FACULDADE DE CIÊNCIAS AGRÁRIAS E VETERINÁRIAS

UNIVERSIDADE ESTADUAL PAULISTA

CÂMPUS DE JABOTICABAL

EPISTATIC INTERACTIONS ASSOCIATED WITH FATTY ACID PROFILE OF BEEF FROM NELLORE CATTLE

Sabrina Thaise Amorim

Zootecnista

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TÍTULO DA DISSERTAÇÃO: EPISTATIC INTERACTIONS ASSOCIATED WITH FATTY ACID PROFILE OF BEEF FROM NELLORE CATTLE

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DADOS CURRICULARES DO AUTOR

Sabrina Thaise Amorim, nascida em 02 de julho de 1995 na cidade de Brusque – Santa Catarina, filha de Alexandre Adriano Amorim e Liliane Raquel Pavesi Amorim. Iniciou em março de 2013 o curso de graduação em Zootecnia na Universidade Federal de Santa Catarina, obtendo o título de Zootecnista em dezembro de 2017. Durante a graduação foi bolsista de Iniciação Científica do CNPq, monitora da disciplina “Genética Aplicada à Zootecnia”, integrante do Grupo de Pesquisa em Produção Animal e integrante do Laboratório de Pesquisa e Ensino em Genética Animal (LEPGA) da mesma instituição de fomento, sob a orientação do Prof. Dr. André Luís Ferreira Lima. Em março de 2018, ingressou no Programa de Pós-graduação em Genética e Melhoramento Animal da Faculdade de Ciências Agrárias e Veterinárias da Universidade Estadual Paulista “Júlio de Mesquita Filho”, campus de Jaboticabal, sob a orientação do Prof. Dr. Fernando Sebastián Baldi Rey, inicialmente como bolsista CAPES e posteriormente como bolsista FAPESP (Processo FAPESP 2018/19463-4). Realizou Estágio de Pesquisa no Exterior (BEPE-MS, Processo FAPESP:2019/04929-0) na Virginia Polytechnic Institute and State University sob supervisão do Dr. Gota Morota.

“It was the best of times, it was the worst of times, it was the age of wisdom, it was the age of foolishness, it was the epoch of belief, it was the epoch of incredulity, it was the season of light, it was the season of darkness, it was the spring of hope, it was the winter of despair, we had everything before us, we had nothing before us” (…).

(Charles Dickens - A Tale of Two Cities)

Dedico

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SUMÁRIO

EPISTATIC INTERACTIONS ASSOCIATED WITH FATTY ACID PROFILE OF BEEF FROM NELLORE CATTLE

RESUMO- A carne é uma fonte importante de aminoácidos, vitaminas, minerais e ácidos graxos. A seleção genômica tem sido sugerida como uma alternativa para lidar com características complexas como o perfil de ácidos graxos da carne. A epistasia é um efeito genético não aditivo e ocorre entre SNPs (polimorfismos de nucleotídeo único), genes ou QTLs (loci de característica quantitativa). Abordagens para modelar efeitos epistáticos já foram desenvolvidas na seleção genômica, no entanto, estudos que consideram a identificação de efeitos epistáticos entre regiões genômicas em animais de produção ainda são raros. A variação na composição dos ácidos graxos pode ser explicada pela caracterização e identificação de interações epistáticas que podem ser úteis para a predição de fenótipos. Assim, o objetivo deste estudo foi identificar interações epistáticas para as características do perfil de ácidos graxos (AG) da carne em animais Nelore. Foram utilizados dados de 963 machos Nelore de fazendas que integram os programas de melhoramento DeltaGen, CRV PAINT e Nelore Qualitas. O perfil de AG foi analisado em amostras de Longissimus thoracis por cromatografia gasosa, com coluna capilar de 100 m. Este estudo teve como objetivo realizar um Estudo de Interação Genômica para identificar interações gene-gene usando o método empírico Critério de Independência de Hilbert-Schmidt (HSIC) para testar epistasia em características do perfil de ácidos graxos. O controle de qualidade dos marcadores SNP consistiu em excluir aqueles com posição genômica desconhecida, localizados nos cromossomos sexuais; monomórficos e marcadores com frequência do alelo menor que 0,10; SNP que estavam fora do equilíbrio de Hardy-Weinberg, e call rate de 90%, marcadores com excesso de heterozigosidade e SNP muito correlacionados. Após o controle de qualidade, 347.393 SNP de 890 amostras de animais sobraram para análise. Os genótipos ausentes foram imputados usando estimativas de frequência de alelos de uma distribuição binomial dos dados. SNPs nas interações $\mathrm{P} < 1 \times 10^{-8}$ foram mapeados individualmente e usados para pesquisar genes candidatos. 602, 3, 13, 23, 13, 215 e 169 genes candidatos foram identificados para a soma de ácidos graxos saturados (AGS), ácidos graxos monoinsaturados (AGMI), ácidos graxos poliinsaturados (AGPI), ômega-3 (OM3), ômega-6 (OM6) e as razões AGS: AGPI e OM3: OM6, respectivamente. Os genes candidatos encontrados estavam associados ao colesterol, diferenciação de células de gordura, processo e regulação metabólica de lipídios, desenvolvimento do músculo esquelético, receptores de lipoproteínas de baixa densidade, eficiência alimentar e resposta inflamatória. A análise de enriquecimento revelou 75 termos significativos (P <0,05), a maioria relacionada à qualidade da carne e termos complementares. O número de interações significativas por característica de ácido graxo variou amplamente. Isso pode indicar que algumas características de ácidos graxos podem exigir quantidades variáveis de redundância genética ou controle para criar ácidos graxos essenciais para o condicionamento físico. A visualização de interações epistáticas significativas revelou muitas regiões de controle regulatório potencial em vários cromossomos. Nossos resultados mostraram interações genéticas substanciais associadas ao perfil lipídico, qualidade da carne, características de carcaça e eficiência alimentar, indicando que as interações gene-gene podem desempenhar

papéis muito diferentes no controle da variação fenotípica de caracteres relacionados aos lipídeos em bovinos.

Palavras-chave: *epistasia, GWIS, ácidos graxos, Nelore.*

EPISTATIC INTERACTIONS ASSOCIATED WITH FATTY ACID PROFILE OF BEEF FROM NELLORE CATTLE

ABSTRACT - Meat is an important source of amino acids, vitamins, minerals, and fatty acids. Genomic selection has been suggested as an alternative to deal with complex characteristics such as the fatty acid profile of the meat. Epistasis is a non-additive genetic effect and occurs between SNPs (single nucleotide polymorphisms), genes, or QTLs (quantitative trait loci). Approaches to model epistatic effects have also been developed in genomic selection, however, studies that consider the identification of epistatic effects between genomic regions in farm animals are still rare. The variation in the fatty acid composition can be explained by the characterization and identification of epistatic interactions that can be useful for the prediction of phenotypes. Thus, the objective of this study was to identify epistatic interactions for the meat fatty acid (FA) profile traits in Nellore animals. Data from 963 Nellore males from farms that integrate the breeding programs DeltaGen, CRV PAINT, and Nelore Qualitas were used. The FA profile was analyzed in Longissimus thoracis samples using gas chromatography, with a 100 m capillary column. This study aimed to conduct a Genomic Interaction Study to identify gene-gene interactions using the Hilbert-Schmidt Independence Criterion (HSIC) empirical method to test epistasis in fatty acid profile traits. The quality control of the SNP markers consisted of excluding those with an unknown genomic position, located on the sex chromosomes; monomorphic and markers with a minor allelic frequency less than 0.10; SNP that were out of the Hardy-Weinberg balance, call rate below 90%, markers with excess heterozygosity and SNP very correlated. After quality control, 347,393 SNP of 890 animal samples were left. The missing genotypes were imputed using allele frequency estimates from a binomial distribution of the data. SNPs in interactions $P < 1 \times 10^{-8}$were individually mapped and used to search for candidate genes. 602, 3, 13, 23, 13, 215, and 169 candidate genes were identified for the sum of saturated fatty acids (AGS), monounsaturated fatty acids (AGMI), polyunsaturated fatty acids (AGPI), omega-3 (OM3), omega-6 (OM6), and the AGS: AGPI and OM3: OM6 ratios, respectively. The candidate genes found were associated with cholesterol, differentiation of fat cells, lipid metabolic process and regulation, skeletal muscle development, low-density lipoprotein receptors, food efficiency, and inflammatory response. The enrichment analysis revealed 75 significant terms (P <0.05), most related to the quality of the meat and complementary terms. The number of significant interactions per fatty acid characteristic varied widely. This may indicate that some fatty acid traits may require varying amounts of genetic redundancy or control to create fatty acids essential for fitness. Visualization of significant epistatic interactions revealed many regions of potential regulatory control on several chromosomes. Our results showed substantial genetic interactions associated with lipid profile, meat quality, carcass characteristics, and feed efficiency, indicating that gene-gene interactions can play very different roles in controlling the phenotypic variation of lipid-related characters in cattle.

Keywords: *epistasis, GWIS, fatty acids, Nellore cattle.*

CHAPTER 1 – State of the Art

1.0 Lipids in Human Diet

Nutrition is the most important environmental factor in human health as it constitutes one of the basic requirements for the promotion and protection of health, enabling the affirmation of growth and development with quality of life (Rizzolo De Oliveira Pinheiro, 2005). In the human diet, fat is an essential nutrient which supplies the body with energy, essential fatty acids and provides transport for fat-soluble vitamins (A, D, E, and K and carotenoids)(Martin and Coolidge, 1978). Also, oils and fats participate in the regulation of the expression of genes that encode several enzymes involved in the metabolism of lipids and carbohydrates, besides participating in vital cellular processes (Khan and Vanden Heuvel, 2003).

Over the past century, dietary recommendations have changed to keep pace with the current research on health and nutrition. In the past, nutritional deficiencies and infectious diseases have been a concern in nutrition-related problems. Excessive total fat intake was not a problem due to most populations not even getting enough food to eat previous to technological advances in agriculture. However, in the 21st century, the problem seems to be another: Fast-food franchising's everywhere, high-calorie sodas, candies, snack foods, package foods, and every day's rush is making several populations experience an epidemic of overweight and obesity-related poor diet which and physical inactivity, which may result in diseases (World Health Organization, 2003; Walker et al., 2005).

With the increasing amount of packaged and fast food items in the human diet, people are now started to be concerned with the amount of fat in their diets, especially after studies like Stamler et al. (1999) who showed that about 75% of new cases of chronic non-communicable diseases that occurred in developed countries between 1970 and 1980 can be explained by high levels of unfavorable lipids in food combined with inadequate physical activity, obesity, and increased blood pressure.

Animal fats are mostly mentioned first when it comes to reducing the proportion of fat in the diet. Meat comprises roughly 10-20% of energy intake in most meat consuming countries (Food and Agriculture Organization of the United Nations, 2002; World Health

Organization, 2003) and its consumption has increased more than 10% worldwide since the beginning of the 1960s. Meat and meat products have the reputation of being high-fat, but options of lower fat content and higher muscle content has been obtained using breeding measures, changes in environmental conditions, feed, slaughter at younger ages, and leaner cuts of meat (Jakobsen, 1999; Jakobsen et al., 2006) High-fat and low-fat variants are available in almost all foods. Meat and meat products vary greatly in their fat content, although the general tendency (towards high-fat or low-fat) is the same.

Data on the average fat content and fatty acid composition of meats and meat products are published as part of food composition tables throughout the world. The World Health Organization recommends that fat should provide between 15 and 30% of the calories in the diet, saturated fat should not provide more than 10% of these calories, and cholesterol intake should be limited to 300 mg/day and emphasizing on keeping trans-fat as low as possible. These limitations take into account not only the total amount of fat but also the cholesterol levels and fatty acid composition in foods, of which meat and its products constitute a major part (World Health Organization, 2003).

1.1 Classification of Dietary Lipids

To understand how certain kinds of fats are associated with certain diseases and how they can be used in the food industry, one must know the basics of the biochemistry of fats. In most foods, fat is a mixture of triglycerides, phospholipids, sterols, and related compounds (Paul and Southgate, 1979).

Chemically, fats are triglycerides. Triglycerides are esters compounds that form from a reaction of an alcohol and an acid by the removal of water, in other words, three fatty acids (organic acid containing usually between 4 and 24 carbon atoms) joined to glycerol (polyhydric alcohol containing three carbon atoms, each of which is joined to a hydroxyl group) (McWilliams, 2008).

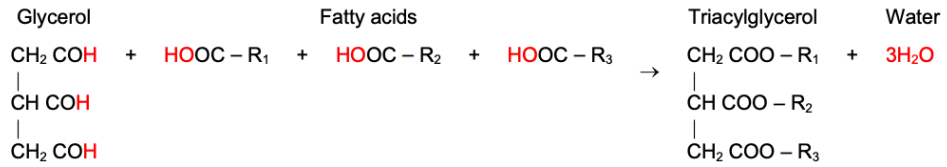

Figure 1 - The formation and structure of a triglyceride (Ockerman, 1996; Purves et al., 2005). The -OH group is removed from the fatty acid along with a -H from the OH group of glycerol, resulting in a triglyceride and three water molecules.

In Figure 1, R1, R2, and R3 represent fatty acids. If R1, R2, and R3 are the same, the triglyceride is termed a simple triglyceride, whereas if one of the three fatty acids are different, the triglyceride is said to be mixed (Ockerman, 1996). If the glycerol only has one or two fatty acids attached, we call them mono or diglycerides (Gurr and Harwood, 1991).

Fats can be solid or liquid at room temperature, depending on their composition and structure - the combination of three fatty acids in the triglyceride determines that. The term “oil” is used for fats that are liquids at room temperature, usually unsaturated. Saturated fats are solids at room temperature, like lard, butter or any other animal fat. Fatty acids constitute greater than 90% of a fat molecule, so the types of fatty acids present in food can determine certain properties of the fat (Martin and Coolidge, 1978; Warriss, 2000). Properties that fat contributes to food products include physical state solid or liquid at room temperature, shelf-life stability, aroma, and flavor.

1.2 Fatty Acids

Fatty acids vary in the number of carbon atoms, whereas the simplest organic acid is acetic acid ($\mathbf{C}\mathrm{H}_3\mathbf{C}\mathrm{OOH}$) with only two carbon atoms (Gurr and Harwood, 1991; Warriss, 2000). Fatty acids are classified as saturated (SFA), mono-unsaturated (MUFA), and polyunsaturated fatty acids (PUFA) (Gurr and Harwood, 1991; Ockerman, 1996; Warriss, 2000; Purves et al., 2005).

1.2.1 Saturated Fatty Acids

Chemically, saturated fats are fatty acids in which all available carbon binding sites are saturated with hydrogen, therefore giving them the name “saturated” fats, so the name “saturated fatty acids” (SFA) derive from the chemical structure (Martin and Coolidge,

1978). Saturated fats are mostly solid at room temperature, including lard, coconut oil, palm oil, palm kernel, butter, and other animal fats. The human body uses SFA for physiological and structural purposes, but these structures can be synthesized endogenously, and therefore, SFA is not essential in the diet (Valsta et al., 2005; Hooper et al., 2015).

Meat is an important source of saturated and unsaturated fatty acids (Ponnampalam et al., 2001). The predominant SFA in cattle fat are myristic (C14:0), palmitic (C16:0) and stearic (C18:0) (Lawrie, 2006), which are related to the increase in cardiovascular diseases in the population (Katan et al., 1994; Katan et al., 1995; Sacks and Katan, 2002). A high consumption of these SFA is associated with an increase in serum cholesterol and low-density lipoprotein (LDL) levels, known as risk factors for the occurrence of cardiovascular diseases (Katan et al., 1994). Myristic acid (C14: 0) has the potential to increase serum cholesterol concentrations four to six times higher than C16:0 (Mensink and Katan, 1992).

To reduce saturated fats, solid fats can be replaced with vegetable oils that are rich in mono and polyunsaturated fatty acids. However, these oils can give some food products different consistency and flavor as the former saturated fats.

1.2.2 Unsaturated Fatty Acids

Based on the number of double bonds present, unsaturated fatty acids are further divided into monounsaturated fatty acids (MUFA) with only one double bond and polyunsaturated fatty acids (PUFAs) with two or more double bonds (Ockerman, 1996). The presence of double bonds makes these fatty acids chemically more unstable when compared to saturated ones. They are present in cell membranes and contribute to the maintenance of the structure, chemical functions and integrity by the formation of phospholipid monolayers in the cell membranes. MUFA are used as energy substrates, while PUFA are precursors for phospholipids and prostaglandins (Lehninger, 2004).

PUFA are further grouped into two series: n-3 series (most abundant in fish oils) represents the FA that has the first double bond between C3 and C4 from the methyl end of the carbon chain, like α-linolenic acid (C18:3), eicosapentaenoic acid (EPA, C20:5), and docosahexaenoic acid (DHA, C22:6); the n -6 series, represented by linoleic acid

(C18:2), γ-linolenic acid (C18:3), dihomo--linolenic acid (C20:3), and arachidonic acid (C20:4) present the first double bond between C6 and C7 from the methyl end of the carbon chain are the main PUFA found in vegetable oils, seeds, and nuts (Lehninger, 2004; Tokuyama and Nakamoto, 2011).

Usually, unsaturated fatty acids have a *cis* configuration, and most *trans*-fatty acids are not found in nature. When fats undergo through artificial processes, they become *trans*-fats, especially as a minority product of unsaturated fat hydrogenation consisting of reducing double bonds of *cis* acids to single bonds (Ockerman, 1996; Lehninger, 2004). The *trans* configuration means that the two carbon atoms at both ends of the double bond are opposite.

They are the result of the interaction between the FA double bonds, hydrogen and the catalyst during partial hydrogenation reactions. As a consequence, there is no chain bending and its conformation is very similar to a saturated fatty acid. Naturally occurring *trans* fats are found in meats and dairy products produced from biohydrogenation process in ruminant animals (Mozaffarian et al., 2006).

Plant sources of fats include olives, cottonseeds, soybeans, rapeseed (canola oil), corn, sunflower seeds, safflower seeds, grape seeds, walnuts, macadamia nuts, and coconuts (McWilliams, 2008). Oils rich in monounsaturated fatty acids include canola, olive, and safflower oils. In animals, sources of unsaturated fatty acids are fish oils, milk, and meat. Strychar et al. (2003) reported that diets rich in monounsaturated fatty acids help lower total cholesterol and support a healthy heart. An example of polyunsaturated fats sources are soybean, corn, and cottonseed oils.

1.2.3 Phospholipids and Cholesterol

Similar to triglycerides, phospholipids have a glycerol backbone. Phospholipids are esters of fatty acids that include phosphoric acid and other constituents (Martin and Coolidge, 1978). To form a phospholipid, the glycerol esterifies only two fatty acids along with a phosphate and an alcohol (Romans et al., 2001; Lehninger, 2004).

Phospholipids are major components of the plasma membrane, the outermost layer of animal cells. Unlike triglycerides, which have three fatty acids, phospholipids have two fatty acids that help form a diacylglycerol. The third carbon of the glycerol backbone is

also occupied by a modified phosphate group modified by an alcohol. Phosphatidylcholine and phosphatidylserine are examples of two important phospholipids that are found in plasma membranes (McCormick, 1994).

Cholesterol is known as a precursor for many hormones, an essential constituent of cell membranes, and the precursor for bile salts necessary for the digestion of lipids (Martin and Coolidge, 1978; Godber, 1994). The human body synthesizes cholesterol in sufficient quantities, so it is not a dietary essential nutrient (USD HHS, 2005). The major sources of cholesterol in the diet are eggs (25% of total), chicken meat (12% of total), beef (6% of total), and beef burgers (5% of total) (Jakobsen, 1999; USD HHS, 2005; Valsta et al., 2005).

1.3 Meat Composition

Skeletal muscle is quantitatively the most important tissue of a bull's or cow's body. At a commercial slaughtering weight of 450 kg of carcass weight (CW), muscular tissue represents around 70% and adipose tissue the 20-25 % of the CW (Nürnberg et al., 1998). Meat is composed of muscular fibers, connective, adipose, vascular and nervous tissues. The muscular adipose tissue is distinguished between intermuscular fat and intramuscular fat (IMF) (Nürnberg et al., 1998).

The fat deposition on adipose tissue and skeletal muscle is strongly influenced by the balance between energy intake and expenditure, as well as energy intake and mobilization (Nürnberg et al., 1998). Adipose tissue deposition patterns in mammals are different within species, diet, age, gender, weight, breed, hormone concentrations, and maintenance requirements can all influence the rate and site of deposition (Gutiérrez-Gil et al., 2008).

Meat quality traits like IMF play an important role in consumer acceptance, because it affects the sensory quality of meat, in terms of juiciness, tenderness and flavor (Lawrie, 2006). The main goal in the meat industry is increasing IMF enough to cover the eating quality demand, but avoiding an excess of fat that would affect human health when consuming meat. Fatty acids contribute to various aspects of meat quality and ultimately, to the nutritional value of the final product.

1.3.1 Lipids in beef

Muscle lipids are composed primarily of phospholipids located in cell membranes and neutral lipids consisting of mainly triacylglycerols in the adipocytes (De Smet et al., 2004). The phospholipids content in the muscle is relatively independent of the total fat content, and it can range between 0.2 and 1% of muscle weight. Nonetheless, the muscle triacylglycerols content is strongly related to the total fat content, and ranges from 0.2% to more than 5% (Leseigneur-Meynier and Gandemer, 1991; Fernandez et al., 1999). Phospholipids are high in PUFA, while triacylglycerols contain small amounts of PUFA. Phospholipids are membrane components, so the PUFA proportion is highly conserved to maintain cell membrane integrity. Although, it is important to highlight that the PUFA content of the triacylglycerols is highly influenced by dietary fat compositions, especially in monogastric (Leseigneur-Meynier and Gandemer, 1991; De Smet et al., 2004).

For many years the composition of fatty acids in meat-producing animals has received considerable interest given its implications for human health and meat quality traits (Xie et al., 1996; Wood et al., 2004). The nutritional quality of meat takes into account the human nutritionist recommendations of fat ingestion, where lower levels of SFA are recommended, and increased levels of PUFA, particularly the n-3 family fatty acids such as linolenic acid (C18:3 n-3), eicosapentaenoic acid (EPA; 20:5 n-3) and docosahexaenoic acid (DHA; C22:6 n-3) are desirable. In this sense, the nutritional properties of meat are strongly related to its fat content and its fatty acid composition (Wood et al., 2004).

Wood et al. (2008) reported that the beef composition is on average, 50% of saturated (SFA), 40% of monounsaturated (MUFA) and 10% of polyunsaturated fatty acids (PUFA). The most ubiquitous fatty acids are palmitic (C16:0), oleic (C18:1), and stearic (C18:0) acids. The predominant PUFA is the Linoleic acid (C18:2) (~0.5–7%), followed by alpha-linolenic acid (up to 0.5%). PUFA like the group of conjugated linoleic acids (CLA) can be found at low mg-levels in meats, and they have beneficial effects on health (Wilson et al., 2000; Whigham et al., 2007; Campbell and Kreider, 2008). *Trans*-fatty acids comprise about ~2–4% of total fatty acids in ruminant meats. Hence, the fatty acid composition has a considerable effect on the diet/health relationship, since each fatty acid affects the plasmatic lipids differently.

1.4 Effects of fatty acid composition on human health

Evidence that different fatty acids have varying effects on human health and disease prevention is well documented, and therefore, particular attention should be placed on the fatty acid composition of a food (Grundy and Denke, 1990; Valsta, 1999; Kremer, 2000; Walker et al., 2005). Through the years, several studies showed different fatty acid concentrations in meat. In 1986, Eichhorn et al. (1986) analyzed steer longissimus muscle samples. The authors reported that the samples had approximately 47.9% SFA, 46.1% MUFA, and 5.4% PUFA. Twenty-three years later, Leheska et al. (2008) study showed variations, they reported 45.1% SFA, 51.6% MUFA, and 3.4% PUFA. The difference in their studies may be because fatty acid composition can be altered through feeding, which includes manipulating rumen fermentation patterns. Also, different meat muscles and/or cuts differ in fat content and the type of muscle fibers. Muscle fibers are classified according to their metabolic, contractile and color properties (Klont et al., 1998). Three major fiber types can be identified using a method by Gauthier (1969): red, intermediate, and white. This method consists of fiber typing based on histochemical reactions of aerobic oxidative capacity, using the reference enzyme succinate dehydrogenase (SDH). This method reflects differences in mitochondrial content in the fiber, differences in muscle fiber type between muscles are reflected in differences in fatty acid composition: “red” muscles have a higher proportion of phospholipids; “white” muscles have a higher percentage of PUFA (Wood et al., 2008).

Saturated fatty acids are known to increase low-density lipoprotein (LDL) cholesterol (bad cholesterol) content of blood while PUFA tends to lower LDL cholesterol concentrations in blood (Katan et al., 1994). Stearic, myristic, and lauric acids are the major SFA found in human diets (Katan et al., 1994). However, these SFA have varying effects on human health. Lauric, myristic and palmitic acids all raise LDL cholesterol compared to PUFA, in contrast, stearic acid tends to have a neutral effect (Katan et al., 1994; Romans et al., 2001).

The fatty tissue of ruminant animals is a natural source of conjugated linoleic acid (CLA) isomers, such as *cis*-9, *trans*-11 (French et al., 2000). These fatty acids are synthesized in the rumen as a result of the biohydrogenation process of fatty acids carried out by microorganisms (Tamminga and Doreau, 1991) and its effects are favorable to

human health. The CLA has a major role in lipid metabolism, especially in the oxidative cellular system, which explains many physiological properties of fatty acids. Several studies evaluated the influences of CLA on the energetic metabolism, highlighting significant changes in lipid metabolism and body composition in rats and humans. Their action on lipid metabolism is associated with the inhibition of the entry of glucose into the adipocytes, which may lead to changes in insulin metabolism. The consumption of products containing CLAs presents health benefits, many studies are indicating that CLA consumption may prevent certain diseases and cancers. In particular, CLA is anticarcinogenic and antiatherosclerosis in regularly exercising individuals (Kramer et al., 1997; Churruca et al., 2009; Benjamin et al., 2015).

The main MUFA in meat is oleic acid (C18:1 n-9), and the main PUFA are Linoleic (C18:2 n-6), arachidonic (C20:4 n-6) and linolenic (C18:3 n-3). It is important to elucidate that meat from ruminants presents higher amounts of PUFA in comparison to monogastric, this fact is explained by the extensive hydrogenation of dietary unsaturated fatty acids by rumen microorganisms (Wood et al., 2008). The presence of MUFA and PUFA in the diet reduces the level of low-density lipoproteins-cholesterol plasma, although PUFA also depresses the high-density lipoproteins-cholesterol (Mattson and Grundy, 1985). Besides, n-3 PUFA has gathered considerable attention because of their roles in homeostasis and inflammatory response.

Among the most important unsaturated fatty acids for human health are the polyunsaturated fatty acids of the omega-3 (n-3) and omega-6 series (n-6), as these present beneficial effects in the immune and inflammatory response (Grundy and Denke, 1990; Youdim et al., 2000). Omega-6 and Omega-3 fatty acids are called essential because organisms are unable to produce them. They are fundamental in reactions such as the transfer of atmospheric oxygen to blood plasma, the synthesis of hemoglobin, and as hormone precursors (Ulbricht and Southgate, 1991; C. Martin et al., 2006). The n-6 / n-3 ratio in the human diet during the period before industrialization was estimated from 1:1 to 2:1. It was estimated that the Western diet was deficient in omega-3 fatty acids with an omega-6 to omega-3 ratio of 15-20/1, instead of 1/1, which is the ideal (Simopoulos, 1991). The balance of omega-6 / omega-3 fatty acids is an important determinant in decreasing the risk of coronary heart disease (Simopoulos, 2008). However, with

industrialization, there was a progressive increase in this ratio, mainly with the increase in the consumption of refined oils from oleaginous plants, resulting in diets with inadequate amounts of n-3 fatty acids. It is currently suggested that this ratio should not be greater than 4:1 (Dinicolantonio and O'Keefe, 2018).

1.5 Genetic Variation for beef fatty acid profile and genomic selection

The development of thousands of markers that cover the bovine genome with greater coverage, the chances of finding markers associated with productive traits has increased. As a consequence, the use of genomic information to predict the genetic values of economic important traits can contribute to the improvement of traits related to meat quality.

The difficulty and high cost of obtaining phenotypic measures of the beef fatty acid profile is a limiting factor in its implementation in breeding programs. However, with the growing worldwide demand for healthier products, many research groups show that the fatty acid profile of beef has genetic variation to respond to selection, this is important because the availability of genetic parameters is essential to verify the possibility of genetic improvement for the fatty acid profile of the meat. Malau-Aduli et al. (2000) estimated genetic parameters for the fatty acid profile of animals at two stages (weaning and slaughter). At weaning, heritability estimates for individual fatty acids, SFA, MUFA, and PUFA were low to moderate, ranging from 0.03 to 0.31. However, at slaughter, heritability estimates for the same group of traits were slightly higher, ranging from 0.02 to 0.44. The authors emphasized that PUFA are highly desirable in the human diet, given their ability to reduce serum cholesterol, have sufficient genetic variability to respond to selection.

Tait et al. (2007) estimated genetic parameters for the fatty acid profile in lipid tissue and meat in Angus cattle using the sire model. Heritability estimates for individual fatty acids in meat and lipid tissue ranged from 0.06 to 0.27, and from 0.20 to 0.49, respectively. Subsequently, Nogi et al. (2011) estimated genetic parameters for the fatty acid profile in the Wagyu breed. Heritability estimates for individual fatty acids ranged from 0.00 to 0.78. The heritability estimates for MUFA, SFA and MUFA were 0.68, 0.66, and 0.47, respectively. According to the authors, the heritability estimates found indicate

that there is a possibility of genetic improvement for fat content in meat. Still working with the Wagyu breed, Inoue et al. (2011) obtained h^2 estimates of 0.66 for MUFA and 0.75 for the relationship between SFA and MUFA. The authors suggested that to improve the fatty acid profile, an appropriate strategy would be to use the SFA:MUFA ratios as a selection criterion.

Genomic selection has been suggested as an alternative to deal with complex and difficult to measure traits that are regulated by many genes in plants and animals (Hayes et al., 2009; Zhao et al., 2015). Genomic prediction (GP), proposed by Meuwissen et al. (2001) aims to predict the genetic merit of individuals in a population using information from genetic markers. In particular, GP uses dense genome-wide single-nucleotide polymorphisms (SNPs) panels assuming that causal genes that affect a trait of interest are in linkage disequilibrium with at least one marker. In GP, various models are available to deal with different genetic architecture. These models use different assumptions about the distribution of marker effects: some models assume that there are markers with major effects and other models assume that all markers have similar effects (e.g., infinitesimal assumptions)(Gianola, 2013; De Los Campos et al., 2013). It is possible to include non-additive effects such as dominance (Toro and Varona, 2010; Z. G. Vitezica et al., 2013; Zeng et al., 2013; Nishio and Satoh, 2014) and epistasis (Wittenburg et al., 2011a; Su et al., 2012; Muñoz et al., 2014). Also, there are GP models that consider additive and non-additive effects nonparametrically (Gianola et al., 2006; Gianola and Van Kaam, 2008).

Like most economically important traits in animal production, beef fatty acid composition is influenced by environmental and genetic factors (Feitosa et al., 2017a; Aboujaoude et al., 2018). The fatty acid composition can be altered through genetics (Huerta-Leidenz et al., 1993; Pitchford et al., 2002a), but the genetic factors affecting fatty acid composition in cattle have been less investigated when compared to monogastric animals, although several studies report differences between breeds for fatty acid composition (Gillis et al., 1973; Mills et al., 1992; Huerta-Leidenz et al., 1993; Siebert et al., 1996; Rule et al., 1997; Malau-Aduli et al., 1998; Pitchford et al., 2002). Differences due to crossbreeding and breeds can change the fatty acid composition of the meat (Fisher et al., 2000), but generally, the nature and level of deposition of fatty acids in the

muscle depends on the diet, ingestion, intestinal absorption, hepatic metabolism, and lipid transportation (Geay et al., 2001).

Genomic selection studies for the fatty acid profile in cattle is still limited due to many factors, it is worth mentioned that usually fatty acid data sets are small, which difficult genetic predictions, but the number of published researches is growing over the years.

Genomics is an alternative to help us to understand how traits like fatty acid profile are regulated. Among the genomic studies, it is worth mentioning Reecy et al. (2010). The authors analyzed the fatty acid concentration in Angus bull's meat. The authors estimated the effects of SNP on each trait using a Bayes C module. One of the author's conclusions was that a large proportion of variation in fatty acid composition is associated with a relatively low number of SNPs. Therefore, genetic progress can be achieved by the implementation of whole-genome selection to improve fatty acid composition in meat. Later, Yeon et al. (2013) carried out studies to identify genetic variants of Fatty acid synthase (*FASN*) and association with fatty acid composition in Hanwoo cattle. The authors observed 6 genetic variants located in exons 20, 24, 32, 34 and 39, and concluded that SNPs located in *FASN* coding regions (exons 20, 32 and 39) have a significant association with the composition of fatty acids.

In the Angus breed, Saatchi et al. (2013) carried out studies of association and broad genomic selection to determine the extent to which molecular markers could be responsible for variation in fatty acid composition in skeletal muscle and to identify the genomic regions associated with this variation. The authors reported that when using 54k markers, up to 54% of the variation in the fatty acid composition of the meat was explained by SNP markers. Also, 57 genomic regions associated with the fatty acids profile were found. The authors concluded that this large number of genomic regions might indicate the presence of an elaborate molecular mechanism that controls fatty acid content in skeletal muscle.

Using Nellore cattle genomic data, Cesar et al. (2014) obtained null to moderate heritabilities, ranging from 0.11 to 0.17 for SFA, MUFA, and PUFA. These authors were pioneers in GWAS for these traits in this breed. Since then, genomic studies for Nellore cattle increased. Feitosa et al. (2017) estimated genetic-quantitative relationships between the beef fatty acid profile with the carcass and meat traits of Nellore cattle using

the single-step genomic BLUP (ssGBLUP) procedure. The study reported that heritability estimates for OM3, OM6, SFA, MUFA, and PUFA sums were low to moderate, varying from 0.09 to 0.20. Later, Chiaia et al. (2017), working with *Longissimus thoracis* muscle samples of Nellore cattle aimed to predict the direct genomic value for SFA, MUFA, PUFA, OM3 and OM6 using different methodologies such as SNP-BLUP, BayesCπ, BayesC and Bayesian Lasso. The authors results indicated that none of the methods excelled in terms of accuracy, but SNP-BLUP showed the less biased genomic evaluations.

The above mentioned studies shows us that the identification of regions of the genome responsible for genetic variation in the meat fatty acid profile should contribute to the understanding of meat fatty acid synthesis, as well as increase opportunities to improve meat fat composition through genomic selection (Bouwman et al., 2011).

With the changes in the world economy, the intensification of livestock production in Brazil and the public appeal of organs and entities (Eilander et al., 2015), there were changes in the habits and preferences of consumers, who are now looking for increasingly healthier foods. This shows that technicians, breeders, and industry must pay attention to the demands of their consumers (Barcellos, 2007), and the industry must seek technical and practical solutions for the production of healthier and processed foods. Therefore, genomic selection and prediction for meat quality traits like the fatty acid profile may play an important role in identify genes associated with lipid and fatty acids metabolism for healthier meat without affecting consumers' acceptance

1.6 Genome-Wide Association Studies and Epistasis

A Genome-Wide Association study (GWAS) aims to detect variants at genomic loci associated with complex traits or diseases in a population, in particular, at detecting associations between common SNPs and phenotypes (Visscher et al., 2012a).

1.6.1 Genetic variants

The DNA term refers to a double-stranded molecule located within the cell nucleus. A genetic variation occurs when a nucleotide on a certain genomic position in the DNA is exchanged, called single nucleotide polymorphism (SNP). Chemically, the exchange

happens between four nitrogenous bases: adenine (A) and guanine (G) or between cytosine (C) and thymine (T). For each SNP, there is two possible alleles.

SNPs may fall within non-coding regions, coding sequences or in regions between genes (intergenic regions). In most cases, SNPS are more frequent in non-coding regions than in coding regions (Castle, 2011). A SNP within a coding sequence do not necessarily change the sequence of amino acids of a protein that is produce, due to the degeneracy of the genetic code. However, SNPs in non-coding regions may affect gene splicing mechanism, transcription factor binding, the degradation of ribonucleic acid (RNA) or the sequence of non-coding RNA. Lastly, a SNPS down or upstream of a gene may affect the gene expression, also referred to as an expression single nucleotide polymorphism (eSNP)(Castle, 2011; Visscher et al., 2012a). Therefore, SNPs are considered as the biological markers in GWAS to map the association between measurable phenotypes and genetic factors.

1.6.2 Linkage and association analysis

Linkage can be defined as the phenomenon in which adjacent alleles located on the same chromosomal segment are transmitted together (in blocks) during meiosis more frequently than would be expected by chance. This set of linked alleles is called a haplotype (Meuwissen and Goddard, 1996; Hafler and De Jager, 2005; Calus et al., 2008).

Consider two markers, A and B, that are on the same chromosome. A has two alleles A1 and A2, and B has alleles B1 and B2. Four haplotypes of markers are possible i) A1_B1, ii) A1_B2, iii) A2_B1, and iv) A2_B2. It is expected, in a balanced situation, where the frequency of all alleles is 0.5, that the four haplotypes occur with a frequency equal to 0.25. Any deviation from 0.25 is called linkage disequilibrium (LD). LD is defined as the difference between the observed haplotypic proportions and those expected if the alleles segregated independently (Hill, 1981). LD will be discussed in the following sections of this thesis.

Within a family, linkage occurs when two genetic markers remain linked on a chromosome rather than being broken apart by recombination events during meiosis (red lines). In a population, contiguous stretches of founder chromosomes from the initial

generation are sequentially reduced in size by recombination events. As times passes, a pair of markers or points on a chromosome in the population move from LD to linkage equilibrium, as recombination events eventually occur between every possible point on the chromosome (Bush and Moore, 2012).

A linkage analysis relies on segregation of alleles within the family, it uses the recombination events only within a recorded pedigree, which results in a typically large confidence interval for the position of the quantitative trait loci (QTLs). On the other hand, association analysis simply correlates markers with phenotypes across a population (Darvasi et al., 1993; Darvasi and Pisanté-Shalom, 2002).

1.6.3 Heritability and the case of missing heritability

A century ago, Fisher (1918) and Wright (1920) introduced the concept of heritability. Heritability measures the proportion of the phenotypic variance in a population attributable to genetic differences. This concept relies on the idea that the total phenotypic variance V_P in a population can be partitioned into two components, a genetic component V_G and an environmental component V_E.

The broad sense heritability H^2 is defined as the ratio of V_G/V_P. However, in most studies, what is reported is the narrow sense heritability (h^2) that relies on i) partitioning the genetic variance into an additive component, or the breeding value (V_A), ii) a dominance component (V_D) that takes into account the interaction between alleles at the same locus, iii) and an epistatic component (V_I) that accounts for interactions between alleles at diferent loci. The narrow sense heritability is then the ratio V_A/V_P (Falconer and Mackay, 1996).

With the recent contributions of GWAS studies on different traits, recently we can quantify the contribution of the genetic variants to phenotypic variance and measure the genomic heritability (de los Campos et al., 2015). Genomic heritability infers the proportion of variance that can be explained by a linear regression on a set of markers used as explanatory variables. This can be done using only the significant SNPs associated with traits in GWAS. However, most variants identified by GWAS so far explain only a small proportion (less than 50 %) of estimated heritability, the proportion of variation

in a particular trait that is attributable to genetic factors (Maher, 2008; Sackton and Hartl, 2016).

An example is the heritability of height in humans. The h^2 for height that was estimated to be 80% based on its correlation between relatives. Gudbjartsson et al. (2008) found 27 associated genomic regions, Lettre et al. (2008) reported 12 and Weedon and Frayling (2008) found 20 genomic regions associated with human height. Later, Wood et al. (2014) reported 697 genetic variants with genome-wide significance on adult height. Taken together, the associated loci in each study explained, respectively, 3.7, 2.0, 2.9%, and 20 % of the phenotypic variance.

The unexplained heritability is so-called missing heritability. To understand the problem of missing heritability from GWAS studies and explain where the missing heritability might be found, many explanations were proposed (Maher, 2008; Manolio et al., 2009), including: i) larger numbers of variants with smaller effect yet to be found; ii) structural variants poorly captured by existing arrays; iii) rarer variants that may have larger effects and are poorly detected by available genotyping arrays that focus on variants present in 5% or more of the population; iv) low power to detect gene-gene interactions.

1.7 Epistasis

Gene-gene interaction could be an explanation of the missing heritability, epistasis, defined generally as the interaction between different genes, is a hot topic of discussion in complex trait genetics in recent years (Carlborg and Haley, 2004; Phillips, 2008; Mackay, 2014; Pedruzzi et al., 2018).

1.7.1 Concepts of Epistasis

Quantitative traits are characterized by being controlled by many loci, which may have pleiotropy and can be affected by epistasis. Pleiotropy is defined by the control of more than one trait by the same locus; Epistasis, on the other hand, constitutes interactions between loci that can affect the phenotype (Falconer and Mackay, 1996). Both of these effects are usually disregarded in genetic studies.

Epistasis is an important concept in population and quantitative genetics. Its definition varies somewhat across these fields, but the underlying concept is that the effects of allelic substitution at one gene can be dependent on the allelic state of another gene or genes. In population genetics, the role of epistasis is linked to theories of fitness and adaptation. In quantitative genetics, epistasis has a broader meaning that encompasses any nonadditive interaction among genes and it is often identified with the interaction term in analysis of variance (Falconer and Mackay, 1996).

The term epistasis was initially defined by Bateson (1909) as a way of explaining Mendelian segregation distortions in experiments with chickens, including the inheritance of the crest shape in the *Gallus gallus* species. Bateson used the term “epistatic” to describe a masking effect whereby a variant or allele at one locus prevents the variant at another locus from manifesting its effect on a phenotype. In this sense, the variants must be interacting with one another, at least in the loose sense that they exist within pathways that both influence the same phenotype.

A few years later, Fisher (1918) presented a statistical interpretation, decomposing the genetic variance into additive, dominant and epistatic. Fisher’s quantitative theory of population genetics defined epistatic effects on additive models as a “noise” term. But epistasis is known to play a role in several evolutionary processes. Epistasis in traits related to fitness of an individual can lead to the existence of multiple fitness peals and multiple stable equilibria for gene frequencies in a population – this is the main idea to Wright’s shifting balance theory (Wright, 1932; Chouteau and Angers, 2012). Wright’s theory proposes that population subdivision can lead to the evolution of coadapted gene complexes. When we have incompatibilities among sets of genes this will lead to isolation and speciation.

Based on components of genetic variance, Cockerham (1954) and Kempthorne (1954) divided the different epistatic effects according to the individual action of the locus, they are: additive x additive, additive x dominance, and dominance x dominance. This subdivision can be used as a tool to identify the exact way in which genes interact. Variance components have proven to be useful in predicting the response of a population to selective pressure and have been successfully applied in breeding programs. The

epistatic variance components reflect an average effect over many genes on the phenotype distribution in a population (Young and Durbin, 2014).

Phillips (2008) condensed term “epistasis” into three main categories: functional - the molecular interactions that any genetic elements have with one another; compositional - describes the ways a specific genotype is composed and the influence that this specific genetic background has on the effects of a given set of alleles, and not applied to natural populations; and statistical - attributed to Fisher is the average deviation of combinations of alleles at different loci estimated over all other genotypes present within a population. The first two are considered as physiological epistasis, referring to any situation in which the genotype at one locus modifies the phenotypic expression of the genotype at another locus (Cheverud and Routman, 1995; Sackton and Hartl, 2016). Statistical epistasis mostly relies on the concept of a linear model that describes the relationship between an outcome variable and a predictor variable or variables (Cordell, 2009).

Currently, the most accepted statistical definition of epistasis refers to the interaction between alleles of different loci that result in phenotypic values not predicted by the sum of individual effects of the loci, that is, when the combined effect of different loci on a genotype cannot be predicted by the sum of its individual effects. We can also say that it is a form of action that is not explained by an additive-dominant model (Falconer and Mackay, 1996).

1.7.2 Epistatic Interactions

Interaction between SNPs, genes or QTLs (quantitative trait loci) results in non-linear effects that control the variation of phenotypes (Zhang et al., 2017), and is also the most complex source of non-additive genetic variation. Epistatic interactions are dependent on the average effects of genes and deviations of dominance in the individual locus, as the result is dependent on the average degree of dominance and the allele frequency of the population (Ramalho et al., 1993; Bernardo, 2002). Besides, variation due to non-additive effects manifests itself as additive genetic variance (Hill et al., 2008a), and the potential influence of epistasis depends on genetic details about which we currently know little about it (Hansen, 2013).

The simplest epistatic interactions are duplicated and complementary. Duplicate interactions occur when two or more loci perform the same function. If at least one of the loci is present, the phenotype will be produced, so that the non-expression of a locus will not result in an abnormal phenotype. An example of this effect can was reported by Hatchett et al. (1993), when they described the effects of duplicated epistasis between two genes that condition resistance to the Hessian fly (*Mayetiola destructo say*) in the rye. Complementary interactions occur when more than one locus is needed to produce a phenotype. For example, if two or more loci are involved in a biosynthesis route, both loci must be active and their products must be present to generate the final phenotype.

With that in mind, we can distinguish some scenarios involving epistatic interactions, and which one will be the most important for a trait: (I) systematic positive epistatic interactions between genes, as substitutions of genes that have positive effects on the trait and that increase the effects other potential genetic substitutions with positive effects on the trait studied. In summary, we will have a systematic increase in the phenotypic effects of gene substitutions. (II) systematic negative epistatic interactions, are gene substitutions that have positive effects on the trait worked and tend to diminish the effects of other substitutions with positive effects. (III) Interactions without systematic directions, that is, some gene substitutions will increase and some will decrease, but the net change will be zero. Finally, (IV) negative interactions, where we have different alleles in a locus with different specific interactions, with changes in the order of allelic effects, resulting in complex dynamics (Hansen, 2013).

1.8 Analysis Challenge

The challenges of searching for epistatic effects on a genome-wide level is confronted with the same statistical issues as in GWAS resulting from the genetic part, such as the false positives caused by population heterogeneity, and mostly the computational expense due to the increasing sample sizes and scale of genotyping.

1.8.1 Computational challenge

Epistasis analysis has utility in both animal and human genetics analysis of biosynthetic pathways, developmental pathways and other genetic networks since its first

application conducted by Bateson and Punnett revealed a pattern of epistasis influencing flower color in sweet peas (Bateson, 1909).

Real metabolic networks are very complex. SNP markers can be in coding regions, when they affect the production of proteins or RNA, or in regulatory regions, affecting the amount and times when proteins are produced. Therefore, there are almost infinite ways on how genes can interact. The statistical definition of epistasis is more useful for finding the sets of markers that interact, as it allows fewer tests per group of markers.

A major problem in the study of epistasis is the data dimensionality (Bellman, 1957). If we want to do an exhaustive search of SNP pairs, when we increase the number of SNPs to be analyzed, for example, from one to two, or from two to three, we are adding a new dimension to the analysis, and this ends up resulting in a huge increase in computational time.

In exhaustive methods search for epistasis, at first, it is necessary to analyze each set of markers separately. If there are n SNPs and it is necessary to analyze epistasis of up to p loci, the number of analyzes will be $C_{n,p}$, which is equivalent to:

$$\frac{[\mathrm{n}\ (\mathrm{n}-1)\ldots(\mathrm{n}-\mathrm{p}\ +\ 1)]}{p!}$$

This combinatorial complexity, although not as bad as a factorial, can be considered as a problem, as it is approximately exponential when the number of p loci in each set varies. For common values in the search for epistasis - p between 2 and 10, and n from 300 thousand to one million - for each locus added in p, the processing time is multiplied by approximately one hundred thousand (Motsinger and Ritchie, 2006; Niel et al., 2015).

The search for epistasis in association studies may be seen as a problem of selection of variables that interact and with effect on the variable of interest. Therefore, association studies in genetics need to develop their own methods, suitable for this large number of variables.

Genome-Wide Interaction Studies (GWIS) became popular in the recent years in human studies (van Leeuwen et al., 2014a; Arnau-Soler et al., 2018; Jiao et al., 2019; Zeng et al., 2019). To perform a genome-wide pairwise epistasis study for n SNPs, for

example, it is required $n(n-1)/2$ tests, which means that a GWIS faces a more serious computational challenge compared to GWAS (van Leeuwen et al., 2014).

Most of the studies using epistasis to analyze the structure of genetic pathways utilize a small set of genes that had previously been identified to affect the trait of interest. Nevertheless, the entire premise of epistasis is that genetic interaction can influence phenotypes when found in combination with one another. Phillips (2008) suggests that is better to conduct a large-scale systematic study of the possible pairwise interactions between all genes, and the datasets investigated with GWAS can be appropriate sources to search for pairwise epistasis.

1.8.2 Allele Frequencies and Genotype Call Rate

In GWAS studies, one of the quality control check points is the minor allele frequency (MAF) (Bush and Moore, 2012; Uitterlinden, 2016). MAF refers to the frequency of the allele in a locus appearing with a low rate in the population. It is important to filter the markers based on MAF, because the statistical power is extremely low for rare SNPs. According to Turner et al. (2011), SNP that present extremely low MAF have the potential to lead to spurious associations, it may be due to either genotyping errors or population stratification. Hence, to avoid high rates of false positives or negatives and bias, the threshold of 1% or 5% is applies to remove SNP with low MAF (Anderson et al., 2010). In some quality controls of SNP there is the option to exclude high correlated SNP based on the MAF percentage: each SNPS is compared with every other SNP that has a MAF within 2.5 percentage units. Two SNP are declared high correlated if the genotypes are all the same (0–0, 1–1, and 2–2) or all opposite (0–2, 1–1, and 2–0) (Wiggans et al., 2009).

The genotype call rate is a good indicator of maker quality, being calculated as:

$$Genotype\ Call\ Rate = \frac{number\ of\ genotyped\ individuals}{total\ number\ of\ individuals}$$

In a DNA sample several variations may exists that can have substantial effects on the genotyping accuracy and on the genotype call rate. Below average call rates and accuracy indicates samples of low DNA concentration and/or quality, which can affect the

genotype failure rate, resulting in missing genotypes. In most GWAS studies in livestock species, SNP with lower call rate than 90% are removed from the analysis, but the threshold may vary from study to study (Cooper et al., 2013; Reed et al., 2015; McClure et al., 2018).

1.8.3 Linkage Disequilibrium

The classical definition of LD refers to the non-random association of alleles between two loci. The concept is related to chromosomal linkage, where two variants on a chromosome remain physically joined on a chromosome through generations of a family. From generation to generation, chromosomal segments are broken apart by recombination events within a family. Through generations, this effect is amplified. Until all alleles in the population are in linkage equilibrium or are independent, repeated random recombination events keep breaking apart segments of a contiguous chromosome (containing linked alleles) (Bush and Moore, 2012). The rate of LD decay decreases according to the increasing number of generations. LD can arise due to migration, mutation, small finite population size, selection or other genetic events which the population experiences [eg. Lander and Schork (1994)].

One way to measure LD is using $\boldsymbol{D}$ proposed by Hill (1981):

$$D = freq(A1\_B1) * freq(A2\_B2) - freq(A1\_B2) * freq(A2\_B1)$$

Where $\boldsymbol{freq(A1\_B1)}$ is the frequency of the haplotype A1_B1 in the population, and likewise for the other haplotypes. The $\boldsymbol{D}$ statistic, however, is very dependent on the frequency of the individual alleles, so when we want to compare the extent of LD among multiple pairs of loci at different points along the genome $\boldsymbol{D}$ is not particularly useful. The $\boldsymbol{r^2}$ statistic proposed by Hill and Robertson (1968) is less depended on allele frequencies:

$$r^2 = \frac{D^2}{freq(A1) * freq(A2) * freq(B1) * freq(B2)}$$

Where $\boldsymbol{freq(A1)}$ represents the frequency of A1 allele in the population, and likewise for the other alleles in the population. The $\boldsymbol{r^2}$ statistic ranges from 0 to 1, where 0 implies frequent recombination between two variants and statistical independence under principles of Hardy-Weinberg Equilibrium (HWE) and 1 means a complete LD, indicating no recombination between the two variants within the population.

In livestock populations, it was suggested that LD can also be deliberately created (Goddard, 1991; Lander and Schork, 1994). The finite population size is generally implicated as the key cause of LD in livestock, this is because: i) the relatively small effective population size, which causes relatively large amounts of LD; ii) LD due to crossbreeding is large when crossing inbred lines, but small when crossing breeds that do not differ as markedly in gene frequencies and it disappears after only a limited number of generations (Goddard, 1991); iii) selection effect is likely to be localized around specific genes, and so has relatively little effect on the amount of LD "averaged" over the genome; iv) and mutations are likely to have occurred many generations ago.

A GWAS based upon the principle of LD at the population level performs an unbiased scan of the genome compared with linkage analysis (that only considers that LD exists within families and is broken down by recombination after only a few generations) in which only a few genetic markers per chromosome is used to tag a causal variant since the number of recombination events per meiosis is relatively small (Visscher et al., 2012). GWAS represents an important step beyond family-based linkage studies and a powerful tool for investigating the genetic architecture of complex traits.

1.9 Genome-Wide Interaction Studies for lipid composition in livestock

The advent of SNP arrays and genomics made it relatively easy to genotype a wide array of individuals. As a result, many GWAS studies have been carried out in the past several years (Visscher et al., 2017). Most of GWAS studies have only reported additive effects of SNPs and additive genetic variance on single-locus tests. However, this is not that only type of genetic association. An alternative that may provide new insights into the genetic architecture that underlies variation in complex traits is the genome wide SNP-SNP interaction analysis (GWIS). GWIS has been applied recently in both humans and livestock species(Li et al., 2013; Kogelman and Kadarmideen, 2014; Hibar et al., 2015; Li

et al., 2015; Kramer et al., 2016). Perform a GWIS represents the next step for detecting the variations in complex traits, because single-locus tests cannot identify the interactions among SNPs, genes or other genetic or environmental factors. So, identifying markers that show interactions would help to explain a higher proportion of the heritable variance (Cordell, 2009).

GWAS studies in humans revealed many genetic loci associated with lipid levels in human plasma (Kathiresan et al., 2008; Aulchenko et al., 2009; Teslovich et al., 2010; Willer et al., 2013). However, it is still difficult to find evidence for SNP x SNP interactions. Ma et al. (2012) identified a significant association interaction between the HMGCR gene (also known as a transmembrane glycoprotein that is the rate-limiting enzyme in cholesterol biosynthesis) and the LIPC gene in relation to HDL cholesterol. Turner et al. (2011) reported eight SNP x SNP interactions associated with HDL cholesterol levels, and recently, van Leeuwen et al. (2014) performed a GWIS resulted in the consistent finding of a possible interaction between a SNP in ARMC8 gene and a SNP in SPATA8 gene related to HDL levels in humans.

In livestock species, GWIS studies have been performed in cattle and poultry. Studies from Ek et al. (2012) and Carlborg et al. (2006) suggested that epistatic interactions between genes (or QTLs) are important for quantitative traits such as growth and fatness traits in chickens (*Gallus gallus*). Hu et al. (2010) previously identified epistatic interactions among 10 candidate genes for chicken abdominal fat traits, and constructed networks of the interacting genes. Kramer et al. (2016) identified many epistatic interactions were identified in two fractions of fatty acid content, triglycerides and phospholipids. The authors reported that the number of significant interactions per fatty acid trait varied greatly, and although the large numbers of interactions identified, the validation of these interactions was a difficult task.

Fatty acid composition is of paramount importance due to their role in human health. The genetic dissection of fatty acid composition could lead to a better understanding of the molecular mechanisms that control fatty acid content in meat. Results from a GWIS may indicate the presence of an elaborate molecular mechanism that control fatty acid content in skeletal muscle.

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CAPÍTULO 2 – Genome-Wide Interaction Study for fatty acid profile of beef from Nellore Cattle.

Abstract - Epistasis, or the interaction between genes, have not always been taken fully into account in genomic studies. Gene-gene interactions cause hidden quantitative genetic variation in natural populations and could be responsible for the small effects, missing heritability, and the lack of replication that are typically observed for complex traits. Epistasis should be considered in genetic analysis to better understand the genotype-phenotype relationship, by allowing for epistatic interactions between potential genetic loci, researchers may succeed in identifying genetic variants which might otherwise have remained undetected. The aim of this study was to perform a Genome-Wide Interaction Study to identify gene-gene interactions in beef fatty acid profile traits Nellore cattle. The investigated dataset contained records from 963 bulls, finished in feedlot (90 days) and slaughtered with approximately 24 months. Meat samples of Longissimus muscle, were taken to measure fatty acids composition (FA). FA were quantified by gas chromatography. The quality control of the SNP markers consisted of excluding those with unknown genomic position, located on sex chromosomes; monomorphic and markers with minor allele frequency less than 0.10; SNP that were out of Hardy-Weinberg equilibrium ($P < 10^{-6}$); call rate less than 90%, markers with excess heterozygosity and high correlated SNP. After quality control, 347,393 SNP from 890 animal samples were left. Missing genotypes were imputed using allele frequency estimates from a binomial distribution from the data. The SNP in the interactions $P < 10^{-8}$ were mapped individually, and used to search for candidate genes. A total of 602, 3, 13, 23, 13, 215 and 169 candidate genes for the sum of saturated fatty acids (SFA), monounsaturated fatty acids (MUFA), polyunsaturated fatty acids (PUFA), omega-3 (OM3), omega-6 (OM6), SFA:PUFA ratio and OM3:OM6 ratio were identified, respectively. The candidate genes found were associated to cholesterol, fat cell differentiation, lipid metabolic process and regulation, skeletal muscle development, low-density lipoprotein receptors, feed efficiency and inflammatory response. Enrichment analysis revealed 75 significant terms ($P < 0.05$), most of them related to meat quality and complementary terms. The number of significant interactions per sum of fatty acid trait varied greatly. This may indicate that some sum of fatty acid traits may need varying amounts of genetic redundancy or control for the creation of fatty acids critical to fitness. Visualization of significant epistatic interactions revealed many regions of potential regulatory control across multiple chromosomes. Our results showed substantial genetic interactions associated with lipid profile, meat quality, carcass and feed efficiency traits, indicating that gene-gene interactions may play very different roles in the control of phenotypic variation for lipid related traits in cattle.

Keywords: *Bos indicus, epistasis, GWIS, fatty acids, meat*

Resumo - A epistasia, ou a interação entre genes, nem sempre foi levada em consideração em estudos genômicos. As interações gene-gene causam variação genética quantitativa oculta em populações naturais e podem ser responsáveis por pequenos efeitos genéticos, pela "*missing heritability*", além de serem responsáveis por problemas de replicação que normalmente são observadas para características complexas. A epistasia deve ser considerada em análises genéticas para melhor entender a relação genótipo-fenótipo, ao permitir interações epistáticas entre os loci genéticos em potencial, os pesquisadores podem conseguir identificar variantes genéticas que, de outra forma, poderiam não ter sido detectadas. O objetivo deste estudo foi realizar um Estudo de Interação Genômica para identificar interações gene-gene para testar a epistasia em características de perfil de ácidos graxos da carne de bovinos Nelore. O conjunto de dados investigado continha registros de 963 touros, terminados em confinamento (90 dias) e abatidos com aproximadamente 24 meses. Amostras de carne do músculo *Longissimus* foram coletadas para medir a composição de ácidos graxos (AG). Os AG foram quantificados por cromatografia gasosa. O controle de qualidade dos marcadores SNP consistiu em excluir aqueles com posição genômica desconhecida, localizados nos cromossomos sexuais; monomórficos e marcadores com menor frequência alélica menor que 0,10; SNP que estavam fora do equilíbrio de Hardy-Weinberg ($P < 10^{-6}$), *call rate* inferior a 90%, marcadores com excesso de heterozigosidade e SNP muito correlacionados. Após o controle de qualidade, 347.393 SNP de 890 amostras de animais foram deixados. Os genótipos "ausentes" foram imputados usando estimativas de frequência de alelos a partir de uma distribuição binomial dos dados. Os SNP nas interações $P < 10^{-8}$ foram mapeados individualmente e usados para procurar genes candidatos. Foram identificados 602, 3, 13, 23, 13, 215 e 169 genes candidatos para a soma de ácidos graxos saturados (AGS), ácidos graxos monoinsaturados (AGMI), ácidos graxos poli-insaturados (AGPI), ômega-3 (OM3), ômega-6 (OM6), e as relações AGS: AGPI e OM3: OM6, respectivamente. Os genes candidatos encontrados foram associados ao colesterol, diferenciação de células adiposas, processo e regulação metabólica lipídica, desenvolvimento muscular esquelético, receptores de lipoproteínas de baixa densidade, eficiência alimentar e resposta inflamatória. A análise de enriquecimento revelou 75 termos significativos ($P <$ 0.05), a maioria relacionada à qualidade da carne e termos complementares. O número de interações significativas por característica de ácido graxo variou muito. Isso pode indicar que algumas características de ácidos graxos podem precisar de quantidades variáveis de redundância genética ou de controle para a criação de ácidos graxos essenciais para a aptidão. A visualização de interações epistáticas significativas revelou muitas regiões de controle regulador potencial em vários cromossomos. Nossos resultados mostraram interações genéticas substanciais associadas ao perfil lipídico, qualidade da carne, características de carcaça e eficiência alimentar, indicando que as interações gene-gene podem desempenhar papéis muito diferentes no controle da variação fenotípica de caracteres relacionados a lipídios em bovinos.

Palavras-chave: *Bos indicus, epistasia, GWIS, ácidos graxos, carne*

2.1 Introduction

In ruminant animals, the lipid profile is a reflection of the microbial transformations and synthesis that occur in the rumen (Church, 1974; De Smet et al., 2004). The adipose tissue of these animals is rich in triglycerides, with a predominance of saturated fatty acids and a small amount of polyunsaturated fatty acids(Mahgoub et al., 2002; Bas et al., 2007). Typically, 80% of fatty acids are myristic (C14:0), palmitic (C16:0), stearic (C18:0) and oleic (C18:1), the first two are known to raise blood levels of low-density lipoproteins, increasing the risk of heart attacks. To a lesser extent, there are still *trans* and *cis* isomers of unsaturated fatty acids. Several studies have portrayed the implication of certain constituents of meat in some of the most prevalent diseases in modern society, such as cancers, obesity, cardiovascular diseases, and hypertension. The quantity and quality of fat eaten is strongly related to human health (Rioux and Legrand, 2007).

The amount of intramuscular fat deposited in the *Longissimus* muscle is the main determinant of the carcass value and a predictor of palatability in the international scenario (Ferraz and Felício, 2010). Different muscles differ in fat content and may also differ in fatty acid composition (Webb et al., 1998), and generally, the nature and composition of fatty acids in muscle depend on the diet, intake, intestinal absorption, liver metabolism, and lipid transport (Geay et al., 2001).

The genetic factors that affect the composition of fatty acids in cattle have gained importance in recent years, and the number of studies has grown with the advent of genomic selection (Saatchi et al., 2013; A.S. Cesar et al., 2014; Kramer et al., 2016; Lemos et al., 2016a; Lemos et al., 2017). Genomic selection has been very successful in livestock species, it provides more genetic gain at a similar or lower cost than traditional selection (Goddard et al., 2010). Besides, it offers opportunities to study traits that are difficult/expensive to select and/or measure. The genomic selection allows us to work with different models to deal with different genetic architectures. It is possible to include non-additive effects such as dominance (Toro and Varona, 2010; Z. G. Vitezica et al., 2013; Zeng et al., 2013; Nishio and Satoh, 2014) and epistasis (Wittenburg et al., 2011b; Su et al., 2012; Muñoz et al., 2014).

In genomic selection, a Genome-Wide Association Study (GWAS) is an observational study of a genome set of genetic variants in different individuals to see if

any of the variants are associated with a trait of interest (Uitterlinden, 2016). GWAS using high-throughput technologies and phenotyping technologies have enabled the study of the relationship between phenotype and genotype at an unprecedented level of detail. Genomic studies have detected a large number of genetic variants associated with several complex traits. However, they fail to explain much of the phenotypic variation. The variants found through GWAS only explain a small portion of the heritability of complex traits (Maher, 2008; Manolio et al., 2009). The additional variation may be explained by the increased number of contributing loci, environmental effects, and the identification and characterization of genetic interactions.

Meanwhile, epistasis has been suggested to be considered for a better understanding of the genetic architecture of complex traits. Epistasis, generally defined as the interaction between different genes, has been a hot topic in quantitative genetics for a long time (Fisher, 1930; Wright, 1931; Cordell, 2009). There still is a controversy about the role of epistasis because the majority of researchers only concentrate on additive effects and most genetic variation is currently assumed additive (Falconer and Mackay, 1996; Hill et al., 2008b). Non-additive genetic effects have been largely ignored in the genetic evaluation of livestock for several reasons: the lack of informative pedigrees, the complex calculations, and statistical additive variance that captures biological dominance or higher-order interaction effects (Varona et al., 2018).

Following the traditional GWAS approach, genome-wide interaction analyses (GWIS) are used to investigate SNP-SNP interactions. This method does not need the selection of candidate sites; however, the computational time is a huge barrier. With the advancement of computing technology, the major barrier has been overcome, and SNP-SNP interaction studies gradually focused on the whole genome level (Goudey et al., 2013). Most of GWIS until now were performed in human genetics (Wei et al., 2012). SNP-SNP interactions have always been explained by mapping to gene-gene interactions, further genome-wide pathway-based association analysis will further support the interpretation of gene-gene interactions. This approach can provide additional biological insights and allow one to explore new candidate genes (Wang et al., 2011).

Despite the recent achievements in the study of fatty acids using genomic selection (Chiaia et al., 2017; Feitosa et al., 2017a; Lemos et al., 2017; Aboujaoude et al., 2018;

Dos Santos Silva et al., 2019), there is still a drastic shortage of non-additive gene action studies in cattle breeds. Although selection on fatty acid content in cattle with the inclusion of epistatic effects may not be very advantageous due to the breakdown of epistasis during selection (Huang et al., 2012), the ability to identify the interactions may lead to further understanding of the molecular mechanisms underlying variation in lipid profile, as well as to better phenotype predictions. This study aimed to identify epistatic interactions that could account for additional genetic variation in the fatty acid profile of beef of Nellore cattle.

2.2 Material and Methods

This study was approved by Ethics Committee of the Faculty of Agrarian Sciences and Veterinary, São Paulo State University (FCAV-UNESP, Jaboticabal, São Paulo, Brazil).

2.2.1 Animals and Management

The dataset comprises phenotypic and genotypic information from 963 Nellore bulls from eight farms located in the Southeast, Northeast and Midwest of Brazil, which participate in three beef cattle breeding programs (Nelore Qualitas, Paint and DeltaGen). The three breeding programs are focused in select animals based on growth, finishing and sexual precocity traits.

Farms located in the Midwest and Southeast regions, concentrated their breeding season between August and October, coinciding with the period of greatest grazing digestibility. The farms located in the Northeast region adopted different breeding seasons from November to January. The choice of the breeding season depends on several factors, such as climatic conditions, availability of pastures, labor, and adequate time for calves to be born. Normally, the breeding season lasts from 60 to 120 days. The weaning was performed at seven months of age. Animals were raised on grazing conditions using *Brachiaria sp*. and *Panicum sp.* forages, and free access to mineral salt, at density varying from 1.2 to 1.6 animal unit/hectare (AU/ha). After yearling, the breeding animals were selected and the rest remained in feedlot (approximately 90 days). During feedlot, the forage: concentrate ratio ranged from 50:50 to 70:30, depending on the farm.

In general, whole-plant corn or sorghum silage was used as high quality forage. Grains of corn and/or sorghum, soybeans, soybean meal, or sunflower seeds were used as feed protein concentrate.

Animals were slaughtered based on weight (500-550 Kg), and they had an average of 24 months when slaughtered. The slaughter occurred in commercial slaughterhouses under Brazilian Federal Inspection Service (SIF), in accordance with the commercial standard procedure. After stored for 48 hours at 0-2°C, beef samples from *Longissimus thoracis* muscle, between the 12th and 13th ribs of the left half-carcasses were collected and placed in plastic bags to be stored at −80°C to further measure the fatty acids profile.

2.2.3 Determination of fatty acid profile

The total lipid concentration was quantified at the Animal Product Technology Laboratory in the Technology Department of FCAV/UNESP using the Bligh and Dyer (Bligh et al., 1959) method.

The fatty acid profile was determined at the Meat Science Laboratory (LCC) in the Department of Animal Nutrition and Production at FMVZ/USP (Pirassununga, São Paulo, Brazil). Meat fatty acids were extracted from intramuscular fat of the *Longissimus thoracis* muscle as described by Folch et al. (1957). Muscle samples (~100 g) were collected and ground to determine the fatty acid profile. The lipids were extracted by homogenizing the sample with a chloroform and methanol (2:1) solution. NaCl at 1.5% was added to isolate the lipids.

The separated fat was methylated, and the methyl esters were formed according to Kramer et al. (1997). The fatty acids were quantified by gas chromatography (GC-2010 Plus AOC 20i auto-injector, Shimadzu, Kyoto, Japan) with a SP-2560 capillary column (100 m x 0.25 mm I.D. x 0.02 mm, Supelco, Bellefonte, PA). Gas chromatography is extensively used technique for identifying and detecting the components of a mixture of compounds. It is very similar to fractional distillation and separates the components of a mixture primarily based on boiling point differences, but at a fine micro level. Gas chromatography is an efficient and cost-effective method of separation, requires small sample and provides fast and highly accurate quantitative analysis. The initiating temperature of 70°C was increased gradually up to 175°C (13°C/min), held for 27

minutes, and increased further up to 215°C (4°C/minute) and held for 31 minutes. Hydrogen (H_2) was the carrier gas, flowing at 40cm^3/s. The temperature used by the flame ionization detector (FID) was 250°C, H_2 flow of 40 mL/min, air flow of 400 mL/min, Make-up of 30 mL/min kPa (N_2), and Sampling Rate of 40 msec.

The total running time of each sample (stop time) was 86 minutes. Fatty acids were identified by comparing the retention time of sample methyl esters with the fatty acids standard C4-C24 (F.A.M.E mix Sigma®) and GLC 463 Reference Mixture Nu Check, vaccenic acid C18:1 trans-11 (V038-1G, Sigma®) C18:2 trans-10 cis-12 (UC-61M 100mg), CLA and C18:2 cis9, trans-11 (UC- 60M 100mg), (Sigma®), tricosanoic acid (Sigma®), and nonadecanoic acid (Sigma®).

Fatty acids were quantified by normalizing the area under the curve of methyl esters using the GS 2.42 software. Fatty acids contents were expressed as percentage of total fatty acid methyl ester quantified. The sum of saturated fatty acid (SFA) (C10:0 + C11:0 + C12:0 + C13:0 + C14:0 + C15:0 + C16:0 + C17:0 + C18:0 + C21:0 + C24:0), monounsaturated (MUFA) (C16:1 + C17:1 c10 + C18:1 t11 + C15:1 c10 + C20:1 c11 + C24:1 + C22:1 n9 + C18:1n9c + C14:1 + C18:1 n9t), polyunsaturated (PUFA) (C18:2 n6 + C18:3 n3 + C18:3 n6 + C20:3 n3 cis-11, 14, 17 + C20:3 n6 cis-8, 11, 14 + C20:4 n6 + C20:5 n3 + C22:6 n3), ômega-6 (n-*6*) (C18:3 n6 + C20:3 n6 c8, c11, c14 + C18:2 n6 + C20:4 n6) and omega-3 (n-*3*) (C18:3 n3 + C20:3 n3 c11, c14, c17 + C22:6 n3 + C20:5 n3) were calculated. The polyunsaturated/saturated fatty acids and n-6/ n-3 ratios were also quantified. The following phenotypes were chosen in this study: sum of i) SFA, ii) MUFA, iii) PUFA, iv) Omega-3 (OM3), v) Omega-6 (OM6), the ratios of vi) SFA: PUFA, and vii) OM3-OM6.

2.3.4 Fatty acid content and heritability estimates

The descriptive statistic for intramuscular fat (IMF %), sum of SFA, MUFA, PUFA, OM3, OM6 and the ratios of SFA:PUFA and OM3:OM6 and the heritability estimates were previously estimated in Lemos et al. (2016)) GWAS study. Table 1 presents their results.

The sum of fatty acids that presented the highest content in the samples of *Longissimus thoracis* analyzed was the sum of SFA (40.621± 6.117), followed by MUFA (37.581± 8.144), PUFA (13.457± 5.586), OM6 (9.374± 4.450), OM3 (3.820± 1.558).

The heritability was estimated considering a linear animal model (ssGBLUP). The Gibbs sampling approach was used to estimate de (co)variance components. To check the convergence for all estimated parameters, the author's verified the convergence of the chain by inspecting the trace-plots combined with a the Geweke's and Heidelberger and Welch convergence diagnostic (Heidelberger and Welch; Gianola and Fernando, 1986; Geweke, 1991). The heritability estimates for the sum SFA, MUFA, PUFA, OM3, OM6 and their ratios were low to moderate, ranging from 0.07 (SFA:PUFA ratio) to 0.23 (OM6). The sum of OM3, the OM3:OM6 ratio, and the sum of SFA and PUFA (<0.12). However, moderate heritability estimates were obtained for MUFA (0.20) and OM6.

Table 1. Descriptive statistics and heritability estimates for fatty acid profile[1]

Trait	[b]N	Min	Max	Mean ± SD[c]	h^2 ± *SD*
[a]Intramuscular fat (%)	943	-	-	0.83±0.42	-
Sum of SFA	891	2.559	52.357	40.621± 6.117	0.12±0.07
Sum of MUFA	891	3.751	64.930	37.581± 8.144	0.20±0.15
Sum of PUFA	891	2.067	37.623	13.457± 5.586	0.08±0.05
Sum of OM3	891	0.240	11.356	3.820± 1.558	0.11±0.07
Sum of OM6	891	0.742	33.870	9.374± 4.450	0.23±0.10
SFA:PUFA ratio	891	0.041	3.030	0.348± 0.201	0.07±0.05
OM3:OM6 ratio	891	0.123	11.154	2.535± 0.972	0.11±0.08

[1] Fatty acid content and heritability estimated by Lemos et. al. (2016). [a]The concentration of fatty acids are expressed as a percentage of total fatty acid methyl esters (FAME) quantified. [b] *N* number of animals with records. [c] *SD* standard deviation. SFA: saturated fatty acids. MUFA: monounsaturated fatty acids. PUFA: polyunsaturated fatty acids.

2.3.5 Genotyping of Animals

A total of 963 animals were genotyped using 777,962 SNPs (Bovine high-density chip, Illumina, San Diego, CA, USA). The quality control of the SNP markers consisted of excluding those with unknown genomic position, located on sex chromosomes; monomorphic and markers with minor allele frequency (MAF) less than 0.10; SNP that were out of Hardy-Weinberg equilibrium with very low probability (*p*-value < 10^{-6}); call

rate less than 90%, markers with excess heterozygosity and high correlated SNP. After quality control, 347,393 SNP from 890 animal samples were left. PREGSF90 software was used for SNP quality control (Misztal et al., 2002). Missing genotypes were imputed using allele frequency estimates from a binomial distribution from the data. All SNP markers were assigned a UMD3.1 bovine genome build position.

2.3.6 Genome-Wide Interaction Analysis

The method used in this study was a fast approach derived from Hilbert-Schmidt Independence Criterion (HSIC) to test whether the two variants are independent of the phenotype (Gretton et al., 2005). This approach was first applied by Kam-Thong et al. (2011) on epistasis detection, showing the close relationship between HSIC and linear regression by examining the derivation of estimates using the least squares regression method. Intuitively, HSIC can be thought of as a squared correlation coefficient between two random variables x and y computed in feature spaces $\mathcal{F}$ and $\mathcal{G}$. Given a finite number of observations $\boldsymbol{m}$ and for each $x \in \mathcal{X}$, $y \in \mathcal{Y}$, empirical HSIC as reported by Gretton et al. (2005) as:

$$\mathrm{HSIC}(Z,\mathcal{F},\mathcal{G}) := (m-1) - 2\mathbf{tr}(\mathbf{KHLH})$$

Where $\mathcal{F}$ and $\mathcal{G}$ are reproducing kernel Hilbert spaces on X and Y with associated Kernels $k: \mathcal{X} \times \mathcal{X} \to \mathbb{R}$ and $\mathcal{Y} \times \mathcal{Y} \to \mathbb{R}$. $Z := \{(x_1, y_1), \ldots, (x_m, y_m)\} \subseteq \mathcal{X} \ltimes \mathcal{Y}$. $\mathbf{H},\mathbf{K},\mathbf{L} \in \mathbb{R}^{m \times m}$, $\mathbf{K}_{ij} := k(x_i, x_j)$, $\mathbf{L}_{ij} := l(y_i, y_j)$ and $\mathbf{H}_{ij} := \delta_{i,j} - m^{-1}(\delta_{i,j} = 1 \text{if } i = j;\ \delta_{i,j} = 0$, otherwise). Here, i and j are the indices in the matrix. HSIC is zero if and only if the random variables are independent (Gretton et al., 2005).

The dependence between the pair of variants and the phenotype, the kernels for the phenotype and the potential epistatic variants are measured by:

$$k(x_i, x_i) = \emptyset(x_i)\emptyset(x_i) = \tilde{x}_{A_i}\tilde{x}_{B_i}$$
$$l(y_i, y_i) = \varphi(y_i)\varphi(y_i) = \tilde{y}_i$$

Where $\tilde{x}_{A_i}$, $\tilde{x}_{B_i}$ and $\tilde{y}_i$ represents variant A, variant B and the phenotype, which are all Z-score normalized. Then, the empirical HSIC for epistasis is described as:

$$\mathrm{epiHSIC}\big((\mathrm{X},\mathrm{Y}),\mathcal{F},\mathcal{G}\big) \propto \sum_{i=1}^{m} \tilde{x}_{A_i}\tilde{x}_{B_i}\tilde{y}_i$$

where X is the epistatic effect, and Y represents the phenotypic score. A Z-test is further performed on the correlation and $\mathrm{epiHSIC}$ values to obtain the significance for each pair of SNP. The significance threshold (*Z-score*) to select variant pairs for output was 1×10^{-5}.

A total of 1,554,267 (SFA), 1,251,284 (MUFA), 1,048,575 (PUFA), 1,139,090 (OM3), 1,566,967 (OM6), 7,138,754 (SFA: PUFA), 4,481,479 (OM3:OM6) valid SNP-SNP tests were performed. We then selected the SNP with interaction $\mathrm{P} < 1 \times 10^{-8}$. For the sum of SFA 744 interactions were left, 15 for MUFA, 640 for PUFA, 141 for OM4, 87 for OM6, 1210 for SFA:PUFA ratio, and 724 for OM3:OM6 ratio. After that, we applied a Bonferroni-corrected test with a significant threshold $P = 4.05 \times 10^{-13}$. After the Bonferroni correction 61, 2, 29, 15, 5, 724, 154 valid SNP-SNP interactions were left for the sum of SFA, MUFA, PUFA, OM3, OM6, SFA:PUFA ratio and OM3:OM6 ratio were left for further analysis.

In order to rule out the possibility of an accidental finding, we mapped these SNPs to genes, then excluded the SNP-SNP interactions by the following criteria: i) neither SNPs exist in genes; ii) either of the two SNPs exist independently in a gene.

Throughout this study, we used R statistical computing environment (R Development Core Team, 2019) to scan for genomic interactions with Hilbert–Schmidt Independence Criterion with the ***episcan*** R package (Beibei and Putz, 2018).

2.3.7 Gene searching

The SNP pairs from significant interactions were placed in the cattle UMD3.1 genome assembly by the Ensembl Biomart tool with the Genes 94 database (Haider et al., 2009). The classification of genes regarding their biological function and appropriate analysis of metabolic pathways was performed by The Database for Annotation, Visualization and Integrated Discovery (DAVID) v. 6.8, (Huang et al., 2007; Huang et al., 2009a) using all annotated genes in the cattle genome as background. Kyoto Encyclopedia of Genes and Genomes (KEGG), and Gene Ontology (GO) databases were used for functional enrichment analysis considering a *p*-value < 0.1 threshold for

significance. Terms associated with fatty acids were used to identify genes associated with fatty acid epistasis.

3.0 Results

3.1 Genome-Wide Interaction Studies

GWIS based on Z-score for sum of SFA, MUFA, PUFA, OM3, OM6, SFA: PUFA and OM3:OM6 ratio determined 744, 15, 640, 141, 87, 1210, 724 SNP-SNP interactions with $P < 10^{-8}$, respectively. After the Bonferroni criteria, 61 interactions remained for SFA, 2 for MUFA, 29 for PUFA, 15 for OM3, 5 for OM6, 724 for SFA:PUFA ratio, and 154 for OM3:OM6 ratio (Figures 2-).

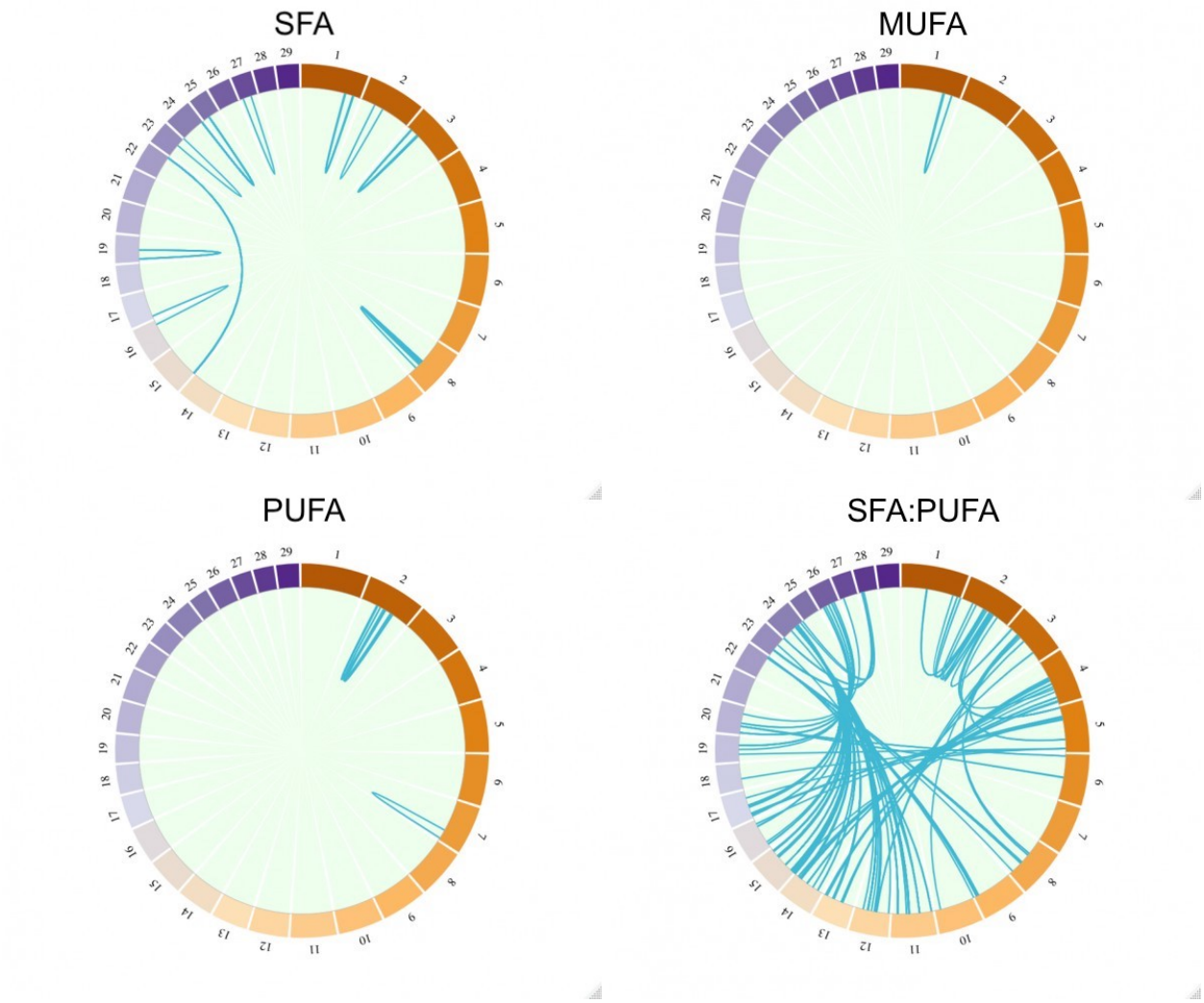

Figure 2 - Circos visualization of mapped SNP-SNP interactions for the sum of SFA, MUFA, PUFA and SFA:PUFA ratio ($P < 1 \times 10^{-8}$)). The curves represent the interactions between the two SNPs.

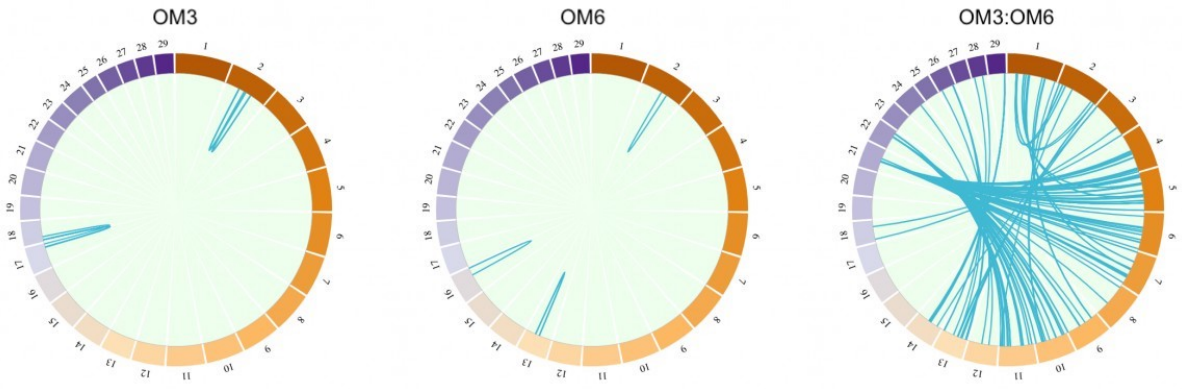

Figure 3 - Circos visualization of mapped SNP-SNP interactions for the sum of OM3, OM6, and OM3:OM6 ratio ($P < 1 \times 10^{-8}$). The curves represent the interactions between the two SNPs.

3.2 SNP-SNP interactions

Interactions are placed in the Appendix Section of this study. Markers exact positions (in *bp)* on the genome can be found in Tables 2-8.

The interaction between marker 299840 (*PIGN*)-299962 (*PHLPP1*) in chromosome 24 presented the lowest *p*-value (3.92×10^{-14}) after screening by exclusion criteria for SFA (Table 2). For PUFA, the interaction with the lowest *p*-value (6.39×10^{-14}) was between markers 29329 (*ZRANB3*)-29951 (*DPP10*) in chromosome 2 (Table 3). MUFA only has two significant interactions (both in chromosome 1), the first one between marker 14329 (*KCNAB1*)-14852 (*MBNL1*) showed a *p*-value of 2.66×10^{-10} after the Bonferroni correction, and the second, between markers 14329 (*KCNAB1*)-14398 (*PLCH1*) presented a p-value of 1.65×10^{-9} (Table 4).

When we evaluated the fifteen interactions of the sum of OM3 (Table 5), markers 26767 (*FMNL2*)- 26824 (*CACNB*4) in chromosome 2 had the lowest *p*-value (2.86×10^{-14}). The sum of OM6 (Table 6) had five significant interactions in chromosomes 2, 13 and 16. Markers 190071 (*FRMD4A*) and 191553 (*RALGAPA2*) in chromosome 13 had the smallest *p*-value (1.04×10^{-12}). Two other interactions were mapped in chromosome 13 between markers in the same genes as mentioned before (190067-191553 and 190068-191553).

The SFA:PUFA and OM3:OM6 ratios kept the highest number of interactions among the traits evaluated in this study. Starting with the SFA:PUFA ratio (Table 7), we found interactions in all chromosomes except chromosomes 7, 21, 25 and 29 (Figure 2). The most significant interaction for SFA:PUFA ratio was between markers 252170 (*ASIC2*) in chromosome 19 and 306894 (*ATAD1*) in chromosome 26 (*p*-value = 1.91×10^{-30}). Finally, for the OM3:OM6 ratio (Table 8, Figure 3) the first two interactions between markers 157768 (*FUT8*) – 281870 (*FHIT*), and the second between 157769 (*FUT8*) – and 281870 (*FHIT*).

3.3 Gene searching

The SNP in the significant interactions $P < 10^{-8}$ were mapped individually, and used to search for candidate genes (CG), which were described in Supplementary Material. A total of 602, 3, 13, 23, 13, 215 and 169 candidate genes for SFA, MUFA,

PUFA, OM3, OM3, SFA:PUFA ratio and OM3:OM6 ratio were identified, respectively. 66 genes were common with at least one trait, up to four traits.

The candidate genes found for SFA were associated to the regulation of cholesterol biosynthetic process, cholesterol metabolic process, fat cell differentiation, metabolic process of glycolipids, lipid metabolic process and regulation, regulation of lipolysis in adipocytes, lipid transport, regulation of sequestering of triglyceride, lipoprotein lipid oxidation, white fat cell differentiation, and regulation of skeletal muscle fiber development. For MUFA, the genes were related to oxidation-reduction process, and transport and activity of potassium ion.

The genes found for PUFA were associated to the muscle fiber development, and protein lipidation. As for the sum of OM3, triglyceride metabolic and cholesterol biosynthetic process, fat cell differentiation and regulation of fatty acid oxidation were the functions of some genes found in the SNP marked. The SFA:PUFA and OM3:OM6 ratio showed many genes associated to the cholesterol metabolic and biosynthetic process, cholesterol transport, metabolism of lipids, high-density lipoprotein particle remodeling, low-density lipoprotein (LDL) receptors, regulation of cholesterol storage, response to LDL particle, regulation of fat cell differentiation and triglyceride metabolic process.

3.4 Enrichment Analysis

The analysis performed using the DAVID v.6.8 (Huang et al., 2009b; Huang et al., 2009a) comprised 912 protein-coding genes identified using the bovine reference genome assembly UMD3.1. We found 75 significant terms ($P<0.05$) most of them related to meat quality and complementary terms. The functional enrichment analysis revealed 28 gene ontology biological processes, 13 gene ontology molecular functions, 16 gene ontology cellular components, and 18 KEGG pathways as significant (Table 9, Appendix Section)

Some of the significant terms were insulin secretion (bta04911), glucose metabolic process (GO:0006006), smooth muscle tissue development (GO:0048745), small GTPase mediated signal transduction (GO:0007264) and carbohydrate digestion and absorption (bta04973).

4.0 Discussion

4.2 Genome-Wide Interactions

GWAS are hypothesis-free methods for identifying associations between genetic regions (or loci) and traits (Uitterlinden, 2016). It has long been known that genetic variation between individuals can cause differences in phenotypes. Commonly-used univariate analysis techniques have been able to detect a number of significantly associated loci, for many conditions these discovered variants do not account for a majority of the theoretical estimates of genetic heritability (Zuk et al., 2012). One hypothesis for missing heritability is that the additive modelling used in GWAS analysis is insufficient to model the interaction between genotype and phenotype (Zuk et al., 2012), under this hypothesis the interactions between variants needs to be considered in order to accurately the influence of genetic variation and model complex traits. It has been suggested that 2-way and 3-way SNP interactions can explain up to ~ 50% and ~ 100% of trait variance while each SNP involved explains none, indicating that critical SNP pairs may be ignored by standard analysis predominantly applied to GWAS so far (Culverhouse et al., 2002). In this sense, GWIS are becoming a more widely used method to provide valuable evidence of potential gene–gene and gene-environment interactions contributions towards phenotypes of interest (Goudey et al., 2013).

In the case of FA and meat quality markers, testing for potential interactions between SNPs and FA on biomarker levels may provide a more complete picture as to how an individual's genetics modulate FA levels leading to differential effects on biomarker levels. Here we report the interactions and candidate genes related to lipids, fatty acids, and meat quality traits found.

4.2.1 Sum of SFA

Oils and fats, both animal and vegetable, are part of the human diet triangle, the other two being proteins and carbohydrates. Ideas and recommendations about the importance of oils and fats in human nutrition have undergone a continuous change

during the last few years. Red meat has been classified within the category of foods rich in fat, and is very critically pointed out when it comes to healthy eating - some tables of the chemical composition of meat are very outdated, and may have a high-fat content, that is not currently observed. Beef provides essential nutrients with high biological value, such as proteins, vitamins, essential fatty acids, and minerals, but it has been associated with the appearance of cardiovascular diseases, due to the characteristics of its fat, which has higher concentrations of SFA and lower concentrations unsaturated fatty acids compared to non-ruminant fat (Lopes et al., 2012).

PIGN – PHLPP1 gene interaction

In the present study, our GWIS for SFA found 25 interactions between *PIGN* and *PHLPP1* genes that may contribute to the inflammatory response. The *PIGN* gene plays a role in the glycosylphosphatidylinositol (GPI) biosynthesis. The GPI is a phosphoglyceride linked to a wide variety of biological processes (Paulick and Bertozzi, 2008). It is worth mention the role of GPI in glycosylation, which is the process where a carbohydrate is attached to a target macromolecule (protein or lipid), affecting cell-to-cell adhesion, an important mechanism of the immune system, which recognize specific carbohydrate moieties (Grab et al., 1987).

Inflammation is one of the body's response to infection or injury, and it is the beginning of the immunological process of elimination of pathogens, and reparation of damaged tissues. It is typically identified by swelling, pain, redness and heat – these responses occur as a result of increased blood flow into the injured region, and permits antibodies to leave the bloodstream and cross the endothelial wall. Although much is known about the kinases that control inflammatory signaling, little is known about the opposing phosphatases like the PH domain Leucine-rich repeat Protein Phosphatase 1 (*PHLPP1*). *PHLPP1* was originally discovered for its function in suppressing growth factor, but it is also involved in the immune response (Gao et al., 2005; Li et al., 2011; Patterson et al., 2011; Bradley et al., 2015). Katsenelson et al. (2019) reported an interaction between *PHLPP1* and *STAT-1* in mice, where *PHLPP1* acts on the macrophages, controlling the duration of inflammatory signaling by dephosphorylating the transcription factor STAT1 reducing the expression of target genes involved in innate immunity and cytokine signaling.

In cattle, *PHLPP1* was reported by Pereira et al. (2016) as a growth-related gene, as well as Hartati et al. (2015), who reported the same gene in Indonesian Peranakan Ongole cattle. A genome-wide association study for the growth and feed intake trajectory found regions associated with *PHLPP1* (Howard et al., 2015) on Duroc Boars, and Andreozzi et al. (2011) reported that *PHLPP1* abundance is increased in adipose tissue and skeletal muscle of obese individuals, and is also significantly related to body mass index and insulin resistance. Obesity is associated with macrophage accumulation in adipose tissue, being directly proportional to measures of adiposity in mice and humans. When the adipose tissue becomes inflamed, it results in insulin resistance. Studies have shown that diets with a high content of SFA results in insulin resistance, because SFA activates the Toll Like Receptor 4 (*TLR4*), increasing the expression of a number of inflammatory genes in adipocytes. This mechanism by which SFA activates *TLR4* involve the balance between saturated and unsaturated fats (Lee et al., 2001; Lee et al., 2003; Weisberg et al., 2003; Shi et al., 2006).

STK3 – RAB7A gene interaction

The interaction between STK3 and RAB7A genes had a *p*-value of 2.26×10^{-11}. *STK3* gene encodes a serine/threonine protein kinase activated by proapoptotic molecules indicating the encoded protein functions as a growth suppressor (Camgoz et al., 2018). A GWAS study on the body temperature changes of a broiler-type strain chickens under acute heat stress (Zhuang et al., 2019) revealed that *STK3* is involved in signal transduction and programmed cell death pathway, also it was located in body temperature QTL region, suggesting that SNPs are suitable markers for the genetic selection of thermotolerance. The role of *STK3* in livestock species is still unclear, these gene was also related to milk protein yield in Nordic Holstein cattle (Cai et al., 2018). In a fine QTL mapping for meat quality traits in French Charolaise breed, *STK3* was reported as a gene implying in fat metabolism, expressed in bovine muscle (low) and adipose (medium) tissue (El Hou et al., 2019). The gene was also reported in traits like feed efficiency, performance, carcass weight and marbling in beef cattle (Lee et al., 2011; Abo-Ismail et al., 2014) , and milk yield and fat yield in dairy cattle (Cai et al., 2020).

RAB7A gene is a key regulator in endo-lysosomal trafficking, that plays a central role, in many other cellular and physiological events, such as growth-factor-mediated cell

signaling, nutrient-transporter mediated nutrient uptake, and lipid metabolism (Edinger et al., 2003). An alternative pathway of lipid metabolism through the lysosomal degradative pathway of autophagy has been described as lipophagy, which *RAB7A* impacts (Liu and Czaja, 2013). Lipophagy generates free fatty acids from breaking triglycerides of mitochondrial *β*-oxidation. This process regulates intracellular lipid stores levels of free lipids such as fatty acids and energy homeostasis. The quantity of metabolized lipids varies in response to the nutrients extracellular supply. Lipophagy can lead to excessive lipid accumulation in tissues, such as hepatic steatosis, altering hypothalamic neuropeptide release that affects body mass and may lead to diseases like Type 1 diabetes (Regnell et al., 2017). Exogenous fatty acids regulate lysosomal (lysosomes are a major site of nutrient sensing in cells) nutrient sensing depending on their structure, Kwon and Querfurth (2015). Yasuda et al (2014) reported that saturated fatty acids activate and unsaturated fatty acids inhibit, although caution must be noted in interpreting data involving exposure to a high concentration of a single fatty acid as this is unphysiological compared to the diverse mixture of fatty acids cell are exposed to *in vivo*. A longitudinal analysis of hepatic transcriptome and serum metabolome demonstrated altered lipid metabolism on diabetic rats by Regnell et al. (2017) reported *RAB7A* in the functional analysis for lipid metabolism in the concentration of cholesterol and sterol.

Genes involved in inflammation process, efficiency and lipid metabolism

From the genes found in each interaction of SFA, it is worth mention *ARHGEF26* gene, that was associated to the progression of coronary artery disease, because it may regulate vascular inflammation by affecting the function of vascular cells (Klarin et al., 2017; Zhu et al., 2018). In cattle, a meta-analysis of the mesenteric fat from crossbred beef steers reported *ARHGEF26* associated with body weight gain and feed intake, specifically involved in cellular development and growth (Lindholm-Perry et al., 2020).

The orphan nuclear receptors of the NR4A family play an important role in maintaining cellular homeostasis (Holla et al., 2011). The NR4A family includes three members: *NR4A1*, *NR4A2*, and *NR4A3*. Pearen et al. (2008) showed that the NR4A3 expression increased by the β-adrenergic receptor in skeletal muscle, which modulates lipolysis, energy expenditure, and fatty acid utilization in skeletal muscle. The studies have associated that the expression of *NR4A3* reduced the expression of genes

associated with glucose and fatty acid in a skeletal muscle cell line. When *NR4A3* was hyper expressed, it increased the ability of insulin to stimulate glucose transport. Thus, *NR4A3* gene expression is thought to be involved in the regulation of genes that control glucose and fatty acid utilization in skeletal muscle. *NR4A3* gene expression was reduced in the skeletal muscle and in the adipose tissue of diabetic and insulin-resistant rodents (Fu et al., 2007). *NR4A3* was reported in a feed efficiency study in pigs using a transcriptome analysis of the adipose tissue and was identified as one of the key genes involved in fatty acid oxidation in high feed efficient pigs (Xu et al., 2018).

The *PIP5K1B* gene encodes the Phosphatidylinositol-4-Phosphate 5-kinase, Type1, Beta enzyme. Although the poor knowledge of the enzyme function, the produced bioactive lipid is an active signaling molecule involved in cell survival and apoptosis, epithelial cell morphogenesis. In Norwegian-Swedish horses, a GWAS study linked *PIP5K1B* gene to the negative regulation of oxidative stress (Velie et al., 2018). Oxidation is a normal and necessary process that takes place in every organism. Oxidative stress, on the other hand, occurs when there's an imbalance between free radical activity and antioxidant activity that can lead to a vast number of diseases, such as hypertension, diabetes, and atherosclerosis (Storz and Imlay, 1999; Burton and Jauniaux, 2011). When exploring genomic variants related to residual feed Intake in chickens, Liu et al. (2018) reported PIP5K1B participated in in carbohydrate and lipid metabolism in the liver of Beijing-You chickens.

4.2.2 Sum of PUFA

FMNL2 interactions with GALNT13 and NEB

Meat tenderness is considered to be the most important trait affecting consumer satisfaction (Miller et al., 2001; Shackelford et al., 2001). One of the factors that can influence meat tenderness is myofibrillar proteins. Actomyosins make up most of the proteins in the post-mortem muscle, and the often-observed stiffness can be associated with the formation of actomyosin bridges, a process similar to muscle contraction. The number of actomyosin bridges formed during rigor, however, is much greater than in muscle contraction (Lawrie, 2006). When there is not enough energy in the muscle to break the actomyosin bonds, relaxation in the case of rigor mortis is not possible. The

meat tenderization process occurs during refrigerated storage, it consists of the proteolysis of the structural components of the myofibrils, promoting the degradation of the Z line (which leads to the degradation of the myofibrils). This and other changes cause a decrease in stiffness and a gradual increase in meat tenderness (Lian et al., 2013).

The Formin like 2 (*FMNL2*) gene is part of the diaphanous-related formin family, that consists of proteins that controls cytoskeletal organization. As most formins, the mouse *FMNL2* orthologue is known to polymerize actin filaments. Actin-based processes are regulated by diaphanous-related formin, which influences meat tenderness associated proteolysis. Proteolysis contribute to disruption of myofibrils and the overall structural integrity of the muscle, resulting in increased meat tenderness (Hopkins and Thompson, 2001). Studies by Olson (2003) and Zhao et al. (2012) showed that specific GTPases are affected by diaphanous-related formins, affecting meat tenderness in cattle.

GALNT13 is one of 20 enzymes known to initiate O-glycosylation. This process of the modification a serine or threonine residues on proteins by addition of an amino sugar derivative of galactose, has several functions in organism, especially in the immune system and cell metabolism control (Van Den Steen et al., 1998; Goettig, 2016). Functions of *GALNTs* are not yet fully understood, but studies point that they may affect lipoprotein metabolism and fat deposition in intramuscular space (Schjoldager et al., 2010; Schjoldager et al., 2012) . A study in humans suggested that an allele of *GALNT13* is involved in energy pathways (Wang et al., 2014). In cattle, *GALNT13* was reported by Abo-Ismail et al (2018) as associated with metabolic weight and back fat in beef catle using genotypic effect and additive and dominance models (Abo-Ismail et al., 2018).

The *NEB* gene codifies the nebulin protein, that plays an important role in skeletal muscles (Stedman et al., 1988). Within skeletal muscle cells, nebulin is found sarcomeres – necessary for muscle contraction. Nebulin accounts for 3 to 4% of the total myofibrillar protein, Root and Wang (2001) suggested that *NEB* gene plays a different role in muscle sarcomeres, acting as a regulator of muscle contraction by inhibiting cross-bridge formation of thin filaments. A previous study by Anderson and Kunkel (1992) reported that the loss of several myofibril proteins such as nebulin, actin, and filamin can an increase of the adipose mass in muscle. The variation in the *NEB* gene in their study was associated with the different amounts of fatty acids in muscle, such as lauric acid (C12:0),

palmitic acid (C16:0), palmitoleic acid (C16:1), gondoic acid (C20:1), conjugated linoleic acid (C18:2c9t11), linoleic acid:γ-linolenic acids (18:2/18:3 ratio), suggesting that an increase in the cytoskeletal matrix can cause a decrease in adipose tissue. Lee et al. (2007) identified differentially expressed genes related to intramuscular fat development in Hanwoo cattle, and found that *NEB* gene was highly expressed in individuals that produce meat with low marbling. Finally, Dunner et al. (2013) reported pleiotropic effects in the *NEB* gene in 15 European *Bos taurus* breeds affecting several beef fatty acids. This gene was also reported and associated with lipid composition in the genomic study of beef fatty acid profile in Nellore cattle by Lemos et al. (2016).

DPP10 – MGAT5

Inactive dipeptidyl peptidase 10 is a protein encoded by the *DPP10 gene*, associated to the docosahexaenoic acid measurement in humans. Docosahexaenoic acid (DHA) is an omega-3 fatty acid mainly found in seafood, such as fish, shellfish, and fish oils. It comprises 40% of the polyunsaturated fatty acids (PUFA) in the brain and 60% of the PUFA in the retina (Allaire et al., 2016; Allaire et al., 2017; Guo et al., 2017; Sekikawa et al., 2019). Fatty acids from the Omega-3 family are linked to a reduced risk of heart disease, especially the long-chain omega-3 fatty acids from fish oils, such as DHA. Xu et al. (2019) showed the beneficial effects of *n*-3 PUFAs on pulmonary function in humans by modeling *n*-3 PUFA genome-wide interactions, and identified a novel *DPP10* SNP association with pulmonary health.

MGAT5 gene encodes a protein of the glycosyltransferase family. It is one of the most important enzymes involved in the regulation of the biosynthesis of glycoprotein oligosaccharides. Oligosaccharides alterations on cell surface glycoproteins cause significant changes in the adhesive or migratory behavior of a cell. *MGAT5* is involved in the biosynthesis of N-glycan that have been implicated as positional candidate for large-effect gene for DMI in Angus and Hereford (Do et al., 2013; Seabury et al., 2017) . Johswich et al. (2014) reported that *MGAT5* deficient mice shown to experience diminished glycemic response to exogenous glucagon, and increased insulin sensitivity. *MGAT5* was also reported in both pigs and dairy cattle. In Duroc pigs, *MGAT5* was pointed as a candidate gene for intramuscular fat content (Ding et al., 2019). In Chinese

Holstein cattle, the gene was reported as a suggestive candidate gene for stearic acid (C18:0), a long-chain saturated fatty acid (Li et al., 2014).

4.2.3 Sum of MUFA

Monounsaturated fatty acids are considered non-essential, since they can be synthesized de novo. However, MUFA such as oleic acid, make a major contribution to dietary fat intake in many populations, often constituting at least a third of the total fatty acid intake (ex. Mediterranean diets). Studies investigating the effects of MUFA-rich diets on immune function have often been overshadowed by those which investigate the feeding of diets rich n-3 and n-6 PUFA, known by its effects on suppress immune and enhance immune functions, respectively (Calder, 2002). However, there is evidence that MUFA have effects which are similar to fish oils in animals.

An interesting study in humans by Mata et al. (1996) proposed to test four isocaloric dietary periods different in fat content (SFA, MUFA, n-3 and n-6) in healthy man and women. The authors reported that during the MUFA period, the monocyte adhesion to endothelian cells was lower, and the resistance of LDL to oxidation was greatest during this diet. The differences in adhesion was suggested to be related to the modulation of LDL fatty acid composition, because it showed a negative correlation between monocyte adhesion to endothelial cells and the oleic acid content of LDL. Studies in animals support the idea that MUFA are capable of modulating cells of the immune system (Yaqoob et al., 1994; Sanderson et al., 1995; Jeffery et al., 1997; Yaqoob, 1998; Moussa et al., 2000). Therefore, the effects of MUFA on adhesion molecules are potentially important, since they appear to have a role in the immune system.

KCNAB1* interactions with *MBLN1* and *PLCH1

Potassium channels are ion channels distributed and found in all living things. They form pores in the cell membrane that are selective to potassium, these formed channels are found in most cells, controlling a huge variety of cellular functions. In mammals, there are more than eighty genes that encode potassium channel subunits (Doyle et al., 1998; Littleton and Ganetzky, 2000). *KCNAB1* is a potassium channel, voltage-gated ion channel with diverse functions including regulating neurotransmitter release, blood pressure insulin secretion smooth muscle contraction, lymphocyte activation as well as

naïve and regulatory T cell development and cell volume (Toppin et al., 2010; McCarthy et al., 2014). In a GWAS for carcass merit traits like hot carcass weight, average backfat thickness, rib eye area, lean meat yield and carcass marbling score from multiple beef cattle breeds, *KCNAB1* gene was reported in the five top most significantly enriched biological functions for rib eye area. In humans, *KCNAB1* was reported in estrogen receptor-dependent expression in response to eicosapentaenoic acid (C20:5) in estrogen-receptor cells.

Muscleblind-like proteins (*MBNL*) belong to a family of tissue-specific RNA metabolism regulators encoded by three genes *MBNL1*, *MBNL2* and *MBNL3* in mammals. *MBNL1* is the best-studied family member of *MBNL* proteins due to its predominant expression in muscle tissue (Konieczny et al., 2014). The *MBNL1* gene is located on chromosome 1, and acts in a metabolic part of adipogenesis, where there are processes of cell differentiation from pre-adipocyte to adipocyte (fat cells, and energy reserves). There are two types of adipocytes: white, responsible to store excess energy as triglycerides; brown adipocytes, specialized in the dissipation of energy through the production of heat. Brown adipose tissue is composed of brown adipocytes, and it is abundant in small mammals and infants. Hung and Lin (2020) identified differential splicing profiles of *MBNL1* gene during brown adipogenesis and they also reported that *MBNL1* isoform exhibited more-prominent influence on brown adipose tissue development. From a clinical point of view, brown adipose tissue is considered as a potential target to treat obesity and associated metabolic disorders in humans. Modulation of *MBNL* proteins extensively demonstrated to interfere with the development or maintenance of skeletal muscles which are derived from the same lineage as brown adipocytes (Saely et al., 2011; Hemani et al., 2014). *MBNL1* was reported by Bolormaa et al. (2015) as one of the candidate genes that could interact with *PLAG1* gene in a beef cattle research on non-additive genetic variation in growth, carcass and fertility traits. Hamill et al. (2012) reported *MBNL1* was upregulated, in other words, increased gene expression, on a system biology analysis of differentially expressed genes — gene interaction networks for meat tenderness in pork.

Phospholipases are a superfamily of enzymes that act on cell membranes, cleaving phospholipids into fatty acids and lysophospholipids. There are different types of

phospholipases, depending on the hydrolysis site. In the first group are phospholipases A1 and A2, phospholipase B (lysophospholipase), while the second group contains phospholipase C and phospholipase D. Phosphoinositide phospholipase C (*PLCH1*) is an enzyme that cleaves phosphatidylinositol 4,5-bisphosphate to generate inositol 1, 4,5-trisphosphate and diacylglycerol. Phospholipases are usually expressed and have diverse biological functions, including a role in inflammation (Goñi et al., 2012; Yang et al., 2013) . *PLCH1* gene was reported in a network analysis of the genetic determination conformation, growth and fatness in pigs (Puig-Oliveras et al., 2014a) and Lemos et al. (2016) found a region on BTA1 at 112 Mb associated with *PLCH1*, both studies associated the gene with lipid metabolism.

4.2.4 Sum of OM3

For the sum of OM3, genes related to immune response, insulin stimulus, muscle fiber development, glycosylation, glutamate catabolic process, oxidation-reduction process, and regulation of glucose import in response to insulin stimulus were found.

Fatty acids have several functions on immune cells: they serve as fuel for energy generation; makeup phospholipids in the plasma membrane, contributing to the physical and functional properties of the membranes; they regulate gene expression with effects on receptor activity, intracellular signaling processes or cell transcription factors (Kiecolt-Glaser et al., 2014; Gutiérrez et al., 2019).

A balanced diet is essential for the correct function of every part of an individual, including the immune system. The impact of dietary PUFA on the immune system has been investigated for decades, with a special focus on the omega-3 PUFA, such as α-linolenic acid (C18: 3) eicosapentaenoic acid (C20: 5), and docosahexaenoic acid (C22: 6). The α-linolenic acid is generally the most consumed n-3 fatty acid in most human diets (Calder, 2013), as it is present in green leaves and vegetable oils (soy, canola, flaxseed), nuts and seeds. However, the main PUFA consumed in Western diets is linoleic acid (predominant in vegetable oils), which is normally consumed 5 to 20 times in greater quantities than α-linolenic acid (Gilley et al., 2020).

HECTD4 gene is located on chromosome 17, it belongs to the HECT domain subfamily of a ubiquitin ligase protein helps determine the type of ubiquitin chain formed

during ubiquination (Sheng et al., 2012). Ubiquination is a process that helps to determinate the biological fates of protein by targeting them for degradation, altering their cellular location, or affecting their interactions and activity (Magnani et al., 2005). Magnani et al. (2005) have demonstrated that ubiquination controls the immune response through NFκB regulation, thus alterations in HECT domains could result in altered NFκB regulation and subsequent altered immune response. On a gene co-expression analysis for potential pathways and regulators of beef tenderness in Nellore cattle, Gonçalves et al. (2018) reported that *HECTD4* gene was a target of top differentially hubbed transcripts (a hub gene is defined as gene with high correlation in candidate modules) enriched for terms associated with proteasome GO terms and KEGG pathways. The gene exhibited negative differentially hubbed between the High and Low meat groups for shear force estimated breeding values at 14 days of aging and were enriched for proteasome. Co-expression analysis are a powerful approach to integrate the transcripts (e.g. RNA-Seq data) relationship into different pathways based on phenotypic variation (Reverter et al., 2006; Reverter and Chan, 2008) .

On chromosome 17, we found the protein tyrosine phosphatase, non-receptor type 11 (*PTPN11*), a gene that encodes a tyrosine phosphatase (*SHP2*). *SHP2* plays a role in biological processes such as cell growth and differentiation, mitogenic activation, metabolic control, transcription regulation, besides it is widely expressed in most tissues (Agazie and Hayman, 2003; Huang et al., 2014; Zhang et al., 2015). Studies suggests that S*HP2* affects biological functions in the gastrointestinal tract, and in mice, animals knocked-out for *SHP2* exhibited an attenuated hepatocyte proliferation (Bard-Chapeau et al., 2006; Coulombe and Rivard, 2016).

Collagen accounts for one-third of proteins in animals, it is formed especially by glycine, proline, and hydroxyproline. Collagen is essential to maintain the normal structure and strength of connective tissue, such as bones, skin, cartilage, and blood vessels. Fish, birds and mammals can synthesize proline from arginine, or produce proline from glutamine and glutamate in the small intestine (León-López et al., 2019). Some functions of proline include the regulation of gene expression and cell differentiation, integrating nutrient and growth factor signaling in cells, synthesis of and proteins (collagen and elastin) and scavenging oxidants such as free radical species (Paul et al., 2019). The

PRODH gene encodes proline oxidase, or proline dehydrogenase enzyme, which starts the proline cycle, generating glutamate products. The start of the proline cycle under certain conditions of nutrient stress may be a mechanism by which cells switch to a catabolic mode for maintaining cellular energy levels. Recent studies have related proline with lipid metabolism. Murakami et al. (2017) reported that an N-terminal di-proline motif is essential for fatty acid–dependent degradation of Δ^9-desaturase (DESAT1) in Drosophila. The authors designated the sequential prolines as a "di-proline motif," which plays a crucial role in the regulation of Δ^9-desaturase expression in response to changes in unsaturated fatty acids at cellular level.

The Δ^9-desaturase is an iron-containing microsomal protein that catalyzes the biosynthesis of MUFA by introducing the first cis double bond at the Δ^9position on the carbon chain. Only terminal desaturase activity is sensitive to changes in diet, hormonal balance, developmental processes, and temperature changes (Tocher et al., 1998). There are at least three desaturases in ruminant tissues that favor the synthesis of fatty acids *de novo*: Δ^5, Δ^6 and Δ^9-desaturase. Of these, Δ^9-desaturase acts on the SFA to convert them into MUFA. The expression levels of the Δ^9-desaturase enzyme are associated with the content of MUFA in the muscle of ruminants, as well as with the levels of CLA, as they convert vaccenic acid (C18: 1t11) into conjugated linoleic acid (C18: 2c9t11) - the greater CLA isomer in ruminant fats (Lourenço et al., 2008). Scollan et al. (2006) and (Bartoň et al., 2007) showed in their studies that different breeds reflect differences in fatty acid metabolism, gene expression or enzyme activity. In fact, the enzymes Δ^5, Δ^6, and Δ^9 desaturases were considered as the most important genetic factors associated with the deposition of fatty acids in the carcass fat.

4.3.5 Sum of OM6

The daily consumption of fatty acids from the n-3 and n-6 series has been the subject of recurrent research concerning human health (Budowski and Crawford, 1985; Sinclair et al., 1987; Simopoulos, 2001). Omega-6 fatty acids are found mainly in vegetable oils, corn, sunflower, and soybean seeds, contributing to the production of inflammatory and cancerous eicosanoids. It is important to note that the consumption of n-6 fatty acids should be moderate since in excess they can contribute to the increased

risk of situations such as the development of cancers, sudden death, heart disease, vasoconstriction, increased blood pressure, elevated triglycerides rate, and arthritis (Simopoulos, 2001; C.A. Martin et al., 2006).

The sum of n-6 resulted in five significant interactions between six genes. These genes are related to biological processes like epithelial cell polarity, proteome, GTPase activity, and zinc finger proteins. *RABGAP1L* and *RALGAPA2* genes in particular, are related to GTPase activity and signaling. GTPases are hydrolase enzymes that bind to the nucleotide and hydrolyze it to guanosine diphosphate (Chenette and Der, 2011; Prakash and Gorfe, 2017; Peurois et al., 2018). GTPases have several functions, such as signal transduction, protein biosynthesis, and regulation of cell division, proliferation and differentiation as molecular switches or timers in many fundamental cellular processes. GTPases can also be subdivided into small GTPases. Small GTPases are related to cellular signaling events involving membranes or the cytoskeleton. They can be subdivided according to their functions: Ras (cell proliferation), Rho (cell morphology), Rab (vesicle transport), Arf (vesicle transport), and Ran (nuclear transport) (Hall, 1990).

Small GTPases can be modified by lipids, and these modifications are characterized by the type of lipid and the site of modification in the protein. One example is the myristoylation, where ARF proteins are modified by myristic fatty acid (C14:0). Lipid modifications can facilitate the attachment of soluble proteins to biological membranes, but they also enable protein-protein interactions and protein-lipid interactions. A lipid modification (myristoylation) occurs during the apoptosis in the actin protein. Apoptosis accompanies a dramatic reorganization of the cytoskeleton, which is followed by morphological changes and cellular fragmentation. Actin is one of the main proteins responsible for muscle contraction along with side myosin, affecting the texture of the meat and the ability to retain humidity. In addition to their role in muscle contraction, actin and myosin can affect meat tenderness by denaturing at different temperatures, actin, for example, denatures at a higher temperature, which results in the hardening of meat fibers by loss of humidity in cooking (Kinsella and O'Mahony, 1994; Chenette and Der, 2011; Prakash and Gorfe, 2017; Peurois et al., 2018; Qu et al., 2019).

Some lipid modifications can be reversible and may have regulatory functions in signal transduction, such as providing a structural basis for the assembly of signaling-

protein-complexes on membranes or terminating a signal cascade by hydrolysis of the lipid and dissociation of the protein from the membrane.

4.3.6 OM3:OM6 ratio

The assessment of the amount of total lipids ingested in the diet is based on the ratio of OM3: OM6 and SFA: PUFA. Studies indicate that the diet of western countries has an imbalance in the consumption of fatty acids of the n-3 and n-6 series, due to the high consumption of vegetable fats (sources of n-6). Studies indicate that the excessive amounts of n-6 PUFA found in current diets increase the OM3: OM6 ratio, promoting an increase in cardiovascular, inflammatory, and autoimmune diseases. However, when the relationship between OM3: OM6 (with greater consumption of n-3) is balanced, studies show suppressive effects on these pathogenic states mentioned above (Rustan; Simopoulos et al., 1999; Shahidi and Ambigaipalan, 2018; Manson et al., 2019). The ideal intake of OM3:OM6 ranges from 1:1 to 4:1 (Simopoulos, 2006).

The OM3:OM6 ratio showed a high number of interactions, and the genes found were related to lipid metabolism, catabolic process and biosynthesis; protein glycosylation, fatty acid biosynthesis, fatty acid oxidation, cholesterol regulation and metabolism and regulation of lipolysis.

FUT8-FHIT

α1,6-Fucosyltransferase (*FUT8*) catalyzes the transfer of a fucose residue to N-linked oligosaccharides on glycoproteins by means of an α 1,6-linkage to form core fucosylation in mammals. Fucosylation is a type of glycosylation, and it is the process of adding a fucose sugar units to a molecule, it plays a role in a wide variety of biological processes, including cell adhesion, blood antigens, and some severe diseases including cancer metastasis, congenital disorders of glycosylation, and infections (Wang et al., 2006). *FUT8* has been related to a growth factor receptor (PDGF) by Wang et al. (2005) The authors aimed to define the physiological roles of *FUT8*, and *FUT8*-null using mice and gene targeting technology. Their results showed that disruption of *FUT8* induces severe growth retardation, early death during postnatal development. Most animals manifested severe growth retardation, which was linked to the dysregulation of the *PDGF* receptor activation. *FUT8* was also linked to the Low-density lipoprotein receptor-related

protein 1 (*LRP1*) (Yamaguchi et al., 2000; Ihara et al., 2007; Vanhooren et al., 2011). *LRP1* is a protein expressed in multiple tissues, though it is most abundant in vascular smooth muscle cells, hepatocytes, and neurons.

LRP1 plays a key role in intracellular signaling, lipid and lipoprotein metabolism, protease degradation, platelet derived growth factor receptor regulation cell growth, inflammation, and apoptosis. In Ho Lee et al. (2006) study in mice, the authors have demonstrated that the loss of core fucosylation impairs the function of *LRP1* resulted in a reduction in the endocytosis of insulin like growth factor binding protein-3 (*IGFBP-3*) in the cells derived from *FUT8*-null mice, but it could be restored with the re-introduction of *FUT8*, showing that fucosylation is crucial for the scavenging activity of *LRP1* in vivo. A study in chickens by D'Andre et al. (2013) aimed to identify and characterize genes that controls fat deposition. Their study revealed a network of eleven genes (*LPL, ACSBG2, AACS, FASN, LSS, FDPS, SULT1B1, HMGCR, DPP4, FUT8,* and *PLAU*) that provides strong evidences of their involvement in lipid biosynthesis, cholesterol biosynthesis and fatty acid degradation in fast-growing chickens. *FUT8* was also reported in a whole genome association studies of residual feed intake in low and high residual feed intake selected lines of Yorkshire pigs (Onteru et al., 2013).

FHIT gene is a member of the histidine triad family, and encodes a fragile histidine triad protein and is expressed in different tissues, including stomach, colon, small intestine, and esophagus. The diadenosine P1, P3-bis(5'-adenosyl)-triphosphate adenylohydrolase enzyme encoded by *FHIT* is involved in purine metabolism (Zanesi et al., 2005; Prosseda et al., 2019). Purines are the most abundant metabolic substrates for all living organisms by providing essential components for nucleic acids, regulating enzymatic activity, protein synthesis and energy transfer in cells. Thus, purines and their derivatives widely participate in biological processes, including immune responses. In chicken, *FHIT* was reported to be involved in body weight development and its expression was observed in chicken colon, heart, brain, kidney, liver, lung, and testis. The diadenosine P1, P3-bis(5'-adenosyl)-triphosphate adenylohydrolase enzyme produces adenosine diphosphate in primordial germ cells in chicken (Rengaraj et al., 2013). Reyer et al. (2015) also reported *FHIT* affecting the body weight of a commercial broiler line in a GWAS study. In cattle, *FHIT* was related to milk train in Xinjiang brown breed (Ju et

al., 2020). The gene was isolated, and eight polymorphic insertion/deletion loci significantly related to the milk traits, such as milk yield, milk fat yield, protein percentage in milk, and somatic cell score. In addition, Widmann et al. (2013) showed that *FHIT* gene as one of the central hubs in a differential growth network in a study with 152 male individuals from a Charolais x German Holstein population. In humans, reduced gene expression of *FHIT* in cardiac tissue was reported in obese individuals compared to lean subjects by Beaumont et al. (2016). The authors highlighted that the variant found in their study was not part of an expression quantitative loci, but it showed impact on DNA methylation in adipose tissue. Therefore, *FHIT* was strongly associated with abdominal adiposity, visceral fat mass and subcutaneous fat mass, suggesting that the gene may be associated with cardio-metabolic diseases.

Genes affecting lipid metabolism, and other important traits

Today, one of the biggest challenges to researches into genetics field is to translate their results into practical knowledge, especially gathering numerous data from genome projects into determining a gene's function linked to a phenotype of interest. This approach often involves some intelligent guesswork—searching for homologous sequences in different species as well as characterizing their phenotype. In this section, we choose to highlight four genes (*ACSS3, PPARGC1A, SLC2A2 and EXOC4)* from different interactions associated to lipid metabolism, feed efficiency and other meat quality traits.

Protein kinase, cGMP-dependent, type I (*PRKG1*) is a gene related to several biological processes, such as inflammation, muscle-skeleton diseases in humans, residual feed intake in cattle (Taye et al., 2017; De Souza Fonseca et al., 2018), for intramuscular fat content in pigs (Puig-Oliveras et al., 2014b; Kim et al., 2015), milk fatty acids in dairy cattle (Li et al., 2014). Nisoli et al. (1998) first reported *PRKG1* gene involved in cyclic guanosine monophosphate (cGMP)-PKG signaling pathway, inhibiting rat brown adipocyte proliferation by regulating the lipolysis in adipocytes, releasing fatty acids and glycerol by the hydrolysis of triglycerol. Amieux and McKnight (2010) showed that brown adipocyte tissue from *PRKG1* knockout mice had lower triglyceride stores in their brown adipose tissue. An RNA-Seq analysis in swine by Puig-Oliveras et al. (2014b) showed that the *PRKG1* gene was the differentially-expressed in muscle between high

and low groups for fatty acid (extreme phenotypes) traits. Later, Li et al. (2014) in a GWAS study identified *PRKG1* as one of the twenty promising genes associated with milk fatty acid traits in Chinese Holstein. In a follow-up study in the same research group, Shi et al. (2019) reported for the first time that *PRKG1* gene had significant effects on medium-chain saturated fatty acids in dairy cattle, especially on caprylic acid (C8:0).

Acetate is the major volatile fatty acid produced in ruminal fermentation. It is oxidized throughout most of the body to generate ATP, and it is the major source of acetyl-CoA for lipid synthesis. Fatty acid synthesis starts with acetyl-CoA and NADPH through the action of enzymes. Most of the acetyl-CoA which is converted into fatty acids is derived from carbohydrates via the glycolytic pathway. Acyl-coenzyme A synthetases (ACSs) catalyze the initial reaction in fatty acid metabolism (Lehninger, 2004). The existence of many ACSs suggests that each plays a unique role, directing the acyl-CoA product to a specific metabolic fate. *ACSS3* gene encodes Acyl-CoA synthetase short-chain family member 3 enzyme, which catalyzes the initial reaction in fatty acid metabolism, allowing the thioester produced and CoA in anabolic and catabolic pathways (Watkins et al., 2007). In an RNA-Seq study to elucidate mammary gene regulation in dairy ewes, Suárez-Vega et al. (2019) investigated the reduction of fat in milk caused by conjugated linoleic acids anti-lipogenic effects. Among the genes found in their study, two from the ACS family – *ACSS2* and *ACSS3* were downregulated. In beef cattle, Dalrymple et al. (2014) reported *ACSS3* in their study using muscle gene expression to estimate triglyceride deposition, and relative contributions of fatty acid synthesis and fatty acid import in intramuscular fat in crossbreed cattle (Piedmontese × Hereford, and Wagyu × Hereford). The authors calculated the fold change in expression of the full set of genes between 12 and 25 months and the ratio of the fold changes between crosses and between triglyceride synthesis and storage and fatty acid synthesis pathway genes. The size of the increase in gene expression varied from a small decrease in expression *ACSS3* to an increase in *THRSP* expression in Wagyu × Hereford animals. Thyroid Hormone Responsive (*THRSP*) is known by its role in lipid metabolism, controlled by nutritional and hormonal factors. *THRSP* affects body weight and glucose tolerance by increasing insulin sensitivity, which leads to changes in body fat mass (Anderson et al., 2009). *ACSS3* was also reported in Nellore heifers by Mota et al. (2020) in a GWAS for plasticity for age at

first calving, but the gene was also related to lipid metabolism and energy production. *ACSS3* was considered to be a good candidate gene for capric acid (C10:0) and pentadecylic acid (C15:0) by Buitenhuis et al. (2014) in a GWAS analysis for milk-fat composition in Danish Holstein and Danish Jersey cattle.

PPARGC1A gene is expressed at different levels in a large number of tissues. The key role of *PPARGC1A* is activating a variety of nuclear hormone receptors and transcription factors regulating energy homeostasis. However, studies have shown that this gene is involved in adipogenesis, gluconeogenesis, oxidative metabolism, β-oxidation of fatty acids and adaptive thermogenesis (Chagnon et al., 2003; Semple et al., 2004). Studies in humans link *PPARGC1A* to obesity-related diseases, insulin resistance, susceptibility to type II diabetes and lipodystrophy (Blott et al., 2003; Bouwman et al., 2011; Do et al., 2017). In cattle, *PPARGC1A* gene was previously described as functional candidate gene by its roles in energy, fat, and glucose metabolism (Weikard et al., 2005). In dairy cattle, *PPARGC1A* was related to the mammary gland metabolism because of its dynamic in the regulation of programs linked to energy homeostasis (Bouwman et al., 2011). Lactational performance is influenced by metabolic processes linked to glucose and fat metabolism (Bell and Bauman, 1997). Glucose availability is a limiting factor for milk production, the mammary gland uses approximately 60-70% of glucose for lactose synthesis, and about 20-30% of the remaining glucose to generate NADPH2 - that is used in milk fatty acid synthesis (Lehninger, 2004). In this sense, glucose is a limiting factor for the fatty acid synthesis pathway during lactation. Given its role in energy metabolism, adipogenesis and aspects of glucose, it is plausible that the metabolic adaptive processes modulated during lactation in dairy cattle might be coordinated by *PPARGC1A* gene. *PPARGC1A* was mapped on BTA6 in a region linked to a QTL for milk fat synthesis (Weikard et al., 1997).

Sevane et al. (2013) first reported *PPARGC1A* gene associated with meat quality traits. The authors reported several polymorphisms of the *PPARG1A* gene associated with FA composition in eleven European cattle breeds. In Nellore cattle, Fonseca et al. (2015) also identified *PPARGC1A* gene polymorphisms associated with growth and carcass traits, especially for weight at 378 days in female individuals. The significant effects observed for the gene explained 1.12% of the additive genetic variance of the trait,

corroborating with the Studies by Sevane et al. (2013), Shin and Chung (2013), and Li et al. (2014) that also correlated polymorphisms in the same gene with growth and carcass traits in other beef cattle breeds. The significant association found by Fonseca et al. (2015) is an indication that the fat metabolism participates in weight at 378 days in Nellore cattle. More recently, Bartoň et al. (2016) reported polymorphisms in *PPARGC1A* in Fleckvieh breed. The authors aimed to associate the polymorphisms in the genes studied with intramuscular fat content and the fatty acid composition of muscle in beef cattle. The polymorphism in *PPARGC1A* showed significant effects on myristic acid (C14:0), sum of SFA and atherogenic index (AI= C12:0 + 4 × C14:0 + C16:0)/ (MUFA + PUFA)). The results of the above studies reinforce that further studies are necessary to confirm the effect of the *PPARG1A* gene on the FA composition of beef adipose tissue.

SLC2A2 gene, also known as glucose transporter 2 (*GLUT2*), is a gene that encodes a glucose transporter (transports sugars such as fructose, glucose, mannose and galactose) in the liver, intestine, and kidney epithelium (Roncero et al., 2004; Bedford et al., 2014). In mammals, *SLC2A2* plays an essential role in glucose metabolism, absorption and homeostasis, stimulation of insulin secretion by glucose in pancreatic cells, and food intake. *SLC2A2* acts by lowering the blood glucose levels during hyperglycemia, through transporting glucose from blood into liver, so we can assume that elevated transcription or translation of this gene may lead to a quick decrease in blood glucose level (Wang et al., 2019). In humans, was related to type II diabetes, and in mice deficient in *SLC2A2* individuals were reported as hyperglycemic and had elevated plasma levels of glucagon and free fatty acids (Dai et al., 2016; Wang et al., 2019).

A protein network interaction analyses revealed that *SLC2A2* was an important candidate gene for feed efficiency in pigs. Liang et al. (2015) found that *SLC2A2* gene expression was associated with pig growth rate and could affect fat deposition in Yorkshire and Tibetan pigs. Bedford et al. (2014) and Wang et al. (2019) studies in pigs showed an increased *SLC2A2* gene expression in the small intestine by an epidermal growth factor that promoted pig growth. In addition, a transcriptome analysis for feed efficiency in Duroc × Landrace × Yorkshire pigs showed that an increased expression of *SLC2A2* in the intestinal columnar epithelial cells enhanced glucose uptake, suggesting that higher efficient animals with higher *SLC2A2* gene expression had greater ability to

absorb glucose than the lower efficient subjects (Wang et al., 2019). The authors concluded that high *SLC2A2* expression in highly efficient animals resulted in greater glucose sensitivity and stimulated insulin secretion, which promoted glucose absorption and improved feed efficiency.

Finally, *EXOC4* (exocyst complex component 4), a component of the exocyst (Exo70) complex which participates in temporal and spatial regulation of exocytosis was found in our study related to the OM3:OM6 ratio. Exocysts were reported by Wu et al. (2008) and Tanaka and Iino (2015), showing that they interact indirect and directly with several proteins, including cell membranes, small GTPases, cell cortex and with the cytoskeletal, indicated that the gene modulates cell migration may control MAPK and ERK signaling pathways. In a follow-up study, they also found that *EXOC4* can mediate cell migration and adhesion (Tanaka and Iino, 2015) (*EXOC4* has been connected with various diseases in humans, such as cancer, neuronal disorders and type II diabetes. *EXOC4* also mediates *GLUT4* (glucose transporter 4), responsible for the insulin-stimulated glucose transport in the adipose and muscle tissue. Laramie et al. (2008) identified in a population genetic study that several type II diabetes associated SNP were near *EXOC4* gene. Jiao et al. (2019) investigated gene interactions for body mass index in a European-American adult female cohort via genome-wide interaction analyses and pathway association analyses. The authors most significant interaction associated with body mass index was between rs7800006 and rs10797020 ($P = 2.63 \times 10^{-11}$) in *EXOC4* gene. The GWIS study suggested that *EXOC4* related pathways may contribute to the development of obesity in humans. In livestock species, few studies suggest an association between the *EXOC4* gene and meat quality. Welzenbach et al. (2016) showed that the gene may be a candidate gene associated with rate of drip loss in pigs. *EXOC4* gene was significantly correlated with triiodothyronine and thyroxine concentrations in Chinese Holstein cattle by Gan et al. (2019). Triiodothyronine affects growth and development, metabolism, body temperature, and heart rate. Thyroxine is primarily responsible for the regulation of metabolism. However, more studies are needed to identify the association between *EXOC4* and meat quality in livestock species.

4.3.7 SFA: PUFA ratio

Cholesterol is a sterol found in animal products such meat, eggs, milk, meat products (sausages, ham, cold cuts), poultry and seafood. Among the dietary factors which can affect the plasma cholesterol level, the amount of dietary cholesterol the saturated fatty acids intake perhaps more important when we talk about health dietary habits (Chang and Huang, 1998). SFA:PUFA ratio is used to predict the cholesterolemic effect of dietary fat, especially due it to the relationships between dietary fat, plasma lipid levels, and coronary heart diseases, linked to the increase of LDL by the consumption of saturated and trans fatty acids (Howell et al., 1997). Studied have shown that diets with higher SFA:PUFA ratios were found to have stronger hypocholesterolemic effect than those with lower SFA:PUFA ratios, or in other words, it has the power to reduce serum and hepatic levels of total cholesterol in individuals (Shepherd et al., 1978; Schonfeld et al., 1982; Keys et al., 2009).

Genes involved in lipid profile and carcass merit traits

The genes found for SFA:PUFA ratio were related to glucose metabolism, feed efficiency, carcass trait, skeletal muscles, lipid metabolism, and obesity. Here we chose to describe the candidate genes that could be incorporated into breeding programs.

Adipocytes are the main fat storage cells in living organisms. They primary purpose is to provide free fatty acids from stored energy when the food is limited and energy is required. The primary cellular fuel is glucose, however when glucose is limited, free fatty acids are secreted from adipose tissue to the bloodstream. Moreover, the secreted fatty acids are used as a fuel in the situations of stress or increased activity (Gregoire et al., 1998). *PDE4D* gene belongs to a family of four *PDE4* genes. The genes from this family encodes phosphodiesterase enzyme, which specifically hydrolyzes intracellular cAMP binding (Kim et al., 2008; Lee et al., 2011). cAMP signaling pathways are well known by its roles in controlling adipocyte differentiation and immune system (Sassone-Corsi, 2012). cAMP signaling adipocytes are regulated in multiple ways. The insulin action, for example, acts promoting the excess nutrient utilization in the bloodstream, a mechanism that may inhibit lipolysis. Insulin inhibits lipolysis stimulated by cAMP signaling, with the activation of PDE enzymes (Ravnskjaer et al., 2015). Studies *in vitro* have shown that PDE inhibitors and synthetic cAMP analogs are commonly employed to switch on the

adipogenic program (Russell and Ho, 1976). *PDE4* gene was shown to be regulated by transcriptional and post-translational mechanisms (Houslap et al., 1998; Conti and Jin, 1999; Liu et al., 2000). This mechanism results in a negative feedback for cAMP signaling, in other words, when *PDE4* is inhibited, cAMP levels are increased (Rogne and Taskén, 2014; Schafer et al., 2014; Xu et al., 2017), which suggests that cAMP concentration may influence the process of adipocyte differentiation. Dos Santos Silva et al. (2019) used transcriptome RNA-Seq data *from Longissimus thoracis* muscle of Nellore males to identify hub genes based on co-expression network obtained from differentially expressed genes associated with intramuscular fat content. The authors reported *PDE4D* in highest intramuscular fat content animals, and highlighted the genes known function on lipid metabolism through the regulation of cAMP signaling, which may impact the intramuscular fat content implying directly in meat quality and other organoleptic important traits.

The solute-carrier gene (SLC) superfamily encodes membrane-bound transporters, they are known for their role in transporting glucose, inorganic compounds, amino acids, vitamins, fatty acids, lipids, thyroid hormone and urea. *SLC14A2* gene is mainly expressed in brain (Dahlin et al., 2009), and associated with fat thickness in humans and pigs (Lee et al., 2011). In a combined SNP association study of neuronal genes affecting subcutaneous fat thickness in human and pig, *SLC14A2* was related to fat accumulation. An important concept in carcass classification is to obtain the maximum number of muscles, minimum bone weight number, and an adequate amount of fat. In carcass typification systems, the amount and distribution of fat in the carcass are important factors in determining its value (Jaeger et al., 2004). The animal diet can directly influence the degree of finishing and the fat content of the carcass, and the size/quantity of each fat deposit can be influenced by the breed, physiological, and nutritional status of an animal. Some consequences of fat accumulation are i) the increase in the degree of finishing of the carcasses, important to avoid shortening by cold, ii) reduction of feed efficiency due to the accumulation of visceral fat (Peron et al., 1995). Since the fat accumulation process may affect the body structure of an animal, it would be interesting to investigate further how SLC family genes influence carcass traits.

PLTP belongs to the lipopolysaccharide binding/lipid transfer gene family, the gene is known by its role in the transportation of different lipid molecules. *PLTP* activity with lipid molecules has been extensively studied. Although the divergent results among different human populations, *PLTP* is associated with plasma cholesterol, triglyceride and very-low density lipoprotein (Tahvanainen et al., 1999; Cheung et al., 2006; Cheung et al., 2009). Most studies shown evidence that obesity factors such as hepatic triglyceride enzymes and some hormones can modulate *PLTP* activity. The central role of *PLTP* in lipoprotein metabolism is transferring phospholipids between HDL particles. Furthermore, *PLTP* is essential for the transfer of excess surface lipids from triglyceride-rich lipoproteins to HDL, facilitating smaller lipoprotein formation, contributing to the formation of LDL and assisting and maturating HDL particles (Albers et al., 2012). In bovines, the PLTP gene is located on BTA13, a chromosome that has multiple carcass candidate QTL reported, providing statistical support (Schläpfer et al., 2002; Kim et al., 2003; Cheong et al., 2008) . Two QTL (BM6548 and AGLA232) near the PLTP QTL region have significant effects on marbling score (Schläpfer et al., 2002; McClure et al., 2010). These findings indicate that *PLTP* may influence on lipid profile traits. A study using merged phenotypic and genotypic information to characterize the variation in fatty acid composition and sensory parameters and to represent the diversity among 15 cattle populations of Europe used a polymorphic *PLTP* marker (ss77832104, Btau_4.0 bovine genome sequence) (Williams et al., 2009; Dunner et al., 2013). Dunner et al. (2013) associated *PLTP* with n-6 to n-3 ratio, showing that the polymorphism in *PLTP* affected the amount of fatty acids in muscle. The A allele of the SNP ss77832104 (located in the 3′-UTR) decreased the n-6: n -3 ratio by 8%.

4.4 Enrichment Analysis

For the enrichment analysis we chose to work with all genes from all traits together. The tables containing all epistatic SNP can be found on the supplementary material section of this study.

The smooth muscle tissue development (GO:0048745) is a biological process that encompasses the progression of smooth muscle over time, from its formation until maturation. The *ITGA8, TIPARP, TP63*, and *DLG1* genes were identified as related to

smooth muscle tissue development. Improving feed efficiency is a major challenge in livestock production, with additional benefits on its ecological footprint. An association analyses by Oliveira et al. (2016) pointed that the *TIPARP* is a potential candidate gene influencing residual feed intake variation in Nellore cattle. Residual feed intake has been specifically proposed to capture the efficiency of feed use independent from the production needs, corresponding to feed efficiency (Koch et al., 1963). The RFI can be computed at the genetic or phenotypic levels as the difference between observed feed intake and feed intake predicted from production and maintenance needs. In this sense, as residual feed intake is a measure of feed efficiency, impacting the profitability of cattle herds and potentially reducing methane emission (Gilbert et al., 2017).

Small GTPase mediated signal transduction (GO:0007264) biological process encompasses several molecular signals in which a small monomeric GTPase relays signals, while guanyl-nucleotide exchange factor activity (GO:0005085) molecular function promotes the exchange of guanyl nucleotides associated with a GTPase. Under regular cellular physiological conditions, the concentration of GTP is higher than that of GDP, favoring the substitution of GDP by GTP in association with the GTPase. Regulation of Rho protein signal transduction (GO:0035023) biological process is related to any process that modulates the rate of Rho protein signal transduction, a small GTPase related to cell morphology (Hall, 1990).

Endopeptidase activity (GO:0004175) molecular function encompasses the proteolytic peptidases functions that hydrolysis alpha-peptide bonds of nonterminal amino acids in a polypeptide chain. The endopeptidase activities in skeletal muscle influencing meat tenderness has been described (Sentandreu et al., 2002). The *NCSTN, SENP2, ADAM10, PSMD2*, and *SENP5* genes were identified as related to endopeptidase activity. SENP2 is known by its role in the regulation of myostatin. Myostatin is primarily expressed by skeletal muscle and acts in an autocrine manner to inhibit myoblast proliferation, differentiation, and protein synthesis (McCroskery et al., 2003; Artaza et al., 2005). In mice, disruption of the myostatin gene causes a large and widespread increase in skeletal muscle mass as a result of cell hyperplasia and hypertrophy (Qi et al., 2014). In cattle, NCSTN was associated with growth traits like body height, body weight, body length, chest girth, rump length, and hip circumference in Chinese cattle by Yao et al.

(2020). Koo et al. (2015) investigated the role of *SENP2* in fatty acid metabolism, and reported that the overexpression of *SENP2* increases fatty acid oxidation by upregulating the expression of enzymes linked to this process.

Carboxylic ester hydrolase activity (GO:0052689) molecular function comprises the catalysis of the hydrolysis of ester bonds into alcohols and carboxylic acids. Carboxylic ester hydrolases are important lipolytic enzymes secreted by the pancreas that acts in different substrates and form different products, combining properties of esterases and lipases (Casas-Godoy et al., 2012). The most extensively researched carboxylic ester hydrolases encompass carboxylesterases, triacylglycerol lipases, lysophospholipases, acetylcholinesterases, phospholipase A2s, aminoacyl-tRNA hydrolases, butyrylcholinesterases, and cocaine esterases (Chen et al., 2016). All the carboxylic ester hydrolase hydrolyzes a large variety of substrates including cholesterol esters, acylglycerols, phospholipids, carotenoid esters, and vitamin esters (Van Den Steen et al., 1998; Casas-Godoy et al., 2012). The *NCEH1*, *BCHE*, *NLGN1*, *LIPH*, and *LIPI* genes were identified as related to carboxylic ester hydrolase activity. *NCEH1* (Neutral cholesterol ester hydrolase 1) gene encodes an enzyme which is located in the endoplasmic reticulum which plays a role in the regulation of the levels of platelet activating factor and lysophospholipids, participating in the lipid catabolic process (Sekiya et al., 2009). *NCEH1* plays a key role in the reverse cholesterol transport in macrophages, playing a critical role in the accumulation of cholesterol plaques on the arteries walls, which causes obstruction of blood flow in humans (Igarashi et al., 2010). *BCHE* gene encodes an enzyme already associated with the body mass index (Furtado-Alle et al., 2008). Studies in humans showed that individuals with innate high *BCHE* activity tend to be thinner and that *BCHE* synthesis is increased in individuals that gain weight, suggesting that *BCHE* activity is important in energy balance (Alcântara et al., 2003). In mice, knockout individuals became obese compared to wild-type littermates after an 11% fat diet indicate a role for *BCHE* in fat utilization (Li et al., 2008). *BCHE* lower activity possibly cause an imbalance in lipid metabolism, which may lead to an increased predisposition to obesity and to a lower ability to maintain metabolic homeostasis (Lima et al., 2013).

The insulin secretion (bta04911) pathway is delegated to maintain normal fuel homeostasis in cells. Glucose-induced insulin secretion is the principal mechanism of insulin release. Glucose is transported by its transporter into the pancreatic beta-cell and the metabolism of glucose generates ATP. Insulin is the primary anabolic endocrine signal that plays a vital role in lipid, protein, and carbohydrate metabolism. Insulin enhances cellular glucose uptake, stimulates glycolysis, and promotes the synthesis of glycogen in muscle and liver, adipose triglycerides, and skeletal muscle protein, while also preventing their degradation (Hardie, 2012; Baumgard et al., 2016). The *KCNMB3, GCK, SLC2A2, ADCY5, RYR2, ATP1A4, CAMK2B, ATP1A2, PCLO,* and *KCNMB2* genes were identified as related to insulin secretion pathway. *KCNMB3* promoter has a PPAR-γ receptor complex binding site, through which n-3 PUFA may influence (Zheng et al., 2013). n-3 PUFA and their metabolites are natural ligands for peroxisome proliferator receptor activator (PPAR) γ, and PPAR-γ is reported to regulate insulin sensitivity and glucose homeostasis through different mechanisms (Gregoire et al., 1998; Liao et al., 2007). KCNMB*3* promoter may support the interactions of n-3 PUFA with genetic variants at *KCNMB3* to influence activity of BK channels in the regulation of insulin secretion (Düfer et al., 2011).

ATP binding (GO:0005524) molecular function implicates in interactions selectively and non-covalent with ATP, an important coenzyme and enzyme regulator, while cation-transporting ATPase activity (GO:0019829) enables the transfer of solutes from one side of a cell membrane to the other.

The carbohydrates ingested are digested to monosaccharides, mainly glucose, galactose, and fructose, before absorption in the small intestine. Carbohydrate digestion and absorption (bta04973) pathway has been identified as influencing backfat thickness and fat-related traits in Canchim beef cattle (Mokry et al., 2013). The *SLC2A2, SI, HK2, ATP1A4, PIK3CA,* and *ATP1A2* genes were identified as related to carbohydrate digestion and absorption. The glucose metabolic process (GO:0006006) includes the chemical reactions and pathways involving glucose, an important source of energy for animals. Several studies have been showing the important role of this biological process in meat quality traits such as intramuscular fat content in Nellore (Cesar et al., 2018), Yunling and Chinese Simmental cattle (Zhang et al., 2018), and beef tenderness in Angus

(Zhao et al., 2014). The *GCK, APOD, PGM1, HK2, PIK3CA,* and *ADIPOQ* genes were identified as related to smooth muscle tissue development.

Starch and sucrose metabolism (bta00500) pathway have been identified as affecting feed efficiency in beef and dairy cattle (Li and Guan, 2017; Salleh et al., 2017), tenderness in Qinchuan cattle (Zhang et al., 2011) and intramuscular fat deposition in yak (Wang et al., 2020). The *GBE1, GCK, PGM1, SI,* and *HK2* genes were identified as related to the starch and sucrose metabolism pathway.

Sphingolipid signaling pathway (bta04071) has been associated with a fat deposit in Duroc pigs (Ding et al., 2019). The high-fat diet associated with overnutrition increases sphingomyelin and sphingosine-1 phosphate levels in adipose tissue through the sphingolipid metabolic pathway, resulting in lipid accumulation (Choi and Snider, 2015). The *FCER1A, PLD1, MAP3K5, ADORA3, SPTLC3, BCL2, FCER1G, PIK3CA, NFKB1, PPP2R5E*, and *PPP2R2B* genes were identified as related to the sphingolipid signaling pathway.

The extracellular matrix (ECM) acts an important role in tissue and organ morphogenesis and the maintenance of cell and tissue structure and function. Specific interactions between cells and the ECM are mediated by transmembrane molecules that control several cellular activities such as proliferation, differentiation, adhesion, migration, and apoptosis. ECM-receptor interaction pathway (bta04512) plays an essential role in skeletal muscle development (Thorsteinsdottir et al., 2011) and it is involved in the depot-specific adipogenesis in both subcutaneous and intramuscular fat in cattle (Lee et al., 2013). Genes from ECM-receptor interaction pathway has been identified as related to fatty acid composition and content of *Longissimus thoracis* muscle of Nellore (Berton et al., 2016), Yunling and Chinese Simmental (Zhang et al., 2018), intramuscular adipose tissues in Hanwoo (Lee et al., 2014) and adipogenesis in subcutaneous adipose tissue of Xianan beef cattle (Qu et al., 2019). Afonso et al. (2019) characterizing the biological pathways involved in *Longissimus thoracis* mineral deposition in Nellore, identified the ECM-receptor interaction pathway as overrepresented, which could be explained by the role of tight junction in mineral absorption because the diffusion of minerals can occur through pores in the tight junctions (Goff, 2018). The tight junction (bta04530) pathway was also identified as significant ($P<0.05$), which encompasses its function that creates

a selectively permeable barrier to diffusion through the space between neighboring cells, limiting paracellular movement of solutes and material across epithelia (Van Itallie and Anderson, 2014).

4.5 Future directions

The discovery that many complex traits have genetic variation and can be heritable motivated researchers to search for the genetic variants associated with economical traits through the years. It was not until the beginning of the 21st century that two major developments finally provided cost-effective, thorough coverage of the genome: i) completion of the Human Genome Project (Lander et al., 2001; Dolgin, 2009), resulting in the first detailed maps of the human genome and the patterns of LD for hundreds of SNPs; ii) the production n of DNA arrays. Genome coverage was improved by imputing additional SNP from different reference maps such as the 1000 Genomes Project and HapMap (Belmont et al., 2003; Siva, 2008),

Traditionally, the exploration of genetic variance in plants and livestock species has mostly been limited to the use of additive effects estimated using both pedigree and genomic data. In this sense, the role of genetics in complex traits has been quantified as the proportion of the total phenotypic variance explained by additive genetic variance, or heritability (Visscher et al., 2014). One of the many limitations to better understand the genetic architecture of a complex trait is that typically the data structure does not allow estimation of additive, dominance, and epistatic variance simultaneously (de Vries et al., 1994; Zulma G. Vitezica et al., 2013). Yet, heritability estimation using additive models does not only capture only additive gene action but can potentially also capture part of the dominance effects and epistatic interactions (Falconer and Mackay, 1996; Hill et al., 2008). This may occur through two possible mechanisms, i) by generating real additive variation as marginal effects from higher-order interactions (Evans et al., 2006; Hill et al., 2008; Greene et al., 2009; Hemani et al., 2013); ii) by creating a statistical illusion of additive variance through confounding between non-additive and environment effects. Thus, the proportion of phenotypic variation that is explained by all genetic effects and how much of the total genetic variation is actually due to additive effects is still unclear (Vinkhuyzen et al., 2012).

GWAS has been successful in the discovery of many novel variants related to diseases and economic traits, but despite its success, the variants identified as being statistically significant typically account for minimal fractions of the genetic variance, even for highly heritable traits (Bush and Moore, 2012; Edwards et al., 2013; Uitterlinden, 2016). This is what we call “missing heritability” (proportion of additive genetic variation not captured by common variants on SNP arrays), a hot topic that has been extensively investigated in the area of quantitative genomics (Eichler et al., 2010; Brachi et al., 2011; Visscher et al., 2014). There are several explanations for this phenomenon: insufficient sample sizes, rare variants, over-estimated heritability, sparse genetic coverage, imprecise phenotypic data, and poor modeling (unaccounted epistatic effects) – these factors also imply on genomic prediction accuracy (Hemani et al., 2013). One of the solutions for the problem of “missing heritability” would be to combine GWAS with other large-scale approaches, such as Copy Number Variations, RNA-Seq, gene expression, proteomics, metabolomics, methylation, organellar DNA, among others, to fully explain the genetic variance of complex traits to identify all the remaining genetic variants that affect the phenotype (Edwards et al., 2013; Robinson et al., 2014).

Interaction-based analysis of genomic data is increasingly appealing ways to represent the molecular interactions of complex phenotypes, as we recognize that individual variants may be less important than the joint effects of many variants distributed across the genome (Gilbert-Diamond and Moore, 2011; Fang et al., 2019). This acknowledges the importance of epistasis in explaining the “missing heritability” of complex phenotypes, and also emphasizes the need to revisit heritability estimates to acknowledge the contribution of interactions between loci. In this sense, it is necessary to consider higher-order interactions between variants, gene-gene and gene-environmental interactions in genomic prediction, because the genome does not exist by itself, and interactions between variants, genes and the environment could also be non-additive and exist in the absence of main effects(Sukumaran et al., 2017; Hassen et al., 2018; Li et al., 2019; Tsai et al., 2020) . The contribution of interactions may additionally produce more reproducible research via substantiating the importance of higher-order relationships over individual variants. Different studies have shown the importance of genetic interactions, especially in the determination of polygenic traits (Shao et al., 2008;

Jarvis and Cheverud, 2011; Huang et al., 2012), which support the great potential for network genetics.

Studying epistasis and adopting interaction study approaches to future research is emblematic of embracing because it requires the integration of multi-omics information and powerful computation tools, rather than ignoring or rejecting the inherently interdependent, and complex nature of genetics. Integrating multi-omics data and joint modeling to genomic data analysis may be a more powerful and accurate approach to help us understand the complexity of the genetic architecture of traits related to sustainable animal production.

5.0 Final Considerations

The thesis is intended identify epistatic interactions using an exhaustive search method, Meanwhile, we also aimed to extend our understanding of statistical epistasis and to expose complex synergetic factors underlying fatty acid profile in meat. We have focused on pairwise interactions, but further studies may (and should) include high-order interactions. The correlation method (HSIC) used in this research offers the opportunity to be adapted not only in epistasis studies, but also multi-omics approaches, where researches could seek for the network among genomic, proteomics, transcriptomics and metabolomics.

As discussed in Chapter 2, many studies including ours are trying to understand the role of statistical epistasis in livestock species and on human health by looking at the pathway/network that the variants involved in comparing epistatic effects to the single locus effects on complex traits seeking for generalizability of the interaction presence across populations. The present study reports for the first-time epistatic interactions for fatty acid profile related traits in Nellore cattle. Our results showed substantial genetic interactions associated with lipid profile, meat quality, carcass and feed efficiency traits. Although the large number of genetic interactions associated with lipid profile, further studies should be conducted to verify if the interacting loci have biologically reasonable functions, especially the functional mechanisms. There is still a lack of information on how many genes acts in livestock species, and well-established experimental verification for genetic interactions. The number of significant interactions per sum of fatty acid trait

varied greatly. This may indicate that some sum of fatty acid traits may need varying amounts of genetic redundancy or control for the creation of fatty acids critical to fitness. Visualization of significant epistatic interactions revealed many regions of potential regulatory control across multiple chromosomes.

Nevertheless, our access to this population of animals provided the unique opportunity to set a higher bar for requirements when analyzing some phenotypes. This research combined with previous study helps us to better understand the genetic variation behind fatty acid profile traits, and besides the limitations on identifying epistatic interactions, there are ways to help minimize these shortcomings as well as further identifying what needs to be accomplished to overcome the deficiencies. Our results indicate that epistatic interactions may play very different roles in the control of phenotypic variation for lipid related traits in cattle.

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APPENDIX

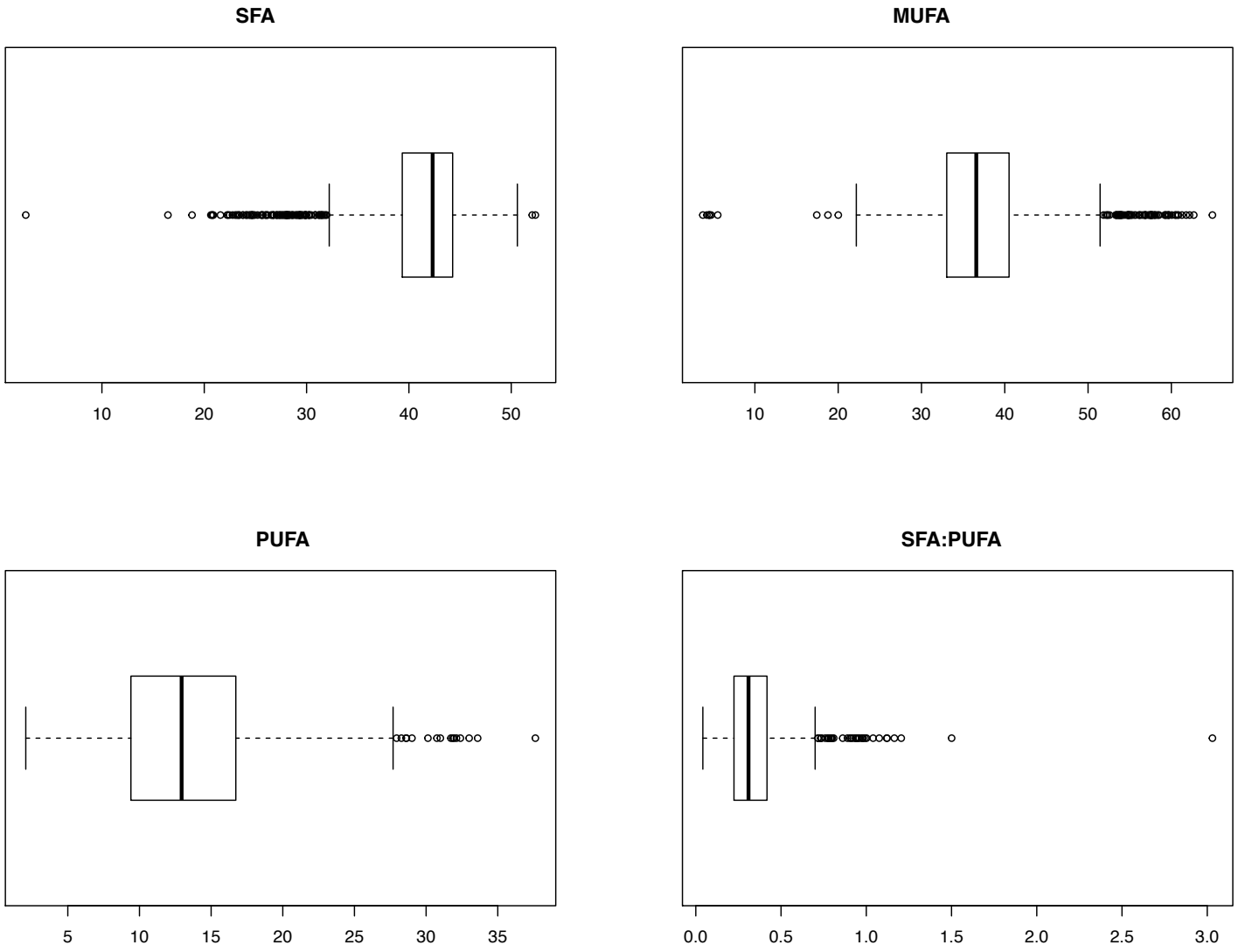

Figure 4 - Distribution of Phenotypes for SFA, MUFA, PUFA and SFA:PUFA ratio

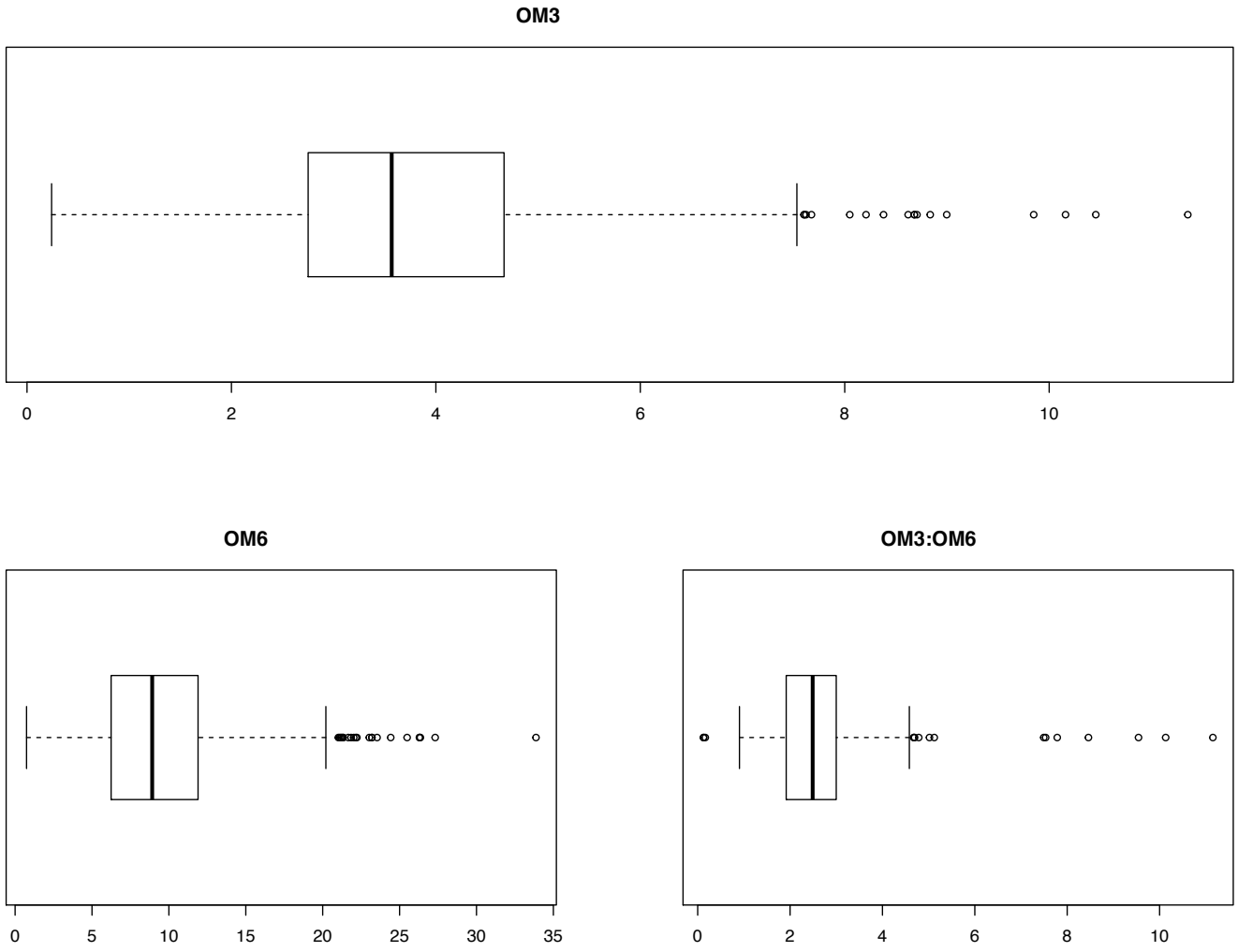

Figure 5 - Distribution of Phenotypes for OM3, OM6 and OM3:OM6 ratio

Table 2. Pairwise interactions between SNP ordered from smallest to largest p-adjusted value (Bonferroni correction) for SFA.

[1]M1	[2]M2	*CHR_M1	**CHR_M2	POS_ M1	GENE 1	POS_M2	GENE 2	Z-score	p-value	p-adjusted
299840	299962	**24**	**24**	60998847	*PIGN*	61767732	*PHLPP1*	9.237605971	2.52E-20	3.92E-14
14592	15033	**1**	**1**	114375154	*ARHGEF26*	118406468	*ERICH6*	-9.210066565	3.26E-20	5.07E-14
299853	299962	**24**	**24**	61059996	*PIGN*	61767732	*PHLPP1*	-9.199994136	3.58E-20	5.56E-14
14857	15033	**1**	**1**	116316049	*MBNL1*	118406468	*ERICH6*	9.195536985	3.73E-20	5.80E-14
299852	299965	**24**	**24**	61057651	*PIGN*	61777439	*PHLPP1*	-9.1818556	4.24E-20	6.59E-14
299846	299962	**24**	**24**	61034374	*PIGN*	61767732	*PHLPP1*	-9.147871027	5.81E-20	9.03E-14
299848	299962	**24**	**24**	61045805	*PIGN*	61767732	*PHLPP1*	9.147871027	5.81E-20	9.03E-14
299853	299961	**24**	**24**	61059996	*PIGN*	61765724	*PHLPP1*	9.13632231	6.46E-20	1.00E-13
299852	299962	**24**	**24**	61057651	*PIGN*	61767732	*PHLPP1*	-9.130181396	6.84E-20	1.06E-13
206092	284062	**14**	**22**	66564630	*RGS22*	59869412	*RAB7A*	-9.117988245	7.65E-20	1.19E-13
299845	299962	**24**	**24**	61032761	*PIGN*	61767732	*PHLPP1*	-9.046383695	1.48E-19	2.30E-13
250534	250536	**19**	**19**	5247847	*TOM1L1*	5251004	*TOM1L1*	-9.017790727	1.92E-19	2.98E-13
25738	25741	**2**	**2**	35978473	*RBMS1*	35988662	*RBMS1*	-8.999439341	2.27E-19	3.53E-13
299849	299961	**24**	**24**	61046286	*PIGN*	61765724	*PHLPP1*	8.984262347	2.60E-19	4.05E-13
299844	299962	**24**	**24**	61023449	*PIGN*	61767732	*PHLPP1*	8.965139248	3.10E-19	4.82E-13
39974	40120	**3**	**3**	9912443	*CFAP45*	10669751	*CADM3*	8.934468504	4.09E-19	6.36E-13
299853	299965	**24**	**24**	61059996	*PIGN*	61777439	*PHLPP1*	-8.870855666	7.26E-19	1.13E-12
299845	299961	**24**	**24**	61032761	*PIGN*	61765724	*PHLPP1*	8.77083076	1.77E-18	2.76E-12
299846	299961	**24**	**24**	61034374	*PIGN*	61765724	*PHLPP1*	-8.77083076	1.77E-18	2.76E-12
39968	40120	**3**	**3**	9884166	*TAGLN2*	10669751	*CADM3*	-8.770335569	1.78E-18	2.77E-12
299849	299962	**24**	**24**	61046286	*PIGN*	61767732	*PHLPP1*	8.634824297	5.88E-18	9.14E-12
206071	284065	**14**	**22**	66497860	*RGS22*	59886531	*RAB7A*	-8.618373854	6.79E-18	1.06E-11
39378	40205	**3**	**3**	6987793	*UHMK1*	11160291	*SPTA1*	-8.542775389	1.31E-17	2.04E-11
299846	299965	**24**	**24**	61034374	*PIGN*	61777439	*PHLPP1*	-8.537820773	1.37E-17	2.13E-11
206218	284065	**14**	**22**	67906207	*STK3*	59886531	*RAB7A*	8.530567822	1.46E-17	2.26E-11
299845	299965	**24**	**24**	61032761	*PIGN*	61777439	*PHLPP1*	8.466784483	2.52E-17	3.92E-11
299848	299961	**24**	**24**	61045805	*PIGN*	61765724	*PHLPP1*	-8.454608035	2.80E-17	4.35E-11
14857	14937	**1**	**1**	116316049	*MBNL1*	117589718	*MED12L*	-8.430913392	3.43E-17	5.33E-11
299852	299961	**24**	**24**	61057651	*PIGN*	61765724	*PHLPP1*	8.428898893	3.49E-17	5.42E-11
299848	299967	**24**	**24**	61045805	*PIGN*	61787493	*PHLPP1*	8.395735599	4.63E-17	7.20E-11
299848	299965	**24**	**24**	61045805	*PIGN*	61777439	*PHLPP1*	8.376909766	5.43E-17	8.45E-11
126629	126690	**8**	**8**	65370495	*NR4A3*	65781685	*TEX10*	8.269314544	1.35E-16	2.09E-10
14592	14857	**1**	**1**	114375154	*ARHGEF26*	116316049	*MBNL1*	-8.216600734	2.09E-16	3.25E-10
39967	40118	**3**	**3**	9868916	*IGSF9*	10664578	*CADM3*	-8.192980531	2.55E-16	3.96E-10
299846	299967	**24**	**24**	61034374	*PIGN*	61787493	*PHLPP1*	8.173960641	2.98E-16	4.64E-10
123886	126690	**8**	**8**	44615758	*CBWD2*	65781685	*TEX10*	-8.135929957	4.09E-16	6.35E-10
206092	284065	**14**	**22**	66564630	*RGS22*	59886531	*RAB7A*	-8.108373259	5.13E-16	7.97E-10
299853	299967	**24**	**24**	61059996	*PIGN*	61787493	*PHLPP1*	8.095745274	5.69E-16	8.85E-10
39967	40119	**3**	**3**	9868916	*IGSF9*	10665945	*CADM3*	-8.072824274	6.87E-16	1.07E-09

124552	126690	**8**	**8**	49174051	*TMC1*	65781685	*TEX10*	8.039165938	9.05E-16	1.41E-09
206092	284064	**14**	**22**	66564630	*RGS22*	59875610	*RAB7A*	-8.015824345	1.09E-15	1.70E-09
39968	39974	**3**	**3**	9884166	*TAGLN2*	9912443	*CFAP45*	-8.003401417	1.21E-15	1.88E-09
123957	126690	**8**	**8**	45213902	*PIP5K1B*	65781685	*TEX10*	7.948827149	1.88E-15	2.93E-09
313414	313524	**27**	**27**	4519557	*MCPH1*	6605494	*WDR17*	-7.94113106	2.00E-15	3.11E-09
125206	126690	**8**	**8**	53209337	*PRUNE2*	65781685	*TEX10*	7.933422877	2.13E-15	3.31E-09
39379	40205	**3**	**3**	6989312	*UHMK1*	11160291	*SPTA1*	7.913934944	2.49E-15	3.88E-09
123896	126653	**8**	**8**	44674742	*PGM5*	65518890	*ERP44*	-7.908964977	2.60E-15	4.03E-09
123896	126690	**8**	**8**	44674742	*PGM5*	65781685	*TEX10*	7.904954234	2.68E-15	4.17E-09
39967	39974	**3**	**3**	9868916	*IGSF9*	9912443	*CFAP45*	7.898303174	2.83E-15	4.39E-09
299849	299967	**24**	**24**	61046286	*PIGN*	61787493	*PHLPP1*	-7.886734931	3.10E-15	4.82E-09
39378	40120	**3**	**3**	6987793	*UHMK1*	10669751	*CADM3*	-7.882089737	3.22E-15	5.00E-09
299852	299967	**24**	**24**	61057651	*PIGN*	61787493	*PHLPP1*	7.871156887	3.51E-15	5.46E-09
39967	40120	**3**	**3**	9868916	*IGSF9*	10669751	*CADM3*	-7.847496867	4.24E-15	6.60E-09
206071	284064	**14**	**22**	66497860	*RGS22*	59875610	*RAB7A*	-7.836787516	4.62E-15	7.18E-09
299845	299967	**24**	**24**	61032761	*PIGN*	61787493	*PHLPP1*	7.832136233	4.80E-15	7.46E-09
39379	40120	**3**	**3**	6989312	*UHMK1*	10669751	*CADM3*	-7.811777336	5.64E-15	8.76E-09

[1]**M1**: first marker; [2]**M2**: second marker. ***CHR_M1**: chromosome number of the first SNP. ****CHR_M2**: chromosome number of the second SNP. **POS_M1**: position of the first marker in the chromosome (bp). **POS_M2**: position of the second marker in the chromosome (bp).

Table 3. Pairwise interactions between SNP ordered from smallest to largest p-adjusted value (Bonferroni correction) for PUFA

[1]M1	[2]M2	*CHR_M1	**CHR_M2	POS_M1	*GENE 1*	POS_M2	*GENE 2*	Z-score	p-value	p-adjusted
29329	29951	**2**	**2**	62337626	*ZRANB3*	67316112	*DPP10*	-9.142718005	6.09E-20	6.39E-14
26811	26842	**2**	**2**	44269847	*CACNB4*	44489753	*ARL5A*	-8.347836082	6.95E-17	7.29E-11
26713	26829	**2**	**2**	43710316	*FMNL2*	44357350	*CACNB4*	8.303182227	1.01E-16	1.06E-10
26543	26713	**2**	**2**	42074259	*GALNT13*	43710316	*FMNL2*	-8.182645133	2.78E-16	2.91E-10
26817	26842	**2**	**2**	44294648	*CACNB4*	44489753	*ARL5A*	-8.162004197	3.30E-16	3.46E-10
26763	26828	**2**	**2**	43960283	*FMNL2*	44352479	*CACNB4*	-8.147964646	3.70E-16	3.88E-10
26543	26712	**2**	**2**	42074259	*GALNT13*	43706505	*FMNL2*	8.14575902	3.77E-16	3.95E-10
26757	26570	**2**	**2**	43916575	*FMNL2*	42208388	*GALNT13*	8.142321638	3.88E-16	4.07E-10
26712	26824	**2**	**2**	43706505	*FMNL2*	44339781	*CACNB4*	-8.116255391	4.81E-16	5.04E-10
26734	26862	**2**	**2**	43818526	*FMNL2*	44706849	*NEB*	8.084261328	6.25E-16	6.56E-10
26570	26757	**2**	**2**	42208388	*GALNT13*	43916575	*FMNL2*	8.05864352	7.71E-16	8.09E-10
26543	26734	**2**	**2**	42074259	*GALNT13*	43818526	*FMNL2*	-8.051743529	8.16E-16	8.56E-10
29329	29553	**2**	**2**	62337626	*ZRANB3*	64897761	*NCKAP5*	8.005737682	1.19E-15	1.25E-09
26755	26862	**2**	**2**	43909122	*FMNL2*	44706849	*NEB*	-8.000629215	1.24E-15	1.30E-09
26543	26755	**2**	**2**	42074259	*GALNT13*	43909122	*FMNL2*	7.946839999	1.91E-15	2.01E-09
26713	26862	**2**	**2**	43710316	*FMNL2*	44706849	*NEB*	7.925016814	2.28E-15	2.39E-09
26763	26841	**2**	**2**	43960283	*FMNL2*	44455898	*CACNB4*	-7.901122914	2.76E-15	2.90E-09
26735	26862	**2**	**2**	43819207	*FMNL2*	44706849	*NEB*	-7.895075954	2.90E-15	3.04E-09
29951	29439	**2**	**2**	67316112	*DPP10*	63425581	*MGAT5*	7.875721713	3.39E-15	3.55E-09
29329	29565	**2**	**2**	62337626	*ZRANB3*	64966864	*NCKAP5*	7.869536487	3.56E-15	3.73E-09
26764	26841	**2**	**2**	43964871	*FMNL2*	44455898	*CACNB4*	7.865493751	3.68E-15	3.86E-09
26820	26766	**2**	**2**	44305088	*CACNB4*	43973183	*FMNL2*	7.858966054	3.87E-15	4.06E-09
26763	26794	**2**	**2**	43960283	*FMNL2*	44237727	*CACNB4*	-7.835138232	4.68E-15	4.91E-09
29095	27769	**2**	**2**	60502926	*THSD7B*	53134549	*ARHGAP15*	-7.819066433	5.32E-15	5.58E-09
26763	26793	**2**	**2**	43960283	*FMNL2*	44236066	*CACNB4*	-7.800384783	6.17E-15	6.47E-09
26820	26762	**2**	**2**	44305088	*CACNB4*	43948826	*FMNL2*	-7.780639412	7.22E-15	7.57E-09
29333	30024	**2**	**2**	62360277	*ZRANB3*	67910381	*DPP10*	7.755070561	8.83E-15	9.26E-09
29092	27769	**2**	**2**	60499160	*THSD7B*	53134549	*ARHGAP15*	7.750765082	9.13E-15	9.58E-09

[1]**M1**: first marker; [2]**M2**: second marker. ***CHR_M1**: chromosome number of the first SNP. ****CHR_M2**: chromosome number of the second SNP. **POS_M1**: position of the first marker in the chromosome (bp). **POS_M2**: position of the second marker in the chromosome (bp).

Table 4. Pairwise interactions between SNP ordered from smallest to largest p-adjusted value (Bonferroni correction) for MUFA

[1]M1	[2]M2	*CHR_M1	**CHR_M2	POS_M1	GENE 1	POS_M2	GENE 2	Zscore	p-value	p-adjusted
14329	14852	**1**	**1**	112344219	*KCNAB1*	116275673	*MBNL1*	-8.214976162	2.12E-16	2.66E-10
14329	14398	**1**	**1**	112344219	*KCNAB1*	112856613	*PLCH1*	7.992588712	1.32E-15	1.65E-09

[1]**M1**: first marker; [2]**M2**: second marker. ***CHR_M1**: chromosome number of the first SNP. ****CHR_M2**: chromosome number of the second SNP. **POS_M1**: position of the first marker in the chromosome (bp). **POS_M2**: position of the second marker in the chromosome (bp).

Table 5. Pairwise interactions between SNP ordered from smallest to largest p-adjusted value (Bonferroni correction) for SFA:PUFA ratio.

[1]M1	[2]M2	*CHR_M1	**CHR_M2	POS_M1	GENE 1	POS_M2	GENE 2	Z-score	p-value	p-adjusted
252170	306894	**19**	**26**	16504251	*ASIC2*	9411712	*ATAD1*	-12.76173211	2.68E-37	1.91E-30
257981	310709	**20**	**26**	3607029	*FBXW11*	39977573	*RGS10*	12.2182307	2.48E-34	1.77E-27
178987	297075	**12**	**24**	33918900	*ATP8A2*	40365618	*ARHGAP28*	-12.21406191	2.62E-34	1.87E-27
179003	297077	**12**	**24**	34048917	*ATP8A2*	40371138	*ARHGAP28*	-11.81711598	3.18E-32	2.27E-25
261110	313686	**20**	**27**	29265631	*HCN1*	7557964	*NEIL3*	-11.1576565	6.57E-29	4.69E-22
255801	309748	**19**	**26**	50614041	*WDR45B*	33500745	*VTI1A*	10.92396064	8.85E-28	6.32E-21
28912	39872	**2**	**3**	59857405	*THSD7B*	9397038	*VANGL2*	10.70914087	9.22E-27	6.58E-20
178999	297075	**12**	**24**	34003865	*ATP8A2*	40365618	*ARHGAP28*	10.69698277	1.05E-26	7.51E-20
163088	296963	**11**	**24**	9757497	*HK2*	39497963	*EPB41L3*	10.60408913	2.85E-26	2.04E-19
289138	319964	**23**	**28**	32765276	*FAM65B*	7177620	*SLC35F3*	-10.58155219	3.63E-26	2.59E-19
213857	297528	**15**	**24**	36072523	*PLEKHA7*	45701736	*SLC14A2*	10.39935754	2.50E-25	1.78E-18
232517	297774	**17**	**24**	9847596	*NR3C2*	47262205	*HDHD2*	-10.37182671	3.33E-25	2.38E-18
225768	297528	**16**	**24**	38923355	*GORAB*	45701736	*SLC14A2*	-10.34903876	4.23E-25	3.02E-18
206118	297526	**14**	**24**	66715114	*VPS13B*	45699025	*SLC14A2*	-10.27708406	8.94E-25	6.38E-18
225769	297528	**16**	**24**	38924807	*GORAB*	45701736	*SLC14A2*	10.18111329	2.41E-24	1.72E-17
144284	284065	**9**	**22**	75440483	*MAP7*	59886531	*RAB7A*	-10.15846958	3.04E-24	2.17E-17
209764	297528	**15**	**24**	4608820	*PDGFD*	45701736	*SLC14A2*	-10.13007967	4.06E-24	2.90E-17
179000	297075	**12**	**24**	34009782	*ATP8A2*	40365618	*ARHGAP28*	10.0509874	9.10E-24	6.49E-17
252266	307518	**19**	**26**	16940017	*ASIC2*	15465636	*PLCE1*	-10.03140374	1.11E-23	7.92E-17
232513	297774	**17**	**24**	9808520	*NR3C2*	47262205	*HDHD2*	10.01076682	1.37E-23	9.76E-17
232515	297774	**17**	**24**	9831087	*NR3C2*	47262205	*HDHD2*	9.949723597	2.53E-23	1.81E-16
207761	297528	**14**	**24**	76866740	*MMP16*	45701736	*SLC14A2*	-9.853834812	6.60E-23	4.71E-16
207753	297528	**14**	**24**	76821399	*MMP16*	45701736	*SLC14A2*	-9.829644444	8.39E-23	5.99E-16
213858	297528	**15**	**24**	36100743	*PLEKHA7*	45701736	*SLC14A2*	-9.823984151	8.88E-23	6.34E-16
207657	297528	**14**	**24**	76119558	*NBN*	45701736	*SLC14A2*	9.800998386	1.11E-22	7.96E-16
190639	297526	**13**	**24**	32686680	*SLC39A12*	45699025	*SLC14A2*	-9.791337711	1.23E-22	8.76E-16
232516	297774	**17**	**24**	9841754	*NR3C2*	47262205	*HDHD2*	9.778146147	1.40E-22	9.98E-16
42934	179090	**3**	**12**	31001943	*CTTNBP2NL*	34701872	*TNFRS19*	-9.733638964	2.17E-22	1.55E-15
166839	296963	**11**	**24**	37162091	*SPTBN1*	39497963	*EPB41L3*	-9.698504984	3.06E-22	2.18E-15
255810	309748	**19**	**26**	50789320	*OGFOD3*	33500745	*VTI1A*	-9.688458842	3.38E-22	2.41E-15
296331	320100	**24**	**28**	32624197	*IMPACT*	8282349	*B3GALNT2*	9.666222258	4.20E-22	3.00E-15
207768	297528	**14**	**24**	76887575	*MMP16*	45701736	*SLC14A2*	9.642298748	5.30E-22	3.78E-15
215777	297528	**15**	**24**	52146178	*NUP98*	45701736	*SLC14A2*	-9.600767855	7.94E-22	5.66E-15
207654	297528	**14**	**24**	76112356	*NBN*	45701736	*SLC14A2*	-9.595932028	8.32E-22	5.94E-15
169844	296963	**11**	**24**	68573047	*PCYOX1*	39497963	*EPB41L3*	-9.554072129	1.25E-21	8.90E-15
252280	308413	**19**	**26**	17027244	*ASIC2*	24079093	*NT5C2*	-9.518322986	1.76E-21	1.26E-14
207649	297528	**14**	**24**	76085697	*NBN*	45701736	*SLC14A2*	9.515657224	1.81E-21	1.29E-14
209773	297528	**15**	**24**	4684295	*PDGFD*	45701736	*SLC14A2*	9.509187719	1.92E-21	1.37E-14
64557	206694	**4**	**14**	87808102	*CADPS2*	70244957	*PTDSS1*	9.481038912	2.52E-21	1.80E-14

166846	296963	**11**	**24**	37230660	*SPTBN1*	39497963	*EPB41L3*	-9.46598291	2.91E-21	2.08E-14
255811	309749	**19**	**26**	50791728	*OGFOD3*	33501573	*VTI1A*	9.456657481	3.18E-21	2.27E-14
216164	297528	**15**	**24**	55287348	*ARRB1*	45701736	*SLC14A2*	-9.449100226	3.42E-21	2.44E-14
289032	319962	**23**	**28**	32310871	*LRRC16A*	7155492	*SLC35F3*	9.393310152	5.81E-21	4.15E-14
284062	319735	**22**	**28**	59869412	*RAB7A*	6000493	*PCNX2*	-9.369616601	7.28E-21	5.20E-14
206218	297526	**14**	**24**	67906207	*STK3*	45699025	*SLC14A2*	-9.359767464	7.99E-21	5.70E-14
70336	232936	**5**	**17**	4520002	*KCNC2*	11960908	*SLC10A7*	9.309104967	1.29E-20	9.20E-14
284065	319735	**22**	**28**	59886531	*RAB7A*	6000493	*PCNX2*	9.282643546	1.65E-20	1.18E-13
178996	297075	**12**	**24**	33986159	*ATP8A2*	40365618	*ARHGAP28*	9.274509649	1.78E-20	1.27E-13
163098	296963	**11**	**24**	9785177	*HK2*	39497963	*EPB41L3*	-9.273755637	1.80E-20	1.28E-13
166922	296963	**11**	**24**	38141105	*PPP4R3B*	39497963	*EPB41L3*	9.268652298	1.89E-20	1.35E-13
123900	283750	**8**	**22**	44692501	*PGM5*	58051122	*FGD5*	-9.266012234	1.93E-20	1.38E-13
29035	39891	**2**	**3**	60295198	*THSD7B*	9579325	*DCAF8*	-9.25010625	2.24E-20	1.60E-13
252171	306894	**19**	**26**	16504941	*ASIC2*	9411712	*ATAD1*	-9.24658364	2.32E-20	1.65E-13
159048	284262	**10**	**22**	89920617	*SLIRP*	61120264	*C22H3orf22*	-9.246239093	2.33E-20	1.66E-13
255797	309747	**19**	**26**	50588800	*RAB40B*	33497380	*VTI1A*	9.236383238	2.55E-20	1.82E-13
284064	319735	**22**	**28**	59875610	*RAB7A*	6000493	*PCNX2*	-9.220882255	2.95E-20	2.10E-13
232630	297774	**17**	**24**	10350076	*ARHGAP10*	47262205	*HDHD2*	-9.213975328	3.14E-20	2.24E-13
220086	297528	**15**	**24**	84541186	*MS4A7*	45701736	*SLC14A2*	9.212850298	3.18E-20	2.27E-13
213898	297528	**15**	**24**	36479695	*SOX6*	45701736	*SLC14A2*	-9.202885835	3.48E-20	2.49E-13
206705	297527	**14**	**24**	70317978	*MTERF3*	45700932	*SLC14A2*	-9.201055665	3.54E-20	2.53E-13
83210	259870	**5**	**20**	108446321	*ERC1*	20079650	*PDE4D*	-9.188799563	3.97E-20	2.84E-13
206133	297526	**14**	**24**	66874853	*VPS13B*	45699025	*SLC14A2*	-9.178299673	4.38E-20	3.13E-13
289129	319962	**23**	**28**	32734822	*FAM65B*	7155492	*SLC35F3*	-9.155950505	5.39E-20	3.85E-13
284063	319735	**22**	**28**	59872350	*RAB7A*	6000493	*PCNX2*	9.149852225	5.70E-20	4.07E-13
219263	297528	**15**	**24**	77573308	*CKAP5*	45701736	*SLC14A2*	-9.134573725	6.57E-20	4.69E-13
64554	206694	**4**	**14**	87800864	*CADPS2*	70244957	*PTDSS1*	-9.097133157	9.27E-20	6.62E-13
190251	297526	**13**	**24**	29984067	*NMT2*	45699025	*SLC14A2*	-9.09712443	9.28E-20	6.62E-13
190793	297526	**13**	**24**	33455505	*EPC1*	45699025	*SLC14A2*	9.075419904	1.13E-19	8.08E-13
206622	297526	**14**	**24**	69827335	*CPQ*	45699025	*SLC14A2*	9.039401531	1.58E-19	1.12E-12
219305	297528	**15**	**24**	78169400	*C15H11orf49*	45701736	*SLC14A2*	-9.019720007	1.89E-19	1.35E-12
144296	284065	**9**	**22**	75481233	*MAP7*	59886531	*RAB7A*	-9.011097408	2.04E-19	1.46E-12
59546	202995	**4**	**14**	38703078	*CACNA2D1*	45478315	*MRPS28*	-8.968573355	3.00E-19	2.14E-12
179005	297077	**12**	**24**	34056537	*ATP8A2*	40371138	*ARHGAP28*	8.963080339	3.16E-19	2.25E-12
289033	319962	**23**	**28**	32311772	*LRRC16A*	7155492	*SLC35F3*	-8.951067886	3.52E-19	2.51E-12
205591	297526	**14**	**24**	63286705	*SLC25A32*	45699025	*SLC14A2*	8.934791618	4.08E-19	2.91E-12
209787	297528	**15**	**24**	4742211	*PDGFD*	45701736	*SLC14A2*	8.928316751	4.33E-19	3.09E-12
69899	232611	**5**	**17**	1495774	*TBC1D15*	10306845	*ARHGAP10*	8.911778666	5.02E-19	3.59E-12
206694	297526	**14**	**24**	70244957	*PTDSS1*	45699025	*SLC14A2*	-8.909693451	5.12E-19	3.65E-12
39379	80730	**3**	**5**	6989312	*UHMK1*	88426925	*ST8SIA1*	8.906512986	5.27E-19	3.76E-12
296963	320432	**24**	**28**	39497963	*EPB41L3*	10056945	*RYR2*	-8.903388065	5.42E-19	3.87E-12
40206	159045	**3**	**10**	11180421	*SPTA1*	89900420	*ALKBH1*	8.900260689	5.57E-19	3.98E-12

207655	297528	**14**	**24**	76114413	*NBN*	45701736	*SLC14A2*	8.897746189	5.70E-19	4.07E-12
169871	296963	**11**	**24**	68690415	*CAPN14*	39497963	*EPB41L3*	8.867539321	7.48E-19	5.34E-12
202989	297526	**14**	**24**	45427353	*MRPS28*	45699025	*SLC14A2*	8.83447754	1.01E-18	7.18E-12
89840	259870	**6**	**20**	36178233	*MMRN1*	20079650	*PDE4D*	-8.833375055	1.02E-18	7.25E-12
219268	297528	**15**	**24**	77614397	*CKAP5*	45701736	*SLC14A2*	8.830842915	1.04E-18	7.42E-12
178994	297075	**12**	**24**	33978213	*ATP8A2*	40365618	*ARHGAP28*	8.83010029	1.05E-18	7.47E-12
178995	297075	**12**	**24**	33982820	*ATP8A2*	40365618	*ARHGAP28*	8.803652125	1.32E-18	9.45E-12
166831	296963	**11**	**24**	37053202	*SPTBN1*	39497963	*EPB41L3*	8.803288243	1.33E-18	9.48E-12
231768	297529	**17**	**24**	4973973	*TMEM154*	45707035	*SLC14A2*	-8.802438562	1.34E-18	9.56E-12
252267	308413	**19**	**26**	16948138	*ASIC2*	24079093	*NT5C2*	-8.796506257	1.41E-18	1.01E-11
128666	284033	**8**	**22**	79703780	*NTRK2*	59632519	*KIAA1257*	-8.790414254	1.49E-18	1.06E-11
232158	297529	**17**	**24**	7679873	*DCLK2*	45707035	*SLC14A2*	8.776137255	1.69E-18	1.21E-11
159045	284262	**10**	**22**	89900420	*ALKBH1*	61120264	*C22H3orf22*	-8.766011638	1.85E-18	1.32E-11
22563	31350	**2**	**2**	14467327	*PDE1A*	80271418	*MYO1B*	8.761969242	1.92E-18	1.37E-11
169870	296963	**11**	**24**	68688726	*CAPN14*	39497963	*EPB41L3*	-8.761816341	1.92E-18	1.37E-11
219265	297528	**15**	**24**	77584004	*CKAP5*	45701736	*SLC14A2*	-8.74957617	2.14E-18	1.53E-11
163094	296963	**11**	**24**	9765392	*HK2*	39497963	*EPB41L3*	-8.74746246	2.18E-18	1.56E-11
70330	232930	**5**	**17**	4485709	*KCNC2*	11932668	*SLC10A7*	8.746295214	2.20E-18	1.57E-11
123906	283750	**8**	**22**	44732966	*PGM5*	58051122	*FGD5*	8.745011196	2.23E-18	1.59E-11
59544	202989	**4**	**14**	38691328	*CACNA2D1*	45427353	*MRPS28*	-8.706576711	3.13E-18	2.24E-11
205470	297526	**14**	**24**	62449702	*DCSTAMP*	45699025	*SLC14A2*	-8.694748349	3.48E-18	2.48E-11
144232	284065	**9**	**22**	75113906	*PDE7B*	59886531	*RAB7A*	-8.694331264	3.49E-18	2.49E-11
143674	284064	**9**	**22**	70072826	*EPB41L2*	59875610	*RAB7A*	-8.688939599	3.66E-18	2.61E-11
196250	297526	**13**	**24**	80021463	*NFATC2*	45699025	*SLC14A2*	-8.679238609	3.98E-18	2.84E-11
213915	297528	**15**	**24**	36624829	*SOX6*	45701736	*SLC14A2*	-8.673840455	4.18E-18	2.98E-11
69898	232508	**5**	**17**	1494203	*TBC1D15*	9797631	*NR3C2*	8.671617144	4.26E-18	3.04E-11
206703	297527	**14**	**24**	70310521	*MTERF3*	45700932	*SLC14A2*	8.668955752	4.36E-18	3.11E-11
63185	206092	**4**	**14**	77782748	*CAMK2B*	66564630	*RGS22*	-8.666253154	4.47E-18	3.19E-11
166837	296963	**11**	**24**	37112612	*SPTBN1*	39497963	*EPB41L3*	-8.658467266	4.78E-18	3.41E-11
64561	206705	**4**	**14**	87826794	*CADPS2*	70317978	*MTERF3*	-8.657928027	4.80E-18	3.43E-11
206702	297527	**14**	**24**	70296507	*PTDSS1*	45700932	*SLC14A2*	-8.644160385	5.42E-18	3.87E-11
178991	297075	**12**	**24**	33952558	*ATP8A2*	40365618	*ARHGAP28*	-8.643332934	5.46E-18	3.90E-11
202440	297526	**14**	**24**	40878794	*HNF4G*	45699025	*SLC14A2*	-8.625223198	6.40E-18	4.57E-11
70800	232981	**5**	**17**	7006175	*NAV3*	12140301	*SLC10A7*	-8.624668712	6.43E-18	4.59E-11
213914	297528	**15**	**24**	36623920	*SOX6*	45701736	*SLC14A2*	-8.618003037	6.81E-18	4.86E-11
209766	297528	**15**	**24**	4615760	*PDGFD*	45701736	*SLC14A2*	8.61097665	7.24E-18	5.17E-11
209768	297528	**15**	**24**	4646805	*PDGFD*	45701736	*SLC14A2*	-8.61097665	7.24E-18	5.17E-11
163087	295299	**11**	**24**	9756458	*HK2*	24442137	*CCDC178*	8.602952447	7.77E-18	5.55E-11
21116	30332	**2**	**2**	4311198	*UGGT1*	71610210	*TMEM37*	-8.588330584	8.82E-18	6.30E-11
144297	284065	**9**	**22**	75481682	*MAP7*	59886531	*RAB7A*	-8.583869279	9.17E-18	6.55E-11
83215	259870	**5**	**20**	108471989	*ERC1*	20079650	*PDE4D*	8.578786652	9.59E-18	6.84E-11
70333	232930	**5**	**17**	4489950	*KCNC2*	11932668	*SLC10A7*	-8.576881885	9.75E-18	6.96E-11

159051	284265	**10**	**22**	89947271	*SNW1*	61128608	*CHST13*	-8.560772568	1.12E-17	8.00E-11
190786	297526	**13**	**24**	33423542	*EPC1*	45699025	*SLC14A2*	-8.559648348	1.13E-17	8.08E-11
202988	297526	**14**	**24**	45423941	*MRPS28*	45699025	*SLC14A2*	8.556703417	1.16E-17	8.29E-11
123897	283750	**8**	**22**	44678053	*PGM5*	58051122	*FGD5*	-8.54731409	1.26E-17	8.99E-11
232620	297774	**17**	**24**	10330387	*ARHGAP10*	47262205	*HDHD2*	-8.54271912	1.31E-17	9.36E-11
30024	69898	**2**	**5**	67910381	*DPP10*	1494203	*TBC1D15*	-8.538274008	1.36E-17	9.73E-11
196873	297526	**14**	**24**	2401335	*ZC3H3*	45699025	*SLC14A2*	8.528504814	1.48E-17	1.06E-10
206700	297526	**14**	**24**	70277874	*PTDSS1*	45699025	*SLC14A2*	-8.517052244	1.64E-17	1.17E-10
48379	190251	**3**	**13**	70583130	*TNNI3K*	29984067	*NMT2*	-8.511665435	1.71E-17	1.22E-10
64809	207649	**4**	**14**	89946180	*POT1*	76085697	*NBN*	-8.510420263	1.73E-17	1.24E-10
143675	284064	**9**	**22**	70082581	*EPB41L2*	59875610	*RAB7A*	8.503363027	1.84E-17	1.31E-10
196880	297526	**14**	**24**	2468020	*RHPN1*	45699025	*SLC14A2*	8.502394469	1.86E-17	1.33E-10
64535	206694	**4**	**14**	87707067	*CADPS2*	70244957	*PTDSS1*	-8.499015349	1.91E-17	1.36E-10
6178	29440	**1**	**2**	42297334	*OR5K4*	63443648	*MGAT5*	-8.498749726	1.92E-17	1.37E-10
59545	202989	**4**	**14**	38698913	*CACNA2D1*	45427353	*MRPS28*	-8.489299004	2.08E-17	1.48E-10
179051	297077	**12**	**24**	34458185	*SPATA13*	40371138	*ARHGAP28*	8.488913448	2.09E-17	1.49E-10
202443	297526	**14**	**24**	40898414	*HNF4G*	45699025	*SLC14A2*	-8.487144621	2.12E-17	1.51E-10
80725	238826	**5**	**17**	88388051	*ST8SIA1*	56004357	*KDM2B*	8.480225378	2.25E-17	1.60E-10
206071	297526	**14**	**24**	66497860	*RGS222*	45699025	*SLC14A2*	-8.480680099	2.24E-17	1.60E-10
177284	297075	**12**	**24**	16345453	*LCP1*	40365618	*ARHGAP28*	8.476008143	2.33E-17	1.66E-10
39919	123886	**3**	**8**	9700042	*IGSF8*	44615758	*CBWD2*	8.456598072	2.75E-17	1.97E-10
63178	206092	**4**	**14**	77744530	*CAMK2B*	66564630	*RGS22*	-8.447264043	2.98E-17	2.13E-10
16048	29486	**1**	**2**	126525570	*SLC9A9*	64254179	*NCKAP5*	8.444993717	3.04E-17	2.17E-10
63184	206092	**4**	**14**	77778258	*CAMK2B*	66564630	*RGS22*	8.444466946	3.05E-17	2.18E-10
213154	297528	**15**	**24**	31418981	*POU2F3*	45701736	*SLC14A2*	-8.43068879	3.44E-17	2.45E-10
232621	297774	**17**	**24**	10331605	*ARHGAP10*	47262205	*HDHD2*	8.421621653	3.71E-17	2.65E-10
232584	297774	**17**	**24**	10203989	*ARHGAP10*	47262205	*HDHD2*	-8.416352392	3.88E-17	2.77E-10
29553	69898	**2**	**5**	64897761	*NCKAP5*	1494203	*TBC1D15*	-8.414759642	3.94E-17	2.81E-10
178988	297075	**12**	**24**	33932009	*ATP8A2*	40365618	*ARHGAP28*	-8.4135498	3.98E-17	2.84E-10
29488	69897	**2**	**5**	64291820	*NCKAP5*	1484381	*TBC1D15*	8.409382013	4.12E-17	2.94E-10
231770	297529	**17**	**24**	5018949	*TMEM154*	45707035	*SLC14A2*	8.408083976	4.17E-17	2.98E-10
209790	297528	**15**	**24**	4749879	*PDGFD*	45701736	*SLC14A2*	-8.390536928	4.84E-17	3.45E-10
206092	297526	**14**	**24**	66564630	*RGS222*	45699025	*SLC14A2*	8.38854334	4.92E-17	3.51E-10
179053	297077	**12**	**24**	34487427	*SPATA13*	40371138	*ARHGAP28*	8.387518328	4.97E-17	3.54E-10
225767	297528	**16**	**24**	38921915	*GORAB*	45701736	*SLC14A2*	8.373105393	5.61E-17	4.01E-10
167584	296963	**11**	**24**	44501816	*CCDC138*	39497963	*EPB41L3*	8.365514625	5.99E-17	4.27E-10
190789	297526	**13**	**24**	33433599	*EPC1*	45699025	*SLC14A2*	-8.360270386	6.26E-17	4.47E-10
207769	297528	**14**	**24**	76888461	*MMP16*	45701736	*SLC14A2*	-8.36001381	6.27E-17	4.48E-10
60205	202995	**4**	**14**	47354136	*CDHR3*	45478315	*MRPS28*	-8.351962846	6.71E-17	4.79E-10
144229	284064	**9**	**22**	75085763	*PDE7B*	59875610	*RAB7A*	-8.34782984	6.95E-17	4.96E-10
196252	297526	**13**	**24**	80032003	*NFATC2*	45699025	*SLC14A2*	8.347534854	6.97E-17	4.98E-10
232594	297774	**17**	**24**	10235581	*ARHGAP10*	47262205	*HDHD2*	8.33282675	7.89E-17	5.63E-10

207744	297528	**14**	**24**	76769105	*MMP16*	45701736	*SLC14A2*	8.332019579	7.95E-17	5.67E-10
207751	297528	**14**	**24**	76811795	*MMP16*	45701736	*SLC14A2*	-8.332019579	7.95E-17	5.67E-10
205468	297526	**14**	**24**	62446551	*DCSTAMP*	45699025	*SLC14A2*	8.330292854	8.06E-17	5.76E-10
232666	297774	**17**	**24**	10484179	*ARHGAP10*	47262205	*HDHD2*	8.303273714	1.01E-16	7.23E-10
124569	283750	**8**	**22**	49256247	*TMC1*	58051122	*FGD5*	8.299845378	1.04E-16	7.44E-10
163061	289129	**11**	**23**	9648849	*TACR1*	32734822	*FAM65B*	8.299448671	1.05E-16	7.47E-10
232151	297529	**17**	**24**	7663703	*DCLK2*	45707035	*SLC14A2*	8.29295015	1.10E-16	7.89E-10
144272	284065	**9**	**22**	75372772	*MAP7*	59886531	*RAB7A*	8.29128923	1.12E-16	8.00E-10
28897	39872	**2**	**3**	59787779	*THSD7B*	9397038	*VANGL2*	-8.286069682	1.17E-16	8.36E-10
58216	196250	**4**	**13**	27197790	*HDAC9*	80021463	*NFATC2*	8.278201182	1.25E-16	8.93E-10
207658	297528	**14**	**24**	76126581	*NBN*	45701736	*SLC14A2*	8.272611893	1.31E-16	9.36E-10
179061	297077	**12**	**24**	34545672	*C1QTNF9*	40371138	*ARHGAP28*	8.272271247	1.31E-16	9.38E-10
57799	190823	**4**	**13**	23135934	*DGKB*	33633520	*KIF5B*	-8.26873551	1.35E-16	9.66E-10
40858	179073	**3**	**12**	16212831	*IL6R*	34600028	*MIPEP*	-8.268651859	1.35E-16	9.67E-10
166833	296963	**11**	**24**	37107434	*SPTBN1*	39497963	*EPB41L3*	8.265118991	1.40E-16	9.96E-10
80731	252280	**5**	**19**	88428857	*ST8SIA1*	17027244	*ASIC2*	-8.256853813	1.50E-16	1.07E-09
59547	202995	**4**	**14**	38731862	*CACNA2D1*	45478315	*MRPS28*	-8.255753433	1.51E-16	1.08E-09
232149	297529	**17**	**24**	7644804	*DCLK2*	45707035	*SLC14A2*	-8.246959052	1.62E-16	1.16E-09
29951	69898	**2**	**5**	67316112	*DPP10*	1494203	*TBC1D15*	-8.241539073	1.70E-16	1.21E-09
145589	284065	**9**	**22**	84688764	*GRM1*	59886531	*RAB7A*	8.24111482	1.71E-16	1.22E-09
176512	297075	**12**	**24**	11924528	*VWA8*	40365618	*ARHGAP28*	-8.240640606	1.71E-16	1.22E-09
284262	319735	**22**	**28**	61120264	*C22H3orf22*	6000493	*PCNX2*	-8.233241991	1.82E-16	1.30E-09
178986	297075	**12**	**24**	33905997	*ATP8A2*	40365618	*ARHGAP28*	8.227557788	1.91E-16	1.36E-09
166841	296963	**11**	**24**	37171806	*SPTBN1*	39497963	*EPB41L3*	8.226832593	1.92E-16	1.37E-09
89991	259870	**6**	**20**	37272546	*FAM13A*	20079650	*PDE4D*	-8.224304603	1.96E-16	1.40E-09
69792	209790	**5**	**15**	838374	*TSPAN8*	4749879	*PDGFD*	-8.22285734	1.99E-16	1.42E-09
123957	283750	**8**	**22**	45213902	*PIP5K1B*	58051122	*FGD5*	8.2178231	2.07E-16	1.48E-09
250542	306821	**19**	**26**	5276248	*TOM1L1*	8789214	*SGMS1*	-8.217795972	2.07E-16	1.48E-09
16056	29486	**1**	**2**	126539752	*SLC9A9*	64254179	*NCKAP5*	-8.217037433	2.09E-16	1.49E-09
29486	63196	**2**	**4**	64254179	*NCKAP5*	77841970	*GCK*	-8.216768002	2.09E-16	1.49E-09
29489	69897	**2**	**5**	64305441	*NCKAP5*	1484381	*TBC1D15*	8.216939593	2.09E-16	1.49E-09
257979	310709	**20**	**26**	3600078	*FBXW11*	39977573	*RGS10*	8.213327106	2.15E-16	1.54E-09
206121	297526	**14**	**24**	66737614	*VPS13B*	45699025	*SLC14A2*	8.208476483	2.24E-16	1.60E-09
232107	297529	**17**	**24**	7141639	*LRBA*	45707035	*SLC14A2*	8.207143827	2.27E-16	1.62E-09
144288	284065	**9**	**22**	75454006	*MAP7*	59886531	*RAB7A*	8.201482336	2.37E-16	1.70E-09
63175	206092	**4**	**14**	77725458	*CAMK2B*	66564630	*RGS22*	8.188087616	2.65E-16	1.89E-09
31350	69898	**2**	**5**	80271418	*MYO1B*	1494203	*TBC1D15*	8.187732082	2.66E-16	1.90E-09
209770	297528	**15**	**24**	4652065	*PDGFD*	45701736	*SLC14A2*	-8.186995784	2.68E-16	1.91E-09
17198	29486	**1**	**2**	135450925	*EPHB1*	64254179	*NCKAP5*	8.178302283	2.88E-16	2.06E-09
206122	297526	**14**	**24**	66743421	*VPS13B*	45699025	*SLC14A2*	-8.1781206	2.88E-16	2.06E-09
144304	284065	**9**	**22**	75513089	*MAP7*	59886531	*RAB7A*	-8.172980033	3.01E-16	2.15E-09
206134	297526	**14**	**24**	66877367	*VPS13B*	45699025	*SLC14A2*	8.173098147	3.01E-16	2.15E-09

232085	297529	**17**	**24**	6851772	*LRBA*	45707035	*SLC14A2*	-8.17233415	3.02E-16	2.16E-09
178278	297075	**12**	**24**	24256654	*POSTN*	40365618	*ARHGAP28*	8.171993619	3.03E-16	2.17E-09
178989	297075	**12**	**24**	33941387	*ATP8A2*	40365618	*ARHGAP28*	-8.168442939	3.12E-16	2.23E-09
206519	297526	**14**	**24**	69346879	*CPQ*	45699025	*SLC14A2*	-8.165178843	3.21E-16	2.29E-09
14559	29486	**1**	**2**	114221979	*GPR149*	64254179	*NCKAP5*	-8.148944974	3.67E-16	2.62E-09
57800	195584	**4**	**13**	23167092	*DGKB*	75383374	*PLTP*	-8.139167257	3.98E-16	2.84E-09
167590	296963	**11**	**24**	44602418	*LIMS1*	39497963	*EPB41L3*	8.138828991	3.99E-16	2.85E-09
63187	206092	**4**	**14**	77796085	*CAMK2B*	66564630	*RGS22*	8.13383985	4.16E-16	2.97E-09
69912	232620	**5**	**17**	1562117	*TPH2*	10330387	*ARHGAP10*	8.133347278	4.18E-16	2.98E-09
169835	296963	**11**	**24**	68488679	*TIA1*	39497963	*EPB41L3*	8.13194789	4.22E-16	3.02E-09
39890	123014	**3**	**8**	9563094	*DCAF8*	38784045	*IL33*	8.131440843	4.24E-16	3.03E-09
39967	123896	**3**	**8**	9868916	*IGSF9*	44674742	*PGM5*	-8.128603788	4.34E-16	3.10E-09
42930	179079	**3**	**12**	30978898	*ST7L*	34635671	*MIPEP*	8.125761339	4.45E-16	3.17E-09
144230	284064	**9**	**22**	75086283	*PDE7B*	59875610	*RAB7A*	8.121955999	4.59E-16	3.27E-09
176503	297075	**12**	**24**	11900051	*VWA8*	40365618	*ARHGAP28*	8.117870069	4.74E-16	3.39E-09
39891	123051	**3**	**8**	9579325	*DCAF8*	39203385	*RIC1*	-8.114663237	4.87E-16	3.48E-09
179057	297077	**12**	**24**	34505806	*SPATA13*	40371138	*ARHGAP28*	-8.109783951	5.07E-16	3.62E-09
206632	297526	**14**	**24**	69863353	*CPQ*	45699025	*SLC14A2*	8.10991283	5.07E-16	3.62E-09
58406	196250	**4**	**13**	28617684	*TMEM196*	80021463	*NFATC2*	8.100757317	5.46E-16	3.90E-09
144218	284064	**9**	**22**	74974302	*PDE7B*	59875610	*RAB7A*	-8.100142869	5.49E-16	3.92E-09
144306	284065	**9**	**22**	75516637	*MAP7*	59886531	*RAB7A*	8.098894483	5.55E-16	3.96E-09
232156	297529	**17**	**24**	7677338	*DCLK2*	45707035	*SLC14A2*	8.097123244	5.63E-16	4.02E-09
124554	283750	**8**	**22**	49186722	*TMC1*	58051122	*FGD5*	-8.093165064	5.81E-16	4.15E-09
179059	297077	**12**	**24**	34516586	*SPATA13*	40371138	*ARHGAP28*	-8.084377278	6.25E-16	4.46E-09
17207	29486	**1**	**2**	135466589	*EPHB1*	64254179	*NCKAP5*	8.08346483	6.30E-16	4.49E-09
172882	297075	**11**	**24**	92913599	*TTLL11*	40365618	*ARHGAP28*	8.081371394	6.40E-16	4.57E-09
143679	284064	**9**	**22**	70089340	*EPB41L2*	59875610	*RAB7A*	8.077380244	6.62E-16	4.72E-09
16043	29486	**1**	**2**	126515676	*SLC9A9*	64254179	*NCKAP5*	-8.076677066	6.66E-16	4.75E-09
39378	80699	**3**	**5**	6987793	*UHMK1*	88188462	*C2CD5*	-8.061803494	7.52E-16	5.37E-09
166921	296963	**11**	**24**	38132585	*PPP4R3B*	39497963	*EPB41L3*	8.060156877	7.62E-16	5.44E-09
180220	297077	**12**	**24**	46672861	*DACH1*	40371138	*ARHGAP28*	8.059489438	7.66E-16	5.47E-09
29049	39919	**2**	**3**	60361987	*THSD7B*	9700042	*IGSF8*	-8.057630058	7.78E-16	5.55E-09
176502	297075	**12**	**24**	11899090	*VWA8*	40365618	*ARHGAP28*	-8.052517993	8.11E-16	5.79E-09
206125	297526	**14**	**24**	66783251	*VPS13B*	45699025	*SLC14A2*	8.045216341	8.61E-16	6.15E-09
179109	297077	**12**	**24**	34944506	*SGCG*	40371138	*ARHGAP28*	-8.044903584	8.63E-16	6.16E-09
80730	244912	**5**	**18**	88426925	*ST8SIA1*	25017398	*SLC12A3*	8.044195413	8.68E-16	6.20E-09
209772	297528	**15**	**24**	4679251	*PDGFD*	45701736	*SLC14A2*	-8.042257233	8.82E-16	6.30E-09
48380	190251	**3**	**13**	70587724	*TNNI3K*	29984067	*NMT2*	8.041535778	8.87E-16	6.33E-09
232155	297529	**17**	**24**	7673701	*DCLK2*	45707035	*SLC14A2*	-8.040603148	8.94E-16	6.38E-09
169837	296963	**11**	**24**	68505012	*TIA1*	39497963	*EPB41L3*	-8.038759126	9.08E-16	6.48E-09
29024	39890	**2**	**3**	60259782	*THSD7B*	9563094	*DCAF8*	-8.037870493	9.14E-16	6.53E-09
229284	297528	**16**	**24**	70893860	*ARL8A*	45701736	*SLC14A2*	8.035366556	9.33E-16	6.66E-09

144316	284065	**9**	**22**	75559419	*MAP3K5*	59886531	*RAB7A*	-8.0340211	9.43E-16	6.73E-09
190788	297526	**13**	**24**	33429123	*EPC1*	45699025	*SLC14A2*	8.027626298	9.94E-16	7.09E-09
6018	29333	**1**	**2**	41202669	*EPHA6*	62360277	*ZRANB3*	8.026483997	1.00E-15	7.16E-09
206129	297526	**14**	**24**	66835939	*VPS13B*	45699025	*SLC14A2*	-8.024465687	1.02E-15	7.28E-09
176499	297075	**12**	**24**	11894765	*VWA8*	40365618	*ARHGAP28*	-8.022793794	1.03E-15	7.38E-09
206126	297526	**14**	**24**	66803645	*VPS13B*	45699025	*SLC14A2*	8.019725663	1.06E-15	7.57E-09
6019	29437	**1**	**2**	41211602	*EPHA6*	63407064	*MGAT5*	-8.019116545	1.07E-15	7.60E-09
206120	297526	**14**	**24**	66725671	*VPS13B*	45699025	*SLC14A2*	-8.018946976	1.07E-15	7.61E-09
58419	196252	**4**	**13**	28742888	*TMEM196*	80032003	*NFATC2*	-8.014823048	1.10E-15	7.87E-09
69835	229285	**5**	**16**	1152296	*LGR5*	70897141	*ARL8A*	8.013237331	1.12E-15	7.98E-09
196246	297526	**13**	**24**	79998437	*NFATC2*	45699025	*SLC14A2*	8.012402729	1.12E-15	8.03E-09
144221	284064	**9**	**22**	75017955	*PDE7B*	59875610	*RAB7A*	-8.009787382	1.15E-15	8.20E-09
232150	297529	**17**	**24**	7652242	*DCLK2*	45707035	*SLC14A2*	8.009675579	1.15E-15	8.21E-09
169843	296963	**11**	**24**	68565378	*PCYOX1*	39497963	*EPB41L3*	8.007557908	1.17E-15	8.35E-09
232108	297529	**17**	**24**	7144481	*LRBA*	45707035	*SLC14A2*	-8.007427857	1.17E-15	8.36E-09
61570	206071	**4**	**14**	61839655	*HERPUD2*	66497860	*RGS22*	-8.007014489	1.18E-15	8.39E-09
284059	319449	**22**	**28**	59807304	*COPG1*	4596283	*DISC1*	8.001509355	1.23E-15	8.77E-09
178990	297075	**12**	**24**	33943940	*ATP8A2*	40365618	*ARHGAP28*	-8.00109633	1.23E-15	8.80E-09
232504	297769	**17**	**24**	9780741	*NR3C2*	47202356	*KATNAL2*	7.999425929	1.25E-15	8.92E-09
57459	190793	**4**	**13**	20159988	*SCIN*	33455505	*EPC1*	-7.997253408	1.27E-15	9.08E-09
26543	31350	**2**	**2**	42074259	*GALNT13*	80271418	*MYO1B*	-7.996447784	1.28E-15	9.14E-09
63198	206218	**4**	**14**	77855883	*GCK*	67906207	*STK3*	-7.996041427	1.28E-15	9.17E-09
284265	319921	**22**	**28**	61128608	*CHST13*	6988291	*SLC35F3*	7.994842491	1.30E-15	9.26E-09
166838	296963	**11**	**24**	37158203	*SPTBN1*	39497963	*EPB41L3*	-7.993740526	1.31E-15	9.34E-09
71244	232982	**5**	**17**	10277595	*PTPRQ*	12141089	*SLC10A7*	7.992105327	1.33E-15	9.47E-09
213892	297528	**15**	**24**	36388517	*SOX6*	45701736	*SLC14A2*	-7.991418298	1.33E-15	9.52E-09
232593	297774	**17**	**24**	10232184	*ARHGAP10*	47262205	*HDHD2*	7.988991251	1.36E-15	9.71E-09
229285	297528	**16**	**24**	70897141	*ARL8A*	45701736	*SLC14A2*	-7.986592024	1.39E-15	9.90E-09

[1]**M1**: first marker; [2]**M2**: second marker. ***CHR_M1**: chromosome number of the first SNP. ****CHR_M2**: chromosome number of the second SNP.
POS_M1: position of the first marker in the chromosome (bp). **POS_M2**: position of the second marker in the chromosome (bp).

Table 6. Pairwise interactions between SNP ordered from smallest to largest p-adjusted value (Bonferroni correction) for OM3.

[1]M1	[2]M2	*CHR_M1	**CHR_M2	POS_M1	GENE 1	POS_M2	GENE 2	Z-score	p-value	p-adjusted
26767	26824	**2**	**2**	43977791	*FMNL2*	44339781	*CACNB4*	-7.9934735	1.31E-15	2.86E-14
240075	240162	**17**	**17**	63709283	*RPH3A*	64154326	*HECTD4*	8.851485022	8.64E-19	1.43E-13
26841	26862	**2**	**2**	44455898	*CACNB4*	44706849	*NEB*	8.318311167	8.92E-17	8.40E-13
240068	240073	**17**	**17**	63656132	*OAS1X*	63700047	*RPH3A*	-9.994633956	1.61E-23	1.35E-12
26766	26817	**2**	**2**	43973183	*FMNL2*	44294648	*CACNB4*	7.769645476	7.87E-15	4.80E-12
240074	240162	**17**	**17**	63707713	*RPH3A*	64154326	*HECTD4*	8.797732816	1.40E-18	4.83E-11
29439	30024	**2**	**2**	63425581	*MGAT5*	67910381	*DPP10*	7.853206338	4.06E-15	5.45E-11
29333	29429	**2**	**2**	62360277	*ZRANB3*	63246684	*MGAT5*	8.505277407	1.81E-17	8.10E-11
240073	240158	**17**	**17**	63700047	*RPH3A*	64030042	*PTPN11*	8.843096766	9.31E-19	1.01E-10
26829	26862	**2**	**2**	44357350	*CACNB4*	44706849	*NEB*	8.318974951	8.87E-17	1.40E-10
26824	26862	**2**	**2**	44339781	*CACNB4*	44706849	*NEB*	-8.274442004	1.29E-16	2.60E-10
241368	241420	**17**	**17**	73661326	*SPECC1L*	74402265	*PRODH*	-7.904748221	2.68E-15	1.43E-09
26820	26862	**2**	**2**	44305088	*CACNB4*	44706849	*NEB*	7.77966768	7.27E-15	4.76E-09
240069	240074	**17**	**17**	63659471	*OAS1X*	63707713	*RPH3A*	9.380832575	6.55E-21	6.34E-09
240072	240075	**17**	**17**	63671838	*OAS1X*	63709283	*RPH3A*	-8.681525323	3.90E-18	8.76E-09

[1]**M1**: first marker; [2]**M2**: second marker. ***CHR_M1**: chromosome number of the first SNP. ****CHR_M2**: chromosome number of the second SNP. **POS_M1**: position of the first marker in the chromosome (bp). **POS_M2**: position of the second marker in the chromosome (bp).

Table 7. Pairwise interactions between SNP ordered from smallest to largest p-adjusted value (Bonferroni correction) for OM6.

[1]M1	[2]M2	*CHR_M1	**CHR_M2	POS_M1	GENE 1	POS_M2	GENE 2	Z-score	p-value	p-adjusted
190071	191553	**13**	**13**	28747767	*FRMD4A*	40321655	*RALGAPA2*	8.881106757	6.62E-19	1.04E-12
227175	227188	**16**	**16**	56776044	*RC3H1*	56972389	*RABGAP1L*	-8.344137091	7.17E-17	1.12E-10
190067	191553	**13**	**13**	28729880	*FRMD4A*	40321655	*RALGAPA2*	8.336972788	7.62E-17	1.19E-10
190068	191553	**13**	**13**	28731428	*FRMD4A*	40321655	*RALGAPA2*	8.307371932	9.78E-17	1.53E-10
29329	30024	**2**	**2**	62337626	*ZRANB3*	67910381	*DPP10*	7.815741036	5.46E-15	8.56E-09

[1]**M1**: first marker; [2]**M2**: second marker. ***CHR_M1**: chromosome number of the first SNP. ****CHR_M2**: chromosome number of the second SNP. **POS_M1**: position of the first marker in the chromosome (bp). **POS_M2**: position of the second marker in the chromosome (bp).

Table 8. Pairwise interactions between SNP ordered from smallest to largest p-adjusted value (Bonferroni correction) for OM3:OM6 ratio.

M1	M2	CHR_M1	CHR_M2	POS_M1	GENE 1	POS_M2	GENE 2	Z-score	p-value	p-adjusted
157768	281870	**10**	**22**	77913146	*FUT8*	40951980	*FHIT*	-11.50906115	1.19E-30	5.32E-24
157769	281870	**10**	**22**	77924683	*FUT8*	40951980	*FHIT*	-10.93746838	7.63E-28	3.42E-21
112654	271061	**7**	**21**	83780501	*SSBP2*	33764430	*PTPN9*	-10.79362878	3.69E-27	1.65E-20
66011	270922	**4**	**21**	97870048	*EXOC4*	32230567	*SCAPER*	-10.19711134	2.04E-24	9.15E-18
148212	281869	**9**	**22**	101954614	*C9H6orf118*	40935639	*FHIT*	-10.13776626	3.76E-24	1.68E-17
34174	188387	**2**	**13**	106695959	*TNS1*	17402371	*PFKFB3*	-9.97945258	1.87E-23	8.40E-17
178419	306587	**12**	**26**	25600414	*DCLK1*	6971764	*PRKG1*	9.914751258	3.59E-23	1.61E-16
71375	270961	**5**	**21**	10886649	*ACSS3*	32767935	*PEAK1*	9.761003197	1.66E-22	7.42E-16
148213	281869	**9**	**22**	101956568	*C9H6orf118*	40935639	*FHIT*	-9.727294219	2.31E-22	1.03E-15
108862	271061	**7**	**21**	60518957	*STK32A*	33764430	*PTPN9*	-9.556343095	1.22E-21	5.47E-15
78517	270975	**5**	**21**	71429197	*BPIFC*	32912731	*PEAK1*	9.5534703	1.25E-21	5.62E-15
105797	271019	**7**	**21**	32196151	*SNX24*	33295740	*LINGO1*	-9.484096896	2.44E-21	1.10E-14
168128	281882	**11**	**22**	48872358	*ST3GAL5*	41051553	*FHIT*	-9.46631472	2.90E-21	1.30E-14
148408	281869	**9**	**22**	103037848	*RPS6KA2*	40935639	*FHIT*	-9.433378504	3.97E-21	1.78E-14
849	37352	**1**	**2**	5543434	*GRIK1*	132103255	*EIF4G3*	-9.397089716	5.61E-21	2.51E-14
199297	331184	**14**	**29**	17103116	*MTSS1*	49350297	*SLC22A18*	-9.296736423	1.45E-20	6.49E-14
58892	270921	**4**	**21**	33822113	*GRM3*	32227359	*SCAPER*	-9.233472738	2.62E-20	1.17E-13
108285	271061	**7**	**21**	54755394	*GNPDA1*	33764430	*PTPN9*	9.205137535	3.41E-20	1.53E-13
168128	281977	**11**	**22**	48872358	*ST3GAL5*	41615542	*FHIT*	9.120702496	7.46E-20	3.34E-13
78517	270976	**5**	**21**	71429197	*BPIFC*	32917837	*PEAK1*	9.118216744	7.64E-20	3.42E-13
183573	311566	**12**	**26**	76881130	*DZIP1*	45676546	*BCCIP*	9.108595236	8.35E-20	3.74E-13
198957	331182	**14**	**29**	13770793	*MYC*	49274511	*NAP1L4*	9.090821854	9.83E-20	4.40E-13
75762	270972	**5**	**21**	44718715	*LYZ*	32892649	*PEAK1*	9.087659344	1.01E-19	4.53E-13
77681	270972	**5**	**21**	64453373	*UHRF1BP1L*	32892649	*PEAK1*	-9.039182979	1.58E-19	7.07E-13
36151	190327	**2**	**13**	121146021	*PHC2*	30487621	*ITGA8*	-9.033938582	1.66E-19	7.42E-13
68786	270926	**4**	**21**	114746264	*WDR86*	32313259	*SCAPER*	9.028376324	1.74E-19	7.81E-13
34174	189124	**2**	**13**	106695959	*TNS1*	21832074	*PLXDC2*	9.028271304	1.74E-19	7.82E-13
155705	281870	**10**	**22**	57927405	*ARPP19*	40951980	*FHIT*	9.025202168	1.79E-19	8.04E-13
76528	270972	**5**	**21**	50980926	*PPM1H*	32892649	*PEAK1*	9.00156816	2.23E-19	9.97E-13
59421	270921	**4**	**21**	37737369	*PCLO*	32227359	*SCAPER*	8.985511373	2.58E-19	1.15E-12
59419	270921	**4**	**21**	37734105	*PCLO*	32227359	*SCAPER*	-8.968804883	3.00E-19	1.34E-12
198957	331181	**14**	**29**	13770793	*MYC*	49266279	*NAP1L4*	8.964955424	3.10E-19	1.39E-12
71031	270961	**5**	**21**	8682205	*SYT1*	32767935	*PEAK1*	-8.948995024	3.59E-19	1.61E-12
184229	311566	**12**	**26**	79743911	*DOCK9*	45676546	*BCCIP*	8.943787492	3.76E-19	1.69E-12
69222	270926	**4**	**21**	117002052	*DPP6*	32313259	*SCAPER*	-8.917645963	4.76E-19	2.13E-12
68197	270926	**4**	**21**	111665351	*CNTNAP2*	32313259	*SCAPER*	-8.913930818	4.93E-19	2.21E-12
108461	271061	**7**	**21**	57431076	*KCTD16*	33764430	*PTPN9*	8.911613377	5.03E-19	2.25E-12
5964	105816	**1**	**7**	40862487	*EPHA6*	32296464	*SNX24*	-8.880826556	6.64E-19	2.97E-12
76533	270972	**5**	**21**	51010332	*PPM1H*	32892649	*PEAK1*	8.873530012	7.09E-19	3.18E-12
150518	281869	**10**	**22**	10675747	*CMYA5*	40935639	*FHIT*	8.816501913	1.18E-18	5.29E-12

124017	272064	**8**	**21**	45682215	*TJP2*	42387666	*DTD2*	8.803234847	1.33E-18	5.96E-12
148411	281869	**9**	**22**	103059185	*RPS6KA2*	40935639	*FHIT*	-8.803088324	1.33E-18	5.96E-12
93506	270979	**6**	**21**	61490648	*APBB2*	32947984	*PEAK1*	-8.800509827	1.36E-18	6.10E-12
186856	311566	**13**	**26**	6849129	*SPTLC3*	45676546	*BCCIP*	-8.79602666	1.42E-18	6.35E-12
105816	271019	**7**	**21**	32296464	*SNX24*	33295740	*LINGO1*	-8.792167441	1.47E-18	6.57E-12
159448	281870	**10**	**22**	91716830	*NRXN3*	40951980	*FHIT*	-8.763613177	1.89E-18	8.47E-12
168128	282952	**11**	**22**	48872358	*ST3GAL5*	52807513	*SCAP*	8.754908906	2.04E-18	9.15E-12
136341	281868	**9**	**22**	19022716	*HMGN3*	40933096	*FHIT*	-8.753957222	2.06E-18	9.23E-12
74129	270962	**5**	**21**	30973918	*PRKAG1*	32775240	*PEAK1*	-8.734901432	2.44E-18	1.09E-11
168128	281884	**11**	**22**	48872358	*ST3GAL5*	41081142	*FHIT*	8.735454611	2.43E-18	1.09E-11
71027	270961	**5**	**21**	8669035	*SYT1*	32767935	*PEAK1*	-8.73343108	2.47E-18	1.11E-11
18658	157768	**1**	**10**	145465950	*ADARB1*	77913146	*FUT8*	-8.719719421	2.79E-18	1.25E-11
147267	281869	**9**	**22**	96588973	*SYTL3*	40935639	*FHIT*	8.710878833	3.02E-18	1.35E-11
78542	270976	**5**	**21**	71591197	*SYN3*	32917837	*PEAK1*	8.689003442	3.66E-18	1.64E-11
199240	331184	**14**	**29**	16675992	*KIAA0196*	49350297	*SLC22A18*	8.679203006	3.99E-18	1.79E-11
199853	331184	**14**	**29**	20950649	*SPIDR*	49350297	*SLC22A18*	8.676807639	4.07E-18	1.82E-11
66092	270922	**4**	**21**	98308116	*EXOC4*	32230567	*SCAPER*	8.650068405	5.15E-18	2.31E-11
168635	282954	**11**	**22**	55034976	*CTNNA2*	52892264	*KLHL18*	8.637968365	5.72E-18	2.56E-11
58185	270921	**4**	**21**	26681090	*HDAC9*	32227359	*SCAPER*	-8.59538892	8.30E-18	3.72E-11
139555	281868	**9**	**22**	38810405	*LAMA4*	40933096	*FHIT*	-8.593951027	8.40E-18	3.77E-11
87676	270977	**6**	**21**	20502484	*NPNT*	32923672	*PEAK1*	-8.592909631	8.48E-18	3.80E-11
145360	281869	**9**	**22**	83195737	*UTRN*	40935639	*FHIT*	-8.57000191	1.03E-17	4.64E-11
91115	270979	**6**	**21**	45371880	*PPARGC1A*	32947984	*PEAK1*	-8.555132916	1.18E-17	5.28E-11
193063	322556	**13**	**28**	56114145	*CDH4*	32080047	*C28H10orf11*	-8.547848931	1.25E-17	5.62E-11
81598	270977	**5**	**21**	95737878	*PLBD1*	32923672	*PEAK1*	-8.54588473	1.28E-17	5.72E-11
94589	270979	**6**	**21**	69424221	*DCUN1D4*	32947984	*PEAK1*	8.5407533	1.33E-17	5.98E-11
78549	270976	**5**	**21**	71669255	*SYN3*	32917837	*PEAK1*	8.481342712	2.23E-17	9.98E-11
60535	270921	**4**	**21**	50796862	*CTTNBP2*	32227359	*SCAPER*	-8.465775386	2.54E-17	1.14E-10
174928	297623	**12**	**24**	2377438	*DIAPH3*	46183326	*PSTPIP2*	8.453377614	2.83E-17	1.27E-10
88142	270977	**6**	**21**	23650591	*NFKB1*	32923672	*PEAK1*	-8.419482107	3.78E-17	1.69E-10
139787	281868	**9**	**22**	40336144	*METTL24*	40933096	*FHIT*	8.406309913	4.23E-17	1.90E-10
91115	270977	**6**	**21**	45371880	*PPARGC1A*	32923672	*PEAK1*	-8.386185809	5.02E-17	2.25E-10
93480	270979	**6**	**21**	61388744	*APBB2*	32947984	*PEAK1*	-8.381012021	5.25E-17	2.35E-10
198957	331183	**14**	**29**	13770793	*MYC*	49294582	*NAP1L4*	8.380087315	5.29E-17	2.37E-10
12250	136341	**1**	**9**	95064999	*SPATA16*	19022716	*HMGN3*	8.37490877	5.53E-17	2.48E-10
198957	331036	**14**	**29**	13770793	*MYC*	48252404	*SHANK2*	8.374888146	5.53E-17	2.48E-10
60382	270921	**4**	**21**	49077684	*SLC26A3*	32227359	*SCAPER*	-8.360555463	6.24E-17	2.80E-10
150641	281869	**10**	**22**	11112394	*SERINC5*	40935639	*FHIT*	8.355962785	6.49E-17	2.91E-10
94788	270979	**6**	**21**	70659649	*LNX1*	32947984	*PEAK1*	8.342907322	7.25E-17	3.25E-10
34174	188391	**2**	**13**	106695959	*TNS1*	17409056	*PFKFB3*	-8.329648833	8.11E-17	3.63E-10
64644	270922	**4**	**21**	88624061	*IQUB*	32230567	*SCAPER*	-8.314970639	9.18E-17	4.11E-10
162627	281870	**11**	**22**	7383097	*SLC9A2*	40951980	*FHIT*	-8.313851385	9.26E-17	4.15E-10

64328	270921	**4**	**21**	85966698	*KCND2*	32227359	*SCAPER*	8.312502896	9.37E-17	4.20E-10
12508	136341	**1**	**9**	97246352	*SLC2A2*	19022716	*HMGN3*	8.312089849	9.40E-17	4.21E-10
97485	270980	**6**	**21**	91692544	*PARM1*	32952389	*PEAK1*	-8.311584732	9.44E-17	4.23E-10
78544	270976	**5**	**21**	71639944	*SYN3*	32917837	*PEAK1*	8.305401999	9.95E-17	4.46E-10
6519	108789	**1**	**7**	45554067	*ABI3BP*	59845840	*PPP2R2B*	-8.290189207	1.13E-16	5.07E-10
152979	281869	**10**	**22**	32396887	*C10H15orf41*	40935639	*FHIT*	8.262087402	1.43E-16	6.42E-10
108863	271061	**7**	**21**	60519494	*STK32A*	33764430	*PTPN9*	8.258185817	1.48E-16	6.63E-10
6110	105816	**1**	**7**	41757639	*CRYBG3*	32296464	*SNX24*	-8.242757391	1.68E-16	7.54E-10
3765	74134	**1**	**5**	25941126	*ROBO1*	31092798	*RND1*	-8.233053409	1.83E-16	8.18E-10
18660	157769	**1**	**10**	145479952	*ADARB1*	77924683	*FUT8*	-8.22784308	1.91E-16	8.54E-10
58501	270921	**4**	**21**	29525034	*ABCB5*	32227359	*SCAPER*	8.215395574	2.11E-16	9.48E-10
99459	270986	**6**	**21**	103529445	*PTPN13*	33007458	*PEAK1*	8.210385416	2.20E-16	9.88E-10
58295	270921	**4**	**21**	27624869	*HDAC9*	32227359	*SCAPER*	8.20718949	2.26E-16	1.01E-09
189124	311566	**13**	**26**	21832074	*PLXDC2*	45676546	*BCCIP*	8.207639624	2.26E-16	1.01E-09
64655	270922	**4**	**21**	88710361	*ASB15*	32230567	*SCAPER*	-8.203903423	2.33E-16	1.04E-09
159550	281870	**10**	**22**	92135297	*NRXN3*	40951980	*FHIT*	8.199859608	2.41E-16	1.08E-09
69247	270926	**4**	**21**	117085527	*DPP6*	32313259	*SCAPER*	8.198588901	2.43E-16	1.09E-09
36151	190666	**2**	**13**	121146021	*PHC2*	32882712	*CACNB2*	-8.197880491	2.45E-16	1.10E-09
763	36159	**1**	**2**	4894678	*KRATAP27-1*	121248207	*ZNF362*	-8.17632813	2.93E-16	1.31E-09
48289	242486	**3**	**18**	69756955	*SLC44A5*	5980812	*WWOX*	8.173910322	2.99E-16	1.34E-09
99509	270986	**6**	**21**	103778153	*AFF1*	33007458	*PEAK1*	-8.163177632	3.26E-16	1.46E-09
150589	281869	**10**	**22**	10959145	*THBS4*	40935639	*FHIT*	-8.157776849	3.41E-16	1.53E-09
16487	157768	**1**	**10**	129711930	*CLSTN2*	77913146	*FUT8*	8.156997353	3.43E-16	1.54E-09
170591	282956	**11**	**22**	73815846	*DTNB*	52895185	*KLHL18*	8.157210949	3.43E-16	1.54E-09
169039	282955	**11**	**22**	59020977	*LRRTM4*	52894258	*KLHL18*	8.155051411	3.49E-16	1.56E-09
169841	282955	**11**	**22**	68552767	*PCYOX1*	52894258	*KLHL18*	8.149647468	3.65E-16	1.64E-09
162954	281870	**11**	**22**	9158460	*MRPS9*	40951980	*FHIT*	-8.136833465	4.06E-16	1.82E-09
67470	270922	**4**	**21**	108470321	*ARHGEF5*	32230567	*SCAPER*	8.13173353	4.23E-16	1.90E-09
171462	282960	**11**	**22**	82010353	*FAM49A*	52900206	*KLHL18*	-8.129545023	4.31E-16	1.93E-09
67469	270922	**4**	**21**	108469458	*ARHGEF5*	32230567	*SCAPER*	8.127556944	4.38E-16	1.96E-09
51515	247859	**3**	**18**	97401975	*AGBL4*	47099724	*ZNF567*	-8.123631355	4.52E-16	2.03E-09
115301	271061	**7**	**21**	102483863	*ST8SIA4*	33764430	*PTPN9*	8.115778895	4.83E-16	2.16E-09
105817	271019	**7**	**21**	32299202	*SNX24*	33295740	*LINGO1*	8.10978041	5.07E-16	2.27E-09
4970	78544	**1**	**5**	33455787	*CADM2*	71639944	*SYN3*	-8.109543482	5.08E-16	2.28E-09
333	22574	**1**	**2**	2389877	*URB1*	14543048	*PPP1R1C*	8.109053689	5.10E-16	2.29E-09
161025	281870	**10**	**22**	101421084	*EML5*	40951980	*FHIT*	-8.108672324	5.12E-16	2.29E-09
78517	270972	**5**	**21**	71429197	*BPIFC*	32892649	*PEAK1*	8.107770828	5.16E-16	2.31E-09
4859	78542	**1**	**5**	32881084	*CADM2*	71591197	*SYN3*	-8.106597623	5.21E-16	2.33E-09
170707	282956	**11**	**22**	75222305	*ATAD2B*	52895185	*KLHL18*	8.105514448	5.25E-16	2.35E-09
78548	270976	**5**	**21**	71662302	*SYN3*	32917837	*PEAK1*	8.095939611	5.68E-16	2.55E-09
89134	270977	**6**	**21**	30409888	*UNC5C*	32923672	*PEAK1*	-8.080832591	6.43E-16	2.88E-09
94685	270979	**6**	**21**	70220658	*SCFD2*	32947984	*PEAK1*	8.077171869	6.63E-16	2.97E-09

65256	270922	**4**	**21**	92240887	*GRM8*	32230567	*SCAPER*	8.057004355	7.82E-16	3.50E-09
163633	281870	**11**	**22**	13480698	*ATP6V1B1*	40951980	*FHIT*	8.057211235	7.81E-16	3.50E-09
83719	270977	**5**	**21**	114953550	*SAMM50*	32923672	*PEAK1*	8.055888097	7.89E-16	3.54E-09
65257	270922	**4**	**21**	92242004	*GRM8*	32230567	*SCAPER*	-8.052141844	8.14E-16	3.65E-09
109282	271061	**7**	**21**	63151011	*PPARGC1B*	33764430	*PTPN9*	-8.050484996	8.25E-16	3.70E-09
332	22574	**1**	**2**	2388346	*URB1*	14543048	*PPP1R1C*	-8.048752933	8.36E-16	3.75E-09
88138	270977	**6**	**21**	23637572	*NFKB1*	32923672	*PEAK1*	-8.035150134	9.35E-16	4.19E-09
78535	270976	**5**	**21**	71529654	*SYN3*	32917837	*PEAK1*	8.033776974	9.45E-16	4.24E-09
81558	270976	**5**	**21**	95381490	*ARHGDIB*	32917837	*PEAK1*	-8.027453896	9.95E-16	4.46E-09
89839	270977	**6**	**21**	36171334	*MMRN1*	32923672	*PEAK1*	8.013883092	1.11E-15	4.98E-09
74134	270972	**5**	**21**	31092798	*RND1*	32892649	*PEAK1*	8.003241443	1.21E-15	5.43E-09
155052	281870	**10**	**22**	51653740	*ADAM10*	40951980	*FHIT*	-7.995612688	1.29E-15	5.78E-09
155706	281870	**10**	**22**	57929873	*ARPP19*	40951980	*FHIT*	7.990729485	1.34E-15	6.01E-09
3963	74134	**1**	**5**	26795651	*ROBO1*	31092798	*RND1*	-7.986987859	1.38E-15	6.20E-09
36160	198957	**2**	**14**	121254484	*ZNF362*	13770793	*MYC*	-7.980704525	1.46E-15	6.52E-09
284	22574	**1**	**2**	2179671	*SYNJ1*	14543048	*PPP1R1C*	7.977224661	1.50E-15	6.71E-09
150483	281869	**10**	**22**	10599690	*PAPD4*	40935639	*FHIT*	7.976854614	1.50E-15	6.73E-09
69202	270926	**4**	**21**	116938805	*DPP6*	32313259	*SCAPER*	-7.971261196	1.57E-15	7.04E-09
77557	270972	**5**	**21**	63458534	*ANKS1B*	32892649	*PEAK1*	-7.970954209	1.57E-15	7.06E-09
184237	311566	**12**	**26**	79763832	*DOCK9*	45676546	*BCCIP*	-7.96966218	1.59E-15	7.13E-09
162549	281870	**11**	**22**	6733574	*IL1R2*	40951980	*FHIT*	7.963537964	1.67E-15	7.49E-09
71376	270961	**5**	**21**	10887603	*ACSS3*	32767935	*PEAK1*	7.95244656	1.83E-15	8.19E-09
337	23903	**1**	**2**	2394765	*URB1*	24092026	*PDK1*	7.948944609	1.88E-15	8.43E-09
334	23903	**1**	**2**	2390877	*URB1*	24092026	*PDK1*	-7.942262597	1.99E-15	8.90E-09
23903	174055	**2**	**11**	24092026	*PDK1*	102944335	*AK8*	7.941620388	2.00E-15	8.94E-09
145233	281869	**9**	**22**	82237462	*PHACTR2*	40935639	*FHIT*	7.939839468	2.02E-15	9.07E-09
11230	136341	**1**	**9**	87004658	*CCDC39*	19022716	*HMGN3*	-7.93561182	2.09E-15	9.39E-09
6314	108789	**1**	**7**	43546877	*COL8A1*	59845840	*PPP2R2B*	-7.933006865	2.14E-15	9.59E-09
192924	319615	**13**	**28**	55121443	*NTSR1*	5421874	*SIPA1L2*	7.93178302	2.16E-15	9.68E-09
154382	281870	**10**	**22**	46136026	*DAPK2*	40951980	*FHIT*	7.928528349	2.22E-15	9.94E-09

[1]**M1**: first marker; [2]**M2**: second marker. ***CHR_M1**: chromosome number of the first SNP. ****CHR_M2**: chromosome number of the second SNP.
POS_M1: position of the first marker in the chromosome (bp). **POS_M2**: position of the second marker in the chromosome (bp).

Table 9. Significant (P<0.05) Gene Ontology terms and KEGG pathways revealed by DAVID analyses.

Term	P-value	Genes
GO Biological Process		
GO:0007157~heterophilic cell-cell adhesion via plasma membrane cell adhesion molecules	8.564 E-5	*ALCAM, TIGIT, CADM3, CADM2, NLGN1, NECTIN3, ADGRL1, NECTIN4, CXADR, CD200*
GO:0008037~cell recognition	3.812 E-4	*TIGIT, CADM3, CADM2, NECTIN3, NECTIN4, CD200*
GO:0035023~regulation of Rho protein signal transduction	0.0020378	*MCF2L2, ARHGEF4, VAV3, ARHGEF26, TIAM1, ARHGEF5, ITSN1, FGD5, ECT2, KALRN, ARHGDIB*
GO:0097503~sialylation	0.0035138	*ST3GAL5, ST8SIA4, ST3GAL6, ST8SIA1*
GO:0040014~regulation of multicellular organism growth	0.0036222	*FGFR2, APP, DRD3, PIK3CA, SOD1, PTPN11*
GO:0031954~positive regulation of protein autophosphorylation	0.0038283	*RAP2B, NBN, TOM1L1, PDGFC, PDGFD*
GO:0048745~smooth muscle tissue development	0.0078352	*ITGA8, TIPARP, TP63, DLG1*
GO:0032869~cellular response to insulin stimulus	0.0085035	*KAT2B, PPARG, PRKCI, HDAC9, GHSR, ADIPOQ, USF1*
GO:0007264~small GTPase mediated signal transduction	0.0088587	*RAB7A, RABL3, VAV3, RASEF, DOCK9, DOCK8, ARL6, ITSN1, RAB40B, ARHGAP30, ARHGAP31, PLCE1, ARL5A, RND1, ARL14, RAB5A, ARL8A, DOCK10, ARL13B*
GO:0045184~establishment of protein localization	0.0106160	*LIMS1, NPNT, ITGA8, DZIP1, NLGN1, PHLDB2*
GO:0050730~regulation of peptidyl-tyrosine phosphorylation	0.0143049	*HRG, PDGFC, PDGFD, IFNAR1*
GO:0007156~homophilic cell adhesion via plasma membrane adhesion molecules	0.0200228	*TIGIT, CADM3, CADM2, ROBO1, CDHR3, NECTIN3, ROBO2, NECTIN4, IGSF9, CD200, CDH23*
GO:0006470~protein dephosphorylation	0.0209936	*PPP4R3B, CTTNBP2NL, NCEH1, BCL2, PPM1L, FBXW11*
GO:0046825~regulation of protein export from nucleus	0.0216340	*PTPN14, UHMK1, PTPN11*
GO:0051965~positive regulation of synapse assembly	0.0260813	*SLITRK3, NTRK2, IL1RAP, NLGN1, ADGRL1, EPHB3, EPHB1*
GO:0007160~cell-matrix adhesion	0.0264449	*CD96, MKLN1, ITGA6, TIAM1, FREM1, NPNT, ITGA8, ITGB5*
GO:0097120~receptor localization to synapse	0.0314093	*NLGN1, RELN, DLG1*
GO:0006006~glucose metabolic process	0.0364138	*GCK, APOD, PGM1, HK2, PIK3CA, ADIPOQ*
GO:0007224~smoothened signaling pathway	0.0371896	*HES1, IFT80, IFT57, DZIP1, BOC, IQUB, ARL13B*
GO:0061003~positive regulation of dendritic spine morphogenesis	0.0425675	*CAMK2B, RELN, KALRN*
GO:0010745~negative regulation of macrophage derived foam cell differentiation	0.0425675	*CRP, PPARG, ADIPOQ*
GO:0007613~memory	0.0441155	*ITGA8, SLC24A2, LMX1A, SHANK2, ATAD1, KALRN*
GO:0007155~cell adhesion	0.0444411	*F11R, TNC, NLGN1, ITGB5, CLDN11, CTNNA2, HES1, ALCAM, IGSF11, CD47, NCAM2, CD96, LAMA4, CNTN1, RELN*
GO:0007626~locomotory behavior	0.0446052	*APP, DRD3, ADCY5, RELN, LMX1A, SOD1, ETV5, CDH23*
GO:0031290~retinal ganglion cell axon guidance	0.0474497	*ALCAM, RPL24, EPHB3, EPHB1*

GO:0006612~protein targeting to membrane	0.0474497	*RTP2, RTP4, RTP1, ARL6*
GO:0002053~positive regulation of mesenchymal cell proliferation	0.0474497	*FGFR2, SHOX2, TP63, CHRD*
GO:0048240~sperm capacitation	0.0474497	*SLC26A3, CATSPERD, CFTR, ROPN1*
GO Cellular Component		
GO:0005923~bicellular tight junction	0.0016908	*RAP2B, CLDN8, CLDN16, CLDN17, F11R, FRMD4A, CLDN1, CLDN11, CXADR, ECT2, TJP2, DLG1*
GO:0005829~cytosol	0.0036517	*BACH1, FHIT, KYNU, KCNAB1, WWC1, TP63, NFKB1, PRKG1, MKLN1, TIAM1, HTRA1, ABHD10, MUC13, WWOX, RAP2B, PRKCI, CFTR, ARL6, GMPS, ATP6V1A, PFDN2, PLCE1, COPG1, ARRB1, PGM1, RAB5A, MVK, KPNA4, KPNA1, RAB7A, SOX2, HK2, PAXBP1, ATP6V1B1, PEX5L, WDR45B, RIC1, CHMP2B, ALDH1A1, RGS10, PEX19, PACRG, BCL2, PLCH1, LSG1, HSPA6, UCK2, NFATC2, PPP2R2B, FBXW11, PSTPIP2, ARHGDIB, PTPN7, DVL3, UAP1, PDCD10, SLC12A3, OSBPL6, ARFIP1, SOD1, SAMSN1, CAMK2N2, ATG3, HDAC4, NR1I3, TOM1L1, GCK, NTRK2, AOX4, CRYZL1, ARHGAP10*
GO:0070062~extracellular exosome	0.0049895	*FHIT, RARRES1, GNPDA1, NIT1, GABRB2, NIT2, LSM6, UTRN, FSTL1, TSPAN8, TOMM70, CD47, TBC1D15, GP5, APP, APOA2, APOD, HTRA1, PLA1A, DHX36, PDGFC, ROBO2, PDGFD, MUC13, F11R, TNIK, SPARCL1, HDAC11, PCLO, OGFOD3, IGSF8, MELTF, PGM1, RYR2, IQCG, ARL8A, MVK, CSTA, MGAT5, UGGT1, KALRN, IQCB1, RAB7A, ITGB5, MME, GSTCD, TAGLN2, ATP6V1B1, EPHB1, AHSG, CHMP2B, ALCAM, NDRG3, NAA50, UPK1B, FAM162A, ARHGDIB, AP2M1, ST6GAL1, CACNA2D1, VAV3, MYO1B, SLAMF6, DUSP23, HGD, SERPINI1, SLAMF1, GART, TNFSF10, GBE1, SPTBN1, HSPA13, LXN, PRKAG1, NPNT, CRP, UFC1, LRRC15, DDR2, MSRA, CREG1, PSMD2, DOCK10, CNTLN, DLG1, RAP2B, APCS, ADAM10, PRKCI, CFTR, NECTIN4, ARL6, EML5, POGLUT1, CD84, ATP6V1A, CD86, CARMIL1, SCIN, PLXDC2, RAB5A, GPA33, CNTN1, PPM1L, GNB4, PCYOX1, KPNA4, PROS1, ALDH9A1, AP1M1, SAMM50, CPQ, CLDN11, CPN2, ALDH1A1, IGSF11, HSPA6, HRG, COL8A1, THBS4, MAGEF1, PDCD10, SLC12A3, FETUB, SI, RPL24, ADIPOQ, NCSTN, MGST3, ERP44, TFRC, TOM1L1, DNM1*
GO:0000118~histone deacetylase complex	0.0068073	*HDAC4, TBL1XR1, HDAC11, HDAC9, MECOM, NRIP1*
GO:0016324~apical plasma membrane	0.0069520	*CFAP126, PLD1, SLC22A18, TNIK, SLC12A3, VANGL2, PRKCI, CFTR, IL6R, ATP6V1B1, ATP6V1A, SLC2A2, UPK1B, ATP8B1, CLDN1, CLDND1, MUC13*
GO:0009986~cell surface	0.0079425	*FGFR2, DCBLD2, RTP2, MPZL1, RTP1, ITGB5, LY9, CXADR, APP, ROBO1, P2RY1, PDGFC, ROBO2, ADAM10, NLGN1, CFTR, IL6R, ADIPOQ, DCSTAMP, ADAMTS7, P2RY12, TIGIT, ERP44, TNS1, MELTF, ITGA8, GPA33, GHSR*
GO:0005887~integral component of plasma membrane	0.0088222	*CADM3, MPZL1, GABRB2, CADM2, LRRC8D, KCNJ10, TSPAN8, LGR5, DDR2, CD47, SLC24A2, BOC, HTR1F, SLC33A1, OR10J5, NECTIN3, NECTIN4, NTSR1, SLC7A14, IFNAR1, IFNAR2, SLC26A3, GRM3, CLDN1, FGFR2, DCBLD2, CLCN2, DRD3, GPR149, SLC39A12, EPHB3, EPHB1, ALCAM, BEST3, UPK1B, FCER1G, FCER1A, HCN1, MPZ, NLGN1, ATP1A4, ATP1A2,*

		ATP13A3, ABCB5, ATP13A5, DCSTAMP, ATP13A4, EPHA3, NCSTN, P2RY12, TIGIT, SLC16A7, KCNJ9, TFRC, NTRK2, SLC14A1, CD200
GO:0072562~blood microparticle	0.0114656	*KNG1, APOA2, APCS, TFRC, BCHE, HSPA6, HRG, PROS1, CPN2, AHSG*
GO:0005604~basement membrane	0.0122637	*P3H2, ITGA6, FREM1, NPNT, TNC, CCDC80, ADAMTS1, THBS4*
GO:0005783~endoplasmic reticulum	0.0144217	*CLDN8, HACD2, MRAP, CPQ, PDIA5, ZDHHC19, ANKLE2, SEC62, FDFT1, TMEM50B, P3H2, APOD, BCHE, UPK1B, B3GALNT2, LSG1, POU2F1, ATP8B1, NRROS, IFNGR2, HSD17B7, DLG1, THBS4, PIGZ, NCEH1, ATP11B, LPCAT2, ADIPOQ, USF3, NCSTN, MGST3, ERP44, TRIM59, ITGA8, HSPA13, USP25, UGGT1*
GO:0005913~cell-cell adherens junction	0.0153856	*TIGIT, F11R, CADM3, CADM2, NECTIN3, RPL24, NECTIN4, TAGLN2, CD200, CTNNA2, CHMP2B*
GO:0005622~intracellular	0.0198097	*RTP2, CASR, ATG10, RTP1, ADCY5, CLNK, RAB40B, TBC1D15, ARL5A, FAM49A, STK32A, DGKG, PLCH1, ARL14, MYZAP, ZNF596, PIK3CA, ANO5, DOCK10, DCLK1, FCER1A, ARHGEF4, RABL3, ZNF567, RASEF, ARHGEF5, PRKCI, DOCK9, DOCK8, RPH3A, ADPRH, RDH12, ARHGAP31, NYAP2, POGK, TOM1L1, FYTTD1, VSIG8, PPM1L, ASB2, ADGRL1, ASB5, UNC13C, SSR3, ARL13B*
GO:0009897~external side of plasma membrane	0.0238847	*FCER1A, CD244, NLGN1, TMC1, FCRL6, SLAMF1, CD48, ALCAM, CD86, ITGA6, TFRC, CD80, HEG1, FCER1G, CD200R1L*
GO:0030424~axon	0.0241454	*ALCAM, HCN1, NCAM2, APP, GRM3, MME, ADGRL1, IGSF9, EPHB1, GAP43, UHMK1, FXR1*
GO:0043197~dendritic spine	0.0380434	*APP, GRM3, KCND2, DGKI, SHANK2, FXR1*
GO:0098793~presynapse	0.0390767	*CADPS, NLGN1, KCNJ10, ADGRL1, SYTL3, DGKI, RPH3A*
GO Molecular Function		
GO:0050839~cell adhesion molecule binding	1.150 E-4	*TIGIT, CADM3, CADM2, NECTIN3, ADGRL1, NECTIN4, CXADR, CD200*
GO:0004872~receptor activity	5.501 E-4	*CD244, CADM3, CADM2, NLGN1, ITGB5, NECTIN3, NECTIN4, SLAMF1, TIGIT, CD48, TNFRSF19, CD200R1L, CD200*
GO:0019864~IgG binding	0.0064621	*FCGR2B, FCER1G, FCGR3A*
GO:0005524~ATP binding	0.0075463	*ABCF3, MYH15, PRKAG1, PRKG1, ACSS3, DDR2, MAP3K5, PAK2, VWA8, MCCC1, ATP8B1, PIK3CA, DHX36, TNIK, KIF5B, PRKCI, CFTR, GMPS, STK3, DAPK1, HUNK, ATP6V1A, RFC4, EIF4A2, SMARCA5, TNNI3K, MVK, MAP3K13, KALRN, FGFR2, PFKFB3, OAS1X, PEAK1, HK2, EPHB3, ATP6V1B1, DNAH8, UHMK1, EPHB1, STK32A, SYN3, DGKG, SYN2, HSPA6, DCLK2, CAMK2B, UCK2, POLQ, DCLK1, MYO1B, ATP11B, ATP1A4, DGKI, ATP1A2, ATP13A3, ABCB5, ATP13A5, ATAD1, ATP13A4, EPHA3, SMC4, AK8, GART, ABCC9, DDX55, GCK, RPS6KA2, GSK3B, CCT8, NTRK2, ATP8A2, TNK2, HSPA13, ZRANB3, KATNAL2, ABCC5, ATAD2B*
GO:0042803~protein homodimerization activity	0.0102445	*CADM3, ADAM10, CADM2, CPQ, NECTIN3, NECTIN4, SOD1, ADIPOQ, USF1, DMRTB1, FXR1, HES1, TIGIT, ECE2, APOA2, BCL2, CAMK2B, SLC51A, MAP3K13, CD200*
GO:0004222~metalloendopeptidase activity	0.0194552	*ADAMTS7, ECE2, ADAM10, PAPPA, MME, ADAMTS1, MMP16, MIPEP, ADAMTS2, ADAMTS5, ADAMTS4*

GO:0031849~olfactory receptor binding	0.0202182	*RTP2, RTP4, RTP1*
GO:0019829~cation-transporting ATPase activity	0.0202182	*ATP13A3, ATP13A5, ATP13A4*
GO:0005085~guanyl-nucleotide exchange factor activity	0.0245549	*PLCE1, TIAM1, DOCK9, DOCK8, DOCK10, ELMO1, EIF2B5*
GO:0004175~endopeptidase activity	0.0282275	*NCSTN, SENP2, ADAM10, PSMD2, SENP5*
GO:0052689~carboxylic ester hydrolase activity	0.0363817	*NCEH1, BCHE, NLGN1, LIPH, LIPI*
GO:0004869~cysteine-type endopeptidase inhibitor activity	0.0363817	*KNG1, FETUB, HRG, CSTA, AHSG*
GO:0005102~receptor binding	0.0393575	*TIGIT, CADM3, RND1, CADM2, HRG, NECTIN3, TNK2, NECTIN4, ADIPOQ, CD200*
KEGG pathway		
bta04514: Cell adhesion molecules (CAMs)	4.299 E-5	*CLDN8, CLDN16, F11R, CLDN17, CADM3, MPZL1, MPZ, NLGN1, NECTIN3, CLDN11, ALCAM, TIGIT, NCAM2, CD86, ITGA6, CD80, ITGA8, CLDN1, CNTN1, JAM2*
bta04650: Natural killer cell mediated cytotoxicity	5.975 E-4	*CD244, VAV3, CD247, IFNAR1, PTPN11, CD48, IFNAR2, TNFSF10, PLCG2, FCER1G, PIK3CA, NFATC2, FCGR3A, SH2D1B, IFNGR2*
bta04530: Tight junction	0.0014445	*CLDN8, CLDN16, CLDN17, F11R, MYH15, HCLS1, CLDN1, PRKCI, CLDN11, PPP2R2B, JAM2, TJP2*
bta00601: Glycosphingolipid biosynthesis - lacto and neolacto series	0.0068876	*B4GALT3, B3GNT5, ST3GAL6, ST8SIA1, ABO, B4GALT4*
bta04066: HIF-1 signaling pathway	0.0094477	*PDK1, TFRC, PFKFB3, BCL2, PLCG2, HK2, PIK3CA, CAMK2B, NFKB1, IL6R, IFNGR2*
bta04911: Insulin secretion	0.0097342	*KCNMB3, GCK, SLC2A2, ADCY5, RYR2, ATP1A4, CAMK2B, ATP1A2, PCLO, KCNMB2*
bta05412: Arrhythmogenic right ventricular cardiomyopathy (ARVC)	0.0236456	*CACNA2D1, ITGA6, SGCG, ITGA8, RYR2, CACNB2, ITGB5, CACNB4*
bta05410: Hypertrophic cardiomyopathy (HCM)	0.0241281	*CACNA2D1, ITGA6, SGCG, PRKAG1, ITGA8, RYR2, CACNB2, ITGB5, CACNB4*
bta04670: Leukocyte transendothelial migration	0.0316977	*CLDN8, CLDN16, CLDN17, F11R, VAV3, PLCG2, CLDN1, PIK3CA, CLDN11, JAM2, PTPN11*
bta05160: Hepatitis C	0.0325732	*CLDN8, CLDN16, CLDN17, IFNAR2, OAS1X, GSK3B, CLDN1, PIK3CA, NFKB1, CLDN11, PPP2R2B, IFNAR1*
bta04973: Carbohydrate digestion and absorption	0.0330142	*SLC2A2, SI, HK2, ATP1A4, PIK3CA, ATP1A2*
bta05414: Dilated cardiomyopathy	0.0352704	*CACNA2D1, ITGA6, SGCG, ADCY5, ITGA8, RYR2, CACNB2, ITGB5, CACNB4*
bta04512: ECM-receptor interaction	0.0374169	*CD47, LAMA4, GP5, ITGA6, TNC, ITGA8, ITGB5, RELN, THBS4*
bta04071: Sphingolipid signaling pathway	0.0387191	*FCER1A, PLD1, MAP3K5, ADORA3, SPTLC3, BCL2, FCER1G, PIK3CA, NFKB1, PPP2R5E, PPP2R2B*
bta00500: Starch and sucrose metabolism	0.0412163	*GBE1, GCK, PGM1, SI, HK2*
bta05162: Measles	0.0447350	*IFNAR2, TNFSF10, FCGR2B, OAS1X, GSK3B, IL12A, HSPA6, PIK3CA, NFKB1, SLAMF1, IFNGR2, IFNAR1*
bta04722: Neurotrophin signaling pathway	0.0489176	*MAP3K5, RPS6KA2, BCL2, GSK3B, NTRK2, PLCG2, PIK3CA, CAMK2B, NFKB1, ARHGDIB, PTPN11*
bta04360: Axon guidance	0.0489176	*SEMA5B, EPHA6, RND1, PAK2, ROBO1, GSK3B, ROBO2, EPHB3, NFATC2, EPHB1, EPHA3*

CAPÍTULO 3 - An assessment of genomic connectedness measures in Nellore cattle[1]

ABSTRACT - An important criterion to consider in genetic evaluations is the extent of genetic connectedness across management units (MU), especially if they differ in their genetic mean. Reliable comparisons of genetic values across MU depend on the degree of connectedness; the higher the connectedness, the more reliable the comparison. Traditionally, genetic connectedness was calculated through pedigree-based methods; however, in the era of genomic selection, this can be better estimated utilizing new approaches based on genomics. Most procedures consider only additive genetic effects, which may not accurately reflect the underlying gene action of the evaluated trait, and little is known about the impact of non-additive gene action on connectedness measures. The objective of this study was to investigate the extent of genomic connectedness measures, for the first time, in Brazilian field data by applying additive and non-additive relationship matrices using a fatty acid profile dataset from seven farms located in the three regions of Brazil, which are part of the three breeding programs. Myristic acid (C14:0) was used due to its importance for human health, and reported presence of non-additive gene action. The pedigree included 427,740 animals and 925 of them were genotyped using the Bovine high-density genotyping chip. Six relationship matrices were constructed, parametrically and non-parametrically capturing additive and non-additive genetic effects from both pedigree and genomic data. We assessed genome-based connectedness across MU using the prediction error variance of difference (PEVD) and the coefficient of determination (CD). PEVD values ranged from 0.540 - 1.707, and CD from 0.146 - 0.456. Genomic information consistently enhanced the measures of connectedness compared to the numerator relationship matrix by at least 63%. Combining additive and non-additive genomic kernel relationship matrices or a non-parametric relationship matrix increased the capture of connectedness. Overall, the Gaussian kernel yielded the largest measure of connectedness. Our findings showed that connectedness metrics can be extended to incorporate genomic information and non-additive genetic variation using field data. We propose that different genomic relationship matrices can be designed to capture additive and non-additive genetic effects, increase the measures of connectedness, and to more accurately estimate the true state of connectedness in herds.

Keywords: *Genomic connectedness, Kernel matrices, Nellore cattle, Non-additive gene action.*

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3.1 Introduction

Genetic connectedness is a statistical measurement that allows reliable comparisons of the genetic values across management units (MU) by capturing the linkage among herds. The genetic values of animals from different MU (e.g., contemporary groups, farms, and herds) can be ranked using best linear unbiased prediction (BLUP). However, the accuracy of these comparisons depends on the degree of connectedness among MU, the higher the connectedness, the more reliable the comparison. Genetic connectedness has traditionally been calculated through pedigree-based methods (Lewis et al., 1999b; Kuehn et al., 2007a); however, these methods may underestimate connectedness in production systems such as the beef cattle industry, where commercial herds are poorly registered and multi-sire mating is practiced (Caires et al., 2012; Barbosa et al., 2013; Tonussi et al., 2017; Cavani et al., 2018). A lack of connectedness occurs: when the MU are genetically isolated (or semi-isolated) or there is limited sharing of genetic material; with the use of an incomplete numerator relationship matrix based on poor pedigree data (Carneiro et al., 2001); and with poor use of artificial insemination (AI). Genomic data are expected to more accurately estimate the relationship between individuals using information from genetic markers, such as single nucleotide polymorphisms (SNP), by measuring covariance among relatives and distant relatives previously ignored by a pedigree-based method (Habier et al. 2007).

Yu et al. (2017) evaluated the utility of genome-based connectedness in mice and cattle and noted that genomic relatedness could improve the extent of genetic connectedness measures compared with the pedigree when additive inheritance was assumed. The gain in connectedness measures was later shown to be associated with increased prediction accuracy based on cross-validation (Yu et al., 2018). Genetic connectedness studies were subsequently extended to account for non-additive genetic effects (Momen and Morota, 2018). Those authors performed a computer simulation and found the increased measures of connectedness using additive and non-additive genomic relationship matrices under non-additive gene action. Collectively, those studies demonstrated that genomics can be used to enhance measures of connectedness. However, evaluations of genetic connectedness from field data remain limited.

In Brazil, cattle herds are often separated by large distances, and the rates of AI are low. A recent study by the Brazilian Association of Artificial Insemination (ASBIA, 2019) showed that only 16% of Brazilian dams are inseminated, with just a few farms available to measure expensive traits that require specific techniques or tests, such as post-mortem beef quality traits.

The fatty acid (FA) profile of beef is a trait of interest due to its association with cardiovascular disease in humans (Mensink and Katan, 1992). According to Lawrie (2006), C14:0 is one of the most predominant saturated FA in cattle meat, which interferes with hepatic low-density lipoprotein receptors and consequently increases the amount of circulating low-density lipoprotein cholesterol (Grundy and Denke, 1990; Katan et al., 1994; Katan et al., 1995; Sacks and Katan, 2002). Considering the growing consumer demand for protein sources with a healthy lipid profile, several strategies have been applied to identify and manage the FA profile of beef (Faucitano et al., 2008; Liu et al., 2010; Aboujaoude et al., 2016; Berton et al., 2016; Chiaia et al., 2017).

Non-additive genetic variation for FA has been previously reported in cattle. For example, Malau-Aduli et al. (1998, 2000) reported significant dominance effects in Jersey, Limousin, and Jersey × Limousin crossbred cattle. Li et al. (2012) detected significant additive and dominant effects for 19 individual FA in commercial beef steers. Kramer et al. (2016) identified epistatic interactions associated with FA concentrations in Angus cattle. Thus, the use of connectedness metrics including additive and non-additive gene effects may help to improve the quality of genetic value comparisons in breeding programs.

To date, few connectedness studies have been performed in Brazil (Carneiro et al., 2001; Pegolo et al., 2012), and the impact of genomic relatedness on connectedness measures in Nellore cattle have not been reported. Assessing connectedness statistics through genomic information may be useful for designing breeding programs and effectively linking units to improve the quality of across unit genetic evaluations, which in turn enhance the genetic improvement of Brazilian beef cattle. Therefore, the aim of this study was to investigate the extent of genomic connectedness measures in Nellore cattle by applying additive and non-additive relationship matrices, and to estimate variance

components considering additive, dominance, and epistatic effects for myristic acid (C14:0).

3.2 Material and methods

3.2.1 Data

Fatty acid profile dataset

The dataset included animals from seven farms located in the southeast, northeast, and midwest of Brazil, which are part of three beef cattle breeding programs: DeltaGen (F1 [*n* = 200], F2 [*n* = 22], F3 [*n* = 80]); Paint (F4 [*n* = 190], F6 [*n* = 292], F7 [*n* = 51]); and Nelore Qualitas (F5 [*n* = 90]). These seven farms only collect samples post-mortem for analysis of beef quality traits due to the costs of collecting these phenotypes.

The GPS location of each farm was provided by the respective breeding program. The FA profile phenotypes were obtained for Nellore bulls with an average age of 24 months. The methodology used to determinate FA profiles was consistent with that used in a previous study by Lemos et al. (2016). We analyzed myristic acid (C14:0) because of its importance to human health and high content in animals from feedlots (Zock et al., 1994). The pedigree included 427,740 animals born between 1977 and 2011. A total of n= 925 animals having C14:0 phenotype were genotyped using 777,962 SNP (Illumina, San Diego, CA, USA). Following the removal of markers with a minor allele frequency less than 0.05, 505,367 SNP remained for further analysis. Missing genotypes were imputed using allele frequency estimates from a binomial distribution from the data. The seven farms were treated as MU.

3.3 Connectedness statistics

Genetic connectedness statistics are mostly defined as a function of the inverse of the coefficient matrix, which can be obtained from Henderson's mixed model equations (MME) (Henderson, 1984). In this study, we assessed genome-based connectedness across management units by applying the prediction error

variance of difference (PEVD) (Kennedy and Trus, 1993) and coefficient of determination (CD) (Laloë, 1993).

3.3.1 Prediction error variance of difference

Prediction error variance (PEV) was obtained by fitting the following standard linear mixed model:

$$\boldsymbol{y} = \boldsymbol{Xb} + \boldsymbol{Zu} + \boldsymbol{\varepsilon}$$

where $\boldsymbol{y}$ is a vector of phenotypes, $\boldsymbol{X}$ is an incidence matrix of systematic effects including management units, $\boldsymbol{b}$ is a vector of systematic effects (contemporary group including animals born in the same farm, year, and from the same management group at yearling), $\boldsymbol{Z}$ is an incidence matrix relating individuals to phenotypic records, $\boldsymbol{u}$ is a vector of random additive genetic effects, and $\boldsymbol{\varepsilon}$ is a vector of residuals. The joint distribution of random effects for this model is:

$$\begin{pmatrix} \boldsymbol{y} \\ \boldsymbol{u} \\ \boldsymbol{\epsilon} \end{pmatrix} \sim N\left[\begin{pmatrix} \boldsymbol{Xb} \\ 0 \\ 0 \end{pmatrix}, \begin{pmatrix} \boldsymbol{ZK}\sigma_u^2\boldsymbol{Z}' + \boldsymbol{I}\sigma_\epsilon^2 & \boldsymbol{ZK}\sigma_u^2 & \boldsymbol{I}\sigma_\epsilon^2 \\ \boldsymbol{K}\sigma_u^2\boldsymbol{Z}' & \boldsymbol{K}\sigma_u^2 & 0 \\ \boldsymbol{I}\sigma_\epsilon^2 & 0 & \boldsymbol{I}\sigma_\epsilon^2 \end{pmatrix}\right]$$

where σ_u^2 is the additive genetic variance, σ_e^2 is the residual variance, and $\boldsymbol{K}$ is one of the positive (semi) definite relationship matrices defined later.

The inverse of MME coefficient matrix of Henderson (1984) is represented as:

$$\begin{aligned} \boldsymbol{C^{-1}} &= \begin{bmatrix} \boldsymbol{X'X} & \boldsymbol{X'Z} \\ \boldsymbol{Z'X} & \boldsymbol{Z'Z} + \boldsymbol{K^{-1}}\lambda \end{bmatrix}^{-1} \\ &= \begin{bmatrix} \boldsymbol{C}^{11} & \boldsymbol{C}^{12} \\ \boldsymbol{C}^{21} & \boldsymbol{C}^{22} \end{bmatrix}, \end{aligned}$$

where λ is the ratio of σ_e^2/σ_u^2. The PEV for ith individual ($\hat{u}_i$) is written as:

$$\begin{aligned} \text{PEV}_i &= \text{Var}(\hat{u}_i - u_i) \\ &= \text{Var}(u_i|\hat{u}_i) \\ &= \text{Var}(\hat{u}_i|u_i) \\ &= \boldsymbol{C}_{ii}^{22}\sigma_\epsilon^2, \end{aligned}$$

where $\boldsymbol{C}_{ii}^{22}$ represents the ith diagonal element of the $\boldsymbol{C}^{22}$ coefficient matrix. Then, the PEVD of genetic values between individuals from different MU (Kennedy and Trus, 1993) is given by:

$$\begin{aligned} \text{PEVD}(\hat{u}_i - \hat{u}_j) &= [\text{PEV}(\hat{u}_i) + \text{PEV}(\hat{u}_j) - 2\text{PEC}(\hat{u}_i, \hat{u}_j)] \\ &= (\boldsymbol{C}_{ii}^{22} - \boldsymbol{C}_{ij}^{22} - \boldsymbol{C}_{ji}^{22} + \boldsymbol{C}_{jj}^{22})\sigma_\epsilon^2 \\ &= (\boldsymbol{C}_{ii}^{22} + \boldsymbol{C}_{jj}^{22} - 2\boldsymbol{C}_{ij}^{22})\sigma_\epsilon^2, \end{aligned}$$

where ii and jj refer to the diagonal elements of $\boldsymbol{C}^{22}$ matrix, corresponding to the ith and jth individuals, respectively, and the off-diagonal elements of $\boldsymbol{C}^{22}$ are denoted by ij. PEC_{ij} is the prediction error covariance between the errors of genetic values, which is the off-diagonal element of the PEV matrix. Smaller PEVD indicates that the individuals are more connected.

The average PEVD between individuals across two MU was defined as follows:

$$PEVD_{i'j'} = \frac{1}{n_{i'}.n_{j'}} \sum_{i \in i'} \sum_{j \in j'} PEVD_{ij}$$

where i is an animal in MU i', j is an animal in MU j', $n_{i'}$and $n_{j'}$ represent the total number of records in i' and j' units, and the sum of all pairwise differences between two units is $\sum_{i \in i'} \sum_{j \in j'} PEVD_{ij}$.

3.3.2 Coefficient of determination

CD is defined by scaling the inverse of the coefficient matrix by corresponding coefficients from the relationship matrix, in other words, CD accounts for the reduction of connectedness due to relationship variability between individuals under comparison. The extent of CD ranges between 0 and 1, with larger values indicating increased connectedness.

A pairwise CD between individuals i and j is given by (Laloë et al., 1996):

$$\text{CD}_{ij} = 1 - \lambda \frac{\boldsymbol{C}_{ii}^{22} + \boldsymbol{C}_{jj}^{22} - 2\boldsymbol{C}_{ij}^{22}}{\boldsymbol{K}_{ii} + \boldsymbol{K}_{jj} - 2\boldsymbol{K}_{ij}},$$

where K_{ii} and K_{jj} are the ith and jth diagonal elements of $\boldsymbol{K}$.

The CD between two units can be scaled using the individual average PEVD with the average pairwise relationship differences across individuals to compute the individual average CD as described by Yu and Morota (2019):

$$CD_{i'j'} = 1 - \lambda \frac{\frac{1}{n_{i'}.n_{j'}} \sum_{i \in i'} \sum_{j \in j'} (\boldsymbol{C}_{ii}^{22} + \boldsymbol{C}_{jj}^{22} - 2(\boldsymbol{C}_{ij}^{22}))}{\frac{1}{n_{i'}.n_{j'}} \sum_{i \in i'} \sum_{j \in j'} (\boldsymbol{K}_{ii} + \boldsymbol{K}_{jj} - 2\boldsymbol{K}_{ij})}$$

$$= 1 - \frac{\frac{1}{n_{i'} \cdot n_{j'}} \sigma_e^2 \sum_{i \in i'} \sum_{j \in j'} (\boldsymbol{C_{ii}^{22}} + \boldsymbol{C_{jj}^{22}} - 2(\boldsymbol{C_{ij}^{22}}))}{\frac{1}{n_{i'} \cdot n_{j'}} \sigma_u^2 \sum_{i \in i'} \sum_{j \in j'} (\boldsymbol{K_{ii}} + \boldsymbol{K_{jj}} - 2\boldsymbol{K_{ij}})}$$

$$= 1 - \frac{\frac{1}{n_{i'} \cdot n_{j'}} \sum_{i \in i'} \sum_{j \in j'} PEVD_{ij}}{\frac{1}{n_{i'} \cdot n_{j'}} \sigma_u^2 \sum_{i \in i'} \sum_{j \in j'} (\boldsymbol{K_{ii}} + \boldsymbol{K_{jj}} - 2\boldsymbol{K_{ij}})}$$

$$= 1 - \frac{\sum_{i \in i'} \sum_{j \in j'} PEVD_{ij}}{\sigma_u^2 \sum_{i \in i'} \sum_{j \in j'} (\boldsymbol{K}_{ii} + \boldsymbol{K}_{jj} - 2\boldsymbol{K}_{ij})}$$

3.3.3 Genomic kernel relationship matrices

Parametric relationship matrices: The extent of connectedness measures depends on the choice of relationship matrix $\boldsymbol{K}$. In this study, we evaluated six $\boldsymbol{K}$ matrices. The pedigree-based relationship matrix ($\boldsymbol{K} = \boldsymbol{A}$) was calculated to obtain the additive numerator relationship, reflecting the probability that alleles are identical by descendent inherited from a common ancestor (Wright, 1922). The diagonal element a_{ij}, which is the numerator relationship coefficient between two animals i and j for a population of n individuals is equal to $1 + F_j$, where F_j is the inbreeding coefficient of animal i.The off-diagonals of this matrix are twice the kinship coefficients and are equivalent to the numerators of Wright's correlation coefficients (Wright, 1922; Malécot, 1948). The $\boldsymbol{A}$ matrix among 925 animals was constructed using the pedigree records of 427,740 animals.

The genomic relationship matrix ($\boldsymbol{K} = \boldsymbol{G}$) was used to capture the genomic similarity among individuals, estimating the proportion of the genome between individuals that is identical by state. The $\boldsymbol{G}$ matrix was obtained as a function of the allele content including elements of 0, 1, and 2 representing the copies of reference alleles according to VanRaden (2008) as follows:

$$\mathbf{G} = \mathbf{W_a} \mathbf{W_a'} / 2 \sum_{k=1}^{m} p_k(1 - p_k),$$

where $\mathbf{W_a}$ is a centered incidence matrix taking values $0 - 2p_k$ for zero copies of the reference allele; $1 - 2p_k$ for one copy of the reference allele; and $2 - 2p_k$ for two copies of the reference allele. Here p_k is the allele frequency at SNP $\mathrm{k} = 1, \ldots, \mathrm{m}$.

To capture dominance genetic effects, we constructed a dominance relationship matrix ($\boldsymbol{K} = \boldsymbol{D}$) according to Vitezica et al. (2013):

$$\mathbf{D} = \mathbf{W_d}\mathbf{W_d'}/\sum_{k=1}^{m} (2p_k(1-p_k))^2,$$

where $\mathbf{W_d}$ is the dominance marker incidence matrix, taking values of $-2{p_k}^2$ for zero copies of the reference allele; $2p_k(1-p_k)^2$ for one copy of the reference allele; and $-2(1-p_k)^2$ for two copies of the reference allele.

By combining the aforementioned $\boldsymbol{G}$ and $\boldsymbol{D}$, we considered the following scenarios: pedigree ($\boldsymbol{A}$), additive ($\boldsymbol{G}$), additive and dominance ($\boldsymbol{G}+\boldsymbol{D}$), additive and additive by additive epistasis ($\boldsymbol{G}+\boldsymbol{G}\#\boldsymbol{G}$), and additive, dominance, and additive by additive epistasis ($\boldsymbol{G}+\boldsymbol{D}+\boldsymbol{G}\#\boldsymbol{G}$), where # denotes the Hadamard product (Henderson, 1985). For a multi-kernel approach, a single kernel matrix was derived by weighting each of these kernels by its relative contribution to the total genetic variation according to Momen and Morota, (2018).

3.3.4 Gaussian kernel

In a Gaussian kernel ($\boldsymbol{K} = \boldsymbol{GK}$), the relationship between individuals is defined as distances on the Euclidean space, creating genetic relatedness in terms of spatial distance (de los Campos et al., 2010). The relationship between a pair of individuals i and j with their genotype vectors $\mathbf{w}_i \in (0,1,2)$ and $\mathbf{w}_j \in (0,1,2)$ is given by:

$$\begin{aligned}\mathbf{GK}(\mathbf{w}_i, \mathbf{w}_j) &= \exp(-\theta d_{ij}^2) \\ &= \prod_{k=1}^{m} \exp\left(-\theta(w_{ik}-w_{jk})^2\right),\end{aligned}$$

where $d_{ij} = \sqrt{(w_{i1}-w_{j1})^2 + \cdots + (w_{ik}-w_{jk})^2 + \cdots + (w_{im}-w_{jm})^2}$ is the Euclidean distance between two individuals, and θ is a positive bandwidth parameter that controls the overall smoothness of the kernel function. A small Euclidean distance between two individuals means that their genotypes are similar in state, or in other words, that they have a strong relationship. The parameter θ is what controls the extent of genomic similarity between individuals. As θ increases, the kernel approaches zero (i.e., local kernel); and smaller θ produces entries closer to 1, in other words, two individuals match perfectly (i.e., global kernel) (Morota et al., 2013). We employed a kernel averaging approach

(de los Campos et al., 2010) by integrating two kernel matrices ($\boldsymbol{GK1}$ and $\boldsymbol{GK2}$) using two extreme values of θ, so the mean of the off-diagonals elements in each kernel were 0.2 and 0.8, respectively. The averaged ***GK*** was obtained by:

$$\boldsymbol{GK} = \frac{\sigma^2_{GK1}}{\sigma^2_{GK1} + \sigma^2_{GK2}}\ \boldsymbol{GK1} + \frac{\sigma^2_{GK2}}{\sigma^2_{GK2} + \sigma^2_{GK1}}\ \boldsymbol{GK2}\ ,$$

where σ^2_{GK1} and σ^2_{GK2} are variance components attached to kernels $\boldsymbol{GK1}$ and $\boldsymbol{GK2}$, respectively. A BLUP model coupled with a Gaussian kernel matrix is known as reproducing Hilbert spaces regression (Morota and Gianola, 2014).

Throughout this study, we used R statistical computing environment (R Development Core Team, 2019) to estimate variance components and connectedness metrics with the following packages: the BGLR package (Pérez and de Los Campos, 2014) to estimate variance components and the GCA package (Yu and Morota, 2019) to compute connectedness.

4.0 Results

4.1 Heritability estimates

Descriptive statistics and genetic parameter estimates for C14:0 are presented in Table 1 for parametric kernel matrices. In this study, we estimated narrow-sense heritability (h^2) accounting for additive effects only ($\boldsymbol{K} = \boldsymbol{A}$ or $\boldsymbol{G}$) and broad-sense heritability (H^2) accounting for additive, dominance, and epistasis ($\boldsymbol{K}$= $\boldsymbol{G} + \boldsymbol{D}$, $\boldsymbol{G} + \boldsymbol{G}\#\boldsymbol{G}$, **or** $\boldsymbol{G} + \boldsymbol{D} + \boldsymbol{G}\#\boldsymbol{G}$**)**.

Narrow- and broad-sense heritability estimates for C14:0 ranged from 0.142 (±0.095) to 0.462 (±0.092). Heritability estimates were lower when using the pedigree relationship matrix compared with the other relationship matrices, likely because of incomplete pedigree data. The h^2 estimate from $\boldsymbol{G}$ was approximately two-fold higher than that from $\boldsymbol{A}$. Moreover, the inclusion of dominance ($\boldsymbol{G} + \boldsymbol{D}$) and epistatic effects ($\boldsymbol{G} + \boldsymbol{G}\#\boldsymbol{G}$ and $\boldsymbol{G} + \boldsymbol{D} + \boldsymbol{G}\#\boldsymbol{G}$) increased the heritability estimates. These findings suggest that C14:0 may be controlled by additive as well as non-additive gene action.

Table 1. Descriptive statistics and heritability estimates for each gene action scenario.

			h²		H²		
Trait	**N**	**Mean± SD**	**A**	**G**	**G+D**	**G+G#G**	**G+D+G#G**
C14:0	925	[1]0.26± 0.16	0.142 (0.095)	0.268 (0.081)	0.390 (0.089)	0.420 (0.076)	0.462 (0.092)
Genetic Variance			$\sigma_u^2 = 0.120\ (0.083)$	$\sigma_g^2 = 0.225\ (0.073)$	$\sigma_g^2 = 0.201\ (0.067)$ $\sigma_d^2 = 0.150\ (0.056)$	$\sigma_g^2 = 0.183\ (0.064)$ $\sigma_{g\#g}^2 = 0.175\ (0.069)$	$\sigma_g^2 = 0.155\ (0.059)$ $\sigma_d^2 = 0.123\ (0.049)$ $\sigma_{g\#g}^2 = 0.135\ (0.053)$
Residual Variance			$\sigma_e^2 = 0.725\ (0.086)$	$\sigma_e^2 = 0.615\ (0.071)$	$\sigma_e^2 = 0.550\ (0.078)$	$\sigma_e^2 = 0.494\ (0.085)$	$\sigma_e^2 = 0.481\ (0.080)$

[1]The concentration of fatty acids is expressed as a percentage of total fatty acid methyl esters (FAME). **A**: pedigree. **G**: additive genomic kernel relationship matrix. **G + D**: additive × dominance genomic kernel relationship matrix. **G + G#G**: additive × epistasis genomic kernel relationship matrix. **G + D + G#G**: additive × dominance × epistasis genomic kernel relationship matrix. σ_u^2 : additive genetic variance; σ_g^2: additive genomic variance; σ_d^2: dominance genomic variance; $\sigma_{g\#g}^2$ additive by additive epistasis genomic variance; σ_e^2: residual variance. Posterior standard errors are shown in the parentheses.

4.2 Prediction error variance of difference (PEVD)

Figure 1 (Appendix Section) shows the prediction error variance of difference (PEVD) estimates across MU derived from $\boldsymbol{A}$, $\boldsymbol{G}$, $\boldsymbol{G}+\boldsymbol{D}$,$\boldsymbol{G}+\boldsymbol{G}\#\boldsymbol{G}$, and $\boldsymbol{G}+\boldsymbol{D}+\boldsymbol{G}\#\boldsymbol{G}$ for C14:0. The smaller the PEVD, the higher the connectedness. The smallest PEVD connectedness measures were found in $\boldsymbol{A}$, ranging from 1.654 to 1.707. When comparing $\boldsymbol{A}$ with $\boldsymbol{G}$, enhanced connectedness measures were observed by reduced PEVD (1.387 – 1.422). The MU were more connected when genomic information was used. The inclusion of dominance ($\boldsymbol{G}+\boldsymbol{D}$**)** moderately enhanced the PEVD across MU, with estimates ranging from 1.288 to 1.315. Accounting for additive and additive epistasis ($\boldsymbol{G}+\boldsymbol{G}\#\boldsymbol{G}$) in the model marginally increased the measures of connectedness. Including additive, dominance, and additive by additive epistasis ($\boldsymbol{G}+\boldsymbol{D}+\boldsymbol{G}\#\boldsymbol{G}$) resulted in the highest connectedness estimates among the parametric kernel relationship matrices. The use of $\boldsymbol{GK}$ significantly increased estimates of genetic connectedness across all MU. Overall, $\boldsymbol{A}$ yielded the least connected measures, while $\boldsymbol{GK}$ produced most connectedness estimates that were considered to be less connected.

4.3 Coefficient of determination (CD)

Individual average CD for each of the six scenarios is presented in Figure 2. The higher the CD, the higher the connectedness, and a similar pattern was found as reported for PEVD. The largest measured CD (0.456) was obtained with $\boldsymbol{GK}$, and the smallest CD (0.146) was obtained with $\boldsymbol{A}$. The MU presented low levels of connectedness in $\boldsymbol{A}$, in which the highest estimate was 0.171. Compared to the pedigree-based method, genetic relatedness inferred from $\boldsymbol{G}$ increased the estimates of genetic connectedness across MU, and this trend was enhanced when dominance ($\boldsymbol{G}+\boldsymbol{D}$) and epistasis ($\boldsymbol{G}+\boldsymbol{G}\#\boldsymbol{G}$) were included. Combining additive, dominance, and epistasis in $\boldsymbol{G}+\boldsymbol{D}+\boldsymbol{G}\#\boldsymbol{G}$ resulted in greater measures of connectedness than any of the parametric relationship kernel matrices. The estimates of CD for $\boldsymbol{G}+\boldsymbol{D}+\boldsymbol{G}\#\boldsymbol{G}$ ranged from 0.427 to 0.445. $\boldsymbol{GK}$ presented the highest CD estimates, ranging from 0.442 to 0.456. These results

demonstrated the importance of accounting for additive, dominance, and epistasis when the trait is also controlled by non-additive gene action.

4.4 Connectedness within and across breeding programs

Farms F1–F3 belonged to the DeltaGen breeding program. F1 and F3 farms were found to be the most connected across all scenarios. Farms F4, F6, and F7 belonged to the Paint breeding program. Although all MU presented similar connectedness values for CD, F4 and F6 were found to be the most connected, whereas F7 was well connected with the other two MU. Finally, we investigated the connectedness of the MU between the three different breeding programs and found that only one MU from Nelore Qualitas (F5) was adequately connected with the other MU. This pattern also appeared when we analyzed MU from DeltaGen and Paint, suggesting that these three breeding programs are connected, probably because of the use of AI in recent years.

5.0 Discussion

This study presents genome-based connectedness estimates in Nellore cattle using phenotype and genotype samples of commercial herds from the three breeding programs. The phenotype studied in this analysis is not routinely measured in breeding programs. Phenotypic information relating to the C14:0 trait was only available for a small number of contemporary groups in the MU, which could limit the data connection.

In Brazil, the beef cattle industry is concentrated in two main regions, the southeast and the mid-west. The recent expansion of agriculture has introduced animal husbandry to new regions. Figure 3 shows the distance (in kilometers) between the farms evaluated in our study. Farms F1–F3 are part of the DeltaGen Program, and are spread across three regions (mid-west, southeast, and northeast). F1 and F3 are the most distant units in this program. Within the Nellore Paint program, farms F4, F6, and F7 are located in the mid-west and southeast regions in Brazil. The distance between F4 and F6 is the longest, despite being located in the same region.

Overall, considering each farm apart from its breeding program, F4 was the closest to any other farm, while F6 was the most distant. The average distance

between two farms was 1,188 km. The extent of connectedness may be partially explained by the geographic position of each farm, since Brazilian herds consist of many subpopulations isolated by geographical distance, limiting the sharing of genetic material across MU. Overall, we did not find a clear relationship between connectedness and distance. Thus, geographical location alone does not explain the extent of connectedness.

The degree of connectedness reflects the reliability of comparisons between animals of different MU (Kennedy and Trus, 1993; Tosh and Wilton, 1994; Hanocq et al., 1999; Carneiro et al., 2001; Mathur et al., 2002; Roso et al., 2004). Low connectedness implies that the reliability of genetic value comparisons and animal rankings across MU are not sufficiently reliable (Lewis et al., 1999a; Lewis and Simm, 2000; Kuehn et al., 2008). Theoretically, the extent of connectedness may be of less concern (Fernando et al., 1983; Kennedy and Trus, 1993; Fries and Roso, 1997). Kennedy and Trus (1993), and Tosh and Wilton (1994) reported that disconnected MU may not lead to biased predictions if the genetic values of the base animals are randomly and identically distributed throughout the population. However, this is less true for field populations such as beef cattle in Brazil, because of genetic selection, drift, limited use of AI, and low phenotyping rates in hard-to-measure traits, which have an impact on the genetic means and variance component estimates of the MU (Clément et al., 2001; Tosh and Wilton, 1994; Kuehn et al., 2007b; Tarrés et al., 2010).

5.1 Estimates of genetic parameters

Connectedness is often used to design or evaluate the effectiveness of breeding programs prior to phenotyping. However, phenotypic information enters the derivation of connectedness through heritability or the ratio of variance components (λ). In the present study, we evaluated the FA C14:0 (myristic acid). In general, the narrow-sense heritability estimates for individual FA profiles in the *Longissimus thoracis* muscle of beef cattle are low to moderate (Tait et al., 2007; Cesar et al., 2014; Lemos et al., 2016; Feitosa et al., 2017), ranging from 0.17 to 0.64. Our estimates for myristic acid using $\boldsymbol{A}$ (0.141) were lower than those reported by Tait et al. (2007), of 0.23, in Angus cattle, but were closer when $\boldsymbol{G}$ (0.274) was used in our study. Using a population of Nellore Cattle, Aboujaoude

et al. (2016) reported a genomic h^2 estimate of 0.25. Cesar et al. (2014), and Feitosa et al. (2017) reported genomic h^2 estimates of C14:0 in Nellore Cattle of 0.17 and 0.25, respectively.

In the current study, we evaluated six relationship matrices using pedigree and genomic data accounting for additive and non-additive genetic variation into the kernel relationship matrices. We found that the heritability estimates from $\boldsymbol{A}$ were the lowest. In the beef cattle industry, the poor records for herds are problematic, especially for beef quality traits evaluated after slaughter. This affects both the measurement relationships, and the genetic parameter estimates (Fouilloux et al., 2008a). Here, approximately 55% of records contained no sire information. The percentage of animals with known sires in each farm was F1=13%, F2=72%, F3=81.25%, F4=57.36%, F5= 63.15%, F6=27.39%, F7=100%, and for a total 413/925 animals in the data set. This data set is composed by commercial herds, and in Brazil, most farms are under a multiple service sires (MS) mating system, which consists of groups of cows clustered with several bulls in the same paddock during a riding season (Cardoso and Tempelman, 2003). MS improves the conception rate (Lunstra and Laster, 1982), but results in an uncertain paternity scenario. Therefore, incomplete pedigree data due to MS negatively affects the accuracy of genetic evaluations (Cardoso and Tempelman, 2003).

In the breeding programs in our study, the mating season begins with AI in all dams. However, if the dam is not pregnant, the MS mating system is applied. The bulls used in this system are usually from the same farm, and they only produce one generation of progeny. The calf born from the MS mating system is slaughtered because of uncertain paternity. These calves are considered to have unreliable genetic merit, which means that they are not candidates for breeding, while allowing phenotypes to be obtained from the slaughtered animals. The use of genomic information can enhance the feasibility of genetic evaluation and increase the prediction accuracy of these novel traits in the beef cattle industry.

The use of SNP, which capture molecular similarity and Mendelian sampling, determines relationships between individuals at the genomic level, recovering information missing in the pedigree. Cesar et al. (2014) first reported genomic heritability estimates for FA in Nellore cattle, and showed that SNP panels are a promising tool for the genetic improvement of Nellore cattle in Brazil,

mainly because of the effective cost strategy for their application in breeding programs. We found that the heritability estimates from $\boldsymbol{G}$ were larger compared to those from $\boldsymbol{A}$, recovering a greater proportion of additive genetic variance. Our observations are consistent with those of Ishii et al. (2013) in Japanese Black cattle and Saatchi et al. (2013) in US Angus cattle.

It is also critical to account for dominance and/or epistatic variation to optimize breeding designs, such as mate allocation. Several animal studies (Serenius et al., 2006; Sun et al., 2014; Moghaddar and van der Werf, 2017; Joshi et al., 2018; Ebrahimi et al., 2019) have reported that dominance heritability is often significantly greater than zero, and its inclusion in prediction models could improve performance in those studies. We observed dominance and epistatic variance for C14:0, suggesting that C14:0 may be controlled by both additive and non-additive genetic variation.

5.2 Connectedness statistics

Because of missing pedigree data in the present study, connectedness statistics using the numerator relationship matrix resulted in the lowest connectedness estimates, indicating that $\boldsymbol{A}$ may provide an incorrect picture of connectedness for C14:0 in the population studied. In contrast, $\boldsymbol{G}$ consistently enhanced the measures of connectedness. The results from $\boldsymbol{G}$ highlight how genomic data can help to better observe the true state of connectedness, particularly when pedigree data are less reliable. This supports the findings of Yu et al. (2017), who reported that genomic relatedness inferred from SNP increases the estimates of genetic connectedness across MU compared to estimates of pedigree information. As noted by Yu et al. (2017, 2018), the availability of genomic information provides an opportunity to improve the quality of genetic value comparisons and revisit a number of critical questions related to connectedness.

The increased interest in non-additive variation (Wolak and Keller, 2014; Varona et al., 2018) suggests that it may be possible to account for such variation in connectedness studies. Momen and Morota (2018) demonstrated that connectedness metrics can be extended to incorporate non-additive genetic

variation of complex traits. They showed an increase up to 25% in the capture of connectedness using additive and non-additive genomic kernel relationship matrices when the trait of interest is controlled by non-additive gene action. Our study investigated how the inclusion of such variation could impact connectedness metrics. Increased estimates of connectedness were observed when dominance and epistatic parametric kernels were included. The non-parametric relationship matrix ($\boldsymbol{GK}$) models higher-order epistatic gene action by taking the Hadamard product between the $\boldsymbol{G}$ matrices when SNP were coded in an additive manner (Jiang and Reif, 2015). In our study, $\boldsymbol{GK}$ was better than all parametric approaches, highlighting the usefulness of $\boldsymbol{GK}$ for incorporating non-additive gene action. Nii et al. (2006) first reported the presence of epistatic quantitative trait loci for perirenal C14:0 in wild boars, and was later reported by Uemoto et al. (2009) on chromosome 16 in swine.

In livestock, the inclusion of dominance effects can be justified by the use of semen from a few genetically superior bulls and reproductive biotechnologies (such as multiple ovulation, embryo transfer, and *in-vitro* fertilization). Consequently, the number of full sibling progenies increases, which increases the relationships within and between generations, as well as dominance genetic relationships (VanRaden, 1992). The inclusion of such effects was reported by Varona and Misztal (1999), who noted that the inclusion of dominance effects into genetic evaluations enables the determination of specific combinations for mating schemes, and the separation of additive variation from the rest, especially in populations containing many full-sibs in their pedigree. Hayes and Miller (2000) and Ishida and Mukai (2004) stated that ignoring non-additive genetic variance in breeds could result in biased predictions of genetic values, which would affect the animal's classification as well as national and international comparisons.

Fouilloux and Laloë (2001) developed the criterion of admission to the group of connected herds (CACO) method to compare the average CD values of all herds and to cluster them. This method was applied by Pegolo et al. (2012) in Brazilian Nellore cattle using registered animal data from elite herds (National Association of Breeders and Researchers, ANCP, Ribeirão Preto, Brazil) . They recorded weight after 210 days (h^2 = 0.25) to investigate the trajectory of connectedness from 1999 to 2003, and from 2004 to 2008. They found moderate estimates of pedigree-based connectedness and attributed the increase in

connectedness to the use of AI, which increased 47% with the semen sales in that period. According to ASBIA (2011), only 10% of dams were inseminated at reproductive age. AI affects the measurement of genetic connectedness among herds (Fouilloux et al., 2008) and strongly influences the quality of pedigree information.

AI can accelerate genetic improvement in a population as it allows semen from animals with a higher genetic value to be utilized, which are not normally available for use in natural matings. It also increases the number of offspring per sire. Despite the growing use of AI, most commercial beef cattle programs in Brazil still use unproven sires for natural mating. In addition, programs that use AI still need a bull to mate with their dams in the case of AI failure, which is common on commercial farms where animals are destined for slaughter. As the MU evaluated in this study were not part of consolidated breeding programs, the use of AI was more than 50% of that in all farms, because of known paternity scenarios needed for the genetic evaluations. Thus, use of the MS mating system in these programs incurs the costs of maintaining a non-pregnant cow in the herd.

Changes in connectedness levels reported by Pegolo et al. (2012) showed that herd descriptors, such as the number of animals in the herd, the number of sires used in the herd, the percentage of connecting sires, the percentage of progeny from connecting sires, and the percentage of calves with unknown sires, cannot fully explain how the herds are connected. The use of different types or combinations of relationship matrices to those used in our study may be a viable approach to understand the complexity of genetic connectedness in livestock species.

Here, we describe the first application of genomic connectedness in Brazilian Nellore cattle. This study shows how genomic information can increase connectedness measures when pedigree information is not complete due to multiple sire systems. Collectively, through the use of genomics and by accounting for non-additive gene action, we can better reflect signals of connectedness not captured by pedigree-based counterparts.

6.0 Final Considerations

Genetic connectedness plays a key role in the quality of genetic value comparisons across MU. We used PEVD and CD to assess genomic connectedness measures in Nellore cattle field data, accounting for the presence of non-additive gene action. Our findings show that genomic information can capture connectedness signals that may be missed from the pedigree, providing a more precise picture of connectedness. Working with novel traits can be challenging when they are hard to measure, because only a few breeding programs possess the infrastructure and logistics to collect phenotypes such as the FA profile of meat. We show that it is possible to capture connectedness signals from samples of different farms within and between different breeding programs using a specific phenotype (C14:0) with low heritability. We also confirm that the use of AI, even though still used at low levels in the country (16%), has an important role in connecting herds. Furthermore, we observed that the use of additive and non-additive genomic kernel relationship matrices can enhance the capture of connectedness measures compared to purely additive counterparts.

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APPENDIX

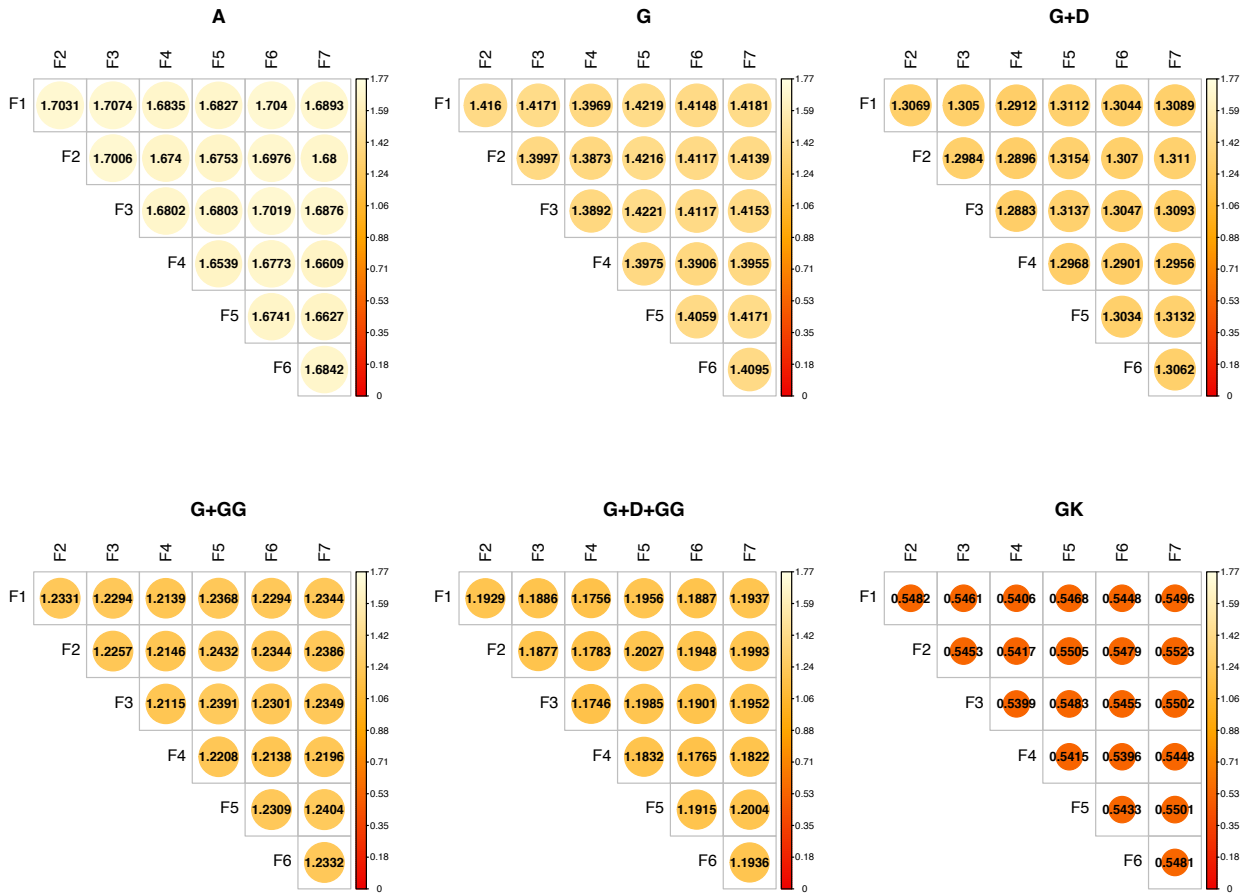

Figure 1. Individual average PEVD for C14:0A: pedigree. G: additive genomic kernel relationship matrix. G × D: additive × dominance genomic kernel relationship matrix. G × G#G: additive × epistasis genomic kernel relationship matrix. G × D × G#G: additive × dominance × epistasis genomic kernel relationship matrix. GK: Gaussian kernel relationship matrix.

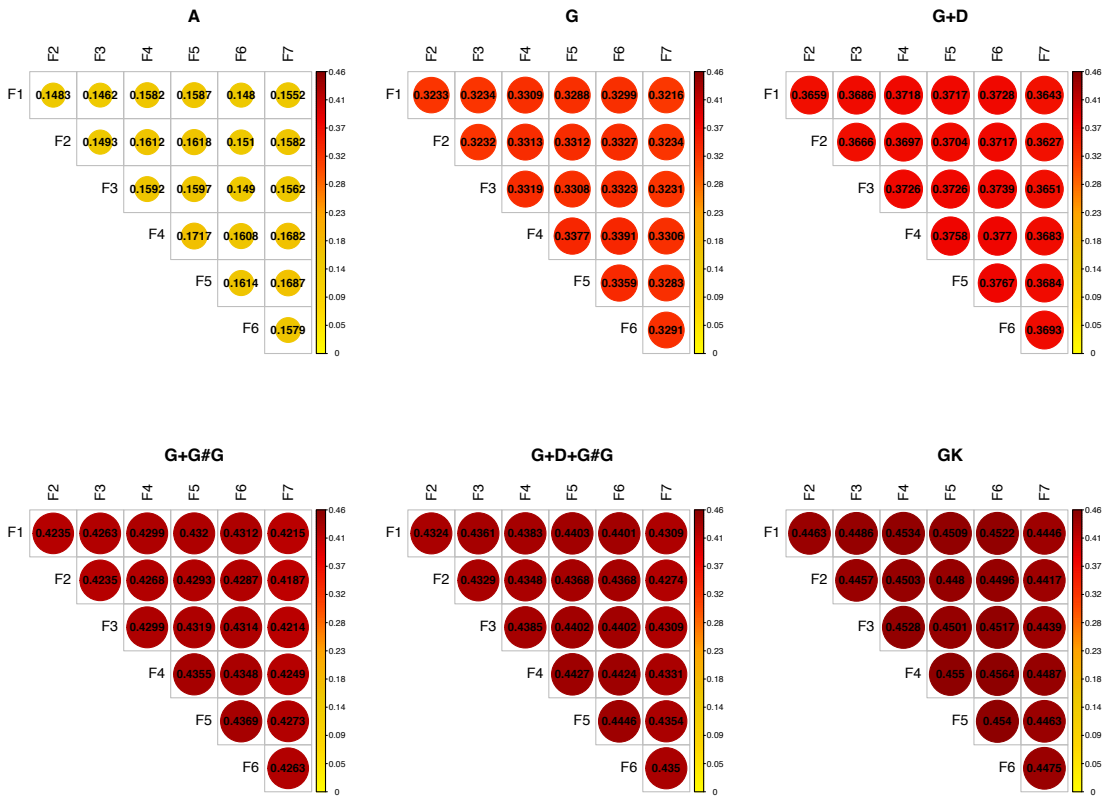

Figure 2. Individual average CD for C14:0. A: pedigree. G: additive genomic kernel relationship matrix. G × D: additive × dominance genomic kernel relationship matrix. G × G#G: additive × epistasis genomic kernel relationship matrix. G × D × G#G: additive × dominance × epistasis genomic kernel relationship matrix. GK: Gaussian kernel relationship matrix.

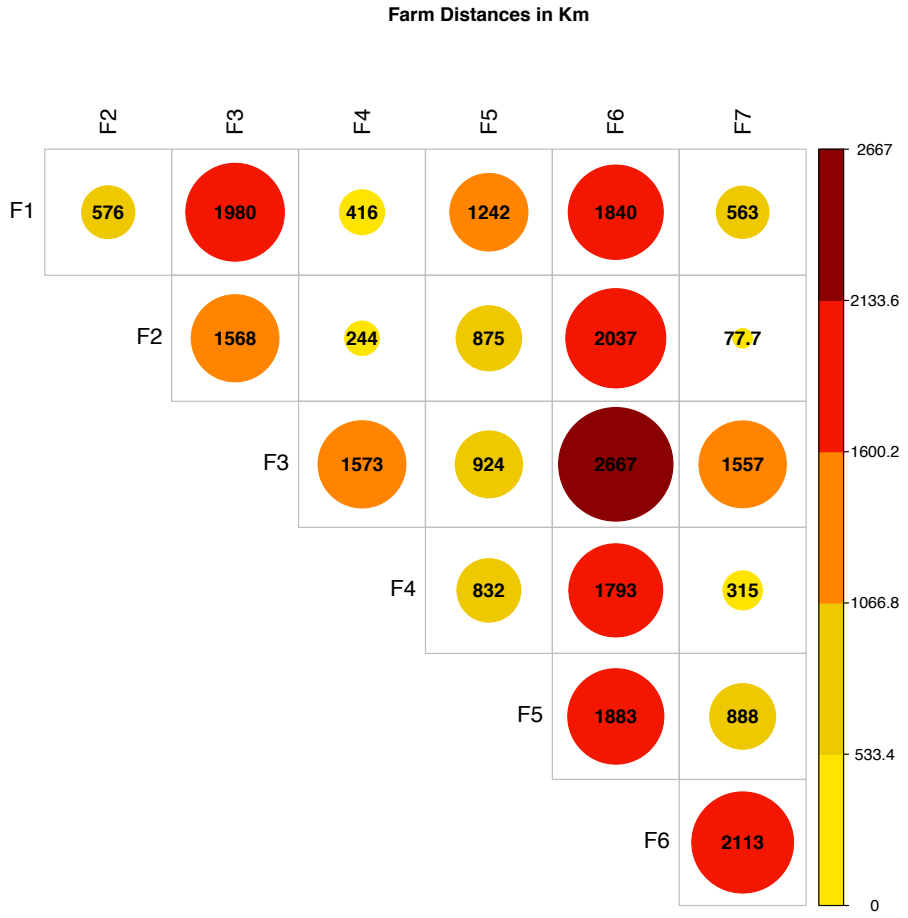

Figure 3. Farm distance in Km. **F1** - Dourados (MS); **F2** - Valparaíso (SP); **F3** - Cotegipe (BA); **F4** - Água Clara (MS); **F5** - Goianésia (GO); **F6** - Juruena (MT); **F7** - Piacatu (SP).