FACULDADE DE CIÊNCIAS AGRÁRIAS E VETERINÁRIAS UNIVERSIDADE ESTADUAL PAULISTA CÂMPUS DE JABOTICABAL

COPY NUMBER VARIATIONS AND SINGLE-NUCLEOTIDE POLYMORPHISMS ASSOCIATED WITH BEEF FATTY ACID PROFILE IN NELLORE CATTLE

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TÍTULO: COPY NUMBER VARIATIONS AND SINGLE-NUCLEOTIDE POLYMORPHISMS ASSOCIATED WITH BEEF FATTY ACID PROFILE IN NELLORE CATTLE

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AUTHOR'S CURRICULAR DATA

MARCOS VINÍCIUS ANTUNES DE LEMOS - born in Mara Rosa - GO, on August 16, 1987, son of Maronita Antunes Clemente Lemos and Devacy Constantino de Lemos. Graduated in Animal Science from the Federal University of Goiás (UFG) - Câmpus Jatai, in September 2011. During the undergraduate course the student was an instructor of the discipline of Animal Genetic Improvement for two years (2009 and 2010), with scholarship granted by UFG. Started the M.Sc. course in Animal Breeding and Genetics at São Paulo State University – FCAV/UNESP – Campus of Jaboticabal in March 2012 and concluded in July 2013. Was granted with a scholarship from CAPES and presented his master with the dissertation entitled "Interação genotipo ambiente para perimetro escrotal em bovinos da raça Nelore utilizando normas de reação" with Prof. Fernando Baldi as advisor. Started the Ph.D. course in Animal Breeding and Genetics at the same university in August 2013. During the doctorate's course, held an intership at Clemson University, under supervision of Ph.D. Susan Duckett, with a scholarship from FAPESP and a project entitled "Analysis of copy number variations in the genome of Nellore and its associations with the fatty acid profile of the meat". Submitted to the defense exam to obtain the Doctorate's degree in Animal Breeding and Genetics in 2017, with Prof. Fernando Baldi as advisor and with the thesis entitled "Copy number variation and single-nuclutide polymorphisms associated with beef fatty acid profile in Nellore cattle".

“Não é o mais forte que sobrevive,
nem o mais inteligente.
Quem sobrevive é o mais
disposto à mudanças.”
Charles Darwin

“Suba o primeiro degrau com fé.
Não é necessário que você veja toda a escada.
Apenas dê o primeiro passo.”
Martin Luther King

À minha base, meu alicerce, meu porto seguro... Meus pais, Devacy e Maronita

Todo o carinho, amor do mundo é pouco para definir a gratidão, o respeito e o amor que tenho por vocês.

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Às minhas avós Maria e Alaíde
Duas vezes mãe... Duas vezes carinho... Duas vezes amor... Duas vezes compreensão...
Amo vocês!

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SUMMARY

COPY NUMBER VARIATIONS AND SINGLE-NUCLEOTIDE POLYMORPHISMS ASSOCIATED WITH BEEF FATTY ACID PROFILE IN NELLORE CATTLE

ABSTRACT - The objective of this study was to identify genomic regions of Nellore cattle that present variations in the number of copies (CNV) and to associate these CNV with the fatty acid profile of the meat. In addition, the objective of this study was to carry out a genome-wid association using the single step method (GWASss) to detect genomic regions associated with saturated, mono and polyunsaturated fatty acids, as well as omega 3, 6 and their relationship. The study of the characterization and distribution of CNVs along the Nellore genome was performed by PennCNV software using genotypic data of 3,794 animals, resulting in 399,361 CNVs identified. After quality control, 2,902 were maintained in the analyzes, resulting in 195,873 CNVs, with an average size of 54,744 pb, maximum of 8.7 Mb and minimum 3 kb. The CNV regions were generated by the overlap of the CNVs by CNVRuler software. The chromosomes that showed the highest incidence of CNVR were BTA19 (24.26%), BTA23 (18.68%) and BTA25 (18.05%). Those with the lowest incidence were BTA29 (1.63%), BTA13 (9.72%) and BTA8 (9.72%). The 9,805 CNV regions estimated in the present study covered approximately 13.05% of the bovine genome and overlaped 5,495 genes known to envolve in biological processes that could be involved in the environmental adaptation of the subspecies to tropical areas. The GWASss study showed 115 windows that explained more than 1% of the additive genetic variation for the 22 fatty acids studied. The identification of these regions and their genes, such as *ELOVL5, ESRRG, PCYT1A* and the *ABC* group genes (*ABCA5, ABCA6* and *ABCA10*) are genes directly and indirectly related to lipid metabolism. The GWAS between AG phenotypes and CNVs resulted in a total of 186 CNVRs that were significant for the saturated (43), monosaturated (42), polyunsaturated (66) and omegas (35) fatty acids groups, in which 278 genes with described function. These results pointed genes associated to AG of various saturations, and the genes *SAMD8* and *BSCL2*, which are related to lipid metabolism; and the *RAPGEF6* gene, related to energetic metabolism. Thus, the numerous genomic regions found in this study, as well as the genes identified in them, should contribute to the formation of a genetic basis of the Nellore beef (*Bos indicus*) fatty acid profile, contributing to a better selection of the traits associated with improvement of human health. The knowledge of these CNVs could improve the understanding of the genetic and physiological mechanisms that contribute to the productive traits, as well as the selection of more productive and efficient animals.
Keywords – Copy number variation, fatty acid, SNP, Nellore, genomic selection

VARIAÇÕES NO NÚMERO DE CÓPIA E POLIMORFISMOS DE NUCLEOTIDEOS SIMPLES ASSOCIADOS AO PERFIL DE ÁCIDO GRAXO DA CARNE DE BOVINOS NELORE

RESUMO - Objetivou-se identificar regiões no genôma de bovinos da raça Nelore que apresentam variações no número de cópias (CNV) e, associar estes CNV com o perfil de ácidos graxos da carne. Além disso, objetivou-se realizar associação genica ampla utilizando os método de single step (GWASss) a fim de detectar regiões genômicas associadas aos ácidos graxos dos grupos saturados, mono e poliinsaturados, assim como os omegas 3, 6 e sua relação. O estudo de caracterização e distribuição dos CNVs ao longo do genoma de bovinos Nelore, foi realizado através do software PennCNV utizando dados genotípicos de 3.794 aniamais, resultando em 399.361 CNVs identificados. Após controle de qualidade, 2.902 foram mantidos nas analises, resultando em 195.873 CNVs, com tamanho medio de 54,744 pb, maximo de 8.7 Mb e minimo 3 kb. As regiões de CNV foram geradas pela sobreposição dos CNVs através do software CNVRuler. Os cromossomos que mostraram maior incidencia de CNVR foram BTA19 (24,26%), BTA23 (18,68%) e BTA25 (18,05%). Ja os que mostraram menor incidencia foram BTA29 (1,63%), BTA13 (9,72%) and BTA8 (9,72%). As 9.805 regiões da CNV estimadas no presente estudo cobrem aproximadamente 13.05% do genoma bovino e sobrepõem-se a 5.495 genes conhecidos que envolvem processos biológicos que poderiam estar envolvidos na adaptação ambiental da subespécie a áreas tropicais. O estudo de GWASss identificou 115 janelas que explicaram mais de 1% da variação genética aditiva para os 22 ácidos graxos estudados. A identificação destas regiões e seus genes genes, tais qual *ELOVL5, ESRRG, PCYT1A* e os genes do grupo ABC (ABCA5, ABCA6 e ABCA10) são genes que estão relacionados direto e inderamente ao metabolismo lipídico. O GWAS entres os fenótipos de AG e os CNVs resultaram em um total de 186 CNVR siginificantivos para os grupos dos ácidos graxos saturados (43), monosaturados (42), poliinsaturados (66) e omegas (35), nas quais foram identificados 278 genes com funçao descrita. Estes resultados apontaram genes associados a AG de varias saturações, podendo ser destacados os genes *SAMD8* e *BSCL2*, os quais estão relacionados ao metabolism lipidico; e o gene *RAPGEF6*, relacionado ao metabolism energetico. Assim os inúmeras regiões genômicas encontradas neste estudo, bem como os genes identificados nas mesmas, devem contribuir para a formação de uma base genética do perfil de ácidos graxos da carne de bovino Nelore (*Bos indicus*), podendo contribuir para uma melhor seleção das características associadas à melhora da saúde humana. saúde. O conhecimento desses CNVs deverá melhorar a compreensão dos mecanismos genéticos e fisiológicos que contribuem para as características produtivas, bem como na seleção de animais mais produtivos e eficientes, contribuindo para o melhoramento genético das características produtivas.

Palavras-chave: Variações no numero de cópias, ácidos gracos, SNP, seleção genômica, Nelore.

CHAPTER 1 – General considerations

In relation to nutritional attributes of beef quality, the composition of fatty acid is important not only because it affects on meat palatability, but also it can affect the human health. The fatty acids harmful to human health have received attenuating attention in recent years. Some studies, with taurine breed, have shown that there is a genetic variation for the trait fatty acid profile of the meat and, therefore, the possibility of genetic improvement of this trait in beef cattle. Meantime, in zebu cattle, the genetic parameter estimates for fatty acid profile are scarce. Furthermore, the trait meat fatty acid profile is something difficult and costly to measure and for this kind of trait is indicated the use of genomic selection, which is a type of marker-assisted selection. The objective of this chapter is show the genetic variability of meat fatty acid profile different cattle breeds, and makes an approach of the implement models and methods that use genomic information, as well the CNV tool, to improve the fatty acid composition of beef.

1. LITERATURE REWIEW

1.1 Introduction

In response to the constant bombardment of health-related stories, there is a continuing and growing concern on the part of the population and public health institutions about excessive consumption of fats, especially fats of animal origin, as well as the type of fat or fatty acid profile in the meat, and their impact on consumer health. The fatty acid profile of intramuscular fat is an important issue for human health, since intramuscular fat cannot be extracted or removed before meat consumption (SMET et al., 2004). The composition of fatty acids of intramuscular fat has been widely studied, as it is also related to the succulence, aroma and tenderness of the meat. For international meat quality standards, the amount of intramuscular fat or marbling deposited on the *Longissimus* muscle is the main determinant of the carcass value and predictor of palatability (FERRAZ AND FELÍCIO, 2010).

Although beef is considered a highly nutritious food, being an important source of proteins, micronutrients and B-complex vitamins, it has a high fat content with undesirable composition, such as high percentage of saturated fatty acids (SFA). A high intake of SFA is associated with an increase in serum cholesterol and low-density lipoprotein levels (LDL), which are risk factors for cardiovascular disease (Katan et al., 1994). The predominant SFA in bovine fat are myristic (C14:0), palmitic (C16:0) and stearic (C18:0) acids (LAWRIE, 2005). It is noteworthy that C14:0 has a potential to raise serum cholesterol concentrations 4 to 6-fold higher than C16:0 (MENSINK and KATAN, 1992).

The fatty ruminant tissue is a natural source of isomers of conjugated linoleic acid (CLA), such as cis - 9, trans - 11 (FRENCH et al., 2000), which are synthesized in the rumen as a consequence of the biohydrogenation process of acids by the microrganisms (TAMMINGA and DOREAU, 1991). CLA has favorable effects on human health, increasing immunostimulatory, antimutagenic and antioxidant activity (IP, 1997). In addition, polyunsaturated fatty acids (PUFAs) present in bovine fat such as linoleic (C18: 2n-6) and linolenic (C18: 3n-3) and monounsaturated fatty

acids (MUFA), such as oleic acid (C18: 1, n-9), which offer protection to the cardiovascular system, since balanced consumption of these drugs is associated with a reduction in serum cholesterol levels and an increase in high-density lipoprotein (HDL) (PENSEL et al., 2002).

For many years the composition of fatty acids in meat-producing animals has received considerable interest in view of its implications for human health and meat quality traits (XIE et al., 1996; WOOD et al., 2004; GANDEMER et al., 1999). Like most traits of economic interest in animal production, the composition of fatty acids is influenced by environmental and genetic factors. A number of studies have demonstrated large changes in fatty acid composition due to alterations in feeding strategies, especially in monogastric animals (NÜRNBERG 1998, JAKOBSEN 1999, DEMEYER et al., 1999) and in ruminants (WOOD et al., 2003). However, genetic factors that affect fatty acid composition in cattle have been less investigated, although several studies report differences between breeds for the composition of fatty acids (GILLIS et al., 1973; HUERTA-LEIDENZ et al., 1993; PITCHFORD et al., 2002; RULE et al., 1997; SIEBERT et al., 1996).

1.2 Copy number variation

Research on DNA has evolved from the discovery of the double-helix structure in 1953 to structural variations today. Copy number variation is a structural genomic variation and all genomic rearrangements are bigger than one base pair (Feuk et al., 2006). This definition includes deletions, insertions, translocations, inversions, and duplications (SCHERER et al. 2007). There are several techniques for measuring variation in number of copies, including the set of comparative genomic hybridization and next generation sequencing (BREHENY et al., 2012). The information obtained in the single nucleotide-polumorphism (SNPs) arrangements allows CNVs to be investigated in large-scale studies.

Compared to the high resolution method of comparative genomic hybridization (CGH) arrays, panels or arrays with thousands of SNPs is a method that detects

changes in the number of copies at the level of 5-10 kb (Log R - LRR ratio), the ratio of allele intensities (BF - BAF frequency) and a better estimation of heterozygosis loss (LOH), making the detection CNV more robust. Several algorithms are able to detect CNVs from the intensity of the fluorescent signal of the SNPs arrays. Cicconardi et al. (2013) applied the two most commonly used algorithms for the identification of CNVs, those implemented in QuantiSNP software (COLELLA et al., 2013) and PennCNV (WANG et al., 2008) to investigate the presence of CNVs in the genome of five *Bos taurus* breeds.

CNVs have been shown to be important in explaining the phenotypic variability of productive characteristics as well as the susceptibility to diseases. Several CNV maps for cattle were constructed using different methods (FADISTA, et al., 2010; BAE et al., 2010; DA SILVA et al., 2016). In these studies CNVs associated with genes have been shown to influence immune functions that affect receptor activities, signal transduction and transcription. The knowledge of these CNVs should improve the understanding of the genetic and physiological mechanisms that contribute to the productive characteristics, as well as the selection of more productive and efficient animals, contributing to the genetic improvement of the productive characteristics.

1.3 CNV studies

Knowledge of the abundance and distribution of CNVs and their association with phenotypes is of great interest. However, until now, a few studies have been investigated for CNVs in cattle (IBEAGHA-AWEMU, et al., 2008, BICKHART et al., 2012, ZHOU et al., 2016; DA SILVA et al., 2016). Thus, little is known about how CNVs contribute to a phenotypic variation of characteristics of economic importance in cattle, in humans, and other model organisms. Some studies have identified regions of CNVs no bovine genome. In this sense, Liu et al. (2008) used three Holstein bulls and identified 25 CNVs through comparative genomic hybridization arrangements (CGH array). A broader systematic review of CNV was reported in 90 CGH cattle, where more than 200 CNVs were identified (LIU et al., 2010). Fadista et al. (2010) reported 304 CNV regions on 20 animals of four bovine breeds using comparative genomic hybridization arrangements (CGH array). In addition to

experiments on CGH arrangements, new evidence of CNV no bovine genome emerged from the results of genotyping, where from bovine HapMap Consortium samples (more than 500 animals of various bovine breeds), where 79 candidate deletions were identified (Matukumalli et al., 2009).

Several strategies to test the association of CNVs with phenotype variation have been proposed, however each strategy can be divided into two phases (BREHENY et al., 2012). In the first phase, called the CNV test or phase I (CNV calling), is to estimate the number of copies present in all segments of the genome for each individual. Several studies in the literature (WILLENBROCK et al., 2005; LAI et al., 2005; MARENNE et al., 2011; PINTO et al., 2011) compared methods of CNV calling. Already, in phase II is to perform a test of genetic association in each segment for which there is variability in the number of copies.

The association studies with CNVs seek to identify associations between phenotype and genetic variation, in the form of changes in the number of copies. In humans, studies have identified associations between variation in copy numbers and various diseases, including Crohn's disease, psoriasis, schizophrenia and autism (FELLERMANN et al., 2006; HOLLOX et al., 2008; SEBAT et al., 2007). Walsh et al., 2008; MORENO et al., 2011). However, such studies in cattle and other species of domestic animals are still rare (HOU et al., 2012a, 2012b; BERTON et al., 2014, LEMOS et al., 2015a, DA SILVA et al., 2016). Recently, Zhou et al., (2016) in a genome-wide association study (GWAS) for CNV and body traits in beef cattle, identified 231 CNVs with high confidence and 17 of them were significantly associated with seven moderately or highly correlated growth traits.

1.4 Fatty acid composition influencing human health and meat quality

The fatty acids (FAs) composition in beef cattle production system has been studied because of its implications for human health and the traits associated with meat quality. There has been interest in to manipulate the fatty acid composition of meat because it has high nutritional value from children to seniors, is a rich source of protein, iron, zinc, complex B vitamins and essential polyunsaturated fatty acids such as linoleic (C18:2), linolenic acid (C18:3) and arachidonic (C20:4; MCNEILL and VAN ELSWYK, 2012). However, meat also is source of fat in the diet and the presence of

cholesterol, low concentration of polyunsaturated fatty acids, and high concentration of saturated fatty acids have been associated with coronary heart disease, diabetes, obesity, and cancer, as well as the ratio of n-6:n-3 polyunsaturated fatty acids, especially in the formation of blood clots leading to a heart attack (ENSER, 2001; JAKOBSEN et al., 2008).

The nutritional properties of meat are largely related to its fat content and its fatty acid composition (WOOD et al., 2004). Different muscles differ in fat content and may also differ in fatty acid composition, which differs between various tissues, including intra- and intermuscular, as well as abdominal and subcutaneous adipose tissue (MARMER et al., 1984; WEBB et al., 1998). Moreover, genetic and environmental factors can influence the fatty acid composition of the meat (DE SMET, 2004; WOOD et al., 2008; DAL BOSCO et al., 2014). Differences due to the crossing of breeds and between animals within breeds, species, breeds or lines can change the fatty acid composition of the meat (FISHER et al., 2000). But generally, the nature and level of deposit of fatty acids in the muscle depends on the diet, ingestion, intestinal absorption, hepatic metabolism, and lipid transportation (GEAY et al., 2001). Fatty acids composition can influence the meat quality in the fat tissue firmness (hardness), due to the different melting points of the fatty acids; shelf life (lipid and pigment oxidation) due to the propensity of unsaturated fatty acids to oxidize, leading to the development of rancidity and changing the color, flavor due to the production of volatile, odorous, lipid oxidation products during cooking and the involvement of these with Maillard reaction products; and aromas (GANDEMER et al., 1999; CAMPO et al., 2003).

WOOD et al. (2008) showed that beef has, on average, 50% of saturated (SFA), 40% of monounsaturated (MUFA) and 10% of polyunsaturated fatty acids (PUFA). However, in ruminants, linoleic acid (C18:2 n–6) and α-linolenic acid (C18:3 n-3), which are present in many concentrate feed ingredients, are degraded into monounsaturated and saturated fatty acids in the rumen by microbial biohydrogenation and only a small proportion (around 10% of dietary consumption) is available for incorporation into tissue lipids. In addition to their important role is work together to regulate immune responses and anti-inflammatory processes. Linolenic acid is also associated with the reduction of coronary diseases and plasma

cholesterol, and also has anticancer properties. The consumption of saturated fatty acids is associated with an increase in serum cholesterol levels and the risk of coronary heart disease. Especially lauric, myristic and palmitic fatty acids are responsible for increasing plasma total and LDL cholesterol concentrations and palmitic acid (C16:0) has the most impact on cholesterol levels, because it raises the levels of LDL.

Long-chain polyunsaturated fatty acids of the omega 3 (n-3) family also are present in the meat, such as eicosapentaenoic, docosapentaenoic and docosahexaenoic acids (C22: 6n-3). Eicosapentaenoic acid acts by relaxing the blood vessels and preventing the formation of blood clots. Arachidonic acid, resulting from omega 6 (n-6) metabolism, leads to constriction of the vessels and formation of blood clots. Although they perform opposite functions, both are necessary for the maintenance of the balance of the organism. Moreover, prostaglandins are lipid autacoids derived from arachidonic acid. They both sustain homeostatic functions and mediate pathogenic mechanisms, including the inflammatory response (RICCIOTTI, 2011). Therefore, an omega 6/omega 3 ratio of less than four is recommended. The bovine meat analysis has verified values of the omega 6/omega 3 ratio between 1.5 and 10.4, and the lowest values were found in the meat of cattle raised in pasture. Disorders that have been suggested to be linked with lack of omega-3 PUFA include hypertension, inflammatory and immune disorders, depression and neurological dysfunction. Repeatedly, there are a lot of dietary recommendations to reduce the consumption of saturated fatty acids, such as prevention of cardiovascular diseases. On the other hand, some studies have shown beneficial effects of polyunsaturated fatty acids, mainly the n-3 family, CLA, docosahexaenoic acid and docosapentaenoic acid on the level of serum lipids and their antithrombotic action on platelets and protection against some diseases (SIMOPOULOS, 2000; JOHNSON and BRADFORD, 2014; JOHNSON & CHASTITY, 2014). Studies also indicate that stearic acid (C18:0) has been shown not to increase total cholesterol or LDL-cholesterol concentrations, and slightly changes serum cholesterol levels in humans, however it is poorly stored in tissues (Bonanome et al., 1988).

To attend the need of good human health, it is necessary to produce meat with a higher ratio of polyunsaturated to saturated fatty acids and a more favorable balance between n-6 and n-3 PUFA. The ratio of n-6:n-3 PUFA is particularly beneficial in cattle, especially from animals that have consumed grass which contains high levels of 18:3 acid. Dietary intake of PUFA from the n-3 series and especially from the n-6 series by the animals favour the production of conjugated isomers of linoleic acid (CLA c9 t11), such as C18:2 cis-9 trans-11 (FRENCH et al., 2000) which are synthesized in the rumen as a result of biohydrogenation of fatty acids, performed by microrganisms (TAMMINGA and DOREAU, 1991). Some of these fats, such as CLA (conjugated linoleic acid), could be beneficial to human health. CLA is important in the prevention of specific cancers and in the treatment of obesity, immune functions, and potential beneficial effects on coronary heart disease (DUGA et al., 2011; IP et al., 1995.)

In relation to diet, fatty acid composition of concentrate and forage diets are different and lead to different fatty acid compositions in tissues. The presence of the rumen makes fatty acid composition in beef more difficult to manipulate by changing diet, but studies showed that the C18:3 acid, n-3 PUFA concentrations, lipid oxidation, color, and aromas were affected by feeding treatments (CAMPO et al., 2003; VATANSEVER et al., 2000). Some data demonstrated the feasibility of reducing population cholesterol levels through strategies involving alteration of fat quality within the agricultural and food manufacturing chains. Ruminants consuming fresh pasture, in general, have higher content of unsaturated fatty acid in their meat that those receiving a grain-based concentrate diet. Grass lipids contain high proportions of the unsaturated linolenic acid (C18:3 n−3) and the only way to improve the ratio of PUFA in ruminant meats is by preventing ruminal biohydrogenation or by feeding protected PUFA supplements (SCOLLAN et al., 2003). For all these reasons, there is an increase interesting in research intended to modify the fatty acid composition in meat, especially reducing the concentration of SFA and increasing PUFA.

1.5 Meat fatty acids profile variation between and within beef cattle breeds

The genetic variability is characterized as the differences among species, differences between breeds or lines, differences due to the crossing of breeds and differences between animals within breeds. The latter source of variation is estimated by heritabilities and genetic correlations. Breed effects may be influenced by the segregation of major genes, of which the double-muscled gene in cattle is well known example (DE SMET et al., 2004). The major factors that influence the fatty acid composition of beef are age of animal, diet, and breed type (WOOD et al., 2008).

Several studies have demonstrated that adipose tissues from *Bos indicus* cattle breeds are less saturated when compared to *Bos taurus* (HUERTA-LEIDENZ et al., 1993; 1996; PERRY et al., 1998; MENEZES et al., 2009; ROSSATO et al., 2010). In this sense, Rossato et al. (2010), pointed out that Nellore beef is nutritionally healthier than Angus breed, once it has lower percentages of cholesterol and higher amounts of n-3 fatty acids, CLA precursor (C18:1 *trans*). Bressan et al. (2011) showed that the production system has an important influence on beef fatty acid profile when compared animals from *Bos taurus* and *Bos indicus* breeds. These authors reported that *Bos taurus* animals had lower percentage of saturated fatty acids (SFA) and higher percentage for monounsaturated fatty acids (MUFA) in relationship to *Bos indicus* animals in feedlot finishing system. According to these authors, *Bos taurus* cattle finished under feedlot conditions have higher ability to desaturate SFA than *Bos indicus* cattle.

Recently, Lemos et al. (2016) realized a study to identify regions associated with saturated, mono and polyunsaturated and n-6 to n-3 ratios, in the *longissimus* thoracis muscle from confined Nellore, using the single-step method. The individual fatty acids with the highest concentration in the intramuscular fat of *longissimus thoracis* found by theses authors were C16:0, C18:1 cis-9, C18:1 trans-11, and C18:0, representing 67.3% of its fat composition. These results are in agreement with those reported by some authors (PRADO et al., 2003; LAWRIE, 2005; KELLY et al., 2013; CESAR et al., 2014) who observed high levels of palmitic, stearic and oleic FAs. Some authors (LAWRIE, 2005; ROSSATO et al., 2009) also reported that palmitic fatty acid was the predominant FA in beef fat. In Nellore finished in feedlot

(CESAR et al., 2014), oleic acid (37.46%) displayed the highest concentration in intramuscular fat. The myristic and palmitic FAs are associated with an increase in circulating LDL cholesterol due to interference with hepatic LDL receptors (Woollett et al., 1992). The saturated fatty acids were predominant, followed by the MUFAs and PUFAs. Similar results (PRADO et al., 2013) were reported for Nellore cattle, 43.93% (SFA), 42.33% (MUFA) and 12.8% (PUFA). However, studies using taurine (PITCHFORD et al., 2002) and Nellore (CESAR et al., 2014) breeds found similar concentrations for SFA and MUFA, 47% and 47.5%; and 47.23%, and 48.34%, respectively.

Information on genetic parameters for carcass and meat traits, fatty acid composition and genetic-quantitative relationships between these traits is essential to improve meat tenderness and the proportion of fat in the carcass, without harming the fat composition in livestock production. On this concern, some studies have been done to estimate these parameters. In these sense, Feitosa et al. (2016), studied the genetic-quantitative relationships between the beef fatty acid profile with the carcass and meat traits of Nellore cattle, used a total of 1826 bulls finished in feedlot conditions, to analyze the following carcass and meat traits: subcutaneous fat thickness (BF), shear force (SF) and total intramuscular fat (IMF). The fatty acid (FA) profile of the *longissimus thoracis* samples was determined. These authors estimated the heritability for individual saturated fatty acids (SFA), which varied from 0.06 to 0.65, for monounsaturated fatty acids (MUFA) it varied from 0.02 to 0.14 and for polyunsaturated fatty acids (PUFA) it ranged from 0.05 to 0.68. The heritability estimates for Omega 3, Omega 6, SFA, MUFA and PUFA sum were low to moderate, varying from 0.09 to 0.20. The carcass and meat traits, SF (0.06) and IMF (0.07), had low heritability estimates, while BF (0.17) was moderate. The genetic correlation estimates between SFA sum, MUFA sum and PUFA sum with BF were 0.04, 0.64 and −0.41, respectively. Similarly, the genetic correlation estimates between SFA sum, MUFA sum and PUFA sum with SF were 0.29, −0.06 and −0.04, respectively. The genetic correlation estimates between SFA sum, MUFA sum and PUFA sum with IMF were 0.24, 0.90 and −0.67, respectively.

Aboujaoud et al., (2016) in a study to determine genetic parameters for fatty acids in intramuscular fat from feedlot-finished Nellore carcasses, they estimated

heritability for individual FAs ranged from 0.01 to 0.35. The heritability estimates for myristic (0.25 ± 0.09), palmitic (0.18 ± 0.07), oleic (0.28 ± 0.09), linoleic (0.16 ± 0.06) and α-linolenic (0.35 ± 0.10) FAs were moderate. Stearic, elaidic, palmitoleic, vaccenic, conjugated linoleic acid, docosahexanoic, eicosatrienoic and arachidonic FAs had heritability estimates below 0.15. Heritability estimates for beef fatty acids were also estimated by Cesar et al. (2014), in a study with Nellore breed. The estimates varied from low (<0.10 for C12:0, C16:0, C18:1 cis-11, C18:1 cis-12, C18:2 cis-9 trans-11, C20:1, C20:3 n-6, C20:4 n-6, C20:5 n-3 and AI, respectively) to moderate (up to 0.29 for IMF, C14:0, C14:1 cis-9, C16:1 cis-9, C17:0, C17:1, C18:0, C18:1 cis-9, C18:1 trans-6, 7, 8, C18:1 trans-10, 11, 12, C18:2 cis 9 cis- 12 n-6, C18:2 trans-11 cis-15, C18:3 n-6, C18:3 n-3, C22:5 n-3, C22:6 n-3, SFA, MUFA, PUFA, Sn-3, Sn-6 and n-6:n- 3). For C15:0, C18:1 cis-13, C18:1 cis-15, C18:1 trans-16, C20:2 the heritability estimates were zero. Differently of these authors, and working with taurine breed, Tait et al. (2007) (Angus) and Nogi et al. (2011) (Japanese Black cattle) found the estimates of heritability for IMF fat deposition and composition traits, higher when compared with the results above. The lower values of heritability reported for the populations of some studies, could be explained by the reduced sample size (CASAS et al., 2011) or lower amount of genetic variation in the population (CASAS et al., 2013).

Comparing these values with another study that was accomplished with a great number of animals (LEMOS et al., 2016), the estimate of linolenic FA heritability, for example, was similar to that found by Cesar et al. (2014) (0.13) and lower than that reported by Nogi et al. (2011) (0.58). However, higher estimates have been reported for linolenic acid in other studies (0.21) (TAIT et al., 2007), and also for palmitoleic acid (0.15) (CESAR et al., 2014) and (0.49) (SAATCHI et al., 2013). Higher heritability estimates were reported for linoleic FA, 0.34 and 0.58, respectively, in the intramuscular fat of Japanese Black cattle, suggesting that genetic influence on linoleic acid varies among breeds (NOGI et al., 2011; INOUE et al., 2011). Recently, authors also estimated high heritability for SFA (0.54) and MUFA (0.54) and, therefore, concluded that there is sufficient genetic variation in the fatty acid profile of cattle subcutaneous fat to respond to selection (KELLY et al., 2013). Therefore, these results suggest that it is possible to change the beef lipid

composition of intramuscular fat of different cattle breeds' through selection. This information is important for breeding programs that aim at improving the beef fatty acid composition.

1.6 Genetic markers and metabolic pathways associated with meat fatty acids profile

The fatty acid metabolism is a complex process, which includes lipolysis of dietary fat and biohydrogenation in the rumen, *de novo* synthesis of fatty acids by rumen bacteria, absorption and transport of fatty acids by the host animal, *de novo* synthesis in tissues host, elongation and desaturation in animal tissues, hydrolysis of triglycerides and esterification, oxidation of fatty acids or metabolism to other components (BAUCHART, 1993; CHILLIARD, 1993; JENKINS, 1993, LATIOTIS et al., 2010; EKINE-DZIVENU et al., 2014).

In ruminants, the fatty acid synthesis occurs mainly in the adipose tissue, except during the lactation, when the mammary gland becomes the predominant organ (VERNON and FLINT, 1983). The main point about control of the fatty acids synthesis is the acetyl-CoA carboxylase, and it seems that the endocrine control is very similar in, at least, adipose tissue (insulin activation, inhibition of catecholamine) of ruminants and non-ruminants (VERNON and FLINT, 1988).

Acetate is the principal precursor of fatty acid synthesis in ruminants, and must be converted to acetyl-CoA by the action of acetyl-CoA synthetase and then incorporated into fatty acids. The adipose tissue is largely responsible for the conversion of acetate into acetyl-CoA, and consequently, the greatest synthesizer of fatty acids in ruminants (POLIZEL NETO et al., 2008). Some studies have been carried out to evaluate gene expression pattern in cattle for fatty acid composition and also identified genomic regions and metabolic pathways involved in those process, aiming to improve the beef fatty acid profile. In this sense, Berton et al. (2016) studied the gene expression profile in Nellore cattle with extreme phenotypes for intramuscular fatty acid composition, found the *ACSM3* (acyl-CoA synthetase medium-chain family member 3) gene as differentially expressed for linoleic, monounsaturated, polyunsaturated, saturated and omega-3 acids, participates in the

metabolism of lipids and in metabolic pathways that involves the precursor acetyl-CoA metabolism. Also, the *ACSS1* (acyl-CoA synthetase short-chain family member 1) gene acts in the transformation of acetyl-CoA into fatty acids, through chemical reactions and metabolic pathways involving acetyl-CoA, being differentially expressed ($q < 0.05$), for saturated fatty acids such as palmitic, stearic, oleic and total saturated acids.

Some studies has been realized in attempt do identify and describe the genes which plays this important role on the beef fatty acids metabolic pathways**.** In a previously study Lemos et al. (2016) found several nearby areas of major QTL associated with groups of saturated, monounsaturated, and polyunsaturated fatty acids, in Nellore meat. These regions found harbor interesting PCG (putative candidate genes), which are involved in lipid metabolism, as a constituent of cell membranes, receptors for reproductive hormones, biosynthesis and hydrolysis of phospholipids and membrane constituents, synthesis of protein kinases, transport and use of fatty acids and cholesterol, energy metabolism, elongation factors and synthesis of long-chain fatty acids in different species. Among the many genes identified, the *ELOVL5* gene located on chromosome BTA23 at 25 Mb and associated with the C20:4 n-6 (arachidonic acid) is highlighted. The genes responsible for the elongation of very-long-chain fatty acid (*ELOVL*) encode enzymes that play an important role in the elongation of long-chain fatty acids. The fatty acid synthesis involves a number of enzymes, such as fatty acid synthase (*FASN*), which is located on chromosome BTA19 between 51,384,922 and 51,403,614 bp, while its variations have been related to fatty acid composition of Angus beef (Zhang et al., 2008). In mammals, *FASN* synthesizes the fatty acids that contain up to 16 carbon atoms, and the genes of the *ELOVLs* group produce long-chain fatty acids with 18 carbon atoms or more (JAKOBSON et al., 2006; BONET et al., 2011).

In additional, the authors employed the Ingenuity Pathway Analysis (IPA) online software to detect the canonical pathways involving the genes of the above study. None canonical pathway was significant (p-value <0.05). A large proportion of the pathways acted on fucose and cholesterol biosynthesis, and *PPARa* activation, which would provide valuable insights into explaining the molecular mechanism of lipid metabolism. As one of the pathways showed on canonical pathway, the

PPARalpha has a great role in the regulation in the fatty acids metabolism. Peroxisome proliferator-activated receptors (*PPARs*) are nuclear hormone receptors that are activated by fatty acids and their derivatives and play an essential physiological role in the regulation of adipocyte tissue development lipogenesis and skeletal muscle lipid metabolism (BERGER and MOLLER, 2002; HIHI et al., 2002; ABBOTT, 2009). Doran et al. (2014) studied GWAS in Holstein-Friesian cattle identified the PPAR signaling pathway as the most significantly overrepresented biological pathway involved in carcass trait performance, suggesting that *PPAR* would appear also play a key role in controlling carcass weight, carcass fat and carcass conformation traits.

Li et al. (2015) sampled spleen tissues from grass-fed and grain-fed Angus steers and performed a comparative study of gene expression using RNASeq method. Then, based on the differentially expressed genes (DEGs), they implemented a functional analysis and identified potential mechanisms that could contribute to the difference observed between both groups. The authors have detected 123 DEGs between grass-fed and grain-fed spleen of Angus cattle. In the grass-finished group, 87 were up-regulated while the other 36 decreased their gene activity. Based on these genes, they performed an Ingenuity Pathway Analysis (IPA) and identified 9 significant molecular networks and 13 enriched biological pathways. Two of the pathways, *Nur77* signaling in T lymphocytes and calcium-induced T lymphocyte apoptosis which are immune related, contain a pair of genes *HLA-DRA* and *NR4A1* with dramatically altered expression level.

In a recent study, Berton et al. (2016) analyzed the gene expression profile of intramuscular muscle in Nellore cattle with extreme values of fatty acid and identified several genes associated with fatty acid metabolism, such as those involved in intra and extra-cellular transport of fatty acid synthesis precursors in intramuscular fat of *Longissimus thoracis* muscle. The authors found some genes that plays an important whole on the fatty acids metabolic pathways, like: *CSM3* and *ACSS1* genes, which work as a precursor in fatty acid synthesis; *DGAT2* gene that acts in the deposition of saturated fat in the adipose tissue; *GPP* and *LPL* genes that support the synthesis of insulin, stimulating both the glucose synthesis and the amino acids entry into the cells; and the *BDH1* gene, which is responsible for the synthesis and degradation of

ketone bodies used in the synthesis of ATP.

1.7 Genomic selection and genome wide association studies for beef fatty acid composition

Marker assisted selection (MAS) is recommended to increase the annual genetic gain for traits of economic importance in several animal species (DEKKERS, 2004). In this kind of selection, molecular information from markers is used together with phenotypic data of production and pedigree to select the animals. This way, MAS provides possibility to improve difficult and/or high cost measurement traits, such as the meat fatty acids composition. Some studies in several countries have mainly used microsatellite as genetic marker to study the fatty acids composition in taurine breeds (ALEXANDER et al., 2007; ABE et al., 2008; TSHIPULISO et al., 2008; GUTIÉRREZ-GIL et al., 2010; MORRIS et al., 2010). However, genotype using microsatellite markers is expensive and just a small proportion of the total genetic variance can be show for the markers, limiting the progress or genetic gain (SOLBERG et al. 2008).

Sequencing of the bovine genome has led to the discovery of thousands of single nucleotide polymorphism (SNP) markers and subsets of SNPs that can characterize the bovine genome with a wider range and lower cost (MATUKUMALLI et al., 2009). In bovine, genome wide association studies (GWAS) and genomic selection have been done using high-density SNP chips, with thousands of genetic markers for traits related to milk or meat quality, as the fatty acid composition (REECY et al., 2010; UEMOTO et al., 2010; BOUWMAN et al., 2011).

In dairy cattle, Bouwman et al. (2011) studied the association among 50.000 SNPs and the content of saturated fatty acids (C4:0 - C18:0), monounsaturated (C10:1-C18:1) and polyunsaturated (C18:2cis9trans11-CLA), to identify genomic regions associated with individual fatty acids in bovine milk. The authors found 54 regions on 29 chromosomes that were significantly associated with one or more fatty acids. In beef cattle, studies involving genomic association or selection are scarce. Uemoto et al. (2010) found 32 SNPs located on the chromosome 19, associated with the amount of oleic acid (C18:1) in the intramuscular fat of the trapezius muscles in

Japanese Black cattle. The content of oleic acid is positively correlated with the sensorial quality of the meat (MELTON et al., 1982). In the study of Uemoto et al. (2010), the authors used the Illumina BovineSNP50 BeadChip and genotyped only 160 bovines (80 animals with higher values and 80 animals with lower values of oleic acid) selected from 3,356 animals based on corrected phenotype.

Another study with a significantly higher number of animals has been shown for Reecy et al. (2010). They used the BovineSNP50 beadchip (54 k) to genotype 2.285 Angus bulls to analyze the fatty acid concentration of the meat. Effects of SNPs on each trait were estimated using the Bayes C module, considering the probability of a SNP not influencing the trait (π) = 0.90. Depending on the fatty acid considered in the analysis, 2.3% to 48.5% of observed variance could be explained by an animal's 54K genotype. According to the authors, long chain fatty acids appear to be lowly heritable traits with a low proportion of variance accounted by markers, in relation to short chain fatty acids. They concluded 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 implementation of whole genome selection to improve fatty acid composition in meat. Similarly, Saatchi et al. (2013) found in other GWAS with 2,177 Angus cattle, using a 54 K genotyping panel, 57 genomic regions associated with the fatty acids profile trait in meat. The authors concluded that this large number of genomic regions might indicate the presence of an elaborate molecular mechanism that control fatty acid content in skeletal muscle.

The first genome wide association study involving intramuscular fat deposition and fatty acid composition in Nellore cattle (*Bos indicus*) was carried out by Cesar et al. (2014). The authors genotyped 386 Nellore steers using a BovineHD BeadChip (770 k) and used Bayesian methods (Bayes B) to identify genomic regions and putative candidate genes that could be involved with fatty acid composition in Nellore. The authors found eight genomic regions (1 Mb windows) for saturated fatty acids that explained more than 1% of genotypic variation for C12:0, C14:0, C16:0, and C18:0. Ten genomic regions for monounsaturated fatty acids, which relates C14:1 cis-9, C16:1 cis-9, C18:1 cis-9, and C18:1 trans-16. For polyunsaturated fatty acids, nine genomic regions which relates C18:2 cis-9 cis12 n-6, C18:2 trans-11 cis-15, C18:3 n-3, C18:3 n-6, C20:3 n-6, C20:5 n-3, and C22:5 n-3. They concluded that

intramuscular fat composition is affected by many loci with small effects and the identification of genomic regions associated to fatty acid composition can lead to selection to improve human nutrition and health.

Trying to identify regions of the genome associated with saturated, mono and polyunsaturated fatty acids, Lemos et al. (2016) genotyped 1,616 Nellore using the High-Density Bovine BeadChip (770 k) and the single-step method to perform the GWAS. The authors used the single step method because it allows to combine the information of genotyped and non-genotyped animals in the genetic evaluation process, expanding the scope and identification of potential regions associated with loci responsible for variations in the studied traits (KEMPER AND GODDARD, 2012). Interestingly, the results showed that a total of 31 genomic regions that explain more than 1 % of genotypic were found for total saturated fatty acids, C12:0, C14:0, C16:0 and C18:0; 37 genomic regions for monounsaturated fatty acids, C14:1, C16:1, C18:1 trans11, C18:1 cis9 and C18:1 trans9; and 40 genomic regions for the polyunsaturated fatty acids group, as: C20:4 n-6, C18:2 cis-9 cis12 n-6, C18:3 n-3, C22:6 n-3 and C20:3 n-6 cis-8 cis-11 cis-14. Additionally, a total 21 genomic regions accounted for more than 1 % of the genetic variance for n-3 and n-6 fatty acids, and the n-6:n-3 ratio. The authors could conclude that the identification of such regions and the respective candidate genes should contribute to improve the genetic knowledge regarding the fatty acids profile of Nellore cattle and help to improve the selection of such traits to favour human health.

Some authors have been testing different methodologies to predict the direct genomic value for many traits in livestock production, such as SNP-BLUP (Single Nucleotide Polymorphisms-Best Linear Unbiased Predictor) which assumes a normal distribution for SNP effect and common variance for all markers (MEUWISSEN et al., 2001; HAYES and DAETWYLER, 2013); the LASSO (Least Absolute Shrinkage and Selection Operator) assumes a double exponential distribution for the SNPs effect (PARK and CASELLA, 2008; DE LOS CAMPOS et al., 2009); and Bayesian methods - BayesC and BayesCπ, which considered a variable with binomial distribution that reports whether a marker (SNP) has (1) or not (0) effect on the trait under study, with π variable probability to be zero and a normal distribution with probability 1-π, assuming that part of the effect markers follows a normal distribution. These methods

differ in the assumptions about the genetic model associated with quantitative traits, and the best method is the one that reflects the biological nature of polygenic traits, in terms of genic effects (RESENDE et al., 2008).

In this sense, studying an Angus population, Saatchi et al. (2013) concluded that genomic selection for beef FA profile using Bayesian models is feasible. Moreover, Onogi et al. (2015) evaluated the predictive ability of genomic selection in FA composition of Japanese Black cattle, using single-step genomic best linear unbiased method. Recently, Chiaia et al. (2016) evaluated the genomic predictability for beef fatty profile in Nellore breed and concluded that genomic information can assist in improving FA profile in Zebu animals, since the use of genomic information yielded genomic values for FA profile with accuracies ranging from low to moderate. The authors also concluded that the none of the evaluated methods (SNP-BLUP, Bayesian Lasso, BayesC, and BayesCπ) excelled in terms of accuracy, however, the SNP-BLUP method allows obtaining less biased genomic evaluations, thereby; this method is more feasible when taking account the computational cost. The genomic selection has potential to increase the genetic gain for hard measure traits, like the FA profile, however, the most suitable model to evaluate those traits are still being studied. The divergence between studies suggests that the difference within the methods is due to the genetic architecture of the trait i.e., the accuracy tends to increase as the model adjusts itself to the genetic architecture of the trait (LUND et al., 2009). For traits that are affected by moderate to major genes effect, higher accuracies can be reached through Bayesian methods (NEVES et al., 2014). Traits that are controlled by many genes with small effects, polygenic trait, the SNP-BLUP method showed better prediction ability (CLARK et al., 2011).

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CHAPTER 2 - Copy number variation regions in indicine cattle: evidences of environment adaptation

ABSTRACT: The aim of the present study was to analyze the distribution of CNV and CNV regions across Nellore cattle genome. A total of 399,361 CNVs were identified in 3,794 Nellore animals genotyped with Bovine HD BeadChip (770k) array using PennCNV algorithm. The *default* quality control was applied and 2,902 samples and 195,873 CNVs remained. The medium CNV length size was 54,744 kb with a maximum of 8.7 Mb and a minimum of 3 kb. The CNV regions (CNVRs) were generated by overlapping the 195,873 CNVs identified using CNVRuler program. There was a higher incidence of CNVRs at BTA19 (24.26%), BTA23 (18.68%) and BTA25 (18.05%). The chromosomes that showed lower incidence of CNV regions were BTA29 (1.63%), BTA13 (9.72%) and BTA8 (9.72%). According to the type, 38.5%, 28.5% and 33.0% of the CNVRs were characterized as insertion, deletion and mixed (insertion and deletion in the same region), respectively. The 9,805 CNV regions estimated in the present study coverage approximately 13.05% of the cattle genome (UMD_3.1, 2,649,685,063 bp) and overlapped with 5,495 known genes which are related with biological processes that might be involved in the environmental adaptation of the subspecies to tropical areas, such as regulation of vasodilatation, immune system response, hair follicle morphogenesis, among others. This study confirms the existence of large structural variations in the genome of Nellore cattle and contributes to understand differences between cattle subspecies, as well as, guidance for future studies with structural variations were presented.
Keywords: CNVR, natural selection, structural variation, Nellore, sub-speciation

1. INTRODUCTION

The discovery of DNA variations that directly influence quantitative traits is important for beef cattle genetics and production. The high-throughput sequencing approaches resulted in the identification of thousands of SNP (Single Nucleotide Polymorphisms) markers. They assumed a central role over the last years, since it allows multiple applications at a relatively low cost (MATUKUMALLI et al., 2009). The focus of the scientific community is the direct influence of SNPs to assess genetic variation in cattle. However, other types of DNA variations may contribute to a wide range of different phenotypes (REDON et al., 2006).

A structural genomic variation named copy number variation (CNV) is defined as a DNA segment with 1 kb or more in length which presents a variable number of

copies when compared to the reference genome (FEUK et al., 2006), due to insertions, deletions, translocations or inversions (SCHERER et al., 2007).

Like SNPs, the copy number variation (CNV) also represent polymorphic variants present in >1% of the population. Though, based on the number and size of duplications and deletions, the CNVs can influence genetic and phenotypic diversity (ZHANG et al., 2009). Many studies have demonstrated the importance of the CNVs in explaining phenotypic variability of several traits in domestic animals, such as cattle (LIU et al., 2008; SEROUSSI et al., 2010; 2012a,b; CICCONARDI et al., 2013;), horses (ROSENGREN-PIELBERG et al., 2008), pigs (PAUDEL, et al., 2013; PAUDEL et al., 2015), sheep (FONTANESI et al., 2012; LIU et al., 2013;), chickens (CROOIJMANS et al., 2013; WANG et al., 2010) and dogs (ALVAREZ and AKEY, 2012; SALMON HILLBERTZ et al. 2007).

Several CNV maps in cattle were built using different methods such as comparative genomic hybridization arrays (aCGH); single nucleotide polymorphism genotyping arrays (SNP chip); and high-throughput next-generation sequencing (NGS) (LIU et al., 2010; HOU et al., 2011b; BICKHART et al., 2012).

Nellore cattle breed belongs to *Bos taurus indicus* subspecies (zebu) and it is adapted to tropical conditions (MADALENA et al., 1990; HAMMOND et al., 1996). Nellore and its crossbreds are widely used in beef cattle production, especially in Brazil (PRAKASH; STIGLER, 2012; OLIVEIRA et al., 2014). Due to the importance of the Nellore breed and considering the lack of genomic characterization studies, the aim of the present study was to analyze the distribution of CNV and CNV regions across Nellore cattle genome. In addition, the investigation of variability is the first step that will allow the future analysis as well as the incorporation of this tool into programs of genetic improvement of Nellore cattle.

2. MATERIAL AND METHODS

Samples

A total of 3,794 Nellore animals (1,759 bulls and 2,035 dams) were used in the present study. They were raised in 10 farms located in the Southeast, Northeast and Midwest regions of Brazil. They belong to three breeding programs (CRV-Paint, DeltaG and Nellore Qualitas) in which animals were selected for growth, finishing and

sexual precocity. This study was approved by ethics committee of the School of Agricultural and Veterinarian Sciences, Sao Paulo State University (UNESP).

CNV and CNVR detection

The animals were genotyped using the Bovine HD BeadChip (High-Density Bovine Bead Chip - Illumina) with 777,962 SNP markers. The identification of CNVs was carried out with the SNP data file from GenomeStudio 1.0 software. The sex chromosomes were excluded from the analysis. For CNVs detection, PennCNV algorithm (WANG et al., 2007) was used, which incorporates multiple sources of information and it is based on a hidden Markov model for CNV detection from the high-density genotyping data. The PennCNV is the most commonly used algorithm for CNV studies, since it presents low rate of false positives.

Initially the PennCNV algorithm was used without any application of quality control parameters to obtain the number of CNVs in all data. After that, in order to obtain the quality of the results, the *default* PennCNV's quality control was applied, eliminating samples with standard deviation for LRR (log R ratio) > 0.30; BAF (frequency of allele B) > 0.05 and value of the waves factor above of 0.01 (Liu et al., 2013).

The CNV regions (CNVRs) were generated by CNVs overlapping between samples using CNVRuler program (KIM et al., 2012). Genomic areas with density below 10% were excluded ("recurrence 0.1"). The recurrence trims the CNVR based on its frequency was performed to avoid false positive predictions. It defines more robust limits of the start and end regions. The "Gain/Loss separated regions" option was applied, to evaluate the type of event (gain or loss) in each region. Overlapping "gain" and "loss" CNVRs were merged into single regions to account for genomic regions in which both events can occur ("mixed" CNVRs). To evaluate the location of the CNVR, the Ensembl Biomart tool using version UMD 3.1 genome assembly.

Gene annotation and functional enrichment

Gene content of each cattle CNVR, were screened by Ensembl VEP tool (*Bos taurus* UMD3.1). The genes overlapping CNVRs were subjected to an enrichment of gene ontology terms (GO) with DAVID (Huang; Sherman; Lempicki, 2009). The

enrichment analysis was carried out for the subset of regions in which CNVs occurred frequently (per definition in >1% of the population, region of frequent CNV).

3. RESULTS

CNV and CNVR Discovery

We performed CNV calling on the UMD3.1 assembly using PennCNV in 3,794 samples (animals) and a total of 399,361 CNVs were identified. After the default quality control filtering, a total of 2,902 animals were kept for subsequent analyzes. A total of 195,873 CNVs were detected with an average length size of 54,744 kb, where 8.7 Mb was the maximum and 3 kb was the minimum length. The highest frequency CNV size class of this study was between 10 and 30 kb (Figure 1).

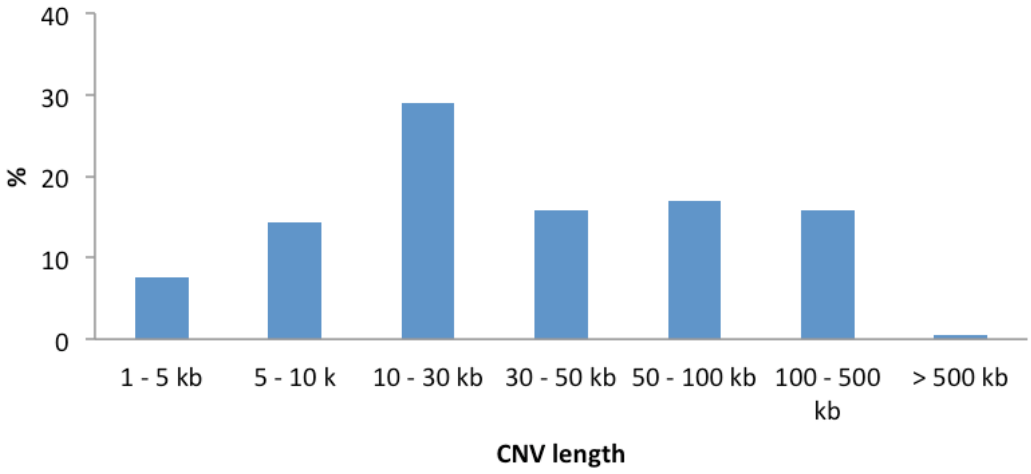


Figure 1 – Percentage of distribution of CNVs across Nellore genome, expressed in kilobase pairs.

Samples of CNVs obtained in this study showed a total of 9,805 CNVR scattered in all 29 chromosomes of the animal population under study. These CNVR correspond approximately 13.05% of the total length of the bovine genome (UMD_3.1, 2.649.685.063 pb) (Table 1).

Table 1 – CNVR distribution, coverage size and coverage percentage of chromosomes by CNVR detected across Nellore genome.

Chromosome	Chromosome	Number of	CNVR mean	CNVR	%

	size (bp)	CNVR	size (bp)	Coverage (bp)	Coverage
1	158,337,067	732	31,496.55	23,055,476	14.56
2	137,060,424	584	33,764.03	19,718,197	14.39
3	121,430,405	559	29,669.44	15,395,899	12.68
4	120,829,699	515	29,891.89	15,394,327	12.74
5	121,191,424	477	39,081.58	18,641,916	15.38
6	119,458,736	475	34,775.71	16,585,218	13.88
7	112,638,659	469	32,827.07	16,518,465	14.67
8	113,384,836	435	32,851.60	10,701,231	9.4
9	105,708,250	387	33,408.40	14,290,463	13.52
10	104,305,016	385	38,737.20	13,107,356	12.57
11	107,310,763	371	28,844.28	14,913,824	13.90
12	91,163,125	355	31,988.35	11,355,867	12.46
13	84,240,350	352	37,525.45	7,782,113	9.24
14	84,648,390	335	33,898.29	11,355,929	13.42
15	85,296,676	316	35,129.38	12,929,053	15.16
16	81,724,687	278	36,158.40	11,100,887	13.58
17	75,158,596	278	32,282.02	10,052,037	13.37
18	66,004,023	272	53,521.74	10,949,639	16.59
19	64,057,457	268	36,134.05	14,557,915	22.73
20	72,042,655	267	41,009.88	8,947,404	12.42
21	71,599,096	226	34,434.12	9,683,926	13.53
22	61,435,874	226	41,343.97	8,128,130	13.23
23	52,530,062	194	32,929.14	9,343,739	17.79
24	62,714,930	194	37,253.85	9,388,255	14.97
25	42,904,170	193	42,114.66	7,222,553	16.83
26	51,681,464	188	34,903.89	7,227,248	13.98
27	45,407,902	177	46,287.58	5,736,358	12.63
28	46,312,546	171	33,545.95	6,561,933	14.17
29	51,505,224	126	57,321.84	819,203	1.59

The CNVRs mean length size was 3,528,473pb, ranging from the 520 pb to 1,476,546 pb. The chromosomes that showed higher rates of CNVRs coverage were the BTA19 (22.73%), BTA23 (17.79%) e BTA25 (16.83%). The pairs of chromosomes that had a lower CNVR coverage were the BTA29 (1.59%), BTA13 (9.24%) and BTA8 (9.4%) (Figure 2).

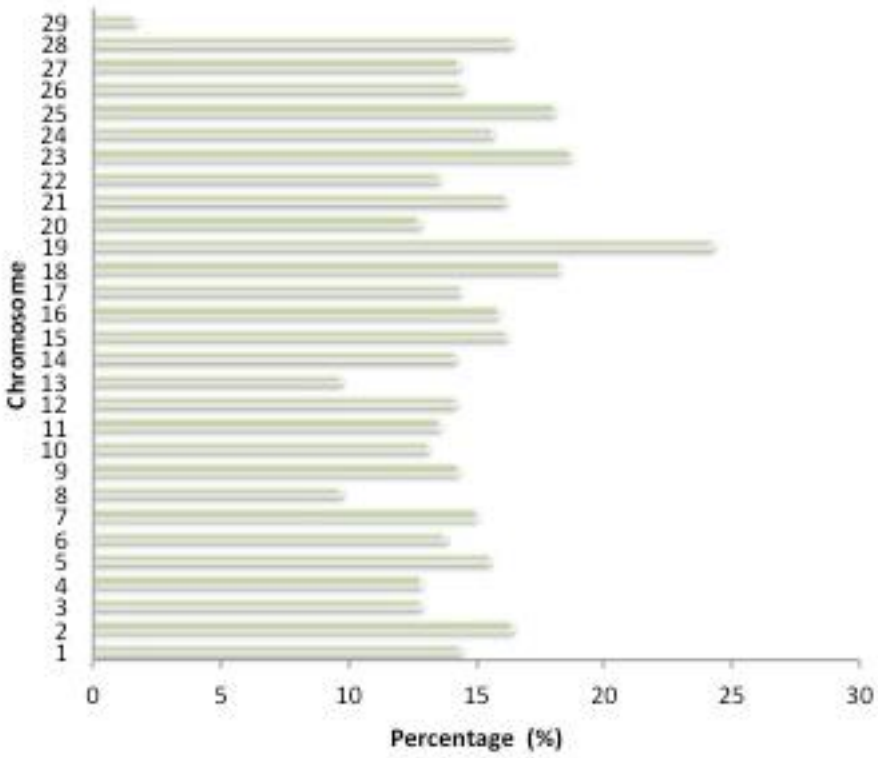


Figure 2 – Percentage of CNVR coverage by autosome chromosome.

From the 9,805 CNVRs obtained, 38.5% of these were characterized as insertion, 28.5% were deletion and 33.0% showed both events in the same region (insertion and deletion). From this total, the BTA28 chromosome showed the highest percentage of insertion (47.87%); on the other hand, the BTA12 and BTA19 chromosomes showed higher percentages of deletion and both events, respectively (Figure 3).

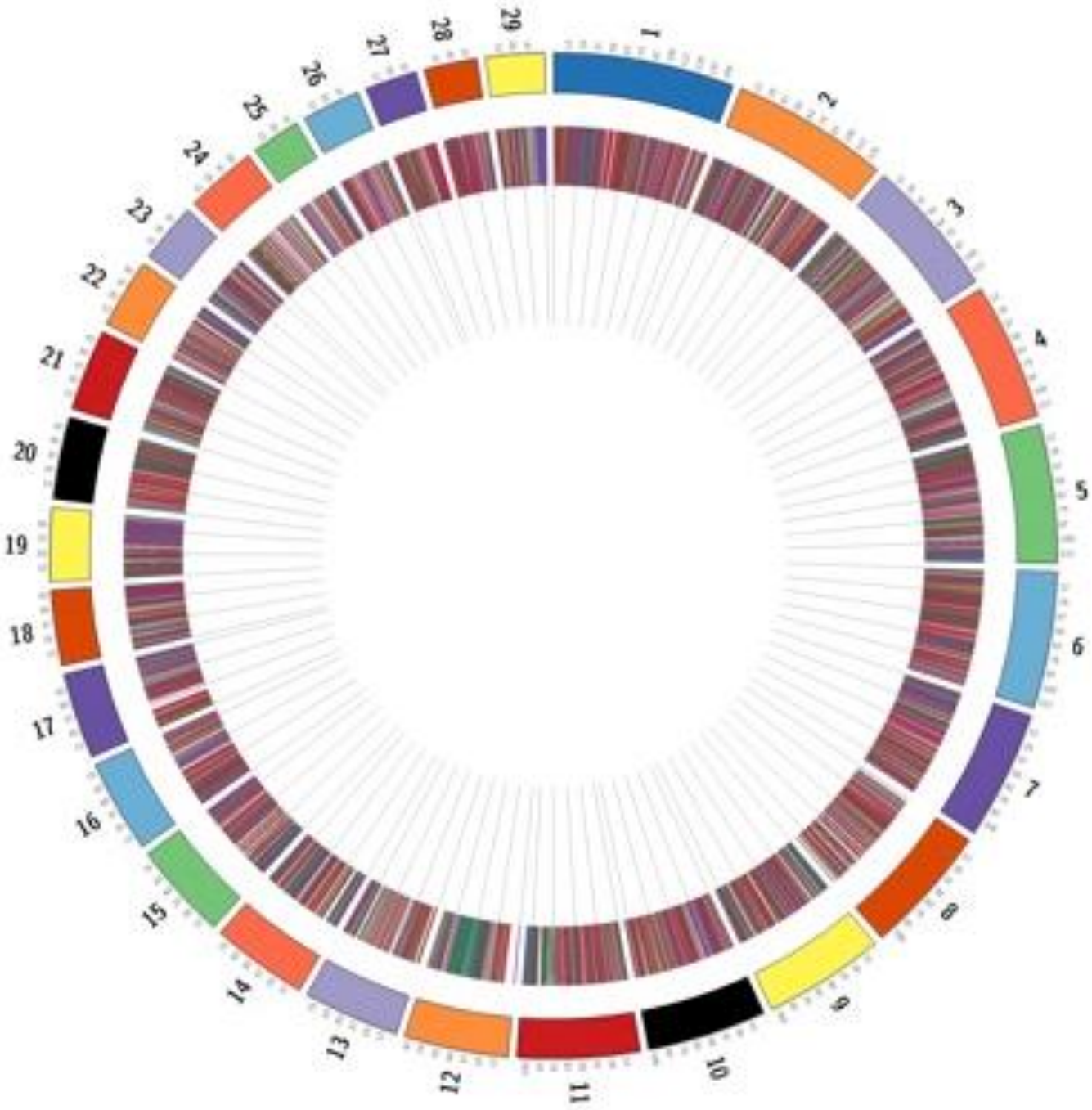
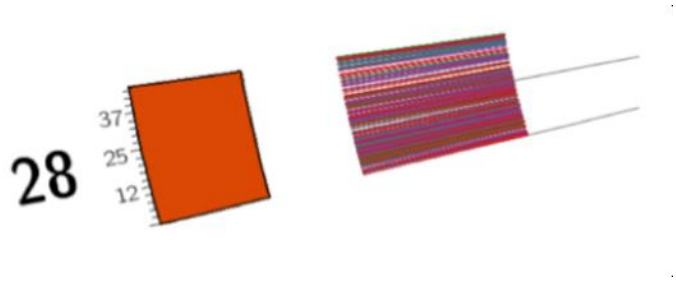
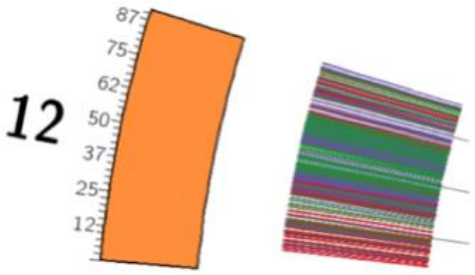


Figure 3 – **Comprehensive circular map of autosomal copy-number variants in *Bos indicus*** generated by Circos software**.** From the outside to the inside of the external circle: Chromosome name; genomic location (in Megabases); bars depicting the CNV regions (loss in green, gain in red and both in purple).

A)



B)



C)

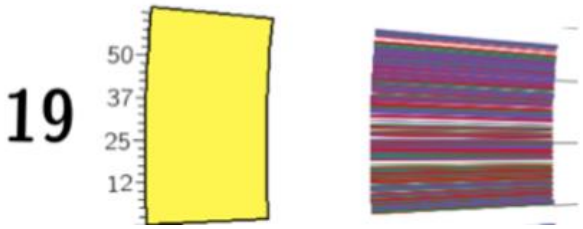


Figure 3 – **Comprehensive circular map of autosomal copy-number variants in *Bos indicus*** generated by Circos software**.** From the outside to the inside of the external circle: Chromosome name; genomic location (in Megabases); bars depicting the CNV regions (loss in green, gain in red and both in purple). Figure 3a, 3b and 3c, are a zoom of the chromosome that showed the highest percentage of insertion and loss regions (BTA28, BTA12, BTA19 respectively).

Gene content and gene ontology

We investigated the gene content of CNVR derived from the UMD3.1 autosomes. The 9,805 non-redundant CNVR found in autosomes overlapped with 5,495 genes with function described. Among them, 15% of CNVRs were within the coding regions, suggesting that the exon regions were duplicated/deleted. In addition, 2% of CNVRs were in upstream (1%) or downstream (1%) regions, which are very close to genes regions. Besides, 17% were intragenic regions, 19% of regions found in introns and 18% in non-coding exon regions (5 'and 3' UTR), which evidences the importance of copy number variation regions (Figure 4).

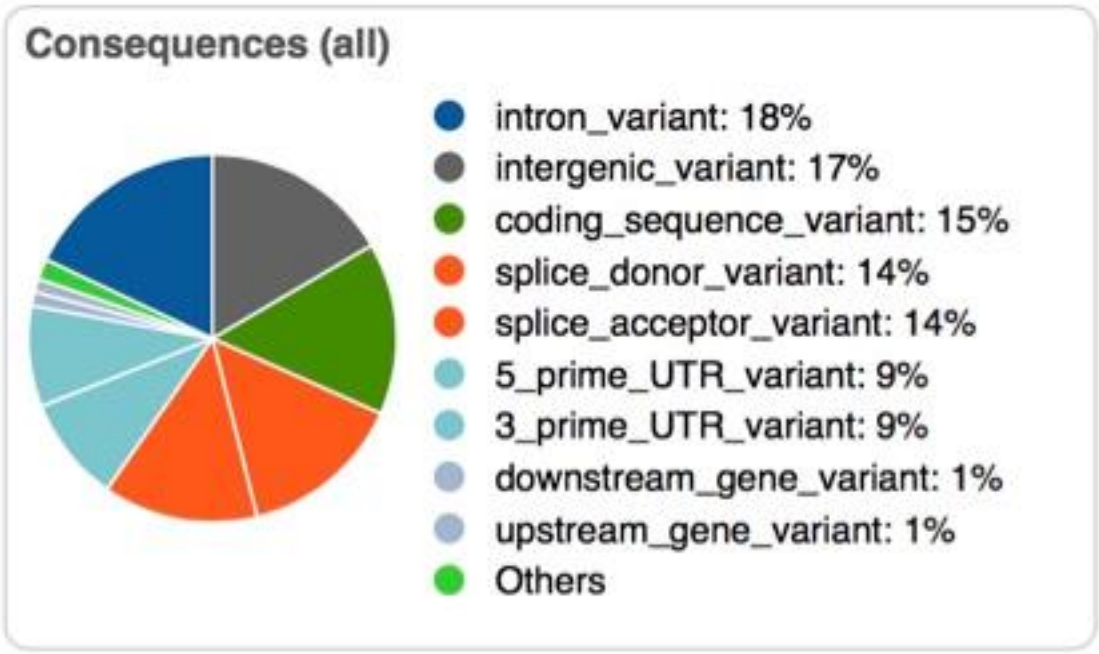


Figure 4 – Graphic representation of CNVR distribution on the bovine genome.

We assigned PANTHER accessions to the overlapped genes with CNVRs identified on UMD3.1 in this study. Multiple categories were statistically significant. This set of copy number variable genes encompasses a wide spectrum of molecular functions, biological processes, cellular components, Panther protein classes and pathways. Several GO terms were found to be significantly over-represented, for example, apoptotic process (GO:0006915), biological regulation (GO:0065007), development process (GO:0032502), growth (GO:0040007), immune system process (GO:0002376), metabolic process (GO:0008152), reproduction (GO:0000003) as

well as several enriched biological process.

The same gene dataset was used on the DAVID tool (HUANG et al., 2009), to analyze the Gene Ontology (GO) functional categories of the protein coding genes located in CNVs (Table 2). The most enriched GO terms are related to biological regulation process presented in Table 2. Four KEEG Pahtways were found to be significant for this gene list. The most enriched pathway was the fatty acids biosynthesis (5.7) followed by Fructose and mannose metabolismo (2.1).

Table 2 – Gene ontology enrichment using DAVID tool

Category	Term	Count	P-Value	Fold Enrichment	FDR
GOTERM_BP_FAT	protein heterotetramerization	3	6.5E-2	6.4	7.0E1
GOTERM_MF_FAT	activin receptor activity	3	6.9E-2	6.2	6.8E1
GOTERM_MF_FAT	peptidyl-lysine 5-dioxygenase activity	3	6.9E-2	6.2	6.8E1
KEEG_PATHWAY	Fatty acid biosynthesis	3	3.9E-3	5.7	4.7E0
GOTERM_BP_DIRECT	negative regulation of cAMP-dependent protein kinase activity	4	2.4E-2	5.2	3.5E1
GOTERM_BP_DIRECT	histone deubiquitination	4	2.4E-2	5.2	3.5E1
GOTERM_BP_DIRECT	regulation of vasodilation	3	9.7E-2	5.2	8.3E1
GOTERM_BP_DIRECT	positive regulation of histone methylation	3	9.7E-2	5.2	8.3E1
GOTERM_BP_DIRECT	positive regulation of cardiac muscle cell proliferation	3	9.7E-2	5.2	8.3E1
GOTERM_BP_DIRECT	protein localization to endosome	3	9.7E-2	5.2	8.3E1
GOTERM_BP_DIRECT	regulation of proteasomal ubiquitin-dependent protein catabolic process	4	5.2E-2	4.2	6.1E1
GOTERM_BP_DIRECT	cellular response to extracellular stimulus	4	5.2E-2	4.2	6.1E1
GOTERM_MF_FAT	calcium-transporting ATPase activity	5	6.5E-2	3.9	6.4E1
GOTERM_MF_FAT	calcium-transporting ATPase activity	5	2.7E-2	3.9	3.5E1
GOTERM_MF_FAT	calcium ion transmembrane transporter activity	5	2.7E-2	3.9	3.5E1
GOTERM_BP_DIRECT	amino acid transmembrane transport	4	8.9E-2	3.5	8.1E1
GOTERM_BP_DIRECT	signal transduction by protein phosphorylation	8	5.5E-3	3.2	9.3E0
GOTERM_CC_FAT	sarcoplasmic reticulum	7	2.2E-2	3.0	2.7E1
GOTERM_BP_DIRECT	cellular response to	5	7.5E-2	2.9	7.5E1

	cAMP				
GOTERM_BP_DIRECT	somitogenesis	5	7.5E-2	2.9	7.5E1
GOTERM_BP_DIRECT	hair follicle morphogenesis	5	7.5E-2	2.9	7.5E1
GOTERM_BP_DIRECT	negative regulation of JAK-STAT cascade	6	4.3E-2	2.8	5.4E1
GOTERM_CC_FAT	clathrin coat	10	7.1E-3	2.7	9.8E0
GOTERM_CC_FAT	chloride channel complex	8	2.0E-2	2.7	2.5E1
GOTERM_BP_DIRECT	retrograde vesicle-mediated transport. Golgi to ER	7	3.6E-2	2.6	4.8E1
GOTERM_BP_DIRECT	protein tetramerization	6	8.6E-2	2.4	7.9E1
GOTERM_BP_DIRECT	regulation of autophagy	6	8.6E-2	2.4	7.9E1
GOTERM_BP_DIRECT	negative regulation of cyclin-dependent protein kinase activity	10	1.5E-2	2.4	2.4E1
GOTERM_BP_DIRECT	protein autophosphorylation	9	3.4E-2	2.2	4.6E1
GOTERM_BP_DIRECT	MAPK cascade	9	3.4E-2	2.2	4.6E1
GOTERM_BP_DIRECT	fatty acid biosynthetic process	7	9.0E-2	2.1	8.1E1
KEEG_PATHWAY	Fructose and mannose metabolism	11	2.6E-2	2.1	2.8E1
GOTERM_BP_DIRECT	chloride transmembrane transport	11	2.3E-2	2.1	3.4E1
KEEG_PATHWAY	Non-small cell lung cancer	18	3.4E3	2.1	4.1E0
GOTERM_BP_DIRECT	cell cycle arrest	9	5.8E-2	2.0	6.5E1
GOTERM_BP_DIRECT	heart development	12	2.5E-2	2.0	3.5E1
KEEG_PATHWAY	Adherens junction	23	1.5E-3	2.0	1.9E0
GOTERM_BP_DIRECT	ubiquitin-dependent protein catabolic process	17	1.1E-2	1.9	1.8E1
GOTERM_CC_FAT	nuclear pore	9	4.2E-3	1.9	7.0E0
GOTERM_BP_DIRECT	protein stabilization	12	5.8E-2	1.8	6.5E1
GOTERM_MF_FAT	enzyme binding	52	3.0E-6	1.8	4.9E-3
GOTERM_BP_DIRECT	cell proliferation	15	3.2E-2	1.8	4.3E1
GOTERM_BP_DIRECT	transcription from RNA polymerase II promoter	25	7.8E-3	1.7	1.3E1
GOTERM_BP_DIRECT	negative regulation of apoptotic process	25	2.7E-2	1.5	3.8E1
GOTERM_BP_DIRECT	intracellular protein transport	20	6.6E-2	1.5	7.0E1
GOTERM_BP_DIRECT	positive regulation of transcription from RNA polymerase II promoter	50	3.0E-3	1.5	5.1E0

Gene ontology (GO) categories significantly over represented, with false discovery rate (FDR)

4. DISCUSSION

We identified a total of 195,873 CNVs, with an average length size of 54,744 kb, maximum of 8.7 Mb and a minimum length of 3 kb. The high number and wide distribution of CNV in the Nellore genome was evidenced before by Da Silva et al. (2016), who detected, by high-density SNP genotyping data from 1,717 Nellore cattle and observed a total of 68,007 CNVs. Similarly, Zhou et al. (2016), in a study with 2,230 Nellore cattle, identified a total of 992,350 CNVs using a multivariate method supplied by Golden Helix SVS 8.3.0.

Several studies have highlighted the distribution and characterization of CNV in the genome of several animals' species (FADISTA et al., 2010; LIU et al., 2010; HOU et al., 2011). SALOMÓN-TORRES et al. (2015), using a high-density SNP chip, reported similar results in a study that identified CNVs in the genome of 12 Holstein cattle., it was found that 29.8% of the CNVs had between 10 and 50 kb.

Fadista et al. (2010) reported 304 CNV regions in 20 animals from four different cattle breeds by CGH array technology. Apart from these preliminary results, new evidences of CNVs in cattle were observed and the Consortium bovine HapMap (over 500 animals of different breeds). It permitted the identification of 79 candidate deletions (MATUKUMALLI et al., 2009). Birckhart et al. (2012) studied the genomic traits of three taurine breeds (Angus, Holstein, and Hereford) and one indicine breed (Nellore) observed that the CNVs overlaps across taurine breeds are higher than the overlap between taurine and indicine cattle. Moreover, Nellore presented the largest CNV diversity among all the studied breeds. These results already suggested that some genetic differences between these subspecies have a CNV contribution.

Comparing the obtained results in this study with the studies already mentioned above, we can note a significant difference in the number of CNVs. It may occur due to the use of different detection methods, which have different platforms or genotyping algorithms (Table 3). Current methodologies, which infer variations in the number of copies from SNP data, lead to some errors at the time of detection (CASTELLANI et al., 2014; REDON et al., 2006). Different factors may influence the accuracy of CNV detection, such as the breakpoint, including batch effects, stratification of the population, experimental differences and the robustness of the statistical model (DELLINGER et al., 2010). The type of genotyping array used can

also interfere with the number of CNVR discovery, increasing or decreasing the detection sensitivity (HOU et al., 2012).

Table 3 - List of CNVs detection studies in cattle using different approaches.

Study	Method	Algorithm	Samples	Bovine sub-specie	CNV
Hou et al. 2012a	SNP chip (777 K)	PennCNV	674	*Bos taurus*	443
Coccinard et al., 2013	SNP chip (648 K)	PennCNV; QuantiSNP	2654	*Bos taurus & Bos indicus*	4839
Salomón-Torres et al., 2015	SNP chip (648 K)	PennCNV; QuantiSNP	12	*Bos taurus*	77
Yang et al., 2015	SNP chip (770 K)	PennCNV; CNVPartition	792	*Bos taurus*	263
Wang et al., 2015	SNP chip (54 K)	PennCNV	231	*Bos taurus x Bos indicus*	433
Zhang et al., 2015	aCGH	seg-MNT	24	*Bos taurus & Bos indicus*	356
Zhou et al., 2016	700,000 SNP probes	Golden Helix SVS 8.3.0	2230	*Bos indicus*	992,350
Da Silva et al., 2016	SNP chip (777 K)	PennCNV;	1,717	*Bos indicus*	68,000
This study	SNP chip (777 K)	PennCNV; CNVRuler	3,794	*Bos indicus*	195,873

The percentage of CNVR coverage found in this study is higher when compared to initial estimates in the literature, including studies with Nellore breed, which ranged from 0.68% to 5.85% of the genome (FADISTA et al., 2010; LIU et al., 2010; ZHOU et al., 2016). Several studies with different taurine breeds reported a percentage of chromosome coverage by CNVR lower than the herein presented (FADISTA et al., 2010; LIU et al., 2010; CHOU et al., 2013). Zhang et al. (2014) identified 486 CNVR, covering 2.45% of the cattle genome using data from 24 animals (*Bos taurus taurus)*. They identified 161 CNVRs in two yaks (*Bos grunniens*) and 163 in three buffaloes (*Bubalus bubalis*). Hou et al. (2011) worked with 539 animals of different breeds, included 366 animals from 14 taurine breeds; 70 animals from three breeds with an predominant indicine background; 46 animals from taurine × indicine composites; and 39 animals from two African groups, one of which (Sheko)

is an ancient hybrid. It was detected 682 CNVRs with 204.4 kb average size, covering a total of 4.6% (139.9 MB) of the genome.

The higher CNVR percentage may also be explained by the larger number of samples used in the present study. Moreover, a different subspecies was used and more CNV loci were predicted in indicine breeds than in European taurine breeds, which is consistent with the known breed divergence and history (LIU et al., 2010). Recent studies report that CNVs evolve 2,5 folds quicker than SNPs and help to promote a quick adaptation to different environment (PAUDEL et al., 2015), being in accordance to cattle sub-speciation.

The relationship between the chromosome size and the number of CNV regions across them is not straightforward (Figure 3). Some chromosomes revealed higher proportion of CNVR than others (relative to their size). The results are not in accordance to the ones presented by Da Silva et al. (2016) who worked with the same breed. The authors found a high positive correlation (0.98) between the number of detected CNVRs and chromosome size. However, some results were similar: the highest number of CNVR was detected on the BTA1 and the second smallest CNVR mean size, according to the bovine chromosome (46.69 kb), were similar in both studies. Also, the BTA25 had the lowest number of CNVR (131) and one of the biggest CNVR mean size (79.37 kb), which agree with our results.

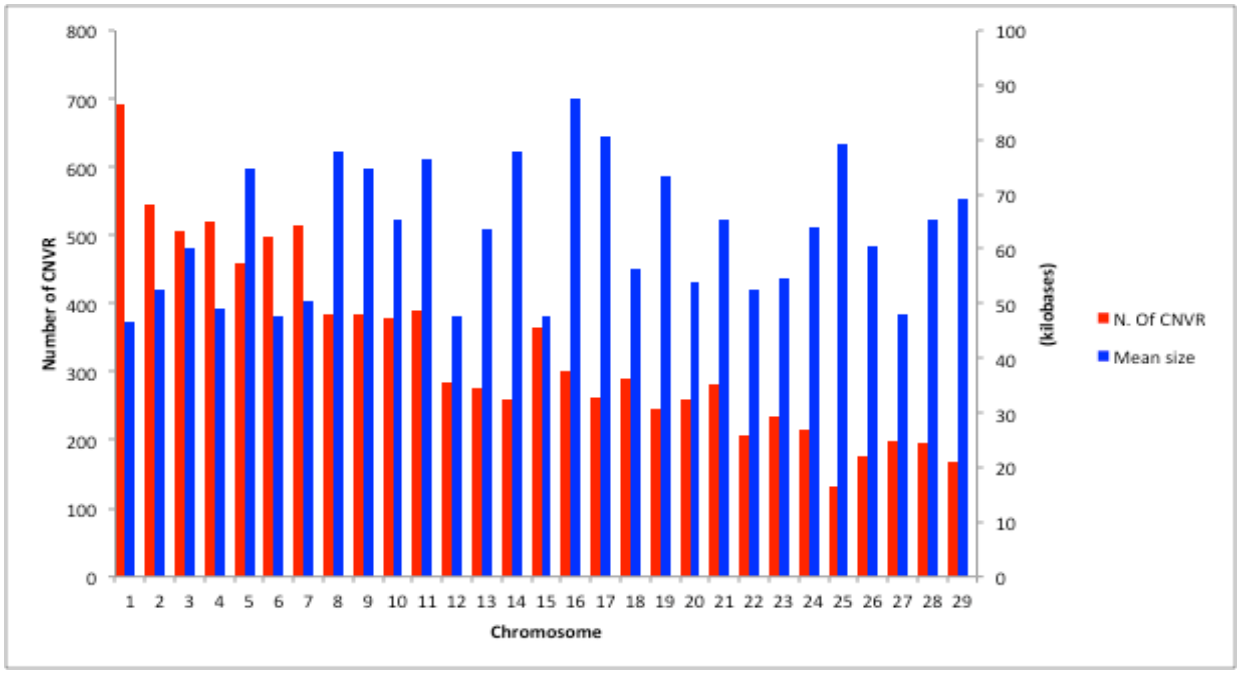


Figure 5 - Number and mean length size of copy number variation regions by chromosome.

Cicconardi et al. (2013), in a study with five different Italian breeds of cattle, found in 1,522 CNV samples of animals Italian Friesian being 27.52% of insertion regions and 75.29% of deletion regions. On the other hand, Da Silva et al. (2016), found 4,801 CNV regions of mixed events in a study with Nellore breed, while the number of deletion and insertion were 212 and 2,306, respectively. In a similar way, from the total of CNVR obtained in our study (9,805), 38.5% of these were characterized as insertion regions of copy number, 28.5% were deletion regions and 33.0% showed both events in the same region (insertion and deletion), what makes our results an approach with a greater number of CNVR and insertion regions when compared to previous studies (FADISTA et al, 2010; BAE et al., 2010; SEROUSSI et al., 2010; LIU et al., 2010; HOU et al., 2010; COCCINARDI et al., 2013; DA SILVA et al., 2016). The insertion or deletion events of one or more copies of a complete gene could result in a proportion increase, reduction or absence of the gene product. This will be determined according to the distance that the CNV regions are from the genes (IAFRATE et al., 2014). Thus, once we have a larger proportion of insertion regions, this may indicate a greater chance of these CNVs effect gene products.

The analyses of the genes affected by the CNVR revealed a higher or the same proportion when compared to some studies (HOU et al., 2011; BAE et al., 2010; CICCONARDI et al., 2013).

The gene ontology analyses showed several terms enriched for the CNVR gene list. One of these terms was the "immune system response" (GO:0002376), which is defined as the process involved in the development or functioning of the immune system: i.e., an organismal system for calibrated responses to potential internal or invasive threats. Some authors have been reported genes related to immune system and sensory response as overrepresented in cattle and human. This fact suggests that survivability benefit can be achieved with the increased dosage of genes related to infection response and sensing the environment (SOTHTARD et al, 2011; LIU et al., 2010; NGUYEN et al., 2006).

Bos taurus indicus breeds are preferred in tropical regions because of their tolerance to heat and resistance to ecto and endoparasites, and it has been the focus of studies that seek to compare the immunological response to various

environmental factor. In this prerogative, O'Kelly (1980) found greater resistance of *B. taurus indicus* crossbreeds raised in tropical conditions, mainly to *Haemonchus* and *Cooperia*, when compared with purebred animals (*B. taurus taurus*). The animal's resistance to internal and external parasites, which correspond to immune response system efficiency, was "acquired" by natural selection in the Indian subcontinent. The first speculations of genetics mechanisms underlying the characteristics start to come over.

The term "regulation of vasodilatation" was enriched by the gene list (5,2) through DAVID analyzes. "Regulation of vasodilatation" is defined as any process that modulates the frequency, rate or extent of increases in the diameter of blood vessels. Indicine cattle show a high thermo tolerance when compared to taurine cattle. Although the physiological basis for this fact has not yet been identified, one possibility is that the density of arteriovenous anastomoses is higher in *B. taurus indicus* (FINCH, 1985). These structures have lower resistance to flow than vascular passages, they involve capillary networks and facilitate blood flow to the skin during heat stress (HALES et al., 1978), being a more efficient way to dissipate heat. Another term signalized that was associated to heat tolerance was "hair follicle morphogenesis". Different from taurine cattle, the indicine cattle has thin, short, smooth and shiny hair coats. The light color hair reflects a large proportion of solar incident radiation and also acts to reduce heat exchange via radiation (CARDOSO et al 2015, HUTCHINSON and BROWN, 1969; FINCH et al., 1984). To better dissipate heat, they also have greater hair angle and a low density of hairs per skin area (CRUZ et al 2016). All these characteristics may be associated to the term pointed out, as well as, to the evidences of environmental adaptation.

Terms related to performance were also identified (PANTHER analyses). The significant term "growth", defined as the increase in size or mass of an entire organism or a part of an organism, was previously reported in a CNV study realized by Sothtard et al. (2011) with *Bos taurus taurus* cattle. This term could appear due to the selection progress for weight in Nellore cattle over the past years; or even due to the lower mass gain that zebu breeds still have when compared to taurine breeds. The term "reproduction" was also significant. It is defined as the production of new individuals that contain some portion of genetic material inherited from one or more

parent organisms (SOTHTARD et al., 2011; ASHBURNER et al., 2000). It is fully described that *Bos taurus indicus* have lower reproductive performance when compared to taurine breeds, specially related to puberty onset. The CNVRs herein identified may be associated to these phenotypes and differences between subspecies.

Some others relevant biological process were enriched by the gene list like fatty acid biosynthesis process together with the KEEG pathway fatty acid biosynthesis (Figure 6). For definition, fatty acid biosynthesis (GO:0006633) is the chemical reactions and pathways resulting in the formation of a fatty acid, any of the aliphatic monocarboxylic acids that can be liberated by hydrolysis from naturally occurring fats and oils (ASHBURNER et al., 2000). An important group of genes belonging to the family of ELOVL are responsible for performing the function of biosynthesis of fatty acids has been linked previously to the fatty acid profile of the meat of Nellore cattle (LEMOS et al., 2016). The authors, working with the same group of animals of this study, associated the gene *ELVOL5* to the arachidonic fatty acid, which is a polyunsaturated fatty acid present in the phospholipids of membranes of the body's cell, is abundant in the brain, muscles and live and is the product of their oxygenation mediate or modulate inflammatory reactions (SMITH et al., 1979; SAMUELSON, 1991). Enrichment of theses terms could be imagined to reflect selection for production of muscle mass with improved fatty acid profile associated with a better resistance of these animals to inflammatory processes.

The other terms like biological regulation process, development process, apoptotic process, among others, were enriched in these DAVID analyses, further confirming the Panther results. The results of our functional analyses are in agreement with results reported of previously CNV studies (FADISTA et al, 2010; HOU et al., 2012; CICCONARDI et al., 2013; DA SILVA et al., 2016).

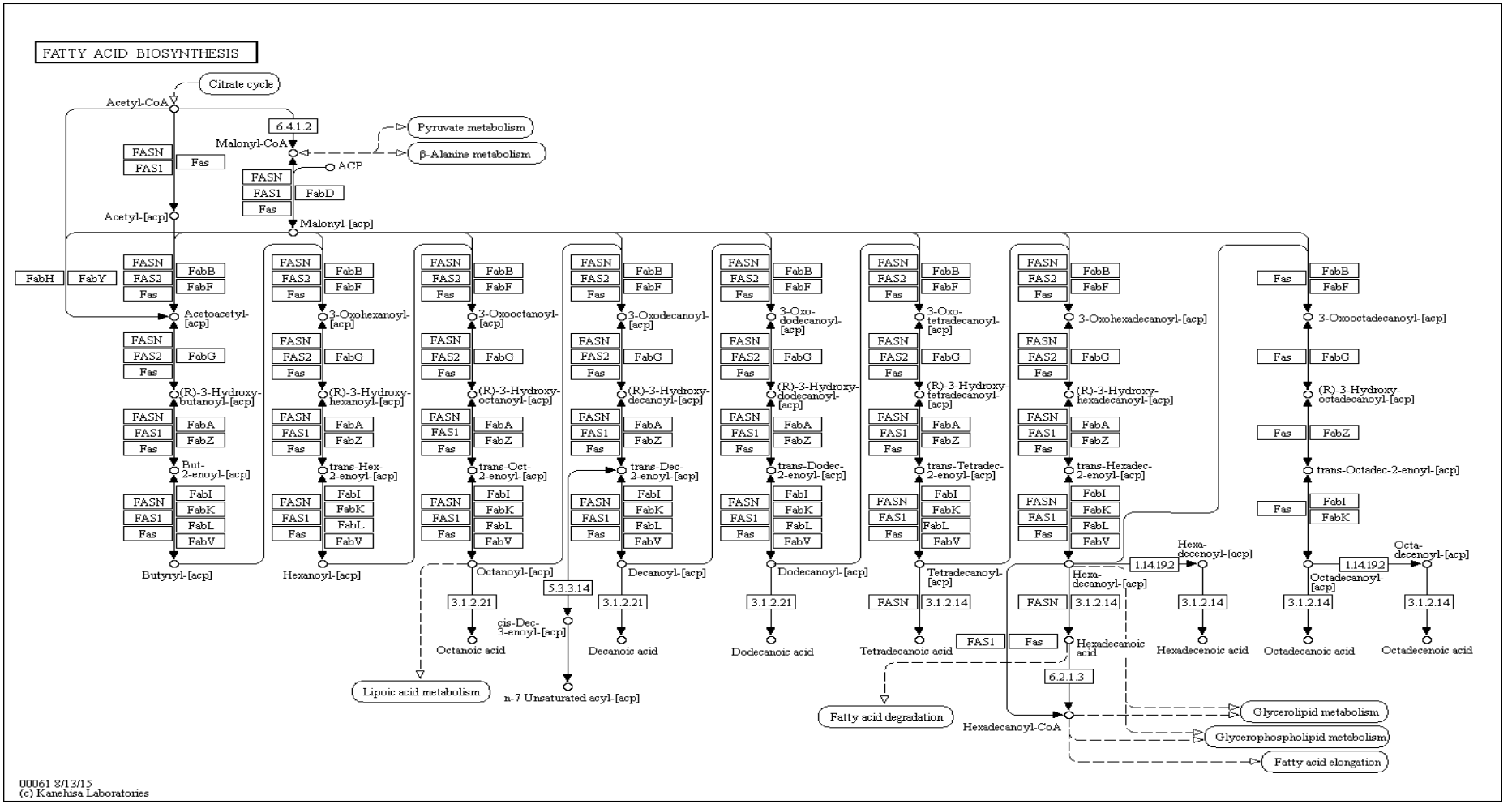


Figure 6 – KEEG pathway fatty acid biosynthesis.

Several studies analyzed the CNV characterization of cattle breeds and most of them were realized with taurine animals (SALOMON-TORRES et al., 2016; JIANG et al., 2012; LIU et al., 2010) and some with indicine (ZHOU et al., 2016; DA SILVA et al., 2016). Previous studies reported a lower number of CNV and CNV regions than the present study (even the ones with Nellore breed). This result is probably may be related to the higher number of samples that we used for this study, since, through preliminary analysis, it was observed that the larger number of samples was proportional to the CNVR increase. However, we have to consider the possibility of a bias occasioned due to the reference genome that is from a taurine animal. A *Bos taurus indicus* public reference genome would better guide the indice genetic studies.

The understanding of the genetic basis in productive and functional traits in zebu cattle has great economic and biological importance. The broad identification of CNVs segregating within the breed provided crucial information to infer that CNVs may be under natural and/or artificial selection.

The variations in the number of copies of the Nellore cattle genome brings important information for the investigation of genetic variations associated to phenotypes of interest, such as production traits and meat quality, which often are restricted to the use of SNPs.

The information generated in this study should assist the detection of deletions, insertions or mutations. It also contributes to conduct further studies of association and fine mapping whose aim was to understand the genetic mechanisms and biological pathways that determine the phenotypes of interest traits for the animal production.

5. CONCLUSION

The present study reported the presence of a high variability of copy number variations in Nellore cattle (*Bos taurus indicus*). The gene enrichment of the CNV regions revealed biological processes that might be involved in the environmental adaptation of the subspecies to tropical areas. The investigation of variability is the first step that will allow the future analysis as well as the incorporation of this tool into programs of genetic improvement of Nellore cattle.

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CHAPTER 3 - GENOME-WIDE ASSOCIATION BETWEEN SINGLE NUCLEOTIDE POLYMORPHISMS WITH BEEF FATTY ACID PROFILE IN NELLORE CATTLE USING THE SINGLE STEP PROCEDURE

ABSTRACT - Saturated fatty acids can be detrimental to human health and have received considerable attention in recent years. Several studies using taurine breeds showed the existence of genetic variability and thus the possibility of genetic improvement of the fatty acid profile in beef. This study identified the regions of the genome associated with saturated, mono- and polyunsaturated fatty acids, and n-6 to n-3 ratios in the Longissimus thoracis of Nellore finished in feedlot, using the single-step method. The results showed that 115 windows explain more than 1% of the additive genetic variance for the 22 studied fatty acids. Thirty-one genomic regions that explain more than 1% of the additive genetic variance were observed for total saturated fatty acids, C12:0, C14:0, C16:0 and C18:0. Nineteen genomic regions, distributed in sixteen different chromosomes accounted for more than 1% of the additive genetic variance for the monounsaturated fatty acids, such as the sum of monounsaturated fatty acids, C14:1 cis-9, C18:1 trans-11, C18:1 cis-9, and C18:1 trans-9. Forty genomic regions explained more than 1% of the additive variance for the polyunsaturated fatty acids group, which are related to the total polyunsaturated fatty acids, C20:4 n-6, C18:2 cis-9 cis12 n-6, C18:3 n-3, C18:3 n-6, C22:6 n-3 and C20:3 n-6 cis-8 cis-11 cis-14. Twenty-one genomic regions accounted for more than 1% of the genetic variance for the group of omega-3, omega-6 and the n-6:n-3 ratio. Based on this genes we performed a DAVID enrichment analyze and and Ingenuity pathway analysis and found 5 clusters annotation enriched the pathways, but none with a significant p-value. Pathways acted on fucose and cholesterol biosynthesis, and PPARa activation, would provide valuable insights into explaining the molecular mechanism of lipid metabolism. The identification of such regions and the respective candidate genes, such as ELOVL5, ESRRG, PCYT1A and genes of the ABC group (ABCA5, ABCA6 and ABCA10), should contribute to form a genetic basis of the fatty acid profile of Nellore (Bos indicus) beef, contributing to better selection of the traits associated with improving human health.

Key-words: *Bos indicus*, fatty acid composition, heritability, genetic markers, mapping, ssGWAS

1. INTRODUCTION

High consumption of saturated fatty acids (SFA) is associated with increased serum levels of cholesterol and low-density lipoproteins (LDL), considered risk factors for the occurrence of cardiovascular disease (KATAN et al., 1994). The SFAs prevalent in beef fat are the myristic (C14:0), palmitic (C16:0) and stearic (C18:0) fatty acids (LAWRIE, 2005; ROSSATO et al., 2009). The polyunsaturated fatty acids (PUFA) present in beef, such as linoleic (C18:2 n-6) and linolenic (C18:3 n-3), and monounsaturated (MUFA), as oleic acid (C18:1 n-9) protect the cardiovascular system, since moderate consumption has been linked to decreasing serum cholesterol and increasing high-density lipoprotein (HDL) (DUCKETT, 2014; PENSEL, 1998; SIMOPOULOS, 1999).

Furthermore, the fat of ruminants is a natural source of conjugated isomers of linoleic acid (CLA c9 t11), such as C18:2 cis-9 trans-11 (FRENCH et al., 2000), which are synthesized in the rumen as a result of biohydrogenation of fatty acids, performed by microorganisms (TAMMINGA & DOREAU, 1991). The CLAs have a positive effect on human health, related to anticancer activity, immune functions, and potential beneficial effects on coronary heart disease (IP, 1997; DUGAN et al., 2011). Strategies such as diet (Faucitano et al., 2008) and gene manipulation (DE SMET et al., 2004) have been used to satisfy the growing consumer demand for protein sources with healthier lipid profile. Thus, regions associated with the expression of beef fatty acid profile have been identified aiming to locate key genes in the genome (CESAR et al., 2014; ISHII et al., 2013; SEVANE et al., 2014) that contribute to these features. This genomic tool will assist the use of information that is beneficial to human health.

Recently, several genome-wide association studies using taurine breeds and their crosses (SAATCHI et al. 2013; YAMAJI et al., 2013; AHLBERG et al., 2014; SEAVEN et al., 2014) have identified genetic variants for fatty acid (FA) profile in beef. These results would allow producers to select for desirable nutritional values

with respect to meat FA that could increase beef value and consumer satisfaction. However, there are limited number of genomic association studies with a large sample size that aim to determine genome regions associated with the meat fatty acid profile of zebu cattle reared in tropical conditions (ROSSATO et al., 2009). The study of Rossato et al., 2009, utilized 386 Nellore steers, sired from 34 unrelated sires, from an experimental herd, and applied the Bayes B method to perform the genome-wide association analysis. In the literature, there is some controversy regarding the capacity of different methods to identify genomic regions related to phenotypes due to differences in method presuppositions (ZENG et al., 2012; WANG et al., 2012; MISZTAL et al., 2013). Moreover, it is important to perform genome-wide association studies (GWAS) in indicine populations due to differences in environment and management conditions, and also differences in allele frequency of genetic markers and QTL, that would influence the results. The identification of genomic regions that affect the meat fatty acid composition may become an important and highly applicable tool to improve the nutritional value of zebu meat given the expensive and difficult nature of collecting phenotypic records.

The objective of this study was to identify regions associated with saturated, mono and polyunsaturated and n-6 to n-3 ratios, in the *Longissimus* thoracis muscle from confined Nellore, using the single-step method and describ the metabolic pathways of these genes.

2. MATERIAL AND METHODS

Local, Animals and Management

This study was approved by ethics committee of the Faculty of Agrarian Sciences and Veterinary, Sao Paulo State University (UNESP).

The database contains animal data from eight farms located in the Southeast, Northeast and Midwest of Brazil, which are part of breeding cattle programs. Genotypes (n = 1616) and phenotypes (n = 963) of Nellore bulls, with average initial age of 24 months, were used. In these breeding programs animals are selected based on growth, finishing and sexual precocity traits.

Breeding seasons are adopted at different periods on these farms. Therefore calving seasons concentrate from August to October in some farms and from November to January in others, and weaning was performed at seven months of age. The 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. 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, and soybeans, soybean meal, or sunflower seeds were used as protein concentrate. The criteria used by farmers for slaughtering was weight (500-550 kg). After stored for 48 hours at 0-2°C, meat samples were removed from the *Longissimus thoracis* muscle, between the 12th - 13th ribs from each animal, placed in plastic bags and stored at −80°C until further analysis to determine the fatty acid profile. The percentage of lipids in the *Longissimus thoracis* muscle (IMF) was obtained using the method proposed by (FOLCH et al., 1957).

Determination of the 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 meat fatty acids were extracted using the method of Folch et al. (FOLCH et al., 1957) and the methyl esters were formed according to Kramer et al. (KRAMER et al., 1997). The fatty acid profile was determined at the Meat Science Laboratory (LCC) in the Department of Animal Nutrition and Production at FMVZ/USP, using the extraction method 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 - Shimadzu AOC 20i auto-injector) with a 100 m SP-

2560 capillary column (0.25 mm in diameter with 0.02 mm thickness, Supelco, Bellefonte, PA). 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 (H2) was the carrier gas, with 40 cm3/s. Fatty acids were identified by comparing the retention time of methyl esters of the samples with the standards C4-C24 (F.A.M.E mix Sigma®), vaccenic acid C18:1 trans-11 (V038-1G, Sigma®) C18:2 trans-10 cis-12 (UC-61M 100mg), CLA e C18:2 cis-9, trans-11 (UC- 60M 100mg), (Sigma®) and tricosanoic acid (Sigma®). Fatty acids were quantified by normalizing the area under the curve of methyl esters using the GS solution 2.42 software. Fatty acids were expressed as a percentage of the total fatty acid methyl ester. The fatty acid profile in meat was performed at the Meat Science Laboratory (LCC) in the Department of Animal Nutrition and Production at FMVZ/USP.

The following individual fatty acids were selected: lauric (C12:0), myristic (C14:0), palmitic (C16:0), stearic (C18:0), myristoleic (C14:1), palmitoleic (16:1), oleic (C18:1 *cis*-9), elaidic (C18:1 trans9), CLA-*cis* (C18:2c9t11), CLA-*trans* (C18:2 trans10 cis 12), vaccenic (C18:1 trans11), linoleic (C18:2 cis9Cis12n6), docosahexaenoic (DHA) (C22:6 n3), and eicosatrienoic FA (C20:3 n6 cis-8,11,14). These FA were chosen due to their importance to human health and their high content in animals from confinement, such as the oleic acid. The sum of saturated (C10:0 +C11:0 +C12:0 +C13:0 +C14:0 + C15:0 + C16:0 + C17:0 + C18:0 + C21:0 + C24:0), monounsaturated (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 (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), omega 6 (C18:3 n6 + C20:3 n6 c8, c11, c14 + C18:2 n6 + C20:4 n6) and omega 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 calculated.

Genotyping of animals

A total of 1,616 animals were genotyped using 777,962 SNPs of the Bovine SNP BeadChip (High-Density Bovine BeadChip). The quality control of the SNPs

markers consisted of excluding those with unknown genomic position, located on sex chromosomes; monomorphic and markers with minor allele frequency (MAF) less than 0.05; call rate less than 90%, and markers with excess heterozygosity. Samples with a call rate less than 90% were also excluded. After quality control, 470,007 SNPs from 1,556 animal samples were left.

Quantitative genetic analysis

The contemporary groups included animals born on the same farm and year, and from the same management group at yearling. The contemporary groups that contained less than three observations and those that deviated ± 3 standard deviations from the mean of that group were eliminated. The model used for the variance component estimation included random additive direct genetic effect, the fixed effect of the contemporary groups, and the animal's slaughter age as a covariable (linear and quadratic effect).

The variances components and genetic parameters were estimated using the Bayesian inference (GIANOLA et al., 1986), considering a linear animal model (ssGBLUP), and the GIBBS2F90 computer programs (GEWEKE et al., 1992; HEIDELBERGER & WELCH, 1983). The statistical model can be represented by the following matrix form:

$$y = X\beta + Za + e,$$

where*: y* is the vector of observations; ***β*****,** the vector of fixed effects; *a*, the vector of direct additive genetic effects; *X*, the known incidence matrix***; Z,*** the incidence matrix of the random additive direct genetic effect (associates vector ***β*** with vector *y*); and ***e*****,** the vector of the residual effect. The *priori* distributions of vectors ***y*****,** ***a*** and ***e*** were given by:

$$y \sim MVN(X\beta + Za)$$
$$a|G \sim MVN(0, H \otimes G)$$
$$e|R \sim MVN(0, I \otimes R)$$

Where: *H* is the matrix of kinship coefficients between animals obtained from the single-step analyses; R, the matrix of residual variance; I, the identity matrix; G,

matrix of additive genetic variance; and, ⊗, the Kronecke product. The prior distribution of variance components of the genetic and residual effects was an inverted Wishart. Uniform initial distribution was defined for fixed effects.

The analyses generated chain lengths of 1,000,000 interactions, where the first 80,000 interactions were discarded. To estimate the parameters, the samples were stored at every 100 cycles, building samples with 800,000 samples. The data convergence was verified with the graphical evaluation of sampled values versus interactions according to the criteria proposed by several authors (MIZTAL et al., 2002; AGUILAR et al., 2011; RAFTERY & LEWIS, 1992), using the Bayesian Output Analysis (BOA) of the R 2.9.0 software.

Analysis of the genomic association

To perform the GWAS analyses the single-step GBLUP (ssGBLUP) method was applied. The ssGBLUP model is a modification of BLUP with numerator relationship matrix A^{-1} matrix replaced by H^{-1} (AGUILAR et al., 2010):

$$H^{-1} = A^{-1} + \begin{bmatrix} 0 & 0 \\ 0 & G^{-1} - A_{22}^{-1} \end{bmatrix}$$

Where, A_{22} is a numerator relationship matrix for genotyped animals, and G is a genomic relationship matrix. The genomic matrix can be created following (VANRADEN et al., 2009) as:

$$G = ZDZ'q$$

Where, Z is a matrix of gene content adjusted for allele frequencies; D, a weight matrix for SNP (initially D=I); and q, a weighting/normalizing factor. According to Vitezica et al. (Vietzica et al., 2011), this factor can be derived by ensuring that the average diagonal in G is close to that of A_{22}. The SNP effects and weights for GWAS were derived as follows (WANG et al., 2012):

1. Let D=I in the first step.
2. Calculate $G = ZDZ'q$
3. Calculate GEBVs for the entire data set using ssGBLUP.

4. Convert GEBVs to SNP effects $\hat{\mathbf{u}} = \frac{\sigma_u^2}{\sigma_a^2}\mathbf{DZ'G}*^{-1}\hat{\mathbf{a}}_g = \mathbf{DZ'}\left[\mathbf{ZDZ'}\right]^{-1}\hat{\mathbf{a}}_g$, where $\hat{\mathbf{a}}_g$ is the GEBV of the animals which were also genotyped.
5. Calculate the weight for each SNP: $\mathbf{d_i} = \hat{\mathbf{u}}_i^2 2\mathbf{p}_i(1-\mathbf{p}_i)$,where I is the i-th SNP
6. Normalized SNP weight to remain the total genetic variance constant.
7. Loop to 2.

The SNP weights were calculated iteratively looping trough steps 4-6. The iterations increase the weights of SNP with large effects and decrease those with small effects.

The percentage of genetic variance explained by i-th region was calculated by:

$$\frac{Var(a_i)}{\sigma_a^2} =\times 100 = \frac{Var\left(\sum_{j=1}^{10} Z_j\, \hat{u}_j\right)}{\sigma_a^2} \times 100$$

Where, a_i is genetic value of the *i*-th region that consists of continuous 10 adjacent SNPs, σ_a^2, the total genetic variance; Z_j , the vector of gene content of the *j*-th SNP for all individuals; and $\hat{u}_j$, marker effect of the *i*-th SNP within the *i*-th region.

Searching for genes

The segments that explained values equal to or greater than 1% of the additive genetic variance were selected to determine the possible QTL regions. The selected segments were identified and located in the bovine genome by surveying the database available in the “National Center for Biotechnology Information” in UMD3.1 version of the bovine genome and Ensembl Genome Browser (FLICEK et al., 2013). In these databanks, it was possible to identify segments located within or close to genes that could explain the variability in the expression of such traits. The classification of genes as the biological function and appropriate analysis of metabolic pathways will be performed by the website “The Database for Annotation, Visualization and Integrated Discovery (DAVID) v. 6.7 “(DAVID –

http://david.abcc.ncifcrf.gov/) and the QTL study already described is used the website AnimalQTLdb (http://www.animalgenome.org/cgi-bin/QTLdb/index).

3. RESULTS AND DISCUSSION

Fatty acid profile

The individual fatty acids with the highest concentration in the intramuscular fat of *Longissimus thoracis* were C16:0, C18:1 cis-9, C18:1 trans-11, and C18:0, representing 67.3% of its fat composition (Table 1). These results agreed with those reported by some authors (PRADO et al., 2003; LAWRIE, 2005; KELLY et al., 2013; CESAR et al., 2014) who observed high levels of palmitic, stearic and oleic FAs. Some authors (LAWRIE, 2005; ROSSATO et al., 2009) also reported that palmitic fatty acid was the predominant FA in beef fat. In Nellore finished in feedlot (CESAR et al., 2014), oleic acid (37.46%) displayed the highest concentration in intramuscular fat. The myristic and palmitic FAs are associated with an increase in circulating LDL cholesterol due to interference with hepatic LDL receptors (WOOLLETT et al., 1992). The saturated fatty acid were predominant, followed by the MUFAs and PUFAs. Similar results (PRADO et al., 2013) were reported for Nellore cattle, 43.93% (SFA), 42.33% (MUFA) and 12.8% (PUFA). However, studies using taurine (PITCHFORD et al., 2002) and Nellore (CESAR et al., 2014) breeds found similar concentrations for SFA and MUFA, 47% and 47.5%; and 47.23%, and 48.34%, respectively.

Table 1 – Descriptive statistics for the fatty acids profile of Nellore beef[1]

Trait	Nomenclature	Mean	[2]SD	[3]N
Intramuscular fat	IMF (in percentage)	0.83	0.42	934
Lauric	C12:0	0.06	0.18	538
Myristic	C14:0	2.13	0.54	867
Myristoleic	C14:1	0.32	0.22	824
Palmitic	C16:0	21.03	2.49	867
Palmitoleic	C16:1	2.18	0.78	937
Stearic	C18:0	13.63	3.32	783
Elaidic	C18:1n9t	2.91	5.07	483
Oleic	C18:1n9c	30.64	4.98	934
Vaccenic (TVA)	C18:1t11	1.29	0.01	878
Linoleic (LA)	C18:2n6	8.32	3.63	865
Linolenic	C18:3n3	0.59	0.26	858
CLA-*cis*	C18:2c9t11	0.26	0.16	727
CLA-*trans*	C18:2 trans10 cis 12	0.20	0.12	241
Docosahexaenoic (DHA)	C22:6 n3	0.95	0.39	865
Eicosatrienoic	C20:3 n6 cis-8,11,14	0.49	0.19	862
Sum of SFA		40.66	6.12	868
Sum of MUFA		37.55	8.05	868
Sum of PUFA		13.42	5.57	868
Sum of Omega-3		3.81	1.55	868
Sum of Omega-6		9.35	4.44	868
n6/n3 ratio		2.54	0.97	868
PUFA/SFA ratio		0.35	0.20	868

[1]The concentration of fatty acids are expressed as a percentage of total fatty acid methyl esters (FAME) quantified [2]SD: Standard deviation [3]N: number of animals with records

In the present study, the n-6:n-3 ratio was less than 4:1, the value recommended by the Department of Health and some authors (WOOD & ENSER, 1997). Excessive amounts of n-6 and a high n-6:n-3 ratio can lead to pathogenies, including cardiovascular, inflammatory, cancer and autoimmune diseases while increased levels of omega-3 fatty acids help to suppress such effects (LORGERIL et al., 1994). Studies have associated a 4:1 ratio to 70% decrease in mortality of humans, and also to preventing cardiovascular diseases (SIMOPOULOS, 2002). The Department of Health recommends values above 0.45 for the PUFA/SFA ratio. The average value for this ratio in this study is below this limit (0.35). A PUFA/SFA ratio of 0.11 has been reported in beef purchased at supermarkets in the UK (ENSER et al., 1996). However, this PUFA/SFA ratio may vary depending upon genetic and dietary factors (DE SMET et al., 2004).

Heritability estimates

The Gibbs sampling approach was used to estimate de (co)variance components and the convergence for all estimated parameters was verified through inspection of trace-plots and the Geweke's (GEWEKE et al., 1992) and Heidelberger and Welch convergence diagnostic (HEIDELBERGER & WELCH, 1983) indicated convergence of the chain. Thus, the burn-in period considered was sufficient to reach the convergence in all parameter estimates. The posterior marginal distributions of heritability estimates for fatty acid profile, presented showed accurate values, tending to normal distribution (Table 2). The symmetric distributions of central tendency statistics are indicative that the analyses are reliable.

The heritability estimates for the individual fatty acids profile of intramuscular fat in the *Longissimus thoracis* muscle were mostly moderate, but low for the C18:0, C16:1 and CLA cis-9 trans-11 acids and high for the C12:0 and CLA trans-10 cis-12 acids (Table 2). The linolenic FA heritability estimate obtained in this study was similar to that found by some authors (0.13) (CESAR et al., 2014) and lower than that reported by other studies (0.58) (NOGI et al., 2011). However, higher estimates have been reported for linolenic acid in other studies (0.21) (TAIT et al., 2007), and also for palmitoleic acid (0.15) (CESAR et al., 2014) and (0.49) (SAATCHI et al., 2013). Higher heritability estimates were reported for linoleic FA, 0.34 and 0.58, respectively, in the intramuscular fat of Japanese Black cattle, suggesting that genetic influence on linoleic acid varies among breeds (NOGI et al., 2011;,(INOUE et al., 2011). Lower and similar heritability was estimated for myristic (0.18) and palmitic (0.21) FAs (PITCHFORD et al., 2002), respectively, while studies with Nellore estimated low heritability for these FAs, ranging from 0.08 to 0.17 (CESAR et al., 2014). However, studies (EIKINE-DZIVENU et al., 2014) reported high estimates for the myristoleic FA (0.51) and (NOGI et al., 2011) also found high estimates for myristic (0.70), palmitic (0.65), myristoleic (0.60) and linoleic (0:58). Other authors also estimated low heritability estimates for the stearic (PITCHFORD et al., 2002),(EIKINE-DZIVENU et al., 2014), CLA cis-9 trans-11 (CESAR et al., 2014),(EIKINE-DZIVENU et al., 2014) and arachidonic (CESAR et al., 2014) FAs.

The heritability estimates for the sum of omega-3 series fatty acids, the n-6:n-3 ratio, and the sum of saturated and polyunsaturated fatty acids and their ratios were low (<0.12). However, moderate heritability estimates were obtained for the sum of monounsaturated fatty acids and the sum of n-6 and n-9 fatty acids. Studies estimated low heritability estimates for SFA and MUFA and moderate values for n-3 and n-6 (CESAR et al., 2014; EIKINE-DZIVENU et al., 2014). Low to moderate heritability estimates for PUFA (0.05 to 0.12), MUFA (0.06 to 0.20) and SFA (0.07 to 0.30) have been reported (PITCHFORD et al., 2002; EIKINE-DZIVENU et al., 2014; MALAU-ADULI et al., 2000). Nevertheless, other studies reported higher heritability estimates for these groups of fatty acids, 0.47 for PUFA, 0.35 to 0.66 for SFA and 0.35 to 0.68 for MUFA in Japanese Black cattle (ENSER et al., 1996; INOUE et al., 2011). Recently, authors also estimated high heritability for SFA (0.54) and MUFA (0.54) and, therefore, concluded that there is sufficient genetic variation in the fatty acid profile of cattle subcutaneous fat to respond to selection (KELLY et al., 2013). The results of this study suggest that it is possible to change the lipids composition of intramuscular fat of Nellore meat through selection. This information is important for breeding programs of zebu breeds that aim at improving the beef fatty acid composition.

Table 2 – Descriptive statistics and highest posterior density (HPD) region for fatty acid heritability estimates.

Trait	Nomenclature	Mean	Median	SD[1]	HPD	
					Low limit	Upper limit
Lauric	C12:0	0.64	0.69	0.24	0.17	0.99
Myristic	C14:0	0.27	0.26	0.10	0.04	0.46
Palmitic	C16:0	0.20	0.19	0.09	0.03	0.38
Stearic	C18:0	0.12	0.11	0.09	0.001	0.30
Myristoleic	C14:1	0.17	0.28	0.09	0.0003	0.62
Palmitoleic	C16:1	0.08	0.07	0.06	0.0002	0.21
Elaidic	C18:1 n9t	0.24	0.21	0.16	0.006	0.57
Oleic	C18:1 n9c	0.28	0.16	0.10	0.08	0.48
Vaccenic (TVA)	C18:1 trans-11	0.11	0.14	0.06	0.003	0.24
Linoleic (LA)	C18:2 n6	0.18	0.17	0.08	0.04	0.34
Linolenic acid (LA)	C18:3 n3	0.15	0.15	0.08	0.02	0.31
Eicosatrienoic	C20:3 n6 cis-8,11,14	0.14	0.13	0.09	0.02	0.31
Docosahexaenoic acid (DHA)	C22:6 n3	0.15	0.14	0.10	0.01	0.32
CLA	Cis9 Trans-11	0.09	0.08	0.06	0.004	0.21
	Trans10 Cis-12	0.52	0.54	0.27	0.02	0.94
Arachidonic acid	C20:4 n-6	0.14	0.11	0.11	0.0007	0.38
Total of SFA		0.12	0.12	0.07	0.0001	0.31
Total of MUFA		0.20	0.12	0.15	0.0005	0.31
Total of PUFA		0.08	0.11	0.05	0.0009	0.32
Omega-3		0.11	0.09	0.07	0.00008	0.29
Omega-6		0.23	0.11	0.10	0.0001	0.31
Omega-9		0.20	0.17	0.14	0.0002	0.46
Omega-6/omega-3 ratio		0.11	0.09	0.08	0.002	0.70
PUFA/SFA ratio		0.07	0.06	0.05	0.0001	0.17

[1]SD: Standard deviation

GWAS, genomic regions

The windows of 10 continuous SNPs that accounted for more than 1% of the genetic variance were used to search for putative candidate genes (PCG), which are represented in tables 3-6. The results indicated a total of 115 different windows that explained more than 1% of the genetic variance for the 22 fatty acids studied.

In GWAS studies of intramuscular fat and fat deposition in meat of Nellore cattle, using the same marker density as in this study, the authors found thirty-three genomic regions (windows with 1 Mb SNPs) associated with the traits, deposition of

intramuscular fat and meat fatty acid profile (CESAR et al., 2014). Similarly, other GWAS for Angus cattle, using a 54 K genotyping panel, found fifty-seven genomic regions associated with the fatty acids profile trait in meat (SAATCHI et al., 2013). For any fatty acid, non-overlapping regions were found with the results obtained by Cesar et al., 2014, Ishii et al., 2013 and Saatchi et al., 2013, who also performed GWAS for beef FA profile in several breeds. Even though no overlapping regions were found for the same fatty acids, regions in the same chromosome were found, but at the longer distances (> 1 Mb). This inconsistent between our results and previous studies could be due the differences between studied populations (breed and environment), distribution of linkage disequilibrium among the causal mutations and genetic markers, model applied to perform the GWAS, genetic marker density. In addition, physiological and metabolic factors involved in those populations might help to explain the differences observed by researchers. In this sense, it is important to highlight that the expression of FA profile in beef is probably influenced by many loci of small effect. Thus, it is expected that each PCG contribute differently for the additive genetic variance of each FA in different populations, environments and management conditions.

Saturated fatty acids

A total of thirty-one genomic regions that explain more than 1% of genotypic were found for total saturated fatty acids, C12:0, C14:0, C16:0 and C18:0. These regions are distributed on chromosomes BTA1, BTA2, BTA3, BTA4, BTA5, BTA7, BTA8, BTA9, BTA12, BTA16, BTA17, BTA20, BTA24, and BTA29 (Table 3).

Table 3 - Genomic regions associated with the saturated fatty acids profile in intramuscular fat of the *Longissimus thoracis* muscle of Nellore.

Traits	**QTL window**	**% Variance explained SNP window [1]**	**PCG[2]**
Total of SFA	1:113607033-113638343	1.29	-[3]
	4:8338571-8365876	6.64	*CDK14*
	5:115737350-115767786	2.59	-
	23:51299188-51326222	3.71	*GMDS*
C12:0	7:10826539-10852759	1.25	-
	16:20584009-20601201	1.46	*ESRRG*
	29:35323539-35335022	1.02	-
C14:0	1:71431023-71499402	2.52	*SLC51A PCYT1A TCTEX1D2*
	1:109079423-112985104	1.43	*PLCH1*
	2:12006597-12035487	2.84	-
	2:95199347-95226934	2.84	*ADAM23*
	2:108339996-108355638	3.93	-
	8:4017767-4040618	1.61	*GALNTL6*
	9:5964257-5996599	1.34	-
	9:75828407-75861439	1.34	*PEX7*
	12:1848851-1867387	1.23	-
	17:33139235-33161695	1.38	-
	29:14239868-14284421	1.25	-
C16:0	1:71431023-71499402	2.48	*SLC51A PCYT1A TCTEX1D2*
	2:12009907-12039794	1.95	-
	3:6449321-7462185	1.63	*UAP UHMK1 HSD17B7*
	4:27962298-27989717	1.76	-
	8: 4017767-4040618	2.47	*GALNTL6*
	9:7517866-7545663	1.73	*BAI3*
	12:21267027-22190566	2.90	*ATP7B DHRS12*
	20:1657499-1668052	1.80	*FAM196B DOCK2*
C18:0	1:96012245-96039235	6.14	*FNDC3B*
	2:125065946-125101312	1.45	*EPB41*

	3:115395556-115435819	8.33	-
	8:100281732-100312597	3.90	*TMEM245 MIR32*
	17:66287675-66303849	1.45	*SVOP*

[1] Window that consists of continuous 10 SNPs
[2] Positional/putative candidate gene
[3] No PCG associated with the trait.

The regions identified on BTA1 and BTA5 chromosomes associated with the total saturated fatty acids had no PCG. BTA4 at 83 Mb had a window that explained the highest percentage of additive genetic variance for the saturated fatty acids group (Figure 1) and an associated candidate gene. The gene in this region is the *CDK14* gene of the cyclin-dependent kinase family (*CDK*). This gene is associated with production of kinase protein, enzymes that catalyze the phosphorylation of proteins by transferring one phosphoryl group of ATP and in exceptional cases, from GTP to threonine, serine (Ser/Thr specific kinase) or tyrosine residues (Tyr specific kinase) (SILVA et al., 2002). The region found on chromosome BTA23 (Figure 1) houses the *GMDS* gene and acts on the metabolism of amino sugars and nucleotide sugars.

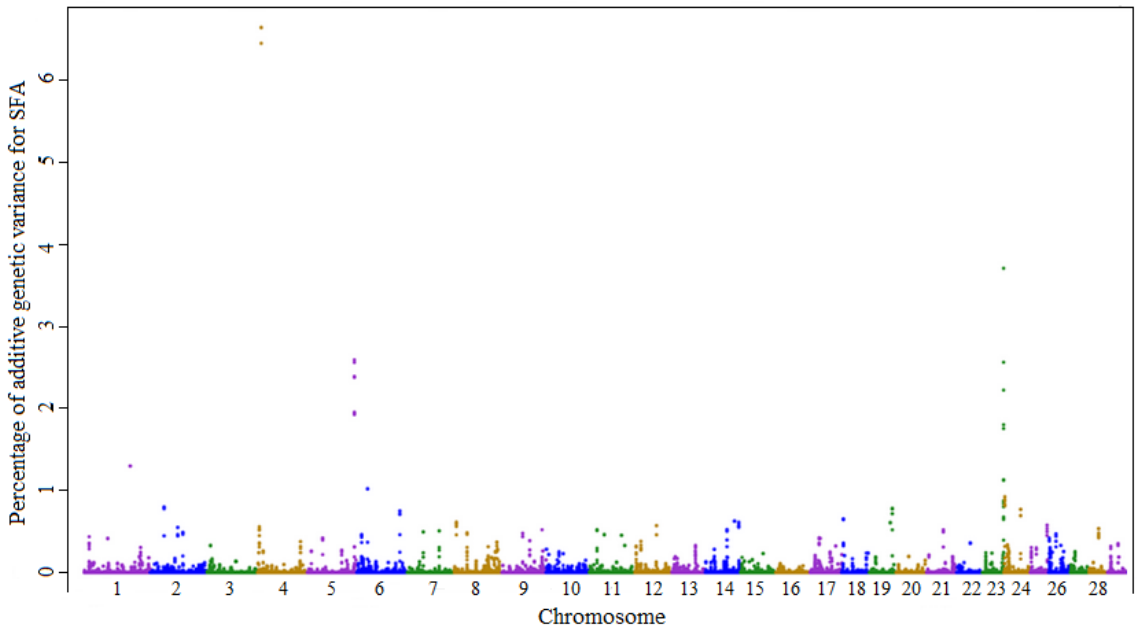

Figure1 – **Manhattan plot of the genome-wide association study for sum of SFA in Nellore**. The X-axis represents the chromosomes, and the Y-axis shows the proportion of genetic variance explained by windows of 10 adjacent SNPs for total saturated fatty acids in Nellore.

Three regions on different chromosomes were associated with lauric (C12:0) acid. The BTA7 at 10 MB and BTA29 at 35 MB with no associated candidate genes and BTA16 at 20 MB associated with the ESRRG gene. The estrogen-related receptor binds to an ERR-alpha response element (ERRE) containing a single consensus half-site, 5'-TNAAGGTCA-3' (Figure 2). Can bind to the medium-chain acyl coenzyme A dehydrogenase (MCAD) response element NRRE-1 and may act as an important regulator of MCAD promoter. Binds to the C1 region of the lactoferrin gene promoter. Requires dimerization and the coactivator, PGC-1A, for full activity.

The *ESRRG* gene has as biological function: transcription, DNA-tamplated (GO:0006351), regulation of transcription, DNA-templated (GO:0006355), transcription from RNA polymerase II promoter (GO:0006366), gene expression (GO:0010467). Some studies show that this gene is strongly correlated with the assembly and regulation of other adipogenic genes, and metabolism and transport of lipids (KUBO et al., 2009; SANOUDOU et al., 2010).

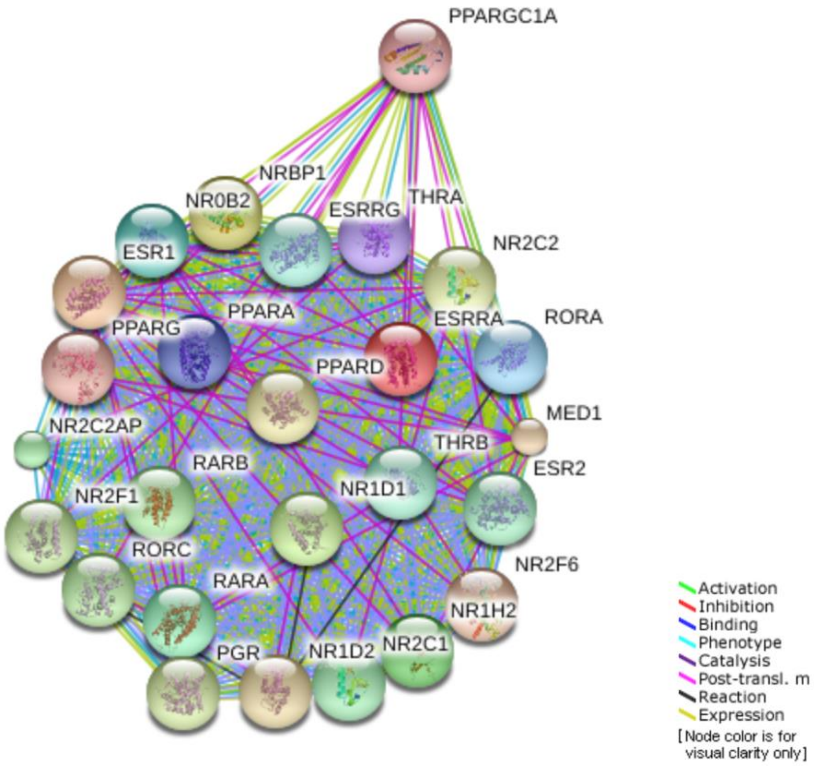

Figure 2 – String interaction network among ESSRG and others 25 genes. Different line colors represent the types of evidence for the association.

A total of eleven areas in seven different chromosomes (BTA1, BTA2, BTA8, BTA9, BTA12, BTA17, and BTA29) were associated with the myristic (C14:0) acid. Two different regions have been identified for this acid. The first, BTA1 at 71 Mb has three associated genes: *SLC51A*, *PCYT1A*, and *TCTEX1D2*. The main candidate is *PCYT1A*, which is a protein-coding gene controlling the phosphatidylcholine, a phospholipid emulsifier with detergent action that reduces surface tension, forming smaller fat particles as triglycerides (GUYTON & HALL, 2002) and has been related to fat percentage and body mass index in humans (SHARMA et al., 2013). This gene is an integrant of the glycerophospholipid metabolism pathway (GO:0006644) (Figure 3); phosphatidylcholine biosynthesis (GO:0006656); phosphatidylcholine from phosphocholine pathways (GO:0006657).

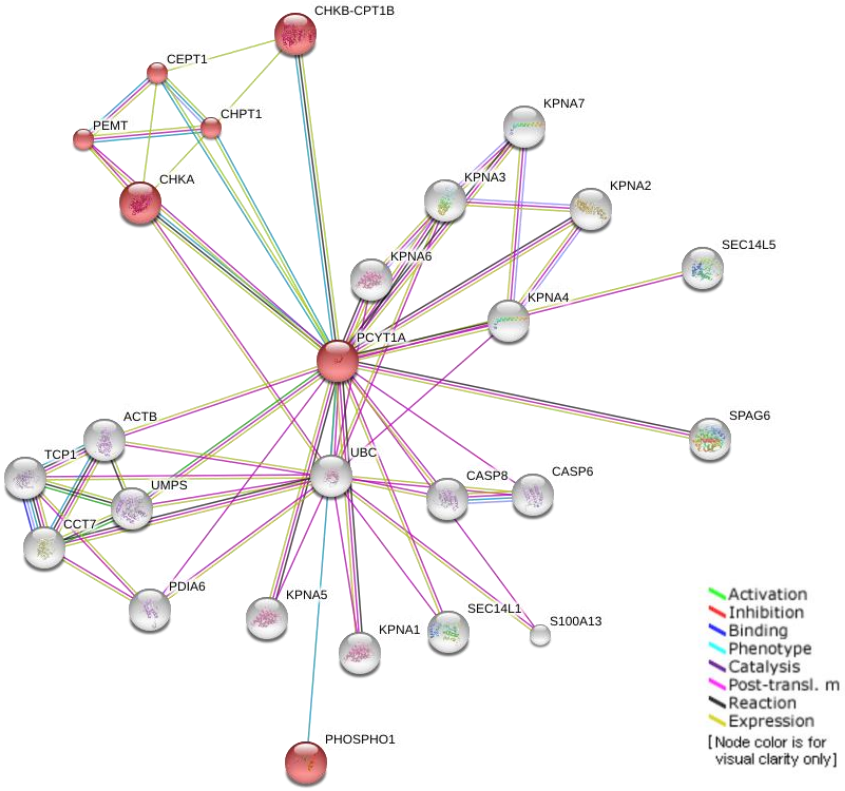

Figure 3 – String interaction network among *PCYT1A* and others 06 genes (highlighted in red), which participate of the glycerophospholipid metabolism pathway.

The second region was identified on BTA1 at 112 Mb and associated with the *PLCH1* gene. This gene is a protein-coding, member of the PLC-eta family of the

phosphoinositide-specific phospholipase C (*PLC*) superfamily of enzymes that cleave phosphatidylinositol 4,5-bisphosphate (PtdIns(4,5)P2) to generate second messengers inositol 1,4,5-trisphosphate (IP3) and diacylglycerol (DAG). Phospholipases are a group of enzymes that hydrolyze phospholipids into fatty acids and other lipophilic molecules. The PLC enzyme is subdivided into beta, gamma, delta, epsilon, zeta, and eta subtypes, which catalyze the hydrolysis of phosphatidylinositol 4,5-bisphosphate (PIP2) into inositol 1,4,5-trisphosphate (IP3) and 1.2-diacilglicerol (DAG). Phospholipases are usually expressed and have diverse biological functions, including a role in inflammation (HWANG et al., 2005). On BTA2 at 95 Mb, a candidate gene was associated with the fatty acid C14:0, *ADAM23*, which, among other functions, was found to suppress adipogenesis in mice (KIM et al., 2015). BTA8 at 4 Mb had a candidate gene (*GALNTL6*) associated with C14:0. This gene catalyzes the initial reaction in the biosynthesis of oligosaccharides, transferring an N-acetyl-D-galactosamine residue to a serine or threonine residue in the protein receptor (PETROVIC et al., 2008). The *PEX7* gene was identified on BTA9 at 75 Mb. This gene encodes cytosolic receptor for the set of enzymes of peroxisomal matrix targeted to the organelle by the PTS2. Mutations in this gene cause disorders in peroxisome biogenesis, which are characterized by multiple modifications in the peroxisome function (WEN et al., 2014).

Eight regions on different chromosomes were identified for the palmitic (C16:0) acid, BTA1 at 71 Mb, BTA2 at 12 Mb, BTA3 at 6 Mb, BTA4 at 27 Mb, BTA8 at 4 Mb, BTA9 at 7 Mb, BTA12 at 21 Mb and BTA20 at 1 Mb. The first region found (BTA1 at 71 Mb) was exactly the same QTL region found for the saturated fatty acid C14:0 and associated with the same genes (*SLC51A*, *PCYT1A*, and *TCTEX1D2*). The larger genomic region found for the group of saturated fatty acids is on BTA3 at 6 Mb (Table 3). This region had only 1.63% of the additive genetic variance for the trait C16:0, in which three candidate genes were located: *UAP1*, *UHMK1*, and *HSD17B7*. The *HSD17B7* encodes an enzyme with the same function of the 17-beta-hydroxysteroid dehydrogenase (EC 1.1.1.62) in sex steroid biosynthesis and 3-ketosteroid reductase (EC 1.1.1.270), and acts on the biosynthesis of cholesterol (MARIJANOVIC et al., 2003). This gene is expressed in most lineages of fast-

growing broilers (D'ANDRE et al., 2013) and is found in the subcutaneous and omental adipose tissue in humans (MACKENZIE et al., 2008).

The region identified on BTA8 at 4 Mb for myristic (C14:0) acid, where the same *GALNTL6* gene described above was located. The *BAI3* gene identified on BTA9 at 7 Mb is known to participate in the myoblast fusion; it is present in the extracellular matrix and plays a role in the adipose tissue formation (HAMOUDA et al., 2014). The BTA12 at 21 Mb QTL was associated with two PCGs: *ATP7B* and *DHRS12*. The *ATP7B* gene participates in the regulation of copper in the body. Mice with this non-functional gene present altered cholesterol and fatty acids synthesis (LUTSENKO, 2014). The *DHRS12* encodes a member of the dehydrogenase/reductase short-chain family. Members of this family are enzymes that metabolize many compounds, such as steroid hormones, prostaglandins, retinoids, lipids and xenobiotics (PERSSON et al., 2009) and a deletion of the region where the gene is associated with lipomas in humans (BARTUMA et al., 2011). BTA20 at 1 Mb was associated with two PCG: *FAM196B* and *DOCK2*, the latter is involved in actin cytoskeleton remodeling through activation of RAC GTPase (KWFIE et al., 2008) and interacts with various lipids (NISHIKIMI et al., 2009).

Five different regions were associated with stearic (C18:0) acid and only one, BTA3 at 115 Mb, was not identified with any PCG. The candidate gene *FNDC3B* was located on BTA1 at 96 Mb while the *EPB41* gene, on BTA2 at 125 Mb. Two PCGs were identified on BTA 8 at 100 Mb: transmembrane protein 245 (*TEMEM245*) and microRNA 32 (*MIR32*). However, none of these genes has a described function in lipid metabolism.

Monounsaturated fatty acids (MUFA)

A total of 37 genomic regions, distributed over sixteen different chromosomes, account for more than 1% of the genetic variance for monounsaturated fatty acids, which relates to total monounsaturated fatty acids, C14:1, C16:1, C18:1 trans11, C18:1 cis9 and C18:1 trans9 (Table 4).

Table 4 - Genomic regions associated with the monounsaturated fatty acids profile in the *Longissimus thoracis* of Nellore

Trait	**QTL window**	**% Variance explained SNP window [1]**	**PCG[2]**
Total MUFA	4:31045067-31056443	1.27	*RAPGEF5*
	4:10646197-10655217	1.10	*CALCR MIR653 MIR489*
	15:23027848-23042930	1.77	*-[3]*
	15:77580057-77608737	1.67	*CKAP5*
	17:19998159-20045296	1.43	-
C14:1	6:20782674-20793003	1.75	*ARHGEF38*
C16:1	3: 93656044-93678760	2.12	*SLC1A7*
	3: 106397884-106406683	1.64	*COL9A2*
	5: 112777768-112833555	1.73	*MIR1281*
	5: 114505769-114536452	1.22	*TTLL1*
	6: 88311624-88350890	1.79	*SLC4A4*
	12: 1698087-1733249	3.27	*TDRD3*
	14: 45588151-45625166	1.12	-
	18: 1131250-1147435	1.35	-
	22:22372870-22388066	1.08	-
	28: 34505791-34523077	1.04	-
C18:1 cis-9	3:6449321-6472465	1.29	
	3:60472950-60520103	1.28	
	4:2070739-2116544	1.12	-
	7:85322486-85362698	1.11	*XRCC4*
	8:101007779-101024030	1.40	*PALM2*
	12:21265917-21282993	2.15	*WDFY2 DHRS12*
	23:44473841-44501022	10.59	*ADTRP*
C18:1 trans-9	4:8337794-8361230	12.00	*CDK14*
	8:68605036-68625678	1.29	-
	9:78101823-78132552	1.87	-
	12:61362119-61385432	1.12	-
	18:56273509-56313047	1.45	*CD37 TEAD2 DKKL1 CCDC155*

	24:56810309-56821447	1.36	*WDR7*
C18:1 trans-11	2: 59116358-59143632	1.78	-
	4: 55957454-55984897	1.23	-
	5: 99332464-99343514	1.06	*YBX3*
	6: 1976981-2001221	1.30	*MARCH1*
	10: 39459983-39493584	2.34	-
	10: 88628112-88637230	1.26	*ESRRB*
	14: 84372868-84383110	1.33	*SNTB1*
	26: 46130255-46142795	3.16	*ADAM12*

[1] Window that consists of continuous 10 SNPs
[2] Positional/putative candidate gene
[3] No PCG associated with the trait.

In the first region of BTA4 at 31 Mb, the *RAPGEF5* gene was associated with total monounsaturated fatty acids. In the second region, all three genes were identified: *CALCR*, *MIR653*, and *MIR489*. The *CALCR* gene expression is associated with the production of various lipids in humans (MIEGUEU et al., 2013) and has SNPs associated with the production of milk fat and body condition in dairy cattle (MAGEE et al., 2010). The BTA15 at 77 Mb was associated with the *CKAP5* gene. No PCG was identified on BTA17 at 19 Mb, and BTA15 at 23 Mb. The C16:1 FA was associated to ten different regions where six PCG were found in. For the first region, BTA3 at 93 Mb, the gene *SLC1A* was identified. The family of this gene includes five high-affinity glutamate transporters, *EAAC1*, *GLT-1*, *GLAST*, *EAAT4* and *EAAT5* (*SLC1A1*, *SLC1A2*, *SLC1A3*, *SLC1A6*, and *SLC1A7*, respectively), also known as excitatory amino acid transporters (EAATs), which are sodium and potassium-dependent members of the solute carrier family 6 (SLC1) found widely distributed in the whole brain (KANAI et al., 2004). On BTA3 at 106 Mb one PCG was identified, COL9A2, which is related to extracellular matrix structural constituent conferring tensile strength according to gene ontology (GO:0030020).

On BTA3, at 6 Mb and 60 Mb, BTA4 at 2 Mb, BTA7 at 85 Mb and BTA8 at 101 Mb, BTA12 at 21 Mb and BTA23 at 44 MB were associated with oleic acid (C18:1 cis 9). BTA7 at 85Mb was associated with the PCG, *XRCC4*. Studies shows that this gene functionally complements XR-1 Chinese hamster ovary cell mutant, which is impaired in DNA double-strand breaks produced by ionizing radiation and restriction enzymes (GIACCIA et al., 1990). On BTA8 at 101 Mb, the PCG *PALM2* was identified. Two genes were identified on BTA12 at 21 Mb: *WDFY2* and *DHRS12*. On

BTA23 at 44 Mb, the *ADTRP*. The genes *DHRS12* and ADTRP were the same reported for C16:0, thus are described above. The genomic region that explained most of the genetic variance of the unsaturated fatty acids group was located on BTA4 at 8 Mb, where the *CDK14* gene was associated with the C18:1 trans-9 (elaidic acid) (Figure 4). The same region was found for total saturated fatty acids (Table 3), where the *CKD14* gene is associated with the same two traits described above. On BTA18 at 56 Mb, four genes associated with the above traits were identified: *CD37*, *TEAD2*, *DKKL1* and *CCDC155*.

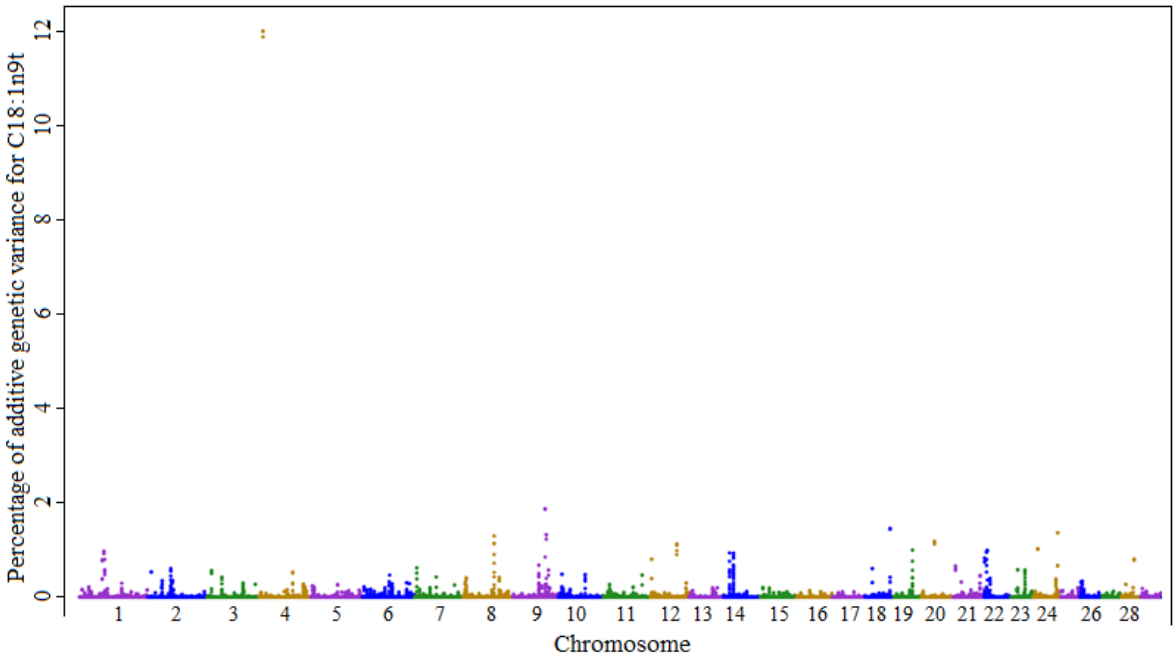

Figure 4 – **Manhattan plot of the genome-wide association study for C18:1n9t (elaidic) in Nellore.** The X-axis represents the chromosomes, and the Y-axis shows the proportion of genetic variance explained by windows of 10 adjacent SNPs elaidic fatty acids in Nellore.

The vacenic fatty acid (C18:1 trans 11) was associated with eight regions in seven different chromosomes. One of these regions, on BTA10 at 88 Mb, was the same associated with lauric (C12:0), thus the PCG *ERSSB* gene was described above.

Polyunsaturated fatty acids (PUFA)

Forty genomic regions explained more than 1% of the additive genetic variation for the polyunsaturated fatty acids group as: C20:4 n-6, C18:2 cis-9 cis12 n-6, C18:3 n-3, C22:6 n-3 and C20:3 n-6 cis-8 cis-11 cis-14 (Table 5).

Table 5 – Genomic regions associated with the polyunsaturated fatty acids in the *Longissimus thoracis* muscle of Nellore.

Trait	**QTL window**	**% Variance explained SNP window [1]**	**PCG[2]**
C20:4 n-6	3:3469461-3492275	2.15	-[3]
	5:4417952-4432872	3.06	-
	9:84138533-84184280	4.95	*FBXO30*
	15:27938668-27964533	1.17	*SIK3*
	16:61647491-61661042	2.34	*RALGPS2 ANGPTL1*
	19:61671067-62341372	5.01	*ABCA5 ABCA6 ABCA10 MAP2K6 PRKAR1A*
	21:70498936-70511360	1.72	-
	23:25179729-25202537	4.20	*ELOVL5*
C18:2 cis9 cis12 n6	1: 71431023-71499402	4.92	*SLC51A PCYT1A TCTEX1D2*
	8: 4017767- 4040618	2.06	*GALNTL6*
	11:82837386-82851713	3.09	*DDX1*
	12: 1850108- 1876467	3.38	-
	21:17398974-17427182	4.04	-
	23:44473841-44501022	9.33	*ADTRP*
C18:3 n3	4:111759995-111777571	2.73	*CNTNAP2*
	12: 1721955-1759560	2.84	*TDRD3*
	12: 1848851-1867387	11.49	-
C22:6 n-3	1:35178519-35188850	1.20	-
	3:24528861-24552342	1.46	-
	7:92496213-92524656	1.20	*GPR98*
	8:108906430-108932062	5.10	-
	12: 1848851-1867387	3.56	-
	23:44473841-	1.63	*ADTRP*

	44501022		
	24:11304045-11346437	1.49	-
	25:12681505-12706195	1.12	-
	26:45184835-45225853	4.12	-
C20:3 n-6 cis-8 cis-11 cis-14	5: 5499399-5512486	1.31	-
	8: 1640176-1659771	1.72	-
	10:97347302-97359563	1.23	-
	11:62497171-62519808	2.96	*PELI1*
	15: 7810022-7842535	1.14	-
	20: 1657499-1668052	1.39	*FAM196B DOCK2*
	25:12681505-12706195	1.80	-
Total PUFA	14:21344791-21376691	1.33	-
	2:63074224-63103605	2.01	*TMEM163*
	10:97347302-97359563	1.52	-
	12:36843604-36886833	6.24	*RNF17*
	14:80616824-80631460	1.97	*RALYL*
	21:17398974-17427182	1.23	*AGBL1*
	23:44473841-44501022	6.79	*ADTRP*

[1] Window that consists of continuous 10 SNPs
[2] Positional/putative candidate gene
[3] No PCG associated with the trait.

On BTA2 at 63 Mb, the *TMEM163* gene was associated with the total PUFA. *TMEM* 163 variants were associated with decreased fasting plasma insulin and also with the homeostatic model assessment of insulin resistance, indicating plausible effect through impaired insulin secretion (TABASSUM et al., 2013). The fatty acid C20:4 n-6 was associated with eight different genomic regions (Table 5). On BTA9 at 84 Mb, the *FBXO30* gene was associated with arachidonic acid. BTA19 at 61 Mb (the largest region found for the group of polyunsaturated fatty acids) had five associated genes: *ABCA5*, *ABCA6*, *ABCA10*, *MAP2K6*, and *PRKAR1A*. The genes of the ABC Group are associated with cholesterol metabolism and lipids homeostasis

(SCHMITZ et al., 2001), as well as *ABCA5*, *ABCA6* and *ABCA10* (YE D et al., 2008; WENZEL et al., 2003). The *MAP2K6* gene was associated with backfat thickness, marbling score and carcass weight in Hanwoo cattle (RUY et al., 2012) and identified in two selection signatures in Galloway and Gelbvieh cattle (ROTHAMMER et al., 2013). The *PRKAR1A* is involved in the regulation of lipid and glucose metabolism and is a component of the signal transduction mechanism of certain GPCRs (G-protein coupled receptor) (MUHN et al., 2013). Mutations in this gene have been associated with obesity phenotypes in humans (LONDON et al., 2014).

On BTA23 at 25 Mb, the *ELOVL* fatty acid elongase 5 (*ELOVL5*) gene was associated with the fatty acid C20:4 n-6. This gene plays an important role in the elongation of saturated and monounsaturated fatty acids up to 24 carbons (GO:0009922), condensing enzymes that catalyze the synthesis of monounsaturated and polyunsaturated fatty acids of very long chains, specifically the current polyunsaturated acyl-CoA with higher activity C18:3(n-6) acyl-CoA (LEONARD et al., 2000). In mammals, seven enzymes have been identified in the *ELOVL* family (*ELOVL1-7*). Each *ELOVL* enzyme has a distinct distribution in different tissues, and different enzymes exhibit different preferences for the fatty acid substrate.

The *ELOVL5* and *ELOVL6* genes are involved in some biological process as fatty acids biosynthetic process (GO:0006633) (Figure 5), unsturated fatty acids biosynthetic process (GO:0006636) (Figure 6), triglyceride biosynthetic process (GO:0019432), unsaturated fatty acid biosynthetic process (GO:0033559) and fatty acid elongation (Figure 7), monosaturated fatty acid (GO:0034625), the production/synthesis of palmitic (C16:0), palmitoleic (C16:1), stearic (C18:0) and oleic (C18:1) fatty acids, important beef fatty acids. Therefore, the role of *ELOVL5* and *ELOVL6* genes in the synthesis of these fatty acids is of great importance in beef breeding programs (GREEN et al., 2010; TAMURA et al., 2009).

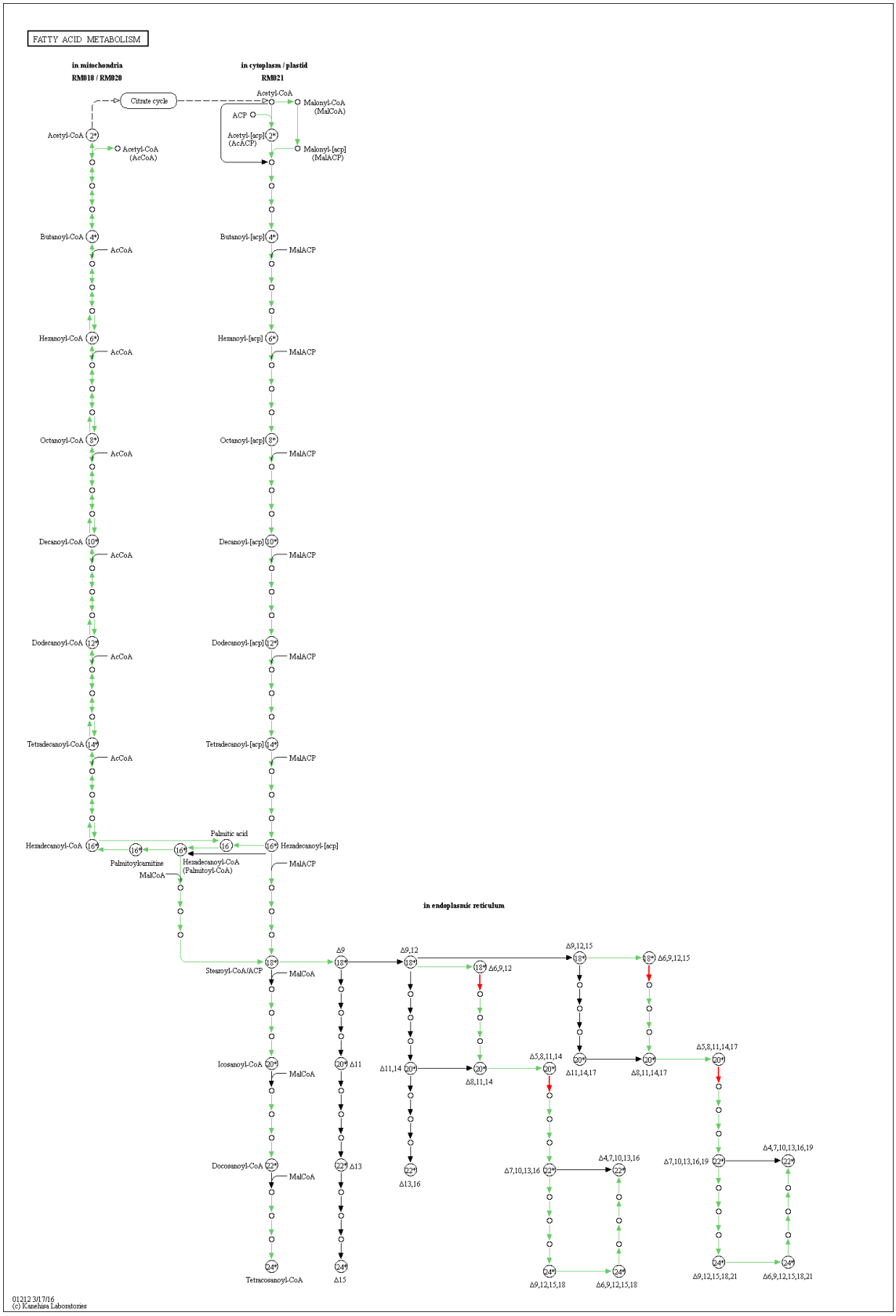

Figure 5 – Fatty acid metabolism pathway (Kyoto Encyclopedia of Genes and Genomes, 2016)

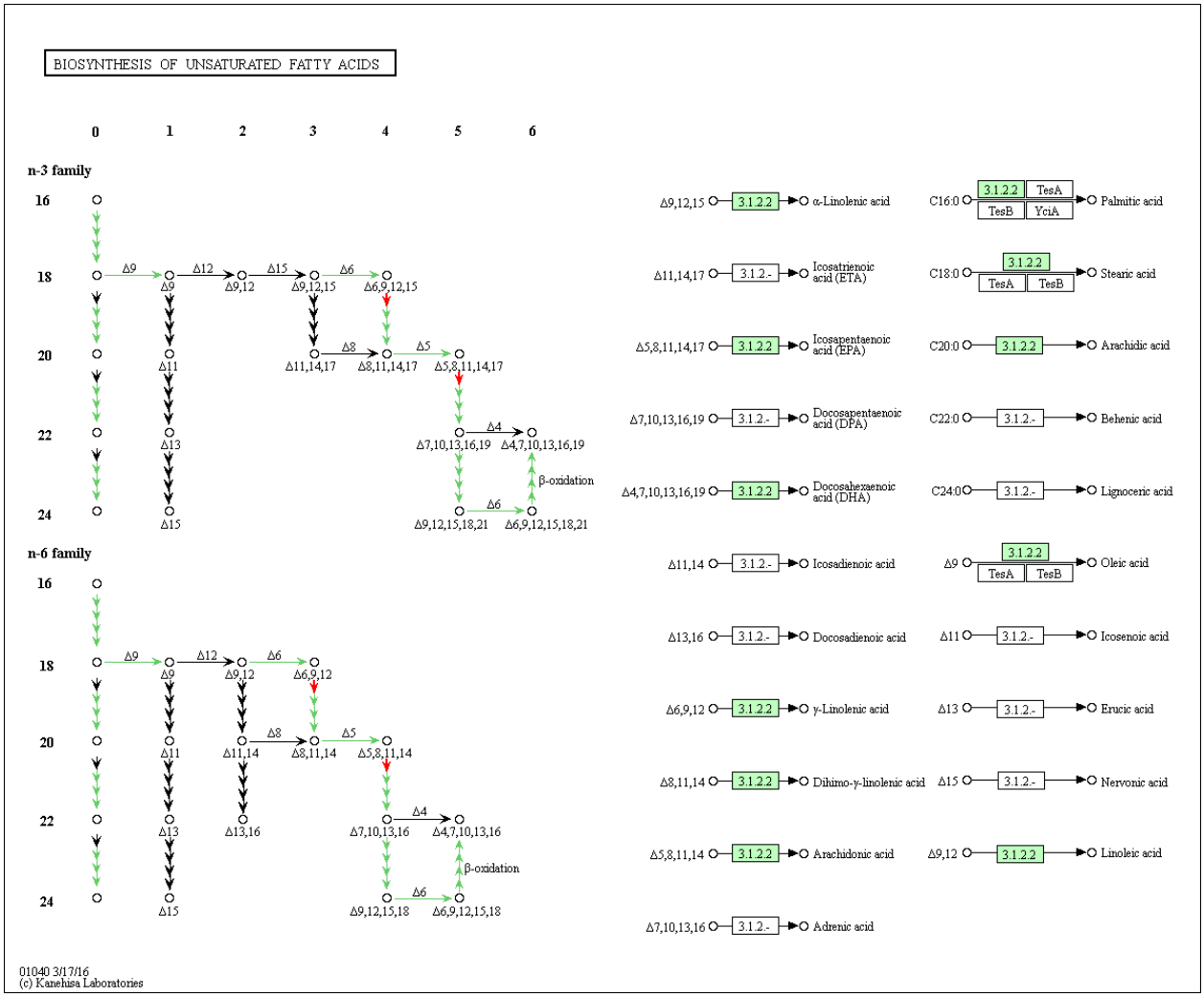

Figure 6- Biosynthesis of unsaturated fatty acids pathway (Kyoto Encyclopedia of Genes and Genomes, 2016)

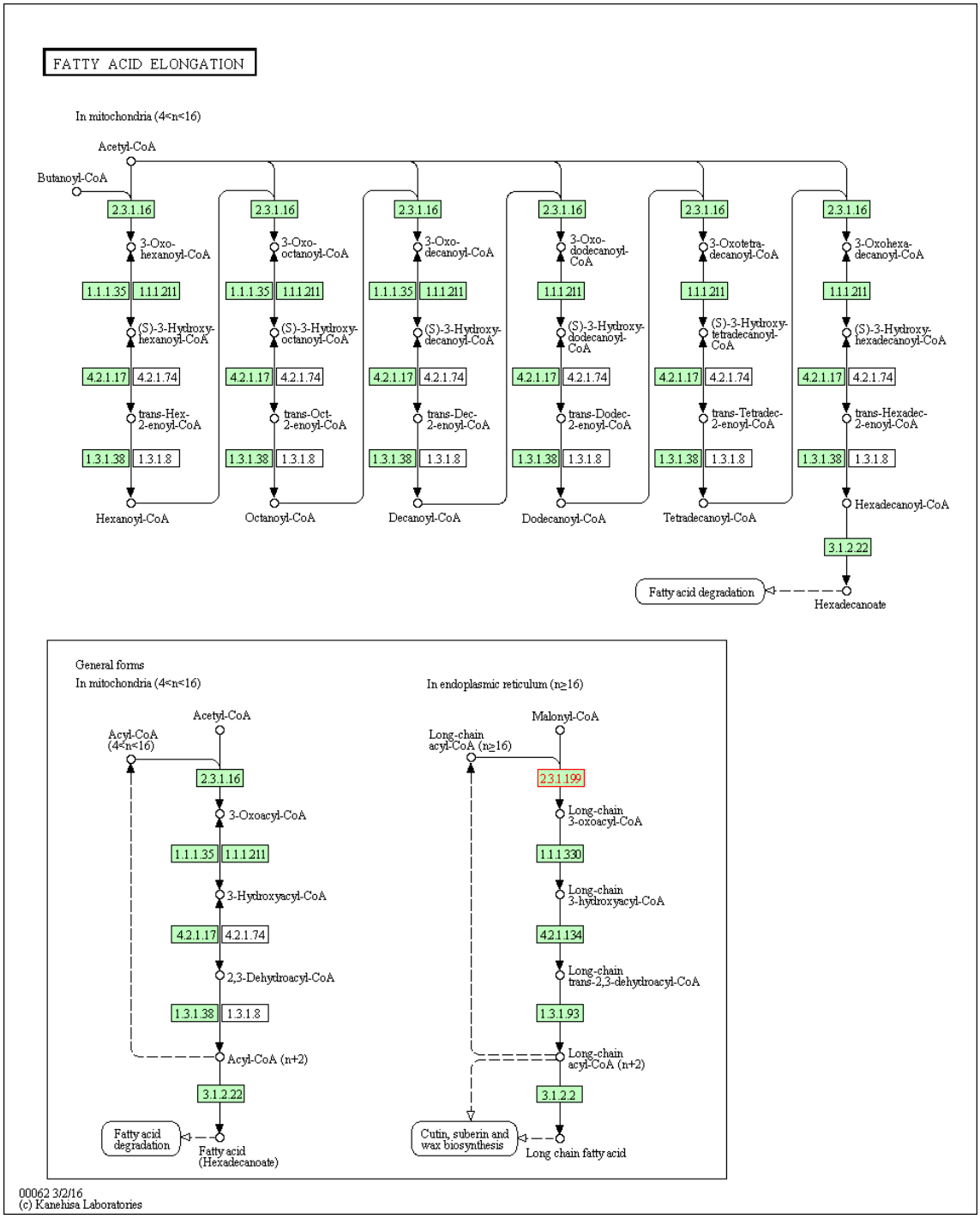

Figure 7 – Fatty acid elongation pathway (Kyoto Encyclopedia of Genes and Genomes, 2016)

The genomic regions of BTA1 at 71 Mb, BTA8 at 4 Mb, BTA11 at 82 Mb, BTA12 at 1 Mb, BTA21 at 17 BM and BTA23 at 44 Mb were associated with the linoleic (C18:2 cis-9 cis-12; n-6) acid. The two regions, BTA1 at 71 Mb and BTA8 at 4 Mb were similar to those observed for the saturated fatty acids C14:0 and C16:0,

where the *SLC51*A, *PCYT1A*, *TCTEX1D*, and *GALNTL6* genes have been previously described. The BTA23 at 44 Mb position had a candidate gene associated and explaining a important proportion of the additive genetic variance of the polyunsaturated fatty acids group (Figure 8). This same region had the *ADTRP* gene associated with total polyunsaturated fatty acids. The BTA11 at 82MB was associated with C18:2 cis-9 cis-12 n-6, (gene: *Asp-Glu-Ala -Asp*) box helicase 1 (DDX1), which acts as a RNA helicase dependent of ATP, able to relax both RNA-RNA and DNA-RNA duplexes (Chen et al., 2002).

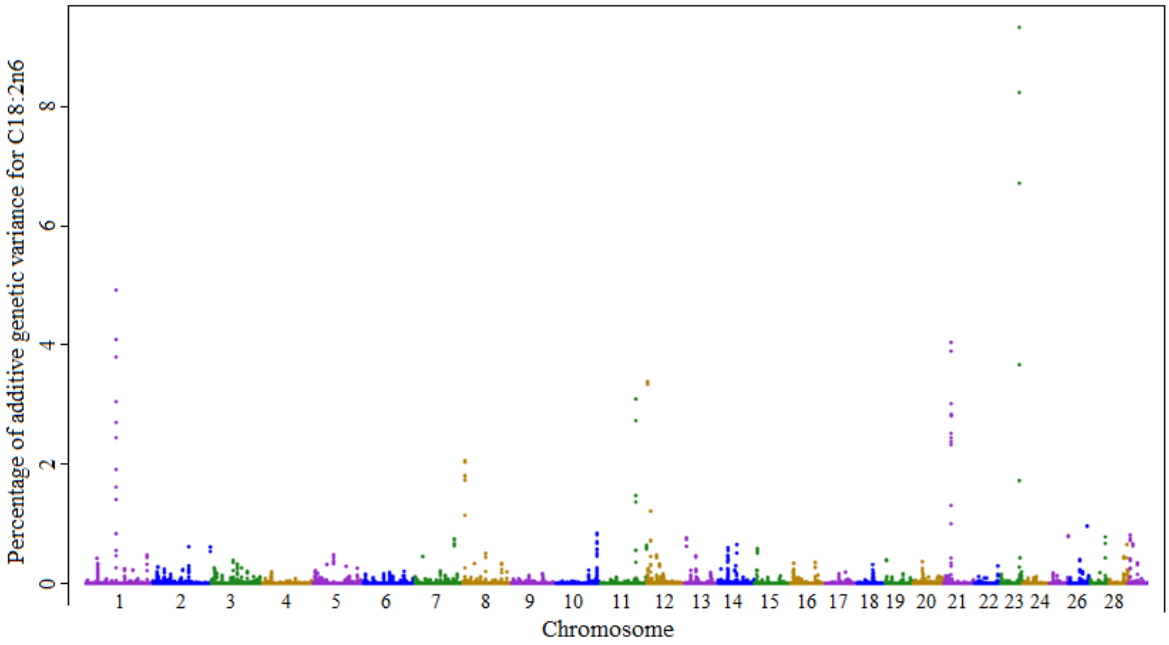

Figure 8 - **Manhattan plot of the genome-wide association study for C18:2n6 (linoleic) in Nellore.** The X-axis represents the chromosomes, and the Y-axis shows the proportion of genetic variance explained by windows of 10 adjacent SNPs linoleic fatty acids in Nellore.

BTA4 at 111 Mb and BTA12 at 1 Mb were associated with the linolenic (C18:3 n-3.) BTA4 at 111 Mb was associated with the *CNTNAP2* gene, which implies diet-induced obesity (Buchner et al., 2012). The gene tudor domain containing 3 (*TDRD3*) was identified on BTA12 at 1 Mb (YANG et al., 2010).

Nine QTL regions were associated with C22:6 n-3 FA, but PCGs were found in only two of these regions: BTA7 at 92 Mb and BTA23 at 44 Mb. BTA7 at 92 Mb was associated with the *GPR98* gene. This gene encodes a member of the receptors superfamily coupled to the G protein. This encoded protein contains a seven-

transmembrane receptor domain bound to calcium. It is expressed in the central nervous system (JACOBSON et al., 2008) and associated with the body condition score in humans (GIACCIA et al., 1990). BTA23 at 44 Mb was also associated with the fatty acid C18:2 cis-9 cis-12 n-6 and, therefore, PCG *ADTRP* has been described above.

The fatty acid C20:3 n-6 cis-8 cis-11 cis-14 was associated with seven regions in seven different chromosomes, but PCGs were identified in only two regions: BTA11 at 62MB and BTA20 at 1 Mb. The PCG *Pellino E3* ubiquitin protein ligase 1 (*PELI1*) was identified on BTA11 at 62 Mb. BTA20 at 1 Mb had two PCGs identified: family with sequence similarity 196, member B (*FAM196BI*) and dedicator of cytokinesis 2 (*DOCK2*). *DOCK2* is involved in cytoskeletal rearrangements necessary for lymphocyte migration in response to chemokines. This PGC activates *RAC1* and *RAC2*, but not the *CDC42*, because it acts as a guanine exchange factor (GEF) nucleotide that changes the *GDP* to free GTP (KWFIE et al., 2008).

Omega 3 and 6 fatty acids

A total twenty-one genomic regions accounted for more than 1% of the genetic variance for n-3 and n-6 fatty acids, and the n-6:n-3 ratio (Table 6).

Table 6 - Genomic regions associated with the omega-3 and omega-6 fatty acids, and the omega-6/omega-3 ratio in the *Longissimus thoracis* muscle of Nellore.

Trait	**QTL window**	**% Variance explained SNP window [1]**	**PCG[2]**
Total n-3	3:7439426-7455843	1.14	*NOS1AP*
	7:92487944-92521811	1.45	*GPR98*
	8:88219918-88234648	9.99	-[3]
	12:1850108-1876467	3.53	-
	21:17398974-17427182	1.13	*LOC100300175*
	25:12775670-12817779	1.22	-
Total n-6	2:63074224-63103605	1.68	*TMEM163*
	3:49887762-49898290	1.41	*BCAR3*
	10:97347302-97359563	1.69	-
	12:36843604-36886833	5.61	*RNF17*
	14:80616824-80631460	1.86	*RALYL*
	23:44473841-44501022	6.68	*ADTRP*
Ratio of n-6:n-3	1:60602819-60636700	1.11	-

	2:131895441-131915476	1.69	-
	3:53399171-53439878	1.39	*ZNF326*
	6:28611816-28631205	1.92	-
	10:52660431-52708192	1.01	*POLR2M*
	10:88145431-88173762	1.01	*TTLL5*
	16:46586463-46598618	1.78	-
	16:63634797-63657084	1.61	*MR1*
	29:10816550-10828285	1.16	*LOC505383*

[1] Window that consists of continuous 10 SNPs
[2] Positional/putative candidate gene
[3] No PCG associated with the trait.

On BTA3 at 7 Mb, BTA7 at 92 Mb, BTA8 at 88 Mb, BTA12 at 1 Mb, BTA21 at 17 Mb, and BTA25 at 12 Mb were associated with total n-3 fatty acids. On the other hand, no PCGs were identified on BTA8 at 88 Mb, BTA12 at 1 Mb, and BTA25 at 12 Mb. Moreover, BTA3 at Mb 7 Mb and BTA7 at 92 were also associated with the C18:1 cis-9 and C22:6 n-3 fatty acids (FA) and, therefore, with the *NOS1AP* and *GPR98* genes described above, respectively.

On BTA2 at 63 Mb, BTA3 at 49 Mb, BTA10 at 97 Mb, BTA12 at 36 Mb, BTA14 at 80 Mb and BTA23 at 44 Mb were associated with total n-6 fatty acids. The BTA2 at 63 Mb harbors the gene transmembrane protein 163 (*TMEM163*). This same region has also been associated with total polyunsaturated fatty acids (PUFA). The BTA3 at 49 Mb was the largest region associated with the omega fatty acids group, where the *BCAR3* gene is found. This gene has been linked to breast cancer in humans (OH et al., 2008). BTA12 at 36 Mb, BTA14 at 80 Mb and BTA23 at 44 Mb have been associated previously with other long-chain fatty acids, whereas the latter region explained the greatest proportion of variance (Figure 9). BTA12 at 36 Mb and BTA14 at 80 Mb were also associated with total polyunsaturated fatty acids and the genes already described. BTA23 at 44 Mb was also associated with the polyunsaturated FAs: C18:2 cis-9 cis-12 n-6, C22:6 n-3 and total PUFA.

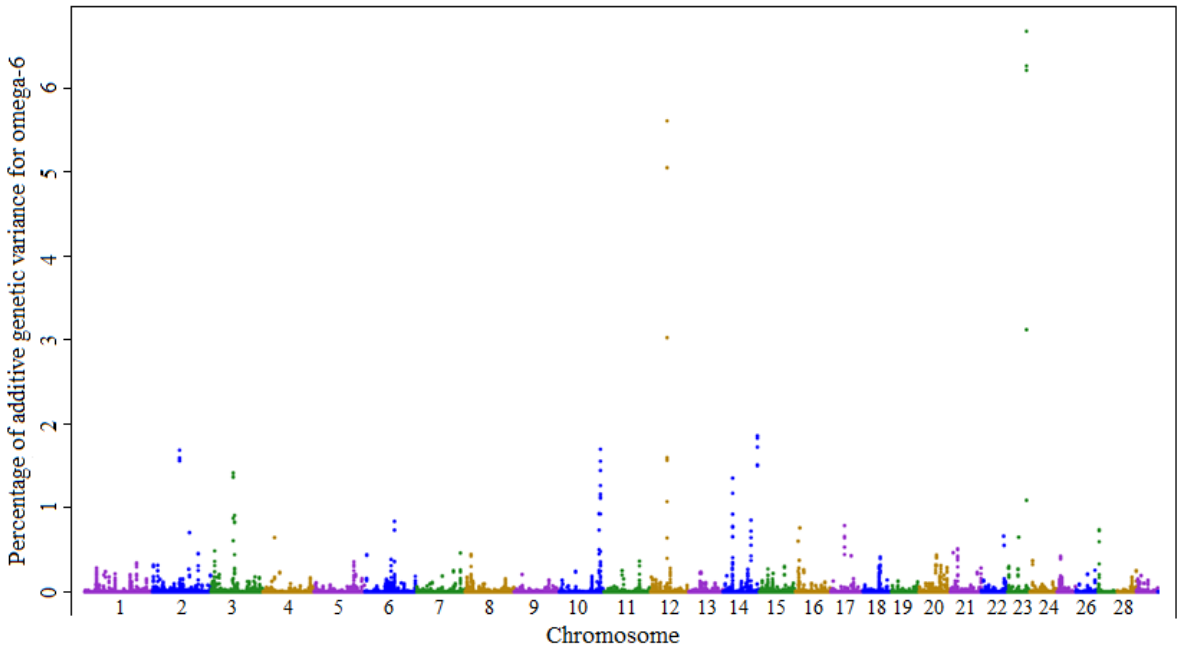

Figure 9 - **Manhattan plot of the genome-wide association study for sum omega-6 in Nellore.** The X-axis represents the chromosomes, and the Y-axis shows the proportion of genetic variance explained by windows of 10 adjacent SNPs omega-6 fatty acids in Nellore.

The single step method method allows to combine the information of genotyped and non-genotyped animals in the genetic evaluation process, thus expanding the scope and identification of potential regions associated with loci responsible for variations in the studied traits (KEMPER et al., 2012). In this sense, some authors (WANG et al., 2014) compared different GWAS methodologies and reported that the single step method method partitions the genetic variance for a particular region among all SNPs markers. On the other hand BayesB or BayesCpi methods, for example, penalizes the zero regions and tends to overestimate the genetic variability explained for each of the identified regions. There is some controversy regarding the capacity or ability of different methods to identify genomic regions associated with the phenotype (ZENG et al., 2012; WANG et al., 2014; MIZTAL et al., 2013). Therefore, caution should be applied when interpreting results from different populations, using various methods because the identified associations depend on the strength or magnitude of the association, methodology, and implementation details.

In this study, we found several nearby areas of major QTL associated with groups of saturated, monounsaturated, and polyunsaturated fatty acids, in Nellore meat. These regions harbor interesting PCG, which are involved in lipid metabolism, as a constituent of cell membranes, receptors for reproductive hormones, biosynthesis and hydrolysis of phospholipids and membrane constituents, synthesis of protein kinases, transport and use of fatty acids and cholesterol, energy metabolism, elongation factors and synthesis of long-chain fatty acids in different species. Among the many genes identified, the *ELOVL5* gene located on chromosome BTA23 at 25 Mb and associated with the C20:4 n-6 (arachidonic acid) is highlighted. The genes responsible for the elongation of very-long-chain fatty acid (*ELOVL*) encode enzymes that play an important role in the elongation of long-chain fatty acids. The fatty acid synthesis involves a number of enzymes, such as fatty acid synthase (*FASN*), which is located on chromosome BTA19 between 51.384.922 and 51.403.614 bp, while its variations have been related to fatty acid composition of Angus beef (ZHANG et al., 2008). In mammals, *FASN* synthesizes the fatty acids that contain up to 16 carbon atoms, and the genes of the *ELOVLs* group produce long-chain fatty acids with 18 carbon atoms or more (JAKOBSON et al., 2006; BONET et al., 2011) (Figure 10)

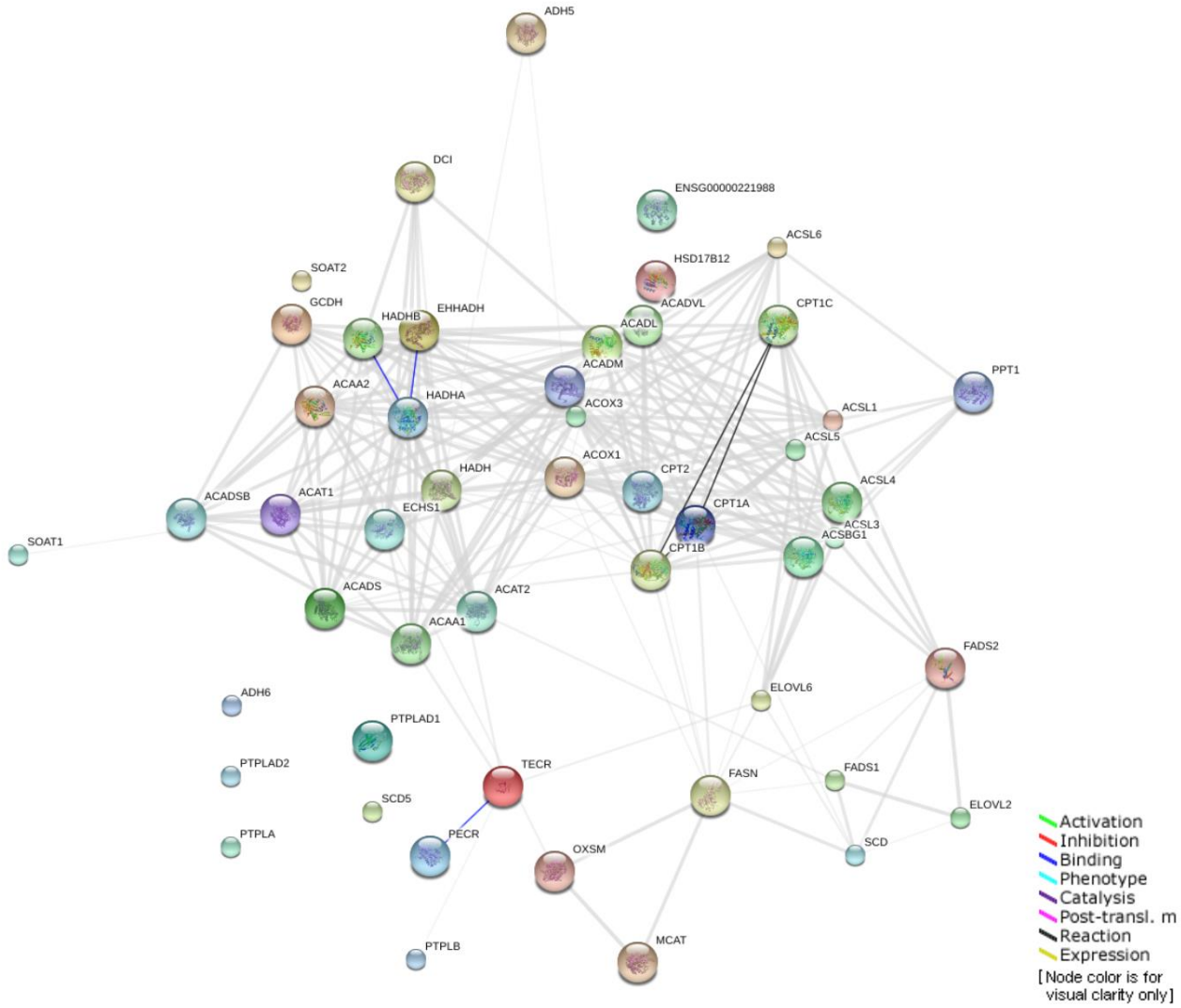

Figure 10 - Figure 3 – String interaction network among ELVOL and others 67 genes including the gene FASN.

Enrichment metabolic pathways

In an attempt to analyze all genes in a general form and see how they work together, we used Database for Annotation, Visualization, and Integrated Discovery (DAVID) v6.7 using cattle as the background, to identified functional gene clusters, which were related to transmembrane helix, integral component of membrane, transport, glycoprotein, transcription regulation, ATP-binding among other (Table 7). Due the number of genes found in this study is considered low, the DAVID statistical

analysis indicated that there are a few genes enriched the clusters annotation and the functional categories were related to membrane functions and some energy functions, but no one has a significant p-value and no one pathway (KEGG) was enriched by the gene group, just GOTERMS and UP_KEYWORDS.

The main function of the first cluster, which has the smallest p-value, was associated with membrane function. One of the membrane function is the secretory membrane system, with allows a cell to regulate delivery of newly synthesized proteins, carbohydrates, and lipids to the cell surface, a necessity for growth and homeostasis (MILLER et al., 2003). Transport of substances across cell membranes is required for several vital functions including digestion, absorption of nutrients, cellular signaling, growth, proliferation, cell death and survival which have previously been reported as influencing feed efficiency traits in beef cattle (SERÃO et al., 2013).

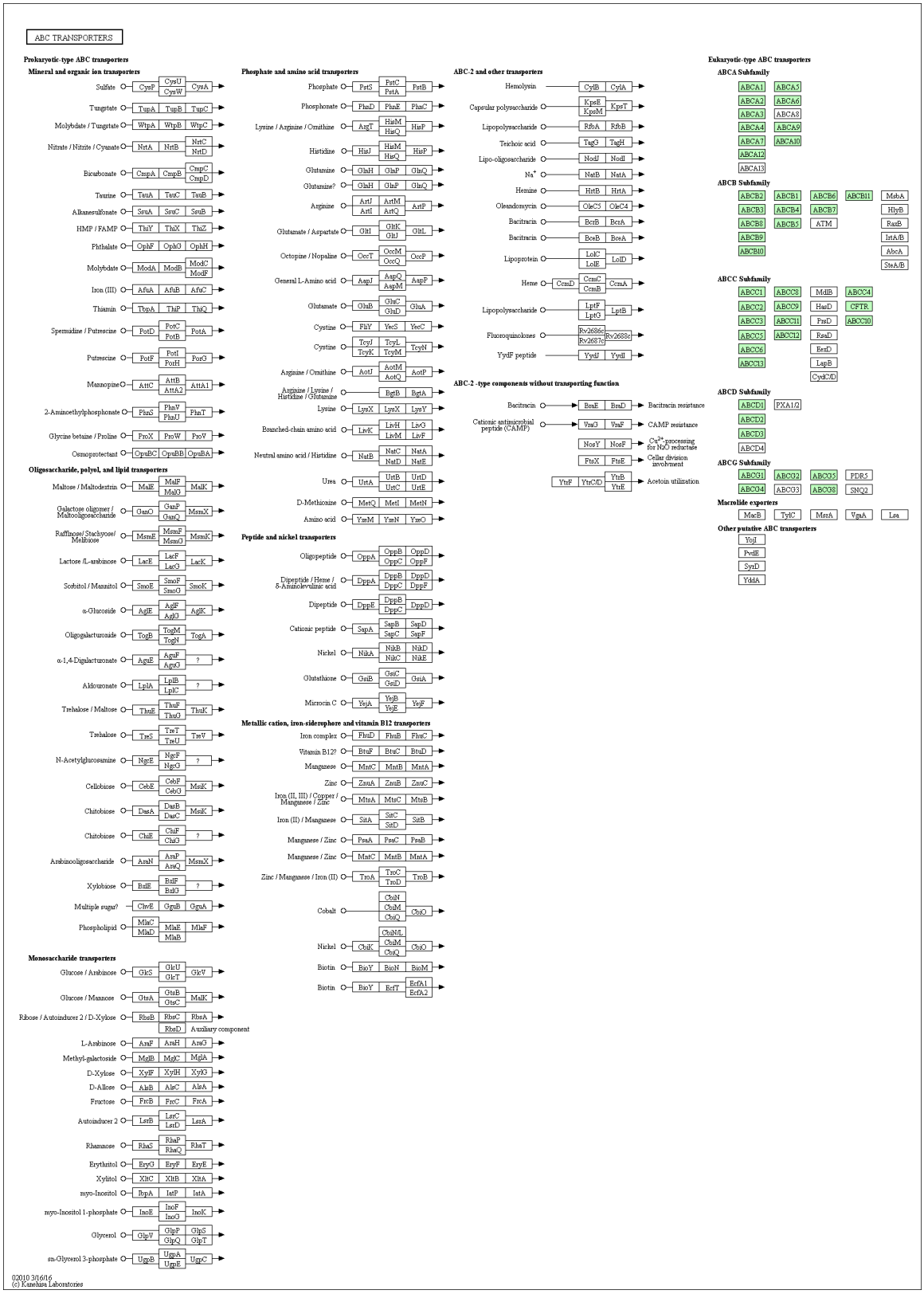

Figure 11- ABC transporters metabolic pathways (Kyoto Encyclopedia of Genes and Genomes, 2016)

We employed the Ingenuity Pathway Analysis (IPA) online software to detect the canonical pathways involving the genes of this study (Figure 12). IPA is a web-based software application for the analysis, integration, and interpretation of data derived from ‘omics experiments, such as RNAseq, small RNAseq, microarrays including miRNA and SNP, metabolomics, proteomics, and small- scale experiments that generate gene and chemical lists. Howerver, this software works just with human information.

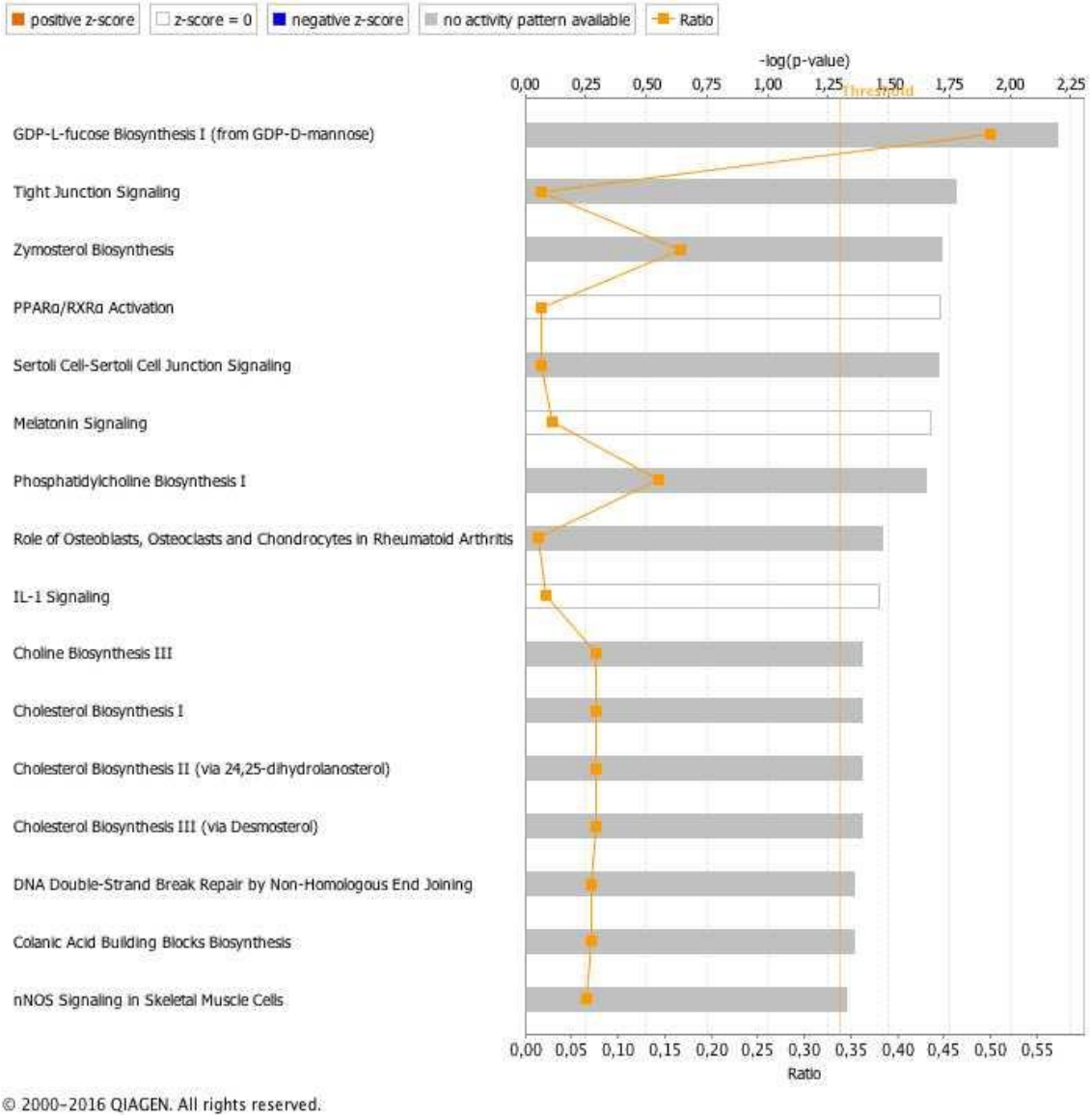

Figure 12 - Canonical pathways enriched with the list of genes by Ingenuity Pathway Analysis (IPA) ($P < 0.05$)

Once again, none canonical pathway was significante (p-value <0.05). A large proportion of the pathways acted on fucose and cholesterol biosynthesis, and PPARa activation, which would provide valuable insights into explaining the molecular mechanism of lipid metabolism.

As one of the pathways showed on canonical pathway, the PPARalpha has a great role in the regulation in the fatty acids metabolism. Peroxisome proliferator-activated receptors (PPARs) are nuclear hormone receptors that are activated by fatty acids and their derivatives and play an essential physiological role in the regulation of adipocyte tissue development lipogenesis and skeletal muscle lipid metabolism (BERGER and MOLLER, 2002; HIHI et al., 2002; ABBOTT 2009). Doran et al. (2014), studying GWAS in Holstein-Friesan cattle identified the PPAR signaling pathway as the most significantly overrepresented biological pathway involved in carcass trait performance, suggesting that PPAR would appear also play a key role in controlling carcass weight, carcass fat and carcass conformation traits.

We examined the gene networks to predict the participation of other interacting molecules in the pathways. The IPA software showed a network of 35 genes interacting each other, of which 13 (ABCA5, ATP7B, CNTNAP2, COL9A2, EPB41, ESRRG, PEX7, PLCH1, POLR2M, RNF17, SLC147, SNTB1, TEAD2) (Figura 13).

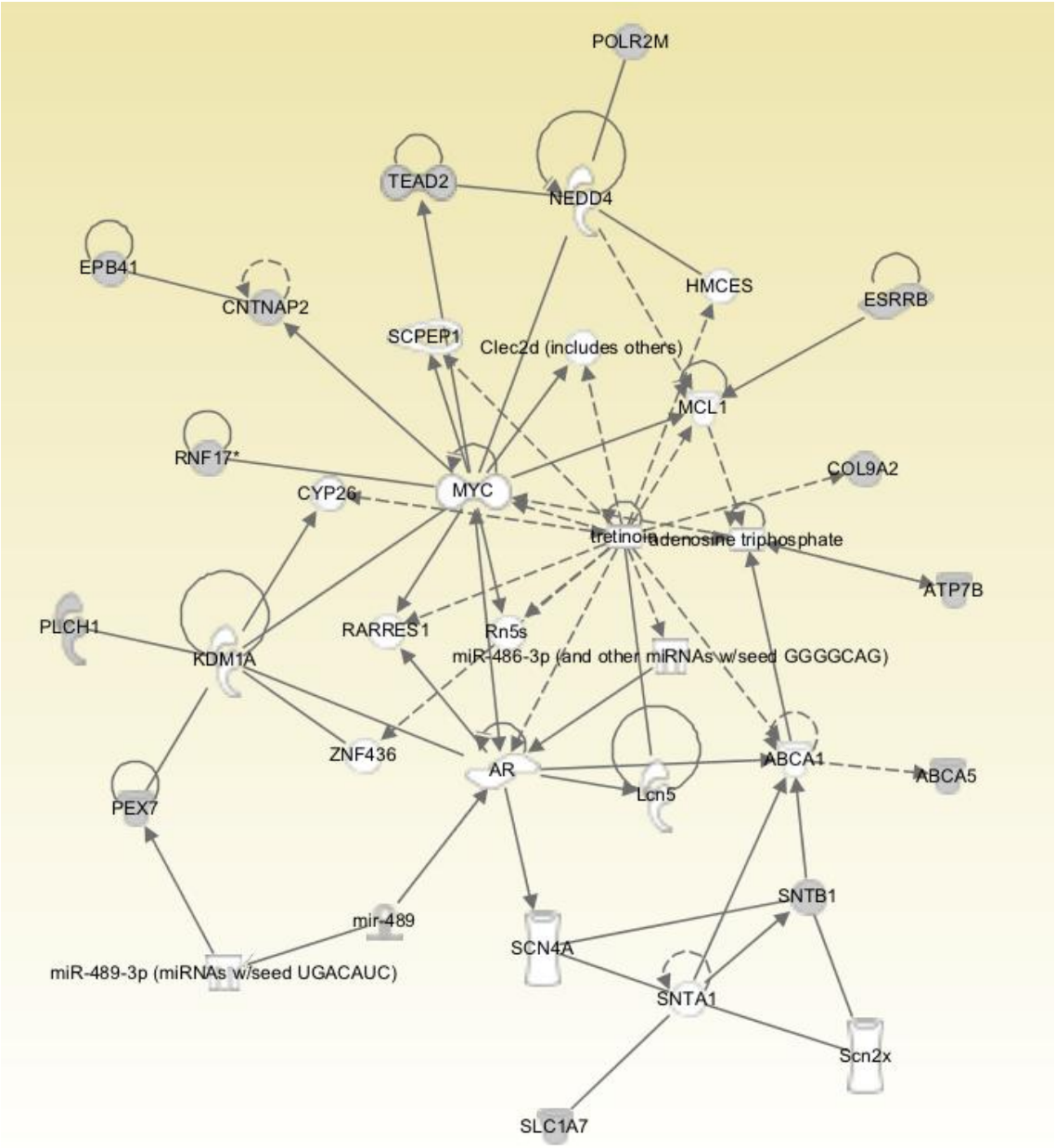

Figure 13 – Molecule network identified by **Ingenuity Pathway Analysis (IPA)**.

The most conssential functions constituinte were gene expression, lipid metabolism and molecular transport. Lipid and protein metabolism were previously related to body composition and protein turnover, are widely known and discussed as important bases for the physiology of feed efficiency (RICHARDSON and HERD 2004; MOORE et al. 2009; HERD and ARTHUR 2009).

Li et al., 2015 sampled spleen tissues from grass-fed and grain-fed Angus

steers and performed a comparative study of gene expression using RNASeq method. Then, based on the differentially expressed genes (DEGs), they implemented a functional analysis and identified potential mechanisms that could contribute to the difference observed between both groups. The authors have detected 123 DEGs between grass- fed and grain-fed spleen of Angus cattle. In the grass-finished group, 87 were up-regulated while the other 36 decreased their gene activity. Based on these genes, they performed an Ingenuity Pathway Analysis (IPA) and identified 9 significant molecular networks and 13 enriched biological pathways. Two of the pathways, Nur77 sig- naling in T lymphocytes and calcium-induced T lymphocyte apoptosis which are immune related, contain a pair of genes HLA-DRA and NR4A1 with dramatically altered expression level.

Our results provide general information regarding the molecular mechanism and functional of the lipid metabolism. However, we recognized that our study suffered some limitations. Identification of the PCG and the follow-up pathway/network analysis were conducted merely relying on the computational strategy; extensive experimental valida- tion work is still needed.

The results of the present study pointed out some PCG that were found associated to several FA of different saturation state: the *CDK14* gene was associated with C18:1 trans-9 and SFA; the *TMEM163* gene related to n-6 fatty acids and PUFA; and the *SLC51*A, *PCYT1A*, *TCTEX1D*, and *GALNTL6* genes that influence the linoleic acid and also some individual saturated fatty acids, like the C14:0 and C16:0. These results could be due to pleitropic effects, where the expression of differents FA could influenced by the same gene which acts in a coordinate manner to contribute to the synthesis of beef FA. Similar findings were reported by (SAATCHI et al., 2013), where both genes, *FASN* and *THRSP*, exhibited pleiotropic effect for the most studided FA in Angus cattle. Genes involved in muscle lipid composition of 15 european Bos taurus breeds were studied by (DUNNER et al., 2013), which reported pleiotropic effects for genes like *CRI1*, *DGAT1*, *FOXO1*, *MMP1*, *SOC2* and *NEB*, affecting several beef FA.

The large number of genomic regions associated with the fatty acid profile found in this study should help to understand the genetic and metabolic mechanisms that determine the fatty acids profile of intramuscular fat, especially in zebu.

According to the results of this study, the meat fatty acid profile of Zebu is probably controlled by several QTL of small effect and, therefore, the identification of relevant genes or large effect seems to be difficult, since for most fatty acids, the contribution of each region or window for the additive genetic variation was small. Therefore, strategies such as genomic selection using or considering the variability among markers at the same time would be more appropriate to improve the fatty acid profile of the bovine meat. The database used in the study is broad since it contains animals that participate in beef cattle breeding programs, and breeders that are sold and used in various regions of the country. Therefore, the results should contribute to the selection and improvement of the meat quality from zebu raised in tropical conditions.

4. CONCLUSION

Several genomic regions associated with QTL related to lipid metabolism and fatty acid composition were identified. The identification of such regions and the respective candidate genes associated with lipid metabolism and energy transport hormones such as, for example, *ELOVL5*, *ESRRG*, *PCYT1A* and genes of the *ABC* group (*ABCA5*, *ABCA6* and *ABCA10*) as well their respective metabolic pathways, should contribute to improve the genetic knowledge regarding the fatty acids profile of Nellore (*Bos indicus*) and help to improve the selection of such traits to favor human health. In addition, these regions can be used in future fine mapping studies, whose primary function is to search for informative causative mutations. These polymorphisms can be inserted into customized low-density chips that assist a more cost-effective genetic evaluation.

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CHAPTER 4 - Association study between copy number variation and fatty acid profile of Nellore beef

ABSTRACT - The aim of this study was to analyze the association between the copy number variations regions (CNVR) and fatty acid profile phenotypes of the saturated (SFA), monounsaturated (MUFA), polyunsaturated fatty acids (PUFA), omega 6, omega 3, the PUFA/SFA and omega 6/omega 3 ratios, in Nellore beef cattle. A total of 963 males were finished in feedlot and slaughtered at approximately two years of age. The fatty acid profile was analyzed on samples of *Longissimus thoracis* muscle using gas chromatography with a 100m capillary column. The BovineHD BeadChip (Illumina) was used to genotype the animals. Samples with a call rate below 90% were excluded. Log R Ratio (LRR) and Allele B Frequency (BAF) were used to estimate copy number variations (CNVs). For quality control, samples with standard deviation values for LRR >0.30, BAF derived as >0.05 and waviness factor >0.01, were eliminated. The CNVR were identified as CNV overlaps in the samples by CNVRuler software. In the analysis of association, the adjusted phenotypic values were considered for the traits, while the data were adjusted for the effects of the farm and year of birth, and management groups at birth, weaning and yearling. A total of 186 CNV regions were significant for the groups of saturated (43), monounsaturated (42), polyunsaturated (66) and omega (35) fatty acids within a 278 genes with function described. The results of this study pointed some genes that were identified associated to several fatty acids of different saturation. The olfactory receptor genes were associated to C12:0, C14:0 and C18:0 traits. The *SAMD8* and *BSCL2* genes, related lipid metabolic process, were associated to C12:0; the *RAPGEF6* gene is related to regulation of GTPase activity and were associated to C18:2 cis-9 cis-12 n-6. Among the results, we highlighted the olfactory receptor activity (GO:0004984), G-protein coupled receptor activity (GO:0004930), potassium:próton antiporter activity (GO:0015386), sodium:proton antiporter activity (GO:0015385), and odorant binding (GO:0005549) molecular functions.This large number of CNVRs identified in this study, and consequently genes identified within them, associated with the fatty acids profile in this study should help to understand the genetic mechanism that influence the fatty acid profile of intramuscular fat in Nellore cattle.

1. INTRODUCTION

The beef fatty acids (FA) profile of intramuscular fat is very important for human health. It is also responsible for the meat flavor and juiciness. The type of FA

has a greater impact on health issues when compared to its total amount (HU, MANSON, and WILLETT, 2001; WOODSIDE and KROMHOUT, 2005). For humans, the ingestion of saturated fatty acids (SFA) present in meat is not healthy due to the increase of serum low-density lipoprotein (LDL) and cholesterol (MENSINK and KATAN, 1990) that is associated to a higher cardiovascular diseases risk. However, monounsaturated (MUFA) and polyunsaturated (PUFA) fatty acids are highly desirable in the human diet, due to their ability to reduce serum cholesterol levels (FELDMAN, 2002), and protection of some degenerative diseases (TAPIERO et al., 2002).

Due to the current demand of consumers for protein sources with a health lipid profile, some livestock dietary manipulation (FAUCITANO et al., 2008) and genetic strategies (DE SMET et al., 2004; BERTON et al., 2016; ABOJOAUD et al., 2016) have been applied. In genetics, several variants, such as SNP (single nucleotide polymorphism) have been employed to identify genetic markers related to expression of fatty acid profile of cattle meat (ISHII et al., 2013; CESAR et al., 2014; SEVANE et al., 2014; LEMOS et al., 2016). Copy number variation (CNV) belongs to another family of genetic variants, which could affect gene expression and consequently phenotypes by changes in gene structure and dosage (ZHANG et al., 2009). However, in livestock, few studies have been conducted, using CNVs, for genetic selection. The traditional CNVs discovery studies try to detect as many variable regions as possible, instead of to focus on CNVs shared by individuals (XU et al. 2014).

The association studies using CNVs identify genetic variations, in number of copies, related to phenotypes. In humans, studies have identified associations between the CNVs and diseases, including Crohn's disease, psoriasis, schizophrenia and autism (FELLERMANN et al., 2006; HOLLOX et al., 2008; SEBAT et al., 2007; WALSH et al., 2008; MORENO et al., 2011). However, genome-wide association studies (GWAS) using CNV, in livestock, are recent. In this sense, Xu et al. (2014a,b) characterized and reported 34 CNVs significantly associated with milk production traits in Holsteins and found one deletion polymorphism associated with resistance to gastrointestinal nematodes in Angus cattle. Yang et al. (2016) detected

17 CNVs significantly associated with seven growth traits Nellore cattle, and one of them (CNV100) may be involved in growth traits through *KCNJ12*.

Therefore, the aim of this study was to associate variations in the number of copies (CNVs) to the profile of fatty acids of *Longissimus thoracis* muscle, in Nellore cattle. To our knowledge, it is first study to detect CNV markers, from SNP microarray data, in association with beef fatty acid profile in cattle.

2. MATERIAL AND METHODS

Local, Animals and Management

This study was approved by ethics committee of the Faculdade de Ciências Agrárias e Veterinárias (FCAV), São Paulo State University (Unesp), Jaboticabal-SP, Brazil.

The database contains records from eight farms located in the Southeast, Northeast and Midwest of Brazil, which are part of cattle breeding programs. Genotypes (n = 3,794) and phenotypes (n = 963) of Nellore steers, with an average age of 24 months, were used. In these breeding programs animals are selected based on growth, finishing and sexual precocity traits.

Breeding seasons are adopted at different periods on these farms. Therefore, calving seasons were from August to October in some farms and from November to January in others. Weaning occurred at seven months of age. The animals were raised on grazing conditions using *Brachiaria sp.* and *Panicum sp* forages, and free access to mineral salt, with a density varying from 1.2 to 1.6 animal unit/hectare. After yearling, the breeding animals were selected and the others went to feedlot. During feedlot, the forage:concentrate ratio ranged from 50:50 to 70:30, according to the farm. In general, whole-plant corn or sorghum silage was used as high quality forage. Grains of corn and/or sorghum, and soybeans, soybean meal, or sunflower seeds were used as protein concentrate. The criteria used by farmers for slaughtering was weight (500-550 kg). After stored for 48 hours at 0-2°C, meat samples were removed from the *Longissimus thoracis* muscle, between the 12th - 13th ribs from each animal and stored at −80°C until analysis to determine the fatty

acid profile. The percentage of lipids in the *Longissimus thoracis* muscle (IMF) was obtained using the method proposed by Folch et al. (1957).

CNV and CNVR detection

The animals were genotyped using the Bovine High-Density BeadChip array (Illumina) with 777,962 SNPs. The identification of CNVs, apart from SNP data, was carried out using GenomeStudio 1.0 software. For CNV's detection the PennCNV algorithm (WANG et al., 2007) was used. It incorporates multiple sources of information and it is based on a hidden Markov model for CNV detection from the high-density genotyping data. The PennCNV is the most commonly used algorithm for CNV studies, since it presents low rate of false positives.

Initially the PennCNV algorithm was used without any application of quality control parameters to obtain the number of CNVs in all data. After that, in order to maintain the quality of the samples, the *default* PennCNV's quality control was applied, eliminating samples with standard deviation for LRR (log R ratio) > 0.30; BAF (frequency of allele B) > 0.05 and value of the waves factor of > 0.01.

The CNV regions (CNVRs) were generated by CNVs overlapping between samples using CNVRuler software (KIM et al., 2012). Genomic regions with density <10% were excluded ("recurrence 0.1"). The recurrence trims the CNVR based on its frequency to avoid false positive predictions. It defines more robust limits of the start and end regions. The option "Gain/Loss separated regions" was applied, to evaluate the type of event (gain, loss) in each region. Overlapping "gain" and "loss" CNVRs were merged into single regions to account for genomic regions in which both events can occur ("mixed" CNVRs).

To evaluate the location of the CNV regions, the Ensembl Biomart tool (THOMAZ et al., 2003) was used with UMD3.1 reference genome assembly.

Determination of the 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 method (BLING and DYER, 1959). The meat fatty acids were extracted using the

method of Folch et al. (1957) and the methyl esters were formed according to Kramer et al. (1997). The fatty acid profile was determined at the Meat Science Laboratory (LCC) in the Department of Animal Nutrition and Production at FMVZ/USP, using the extraction method by Folch et al. (1957). Muscle samples (~100 g) were collected 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 - Shimadzu AOC 20i auto-injector) with a 100 m SP-2560 capillary column (0.25 mm in diameter with 0.02 mm thickness, Supelco, Bellefonte, PA). The initiating temperature of 70°C was increased gradually up to 175°C (13°C/min), held for 27 min., and increased further up to 215°C (4°C/min) and held for 31 min.. Hydrogen (H2) was the carrier gas, with 40 cm3/s. Fatty acids were identified by comparing the retention time of methyl esters of the samples with the standards C4-C24 (F.A.M.E mix, Sigma®), vaccenic acid C18:1 trans-11 (V038-1G, Sigma®) C18:2 trans-10 cis-12 (UC-61M 100mg, Sigma®), CLA e C18:2 cis-9, trans-11 (UC- 60M 100mg, Sigma®) and tricosanoic acid (Sigma®). Fatty acids were quantified by normalizing the area under the curve of methyl esters using the GS solution 2.42 software. Fatty acids were expressed as a percentage of the total fatty acid methyl ester. The fatty acid profile in meat was performed at the Meat Science Laboratory (LCC) in the Department of Animal Nutrition and Production at FMVZ/USP.

The following individual fatty acids were selected: lauric (C12:0), myristic (C14:0), palmitic (C16:0), stearic (C18:0), myristoleic (C14:1), palmitoleic (16:1), oleic (C18:1 *cis*-9), elaidic (C18:1 trans9), CLA-*cis* (C18:2c9t11), CLA-*trans* (C18:2 trans10 cis 12), vaccenic (C18:1 trans11), linoleic (C18:2 cis9Cis12n6), docosahexaenoic (DHA) (C22:6 n3), and eicosatrienoic FA (C20:3 n6 cis-8,11,14). These FA were chosen due to their importance to human health and their high content in feedlot animals, such as the oleic acid. The sum of saturated (C10:0 +C11:0 +C12:0 +C13:0 +C14:0 + C15:0 + C16:0 + C17:0 + C18:0 + C21:0 + C24:0), monounsaturated (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 (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), omega 6 (C18:3 n6 + C20:3 n6 c8, c11, c14 + C18:2 n6 + C20:4 n6) and omega 3 (C18:3 n3 + C20:3 n3 c11, c14, c17 + C22:6 n3 + C20:5 n3) were calculated. The polyunsaturated/saturated fatty acids and omega 6/omega 3 ratios were also calculated.

Association analyzes

The phenotype (Y) was adjusted using an animal model considering the fixed effects of contemporary group (year, farm and management group at yearling) and the covariate age at slaughter in each trait:

$$Y = Xb + e$$

where: Y is a vector of phenotypic values of given quantitative traits, b is a vector of fixed effects and covariate, X is the incidence matrix of fixed effects, and e is the random residual vector with distribution $N \sim (0, \sigma^2_e)$.

Initially, the association study was performed from the entire population of animals that had phenotypes with a linear model. However, few significant CNVRs were identified with FA traits. Thus, we chose to perform a logistic model, where two groups of animals with extreme phenotypes, for each trait, were created. It was considered 25% of the animals with the highest (HIGH) and 25% of the animal with the lowest (LOW) concentrations for each trait, that received the score 1 and 0, respectively.

The CNVRuler software supports the maximum likelihood test (ML), which can be used to evaluate the fit quality of logistic regression models (KIM et al., 2012). False positives (FDR) were controlled using a multiple comparison correction tests, (FDR <0.01). Minor frequency allele (MAF) of less than 0.05 was excluded. The CNVR were generated by the overlap of CNVs, considering only overlaps above 0.01 by the CNVRuler program (KIM et al., 2012).

Genes search

The significant CNVR were placed in the cattle UMD3.1 genome assembly by surveying the National Center for Biotechnology Information (http://www.ncbi.nlm.nih.gov/snp/) database and Ensembl Genome Browser (http://www.ensembl.org/index.html). In these databases, it was possible to identify segments located within or close to genes that could explain the phenotypic variability of studied traits. The classification of genes regarding their biological function was performed by The Database for Annotation, Visualization and Integrated Discovery (DAVID) v. 6.8, (HUANG et al., 2009a; 2009b) using all annotated genes in the cattle genome as background. Gene Ontology (GO) biological process, GO cellular component and GO molecular function annotation data sets were used for functional enrichment analysis considering a P value < 0.1 threshold for significance. Comparisons of the significant CNVRs with described cattle QTLs were done based on the Animal QTLdb (http://www.animalgenome.org/cgi-bin/QTLdb/BT/index) website.

3. RESULTS AND DISCUSSION

Fatty acid profile

The descriptive analysis in table 1 shows that the individuals FA with the highest concentrations in the intramuscular fat of *Longissimus thoracis* were Palmitic acid (C16:0), Oleic acid (C18:1 cis-9), Vaccenic acid (C18:1 trans-11), and Stearic acid C18:0, representing 67.3% of its fat composition. The same behavior were reported by some authors, which observed hig levels of palmitic, stearic and oleic FAs in their studies (Prado et al., 2003; Lawrie, 2005; Kelly et al., 2013), Also, Cesar et al. (2014), in a study with Nellore finished in feedlot, observed that oleic acid (37.46%) displayed the highest concentration in intramuscular fat.

Observing the sum of FA was noted that the group that showed greater concentration was the SAF, followed by MUFAs and PUFAs. These results were in agreement with Prado et al. (2013) reported for Nellore cattle, 43.93% of SFA, 42.33% of MUFA and 12.8% of PUFA.

Table 1 – Descriptive statistics and analysis of variance for the fatty acids[a] for groups of animals with extreme phenotypes (LOW and HIGH)

Trait	**Nomenclature**	**LOW group[b]**				**HIGH group[c]**			
		Min	Max	Mean	SD	Min	Max	Mean	SD
Lauric	C12:0	0.04	0.04	0.04	0.001	0.06	0.06	0.06	0.001
Myristic	C14:0	0.95	1.68	1.38	0.22	2.49	3.73	2.88	0.35
Myristoleic	C14:1	0.09	0.10	0.10	0.001	0.53	0.53	0.52	0.001
Palmitic	C16:0	16.54	20.35	18.84	0.42	23.24	28.57	24.48	0.42
Stearic	C18:0	10.86	12.80	11.80	0.22	15.88	17.78	16.68	0.22
Elaidic	C18:1n9t	0.002	0.13	0.07	0.04	9.24	11.18	10.29	0.83
Oleic	C18:1n9c	25.57	29.51	27.60	0.38	34.51	37.51	35.85	0.38
Vaccenic (TVA)	C18:1t11	0.04	0.12	0.08	0.03	27.33	27.53	27.43	0.05
Linoleic (LA)	C18:2n6	2.47	4.92	4.13	0.28	8.96	11.82	9.98	0.27
Linolenic	C18:3n3	0.23	0.49	0.41	0.03	0.87	1.20	1.00	0.03
CLA-*cis*	C18:2c9t11	0.14	0.20	0.18	0.02	0.33	0.59	0.41	0.02
Docosahexaenoic (DHA)	C22:6 n3	0.64	0.65	0.65	0.003	1.40	1,42	1.41	0.008
Sum of SFA		39.91	42.05	41.10	0.32	45.37	49.62	46.87	0.32
Sum of MUFA		30.14	34.50	32.75	0.39	40.44	43.71	42.20	0.39
Sum of PUFA		4.33	8.24	7.16	0.46	15.79	20.46	17.21	0.46
Sum of Omega-3		2.78	5.39	4.49	0.29	9.77	12.94	10.89	0.29
Sum of Omega-6		1.24	2.65	2.18	0.20	5.14	7.62	6.14	0.20
n6/n3 ratio		1.45	1.64	1.57	0.03	2.15	2.63	2.27	0.03
PUFA/SFA ratio		0.09	0.18	0.16	0.01	0.36	0.51	0.40	0.01

[a]The concentration of fatty acids are expressed as a percentage of total fatty acid methyl esters (FAME); [b]LOW group: ten lowest extreme phenotypes; [c]HIGH group: ten highest extreme phenotypes

Copy number variation and copy number variation regions discovery

The CNV calling CNV calling was performed on the UMD3.1 bovine genome assembly using PennCNV in 3,794 samples (animals) without any quality control and a total of 399,361 CNVs were identified. After the default quality control filtering, a total of 2,902 animals were kept for subsequent analyzes. A total of 195,873 CNVs were detected with an average length size of 54,744 pb, maximum of 8.7 Mb and a minimum length of 3 kb.

Saturated fatty acids

For the saturated fatty acids, 43 CNVR were significant (p value<0.05). The saturated fatty acid that presented the highest number of significant CNVR was the myristic acid (C14:0) with 11 regions, followed by lauric acid (C12:0) and SFA with 10. The myristic acid showed two loss regions and nine mixed regions, while the lauric acid and SFA presented four and three loss regions, one gain region and six mixed regions, respectively (Table 2)

Table 2 – CNVRs associated with the saturated fatty acids profile in intramuscular fat of the *Longissimus thoracis* muscle of Nellore.

Traits	CNVR ID	BTA	Description	Star	End	Size (pb)	Control	Case	p value	FDR
Total of SFA	CNVR_206_2	7	mixed	103154830	103168693	13864	14	28	0.00401	0.24
	CNVR_3436_1	9	mixed	40529468	40667345	137878	18	29	0.00693	0.24
	CNVR_4253_1	21	mixed	18531324	18566035	34712	39	46	0.01737	0.40
	CNVR_4565_1	7	mixed	38359707	38537300	177594	30	28	0.02772	0.44
	CNVR_4752_1	8	mixed	16420855	16924662	503808	26	26	0.00398	0.44
	CNVR_766_1	8	loss	93854223	93863828	9606	15	6	0.01398	0.50
	CNVR_732_1	11	mixed	81414106	81425393	11288	26	30	0.03678	0.50
	CNVR_573_1	13	gain	5750278	5800039	49762	71	54	0.03175	0.50
	CNVR_1052_1	11	loss	53933240	53982995	49756	23	35	0.03294	0.50
	CNVR_2408_1	7	loss	36862487	36865662	3176	17	18	0.01255	0.50
C12:0	CNVR_3143_1	28	loss	28447018	28532009	84992	18	3	0.00251	0.54
	CNVR_760_1	5	mixed	27945443	28025514	80072	17	6	0.02042	0.54
	CNVR_2425_1	18	loss	61894649	61924592	29944	12	3	0.02580	0.54
	CNVR_1785_1	12	mixed	621640	631466	9827	30	17	0.03618	0.52
	CNVR_3102_1	28	mixed	2468624	2860512	391889	59	43	0.03923	0.54
	CNVR_1571_6	10	mixed	24766755	24822212	55458	27	15	0.04343	0.54
	CNVR_2413_1	18	mixed	49995899	50337677	341779	8	1	0.04489	0.52
	CNVR_3212	29	mixed	41533612	41725223	191612	8	1	0.04489	0.54
	CNVR_677_1	4	loss	95780598	95974974	194377	12	4	0.04760	0.54
	CNVR_391_1	3	loss	14360623	14419603	58981	9	2	0.047743	0.54
C14:0	CNVR_1669_1	7	loss	99322117	99328261	6145	19	38	0.00829	0.42
	CNVR_3718_1	20	mixed	44900560	45106413	205854	15	3	0.00929	0.42
	CNVR_1408_1	6	mixed	79932750	79987353	54604	23	9	0.01320	0.43
	CNVR_896_1	4	mixed	36527545	36656495	128951	23	9	0.01320	0.36
	CNVR_1567_1	7	mixed	53453768	53664924	211157	13	3	0.01957	0.42
	CNVR_3550_2	19	mixed	3025966	3029934	3969	19	7	0.01988	0.36

	CNVR_3378_1	17	loss	25056695	25119996	63302	111	135	0.02585	0.42
	CNVR_1001_2	4	mixed	90699813	90705987	6175	25	41	0.03505	0.42
	CNVR_3430_2	17	mixed	51115979	51370688	254710	127	14	0.03783	0.44
	CNVR_1553_2	7	mixed	42945525	43353211	407687	66	87	0.03856	0.42
	CNVR_4411_1	28	mixed	2468624	2860512	391889	91	11	0.04972	0.36
C16:0	CNVR_2226_3	10	mixed	23847193	23854403	7211	55	30	0.00266	0.77
	CNVR_2226_4	10	mixed	24061376	24062806	1431	49	27	0.00569	0.77
	CNVR_335_3	2	mixed	11875973	11974681	98709	13	1	0.01169	0.77
	CNVR_2257_1	10	loss	40734273	40794738	60466	13	4	0.03411	0.77
	CNVR_2819_1	13	loss	14525354	14533369	8016	17	31	0.03798	0.77
	CNVR_2695_1	12	mixed	43164746	43196111	31366	21	36	0.03891	0.77
	CNVR_2045_1	9	mixed	53893533	53899150	5618	14	5	0.04157	0.77
C18:0	CNVR_2505_1	3	mixed	54414408	55017187	602780	43	59	0.01924	0.92
	CNVR_270_1	10	mixed	27086857	27117598	30742	24	10	0.02896	0.92
	CNVR_546_2	11	mixed	93900408	93922796	22389	64	89	0.02582	0.92
	CNVR_952_2	15	mixed	46666188	46975690	309503	41	51	0.02962	0.92
	CNVR_1304_2	19	mixed	2270929	2321350	50422	27	46	0.02750	0.92

*Control: group of animals with the lowest averages obtained for the studied parameter; * Case: Group of animals with the highest averages obtained for each parameter studied.

Within of the 43 significant CNVRs for the saturated fatty acid group harbored a total of 141 genes across the cattle genome (Table 3).

Table 3. Genes associated within significant CNVRs for saturated fatty acids profile in intramuscular fat of the *Longissimus thoracis* muscle of Nellore.

Trait	BTA	Genes
Total of SFA	BTA7	-
	BTA9	*WASF1*
	BTA21	-
	BTA7	-
	BTA8	*C8H9orf72 IFNK MOB3B*
	BTA8	-
	BTA11	-
	BTA13	
	BTA11	*LOC101903989*
	BTA7	-
C12:0	BTA28	*ASCC1 ANAPC16 DDIT4 DNAJB12*
	BTA5	*ATG101 NR4AI GRASP*
	BTA18	*LOC618662*
	BTA12	-
	BTA28	*LOC782694 LOC782769 OR5AS1 OR5L2 LOC513384 LOC787409 LOC100297422 LOC787801 OR5D14 LOC787835 LOC787869 LOC787883 LOC787902 LOC531024 LOC787953*
	BTA10	*LOC767888 LOC101908188*
	BTA18	*PLD3 HIPK4 TRNAG-CCC PRX SERTAD1 SERTAD3 BLVRB SPTBN4 SHKBP1 LTBP4 NUMBL COQ8B ITPKC C18H19orf54 SNRPA MIA RAB4B EGLN2*
	BTA29	*AHNAK EEF1G TRNAG-UCC TUT1 MTA2 EML3 ROM1 B3GAT3 GANAB INTS5 LBHD1 METTL12 UQCC3 UBXN1 LRRN4CL BSCL2 GNG3*
	BTA4	*MKLN1 TRANAE-UCC LOC104972201*
	BTA3	*MEF2D*
C14:0	BTA7	-
	BTA20	-
	BTA6	-
	BTA4	*SEMA3A*
	BTA7	*TMCO6 NDUFA2 IK WDR55 DND1 HARS HARS2 ZMAT2 PCDHA3 PCDHA6 PCDHA10 PCDHA13*
	BTA19	-
	BTA17	*PRAME*
	BTA4	-
	BTA17	-
	BTA7	*LOC788041 LOC788055 LOC616716 LOC788079 OR2L13 OR2T12 OR2M5 OR2M4 OR2M3 OR2T4*
	BTA28	*LOC782694 LOC782769 OR5AS1 OR5L2 LOC513384 LOC787409 LOC100297422 LOC787801 OR5D14 LOC787835*

		LOC787869 LOC787883 LOC787902 LOC531024 LOC787953
C16:0	BTA10	
	BTA10	*LOC10193548*
	BTA2	*ZNF804A*
	BTA10	*LOC100296164 MDGA2*
	BTA13	-
	BTA12	-
	BTA9	-
C18:0	BTA3	*GBP2 LOC785445 LOC781596 LOC781675 LOC781719 GMP4 LOC786500 LOC104968497 GBP6 LOC510382 LOC100336669 LOC510382 LOC100336669 LOC104969803 LOC507055 LOC100336443*
	BTA10	*LOC784260*
	BTA11	*LOC786596 LOC786573*
	BTA15	*LOC100125776 LOC100336980 LOC511622 OR2AG2 LOC783299 OR2AG1 LOC104970024 LOC101903126 LOC506989 LOC783920*
	BTA19	-

A total of ten CNVRs were significant ($p < 0.05$) for the SFA and presented five genes within three different chromosomes. On BTA9, the CNVR_3436_1 has the *WASF1* gene, which is a protein coding gene, widely expressed, strongly in brain. This gene was associated with the Arp2/3 complex, a nucleating core for actin polymerization in vitro, potentially involved in the regulation of RAC induced of actin cytoskeleton required for membrane ruffling (MIKI et al., 1998). The CNVR_4752_1located on BTA8, host *C8H9orf72*, *IFNK* and *MOB3B* genes. *IFNK* gene encodes a member of the type I interferon family, a group of related glycoproteins that play an important role in host defenses against viral infections (LIU et al., 2008).

The CNVRs that were significant for the C12:0, presented the highest number of genes (64) associated when comparing the other saturated fatty acids. The CNVR_3143_1 is located on BTA28 and host the *ASCC1, ANAPC16, DDIT4* and *DNAJB12* genes. The *DDIT4* gene regulates cell growth, proliferation and survival via inhibition of the activity of the mammalian target of rapamycin complex 1 (mTORC1). Besides that, plays an important role in responses to cellular energy levels and cellular stress, including responses to hypoxia and DNA damage (SOFER et al., 2005).

The second significant CNVRs on BTA28 was the CNVR_3102_1, which is a mixed region with all of the genes have olfactory receptor functions. The *OR5AS1, OR5L2*, and *OR5D14* genes codified olfactory receptors, which belong to the family 5. The olfactory receptor genes comprise the largest multigene family in vertebrate genomes (NIIMURA et al. 2007), with more than one thousand coding genes organized in clusters on 26 bovine chromosomes (LEE et al., 2013). They interact with odorant molecules in the snout and after that initiate a neuronal response that triggers the perception of smell, their proteins are members of a large family of G-protein-coupled receptors (GPCR) arising from single coding-exon genes (MALNIC et al., 2004). Other fuction that the olfactory receptors perform is share a 7-transmembrane domain structure with many neurotransmitter and hormone receptors and are responsible for the recognition and G protein-mediated transduction of odorant signals.

In a study to identify genomic regions and metabolic pathways associated with dry matter intake, average daily gain, feed efficiency and residual feed intake in Nellore cattle, Olivieri et al. (2016), reported olfactory receptors genes associated with dry matter intake, wich was reported as a critical factor that could influence the development of fatty liver in pregnant cows (BERTIC et al., 1992). This fact is a great evidence that the olfactory receptors are involved in pathways of FA synthesis.

For the C12:0 trait it was observed another region on BTA18 that was also significant, located at 49 Mb in the CNVR_2413_1 mixed region. This region host a lot of genes and among them we can highlight the *PRX* gene, which encodes a protein involved in peripheral nerve myelin upkeep (SHI et al., 2014); *SPTBN4* gene that codify a protein which acts on the determination of cell shape, arrangement of transmembrane proteins, and organization of organelles (BERGHS et al., 2000); *COQ8B* gene is involved in the biosynthesis of coenzyme Q (ubiquinone), an essential lipid-soluble electron transporter for aerobic cellular respiration, which probably acts as a small molecule kinase, possibly a lipid kinase that phosphorylates a prenyl lipid in the ubiquinone biosynthesis pathway (ASHRAF et al., 2013); and the *MIA* gene that is associated with skin and uveal melanomas in humans (BLESCH et al., 1994). Although several genes associated to C12:0 in this particular region were found, none were described related to lipid metabolism yet.

Other CNVRs significant to the C12:0 was the CNVR_3212, which is a mixed region located on the BTA29 at 41 Mb and presented 17 genes. One of these genes is the *EEF1G,* a protein coding gene related with translation elongation factor activity (GO:0003746) and acts in chain elongation during polypeptide synthesis at the ribosome (SANDERS et al., 1992). Also the *GANAB* gene encodes the alpha subunit of glucosidase II and a member of the glycosyl hydrolase 31 family of proteins (CHI et al., 2006).

The *B3GAT3* gene a encodes a protein belong to the glucuronyl transferase gene family, that catalyzes the formation of the glycosaminoglycan-protein linkage by way of a glucuronyl transfer reaction in the final step of the biosynthesis of the linkage region of proteoglycans (KOIKE et al., 2014). Besides that, this gene acts to carbohydrate metabolic (GO:0005975) and glycosaminoglycan biosynthetic (GO0006024) processes.

The *BSCL2* gene, also located on CNVR_3212, is related to the lipid metabolic process (GO:0006629), lipid catabolic process (GO:0016042) lipid storage (GO:0019915), lipid particle organization (GO:0034389) and fat cell differentiation (GO:0045444). This gene codifies a regulator of lipid catabolism essential for adipocyte differentiation and may also be involved in the central regulation of energy homeostasis. Payne et al. (2008), reported that *BSCL2* gene may affected the expression of key genes which mediate triglyceride synthesis, including *AGPAT2*, *LP1N1*, and *DGAT2*, in humans, thus inhibiting the lipid accumulation of adipose tissue.

Eleven CNVRs were significant to C14:0 FA, among them, five presented genes described function. The *SEMA3A* gene, located on BTA4 at 36 Mb is the CNVR_896_1, a mixed region, is involved in the development of the olfactory system (HANCHATE et al., 2012). The *PRAME* gene was identified on the CNVR_3378_1, a loss region on BTA17 at 25 Mb, encodes an antigen that is preferentially expressed in human melanomas and is expressed in testis (EPPING et al., 2005). In a similar way, Zhou et al. (2016) also identified this gene associated to weaning gain, conformation at weaning and conformation at yearling in a GWAS study for CNV and body traits in Nellore cattle.

The CNVR_1553_2, a mixed region, on BTA7 at 42 Mb, was also significant for the C14:0. This CNVR host six olfactory receptor genes: *OR2L13, OR2T12, OR2M5, OR2M4, OR2M3* and *OR2T4*. All of these genes belongs the olfactory receptor family 2 and are protein coding, and has the same main function of the olfactory receptors genes previously described. The last significant CNVR found for the C14:0 was the CNVR_4411_1, which is a mixed region located on BTA28 at 2.4 Mb, wich have a similar size of the CNVR found for the C12:0, thus in both regions present the same genes identified, which already have been described.

Although seven CNVRs were found to be significant for the C16:0 FA, none presented genes described to be related/associated with lipid or energy metabolism yet. While, for the C18:0, five CNVR were significant and a total of 29 genes was identified. One of the significant CNVR for this trait, the CNVR_2505_1, located on BTA3 at 54 Mb, where was identified the *GBP6* gene, which had previously been associated with conformation at weaning in a genome-wide association study for CNV and body traits in Nellore cattle (Zhou et al., 2016). Among the others CNVR, on the BTA15 at 46 Mb, in a mixed region named CNVR_952_2, is possible to highlight the olfactory receptors group, which have been presented in a few CNVRs reported above and showed important role in the synthesis of fatty acids.

Monounsaturated fatty acids

For the monounsaturated fatty acids group, a total of 42 CNVRs were significant (p value<0.05). The monounsaturated fatty acid that presented the highest number of significant CNVR was the myristoleic acid (C14:1) with 12 regions, followed by vaccenic acid (C18:1 trans11) and MUFA with 10 regions each. The myristoleic acid showed two loss regions and ten mixed regions, while the vaccenic acid presented two loss regions and eight mixed regions and MUFA only presented mixed regions (Table 4).

In table 5 were described the associated genes within significant CNVRs for monounsaturated fatty acid group profile in intramuscular fat of the *Longissimus thoracis* muscle of Nellore. .

A total of 52 genes with functions were described on literature. For the trait MUFA, a total of ten CNVRs were significant ($p < 0.05$), but only two regions, located

in different chromosomes showed associated genes. The exactly same region that was associated with the fatty acid C14:0 was equally found for this trait, the CNVR_3391_2, This region, although it does not contain associated genes relevant to the present study, is important for the study because it was detected in more than one studied trait being an initial point of future studies related to fatty acid profile and fat synthesis.. On BTA2 at 11 Mb, the CNVR_315_1 was also associated with MUFA, and the *ZNF804A* gene was identified. The *ZNF804A* gene is associated with some disease in humans, like bipolar disorder and schizophrenia (Riley et al., 2010).

The C14:1 trait presented the highest number of CNVRs significant ($p < 0.05$) for the monounsaturated group, and a total of 31 genes was identified among 11 different chromosomes. The first CNVR with genes identified was the CNVR_5095_1, which was the same CNVR associated with de fatty acid C12:0, therefore, described above. The second CNVR with gene identified was the CNVR_7642_1, a mixed region located on BTA9 at 31 Mb, that contain the *SAMD8* gene, which is related with lipid metabolic (GO:0006629) and sphingolipid metabolic (GO:0006665) processes. Sphingolipid are a class of lipids containing a backbone of sphingoid bases, a set of aliphatic amino alcohols that includes sphingosine.

Table 4 - CNVRs associated with the monounsaturated fatty acids profile in the *Longissimus thoracis* of Nellore cattle.

Traits	CNVR ID	BTA	Description	Star	End	Size	Control	Case	p value	FDR
Total MUFA	CNVR_2199_3	10	mixed	23847193	23854403	7211	51	29	0.00637	0.50
	CNVR_2498_2	11	mixed	58249719	58427956	178238	23	44	0.00686	0.50
	CNVR_2742_1	13	mixed	14525354	14533369	8016	14	31	0.00988	0.50
	CNVR_3391_2	17	mixed	25056695	25119996	63302	134	108	0.01023	0.50
	CNVR_3012_1	14	mixed	81841283	81878266	36984	17	5	0.01276	0.50
	CNVR_315_1	2	mixed	11825478	11958282	132805	12	2	0.01636	0.50
	CNVR_134_1	1	mixed	83218713	83238102	19390	43	25	0.01707	0.58
	CNVR_1987_2	9	mixed	31107969	31122388	14420	48	69	0.02815	0.58
	CNVR_3551_1	18	mixed	45300008	45330206	30199	60	41	0.02854	0.58
	CNVR_1386_2	6	mixed	80232706	80244389	11684	22	10	0.02973	0.58
C14:1	CNVR_7602_2	9	mixed	16420855	16924662	503808	17	31	0.00623	0.35
	CNVR_5095_1	28	mixed	28447018	28532009	84992	25	7	0.00721	0.35
	CNVR_7642_1	9	mixed	31075126	31122388	47263	73	58	0.01741	0.51
	CNVR_3551_1	20	loss	15371013	15381710	10698	34	13	0.02017	0.51
	CNVR_6779_1	6	mixed	114705514	114716539	11026	24	9	0.03107	0.51
	CNVR_4209_1	23	mixed	25747610	25881173	133564	45	48	0.03271	0.51
	CNVR_361_2	1	mixed	124159079	124420643	261565	18	11	0.03286	0.51
	CNVR_3474_1	2	mixed	126262898	126731462	468565	14	5	0.03839	0.51
	CNVR_7530_1	8	mixed	103917356	104025778	108423	20	10	0.03952	0.51
	CNVR_1849_1	14	loss	79699163	79706075	6913	15	8	0.04617	0.51
	CNVR_230_1	1	mixed	83218713	83238102	19390	22	56	0.04712	0.51
	CNVR_6050_1	4	mixed	106642849	106765834	122986	40	18	0.00577	0.52
C18:1 cis-9	CNVR_2001_1	12	mixed	61223484	61229440	5957	20	45	0.00103	0.22
	CNVR_1663_1	10	mixed	27106916	27117598	10683	25	11	0.01766	0.69
	CNVR_1365_1	8	loss	54371946	54435508	63563	14	4	0.02381	0.69
	CNVR_3033_1	X	mixed	28434257	28494393	60137	54	35	0.02505	0.69
	CNVR_2262_1	15	mixed	3275242	3280326	5085	24	11	0.02527	0.69

	CNVR_2628_4	18	mixed	49074618	49109287	34670	30	16	0.03162	0.77
	CNVR_1349_1	8	mixed	46895749	46925080	29332	16	6	0.03526	0.77
C18:1 trans9	CNVR_3597_1	7	mixed	6708218	6718398	10181	9	16	0.00608	0.78
	CNVR_2280_1	23	mixed	30441844	30472870	31027	12	17	0.02612	0.78
	CNVR_3204_1	5	mixed	27945443	28025514	80072	18	6	0.04091	0.78
	CNVR_4225_1	26	loss	50795857	50962176	166320	13	2	0.01187	0.14
	CNVR_4135_5	26	mixed	3968813	3977095	8283	13	2	0.01187	0.14
	CNVR_4128_2	25	mixed	41250992	41371975	120984	15	5	0.02871	0.16
C18:1 trans11	CNVR_627_1	3	mixed	11962808	11975091	12284	44	64	0.03038	0.16
	CNVR_2756_2	14	mixed	2382595	2468020	85426	13	4	0.03472	0.17
	CNVR_1765_2	8	mixed	33719567	33725198	5632	49	32	0.03666	0.18
	CNVR_671_6	3	mixed	38653612	38799108	145497	14	5	0.04237	0.20
	CNVR_1332_4	6	mixed	33486467	33496920	10454	14	5	0.04237	0.20
	CNVR_2614_1	12	loss	77799528	77803453	3926	20	34	0.04652	0.21
	CNVR_317_1	2	mixed	2527718	2535261	7544	51	70	0.04784	0.21

* Control: group of animals with the lowest averages obtained for the studied parameter; * Case: Group of animals with the highest averages obtained for each parameter studied.

Tabela 5. Genes associated within significant CNVRs for monounsaturated fatty acids profile in intramuscular fat of the Longissimus thoracis muscle of Nellore.

Trait	BTA	Genes
Total of MUFA	BTA10	-
	BTA11	-
	BTA13	-
	BTA17	*PRAME*
	BTA14	-
	BTA2	*ZNF804A*
	BTA1	-
	BTA9	
	BTA18	
	BTA6	-
C14:1	BTA9	-
	BTA28	*ASCC1 ANAPC16 DDIT4 DNAJB12*
	BTA9	*SAMD8*
	BTA20	-
	BTA6	-
	BTA23	-
	BTA1	-
	BTA2	*FGR LOC104971347 AHDC1 WASF2 GPR3 CD164L2 MAP3K6 SYTL1 TMEM222 WDTC1 LOC104971348 TRNAE-UUC SLC9A1*
	BTA8	*LOC783399 LOC104969451 ZFP37 LOC104972947*
	BTA14	*CA13*
	BTA1	-
	BTA4	*LOC101904045 LOC101903865 LOC101903933 LOC104972267 LOC509513 LOC101903672 LOC101903590 LOC101903755*
C18:1 cis-9	BTA12	-
	BTA10	*LOC784260*
	BTA8	*CEP78*
	BTAX	-
	BTA15	-
	BTA18	*LOC614926 ACP7*
	BTA8	*KLF9*
C18:1 trans9	BTA7	-
	BTA23	*LOC100296164 MDGA2*
	BTA5	*LOC100337366 LOC100849008*
C18:1 trans11	BT26	*LOC536342 ADGRA1*
	BTA26	*PCDH15*
	BTA25	*IQCE TTYH3 LFNG*
	BTA3	*LOC100139973*
	BTA14	*ZC3H3 MAFA*
	BTA8	-
	BTA3	*LOC787637*
	BTA6	-

BTA12	*HS6ST3*
BTA2	-

On BTA2 at 126 Mb the CNVR_3474_1, a mixed region was significant for the C14:1 fatty acid and 13 genes (*FGR, LOC104971347, AHDC1, WASF2, GPR3, CD164L2, MAP3K6, SYTL1, TMEM222, WDTC1, LOC104971348 TRNAE-UUC* and *SLC9A1*) were identified. Among theses genes, we can highlight the *FGR* gene which is related with immune system process (GO:0002373) and protein phosphorylation (GO:0006468) and was associated with sarcoma in humans (Chen et al., 2001). The *SLC9A1* gene, is a protein coding gene related with transport of glucose and other sugars, bile salts and organic acids, metal ions and amine compounds and metabolism pathways (SLEPKOV et al., 2005).

BTA8 at 103 Mb, the CNVR_7530_1 mixed region was associated with the C14:1 trait. In this CNVR the *LOC783399, LOC104969451, ZFP37* and *LOC104972947* genes were identified. The *ZFP37* gene encodes a transcription factor that plays a role regulating the structures of the nucleolus and centromere in neurons in mouse (PAYEN et al., 1998). The CNVR_1849_1 was also associated to C14:1 trait on BTA14 at 79 Mb position and the *CA13* gene was identified. The *CA13* gene participates to metabolism (GO:006730) and nitrogen metabolism (GO:0015701).

The C18:1 cis-9 fatty acid presented seven CNVRs significant ($p<0.05$) and within these regions we identified a total of five genes, but none showed relation with lipid or energy metabolism process. The same happened with the C18:1 trans-9 FA, which has tree CNVRs associated with this trait and six genes. A total of 11 CNVRs were associated with C18:1 trans-11 and 11 genes were identified among them. The *TTYH3* gene was identified within the CNVR_4128_2, a mixed region on BTA25 at 41 Mb, and is related to pathways like ion channel transport and transport of glucose and other sugars, bile salts and organic acids, metal ions and amine compounds (ZHU et al., 2013). The *LFNG* gene was also identified in this region which is related to ovarian follicle development (GO:0001541).

Polyunsaturated fatty acids

Sixty-six CNVRs were significant (p value<0.05) for the polyunsaturated fatty acids group. The fatty acid docosahexaenoic (C22:3 n-6) presented the highest number of significant CNVRs associated seventeen regions of which were four loss, two gain and 11 mixed regions. The linolenic acid (C18:3 n-3) and the sum of PUFA presented 14 CNVRs associated and both presented great number of mixed regions (12 and 10, respectively) (Table 6).

Table 6 - CNVRs associated with the polyunsaturated fatty acids profile in the *Longissimus thoracis* of Nellore cattle.

Traits	CNVR ID	BTA	Description	Star	End	Size	Control	Case	p value	FDR
Total of PUFA	CNVR_3828_2	12	mixed	57649064	57684714	35651	30	3	0.00007	0.01
	CNVR_1465_1	4	mixed	90699813	90705987	6175	23	48	0.00150	0.13
	CNVR_55_1	1	mixed	15844150	16000856	156707	30	10	0.00155	0.13
	CNVR_119_1	1	mixed	31670765	31695808	25044	40	66	0.00412	0.19
	CNVR_2458_1	7	loss	88550049	88553909	3861	17	3	0.00449	0.19
	CNVR_4714_1	16	loss	56458959	56464905	5947	13	2	0.01202	0.38
	CNVR_5419_1	20	mixed	15371013	15381710	10698	18	35	0.01440	0.40
	CNVR_105_3	1	mixed	27836489	27900992	64504	12	2	0.01691	0.42
	CNVR_3780_4	12	mixed	39022698	39098205	75508	13	3	0.01947	0.44
	CNVR_2506_1	7	loss	99776747	99798476	21730	14	4	0.02385	0.50
	CNVR_2798_1	8	mixed	93867246	93875806	8561	35	53	0.03324	0.57
	CNVR_876_1	3	loss	6571668	6587033	15366	23	11	0.03603	0.57
	CNVR_2214_1	7	mixed	6708218	6718398	10181	16	29	0.04373	0.57
	CNVR_6481_3	26	mixed	51104225	51286609	182385	18	8	0.04892	0.57
C20:4 n-6	CNVR_1041_1	4	mixed	106642849	106765834	122986	45	21	0.00680	0.63
	CNVR_4510_1	X	gain	48563085	48586299	23215	22	7	0.01231	0.63
	CNVR_888_2	4	mixed	39270414	39425567	155154	12	28	0.01688	0.63
	CNVR_1734_1	7	loss	99322117	99328261	6145	38	20	0.01889	0.63
	CNVR_2334_5	10	mixed	24279052	24285616	6565	47	28	0.02119	0.63
	CNVR_3820_1	18	mixed	45300008	45330206	30199	71	51	0.02934	0.63
	CNVR_1515_3	7	mixed	9409927	9504448	94522	17	7	0.03129	0.63
	CNVR_1530_1	7	mixed	18531324	18566035	34712	45	29	0.03422	0.63
	CNVR_3493_1	16	mixed	39558124	39589152	31029	62	44	0.03524	0.63
	CNVR_2026_1	9	mixed	4374671	4386831	12161	17	30	0.03667	0.63
	CNVR_2687_1	11	mixed	69975117	70555942	580826	18	8	0.04803	0.72

C18:2 cis9 cis12 n-6	CNVR_3012_2	15	mixed	12046071	12115022	68952	13	30	0.00805	0.72
	CNVR_180_3	1	loss	105018867	105264358	245492	42	65	0.01177	0.72
	CNVR_1346_2	6	mixed	80232706	80249371	16666	22	9	0.01915	0.72
	CNVR_2166_4	10	mixed	24061376	24070828	9453	50	31	0.02115	0.72
	CNVR_1672_1	8	mixed	1817817	1860542	42726	18	7	0.02901	0.72
	CNVR_1446_1	7	mixed	24107045	24149485	42441	15	29	0.02910	0.72
	CNVR_2594_1	12	mixed	32054331	32182960	128630	34	19	0.03063	0.74
	CNVR_139_1	1	mixed	83218713	83238102	19390	41	25	0.03505	0.78
	CNVR_4549_1	27	mixed	9036908	9096031	59124	20	34	0.04489	078
	CNVR_4121_2	X	mixed	26287961	26369699	81739	30	17	0.04814	0.87
C18:3 n-3	CNVR_992_1	4	mixed	83429553	83463128	33576	58	31	0.00179	0.12
	CNVR_1042_1	4	mixed	106642849	106765834	122986	45	21	0.00188	0.12
	CNVR_4515_1	X	gain	48563085	48586299	23215	22	7	0.00647	0.23
	CNVR_1524_1	7	mixed	11819446	11876291	56846	52	30	0.00850	0.23
	CNVR_4457_1	X	mixed	28439058	28494393	55336	59	36	0.00919	0.23
	CNVR_2851_2	12	mixed	61223484	61234845	11362	21	40	0.00958	0.23
	CNVR_889_2	4	mixed	39270414	39425567	155154	12	28	0.00962	0.28
	CNVR_1737_1	7	loss	99322117	99328261	6145	38	20	0.01337	0.28
	CNVR_2342_1	10	mixed	27086857	27117598	30742	27	12	0.01489	0.28
	CNVR_2712_1	11	mixed	81414106	81425393	11288	45	26	0.01608	0.47
	CNVR_2338_5	10	mixed	24279052	24285616	6565	47	28	0.01847	0.47
	CNVR_446_1	2	mixed	53991194	53999598	8405	16	6	0.03630	0.47
	CNVR_2030_1	9	mixed	4374671	4386831	12161	17	30	0.04631	0.47
	CNVR_1502_1	7	mixed	114705514	114716539	11026	12	18	0.04074	0.46
C22:6 n-3	CNVR_1044_1	4	mixed	106642849	106765834	122986	45	20	0.00116	0.24
	CNVR_3954_1	20	loss	15371013	15381710	10698	39	18	0.00386	0.27
	CNVR_2335_2	10	mixed	27106916	27117598	10683	27	10	0.00523	0.27
	CNVR_1340_3	6	mixed	28989049	28994000	4952	22	7	0.00649	0.27

	CNVR_4488_1	X	gain	48563085	48586299	23215	22	7	0.00649	0.27
	CNVR_1003_1	4	mixed	90699813	90705987	6175	45	25	0.01096	0.32
	CNVR_1513_2	7	mixed	9409927	9504448	94522	20	7	0.01396	0.32
	CNVR_2024_1	9	mixed	4374671	4386831	12161	18	35	0.01401	0.32
	CNVR_4434_2	X	mixed	28434257	28494393	60137	58	37	0.01745	0.32
	CNVR_3512_1	16	gain	60462670	60502932	40263	13	3	0.01993	0.35
	CNVR_4460_1	X	loss	36544204	36555271	11068	16	5	0.02039	0.35
	CNVR_3152_1	14	mixed	54123999	54126508	2510	40	23	0.02376	0.35
	CNVR_163_1	1	mixed	83218713	83238102	19390	25	42	0.02492	0.37
	CNVR_1739_1	7	loss	99322117	99328261	6145	34	19	0.03187	0.37
	CNVR_4431_1	X	mixed	25335659	25443589	107931	115	92	0.03533	0.44
	CNVR_1868_1	8	loss	44934828	44944671	9844	16	6	0.03633	0.44
	CNVR_1417_1	6	mixed	80232706	80249371	16666	13	25	0.04423	0.44

* Control: group of animals with the lowest averages obtained for the studied parameter; * Case: Group of animals with the highest averages obtained for each parameter studied.

The sum of PUFA presented the 64 genes identified in their CNVRs (Table 7). On BTA16 at 56 Mb there is a loss region named CNVR_4714_1 where the *SLC9C2* gene was identified. This gene belongs to solute carrier family 9, and is related to pathways like transport of glucose and other sugars, bile salts and organic acids, metal ions and amine compounds. This family gene plays an important role to transport the hexose to the mammalian cells, once these sugars are unable to diffuse across cellular membranes, and require transporter proteins to enter into and out of cells (HE et al., 2009).

Table 7 - Genes associated within significant CNVRs for polyunsaturated fatty acids profile in intramuscular fat of the Longissimus thoracis muscle of Nellore

Trait	BTA	Genes
Total of PUFA	BTA12	-
	BTA4	-
	BTA1	-
	BTA1	-
	BTA7	-
	BTA16	*SLC9C2*
	BTA20	-
	BTA1	-
	BTA12	-
	BTA7	-
	BTA8	-
	BTA3	-
	BTA7	-
	BTA26	*NKX6-2 INPP5A*
C20:4 n-6	BTA4	*LOC101904045 LOC101903865 LOC101903933 LOC104972267 LOC509513 LOC101903672 LOC101903590 LOC101903755*
	BTAX	-
	BTA4	*LOC782609*
	BTA7	-
	BTA10	*LOC100295747*
	BTA18	-
	BTA7	*LOC100299465 LOC508826 LOC509641 LOC787503 LOC104972795 LOC504888*
	BTA7	*LOC1019053227 LOC100337044*
	BTA16	*LOC517828*
	BTA9	-
	BTA11	*LOC104968430 ALK*
	BTA15	-
	BTA1	*LOC781650 TRNAS-GGA LOC782622*
	BTA6	-

C18:2 cis9 cis12 n6	BTA10	*LOC101903548*
	BTA8	*LOC520638*
	BTA7	*RAPGEF6*
	BTA12	*LOC100847339 LOC101902112*
	BTA1	-
	BTA27	-
	BTAX	-
C18:3 n3	BTA4	*TRGC3 TRGC4*
	BTA4	*LOC101904045 LOC101903865 LOC101903933 LOC104972267 LOC509513 LOC101903672 LOC101903590 LOC101903755*
	BTAX	-
	BTA7	*LOC100848374 LOC101909051 LOC789203 LOC101906711*
	BTAX	-
	BTA12	
	BTA 4	*LOC782609*
	BTA 7	-
	BTA10	-
	BTA11	-
	BTA10	-
	BTA 2	-
	BTA9	-
	BTA7	*LOC100337366 LOC100849008*
C22:6 n3	BTA4	*LOC101904045 LOC101903865 LOC101903933 LOC104972267 LOC509513 LOC101903672 LOC101903590 LOC101903755*
	BTA20	-
	BTA10	*LOC784260*
	BTA6	-
	BTAX	-
	BTA4	-
	BTA7	*LOC100299465 LOC508826 LOC509641 LOC787503 LOC104972795 LOC504888*
	BTA9	-
	BTAX	-
	BTA16	-
	BTAX	-
	BTA14	-
	BTA1	-
	BTA7	-
	BTAX	-
	BTA8	-

The second CNVR associated with the sum of PUFA, where the NKX6-2 and INPP5A genes were identified, is a mixed region named CNVR_6481_3, which is

located on BTA26 at 51 Mb. The NKX6-2 is a protein gene coding related to multicellular organism development (GO:0007275), while the INPP5A gene codify a protein which mobilizes intracellular calcium and acts as a second messenger mediating cell responses to several stimulations (MILLS et al., 2008).

The C20:4 n-6 trait had 11 CNVR significant and twenty LOC within these CNV regions. The CNVR_1041_1, located on BTA4 at 106 Mb, was exactly the same region that was significant for the C14:1, as described above. Only one gene, *ALK*, was identified with described function was identified in the CNVR_2687_1, located on BTA11 at 66 Mb. The same behavior was observed to the C18:2 cis-9 cis-12 n-6, which presented 10 significant CNVR with a few LOC without functional information and only one gene identified, *RAPGEF6*, in a mixed CNVR named CNVR_1446_1 located on BTA7 at 24 Mb. The *RAPGEF6* gene is related to regulation of GTPase activity (GO:0043087).

The polyunsaturated fatty acid which presented the highest number of CNVRs associated was the C18:3 n-3, but at the same time only sub regions LOC were identified without functional information. It is worth mentioning that the CNVR_1446_1 located on BTA7 at 24 Mb, reported above, was also significant to the C18:3 n-3 and C:226 n-3 traits.

Omega 3 and 6 fatty acids

A total of 35 CNVRs were significant ($p<0.05$) for the n-3, n-6 fatty acids and the n-6:n-3 ratio (Table 6). The total of n-3 presented the highest number of CNVR (13) significant in comparison to n-6 (10) and the n-6:n-3 ratio (12), being that most of the regions were characterized as mixed regions.

Tabela 8 – CNVRs associated with the total of 3, total of 6 and n-6/n-3 ratio fatty acids profile in the *Longissimus thoracis* of Nellore cattle.

Traits	CNVR ID	BTA	Description	Star	End	Size	Control	Case	p value	FDR
Total n-3	CNVR_2257_1	7	mixed	9409927	9504448	94522	21	5	0.00291	0.55
	CNVR_3356_1	10	loss	27086857	27117598	30742	27	12	0.01442	0.55
	CNVR_3212	29	mixed	41592079	41725223	133145	13	3	0.01958	0.55
	CNVR_6294_1	X	gain	48563085	48586299	23215	17	6	0.02425	0.55
	CNVR_5695_2	20	mixed	46839480	47116159	276680	21	9	0.02765	0.55
	CNVR_5694_1	20	mixed	45910295	45990206	79912	18	7	0.02903	0.55
	CNVR_3036_3	9	mixed	31368505	31370192	1688	13	4	0.03546	0.55
	CNVR_3036_2	9	mixed	31316956	31327903	10948	13	4	0.03546	0.55
	CNVR_2749_1	8	loss	44934828	44944671	9844	13	4	0.03546	0.55
	CNVR_4892_1	16	gain	60462670	60502932	40263	13	4	0.03546	0.55
	CNVR_3298_1	10	mixed	6815725	6824247	8523	48	31	0.03709	0.55
	CNVR_6221_1	X	mixed	28434257	28494393	60137	56	38	0.03870	0.55
	CNVR_4852_1	16	mixed	39558124	39589152	31029	67	48	0.04193	0.55
Total n-6	CNVR_4675_1	29	mixed	11800467	11845269	44803	21	34	0.02617	0.82
	CNVR_1026_1	12	mixed	936045	944850	8806	77	109	0.02804	0.82
	CNVR_6852_1	9	mixed	16420855	16924662	503808	20	37	0.03310	0.82
	CNVR_6037_3	6	mixed	77164347	77294195	129849	12	2	0.03727	0.82
	CNVR_956_1	11	mixed	81414106	81425393	11288	37	29	0.03761	0.82
	CNVR_2609_1	19	mixed	2252450	2344351	91902	27	39	0.03918	0.82
	CNVR_5420_1	4	loss	95780598	95974974	194377	15	6	0.03974	0.82
	CNVR_1186_2	12	mixed	57649064	57684714	35651	32	13	0.04137	0.82
	CNVR_3793_1	23	mixed	29385602	29493694	108093	18	29	0.04845	0.82
	CNVR_6759_1	8	mixed	93873095	93875806	2712	34	52	0.04874	0.82

n-6:n-3 ratio	CNVR_2071_3	9	mixed	6225419	6252169	26751	28	6	0.00032	0.07
	CNVR_3175_3	14	mixed	44047994	44134488	86495	25	11	0.01676	0.33
	CNVR_206_1	1	mixed	103098743	103168693	69951	31	16	0.02145	0.33
	CNVR_4228_1	21	loss	36862487	36865662	3176	24	11	0.02402	0.33
	CNVR_2372_5	10	mixed	24275820	24285616	9797	33	52	0.02635	0.33
	CNVR_4508_2	X	mixed	26287961	26369699	81739	20	36	0.02655	0.33
	CNVR_1923_1	8	mixed	46893088	46929974	36887	15	5	0.02813	0.33
	CNVR_4250_1	21	mixed	53293514	53310758	17245	26	13	0.03062	0.33
	CNVR_1534_1	6	loss	114705514	114716539	11026	24	12	0.03849	0.40
	CNVR_2793_1	12	mixed	1167394	1185172	17779	55	38	0.04462	0.41
	CNVR_669_1	3	mixed	40529468	40667345	137878	30	17	0.04484	0.41
	CNVR_4116_2	20	mixed	47008760	47032305	23546	15	6	0.04975	0.42

* Control: group of animals with the lowest averages obtained for the studied parameter; * Case: Group of animals with the highest averages obtained for each parameter studied.

These 35 CNVRs significant for the omega traits showed a total of 48 genes identified between LOC and genes with function described, distributed throughout the Nellore genome (Table 7).

Table 9 - Genes associated within significant CNVRs for omega 3, 6 and n6/n3 ratio fatty acids in intramuscular fat of the Longissimus thoracis muscle of Nellore

Trait	BTA	Genes
Total n3	BTA7	*LOC100299465 LOC508826 LOC509641 LOC787503 LOC104972795 LOC504888*
	BTA10	*LOC784260*
	BTA29	*AHNAK EEF1G TRNAG-UCC TUT1 MTA2 EML3 ROM1 B3GAT3 GANAB INTS5 LBHD1 METTL12 UQCC3 UBXN1 LRRN4CL BSCL2 GNG3*
	BTAX	-
	BTA20	-
	BTA20	-
	BTA9	-
	BTA9	-
	BTA8	
	BTA16	*LOC104974482*
	BTA10	*COL4A3BP*
	BTAX	-
	BTA16	*LOC517828*
Total n6	BTA29	*LOC505383*
	BTA12	-
	BTA9	-
	BTA 6	-
	BTA11	-
	BTA19	-
	BTA4	*MKLN1 TRNAE-UCC LOC104972201*
	BTA12	-
	BTA23	*LOC515704 LOC782379 LOC785479 LOC785557 LOC509155 LOC84614 LOC784652 LOC784681 LOC528343*
	BTA8	-
n-6:n-3 Ratio	BTA9	-
	BTA14	*ZC2HC1A IL7*
	BTA1	*SI*
	BTA21	*LOC101905544*
	BTA10	*LOC100295747*
	BTAX	-
	BTA8	*KLF9*
	BTA21	-

BTA6	-
BTA12	-
BTA3	*COL11A1*
BTA20	-

The total of n-3 presented five CNVR with genes identified. On BTA7 at 9.4 Mb the CNVR_2257_1 was associated with this FA and it is exactly the same region that was associated with C20:4 n-6 and C22:6 n3, therefore are described above. On BTA29 at 41 Mb is the CNVR_3212 where 17 genes were identified and was also associated to C12:0 trait, thus are described above. The other region associated with n-3, which have gene with known function, was CNVR_3298_1, located on BTA10 at 6 Mb. The *COL4A3BP* gene is related to cell morphogenesis (GO:0000902), protein phosphorylation (GO:0006468) and ceramide metabolic (GO:0006672) processes.

A total of 10 CNVR were associated with n-6, but only three presented genes identified. The CNVR located on BTA4 at 95 Mb is a loss region and was also associated with the C12:0 and was described above. A few LOC were identified in the other CNVR, but they do not have function described.

Twelve CNVRs were associated to n-6:n3 ratio and seven genes were identified in six different CNVRs . On BTA4 at 44 Mb is the CNVR_3175_3, a mixed region which present the *ZC2HC1A* and *IL7* genes. The *IL7* gene products a cytokine important for B and T cell development. This cytokine and the hepatocyte growth factor (HGF) form a heterodimer that functions as a pre-pro-B cell growth-stimulating factor. Studies in mice suggested that this cytokine plays an essential role in lymphoid cell survival (MARKLEY and SADELAIN, 2010). On BTA1 at 106 Mb the CNVR_206_1 host the *SI* gene, which encodes a sucrase-isomaltase enzyme that is expressed in the intestinal brush border and is related with carbohydrate metabolic process (GO:0005975), metabolic process (GO:0008152) and polysaccharide digestion (GO:0044245). The *COL11A1* gene was identified in the CNVR_669_1, on BTA3 at 40 Mb, which encodes one of the two alpha chains of type XI collagen, a minor fibrillar collagen and is related to cartilage condensation (GO:0001502) and ossification (GO:0001503).

Functional analysis

The analysis set comprised 213 genes, of which 197 presented DAVID ID and were used to functional analysis. Gene ontology (GO) terms (cellular components, molecular functions and biological processes) and KEGG (Kyoto Encyclopedia of Genes and Genomes) pathways enrichment analysis were performed by DAVID tool to gain insight into the predicted gene networks and the results are summarized on Table 10. Among the results, we highlighted the olfactory receptor activity (GO:0004984), G-protein coupled receptor activity (GO:0004930), potassium:proton antiporter activity (GO:0015386), sodium:proton antiporter activity (GO:0015385), and odorant binding (GO:0005549) molecular functions.

The olfactory receptor activity GO term is defined as combining with an odorant and transmitting the signal from one side of the membrane to the other to initiate a change in cell activity in response to detection of smell (ASHBURNER et al., 2000).

Table 10 – Gene ontology (GO) terms enriched (P value <0.1) with genes the groups of genes identified

GO term	P value	Genes
Biological Process		
GO:0007608~sensory perception of smell	2.47E-12	*LOC784652, LOC528343, OR2AG1, OR2AG2, LOC515704, OR2T4, LOC100849008, OR2L13, OR2M4, OR2M5, LOC100336980, OR2T12, LOC788079, LOC784681, LOC616716, LOC788055*
GO:0007186~G-protein coupled receptor signaling pathway	4.06E-11	*LOC784652, LOC528343, OR2AG1, OR2AG2, LOC504888, OR5D14, LOC515704, OR2T4, LOC509641, OR5L2, LOC100849008, OR2M4, OR2L13, OR2M5, LOC100336980, LOC100299465, LOC786596, OR2T12, LOC788079, OR5AS1, LOC784681, GNG3, LOC513384, LOC788055, LOC616716*
GO:0006427~histidyl-tRNA aminoacylation	0.01297	*HARS2, HARS*
GO:0007156~homophilic cell adhesion via plasma membrane adhesion molecules	0.02536	*PCDHA6, PCDHA3, PCDH15, PCDHA13*
GO:0072673~lamellipodium morphogenesis	0.03843	*WASF1, WASF2*
GO:0050911~detection of chemical stimulus involved in sensory perception of smell	0.04196	*LOC100299465, LOC504888, LOC509641*
GO:0098719~sodium ion import across plasma membrane	0.06324	*SLC9C2, SLC9A1*

GO:0016180~snRNA processing	0.08144	*TUT1, INTS5*
GO:0016601~Rac protein signal transduction	0.08144	*WASF1, WASF2*
GO:0050910~detection of mechanical stimulus involved in sensory perception of sound	0.08144	*PCDH15, COL11A1*
Molecular Function		
GO:0004984~olfactory receptor activity	1.02E-10	*LOC784652, LOC528343, OR2AG1, LOC100125776, OR2AG2, LOC504888, OR5D14, LOC515704, OR2T4, LOC509641, OR5L2, LOC100849008, OR2M4, OR2L13, OR2M5, LOC100336980, LOC100299465, LOC786596, OR2T12, LOC788079, OR5AS1, LOC784681, LOC513384, LOC788055, LOC616716*
GO:0004930~G-protein coupled receptor activity	6.89E-10	*LOC784652, LOC515704, OR5D14, LOC504888, OR2T4, OR5L2, LOC100849008, OR2L13, GPR3, LOC786596, LOC100299465, OR2T12, OR5AS1, LOC788079, LOC784681, LOC788055, LOC528343, OR2AG1, OR2AG2, LOC100125776, LOC509641, OR2M4, OR2M5, LOC100336980, ADGRA1, LOC616716*
GO:0004821~histidine-tRNA ligase activity	0.01309	*HARS2, HARS*
GO:0015386~potassium:proton antiporter activity	0.06994	*SLC9C2, SLC9A1*
GO:0015385~sodium:proton antiporter activity	0.06994	*SLC9C2, SLC9A1*
GO:0005549~odorant binding	0.09453	*OR5D14, OR5AS1, OR5L2, LOC513384*
Cellular Component		
GO:0005886~plasma membrane	3.84E-07	*PRX, PCDHA6, LOC784652, LOC504888, LOC515704, OR5D14, PCDHA3, OR2T4, OR5L2, LOC100849008, OR2L13, LOC100299465, TTYH3, LOC786596, OR2T12, OR5AS1, LOC788079, LOC784681, PCDHA13, LOC788055, LOC528343, OR2AG1, OR2AG2, LOC100125776, KLF9, LOC509641, PCDH15, OR2M4, OR2M5, LOC100336980, BLVRB, SLC9C2, SYTL1, GRASP, LOC616716, SLC9A1*
GO:0016021~integral component of membrane	0.00109	*PCDHA6, LOC784652, LOC504888, PCDHA3, OR5D14, LOC515704, OR2T4, OR5L2, BSCL2, DNAJB12, LOC100849008, OR2L13, GPR3, LOC100299465, LOC786596, OR2T12, OR5AS1, LOC788079, LOC784681, IFNK, LOC513384, PCDHA13, LOC509513, LOC788055, LOC528343, OR2AG1, TMEM222, LRRN4CL, OR2AG2, LOC100125776, MDGA2, LOC509641, SI, PCDH15, ALK, OR2M4, OR2M5, LOC100336980, SLC9C2, ADGRA1, LOC616716, ROM1, SLC9A1*
GO:0031209~SCAR complex	0.04485	*WASF1, WASF2*

GO:0005847~mRNA cleavage and polyadenylation specificity factor complex	0.08770	*TUT1, ZC3H3*

Only one KEGG pathway was identified over represented for this group of genes by DAVID tool, the bta04740:olfactory transduction (P= 9.05E-10) (Figure 1). Functional enrichment analysis of CNVRs in Qinchuan cattle revealed the olfactory transduction pathway as the most enriched (ZHANG et al., 2015). The cellular and molecular machinery for olfactory transduction is located in the olfactory cilia. Odorant transduction begins with odorant binding to specific receptors on the external surface of cilia. Binding may occur directly, or by way of proteins in the mucus (called odorant binding proteins) that sequester the odorant and shuttle it to the receptor (PUERVES et al., 2001). Olfactory transduction pathways act in the perception of odor through olfactory receptors and biochemical signaling events, which influence food consumption (MA et al., 2007). This pathway has been identified as over represented in studies about feed efficiency and performance in crossbred beef cattle (ABO-ISMAIL et al., 2014) and residual feed intake in pigs (DO et al., 2014).

Plasma membranes isolated from bovine olfactory epithelium containing large numbers of olfactory receptors cells are characterized by a high lipid, and especially phospholipid, content odorant molecules are lipoid-soluble, which suggests that the interaction of odorant molecules with lipid layer of the olfactory receptor membrane is important in olfactory reception (KOYAMA and KURIHARA, 1992).

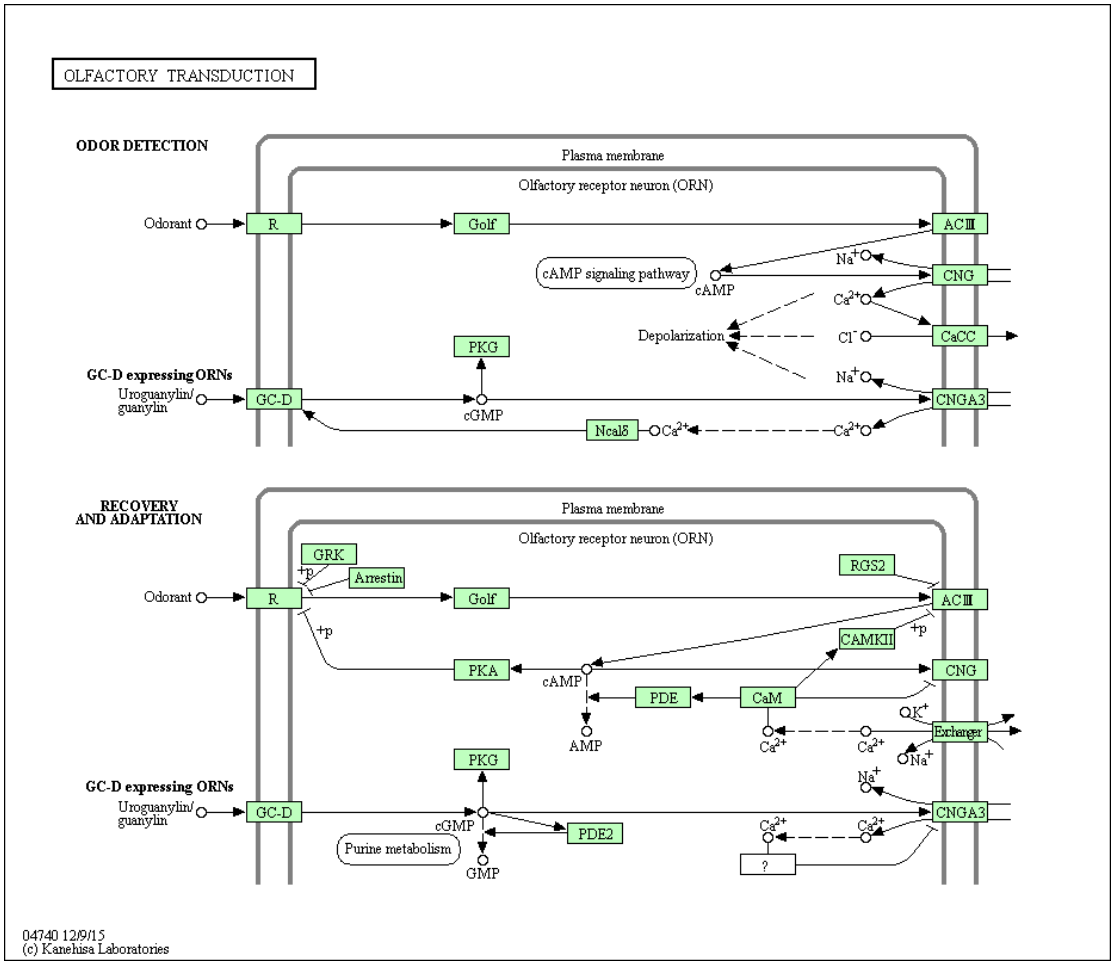

Figure 1 – KEGG olfactory transduction

The importance of OR genes has been previously studied in pigs by Padel et al. (2015), which compared and contrasted the pattern of CNVs among pigs population/species, in order to investigate the role that CNVRs may play in this on-going process of speciation. They reported the majority of the copy number variable genes are ORs known to play a prominent role in food foraging and mate recognition in pig.

Several studies have mainly focused on genome wild-association due to single nucleotide polymorphisms (SNPs) and very few studies have investigated the role that structural variations (SVs) influence on the phenotype trait in livestock (LEMOS et al., 2016; ZHOU et al., 2016). Copy number variation (CNV) belongs to another family of genetic markers, which could affect gene expression and consequently phenotypes by changes in gene structure and dosage (ZHANG et al., 2009). CNV regions can be a major mechanism driving gene and genome evolution

by duplication and deletion of segments of the genome and as a result, create novel gene functions, disrupt gene functions, or affect regulatory mechanisms in the genome (PADEL et al., 2015).

In this study were found several CNVRs nearby QTL regions associated with groups of saturated, monounsaturated and polyunsaturated fatty acids in Nellore *Longissimus thoracis* muscle. Some of these CNVR harbor interesting genes, which are involved in lipid metabolic process, sphingolipid metabolic process, olfactory receptors, metabolic process, cell morphogenesis, transport of lipids, immune system, energy metabolism, nitrogen metabolism, transport of glucose and other sugars, lipid metabolic process, lipid catabolic process, lipid storage, lipid particle organization and fat cell differentiation.

Among the several genes identified in this study, the *BSCL2* associated with C12:0 is highlighted, because it is necessary for correct lipid storage and lipid droplets maintenance and may affect the expression of the *DGAT2* gene in humans, as described by Payne et al. (2008). Once the CNVR, that harbor the *BSCL2* gene is a mixed region, this gene may be acting in a way to increase or decrease the gene dosage.

The *DGAT2* gene is a very important contributor to the triacylglycerol synthesis through their acyltransferase activity. This encodes one of two enzymes which catalyzes the final reaction in the synthesis of triglycerides in which diacylglycerol is covalently bound to long chain fatty acyl-CoAs (KANTARTZIS et al., 2009). Buchanan et al. (2013) reported a positive and moderate correlation between the level of marbling and the expression of *DGAT2* gene in beef meat, and Xie et al., (1996) found negative correlations between marbling and concentrations of stearic, linoleic acid, and PUFA. Berton et al. (2016) reported that the *DGAT2* gene was upregulated for palmitic and downregulated for linoleic acid and PUFA/SFA ratio in a study with Nellore cattle.

The results of this study pointed some genes that were identified associated to several fatty acids of different saturation. The olfactory receptor genes were associated to C12:0, C14:0 and C18:0 traits. The *SAMD8* and *BSCL2* genes, related lipid metabolic process, were associated to C12:0; the *RAPGEF6* gene is related to regulation of GTPase activity and were associated to C18:2 cis-9 cis-12 n-6. This

large number of CNVR identified in this study, and consequently genes identified within them, associated with the fatty acids profile in this study should help to understand the genetic mechanism that influence the fatty acid profile of intramuscular fat in Nellore cattle. Strategies such as genomic selection using or considering the variability among markers at the same time would be more appropriate to improve the fatty acid profile of the bovine meat

4. CONCLUSION

Several CNVRs were associated with the fatty acid profile in this study and pointed some genes that might influence on the fatty acids composition and metabolism. The identification of such CNVRs and the respective candidate genes associated with related lipid metabolic process and regulation of GTPase activity, such as, for example, *SAMD8, BSCL2* and *RAPGEF6* as well their respective metabolic pathways, should contribute to improve the genetic knowledge regarding the fatty acids profile of Nellore (*Bos indicus*) and help to improve the selection of such traits to favor human health. The CNV information found in this study may contribute future fine mapping studies and also can be incorporated in genetic improviment programs.

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CHAPTER 5 - Final considerations

This study presented results on genome wide association study to detect structural variations associated with beef fatty acid profile in Nellore cattle. The data file, formed from information from several farms, located in different regions of the country and, consequently, with the animals under the influence of different breeding environments, has provided a variety of practical situations which may have ensured a broader population-wide coverage of the results obtained.

The results showed important information regarding the application of the CNV identification technique and the genome-wide association using the single-step method its use in genomic selection for Nellore cattle. In the course of this study, it was possible to perceive that, in general, was verified that the animals of this study presented a greater amount of CNV in relation to the other studies that used the same technique adopted by us, which can be justified by the number of animals in our database, the subspecies used (*Bos indicus)* or by the CNV calling method. Thus, the possibility of studying the influence of copy number variations on the phenotype under study becomes easier, but we must take into account that the reference genome used was of a taurine animal. It was also verified that most of the genes found in CNV regions were related to biological processes that might be involved in the environmental adaptation of the subspecies to tropical areas, such as regulation of vasodilatation, immune system response, hair follicle morphogenesis.

When associated with the beef fatty acid profile traits, CNV and SNPs regions have been shown to be involved in lipid metabolic process, sphingolipid metabolic process, olfactory receptors, metabolic process, cell morphogenesis, transport of lipids, immune system, energy metabolism, nitrogen metabolism, transport of Glucose and other sugars, lipid metabolic process, lipid catabolic process, lipid storage, lipid particle organization and fat cell differentiation.

The number of genomic selection studies which use Nellore breed still preliminary, but has been occurring in some regions of the country, being conducted by public research institutions. The expectation is that the results generated by these studies will gradually be incorporated into the genetic evaluations of Brazil's herds. In this regard, the results of this study are encouraging because they show the

feasibility of using structural variations as a selection tool, representing an alternative to be included in beef cattle breeding programs, allowing the genetic improvement of traits with difficult to assess.