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FACTORS AFFECTING THE FATTY ACID PROFILE OF BEEF: SUMMARY AND PROSPECTS FOR COMPLEX TRAIT ANALYSIS

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Introduction

Beef is a nutritious product that provides an excellent source of protein, vitamins, minerals, and lipids in the human diet. The lipid profile of beef contributes to the overall healthfulness and palatability of the final beef product, which indicates it is an economic trait of interest for consumers. Previous studies have characterized the lipid profile in various beef cattle tissues under different dietary conditions, in different breeds, and at various age points. This published collection of fatty acid phenotype data indicates that lipid storage in beef cattle is a dynamic process with individual lipids exhibiting a wide range of phenotypic and genetic variance estimates under different environmental conditions and across many breeds. This range in observed variance can be partially explained by genetic differences among animals for lipid synthesis, desaturation, and deposition, as well as by the environmental interaction of specific lipids and lipid classes in the biological environment of muscle and fat tissues.

The two major lipid depots in beef cattle tissue are represented by the triacylglycerol and phospholipid fractions of the total lipid isolated from both muscle and adipose tissue. The triacylglycerol lipid fraction captures the lipids stored as triglycerides in adipose cells. The phospholipid fraction captures lipids contained in the more diverse phospholipid cellular membrane of both myocytes and adipocytes. The triacylglycerol and phospholipid fractions exhibit the characteristics of quantitative traits. These traits are controlled by many individual genes with many correlated individual lipids composing larger lipid classes such as saturated (SFA), monounsaturated (MUFA), and polyunsaturated (PUFA) fatty acids. There is also a significant environmental component that influences the fatty acid profile. Currently, there is an effort to combine high throughput “omics” data with data from multiple complex phenotypes and systems biology methodology in order to identify the biological drivers behind complex traits of interest. Given these factors, the triacylglycerol and phospholipid fatty acid fractions present an excellent opportunity for an analysis of genetic parameters, the identification of candidate genes, and the application of modern systems biology to analyze overall lipid profile in beef cattle tissues.

The Fatty Acid Profile of Beef Tissues

A wide range of studies have characterized the total fatty acid profile of various beef cattle tissues (Wood et al., 2008; Daley et al., 2010; Hoehne et al., 2012; Pavan and Duckett,

2013; Duckett et al., 2014). The total fatty acid fraction represents the distribution of all lipids present in a biological sample including those derived from the lipid membranes of multiple cell types other than adipocytes. Depending on factors such as diet, age, and tissue, the total fatty acid profile of beef is generally composed of approximately 40-50% of SFA, 40-60% of MUFA, and 5-15% of PUFA (Wood et al., 2008). Two important factors affecting fatty acid profile are maturity of the animal and location of the tissue sampled. Muscle type and adipose location have a significant effect on the fatty acid profile (Pavan and Duckett, 2013; Liu et al., 2015b). Also, as an animal matures and a larger proportion of excess energy is used for fatty acid synthesis there tends to be an increase in the accumulation of SFA in relation to PUFA (Warren et al., 2008). This is likely due to a shift towards lipogenesis and a shift away from adipogenesis as an animal reaches maturity and subsequent the need for more adipocytes is reduced. During lipogenesis the primary fatty acids being produced are saturated in nature. The primary product from the major protein complex driving lipogenesis, which is fatty acid synthase (FASN), is C16:0, which explains this shift in the SFA:PUFA ratio. It is known that this shift occurs as fatty acids are stored as triglycerides in adipocytes, but it is not clear how this shift affects the phospholipid membrane in a maturing animal. This membrane is a dynamic lipid depot and seems to undergo changes in fluidity and composition as adipogenesis proceeds in a maturing tissue (Pietilainen et al., 2011). More research is needed to understand the differences between these two lipid depots and how they change under various conditions and maturity points.

A method developed by Hartman (1967) allowed the separation of polar and non-polar lipids prior to gas chromatography that yields the neutral lipid and phospholipid fatty acid fractions in separate components. The non-polar neutral lipid fraction contains the triacylglycerol, diacylglycerol, ester, and cholesterol components of the tissue. The polar fraction contains the phospholipid bilayer fatty acids which are composed of four major phospholipids in mammals: phosphatidylethanolamine, phosphatidylserine, phosphatidylcholine, and sphingomyelin (Alberts, 2002). Each of these four major phospholipids contain two nonpolar fatty acid chains which compose the individual fatty acids identified as being associated with the phospholipid fatty acid fraction through gas chromatography analysis. Separating the total fatty acid fraction allows for a more detailed analysis of the genes and biological pathways affecting adipogenesis and lipid synthesis when compared to the total fatty acid fraction. When the two fractions are combined it cannot be determined if the fatty acids in the analysis come from the

triacylglycerol or the phospholipid which likely have distinct biological origins and configurations in various cell types.

To date, there have been few studies that have presented a comprehensive separate analysis of the triacylglycerol and phospholipid fatty acid fractions in beef cattle species. Kazala et al. (1999) presented an analysis of the intramuscular fatty acid composition in crossbred Wagyu cattle in which the triacylglycerol fraction was separated from and compared to the total lipid fraction. In this study the triacylglycerol fraction was found to be very similar in composition to the total lipid with no significant difference detected in the MUFA/SFA ratio in *longissimus*. Dannenberger et al. (2007) presented a comprehensive analysis of the fatty acids distributed in multiple phospholipid classes in beef muscle including the phosphatidylethanolamines, phosphatidylcholines, phosphatidylinositols, cardiolipins, sphingomyolins, and lysophosphatidylethanolamines using high performance thin layer chromatography. Analysis of these individual phospholipid classes revealed that pasture feeding to finishing leads to a significant accumulation of omega-3 fatty acids in all classes when compared to finishing on a concentrate diet. The phospholipid membrane is a dynamic lipid depot and is known to undergo changes in fluidity and composition as adipogenesis proceeds in a maturing tissue (Pietilainen et al., 2011). Smith et al. (1998) presented a comprehensive analysis of distribution and saturation of triacylglycerol species in beef cattle in response to different dietary formulas. This study concluded that diet had significant effects on the distribution of saturation and the composition of triacylglycerol species. The previous studies added important data sets for fatty acid analysis, but a future research on the triacylglycerol and phospholipid fractions is needed.

Margetak et al. (2012) presented a complete comparison of the triacylglycerol and phospholipid fatty acid fractions form the *pars costalis* diaphragmatic muscle and subcutaneous fat in beef cattle undergoing different dietary supplementations containing sunflower and flax oils. This study found the triacylglycerol fraction of muscle tissue to contain significantly higher amounts of C14:0 and C16:0. The phospholipid fraction of muscle tissue contained higher amounts of C18:0, C16:1c9, and C18:1c9. The phospholipid fraction of subcutaneous fat was found to contain higher amounts of C14:0, C16:0, C18:0, C16:1c9, and C18:1c9. The study also concluded that oil supplementation increased the absolute amounts of elongated unsaturated fatty acids in both the triacylglycerol and phospholipid fractions. There were no other studies

identified that presented a direct comparison of the triacylglycerol and phospholipid fractions in beef. Future research is needed to determine the effects of breed, maturity, muscle type, and lipid depot on the triacylglycerol and phospholipid fatty acid profiles.

Genetic Parameter Estimates of Fatty Acid Traits

Genetic parameter estimates for individual lipids and lipid classes in beef cattle tissues are available for the total fatty acid fraction. However, there are currently no studies available analyzing genetic parameters in triacylglycerol and phospholipid fractions. Heritability estimates for individual lipids and lipid classes range from 0 to a moderate heritability of approximately 0.6. These heritability estimates indicate the certain lipids would respond well to selection programs. Ekine-Dzivenu et al. (2014) estimated genetic parameters for fatty acids traits in 223 Angus and Charolais crossbred commercial steers. In this study heritability estimates for individual lipids ranged from approximately 0 to 0.51. Heritability estimates for most fatty acids were low, with SFA, MUFA, and PUFA fatty acid classes having a heritabilities less than 0.15. The highest heritability estimates were found for C14:1 and C18:1 with estimates of 0.51 and 0.43, respectively. The relatively low population size likely led to an underestimation of heritability estimates for fatty acid traits in this study.

Inoue et al. (2011) also estimated heritabilities for fatty acid traits in a population of 863 Japanese Black steers. Heritability estimates in this study ranged from approximately 0 to 0.86. Traits with the highest heritability estimates included C14:1 and C14:0 with heritability estimates of 0.86 and 0.82, respectively. Total MUFA also exhibited a high heritability estimate of 0.66. The authors of this study note that the heritability estimates obtained seem high when compared to other breeds and other studies. The authors also note that this difference might be present due to differences in fatty acid synthesis and desaturation enzyme activity in Japanese Black cattle compared to other breeds. Nogi et al. (2011) presented the results of a similar study in a population of 2,275 Japanese Black cattle. Heritability estimates in this study were similar and raged from approximately 0 to 0.78. The highest heritability estimates were obtained for C14:0 and C18:1 with heritabilities of 0.70 and 0.78, respectively. The lipid classes SFA, MUFA, and PUFA had heritability estimates of 0.66, 0.68, and 0.47, respectively. These studies represent the best examples currently present in the literature for fatty acid heritability estimates in a single

breed of cattle due to the large population size, complete reporting of individual lipid heritability estimates, and uniform genetic background of the animals the study.

Pitchford et al. (2002) obtained heritability estimates for fatty acid traits in a population of 1,215 animals with 7 distinct sire breeds. This study also used the percentage of the total lipid as the measurement. Heritability estimates in this population range from approximately 0 to a moderate heritability of 0.27. Fatty classes SFA and MUFA had heritabilities of 0.27 and 0.17, respectively. The fatty acid C16:0 had one of the highest heritability estimates of all individual lipids at 0.21. In contrast to the study by Inoue et al. (2011), this study found relatively lower heritability estimates across all individual lipids and lipid classes. It is possible that using cattle from a variety of genetic backgrounds as opposed to a single breed results in lower heritability estimates.

Ahlberg et al. (2014) obtained posterior mean genomic heritability estimates for various fatty acid classes as a proportion of phenotypic variation explained by a genomic marker panel in a population of 236 crossbred steers and heifers. Heritability estimates for fatty acid classes PUFA and MUFA were 0.7 and 0.4, respectively, when measured on a percentage of total lipid basis. Heritability estimates of PUFA and MUFA were 0.7 and 0.85, respectively, when measured on the basis of mg/100 g of wet tissue.

Heritability estimates for fatty acid traits appear to be variable across the studies estimating these parameters in beef cattle. Genetic background of the animals in the study as well as the measurement system used to determine the fatty acid measurement seem to be the two factors causing the most variation in heritability estimates. In general, studies using a single breed with the percentage of total lipid measurement system yield the highest estimates of heritability for fatty acid traits. The classes SFA and MUFA as well as the individual lipids making up those classes appear to have moderate to high heritabilities. The more unsaturated lipids composing the PUFA class appear to have low to moderate heritabilities. The data gathered in these studies indicates that overall the fatty acid profile has a moderate heritability and certain fatty acids would respond to a marker assisted selection program.

Genetic Correlations Involving Fatty Acid Traits

Genetic correlation estimates have also been well characterized among individual lipids, lipid classes, and carcass traits for the total fatty acid fraction. Fatty acid synthesis and

desaturation occurs through a pathway of related enzyme complexes to produce the many lipids and lipid classes found in mammalian tissues. The central driver of lipid synthesis in mammalian tissues is a large protein complex known as fatty acid synthase (FASN) (Alberts, 2002). The primary products of FASN synthesis are C14:0 and C16:0, which are derived by the addition of 2 carbon acetyl CoA to a growing carbon chain until the final product reaches either 14 or 16 carbons in length. Many other enzymes in addition to FASN work to lengthen individual lipids and add features such as desaturations and isomerizations after the final C14:0 or C16:0 are produced. These include the desaturase class of enzymes, such as steroyl CoA desaturase, and elongation enzymes. It is reasonable to conclude that certain fatty acids would exhibit moderate to high genetic correlations since genetic variation in these biological pathways and networks would affect all lipid products in the assembly line. It also follows that individual lipids and lipid classes should be genetically correlated to carcass traits since the fatty acid profile is known to vary at different levels of tissue maturity (Warren et al., 2008).

Both direction and strength of phenotypic and genetic correlations among fatty acids appear to be highly dependent upon the measurement system used (percent of total lipid vs. mg/100 g tissue). Using percent of total lipid calculation appears to give higher heritability estimates for the majority of lipids and lipid classes (Saatchi et al., 2013; Ahlberg et al., 2014). Phenotypic correlations between SFA and the unsaturated lipid classes MUFA and PUFA are generally negative. SFA is the primary product of *de novo* lipid synthesis, and the newly synthesized saturated fatty acids are then used as precursors for unsaturated fatty acid products derived from that synthesis. Maturity of the animal is also known to drive this association, as fatter animals typically have higher amounts of SFA compared to unsaturated fatty acids (Warren et al., 2008). Multiple studies have identified this phenotypic association (Pitchford et al., 2002; Inoue et al., 2011; Ekine-Dzivenu et al., 2014).

Genetic correlations among fatty acids tend to be less predictable across multiple studies, but the general trend of SFA exhibiting a negative genetic correlation with other fatty acids seems to be a common association. This is likely a reflection of the general pathway of lipid elongation and desaturation that occurs as lipid synthesis and incorporation into various depots proceeds in the adipocyte. Ekine-Dzivenu et al. (2014) found SFA have a negative genetic correlation with MUFA and PUFA, with genetic correlation estimates of -0.99 and -0.41, respectively. MUFA and PUFA were found to have a weak but positive genetic correlation of

0.2. Similarly, Pitchford et al. (2002) found a negative genetic correlation between SFA and other unsaturated fatty acids. The fatty acid C14:0 had negative genetic correlations of -0.61 and -0.27 with MUFA and UFA, respectively. Inoue et al. (2011) also observed the C14:0 to have a negative genetic correlation with MUFA and UFA of -0.74 and -0.81, respectively.

Other individual lipids also exhibit predictable genetic correlations. In general, individual SFA's of different lengths tend to be positively correlated (Inoue et al., 2011; Nogi et al., 2011). A strong negative genetic correlation is also consistently observed between C18:0 and C18:1 (Inoue et al., 2011; Nogi et al., 2011), which is likely a reflection of stearoyl Co-A desaturase (SCD) variation in the catalysis of C18:0 desaturation into C18:1 (Smith et al., 2006). Individual lipids and lipid classes exhibit a wide range of genetic correlation estimations. Some individual lipids exhibit a genetic correlation of almost 1.0 or -1.0 which is likely due to the pathway being highly dependent on the products from each previous step in the synthesis, elongation, or desaturation of lipid products, as well as a likely over-estimation of the parameter.

Genomic Regions of Interest affecting Fatty Acid Profile

Multiple studies have carried out genome-wide association studies in various breeds of cattle for the total fatty acid fraction in order to identify genomic regions, markers, and genes of interest. One of the most important genes involved in *de novo* synthesis of fatty acids is FASN. This protein is a complex of multiple subunits which are transcribed from a region on chromosome 19 starting at approximately 51,384,900 base pairs (bp). Multiple studies have identified this region as having a high association with saturated fatty acids including C14:0, C16:0, and total SFA (Matsuhashi et al., 2011; Uemoto et al., 2011; Ishii et al., 2013; Saatchi et al., 2013; Hayakawa et al., 2015). There have also been multiple detailed studies of this region in relation to the fatty acid profile and there appear to be many different SNP's in the region affecting synthesis of SFA (Li et al., 2012; Oh et al., 2012; Lee et al., 2014). Saatchi et al. (2013) also estimated that markers in this region explain up to 25% of the genetic variance in saturated fatty acids with the highest genetic variance explained in *cis*-9 C18:1. This data suggests there are likely multiple causative mutations in the FASN gene that affect the fatty acid profile in multiple species of cattle (Casas et al., 2001; Casas et al., 2003; McClure et al., 2010).

However, not every species seems to have this association between SFA the FASN loci. Cesar et al. (2014) identified 8 genomic regions explaining approximately 1% of the genetic

variance in SFA's, including C14:0, C16:0, and C18:0. None of these 8 regions were near or overlapped the FASN loci, but they did overlap with previously identified loci affecting marbling score, backfat thickness, and carcass and body weight in Angus cattle. These associations are likely detecting the effect of loci causing variation in carcass fatness, which directly has an effect on percentage of SFA. At different levels of carcass fatness the ratio of SFA to unsaturated fatty acids changes (Warren et al., 2008). This effect can be partially explained by the morphology of adipose cells at different maturity points. In younger animals it can be expected that adipose cells are in a stage of multiplication under conditions of excess energy intake, at which point the ratio of the lipids in the phospholipid membrane to the lipids stored as triacylglycerol is high (Graugnard et al., 2010). As the adipose tissue ages a higher proportion of the lipids synthesized and incorporated are stored in the triacylglycerol as triglycerides which are generally more saturated in nature than the phospholipid membrane (Smith et al., 1998). Given this shift in lipid storage as a tissue ages, it can be expected that loci affecting carcass fatness and adipose cell morphology would also affect the proportion of SFA.

Saatchi et al. (2013) also identified a region on chromosome 29 starting at about the 18th Mb harboring the candidate gene thyroid responsive hormone (THRSP or SPOT14) to explain the second highest amount of genetic variance in C14:0, C16:0, C16:1, cis-9 C18:1, long chain fatty acids (LCFA), and medium chain fatty acids (MCFA). This gene is known to be involved in SFA and LCFA synthesis through transcriptional activity and possibly by acting as a cofactor to FASN (Cunningham et al., 1998; LaFave et al., 2006). Other studies have also identified an association between fatty acid traits and variation and expression of THRSP (Hudson et al., 2014; Oh et al., 2014). Variation in FASN and THRSP appear to be associated with high genetic variance in SFA and fatty acid synthesis in beef cattle tissues.

Genomic regions associated with percentage of MUFA have also been well characterized. A region on chromosome 26 starting at approximately 21,132,700 bp harbors the SCD gene which is known to be involved in lipid desaturation in mammalian tissues (Marchitelli et al., 2013; Estany et al., 2014). Multiple studies have identified this genomic region as having a significant effect on C14:1. C16:1, C18:1 and other elongated MUFA species through GWAS (Ishii et al., 2013; Saatchi et al., 2013; Cesar et al., 2014). Additional regions have been associated with MUFA containing candidate genes for fatty acid related traits. Cesar et al. (2014) identified a region on chromosome 2 in Nellore cattle near two candidate genes, glutamate

decarboxylase 1 (GAD1) and specificity protein 5-transcription factor (Sp5), which are both involved in general energy metabolism, adipogenesis, and lipogenesis pathways. These studies support the hypothesis that SCD is the main candidate gene responsible for variation in MUFA species in beef cattle tissues.

Results from estimates of genetic parameters and GWAS from PUFA in beef tissues have proven to be the most difficult to obtain among the three major lipid saturation classes due to the low variation observed in these traits. Heritability estimates for the PUFA lipids are the lowest for all lipid species (Inoue et al., 2011; Saatchi et al., 2013; Ekine-Dzivenu et al., 2014). This relatively low variance observed for PUFA species is likely a reflection of the biological importance of these lipids in the cell. Since the majority of these elongated and unsaturated lipids are found in the cell membrane it can be reasoned that variance in this lipid depot would be detrimental to the fluidity and function of the phospholipid bilayer. The low genetic variance estimates for these phenotypes also allow for the discovery of fewer candidate genes explaining genetic variance using a GWAS methodology. This does not indicate that there are fewer genes involved in the synthesis or incorporation of these lipids into adipose tissue, but that it is more difficult to identify them using these methods. Another source of difficulty in identifying candidate genes involved with the PUFA species is that a number of them are not synthesized in-vivo, but are instead incorporated from dietary sources. Also, the majority of the PUFA synthesis that does occur in mammalian tissues occurs in the liver rather than in adipose. The main pathway leading to PUFA synthesis relies on the conversion of linoleic and alpha linoleic acids to arachidonic (ARA), eicosapentaenoic (EPA), and docosahexaenoic (DHA) through the activity of fatty acid elongases (ELOVLs) and fatty acid desaturases (FADS) (Jump, 2011).

Studies reporting GWAS results for PUFA species in beef cattle have not found an association with the FAD or ELOVL loci, but rather genomic regions harboring or near candidate genes involved in membrane function, membrane adhesion, adipogenesis, or cell signaling. Cesar et al. (2014 identified 9 genomic regions explaining at least 1% of the genetic variance in multiple PUFA species. Candidate genes in the regions included aquaporin 7 (AQP7), lysil oxidase-like 2 (LOXL2), and RAR-related orphan receptor. These candidate genes are involved in cellular component functions such as the PPAR signaling pathway, lean body mass determination in mice, and cellular receptor pathways but no previous association with bovine adipose tissue has been reported. Saatchi et al. (2013) reported associations between

omega-3 and omega-6 fatty acids and regions on chromosomes 23, 14, 26, and 11, but no candidate genes were reported near these regions that had an association to lipid metabolism. There were also no regions of the genome in this study with a posterior probability of inclusion (PPI) greater than 0.9 for total PUFA or any other individual PUFA lipids. Identification of genomic loci affecting PUFA species needs further investigation. It appears that the low phenotypic and genetic variance estimates for this trait hinder the discovery of candidate genes affecting variation.

Other candidate genes affecting fatty acid profile in livestock species have been described in the literature, but have not shown up in GWAS studies in these traits. Graugnard et al. (2010) described the PPAR gamma signaling axis as a major driver of adipogenesis in response to energy abundance under different dietary conditions. Other lipid metabolism genes implicated in through differential gene expression in this study included adiponectin (ADIPOQ), fatty acid binding protein 4 (FABP4), diglycerol acyltranferase 2 (DGAT2), and sterol regulatory element-binding transcription factor 1 (SREBF1). The role of ADIPOQ has been studied as a regulator of lipid synthesis in milk fatty acid synthesis (Singh et al., 2014; Locher et al., 2015), and it would follow that this hormone would likely have a similar role in signaling lipogenesis in adipose. The binding protein FABP4 has also exhibited differential expression in the muscle of cattle fed differing levels of soybean oil or rumen protected fat (Oliveira et al., 2014). There have been a wide variety of genes described in the literature affecting fatty acid traits in beef cattle and other species. Taken together, this set of candidate genes likely contains a large number of causal mutations contributing variation to fatty acid traits in beef cattle.

Gene Network Theory

Complex trait analysis in livestock species has been assisted by recent advances in the generation of genomic and general “omics” related data sets. However, this generation of extremely large datasets has created a need for more complex analysis systems to detect biological phenomena and relate genotype to phenotype. Some of the primary goals in complex trait analysis using these large datasets are to identify causal genes and causal mutations, interactions among these genes and genomic regions, and to assemble these genes and interactions into networks or pathways in a meaningful way that relates to the underlying biology (Feltus, 2014). Such tasks have been the central goal of disciplines such as systems biology or

systems genetics. These systems biology approaches have been developed to identify a variety of genome features such as copy number variation (Jiang et al., 2015), diagnostic features in the cancer genome (Liu et al., 2015a), and causal mutations underlying traits of interest (Hudson et al., 2009; Chen et al., 2014). Causal mutations are of particular importance to the livestock genomics industry due to the development of selection strategies based on genomic data, which can increase in accuracy when causal mutations are included in the prediction (Druet et al., 2014).

One important development in this area has been the incorporation of GWAS data into the generation of regulatory networks underlying multiple related traits of interest. A method developed by Reverter and Fortes (2013) has utilized the inclusion of SNP identified in GWAS to build gene networks highlighting genes of functional relevance to significant biological pathways, rather than just a single phenotype. The method relies on the generation of SNP networks derived from an association weight network tested for interactions by using an algorithm known as partial correlation and information theory (PCIT) (Reverter and Chan, 2008). The major principal behind this method relies on the assumption that SNP having a high impact on multiple related phenotypes are likely of high importance or contain causal mutations.

The first step in the generation of the association weight matrix is to create a matrix of SNP effects for all phenotypes used in the model. A threshold needs to be chosen that incorporates a number of SNP that have a sufficiently high effect on the phenotype of highest importance. Once the initial set of SNP are chosen, the rest of the phenotypes are populated with the same chosen SNP with effects from each respective GWAS. For Bayesian GWAS methods an appropriate threshold might include a posterior probability of association (PPA) of 0.50 to 0.95, depending on the number of SNP that fall within this range. One benefit of the method is that it can be customized to multiple types of omics data that has been generated in association with multiple related phenotypes of interest. Other methods have successfully been used to generate an association weight matrix from data such as the transcriptome (Lehnert et al., 2006; Fortes et al., 2010; Fortes et al., 2012).

The next step is to identify correlations between all SNP or data points in the association weight matrix. The PCIT algorithm was developed specifically to handle this task of identifying associations or correlations among all data points in a large matrix. The matrix consists of columns that correspond to phenotypes in the analysis, and rows that correspond to the SNP

selected from GWAS results with the highest association to the most important phenotype in the analysis. The algorithm first estimates correlations between every pair of SNP in the dataset across all of the phenotypes. Next, the algorithm identifies a partial correlation between each SNP pair and every other SNP, if such a correlation exists. SNP pairs with a partial correlation of 0 to any other SNP are considered isolated and only associated with one another, and subsequently removed from the final output. The algorithm was optimized for use as an R package (Watson-Haigh et al., 2010) and also optimized to run in parallel for high performance computing applications (Koesterke et al., 2013). The final output of the algorithm is a set of SNP pairs and their associated direct correlations which can be utilized in the final visualization of SNP networks.

Network scoring, annotation, and visualization are the final steps in the association weight matrix approach spanning multiple phenotypes. The are many software packages that can handle gene network visualization, but the Cytoscape software package (Shannon et al., 2003) is particularly useful for its ability to score highly interconnected network clusters. A plugin for the Cytoscape software called MCODE (Bader and Hogue, 2003) was developed to score highly interconnected clusters of genes. The clusters are identified by an analysis of cluster density, which is the product of the number of connections in the network and the number of SNP. Clusters with the highest network density are ranked highest in the scoring criteria. These highly interconnected clusters represent the candidate genes or SNP that have the highest impact on the overall phenotype of interest since they contain associated genes or SNP that affect all phenotypes in the model. Annotation of the final networks is necessary to determine if the captured SNP fall in or near genes of functional significance to the overall phenotype in the analysis.

Multiple studies have utilized the association weight matrix approach to analyze quantitative traits in livestock species. A study by Fortes et al. (2012) utilized transcriptome data to build an association weight matrix to analyze first service conception rates in Brangus heifers. Transcriptome data from 10 related growth and fertility traits were used in the construction of the association weight matrix. This approach identified 5 highly interconnected transcription factors hypothesized to be related to overall fertility as well as markers in multiple genes that have been previously associated with fertility traits in beef cattle.

A study by Ramayo-Caldas et al. (2014b) utilized a SNP effect based association weight matrix to analyze intramuscular fat deposition in approximately 10,000 beef cattle from 3 breeds. The study looked at 29 different traits including intramuscular fat, related fat phenotypes, feedlot performance, and various meat quality traits to identify the markers with the highest impact on fat deposition. The resulting networks produced three transcription factors as key regulators of fat deposition and carcass traits: PPARGC1A, HNF4G, and FOXP3. Multiple other markers were identified within genes of biological importance to the pathways regulating these traits of interest as well. Importantly, it was noted that the transcription factors and major genes of interest were not identified in the GWAS as markers with the highest effect associated with any one individual phenotype. The combination of multiple phenotypes with a high throughput genomic data source incorporated into the association weight matrix allowed these markers to be highlighted.

Another study by Ramayo-Caldas et al. (2014a) used SNP effects to create an association weight matrix for intramuscular fatty acid composition in porcine. This study looked at 15 fatty acid phenotypes to identify key regulators of intramuscular fatty acid metabolism. The final network analysis identified the transcription factors NCOA2, FHL2, and EP300 as central regulators of fatty acid metabolism along with many other individual genes of functional significance. This study was unique in that the authors went on to validate the identified transcription factors as having differential expression at the transcriptomic level using real-time PCR. They found expression differences for extreme fatty acid phenotypes in two breeds in liver tissue for 55 genes involved in their association network, including the three identified transcription factors. Also, approximately 60% of the connections identified in the network analysis were validated at the transcriptomic level. Creating the association weight matrix with multiple types of omics data or validating the networks through expression analysis appears to be a robust method for identifying genes of interest in pathways affecting multiple phenotypes of interest. This method appears to be a promising tool for the dissection of complex traits represented by multiple phenotypes, and the flexibility of this method would allow its application across multiple phenotypes of economic importance for beef cattle.

Conclusion and Implication for Genetic Improvement of Beef Cattle

The fatty acid profile of beef is a complex phenotype that would benefit from a systems biology approach to identify the genes of highest impact regulating overall lipid metabolism. The fatty acid profile is associated with economic traits of interest such as intramuscular fat and healthfulness of the final beef product. Previous research across multiple beef cattle breeds, environmental conditions, and muscle tissues has determined that individual lipids and lipids classes exhibit a wide range of heritability estimates. Lipids of higher abundance such as medium chain SFA and MUFA exhibit a moderate to high heritability, which indicates these traits would respond to a genomic selection program. Given the wide range of observed fatty acid phenotypes in various lipid depots (triacylglycerol vs. phospholipid), muscle types, breeds, and feeding programs there is a need to identify the major pathways, transcription factors, and genes responsible for variation within the overall process of lipid metabolism. The identification of the drivers of lipid metabolism has multiple economic implications for the beef cattle industry since the value of the final beef product is highly dependent on lipogenesis during the finishing phase.

The association weight matrix approach provides a robust methodology that can identify the central regulators of a complex metabolic process such as the fatty acid profile of beef. The application of this method across multiple phenotypes and species has demonstrated its adaptability to multiple data types which is an important feature since the nature of “omics” data is constantly and rapidly changing with technological advances. The fatty acid profile has multiple related individual phenotypes that make up the overall fatty acid profile, including the major lipid classes as well as the various individual lipids contained in muscle and adipose tissue. With the introduction and continued use of high density genotype data into genomic selection programs it is becoming increasingly important to have an understanding of the biology and markers of highest importance for economic traits of interest. The beef cattle industry would benefit from an analysis of the genetic parameters and genetic correlations associated with the intramuscular fatty acid profile from the triacylglycerol and phospholipid fatty acid fractions followed by the implementation of the association weight matrix approach to generate a network analysis of lipid metabolism in Angus beef cattle.

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