Pooled data**	ADG	0.41±0.04	0.33±0.08	0.44±0.06
		DMI	0.30±0.05	0.23±0.05	0.27±0.05
		MWT	0.30±0.05	0.23±0.05	0.31±0.07
		RFI	0.27±0.05	0.20±0.05	0.23±0.05
	Within Elora	ADG	0.41±0.04	0.36±0.05	0.33±0.04
		DMI	0.28±0.05	0.28±0.03	0.26±0.04
		MWT	0.22±0.05	0.21±0.04	0.23±0.03
		RFI	0.27±0.07	0.30±0.06	0.26±0.06
PG1	**Pooled data**	ADG	0.38±0.03	0.32±0.03	0.40±0.04
		DMI	0.49±0.03	0.45±0.03	0.50±0.02
		MWT	0.59±0.03	0.52±0.04	0.67±0.03
		RFI	0.33±0.03	0.29±0.02	0.35±0.03
	Within PG1	ADG	0.37±0.04	0.36±0.03	0.30±0.04
		DMI	0.48±0.02	0.49±0.01	0.46±0.02
		MWT	0.53±0.04	0.52±0.04	0.53±0.04
		RFI	0.33±0.05	0.37±0.05	0.32±0.05
TX	**Pooled data**	ADG	0.32±0.07	0.34±0.06	0.30±0.09
		DMI	0.37±0.05	0.35±0.03	0.39±0.03
		MWT	0.45±0.03	0.39±0.04	0.57±0.02
		RFI	0.31±0.04	0.30±0.03	0.24±0.05
	Within TX	ADG	0.24±0.06	0.29±0.07	0.20±0.06
		DMI	0.36±0.07	0.38±0.05	0.38±0.06
		MWT	0.46±0.06	0.42±0.06	0.48±0.05
		RFI	0.39±0.05	0.33±0.05	0.36±0.06

Within = using animals of the respective breed as training population for evaluation, Pooled data = using data of all the six breeds pooled together as a reference for evaluation. ADG = average daily gain, DMI= dry matter intake, and MWT= metabolic weight and RFI = residual feed efficiency.

Chapter 7. General discussion and recommendation for further research

Feed efficiency (measured as residual feed intake or RFI) and its component traits including dry matter intake (DMI) and average daily growth (ADG) are important production traits in the beef industry with direct impacts on production returns (Fox *et al.* 2001). Indeed, farmers would want to raise an animal that consumes less feed per unit gain and with a faster growth rate, and it has been clearly demonstrated that production profits can significantly increase (18- 43%) even with minimal (of 10%) improvement of either gain or feed efficiency (Fox *et al.* 2001). Although several studies have endeavored to genetically characterize RFI and its component traits through quantitative trait loci (QTL) linkage mapping, DNA marker association studies, and more recently transcriptome studies, their genetic and molecular architecture remains largely unidentified, especially when different breeds are considered. Given the significant economic importance of feed efficiency to the beef industry, RFI and its component traits have started to be included into breeding programs in different countries through genomic selection (Brandebourg *et al.* 2013). However, greater genomic prediction accuracy for the feed efficiency traits are still required for wider adoption of the genomic selection tool. It is expected that knowledge about genomic influence of feed efficiency would help design more effective genomic selection strategies to improve the accuracy of predicting genetic merit of selection candidates for these traits, and hence to improve the rate of response to genomic selection for the traits (Brandebourg *et al.* 2013). Although other gene expression analyses tools are available, RNAseq is a powerful functional genomic method with the ability to simultaneously profile thousands of expressed (coding and noncoding) genes for investigation of associations between the expressed genes and the traits of interest. RNAseq also can profile genes even at a very low level of expression with a

high resolution in a tissue (Marioni *et al.* 2008; Van Den Berge *et al.* 2018). In this thesis project, we employed RNAseq to molecularly characterize residual feed intake and its component traits using steers from three beef populations that were born and raised under similar environmental conditions. We further explored potential of enhancing genomic prediction accuracies of RFI and its component traits through utilizing functional SNP information from transcriptome differential gene expression studies. The thesis included five studies, each with a specific objective. The major results, implications, limitations and recommendation for further research are discussed in this chapter.

7.1 Identification of genes associated with residual feed intake in beef cattle

Through the published literature, it is clear that numerous transcriptomic studies have identified genes associated with residual feed intake (RFI) in beef cattle. However, comparing the identified differentially expressed (DE) genes across the studies, the overlap is low. We hypothesized that this low concordance of DE genes among the studies could be due to the differences in breeds, sex type, age of the animals, the studied tissue, and maybe the environments (including feeds and management) under which animals are raised. In chapter 2, we minimized most of these sources of variation by studying the liver tissue differentially expressed genes between efficient and inefficient steers that were born, raised and tested on the same experimental farm from three beef cattle breed populations. These animals received the same treatments throughout the experiment, and they were on average of similar age at slaughter (i.e. at the time of tissue collection).

We identified multiple genes as associated with RFI within each breed. However, the identified genes were predominantly breed specific. These results implied that the genes that

regulate feed efficiency are probably breed specific. Only five genes including *SCD, LPIN1, TP53INP1, LURAP1L* and *ENSBTAG00000047029* were identified as DE in all the three studied breed populations, and four of these genes (*SCD, LPIN1, TP53INP1* and *LURAP1L*) were downregulated in the efficient animals. *SCD* and *LIPIN1* are key genes involved in lipid synthesis (*SCD* and *LIPIN1*) and accumulation or storage (*LIPIN1*) (Ntambi & Miyazaki 2004; Csaki *et al.* 2013). Therefore, these results highlighted potential significant influence of hepatic lipid synthesis and accumulation on feed efficiency of beef steers across breeds. Functional enrichment analyses in this study identified liver lipid metabolism as one of the major molecular functions associated with RFI. Metabolic processes such as lipid synthesis and accumulation were predicted to be downregulated in more efficient animals, implying that probably more efficient animals prioritize their energy spending away from lipid synthesis (energy costly processes) and spend it more towards protein synthesis and deposition in the muscle. These results are supported by results from some of previous similar studies in the liver tissue and other tissues (Alexandre *et al.* 2015; Weber *et al.* 2016) that identified the association of lipid metabolism with feed efficiency.

We selected the liver as an organ of interest in this thesis project because of its central physiological and metabolic functions in the body (Häussinger 1996), and hence its expected roles to play in regulation of feed efficiency and its component traits. However, we appreciate the fact that there are other important organs/tissues (such the adipose tissue, skeletal muscle, and the digestive tract) in the body whose metabolism potentially influences feed efficiency of the animal. Therefore, further transcriptome studies should be pursued considering these organs and tissues. In the current study we profiled liver tissues collected at one time point, which was at slaughter after the feed intake test period, we recommend that future studies should consider sampling of

tissue at multiple developmental stages of the animal. As highlighted above functional enrichment analysis predicted downregulation of lipid synthesis and accumulation in efficient animals. However, these predictions should have been validated through proteomic, lipid profiling and quantification studies. Despite the highlighted limitations, our results provide further enrichment of our understanding of the molecular basis of feed efficiency in beef cattle.

7.2 Identification of genes associated with growth, dry matter intake, and metabolic weight in beef cattle

Previously several studies have attempted to identify genes associated with growth rate and feed intake through transcriptome studies of different tissues, such as the adipose tissue, rumen epithelia, the duodenum epithelia and jejunum epithelia (Kern *et al.* 2016; Foote *et al.* 2017; Lindholm-Perry *et al.* 2017; Reynolds *et al.* 2017). These studies have identified multiple genes associated with growth or/and feed intake, and some of the enriched biological functions include immune responses, carbohydrate metabolism, lipid metabolism, and amino acid metabolism However, by the time of starting this thesis project no investigation of the liver's differential gene expression as related to body weight gain or feed intake in beef cattle had been reported. Therefore, in chapter three we sought to identify genes associated to average daily gain (ADG), dry matter intake (DMI), and metabolic weight (MWT) and their biological functions through RNAseq analyses of the liver transcriptome of beef steers from the same three breed populations. Within each breed and for each trait, we identified multiple DE genes. As observed in the RFI study in chapter two, the DE gene overlap for each trait between breeds was low, which implied that the growth, feed intake and body weight traits are probably controlled by different genes in different breeds. Results from functional enrichment analyses showed that lipid metabolism was among the top biological functions associated with growth, feed intake, and metabolic weight. These results

suggested a strong relationship between growth, feed consumption and metabolic weight with the animal's lipid metabolic activities. Interestingly in this study key genes involved in cholesterol biosynthesis including *SQLE, PMVK, IDI1, HMGCS1, HMGCR, PMVK, and SQLE* were identified as associated with ADG, DMI or MWT in at least one of the breeds, which revealed the potential to alter beef cholesterol content through genetic selection of these traits as meat cholesterol content is a major health concern of many beef consumers (Li 2017). Additionally, genes involved in different immunological processes were also identified in this study as associated with ADG, DMI or MWT. With these it is hypothesized that differences in the expression of immune related genes in the liver tissue of the animal could be a revelation of the physiological adaptation differences towards feedlot challenges. Nevertheless, differential gene expression analyses in this study were limited by the number of animals to construct divergent phenotypes, as we only had 20 animals per population that were initially selected based on their RFI phenotypes instead of ADG, DMI or MWT , although we lowered the threshold to obtain a substantial number of DE genes. Nevertheless, our results contribute substantially to the molecular characterization for average growth rate, feed intake and metabolic weight in beef cattle. However, future studies with more phenotyped animals should be pursued. Additionally, other tissues (such as the skeletal muscle and adipose tissue) related to direct energy usage and growth should also be studied.

7.3 Identification of MicroRNAs associated with RFI and its component traits

MicroRNAs (miRNAs) are key principle post transcription modulators of gene expression, which have been implicated in regulation of important traits in beef cattle including tenderness, intramuscular fat, fatty acid content and meat yield (Lee *et al.* 2017; Li *et al.* 2018; De Oliveira *et al.* 2019; Kappeler *et al.* 2019). Regarding feed efficiency, only three studies have investigated the

involvement of miRNAs in regulating RFI in beef cattle so far (Al-Husseini *et al.* 2016; De Oliveira *et al.* 2018; Carvalho *et al.* 2019). In the study by Al-Husseini et al. (2016) they only profiled two cDNA libraries composed of pooled RNA of high and low-RFI groups, hence ignoring the individual animal's variability in miRNA expression (Al-Husseini *et al.* 2016). For the study by Carvalho et al. (2019), differential expression of only six miRNAs was investigated through qPCR (Carvalho *et al.* 2019). With respect to the component traits of RFI, only one study that investigated the association of two miRNAs (bta-miR-133b and bta-miR-27a) with growth has been reported (Martin 2017). Therefore, these few studies provide limited knowledge about miRNA molecular control of RFI or its component traits in beef cattle. Therefore, in chapter 4 we aimed to profile hepatic miRNA expression of beef cattle to identify miRNA associated with RFI in beef cattle through RNAseq analyses in the three breed populations. Furthermore, in chapter 5, we explored association of miRNA expression with ADG, DMI and MWT in the liver tissue of beef steers from the same three beef breed populations. We were able to profile expression of the already known miRNAs as well as novel bovine miRNAs expressed in the liver. Results from differential miRNA expression between high and low-RFI steers identified known and novel miRNAs that were associated with RFI and its component traits in each breed. Comparison of the DE miRNAs among the breeds showed that most of the DE miRNAs were breed specific, which corresponded with differential gene (coding) expression results in the RNA sequence analyses, hence, further reaffirming the proposition that the genes regulating RFI in beef cattle are breed specific. Also, for ADG, DMI and MWT we identified multiple differentially expressed miRNA associated with either ADG, DMI or MWT. Similarly, the results showed that majority of the DE miRNAs were breed specific. However, within breed comparison of the DE miRNAs for the four

traits revealed some DE miRNA overlap between the traits, which indicate that these miRNAs play pleotropic effects on the traits.

Prediction of target genes of the identified DE miRNA showed that the majority (55 to 76%) of the DE genes identified in RNAseq analyses (i.e. Chapter two and Chapter three) were potential targets of the DE miRNAs, henceforth providing a link on how the DE miRNA probably regulate RFI or the component traits via influencing target gene mRNAs. In general, most of the DE genes were predicted to be targeted by more than one DE miRNA. Also, for most of the miRNAs, one miRNA was predicted to target more than one gene, which is a characteristic of the complex miRNA-mRNA regulatory interaction (Hashimoto *et al.* 2013). Intuitively, it would be expected that a gene predicted to be targeted by upregulated miRNA should be downregulated in the same tissue. However, we observed that several genes were upregulated despite being predicted to be targeted by multiple upregulated miRNAs. We hypothesized these results could be explained by the difference in the modes regulation miRNAs effect expression of their target genes including translation repression and promoting mRNA degradation (Guo *et al.* 2010). Gene deregulation through increased mRNA degradation can be investigated through abundance profiling of mRNA expression in the tissue through RNAseq analysis as performed in study 2. However, deregulation through translation repression can only be investigated through proteomic studies, since mRNA concentrations remain unaltered by the targeting miRNA (Guo *et al.* 2010). Therefore, we recommend further proteomic based investigation of the regulatory effect of the identified miRNAs on the DE genes. Additionally, we used TargetScan, a popular bioinformatic tool, to predict the potential targets of the DE miRNAs. However future studies should endeavor to validate these target genes through molecular experiments such transfection experiments (Krützfeldt *et al.* 2006; Jin Jung & Suh 2012). Overall, the results in these two chapters provide

more insights into miRNA regulation of RFI and its related traits and contribute to the growing number of the bovine miRNAs in the miRBase database.

7.4 Integration of functional SNPs into genomic prediction of residual feed intake and its component traits

It has been proposed in multiple species that it is possible to improve genomic prediction accuracy with functional enrichment of prediction DNA marker panels (Snelling *et al.* 2013; Lozano *et al.* 2017; Gebreyesus *et al.* 2019). Given the economic importance of RFI and its component traits such as growth rate and feed intake, in chapter 6 we sought to enhance genomic prediction accuracy of these traits through integration of transcriptome analyses results from the four studies of this thesis project (i.e. Chapters: 2, 3, 4 and 5) and those from available literature. We compiled a panel of SNPs from 3753 candidate genes identified through transcriptomic studies, which we called Functional panel, and we compared it to a commercial 50K SNP panel and randomly sampled (Random) SNP panel. Genomic prediction accuracy results showed that in general the three SNP panels did not differ in their prediction accuracy. This implied that on average the Functional panel did not show substantial improvement of the genomic prediction accuracy for the traits. However, in some scenarios, i.e. within Charolais genomic prediction, the Functional panel performed slightly better than both the 50K and Random panel. These results indicate the possibility of improving genomic prediction accuracy of RFI and its component traits through functional enrichment of the SNP panels. However, to make the Functional panel more effective it would require integration of different omics tools (Snelling *et al.* 2013) to identify genes that truly control these traits instead of using all candidate genes that are subjective to false positives. Furthermore, the SNPs used to compile the functional panel in this study were mined from within the candidate genes and 1000bp from the start or end sites of the gene, which implies

that our Functional panel was limited to mainly to within gene SNPs and those in the proximal regulatory regions. However, there is growing evidence that the variants that affect complex traits do not actually reside within genes but rather in distal regulatory regions far from the genes. Genomic information (annotation) of these distal regulatory regions in cattle is currently unavailable, and this is one of the core aim of the Functional Annotation of Animal Genomes (FAANG) project (Andersson et al. 2015). It is expected that the research results from the FAANG project will lead to better SNP functional annotation, which in turn will lead to development of better functional SNP panels to improve genomic prediction of complex traits in beef cattle. In this regard, epigenetics studies including DNA methylation studies via Methyl-Seq or bisulfite sequencing and chromatin modification studies through chromatin immunoprecipitation sequencing or ChIP-Seq and transposase-accessible chromatin sequencing or ATAC-Seq (Dirks et al. 2016) may further aid in identification of genomic transcription regulatory regions or genes associated with feed efficiency and its component traits.

Also, it should be noted that we used imputed genotypes (with imputation error < 5%) to compile the functional SNP panel which we compared against the 50K panel with real genotypes. The possible imputation errors of gene SNPs may compromise the accuracy of genomic prediction of the functional SNP panel. With the emergence of the newly refined bovine reference genome combined with the decreasing costs of whole genome sequencing it is our recommendation that future research should be directed towards more accurate imputed genotypes or real genotypes from sequence data. Additionally, all the four transcriptome studies indicate that the genes controlling RFI and its component traits are majorly breed specific. These results concur with that in a multi-breed feed efficiency and component traits QTL identification study by Saatchi et al. (2014), in which QTLs for the traits are largely breed specific. Therefore more QTL mapping,

eQTL mapping, transcriptome, and SNP functional studies involving multiple beef breeds should be pursued to pinpoint genes and gene SNPs that have common effects across breeds, and genes and gene SNPs that have effects on the trait with a specific breed, which will enable to designing of a more effective DNA marker panel and optimal genomic prediction strategies to improve the prediction accuracy for the traits. In terms of statistical modeling of the SNP marker effects of feed efficiency, methods such as BayesA (Hayes & Goddard 2001), BayesB (Hayes & Goddard 2001), BayesR (Erbe et al. 2012) and BayesRC (MacLeod et al. 2016) should be investigated for integrating transcriptome information into genomic prediction for feed efficiency and its component traits in beef cattle.

In conclusion, future integration of functional information from multiple omics sources into genomic prediction should be pursued to improve genomic prediction accuracy for feed efficiency traits, which would enhance responses to genetic selection of these traits in beef cattle. Accelerated responses to selective breeding for these traits will improve production profitability of beef as well as reduce the negative environmental impacts of beef cattle.

7.5 References

Al-Husseini W., Chen Y., Gondro C., Herd R.M., Gibson J.P. & Arthur P.F. (2016) Characterization and profiling of liver microRNAs by RNA-sequencing in cattle divergently selected for residual feed intake. Asian-Australasian journal of animal sciences 29, 1371.

Alexandre P.A., Kogelman L.J., Santana M.H., Passarelli D., Pulz L.H., Fantinato-Neto P., Silva P.L., Leme P.R., Strefezzi R.F. & Coutinho L.L. (2015) Liver transcriptomic networks

reveal main biological processes associated with feed efficiency in beef cattle. BMC genomics 16, 1073.

Andersson L., Archibald A.L., Bottema C.D., Brauning R., Burgess S.C., Burt D.W., Casas E., Cheng H.H., Clarke L. & Couldrey C. (2015) Coordinated international action to accelerate genome-to-phenome with FAANG, the Functional Annotation of Animal Genomes project. Genome biology 16, 57.

Brandebourg T., Wolfe D. & Foradori C. (2013) US beef industry: a sustainable success story, challenges and priorities. J. Fisheries Livest. Prod 1.

Carvalho E.B., Gionbelli M.P., Rodrigues R.T., Bonilha S.F., Newbold C.J., Guimarães S.E., Silva W., Verardo L.L., Silva F.F. & Detmann E. (2019) Differentially expressed mRNAs, proteins and miRNAs associated to energy metabolism in skeletal muscle of beef cattle identified for low and high residual feed intake. BMC genomics 20, 501.

Csaki L.S., Dwyer J.R., Fong L.G., Tontonoz P., Young S.G. & Reue K. (2013) Lipins, lipinopathies, and the modulation of cellular lipid storage and signaling. Progress in lipid research 52, 305-16.

De Oliveira P.S., Coutinho L.L., Cesar A.S., da Silva Diniz W.J., de Souza M.M., Andrade B.G., Koltes J.E., Mourão G.B., Zerlotini A. & Reecy J.M. (2019) Co-Expression Networks Reveal Potential Regulatory Roles of miRNAs in Fatty Acid Composition of Nelore Cattle. Frontiers in genetics 10.

De Oliveira P.S., Coutinho L.L., Tizioto P.C., Cesar A.S., de Oliveira G.B., Diniz W.J.d.S., De Lima A.O., Reecy J.M., Mourão G.B. & Zerlotini A. (2018) An integrative transcriptome analysis indicates regulatory mRNA-miRNA networks for residual feed intake in Nelore cattle. Scientific reports 8, 17072.

Dirks R.A., Stunnenberg H.G. & Marks H. (2016) Genome-wide epigenomic profiling for biomarker discovery. Clinical epigenetics 8, 122.

Erbe M., Hayes B., Matukumalli L., Goswami S., Bowman P., Reich C., Mason B. & Goddard M. (2012) Improving accuracy of genomic predictions within and between dairy cattle breeds with imputed high-density single nucleotide polymorphism panels. Journal of dairy science 95, 4114-29.

Foote A., Keel B., Zarek C. & Lindholm-Perry A. (2017) Beef steers with average dry matter intake and divergent average daily gain have altered gene expression in the jejunum. Journal of animal science 95, 4430-9.

Fox D., Tedeschi L. & Guiroy P. (2001) Determining feed intake and feed efficiency of individual cattle fed in groups. In: Proc Beef Impr Fed 33rd Ann Res Symp Annu Meet, pp. 80-98.

Gebreyesus G., Bovenhuis H., Lund M.S., Poulsen N.A., Sun D. & Buitenhuis B. (2019) Reliability of genomic prediction for milk fatty acid composition by using a multi-population reference and incorporating GWAS results. Genetics Selection Evolution 51, 16.

Georges M., Charlier C. & Hayes B. (2018) Harnessing genomic information for livestock improvement. Nature Reviews Genetics, 1.

Guo H., Ingolia N.T., Weissman J.S. & Bartel D.P. (2010) Mammalian microRNAs predominantly act to decrease target mRNA levels. Nature 466, 835.

Hashimoto Y., Akiyama Y. & Yuasa Y. (2013) Multiple-to-multiple relationships between microRNAs and target genes in gastric cancer. PloS one 8, e62589.

Häussinger D. (1996) Physiological functions of the liver. In: Comprehensive human physiology (pp. 1369-91. Springer.

Hayes B. & Goddard M. (2001) Prediction of total genetic value using genome-wide dense marker maps. Genetics 157, 1819-29.

Jin Jung H. & Suh Y. (2012) MicroRNA in aging: from discovery to biology. Current genomics 13, 548-57.

Kappeler B.I., Regitano L.C., Poleti M.D., Cesar A.S., Moreira G.C., Gasparin G. & Coutinho L.L. (2019) MiRNAs differentially expressed in skeletal muscle of animals with divergent estimated breeding values for beef tenderness. BMC molecular biology 20, 1.

Kern R.J., Lindholm-Perry A.K., Freetly H.C., Snelling W.M., Kern J.W., Keele J.W., Miles J.R., Foote A.P., Oliver W.T. & Kuehn L.A. (2016) Transcriptome differences in the rumen of beef steers with variation in feed intake and gain. Gene 586, 12-26.

Krützfeldt J., Poy M.N. & Stoffel M. (2006) Strategies to determine the biological function of microRNAs. Nature genetics 38, S14.

Lee S., Park S.J., Cheong J.k., Ko J.Y., Bong J. & Baik M. (2017) Identification of circulating miRNA involved in meat yield of Korean cattle. Cell biology international 41, 761-8.

Li C. (2017) The role of beef in human nutrition and health. Dikeman E, editorEnsuring safety and quality in the production of beef 2, 329-38.

Li N., Zhang Y., Li H.-P., Han L., Yan X.-M., Li H.-B., Du W., Zhang J.-S. & Yu Q.-L. (2018) Differential expression of mRNA-miRNAs related to intramuscular fat content in the longissimus dorsi in Xinjiang brown cattle. PloS one 13, e0206757.

Lindholm-Perry A., Cunningham H., Kuehn L., Vallet J., Keele J., Foote A., Cammack K. & Freetly H. (2017) Relationships between the genes expressed in the mesenteric adipose tissue of beef cattle and feed intake and gain. Animal genetics 48, 386-94.

Lozano R., Del Carpio D.P., Kayondo S.I., Amuge T., Adebo O.A., Ferguson M. & Jannink J.-L. (2017) Leveraging Transcriptomics Data for Genomic Prediction Models in Cassava. bioRxiv, 208181.

MacLeod I., Bowman P., Vander Jagt C., Haile-Mariam M., Kemper K., Chamberlain A., Schrooten C., Hayes B. & Goddard M. (2016) Exploiting biological priors and sequence variants enhances QTL discovery and genomic prediction of complex traits. BMC genomics 17, 144.

Marioni J.C., Mason C.E., Mane S.M., Stephens M. & Gilad Y. (2008) RNA-seq: an assessment of technical reproducibility and comparison with gene expression arrays. Genome research 18, 1509-17.

Martin T.S. (2017) The Study of microRNA in Plasma to Discover Biomarkers Important for Muscle Growth in Beef Cattle.

Ntambi J.M. & Miyazaki M. (2004) Regulation of stearoyl-CoA desaturases and role in metabolism. Progress in lipid research 43, 91-104.

Reynolds J., Foote A., Freetly H., Oliver W. & Lindholm-Perry A. (2017) Relationships between inflammation-and immunity-related transcript abundance in the rumen and jejunum of beef steers with divergent average daily gain. Animal genetics 48, 447-9.

Saatchi M., Beever J.E., Decker J.E., Faulkner D.B., Freetly H.C., Hansen S.L., Yampara-Iquise H., Johnson K.A., Kachman S.D. & Kerley M.S. (2014) QTLs associated with dry matter intake, metabolic mid-test weight, growth and feed efficiency have little overlap across 4 beef cattle studies. BMC genomics 15, 1004.

Snelling W., Cushman R., Keele J., Maltecca C., Thomas M., Fortes M. & Reverter A. (2013) BREEDING AND GENETICS SYMPOSIUM: Networks and pathways to guide genomic selection–. Journal of animal science 91, 537-52.

Van Den Berge K., Hembach K.M., Soneson C., Tiberi S., Clement L., Love M.I., Patro R. & Robinson M.D. (2018) RNA sequencing data: hitchhiker's guide to expression analysis. Annual Review of Biomedical Data Science 2.

Weber K.L., Welly B.T., Van Eenennaam A.L., Young A.E., Porto-Neto L.R., Reverter A. & Rincon G. (2016) Identification of gene networks for residual feed intake in angus cattle using genomic prediction and RNA-seq. PloS one 11, e0152274.

Bibliography

Abo-Ismail M., Squires E., Swanson K., Lu D., Wang Z., Mah J., Plastow G., Moore S. & Miller S. (2010) Fine mapping QTL and candidate genes discovery for residual feed intake on Chromosomes 5, 15, 16, and 19 in beef cattle. In: Proceedings of the 9th World Congress on Genetics Applied to Livestock Production: 1-6 August 2010; Leipzig.

Abo-Ismail M.K., Vander Voort G., Squires J.J., Swanson K.C., Mandell I.B., Liao X., Stothard P., Moore S., Plastow G. & Miller S.P. (2014) Single nucleotide polymorphisms for feed efficiency and performance in crossbred beef cattle. BMC genetics 15, 14.

Agarwal V., Bell G.W., Nam J.-W. & Bartel D.P. (2015) Predicting effective microRNA target sites in mammalian mRNAs. elife 4, e05005.

Ahola J.K. & Hill R.A. (2012) Input Factors Affecting Profitability: a Changing Paradigm and a Challenging Time. In: Feed efficiency in the beef industry (ed. by Hill RA), p. 10. Wiley-Blackwell, Ames, Iowa.

Alexander D.H. & Lange K. (2011) Enhancements to the ADMIXTURE algorithm for individual ancestry estimation. BMC bioinformatics 12, 246.

Alexandre P.A., Gomes R.C., Santana M.H., Silva S.L., Leme P.R., Mudadu M.A., Regitano L.C., Meirelles F.V., Ferraz J.B. & Fukumasu H. (2014) Bovine NR1I3 gene polymorphisms and its association with feed efficiency traits in Nellore cattle. Meta gene 2, 206-17.

Alexandre P.A., Kogelman L.J., Santana M.H., Passarelli D., Pulz L.H., Fantinato-Neto P., Silva P.L., Leme P.R., Strefezzi R.F. & Coutinho L.L. (2015) Liver transcriptomic networks reveal main biological processes associated with feed efficiency in beef cattle. BMC genomics 16, 1073.

Al-Husseini W., Chen Y., Gondro C., Herd R.M., Gibson J.P. & Arthur P.F. (2016) Characterization and profiling of liver microRNAs by RNA-sequencing in cattle divergently selected for residual feed intake. Asian-Australasian journal of animal sciences 29, 1371.

Al-Husseini W., Gondro C., Quinn K., Herd R., Gibson J. & Chen Y. (2014) Expression of candidate genes for residual feed intake in Angus cattle. Animal genetics 45, 12-9.

Anders S., McCarthy D.J., Chen Y., Okoniewski M., Smyth G.K., Huber W. & Robinson M.D. (2013) Count-based differential expression analysis of RNA sequencing data using R and Bioconductor. Nature protocols 8, 1765-86.

Anders S., Pyl P.T. & Huber W. (2015) HTSeq–a Python framework to work with high-throughput sequencing data. Bioinformatics, btu638.

Andersson L., Archibald A.L., Bottema C.D., Brauning R., Burgess S.C., Burt D.W., Casas E., Cheng H.H., Clarke L. & Couldrey C. (2015) Coordinated international action to accelerate genome-to-phenome with FAANG, the Functional Annotation of Animal Genomes project. Genome biology 16, 57.

Andrews S. (2010) FastQC: a quality control tool for high throughput sequence data.

Archer J., Richardson E., Herd R. & Arthur P. (1999) Potential for selection to improve efficiency of feed use in beef cattle: a review. Australian Journal of Agricultural Research 50, 147-62.

Arthur P., Renand G. & Krauss D. (2001) Genetic and phenotypic relationships among different measures of growth and feed efficiency in young Charolais bulls. Livestock production science 68, 131-9.

Baek D., Villén J., Shin C., Camargo F.D., Gygi S.P. & Bartel D.P. (2008) The impact of microRNAs on protein output. Nature 455, 64.

Baldassini W., Bonilha S., Branco R., Vieira J., Padilha P. & Lanna D. (2018) Proteomic investigation of liver from beef cattle (Bos indicus) divergently ranked on residual feed intake. Molecular biology reports 45, 2765-73.

Barendse W., Reverter A., Bunch R.J., Harrison B.E., Barris W. & Thomas M.B. (2007) A validated whole-genome association study of efficient food conversion in cattle. Genetics 176, 1893-905.

Bartel D.P. (2018) Metazoan MicroRNAs. Cell 173, 20-51.

Baruch Y. (2000) The liver: a large endocrine gland. Journal of hepatology 32, 505-7.

Bates D.J., Li N., Liang R., Sarojini H., An J., Masternak M.M., Bartke A. & Wang E. (2010) MicroRNA regulation in Ames dwarf mouse liver may contribute to delayed aging. Aging cell 9, 1-18.

Bell A. (1981) Lipid metabolism in liver and selected tissues and in the whole body of ruminant animals. In: Lipid metabolism in ruminant animals (pp. 363-410. Elsevier.

Benjamini Y. & Hochberg Y. (1995) Controlling the false discovery rate: a practical and powerful approach to multiple testing. Journal of the royal statistical society. Series B (Methodological), 289-300.

Berry D. & Crowley J. (2012) Residual intake and body weight gain: a new measure of efficiency in growing cattle. Journal of animal science 90, 109-15.

Bolormaa S., Hayes B., Savin K., Hawken R., Barendse W., Arthur P., Herd R. & Goddard M. (2011) Genome-wide association studies for feedlot and growth traits in cattle. Journal of animal science 89, 1684-97.

Bourgon S., de Amorim M.D., Miller S. & Montanholi Y. (2017) Associations of blood parameters with age, feed efficiency and sampling routine in young beef bulls. Livestock Science 195, 27-37.

Boxx G.M. & Cheng G. (2016) The roles of type I interferon in bacterial infection. Cell host & microbe 19, 760-9.

Brandebourg T., Wolfe D. & Foradori C. (2013) US beef industry: a sustainable success story, challenges and priorities. J. Fisheries Livest. Prod 1.

Briggs M.H. & Briggs M.D. (1980) Modern breeds of livestock. Macmillan, New York.

Brody S. (1945) Bioenergetics and growth with special reference to the energetic efficiency complex in domestic animals. Reinhold Publ., New York 403.

Brown A.J. & Sharpe L.J. (2015) Cholesterol Synthesis. In: Biochemistry of lipids, lipoproteins and membranes (eds. by Ridgway N & McLeod R), pp. 327-58. Elsevier, Amsterdam, Netherlands.

Carstens G.E. & Tedeschi L.O. (2006) Defining feed efficiency in beef cattle. In: Proceedings of Beef Improvement Federation 38th Annual Research Symposium and Annual Meeting, Choctaw, Mississippi, pp. 12-21.

Carvalho E.B., Gionbelli M.P., Rodrigues R.T., Bonilha S.F., Newbold C.J., Guimarães S.E., Silva W., Verardo L.L., Silva F.F. & Detmann E. (2019) Differentially expressed mRNAs, proteins and miRNAs associated to energy metabolism in skeletal muscle of beef cattle identified for low and high residual feed intake. BMC genomics 20, 501.

CCA (2018) Canadian Beef Industry Statitistics. URL http://www.cattle.ca/cca-resources/industry-stats/.

CCAC (2009) Guidelines on the care and use of farm animals in research, teaching and testing. Canadian Council on Animal Care Ottawa, ON, Canada.

Chen C., Ridzon D.A., Broomer A.J., Zhou Z., Lee D.H., Nguyen J.T., Barbisin M., Xu N.L., Mahuvakar V.R. & Andersen M.R. (2005) Real-time quantification of microRNAs by stem–loop RT–PCR. Nucleic acids research 33, e179-e.

Chen L., Schenkel F., Vinsky M., Crews D. & Li C. (2013) Accuracy of predicting genomic breeding values for residual feed intake in Angus and Charolais beef cattle. Journal of animal science 91, 4669-78.

Chen X., Zhao Y., Wang F., Bei Y., Xiao J. & Yang C. (2015) MicroRNAs in liver regeneration. Cellular Physiology and Biochemistry 37, 615-28.

Chen Y. & Verfaillie C.M. (2014) Micro RNAs: the fine modulators of liver development and function. Liver International 34, 976-90.

Chen Y., Gondro C., Quinn K., Herd R., Parnell P. & Vanselow B. (2011) Global gene expression profiling reveals genes expressed differentially in cattle with high and low residual feed intake. Animal genetics 42, 475-90.

Cheng X.-N., Shao M., Li J.-T., Wang Y.-F., Qi J., Xu Z.-G. & Shi D.-L. (2017) Leucine repeat adaptor protein 1 interacts with Dishevelled to regulate gastrulation cell movements in zebrafish. Nature Communications 8, 1353.

Creighton C.J., Reid J.G. & Gunaratne P.H. (2009) Expression profiling of microRNAs by deep sequencing. Briefings in bioinformatics 10, 490-7.

Crowley J., McGee M., Kenny D., Crews Jr D., Evans R. & Berry D. (2010) Phenotypic and genetic parameters for different measures of feed efficiency in different breeds of Irish performance-tested beef bulls. Journal of animal science 88, 885-94.

Csaki L.S., Dwyer J.R., Fong L.G., Tontonoz P., Young S.G. & Reue K. (2013) Lipins, lipinopathies, and the modulation of cellular lipid storage and signaling. Progress in lipid research 52, 305-16.

Daetwyler H.D., Capitan A., Pausch H., Stothard P., Van Binsbergen R., Brøndum R.F., Liao X., Djari A., Rodriguez S.C. & Grohs C. (2014) Whole-genome sequencing of 234 bulls facilitates mapping of monogenic and complex traits in cattle. Nature genetics 46, 858.

de Oliveira P.S., Cesar A.S., do Nascimento M.L., Chaves A.S., Tizioto P.C., Tullio R.R., Lanna D.P., Rosa A.N., Sonstegard T.S. & Mourao G.B. (2014) Identification of genomic regions associated with feed efficiency in Nelore cattle. BMC genetics 15, 100.

De Oliveira P.S., Coutinho L.L., Cesar A.S., da Silva Diniz W.J., de Souza M.M., Andrade B.G., Koltes J.E., Mourão G.B., Zerlotini A. & Reecy J.M. (2019) Co-Expression Networks Reveal Potential Regulatory Roles of miRNAs in Fatty Acid Composition of Nelore Cattle. Frontiers in genetics 10.

De Oliveira P.S., Coutinho L.L., Tizioto P.C., Cesar A.S., de Oliveira G.B., Diniz W.J.d.S., De Lima A.O., Reecy J.M., Mourão G.B. & Zerlotini A. (2018) An integrative transcriptome analysis indicates regulatory mRNA-miRNA networks for residual feed intake in Nelore cattle. Scientific reports 8, 17072.

Dirks R.A., Stunnenberg H.G. & Marks H. (2016) Genome-wide epigenomic profiling for biomarker discovery. Clinical epigenetics 8, 122.

Donkin S.S. (2012) The role of liver metabolism during transition on postpartum health and performance. In: 2012 Florida Ruminant Nutrition Symposium Proceedings. 23rd Meeting, pp. 97-106.

Drackley J.K., Dann H.M., Douglas N., Guretzky N.A.J., Litherland N.B., Underwood J.P. & Loor J.J. (2005) Physiological and pathological adaptations in dairy cows that may increase susceptibility to periparturient diseases and disorders. Italian Journal of Animal Science 4, 323-44.

Edwards S.M., Sørensen I.F., Sarup P., Mackay T.F. & Sørensen P. (2016) Genomic prediction for quantitative traits is improved by mapping variants to gene ontology categories in Drosophila melanogaster. Genetics 203, 1871-83.

Eisemann J., Hammond A. & Rumsey T. (1989) Tissue protein synthesis and nucleic acid concentrations in steers treated with somatotropin. British Journal of Nutrition 62, 657-71.

Erbe M., Hayes B., Matukumalli L., Goswami S., Bowman P., Reich C., Mason B. & Goddard M. (2012) Improving accuracy of genomic predictions within and between dairy cattle breeds with imputed high-density single nucleotide polymorphism panels. Journal of dairy science 95, 4114-29.

Fabian M.R., Sonenberg N. & Filipowicz W. (2010) Regulation of mRNA translation and stability by microRNAs. Annual review of biochemistry 79, 351-79.

Fang L., Sahana G., Ma P., Su G., Yu Y., Zhang S., Lund M.S. & Sørensen P. (2017) Use of biological priors enhances understanding of genetic architecture and genomic prediction of complex traits within and between dairy cattle breeds. BMC genomics 18, 604.

Fitzsimons C., Kenny D., Deighton M., Fahey A. & McGee M. (2013) Methane emissions, body composition, and rumen fermentation traits of beef heifers differing in residual feed intake. Journal of animal science 91, 5789-800.

Fonseca L.D., Eler J.P., Pereira M.A., Rosa A.F., Alexandre P.A., Moncau C.T., Salvato F., Rosa-Fernandes L., Palmisano G. & Ferraz J.B. (2019) Liver proteomics unravel the metabolic pathways related to Feed Efficiency in beef cattle. Scientific reports 9, 5364.

Foote A., Keel B., Zarek C. & Lindholm-Perry A. (2017) Beef steers with average dry matter intake and divergent average daily gain have altered gene expression in the jejunum. Journal of animal science 95, 4430-9.

Fox D., Tedeschi L. & Guiroy P. (2001) Determining feed intake and feed efficiency of individual cattle fed in groups. In: Proc Beef Impr Fed 33rd Ann Res Symp Annu Meet, pp. 80-98.

Friedländer M.R., Mackowiak S.D., Li N., Chen W. & Rajewsky N. (2011) miRDeep2 accurately identifies known and hundreds of novel microRNA genes in seven animal clades. Nucleic acids research 40, 37-52.

Friedman R.C., Farh K.K.-H., Burge C.B. & Bartel D.P. (2009) Most mammalian mRNAs are conserved targets of microRNAs. Genome research 19, 92-105.

Gailhouste L., Gomez-Santos L., Hagiwara K., Hatada I., Kitagawa N., Kawaharada K., Thirion M., Kosaka N., Takahashi R.u. & Shibata T. (2013) miR-148a plays a pivotal role in the liver by promoting the hepatospecific phenotype and suppressing the invasiveness of transformed cells. Hepatology 58, 1153-65.

Gebreyesus G., Bovenhuis H., Lund M.S., Poulsen N.A., Sun D. & Buitenhuis B. (2019) Reliability of genomic prediction for milk fatty acid composition by using a multi-population reference and incorporating GWAS results. Genetics Selection Evolution 51, 16.

Georges M., Charlier C. & Hayes B. (2018) Harnessing genomic information for livestock improvement. Nature Reviews Genetics, 1.

Gerber P.J., Steinfeld H., Henderson B., Mottet A., Opio C., Dijkman J., Falcucci A. & Tempio G. (2013) Tackling climate change through livestock: a global assessment of emissions and mitigation opportunities. Food and Agriculture Organization of the United Nations (FAO).

Goddard M. (2009) Genomic selection: prediction of accuracy and maximisation of long term response. Genetica 136, 245-57.

Goddard M.E. & Hayes B.J. (2009) Mapping genes for complex traits in domestic animals and their use in breeding programmes. Nature Reviews Genetics 10, 381-91.

Goonewardene L., Wang Z., Price M., Yang R.-C., Berg R. & Makarechian M. (2003) Effect of udder type and calving assistance on weaning traits of beef and dairy× beef calves. Livestock production science 81, 47-56.

Gregory R.I. & Shiekhattar R. (2005) MicroRNA biogenesis and cancer. Cancer research 65, 3509-12.

Griffiths-Jones S., Saini H.K., van Dongen S. & Enright A.J. (2007) miRBase: tools for microRNA genomics. Nucleic acids research 36, D154-D8.

Guo H., Ingolia N.T., Weissman J.S. & Bartel D.P. (2010) Mammalian microRNAs predominantly act to decrease target mRNA levels. Nature 466, 835.

Hartig S.M., Hamilton M.P., Bader D.A. & McGuire S.E. (2015) The miRNA interactome in metabolic homeostasis. Trends in Endocrinology & Metabolism 26, 733-45.

Hashimoto Y., Akiyama Y. & Yuasa Y. (2013) Multiple-to-multiple relationships between microRNAs and target genes in gastric cancer. PloS one 8, e62589.

Häussinger D. (1996) Physiological functions of the liver. In: Comprehensive human physiology (pp. 1369-91. Springer.

Hayes B. & Goddard M. (2001) Prediction of total genetic value using genome-wide dense marker maps. Genetics 157, 1819-29.

Hegarty R., Goopy J., Herd R. & McCorkell B. (2007) Cattle selected for lower residual feed intake have reduced daily methane production. Journal of animal science 85, 1479-86.

Herd R. & Arthur P. (2009) Physiological basis for residual feed intake. Journal of animal science 87, E64-E71.

Herd R. & Bishop S. (2000) Genetic variation in residual feed intake and its association with other production traits in British Hereford cattle. Livestock production science 63, 111-9.

Herd R., Oddy V. & Richardson E. (2004) Biological basis for variation in residual feed intake in beef cattle. 1. Review of potential mechanisms. Australian Journal of Experimental Agriculture 44, 423-30.

Hill R.A. & Ahola J.K. (2012) Feed Efficiency Interactions with Other Traits: Growth and Product Quality. In: Feed efficiency in the beef industry (ed. by Hill RA), p. 148. Wiley-Blackwell, Ames, Iowa.

Hocquette J.-F. & Chatellier V. (2011) Prospects for the European beef sector over the next 30 years. Animal Frontiers 1, 20-8.

Hong C. & Tontonoz P. (2014) Liver X receptors in lipid metabolism: opportunities for drug discovery. Nature reviews Drug discovery 13, 433.

Hu Z.-L., Park C.A. & Reecy J.M. (2018) Building a livestock genetic and genomic information knowledgebase through integrative developments of Animal QTLdb and CorrDB. Nucleic acids research 47, D701-D10.

Hua X., Wu J., Goldstein J.L., Brown M.S. & Hobbs H.H. (1995) Structure of the human gene encoding sterol regulatory element binding protein-1 (SREBF1) and localization of SREBF1 and SREBF2 to chromosomes 17p11. 2 and 22q13. Genomics 25, 667-73.

Huntington G.B. & Eisemann J.H. (1988) Regulation of nutrient supply by gut and liver tissues. Journal of animal science 66, 35-48.

Inoue K., Kobayashi M., Shoji N. & Kato K. (2011) Genetic parameters for fatty acid composition and feed efficiency traits in Japanese Black cattle. Animal 5, 987-94.

Jeremy M.B., John L.T. & Lubert S. (2002) Each Organ Has a Unique Metabolic Profile. In: Biochemistry (ed. by Moran S), p. 1259. W. H. Freeman and Company, New York.

Jin Jung H. & Suh Y. (2012) MicroRNA in aging: from discovery to biology. Current genomics 13, 548-57.

Jordan S.D., Krüger M., Willmes D.M., Redemann N., Wunderlich F.T., Brönneke H.S., Merkwirth C., Kashkar H., Olkkonen V.M. & Böttger T. (2011) Obesity-induced overexpression of miRNA-143 inhibits insulin-stimulated AKT activation and impairs glucose metabolism. Nature cell biology 13, 434.

Kappeler B.I., Regitano L.C., Poleti M.D., Cesar A.S., Moreira G.C., Gasparin G. & Coutinho L.L. (2019) MiRNAs differentially expressed in skeletal muscle of animals with divergent estimated breeding values for beef tenderness. BMC molecular biology 20, 1.

Karisa B., Moore S. & Plastow G. (2014) Analysis of biological networks and biological pathways associated with residual feed intake in beef cattle. Animal Science Journal 85, 374-87.

Karisa B., Thomson J., Wang Z., Stothard P., Moore S. & Plastow G. (2013) Candidate genes and single nucleotide polymorphisms associated with variation in residual feed intake in beef cattle. Journal of animal science 91, 3502-13.

Keel B.N., Zarek C.M., Keele J.W., Kuehn L.A., Snelling W.M., Oliver W.T., Freetly H.C. & Lindholm-Perry A.K. (2018) RNA-Seq Meta-analysis identifies genes in skeletal muscle associated with gain and intake across a multi-season study of crossbred beef steers. BMC genomics 19, 430.

Keiding S. & Sørensen M. (2007) Textbook of Hepatology: From Basic Science to Clinical Practice.

Kellner O.J. (1909) The Scientific Feeding of Animals. Macmillan.

Kennedy B., Van der Werf J. & Meuwissen T. (1993) Genetic and statistical properties of residual feed intake. Journal of animal science 71, 3239-50.

Kern R.J., Lindholm-Perry A.K., Freetly H.C., Snelling W.M., Kern J.W., Keele J.W., Miles J.R., Foote A.P., Oliver W.T. & Kuehn L.A. (2016) Transcriptome differences in the rumen of beef steers with variation in feed intake and gain. Gene 586, 12-26.

Khansefid M., Millen C., Chen Y., Pryce J., Chamberlain A., Vander Jagt C., Gondro C. & Goddard M. (2017) Gene expression analysis of blood, liver, and muscle in cattle divergently selected for high and low residual feed intake. Journal of animal science 95, 4764-75.

Khansefid M., Pryce J., Bolormaa S., Miller S., Wang Z., Li C. & Goddard M. (2014) Estimation of genomic breeding values for residual feed intake in a multibreed cattle population. Journal of animal science 92, 3270-83.

Kim D., Pertea G., Trapnell C., Pimentel H., Kelley R. & Salzberg S.L. (2013) TopHat2: accurate alignment of transcriptomes in the presence of insertions, deletions and gene fusions. Genome biology 14, R36.

Kitaoka Y., Olvera J. & Wool I.G. (1994) The primary structure of rat ribosomal protein S23. Biochemical and Biophysical Research Communications 202, 314-20.

Kleiber M. (1947) Body size and metabolic rate. Physiological reviews 27, 511-41.

Koch R.M., Swiger L.A., Chambers D. & Gregory K.E. (1963) Efficiency of feed use in beef cattle. Journal of animal science 22, 486-94.

Kong R.S., Liang G., Chen Y. & Stothard P. (2016) Transcriptome profiling of the rumen epithelium of beef cattle differing in residual feed intake. BMC genomics 17, 592.

Krämer A., Green J., Pollard Jr J. & Tugendreich S. (2013) Causal analysis approaches in ingenuity pathway analysis. Bioinformatics 30, 523-30.

Krützfeldt J., Poy M.N. & Stoffel M. (2006) Strategies to determine the biological function of microRNAs. Nature genetics 38, S14.

Lackner D.H. & Bähler J. (2008) Translational control of gene expression: from transcripts to transcriptomes. International review of cell and molecular biology 271, 199-251.

Langmead B., Trapnell C., Pop M. & Salzberg S.L. (2009) Ultrafast and memory-efficient alignment of short DNA sequences to the human genome. Genome biology 10, R25.

Lee S., Park S.J., Cheong J.k., Ko J.Y., Bong J. & Baik M. (2017) Identification of circulating miRNA involved in meat yield of Korean cattle. Cell biology international 41, 761-8.

Li C. (2017) The role of beef in human nutrition and health. Dikeman E, editorEnsuring safety and quality in the production of beef 2, 329-38.

Li N., Zhang Y., Li H.-P., Han L., Yan X.-M., Li H.-B., Du W., Zhang J.-S. & Yu Q.-L. (2018) Differential expression of mRNA-miRNAs related to intramuscular fat content in the longissimus dorsi in Xinjiang brown cattle. PloS one 13, e0206757.

Lindholm-Perry A., Butler A., Kern R., Hill R., Kuehn L., Wells J., Oliver W., Hales K., Foote A. & Freetly H. (2016) Differential gene expression in the duodenum, jejunum and ileum among crossbred beef steers with divergent gain and feed intake phenotypes. Animal genetics 47, 408-27.

Lindholm-Perry A., Cunningham H., Kuehn L., Vallet J., Keele J., Foote A., Cammack K. & Freetly H. (2017) Relationships between the genes expressed in the mesenteric adipose tissue of beef cattle and feed intake and gain. Animal genetics 48, 386-94.

Lindholm-Perry A.K., Kern R.J., Keel B.N., Snelling W.M., Kuehn L.A. & Freetly H.C. (2016) Profile of the spleen transcriptome in beef steers with variation in gain and feed intake. Frontiers in genetics 7, 127.

Lkhagvadorj S., Qu L., Cai W., Couture O.P., Barb C.R., Hausman G.J., Nettleton D., Anderson L.L., Dekkers J.C. & Tuggle C.K. (2010) Gene expression profiling of the short-term adaptive response to acute caloric restriction in liver and adipose tissues of pigs differing in feed efficiency. American Journal of Physiology-Regulatory, Integrative and Comparative Physiology 298, R494-R507.

Love M.I., Huber W. & Anders S. (2014) Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. Genome biology 15, 550.

Lozano R., Del Carpio D.P., Kayondo S.I., Amuge T., Adebo O.A., Ferguson M. & Jannink J.-L. (2017) Leveraging Transcriptomics Data for Genomic Prediction Models in Cassava. bioRxiv, 208181.

Lu D., Akanno E., Crowley J., Schenkel F., Li H., De Pauw M., Moore S., Wang Z., Li C. & Stothard P. (2016) Accuracy of genomic predictions for feed efficiency traits of beef cattle using 50K and imputed HD genotypes. Journal of animal science 94, 1342-53.

Lu J. & Clark A.G. (2012) Impact of microRNA regulation on variation in human gene expression. Genome research 22, 1243-54.

MacLeod I., Bowman P., Vander Jagt C., Haile-Mariam M., Kemper K., Chamberlain A., Schrooten C., Hayes B. & Goddard M. (2016) Exploiting biological priors and sequence variants enhances QTL discovery and genomic prediction of complex traits. BMC genomics 17, 144.

Mao F., Chen L., Vinsky M., Okine E., Wang Z., Basarab J., Crews Jr D. & Li C. (2013) Phenotypic and genetic relationships of feed efficiency with growth performance, ultrasound, and carcass merit traits in Angus and Charolais steers. Journal of animal science 91, 2067-76.

Marioni J.C., Mason C.E., Mane S.M., Stephens M. & Gilad Y. (2008) RNA-seq: an assessment of technical reproducibility and comparison with gene expression arrays. Genome research 18, 1509-17.

Markham N.R. & Zuker M. (2008) UNAFold. In: Bioinformatics (pp. 3-31. Springer.

Martin M. (2011) Cutadapt removes adapter sequences from high-throughput sequencing reads. EMBnet. journal 17, 10-2.

Martin T.S. (2017) The Study of microRNA in Plasma to Discover Biomarkers Important for Muscle Growth in Beef Cattle.

Matukumalli L.K., Lawley C.T., Schnabel R.D., Taylor J.F., Allan M.F., Heaton M.P., O'Connell J., Moore S.S., Smith T.P. & Sonstegard T.S. (2009) Development and characterization of a high density SNP genotyping assay for cattle. PloS one 4, e5350.

McDonald P. (2002) Animal nutrition. Pearson education.

Meuwissen T., Hayes B. & Goddard M. (2001) Prediction of total genetic value using genome-wide dense marker maps. Genetics 157, 1819.

Meuwissen T., Hayes B. & Goddard M. (2013) Accelerating improvement of livestock with genomic selection. Annu. Rev. Anim. Biosci. 1, 221-37.

Morimoto A., Kannari M., Tsuchida Y., Sasaki S., Saito C., Matsuta T., Maeda T., Akiyama M., Nakamura T. & Sakaguchi M. (2017) An HNF4α–microRNA-194/192 signaling axis maintains hepatic cell function. Journal of Biological Chemistry 292, 10574-85.

Morris Jr S.M. (2002) Regulation of enzymes of the urea cycle and arginine metabolism. Annual review of nutrition 22, 87-105.

Motameny S., Wolters S., Nürnberg P. & Schumacher B. (2010) Next generation sequencing of miRNAs–strategies, resources and methods. Genes 1, 70-84.

Moutinho C. & Esteller M. (2017) MicroRNAs and epigenetics. In: Advances in cancer research (pp. 189-220. Elsevier.

Mujibi F., Nkrumah J., Durunna O., Grant J., Mah J., Wang Z., Basarab J., Plastow G., Crews D. & Moore S. (2011a) Associations of marker panel scores with feed intake and efficiency traits in beef cattle using preselected single nucleotide polymorphisms. Journal of animal science 89, 3362-71.

Mujibi F., Nkrumah J., Durunna O., Stothard P., Mah J., Wang Z., Basarab J., Plastow G., Crews D. & Moore S. (2011b) Accuracy of genomic breeding values for residual feed intake in crossbred beef cattle. Journal of animal science 89, 3353-61.

Mukiibi R., Johnston D., Vinsky M., Fitzsimmons C., Stothard P., Waters S.M. & Li C. (2019a) Bovine hepatic miRNAome profiling and differential miRNA expression analyses between beef steers with divergent feed efficiency phenotypes.

Mukiibi R., Johnston D., Vinsky M., Fitzsimmons C., Stothard P., Waters S.M. & Li C. (2019b) Differential hepatic micro RNA expression between beef steers with divergent growth rate or feed intake phenotypes.

Mukiibi R., Vinsky M., Keogh K., Fitzsimmons C., Stothard P., Waters S.M. & Li C. (2017) Transcriptome analysis of skeletal muscle tissue in Canadian beef cattle with divergent residual feed intake phenotypes. In: 8th Annual Livestock Gentec Conference, Edmonton, AB, Canada.

Mukiibi R., Vinsky M., Keogh K., Fitzsimmons C., Stothard P., Waters S.M. & Li C. (2019b) Liver transcriptome profiling of beef steers with divergent growth rate, feed intake or metabolic body weight phenotypes. Journal of animal science.

Mukiibi R., Vinsky M., Keogh K., Fitzsimmons C., Waters S.M., Stothard P. & Li C. (2018a) Skeletal muscle transcriptional profiling of Canadian crossbred beef cattle divergent for feed efficiency traits. Journal of animal science 96, 175-6.

Mukiibi R., Vinsky M., Keogh K.A., Fitzsimmons C., Stothard P., Waters S.M. & Li C. (2018) Transcriptome analyses reveal reduced hepatic lipid synthesis and accumulation in more feed efficient beef cattle. Scientific reports 8, 7303.

Murakami Y. & Kawada N. (2017) MicroRNAs in hepatic pathophysiology. Hepatology Research 47, 60-9.

Nafikov R.A. & Beitz D.C. (2007) Carbohydrate and lipid metabolism in farm animals. The Journal of nutrition 137, 702-5.

Nakamoto N. & Kanai T. (2014) Role of toll-like receptors in immune activation and tolerance in the liver. Frontiers in immunology 5, 221.

Nascimento M., Souza A., Chaves A., Cesar A., Tullio R., Medeiros S., Mourão G., Rosa A., Feijó G. & Alencar M. (2016) Feed efficiency indexes and their relationships with carcass, non-carcass and meat quality traits in Nellore steers. Meat science 116, 78-85.

Naylor R., Steinfeld H., Falcon W., Galloway J., Smil V., Bradford E., Alder J. & Mooney H. (2005) Losing the links between livestock and land. Science 310, 1621-2.

Nkrumah J., Basarab J., Wang Z., Li C., Price M., Okine E., Crews D. & Moore S. (2007) Genetic and phenotypic relationships of feed intake and measures of efficiency with growth and carcass merit of beef cattle. Journal of animal science 85, 2711-20.

Nkrumah J., Basarab J., Wang Z., Li C., Price M., Okine E., Crews D. & Moore S. (2007a) Genetic and phenotypic relationships of feed intake and measures of efficiency with growth and carcass merit of beef cattle. Journal of animal science 85, 2711-20.

Nkrumah J., Okine E., Mathison G., Schmid K., Li C., Basarab J., Price M., Wang Z. & Moore S. (2006) Relationships of feedlot feed efficiency, performance, and feeding behavior with metabolic rate, methane production, and energy partitioning in beef cattle. Journal of animal science 84, 145-53.

Nkrumah J., Sherman E., Li C., Marques E., Crews D., Bartusiak R., Murdoch B., Wang Z., Basarab J. & Moore S. (2007) Primary genome scan to identify putative quantitative trait loci for feedlot growth rate, feed intake, and feed efficiency of beef cattle. Journal of animal science 85, 3170-81.

Nkrumah J., Sherman E., Li C., Marques E., Crews D., Bartusiak R., Murdoch B., Wang Z., Basarab J. & Moore S. (2007b) Primary genome scan to identify putative quantitative trait loci for feedlot growth rate, feed intake, and feed efficiency of beef cattle. Journal of animal science 85, 3170-81.

Ntambi J.M. & Miyazaki M. (2004) Regulation of stearoyl-CoA desaturases and role in metabolism. Progress in lipid research 43, 91-104.

O'Brien J., Hayder H., Zayed Y. & Peng C. (2018) Overview of microrna biogenesis, mechanisms of actions, and circulation. Frontiers in endocrinology 9, 402.

O'Kelly J. (1974) The concentrations of lipids in the plasma, liver and bile of genetically different types of cattle. Comparative Biochemistry and Physiology Part B: Comparative Biochemistry 49, 491-500.

Olfert E.D., Cross B.M. & McWilliam A.A. (1993) Guide to the care and use of experimental animals. Canadian Council on Animal Care Ottawa.

Olivieri B.F., Mercadante M.E.Z., Cyrillo J.N.d.S.G., Branco R.H., Bonilha S.F.M., de Albuquerque L.G., de Oliveira Silva R.M. & Baldi F. (2016) Genomic regions associated with feed efficiency indicator traits in an experimental Nellore cattle population. PloS one 11, e0164390.

Paradis F., Yue S., Grant J., Stothard P., Basarab J. & Fitzsimmons C. (2015) Transcriptomic analysis by RNA sequencing reveals that hepatic interferon-induced genes may be associated with feed efficiency in beef heifers. Journal of animal science 93, 3331-41.

Parker G.A. & Picut C.A. (2005) Liver immunobiology. Toxicologic Pathology 33, 52-62.

Pritchard C.C., Cheng H.H. & Tewari M. (2012) MicroRNA profiling: approaches and considerations. Nature Reviews Genetics 13, 358.

Pryce J., Wales W.d., De Haas Y., Veerkamp R. & Hayes B. (2014) Genomic selection for feed efficiency in dairy cattle. Animal 8, 1-10.

Pullen D., Liesman J. & Emery R. (1990) A species comparison of liver slice synthesis and secretion of triacylglycerol from nonesterified fatty acids in media. Journal of animal science 68, 1395-9.

Purcell S., Neale B., Todd-Brown K., Thomas L., Ferreira M.A., Bender D., Maller J., Sklar P., De Bakker P.I. & Daly M.J. (2007) PLINK: a tool set for whole-genome association and population-based linkage analyses. The American Journal of Human Genetics 81, 559-75.

Racanelli V. & Rehermann B. (2006) The liver as an immunological organ. Hepatology 43, S54-S62.

Ramsey R., Doye D., Ward C., McGrann J., Falconer L. & Bevers S. (2005) Factors affecting beef cow-herd costs, production, and profits. Journal of Agricultural and Applied Economics 37, 91-9.

Reynolds C.K. (1992) Metabolism of nitrogenous compounds by ruminant liver. The Journal of nutrition 122, 850-4.

Reynolds J., Foote A., Freetly H., Oliver W. & Lindholm-Perry A. (2017) Relationships between inflammation-and immunity-related transcript abundance in the rumen and jejunum of beef steers with divergent average daily gain. Animal genetics 48, 447-9.

Richardson E. & Herd R. (2004) Biological basis for variation in residual feed intake in beef cattle. 2. Synthesis of results following divergent selection. Animal Production Science 44, 431-40.

Richardson E., Herd R., Archer J. & Arthur P. (2004) Metabolic differences in Angus steers divergently selected for residual feed intake. Australian Journal of Experimental Agriculture 44, 441-52.

Richardson E., Herd R., Oddy V., Thompson J., Archer J. & Arthur P. (2001) Body composition and implications for heat production of Angus steer progeny of parents selected for and against residual feed intake. Australian Journal of Experimental Agriculture 41, 1065-72.

Robinson D. & Oddy V. (2004) Genetic parameters for feed efficiency, fatness, muscle area and feeding behaviour of feedlot finished beef cattle. Livestock production science 90, 255-70.

Robinson M.D. & Oshlack A. (2010) A scaling normalization method for differential expression analysis of RNA-seq data. Genome biology 11, R25.

Robinson M.D., McCarthy D.J. & Smyth G.K. (2010) edgeR: a Bioconductor package for differential expression analysis of digital gene expression data. Bioinformatics 26, 139-40.

Roh S.G., Hishikawa D., Hong Y.H. & Sasaki S. (2006) Control of adipogenesis in ruminants. Animal Science Journal 77, 472-7.

Rolf M., Taylor J., Schnabel R., McKay S., McClure M., Northcutt S., Kerley M. & Weaber R. (2012) Genome-wide association analysis for feed efficiency in Angus cattle. Animal genetics 43, 367-74.

Rui L. (2011) Energy metabolism in the liver. Comprehensive physiology 4, 177-97.

Saatchi M., Beever J.E., Decker J.E., Faulkner D.B., Freetly H.C., Hansen S.L., Yampara-Iquise H., Johnson K.A., Kachman S.D. & Kerley M.S. (2014) QTLs associated with dry matter intake, metabolic mid-test weight, growth and feed efficiency have little overlap across 4 beef cattle studies. BMC genomics 15, 1004.

Sacksteder K.A., Biery B.J., Morrell J.C., Goodman B.K., Geisbrecht B.V., Cox R.P., Gould S.J. & Geraghty M.T. (2000) Identification of the α-aminoadipic semialdehyde synthase gene, which is defective in familial hyperlysinemia. The American Journal of Human Genetics 66, 1736-43.

Saito T. & Sætrom P. (2010) MicroRNAs–targeting and target prediction. New biotechnology 27, 243-9.

Salter A.M. (2017) Improving the sustainability of global meat and milk production. Proceedings of the Nutrition Society 76, 22-7.

Santana M., Freua M., Do D., Ventura R., Kadarmideen H. & Ferraz J. (2016) Systems genetics and genome-wide association approaches for analysis of feed intake, feed efficiency, and performance in beef cattle. Genet. Mol. Res 15, 1-10.

Santana M.H., Utsunomiya Y.T., Neves H.H., Gomes R.C., Garcia J.F., Fukumasu H., Silva S.L., Junior G.A.O., Alexandre P.A. & Leme P.R. (2014) Genome-wide association analysis of feed intake and residual feed intake in Nellore cattle. BMC genetics 15, 21.

Sargolzaei M., Chesnais J. & Schenkel F. (2011) FImpute-An efficient imputation algorithm for dairy cattle populations. J Dairy Sci 94, 421.

Schenkel F., Miller S. & Wilton J. (2004) Genetic parameters and breed differences for feed efficiency, growth, and body composition traits of young beef bulls. Canadian Journal of Animal Science 84, 177-85.

Schenkel F., Miller S., Ye X., Moore S., Nkrumah J., Li C., Yu J., Mandell I., Wilton J. & Williams J. (2005) Association of single nucleotide polymorphisms in the leptin gene with carcass and meat quality traits of beef cattle. Journal of animal science 83, 2009-20.

Seabury C.M., Oldeschulte D.L., Saatchi M., Beever J.E., Decker J.E., Halley Y.A., Bhattarai E.K., Molaei M., Freetly H.C. & Hansen S.L. (2017) Genome-wide association study for feed efficiency and growth traits in US beef cattle. BMC genomics 18, 386.

Seal C. & Reynolds C. (1993) Nutritional implications of gastrointestinal and liver metabolism in ruminants. Nutrition Research Reviews 6, 185-208.

Shalev B. & Pasternak H. (1989) Meat production efficiencies of turkey, chicken and duck broilers. World's Poultry Science Journal 45, 109-14.

Shannon P., Markiel A., Ozier O., Baliga N.S., Wang J.T., Ramage D., Amin N., Schwikowski B. & Ideker T. (2003) Cytoscape: a software environment for integrated models of biomolecular interaction networks. Genome research 13, 2498-504.

Sharpe L.J. & Brown A.J. (2013) Controlling cholesterol synthesis beyond 3-hydroxy-3-methylglutaryl-CoA reductase (HMGCR). Journal of Biological Chemistry, jbc. R113. 479808.

Sherman E., Nkrumah J., Li C., Bartusiak R., Murdoch B. & Moore S. (2009) Fine mapping quantitative trait loci for feed intake and feed efficiency in beef cattle. Journal of animal science 87, 37-45.

Sherman E., Nkrumah J., Murdoch B., Li C., Wang Z., Fu A. & Moore S. (2008) Polymorphisms and haplotypes in the bovine neuropeptide Y, growth hormone receptor, ghrelin, insulin-like growth factor 2, and uncoupling proteins 2 and 3 genes and their associations with measures of growth, performance, feed efficiency, and carcass merit in beef cattle. Journal of animal science 86, 1-16.

Smith S.B., Gotoh T. & Greenwood P.L. (2018) Current situation and future prospects for global beef production: overview of special issue. Asian-Australasian journal of animal sciences 31, 927.

Snelling W., Cushman R., Keele J., Maltecca C., Thomas M., Fortes M. & Reverter A. (2013) BREEDING AND GENETICS SYMPOSIUM: Networks and pathways to guide genomic selection–. Journal of animal science 91, 537-52.

Song G., Sharma A.D., Roll G.R., Ng R., Lee A.Y., Blelloch R.H., Frandsen N.M. & Willenbring H. (2010) MicroRNAs control hepatocyte proliferation during liver regeneration. Hepatology 51, 1735-43.

Spindel J., Begum H., Akdemir D., Collard B., Redoña E., Jannink J. & McCouch S. (2016) Genome-wide prediction models that incorporate de novo GWAS are a powerful new tool for tropical rice improvement. Heredity 116, 395.

Stetson D.B. & Medzhitov R. (2006) Type I interferons in host defense. Immunity 25, 373-81.

Stock K. & Reents R. (2013) Genomic selection: status in different species and challenges for breeding. Reproduction in Domestic Animals 48, 2-10.

Stroynowska-Czerwinska A., Fiszer A. & Krzyzosiak W.J. (2014) The panorama of miRNA-mediated mechanisms in mammalian cells. Cellular and Molecular Life Sciences 71, 2253-70.

Sun H.-Z., Chen Y. & Guan L., Luo (2019) MicroRNA expression profiles across blood and different tissues in cattle. Scientific data 6.

Takagi S., Nakajima M., Mohri T. & Yokoi T. (2008) Post-transcriptional regulation of human pregnane X receptor by micro-RNA affects the expression of cytochrome P450 3A4. Journal of Biological Chemistry 283, 9674-80.

Tao Y., Wang M., Chen E. & Tang H. (2017) Liver regeneration: analysis of the main relevant signaling molecules. Mediators of inflammation 2017.

Tizioto P.C., Coutinho L.L., Decker J.E., Schnabel R.D., Rosa K.O., Oliveira P.S., Souza M.M., Mourão G.B., Tullio R.R. & Chaves A.S. (2015) Global liver gene expression differences in Nelore steers with divergent residual feed intake phenotypes. BMC genomics 16, 242.

Tizioto P.C., Coutinho L.L., Oliveira P.S., Cesar A.S., Diniz W.J., Lima A.O., Rocha M.I., Decker J.E., Schnabel R.D. & Mourão G.B. (2016) Gene expression differences in Longissimus muscle of Nelore steers genetically divergent for residual feed intake. Scientific reports 6.

Tomasini R., Samir A.A., Carrier A., Isnardon D., Cecchinelli B., Soddu S., Malissen B., Dagorn J.-C., Iovanna J.L. & Dusetti N.J. (2003) TP53INP1s and homeodomain-interacting protein kinase-2 (HIPK2) are partners in regulating p53 activity. Journal of Biological Chemistry 278, 37722-9.

Trejo C.O. (2010) Residual feed intake, breed of sire and dam, and individual sire affect performance and carcass characteristics and rates of back fat and intramuscular fat of feedlot steers. University of Illinois at Urbana-Champaign.

USDA (2017) Top U.S. Agricultural Exports in 2017. URL https://www.fas.usda.gov/data/top-us-agricultural-exports-2017.

Van Den Berge K., Hembach K.M., Soneson C., Tiberi S., Clement L., Love M.I., Patro R. & Robinson M.D. (2018) RNA sequencing data: hitchhiker's guide to expression analysis. Annual Review of Biomedical Data Science 2.

Van den Berghe G. (1991) The role of the liver in metabolic homeostasis: implications for inborn errors of metabolism. In: Journal of inherited metabolic disease (pp. 407-20. Springer.

Van Heugten E. (2010) Growing-finishing swine nutrient recommendations and feeding management. National Swine Nutrition Guide (ed. DJ, Meisinger), 80-95.

Wang Y., Zhang F., Mukiibi R., Chen L., Vinsky M., Plastow G., Basarab J., Stothard P. & Li C. (2019) Genetic architecture of quantitative traits in beef cattle revealed by a genome wide association study of imputed whole genome sequence variants: II: carcass merit traits. BMC genomics.

Weber K.L., Welly B.T., Van Eenennaam A.L., Young A.E., Porto-Neto L.R., Reverter A. & Rincon G. (2016) Identification of gene networks for residual feed intake in angus cattle using genomic prediction and RNA-seq. PloS one 11, e0152274.

Yang J., Lee S.H., Goddard M.E. & Visscher P.M. (2011) GCTA: a tool for genome-wide complex trait analysis. The American Journal of Human Genetics 88, 76-82.

Yi P.-S., Zhang M. & Xu M.-Q. (2016) Role of microRNA in liver regeneration. Hepatobiliary & Pancreatic Diseases International 15, 141-6.

Zarek C., Lindholm-Perry A., Kuehn L. & Freetly H. (2017) Differential expression of genes related to gain and intake in the liver of beef cattle. BMC research notes 10, 1.

Zhang F., Ekine-Dzivenu C., Vinsky M., Basarab J., Aalhus J., Dugan M. & Li C. (2017) Phenotypic and genetic relationships of residual feed intake measures and their component traits with fatty acid composition in subcutaneous adipose of beef cattle. Journal of animal science.

Zhang F., Wang Y., Mukiibi R., Chen L., Vinsky M., Plastow G., Basarab J., Stothard P. & Li C. (2019a) Genetic architecture of quantitative traits in beef cattle revealed by a genome wide association study of imputed whole genome sequence variants: I: feed efficiency and component traits. BMC genomics.

Zhang H., Yin L., Wang M., Yuan X. & Liu X. (2019) Factors affecting the accuracy of genomic selection for agricultural economic traits in maize, cattle, and pig populations. Frontiers in genetics 10, 189.

Zhao Q., Kang Y., Wang H.-Y., Guan W.-J., Li X.-C., Jiang L., He X.-H., Pu Y.-B., Han J.-L. & Ma Y.-H. (2016) Expression profiling and functional characterization of miR-192 throughout sheep skeletal muscle development. Scientific reports 6, 30281.

Zhou J., Ju W., Wang D., Wu L., Zhu X., Guo Z. & He X. (2012) Down-regulation of microRNA-26a promotes mouse hepatocyte proliferation during liver regeneration. PloS one 7, e33577.