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CÂMPUS DE JABOTICABAL

LONG NON-CODING RNAS AND MESSENGER RNA ISOFORMS ASSOCIATED WITH MUSCLE FATTY ACID PROFILE IN NELLORE ANIMALS

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UNIVERSIDADE ESTADUAL PAULISTA - UNESP
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Impacto potencial desta pesquisa

O presente estudo identificou lncRNAs e isoformas de mRNAs relevantes, ampliando o entendimento dos processos biológicos relacionados ao perfil de ácidos graxos da carne. Foram descritas regiões genômicas importantes abrindo caminhos para pesquisas futuras com o objetivo de elucidar potenciais marcadores genéticos para perfil de ácidos graxos.

Potential impact of this research

The present study identified relevant lncRNAs and mRNA isoforms, expanding the understanding of biological processes related to meat fatty acid profile. Important genomic regions were described opening avenues for future research with the aim of elucidating potential genetic markers for fatty acid profiling.

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Dados curriculares

Bruna Maria Salatta nascida no dia 11 de janeiro de 1993, na cidade de Itápolis, São Paulo, filha de Donizeti Afonso Salatta e Diva Soares Salatta. Iniciou o curso de Zootecnia em março de 2012 na Faculdade de Ciências Agrárias e Veterinárias, UNESP, campus de Jaboticabal. Foi bolsista de iniciação cientifica (Pibic – CNPq) no período entre 2013 e 2016 sob orientação da professora Dra. Lucia Galvão de Albuquerque. Obteve o título de bacharel em Zootecnia em fevereiro de 2017. Em agosto de 2017, ingressou no curso de mestrado pelo programa Pós-graduação em Genética e Melhoramento Animal na Faculdade de Ciências Agrarias e Veterinárias, UNESP, campus de Jaboticabal, sob orientação da professora Dra. Lucia Galvão de Albuquerque e co-orientação da Dra. Larissa Fernanda Simielli Fonseca. Foi bolsista de CAPES pelo período de 02 de agosto de 2017, até 30 de julho de 2019. Em agosto de 2019 ingressou no curso de doutorado no mesmo programa de Pós-graduação, bolsista da mesma instituição de fomento e sob mesma orientação e co-orientação da Dra. Maria Malane Magalhães Muniz e Dra. Larissa Fernanda Simielli Fonseca.

“*A mais bela experiência que podemos ter é o mistério. É a emoção fundamental que está no berço da verdadeira arte e da verdadeira ciência*.”

Albert Einstein

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LONG NON-CODING RNAS AND MRNAS ISOFORMS ASSOCIATED WITH THE FATTY ACID COMPOSITION OF MUSCLE TISSUE IN NELLORE BREED CATTLE

ABSTRACT – In recent decades, considerable attention has been paid to the increase in beneficial fatty acids (FA) present in foods of animal origin. Traits of economic importance in beef cattle, as well as the meat fatty acid profile, are polygenic in nature and are influenced by genetic and environmental factors. In this sense, the RNA sequencing (RNA-Seq) is a technique employed to examine the expression of functional candidate genes, allowing the identification of significant molecular mechanisms that lead to variations in pathways responsible for distinct tissue phenotypes. Therefore, the objective was to study the transcriptome of Nellore cattle muscle, aiming to identify differentially expressed long non-coding RNAs (lncRNAs) and mRNA isoforms associated with FAs, through RNA-Seq. For this purpose, 48 animals were sequenced and phenotyped to seven individual FAs: myristic, palmitic, stearic, oleic, linoleic, conjugated linoleic, alpha-linolenic; and seven FA groups: sum of saturated FAs, monounsaturated FAs, polyunsaturated FAs, ω3, ω6, PUFA/SFA ratio, and ω6/ω3 ratio. K-means analysis was employed to cluster 48 animals into three groups based on their FA patterns. Cluster C1 exhibited significantly higher proportions ($p \leq 0.05$) of polyunsaturated fatty acids (PUFA), including ω3, ω6, linoleic, and α-linolenic acids compared with C2 and C3. The proportion of monounsaturated fatty acids (MUFA) conjugated linoleic acid, and oleic acid in C2 and C3 was significantly ($p \leq 0.05$) higher compared to C1, while C3 showed significantly higher proportions ($p \leq 0.05$) of saturated fatty acids (SFA), myristic, palmitic, and stearic acids in comparison to the other clusters. For transcriptome analysis, a pairwise comparison experiment between two groups (C1 vs. C2, C1 vs. C3, and C2 vs. C3) was conducted. The results are presented in Chapters 2 and 3. In Chapter 2, a total of 265 long non-coding RNA (lncRNA) transcripts were differentially expressed (p-value < 0.01), being that 75, 113, and 78 identified in the C1 vs. C2, C1 vs. C3, and C2 vs. C3 comparisons, respectively. These lncRNAs were associated with genes belonging to the interferon, interleukin, G protein-coupled receptor, calponin, and apoptotic Bcl-2 families. Functional enrichment analysis revealed that many Gene Ontology (GO) terms were associated with the immune system in genetic profiles with higher saturated fatty acid (SFA) content (e.g, *SERPINE1*). In Chapter 3, a total of 65, 26, and 30 mRNA isoforms were differentially expressed (p-value < 0.01) in the C1 vs. C2, C1 vs. C3, and C2 vs. C3 comparisons, respectively. Additionally, differentially expressed isoforms, such as *C7-203, ADD1-204, OXT-201* and *RBM3-202*, were linked to genes affecting polyunsaturated fatty acid content in the C1 vs. C2 comparions, while other mRNA isoforms important for saturated fatty acid content, such as

EGR1-201, FOSB_*mRNA1*, and SERPINE1_*mRNA1*, were identified in the C1 vs. C2 comparison. Moreover, functional enrichment analysis highlighted various terms associated with fatty acid metabolism, as well as terms related to oxidoreductase activity, lipid transport, and immune response. This thesis provided insights into potential lncRNA and mRNA isoform candidates associated with FA in beef cattle, contributing to a better theoretical understanding of biological processes associated with this feature. This information may contribute to future research focused into identification of genetic markers to be applied in genetic selection of animals with better FA profiles.

Keywords: Fatty Acids, genic lncRNAs, lincRNA, mRNA isoforms, RNA-Seq

RNAS LONGOS NÃO CODIFICANTES E ISOFORMAS DE MRNAS ASSOCIADAS À COMPOSIÇÃO DE ÁCIDOS GRAXOS DO TECIDO MUSCULAR EM BOVINOS DA RAÇA NELORE

RESUMO – Nas últimas décadas, considerável atenção tem sido dada ao aumento de ácidos graxos (AG) benéficos presentes em alimentos de origem animal. Características de importância econômica na pecuária de corte, assim como o perfil de ácidos graxos da carne, são de natureza poligênica e influenciadas por fatores genéticos e ambientais. Nesse sentido, sequenciamento do RNA (RNA-Seq) é uma das abordagens utilizadas para identificar transcritos correspondentes a genes candidatos que provocam variações em vias metabólicas, resultando em diferentes fenótipos. Portanto, o objetivo foi estudar o transcriptoma do músculo de bovinos da raça Nelore, com o propósito de identificar RNAs longos não codificantes (lncRNAs) e isoformas de mRNAs diferencialmente expressas associados à AG, por meio do RNA-Seq. Para tanto, 48 animais foram sequenciados e fenotipados para sete indivíduais: mirístico, palmítico, esteárico, oleico, linoleico, linoleico conjugado, alfa-linolênico; e sete grupos: soma de AG saturados, monoinsaturados, poliinsaturados, ω3, ω6, razão PUFA/SFA e razão ω6/ω3). A análise K-means foi usada para agrupar os 48 animais em três clusters com base em seus padrões de AG. O cluster C1 apresentou proporções significativamente ($p \leq 0,05$) mais altas de AG poliinsaturado, incluindo ω3, ω6, linoléico e α-linolênico. A proporção de AG monoinsaturados, linoleico conjugado e oleico no C2 e C3 foi significativamente ($p \leq 0,05$) maior em relação ao C1, enquanto o C3 teve proporções significativamente ($p \leq 0,05$) maiores para AG saturado, mirístico, palmítico e esteárico em relação aos demais clusters. Para a análise do transcriptoma o experimento de comparação de dois grupos (C1 vs. C2, C1 vs. C3 e C2 vs. C3) foi usada. Os resultados foram apresentados nos capítulos 2 e 3. No capítulo 2, um total de 265 transcritos de lncRNAs foram diferencialmente expressos (p-value < 0,01), dos quais 75, 113 e 78 foram identificados entre as comparações C1 vs. C2, C1 vs. C3 e C2 vs. C3, respectivamente. Os lncRNAs foram associados a genes pertencentes a família de interferon, interleucina, receptores acoplados à proteína G, calponina e apoptóticas Bcl-2. A análise de enriquecimento funcional mostrou que muitos dos termos da ontologia genética (GO) estavam associados com o sistema imune para comparação (C2 vs. C3), que são grupos com maior conteúdo de SFA (p. ex. *IFN-TAU* and *IFNAG*). No capítulo 3, foram identificados 65, 26 e 30 isoformas de mRNA (p-value < 0.01) diferencialmente expressas entre as comparações C1 vs. C2, C1 vs. C3 e C2 vs. C3, respectivamente. As isoformas, *C7-203, ADD1-204, OXT-201* e *RBM3-202*, foram diferencialmente expressa in C1 em ralação a C2 e são relacionadas a genes que afetam o conteúdo de AG poliinsaturado. Enquanto na comparação C1 vs. C2, foram encontradas isoformas de mRNA relacionadas ao conteúdo de AG saturado como, por exemplo, *EGR1-201*, *FOSB_mRNA1*, e *SERPINE1_mRNA1*. Além disso, a análise do enriquecimento funcional aponta diversos termos associados ao metabolismo de ácidos graxos, oxirredutase, transporte lipídico e resposta imune. Esta Tese forneceu insights sobre potenciais candidatos a isoformas de lncRNA e mRNA associados à AG em bovinos de corte, contribuindo para uma melhor compreensão teórica dos processos biológicos associados a esta característica. Estas informações poderão contribuir para futuras pesquisas focadas na identificação de marcadores genéticos a serem aplicados na seleção genética de animais com melhores perfis de AG.

Palavra-Chave: Ácidos Graxos, lncRNAs, lincRNA, isoforms de mRNA, RNA-Seq

CAPÍTULO 1 - CONSIDERAÇÕES GERAIS

1. INTRODUÇÃO

O interesse do consumidor em alimentos que oferecem excelentes propriedades nutricionais e são benéficos à saúde, têm aumentado. Os consumidores estão cada vez mais conscientes das relações entre dieta e bem-estar, dando origem a um mercado crescente de alimentos com benefícios comprovados, ou seja, com propriedades funcionais que auxiliem na prevenção de doenças e na manutenção da saúde (SCOLLAN et al., 2006).

A carne bovina é um alimento de elevado valor nutricional, sendo uma fonte importante de proteínas, aminoácidos essenciais, minerais (ferro, zinco e selênio) e vitaminas A, E, D e do complexo B (B1, B2, B6 e B12) (BRUGIAPAGLIA; LUSSIANA; DESTEFANIS, 2014; DALEY et al., 2010). Nas últimas décadas, considerável atenção tem sido dada ao aumento de ácidos graxos (AG) benéficos presentes em alimentos de origem animal, especialmente na carne e leite (GIVENS, 2010; PARODI, 2016; SALTER, 2013; SCOLLAN et al., 2006; SHINGFIELD et al., 2013). Os ácidos graxos poli-insaturados (AGPI) n-3 presentes na gordura da carne, como o ácido eicosapentaenoico (EPA, 20:5 n-3) e o ácido docosaexaenoico (DHA; 22:6 n-3), demonstraram ter papéis importantes na redução do risco de doença cardiovascular, na prevenção do câncer, no desenvolvimento e manutenção dos tecidos neurais e visuais ao longo da vida (CALDER, 2004; LEAF et al., 2003).

Além dos benefícios dos AG n-3 na saúde humana, os isômeros do ácido linoléico conjugado (CLA), também têm chamado atenção devido à suas propriedades biológicas importantes para a manutenção da saúde (ALDAI et al., 2006). Alguns isômeros de CLA, por exemplo, os cis 9, trans 11 (c9, t11) e t10, c12 apresentam propriedades fisiológicas comprovadas, incluído funções anticarcinogênicas, antiobesidade, antidiabetogênicas, antiaterogênicas, antiarerosclerose, imunomoduladora e melhora a formação óssea (PARK; PARIZA, 2007). As gorduras presentes em ruminantes é uma fonte natural abundante em c9, t11, principal isômero CLA, comumente conhecido como ácido rumênico (KRAMER et al., 1998). Este isômero é produzido principalmente nos tecidos por dessaturação Delta-9 do ácido vacênico (MUFA) (GRIINARI et al., 1999; NUERNBERG et al., 2005; PALMQUIST et al., 2004). Existem relatos de vinte e quatro diferentes tipos de isômeros de CLA presentes naturalmente em alimentos, especialmente de origem de animais ruminantes, incluindo leite, produtos laticínios e carne (SEHAT et al., 1998).

Nesse contexto, pesquisas em todo o mundo têm sido realizadas com o intuito de melhorar o perfil de AG na carne de ruminantes, e aumentar as concentrações de AG benéficos à saúde humana, como a proporção de AGPI (relação n-6/n-3) e reduzir ácidos graxos que podem atribuir algum efeito prejudicial, como o caso dos ácidos hipercolesterolêmicos (AGS como ácido palmítico e ácido mirístico e insaturados trans) (DUNNE et al., 2011; GULATI; GARG; SCOTT, 2005; LOTTENBERG, 2009; POUZO et al., 2015; SCOLLAN et al., 2006, 2014; SINCLAIR, 2007). Contudo, como a maioria das características economicamente importantes em bovinos de corte, a composição de AG é uma característica poligênica e sofre influência de fatores genéticos e ambientais. Além disso, melhorar o perfil de AG da carne bovina é desafiador devido a extensa lipólise e biohidrogenação de lipídios da dieta pelo microbiota ruminal (AHMED et al., 2019; BERTON et al., 2016; FERRINHO et al., 2018). Para contornar isto, e conseguir melhor essa característica em médio e longo prazo tem sido aplicado estratégias alimentares (FERRINHO et al., 2018; MWANGI et al., 2021; NOGOY et al., 2022) combinadas à seleção genética (CHIAIA et al., 2017; ZHU et al., 2017a), buscando obter fontes de proteínas com perfil lipídico mais saudável para atender à crescente demanda dos consumidores mais exigentes e conscientes em relação a uma alimentação saudável e produção sustentável.

No ponto de vista genético, as características complexas são controladas por diversos genes e suas isoformas, que atuam em praticamente todos os níveis de regulação da expressão gênica e de proteínas que induzem as diferenças no perfil de ácidos graxos. Além disso, estudos relataram que a classe de RNAs reguladores, dentre eles, os RNAs longos não codificantes (lncRNAs), desempenham um papel crucial na regulação da expressão gênica eucariótica (DERRIEN et al., 2012; RAPICAVOLI et al., 2013; ZHU et al., 2017b). Vários estudos relatam a frequência de lncRNAs associados à susceptibilidade à mastite clínica (TONG et al., 2017), à resposta imune (GUPTA et al., 2019), mecanismos muscular em bovino (BILLEREY et al., 2014), a qualidade de carne em bovinos Nelore (Muniz et al., 2022). Atualmente, o conhecimento sobre os mecanismos moleculares para a expressão de fenótipos de qualidade da carne bovina regulados por eventos de *splicing* alternativo e regulação gênica envolvendo lncRNAs são ainda limitados, uma vez que se trata de uma característica de difícil mensuração influenciada por diferentes processos biológicos.

A utilização da ferramenta RNA-Seq tem tornado possível quantificar novas isoformas de genes expressos gerados através de eventos de "*splicing*" alternativo, além de auxiliar na identificação de possíveis lncRNA, de maneira mais precisa quando comparado com outras metodologias de análise de expressão gênica, como análise da expressão gênica em série (SAGE, *Serial analysis of gene expression*) e microarranjos (CORCHETE et al., 2020; MALONE; OLIVER, 2011; TANG et al., 2011; WANG et al., 2009). Portanto, RNA-Seq é uma relevante ferramenta para esclarecer os mecanismos subjacentes de características complexas, permitindo uma melhor compreensão da regulação genética do fenótipo pertencente a características interesse (TIZIOTO et al., 2015). Os resultados deste trabalho podem melhorar o conhecimento biológico associado à composição dos AGs da carne bovina e fornecer conhecimento para auxiliar no desenvolvimento de estratégias de melhoramento da qualidade da carne.

2. OBJETIVOS

1.1. Objetivo geral

Estudar o transcriptoma do músculo *Longissimus thoracis* de bovinos da raça Nelore, com o propósito de identificar novas isoformas de mRNA e lncRNA associados à composição de ácidos graxos, fornecendo assim, informações sobre os mecanismos genéticos e moleculares que regulam essa característica.

1.2. Objetivos específicos

- Identificar isoformas de mRNA expressos diferencialmente do músculo *Longissimus thoracis* associadas à diferentes perfis de ácidos graxos usando dados de sequenciamento de RNA (RNA-seq).
- Identificar lncRNA diferencialmente expressos do músculo *Longissimus thoracis* de animais com diferentes perfis de ácidos graxos, utilizando dados de sequenciamento de RNA (RNA-Seq).

2. REVISÃO DE LITERATURA

2.1. Aspectos de qualidade da carne bovina importantes para a manutenção da saúde humana

A composição de nutrientes da carne bovina é de aproximadamente 68,5% de água, 20,9% de proteína e 9,5% de gordura; após o cozimento, os percentuais se alteram para 58,6% de água, 29,4% de proteína e 10,6% de gordura (MCCANCE et al., 2014) . Assim, a carne é considerada uma importante fonte de proteína, especialmente aminoácidos essenciais, que não podem ser sintetizados no corpo humano (WYNESS, 2016; WYNESS et al., 2011). A carne vermelha, em particular, contém proteínas de alto valor nutricional, incluindo todos os oito aminoácidos essenciais exigidos para na alimentação de adultos e todos os nove aminoácidos exigidos para as crianças, sendo eles: isoleucina, valina, leucina, fenilalanina, treonina, metionina, triptofano, histidina e lisina (WYNESS, 2016). As evidências atuais sugerem que a quantidade de proteína é muito semelhante entre a carne orgânica e a produzida convencionalmente (ŘEDNICKA-TOBER et al., 2016; RIBAS-AGUSTÍ et al., 2019). No entanto, o teor de gordura da carne é muito mais variável do que a proteína, devido a influência de fatores de produção e raça. Do ponto de vista nutricional, a gordura é uma fonte rica de energia, mas também de vitaminas e ácidos graxos essenciais, além de contribuir para uma melhor palatabilidade e sabor da carne (WYNESS et al., 2011).

Profissionais da saúde recomendam uma ingestão moderada de gordura total e uma maior ingestão de ácidos graxos poli-insaturados (AGPI), principalmente ômega 3 (n-3), que desempenham papel chave no crescimento e desenvolvimento do embrião, reduz riscos de doenças mentais e apresentam efeitos cardioprotetores (CALDER, 2004; LEAF et al., 2003). O consumo elevado de ácidos graxos saturados (AGS) pode levar a resistência à insulina, estresse oxidativo, bem como estimulação de cascatas inflamatórias, os quais participam da etiologia de doenças cardiovasculares, diabetes e síndrome metabólica (KENNEDY et al., 2009). Por outro lado, os ácidos graxos monoinsaturados (AGMI) e AGPI são benéficos, minimizando o risco de doenças coronarianas, a resistência à insulina, além de promover o aumento/manutenção das concentrações de lipoproteínas de alta densidade (HDL) e diminuição de lipoproteínas de baixa densidade (LDL), ambas responsáveis pelo transporte do colesterol (LOTTENBERG et al., 2012).

As gorduras intramuscular (marmoreio e conteúdo de lipídios), intermuscular (entre músculos) e subcutânea atrelam-se às características ligadas à qualidade de carne, das quais o sabor, maciez e a suculência, sofrem maior interferência do perfil de AG e da quantidade de lipídios (WOOD et al., 2008). Nesse sentido, o perfil de AG da gordura intramuscular presente na carne merece uma maior atenção, sendo de suma importância para a saúde humana. Em geral, a gordura intramuscular corresponde, em média, a 45-48% de AGS, 35-45% de AGMI e até 5% de AGPI dos ácidos graxos totais (SCOLLAN et al., 2006).

Os AGS comumente encontrado na carne bovina são: 14:0 (ácido mirístico), 16:0 (ácido palmítico) e 18:0 (ácido esteárico) (YU et al.,1995). Os principais AGMI incluem palmitoléico (16:1) oleico e vacênico (18:1), e os principais AGPI são ácidos linoléico (18:2) e α-linolênico (18:3), além de conter em proporções menores ácido eicosapentaenóico (EPA), ácido docosahexaenóico (DHA) e ácido araquidônico (AA) de cadeia longa C20/22, ácidos considerados essenciais, uma vez que o organismo é incapaz de sintetizá-los. Além desses, a gordura de ruminantes é uma das principais fontes do conjunto de isômeros do ácido graxo linolênico conjugado (CLA) na dieta humana, como o CLA-cis-9, trans-11 que possuem efeitos benéficos a saúde humana, uma vez que possui ação anticancerígena e previne/controla aterosclerose (BELURY, 2002; WOOD, 2017).

Entretanto, a quantidade e a qualidade do perfil de ácidos graxos na carne bovina são dependentes do metabolismo lipídico que ocorre ao longo da vida do animal. Os processos metabólicos como lipogênese e/ou adipogênese, ou seja, hipertrofia de adipócitos e hiperplasia de células adipogênicas, respectivamente, desempenham um papel chave na deposição de tecido adiposo (HAUSMAN et al., 2009). No entanto, melhorar o perfil de AGs na carne bovina e demais ruminantes tem sido desafiador devido à extensa lipólise e biohidrogenação decorrendo do metabolismo lipídico ruminal dos AGs insaturados provenientes da dieta (SCOLLAN et al., 2017).

A composição de AGs na carne pode ser influenciada por fatores como a genética e sexo do animal, a dieta fornecida e a idade de abate (PARK et al., 2018b; SEXTEN et al., 2012; WARREN et al., 2008; WOOD et al., 2008). Diversos estudos demostraram que a gordura intramuscular de raças zebuínas possui menor saturação quando comparada às de raças taurinas (HUERTA-LEIDENZ et al., 1996; MENEZES et al., 2009; PERRY et al.,1998; ROSSATO et al., 2010). Além disso, existem evidências que a carne de bovinos da raça Nelore é nutricionalmente mais saudável que a carne da raça Angus, por conter porcentagem mais baixas de colesterol e maior quantidade dos AGs, como n-3 e precursor de CLA (C18: 1 trans) (ROSSATO et al., 2010).

2.2. Estrutura e classificação dos ácidos graxos

Os ácidos graxos (AGs) são ácidos carboxílicos classificados pelo comprimento de suas cadeias de carbono, presença e quantidade de ligações duplas e a configuração do átomo de hidrogênio (JENSEN, 2002). Os AGs variam em comprimento de 2 a 80 carbonos; no entanto, na carne bovina, estão presentes cadeias com 14, 16, 18, 20 e 22 átomos de carbono (LUNN et al., 2006).

Os AGS são aqueles que possuem uma ligação simples entre os carbonos, ou seja, não possuem insaturações na molécula, enquanto os ácidos graxos insaturados (AGI) possuem uma ou várias ligações entre os carbonos, sendo subdivididos em dois grupos: AGPI (ácidos graxos poliinsaturados) que apresentam mais de uma dupla ligação e os AGMI (ácidos graxos monoinsaturados) com apenas uma ligação entre os carbonos.

Em quase todos os AGS presente em alimentos, as ligações duplas encontram-se em configuração *cis* (hidrogênios que estão próximos à ligação dupla encontram-se no mesmo lado da cadeia). A presença de uma ligação *cis* em um ácido graxo diminui o seu ponto de fusão, apresentando característica líquida à temperatura ambiente. Ácidos graxos *trans* (hidrogênios estão em lados opostos) são menos comuns na natureza, mas são normalmente encontrados em menor quantidade na gordura de carnes de ruminantes e no leite. Esses ácidos graxos também podem ser produzidos durante a hidrogenação (endurecimento) de óleos insaturados, processo tradicionalmente usado na fabricação de margarina (LUNN et al., 2006).

Os AGPI podem ainda ser classificados como ômega 3 (n-3), ômega 6 (n-6), e ômega 9 (n-9). Estes possuem a primeira dupla ligação, respectivamente 3 e 4, 6 e 7 e entre 9 e 10 respectivamente (LUNN et al., 2006).

2.3. Metabolismo lipídico em ruminantes

O metabolismo lipídico desempenha um papel dinâmico e crítico durante o crescimento dos animais ruminantes, os quais possuem adaptações digestivas e metabólicas que fazem com que sejam animais altamente diferenciados, possuindo uma grande relação de simbiose com a comunidade microbiana anaeróbica indispensável para retirar energia das paredes vegetais (BESSA et al., 2005). Nas últimas décadas, muitos autores (HARFOOT, 1981; JENKINS, 1993a; PALMQUIST et al., 1980) estudaram o destino dos lipídios da dieta durante a fermentação ruminal, enfatizando três principais processos: lipólise ou hidrólise (LP), biohidrogenação (BH) e a síntese de novo, que é realizada majoritariamente pelos microorganismos do ecossistema ruminal.

Os lipídios presentes na dieta de ruminantes, geralmente triglicerídeos, fosfolipídeos e galactolipídeos, são esterificados. Com a exposição ao rúmen, os lipídios esterificados sofrem uma extensa lipólise (LP), ação de fosfolipases, lipases e galactolipases ligadas à membrana celular dos microrganismos presentes (BAUCHART, 1999), liberando galactose, glicerol, ácidos graxos insaturados e ácidos graxos saturados em quantidades reduzidas. A galactose e o glicerol penetram na célula bacteriana e são imediatamente metabolizados e convertidos em ácidos graxos de cadeia curta, que posteriormente são absorvidos por difusão pelo epitélio ruminal naturalmente na forma de proprionato (BAUCHART, 1999). Os AGI liberados são transformados em duas etapas, uma inicial denominada isomerização, seguida da hidrogenação. Esses processos ocorrem na fase de biohidrogenação ruminal (HARFOOT e HAZLEWOOD, 1988).No entanto, estudos evidenciam que diversos fatores podem influenciar na diminuição da extensão da lipólise como: baixo pH (VAN NEVEL; DEMEYER, 1996), nível de lipídios na dieta e a maturidade do volumoso (BEAM et al., 2000), teor de proteína (LOOR et al., 2004), tamanho da partícula do alimento no rúmen e o uso de ionóforos que inibem a atividade e o crescimento bacteriano (BEAM et al., 2000; JENKINS, 1993b; LOOR et al., 2004).

A biohidrogenação ruminal é um processo que consiste na isomerização e hidrogenações (reduções e saturações) de AGP realizadas por algumas bactérias para reduzir a toxicidade sobre o crescimento de microrganismos ruminais. A toxicidade desses ácidos graxos, principalmente dos AGP, é relacionada a natureza anfifílica desses ácidos graxos, isto é, aqueles que são solúveis, tanto em solventes orgânicos como em água, podendo causar rompimento em membranas celulares (PALMQUIST & MATTOS, 2006). Nesse sentido, o mecanismo de defesa dos microrganismos ruminais diante dessa toxicidade é a biohidrogenação ruminal, que converte a gordura insaturada em saturada (PALMQUIST & MATTOS, 2011).

O processo de isomerização é responsável pela conversão de ácidos graxos insaturados *cis*-12 de dupla ligação a isômeros *trans*-11 e algumas modificações posicionais de duplas ligações. Após o mesmo, procede-se à redução de duplas ligações, aumentando o grau de saturação dos AG. Vale ressaltar que fatores que afetem a LP também interferem na BH, uma vez que o requerimento do grupo carboxilado livre é um pré-requisito para que esta aconteça (FRANCISCO et al., 2016; HOFFMANN et al., 2015; ISHLAK et al., 2015).

As bactérias encarregadas pela biohidrogenação são divididas em dois grupos. O primeiro, é responsável pela biohidrogenação do ácido linolênico (C18:3) e ácido linoléico (C18:2) a ácido vacênico (trans-11 C18:1), em menores quantidades outros isômeros. Este grupo é incapaz de biohidrogenar ácido oleico (C18:1) a ácido esteárico (C18:0). Já o segundo grupo de bactérias, são capazes de biohidrogenar completamente ácido linoléico, ácido linolênico e outros isômeros C18:1 a ácido esteárico. No entanto, o processo de biohidrogenação não é completo, originando diversos AG intermediários importantes à saúde humana, como isômeros do CLA e ácido vacênico (KEMP et al., 1984).

Os microrganismos ruminais possuem em sua parede celular lipídios microbianos, estes são produzidos por moléculas precursoras (glicose) presentes nas partículas dos alimentos na dieta

(SHINGFIELD et al., 2008). Dessa forma, a dieta e os tipos de microrganismos presentes no rúmen são fatores que determinam a quantidade de lipídios nos microrganismos. Estes microrganismos, além de serem responsáveis pela modificação de AG dietéticos, também sintetizam diversos ácidos graxos (síntese de novo), em grande parte de cadeia ímpar (15 â 16 átomos de C) e/ou ramificada, estes, AG com ramificações da série ISO e ANTEISO, normalmente responsáveis pela manutenção da fluidez das membranas (BONNET et al., 2007). Dessa forma, os lipídios que chegam ao intestino delgado representam a soma dos lipídios provenientes de origem microbiana e do alimento. Devido a isto a quantidade de lipídios que chegam ao duodeno é maior que a quantidade ingerida pelos ruminantes (SHINGFIELD et al., 2008).

Por meio da ação da bílis os lipídios em fase insolúvel são transferidos para fase micelar, permitindo sua absorção no jejuno. As micelas se formam devido às propriedades anfipáticas de diversas moléculas, tais como ácidos graxos livres, fosfolipídios, colesterol, sais biliares, entre outras. Todos os constituintes da micela se difundem nos enterócitos, exceto os ácidos biliares, que alcançam o íleo e são reabsorvidos através de um processo de co-transporte com sódio. Após a absorção, os ácidos biliares são transportados de volta ao fígado de maneira direta, responsável por extraí-los do sangue, e reciclados pela bile (FURLAN et al., 2006).

Após sua absorção no intestino delgado, os lipídios esterificados são carregados pelas lipoproteínas que são produzidas nas células intestinais e posteriormente liberadas na corrente sanguínea. As lipoproteínas são glóbulos similares às micelas, além de serem responsáveis por transportar ácidos graxos, também carregam o colesterol, formado principalmente no intestino delgado e nas células adiposas (BAUCHART, 1999). Na corrente sanguínea, podemos encontrar uma variedade de lipoproteínas que se distinguem de acordo com características como densidade, tamanho, formato, composição química e função. A incorporação dos lipídios absorvidos na circulação ocorre principalmente por meio das lipoproteínas de densidade extremamente baixa, abreviadas como VLDL (do inglês "Very Low Density Lipoprotein"). Nos ruminantes, a contribuição dos quilomícrons no transporte de lipídios no sangue geralmente é baixa, sendo evidente apenas quando há um aumento significativo nos níveis de gordura na dieta e na absorção intestinal de ácidos graxos (SHINGFIELD; ROUEL; CHILLIARD, 2008).

Após serem transportados para os tecidos periféricos, os ácidos graxos têm a possibilidade de serem depositados de forma intacta ou passar por modificações através dos processos de dessaturação na cadeia carbônica. Isso ocorre por meio da ação de enzimas conhecidas como dessaturases, que introduzem uma dupla ligação oxidando dois carbonos consecutivos na cadeia (MARTINS et al., 2007). Nos tecidos dos ruminantes, especialmente no fígado, no tecido adiposo e na glândula mamária, a enzima Delta-9-dessaturase está presente, desempenhando papel importante na conversão do ácido vacênico em CLA (ácido linoléico conjugado) por meio da dessaturação. (SHINGFIELD et al., 2008).

2.4. Genes associados à deposição de gordura e ao perfil de ácidos graxos na carne bovina

A quantidade de gordura corporal armazenada como depósitos de tecido adiposo subcutâneo, interno e intramuscular e o perfil de ácidos graxos são características quantitativas ou fenótipos complexos na natureza, e influenciados por muitos genes, vias metabólicas complexas e pelo ambiente, principalmente a alimentação (EBERLÉ et al., 2004). Com relação ao fator genético, alguns fatores de transcrição como *SREBPs, RXR/LXR* e *PPAR α/γ/Δ,* foram identificados como como genes-chave no metabolismo lipídico. Estes fatores de transcrição desempenham um papel central na regulação da expressão de vários genes responsáveis pela homeostase energética, promovendo a lipogênese e adipogênese, assim como biossíntese de ácidos graxos como *ACACA, DGAT, SCD, LPL, FANS, CD36* e *FABPs* (HUANG et al., 2017; YIN; ZHANG et al., 2002; ZHANG et al., 2016, 2008).

Adicionalmente, existem mecanismos regulatórios responsáveis por modular a expressão de genes envolvidos na síntese de AGs que compreendem basicamente nas interações epigenéticas desencadeadas por atuações de microRNA (miRNA) (MUROYA et al., 2020), RNAs longo não codificantes (lncRNA) (JIANG et al., 2020), fenômenos de metilação da cadeia de nucleotídeos e modificações covalentes de histonas (BURDGE et al., 2014). Estudos de análise global do transcriptoma realizados para identificar diferenças de expressão gênica nos tecidos adiposos subcutâneos entre bovinos Wagyu e Holandês identificaram genes intimamente relacionados ao metabolismo e acúmulo de gordura (HUANG et al., 2017). Entre esses genes, foram encontrados *Lepitina, EGR1, FOS, SERPINE1, AGT e MMP2* que podem ter grande impacto na diferenciação dos adipócitos e mobilização lipídica (HUANG et al., 2017).

Quanto ao perfil de AGs intramuscular, estudos realizados por Berton et al. (2016) em bovinos Nelore identificaram diversos genes diferencialmente expressos dos quais podemos destacar *ACAT1, FABP7, PPAR α/Δ, ACSM 1 e 3, DGAT2 e ACSS1.* Esses genes estão relacionados ao metabolismo, transporte e oxidação dos ácidos graxos, bem como síntese e degradação dos corpos cetônicos, transporte intracelular de ácidos graxos de cadeia longa e síntese de triglicerídeos e armazenamento intracelular (KERSTEN, 2008; KERSTEN et al., 2000).

Cesar et al. (2016) investigaram genes diferencialmente expressos no músculo *Longissimus dorsi* de bovinos da raça Nelore divergentes para AG palmítico, esteárico, oleico, linoléico, CLA, EPA e DHA. Esses autores observaram que animais com de alto teor de ácido oleico tiveram maior expressão dos genes *SCD*, *SREBF1*, *PPARGC1A/1B*, *FOXO 1 e 3*. No grupo de alto teor de CLA foram apontados os genes *STAT2* e *NFKB1A*, além de compartilhar *SREBF1 e FOXO1* com o grupo do ácido oleico.

Dawood et al., (2021) investigaram novas regiões genômicas associadas à composição de AG em bovinos da raça Angus e encontram regiões que abrigam genes candidatos para o melhoramento da composição de ácidos graxos, como *PFKFB2, PM20D1, BBS4, ACAA2, FADS 2 e 3 e OSBPL5*. Esses genes atuam no perfil de ácidos graxos, transporte, lipólise, acúmulo de gordura e manutenção do equilíbrio do colesterol (AKSANOV et al., 2014; CHUNG et al., 2015; LONG et al., 2018; POLLOCK et al., 2019; PRIETO-ECHAGÜE et al., 2020) e foram associados aos ácidos graxos AGMI, relação AGMI/AGS, ácido palmitoeico (16:1), ácido esteárico (18:0) (DAWOOD et al., 2021).

2.5. Análise de transcriptoma aplicadas à bovinos.

O transcriptoma contém RNAs mensageiros codificantes (mRNAs), bem como RNAs não codificantes (ncRNAs), como microRNA (miRNA, miR), RNA longo não codificante (lncRNA), RNA ribossômico (rRNA) e RNA de transferência (tRNA), etc. Embora várias metodologias tenham sido desenvolvidas para caracterizar esses RNAs, o Sequenciamento de Nova Geração (NGS) surgiu como a plataforma central para o perfil completo e imparcial de genomas e transcriptomas (Marguerat, S., & Bähler, J., 2010). O sequenciamento de RNA (RNA-Seq) é uma tecnologia de alto rendimento usada para fornecer uma visão abrangente de todo o transcriptoma, incluindo detecção de isoformas de mRNA e fusão gênica, perfil de expressão gênica, e análise de célula única (HRDLICKOVA et al., 2017).

O RNA-Seq permitiu descobertas significantes sobre os mecanismos moleculares de diversas características de interesse econômico em animais da raça Nelore. Vários estudos têm usado esta técnica para investigar a expressão gênica em animais de produção, como em bovinos, que possibilitou a identificação de genes diferencialmente expressos no tecido muscular associados a composição de ácidos graxos (BERTON et al., 2016; HUANG et al., 2017; OLIVIERI et al., 2021; SCHETTINI et al., 2022b) e diversas outras características, como maciez da carne (FONSECA et al., 2017; MUNIZ et al., 2021;2022), área de olho de lombo (AOL) e gordura subcutânea (SILVA-VIGNATO et al., 2017), gordura intramuscular (CESAR et al., 2015; SILVA et al., 2019), consumo alimentar residual (TIZIOTO et al., 2015) e conteúdo de ferro da carne (DINIZ et al., 2016).

Outras aplicações do RNA-Seq são as detecções/caracterizações de isoformas de mRNA geradas por eventos de “*splicing”* alternativo, identificar isoformas de mRNA associados à gordura intramuscular (UEDA et al., 2021); composição de ácidos graxos (ZHANG et al., 2018), adipogênese (CAI et al., 2018), a área de olho de lombo e conteúdo de gordura intramuscular (SILVA et al., 2020), marmoreio, maciez e cor da carne (MUNIZ et al., 2021, 2022a) em bovinos.

Além disso, a técnica permite detecção de lncRNAs, estes são estruturalmente semelhantes aos mRNAs e são gerados através da transcrição do DNA (Zhang et al., 2013). Curiosamente, o número de lncRNAs excede em muito o número de genes codificadores de proteínas e participam de uma variedade de processos biológicos complexos, interagindo com proteínas, DNA, bem como RNAs (Wang et al., 2011; Iyer et al., 2015; Schmitt et al., 2016; Rao et al., 2017). A alta versatilidade e a capacidade de regular a expressão gênica dos lncRNAs podem ser utilizadas para agrupar lncRNAs que sejam similares em seus mecanismos moleculares de atuação. Desse modo, os diferentes tipos de ação dos lncRNAs foram agrupados em quatro arquétipos moleculares que buscam ilustrar sua ampla capacidade de atuação: sinal, dissipadores moleculares, guias, e esqueleto molecular (Gao et al., 2020) (Figura 1).

Como moléculas sinalizadoras, os lncRNAs podem atuar sozinhos ou combinados com algumas proteínas (fatores de transcrição) para mediar a transcrição de genes (Figura 1, A). Os lncRNAs como “dissipadores moleculares” atuam bloqueando uma determinada via molecular. Ele se liga a proteínas funcionais para bloquear as proteínas de regularem as moléculas de DNA e mRNA ou se ligam a moléculas de miRNA competitivamente com moléculas de mRNA para bloquear o efeito inibitório do miRNA nas moléculas de mRNA (Figura 1, B). Os lncRNAs podem atuar como moléculas guias, auxiliando proteínas específicas a atingir seu local alvo e exercer suas funções biológicas. Eles carregam algumas moléculas de proteínas funcionais e as localizam na área alvo para desempenhar funções (Figura 1, C). Os lncRNAs como “esqueletos moleculares” em que outras moléculas podem se acoplar para montagem de complexos funcionais e tralharem juntos na área alvo (Figura 1, D).

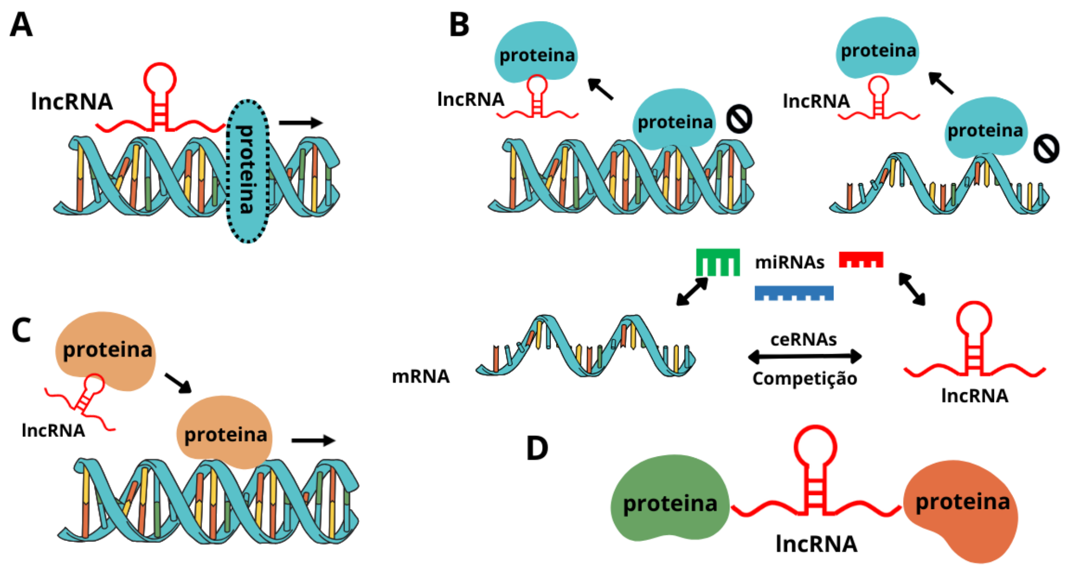

Figura 1. Arquétipos moleculares dos lncRNAs de acordo com seus mecanismos de ação. (A) Sinalizadora; (B) Dissipadores moleculares; (C) Guias (D) Esqueletos moleculares. Figura adaptada de Gao et al. (2020).

Em eucariotos, os lncRNAs atuam praticamente em todos os níveis de regulação da expressão gênica de proteínas, interferindo no epigenoma e regulando os processos transcricionais e pós-transcricionais dos genes alvo (Gao et al., 2020). Os lncRNAs envolvidos na regulação transcricional atuam como ligantes e frequentemente interagem com fatores de transcrição para formar complexos e controlar a transcrição genética (Kurokawa et al., 2011). Vários estudos relatam a frequência de lncRNAs associados à susceptibilidade à mastite clínica (TONG et al., 2017), à resposta imune (GUPTA et al., 2019), à característica de crescimento (JIN et al., 2019) e ao músculo bovino (BILLEREY et al., 2014) e ao marmoreio e cor da carne (MUNIZ et al., 2022a). Em animais de origem indiana, o conhecimento sobre os mecanismos moleculares regulados por isoformas de mRNA e lncRNAs, principalmente no contexto de diferenças individuais na composição de ácidos graxos na carne, ainda é limitado, especialmente em bovinos da raça Nelore.

Através da utilização da tecnologia de RNA-Seq, é possível identificar isoformas de mRNA e lncRNA, o que proporciona um maior esclarecimento da fisiologia subjacente a características de significado econômico. Isso permite a identificação de genes, mecanismos regulatórios e moduladores previamente não documentados. Essas descobertas têm o potencial de contribuir para a produção de produtos cárneos com maior qualidade e mais saudáveis para o mercado consumidor, além de apoiar os esforços de programas de seleção e melhoramento genético no Brasil.

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CHAPTER 2 -SKELETAL MUSCLE LNCRNA PROFILE ASSOCIATED WITH FATTY ACIDS IN BEEF CATTLE

ABSTRACT- This study aimed to identify differentially expressed (DE) long non-coding RNAs (lncRNAs) in muscle tissue of Nellore cattle clustered by their fatty acid profile. *Longissimus thoracis* muscle samples from 48 young bulls were used to quantify fatty acid (FA) (myristic, palmitic, stearic, oleic, linoleic, conjugated linoleic (CLA), α-linolenic and the groups of saturated fatty acids (SFA), monounsaturated (MUFA), polyunsaturated (PUFA), ω3, ω6, PUFA/SFA ratio and ω6/ω3) and to generate RNA-Sequencing data for transcriptomic analyses. The *K*-means analysis was used to classify the 48 animals into three clusters based on their FA patterns. The C1 had significantly ($p \leq 0.05$) higher PUFA, ω3, ω6, linoleic and α-linolenic content than C2 and C3. The proportion of MUFA, CLA and oleic in the C2 and C3 were significantly ($p \leq 0.05$) higher in relation to C1, while C3 had significantly ($p \leq 0.05$) higher proportions of ω6/ω3, SFA, myristic, palmitic and stearic proportion than C1 and C2. DE analyses were performed on three different comparisons, C1 vs. C2, C1 vs. C3 and C2 vs. C3, and 25, 28 and 22 DE lncRNAs (fold change > | 2 |, p-value < 0.01 and false discovery rate (FDR) < 0.05) were found, respectively. For C1 vs. C2 comparison, a new transcript "*lncRNA_16456.3*" was found and was interacted with the genes *FAM126A* (Family with sequence similarity 126 member A) and *IL6* (Interleukin-6). These genes were enriched by GO biological function terms related to cellular response to lipid pathway. For the C1 vs. C3 comparison, the lncRNA "*lncRNA_13894.1*" interacting with the *BNIP3* gene (BCL2/Adenovirus E1B 19 kDa protein-interacting protein 3) was enriched by GO biological function terms related to fat cell differentiation. For the C2 vs. C3 comparison, a new transcript "*lncRNA_16618.6*" interacted with genes involved in G protein-coupled receptors (GPCRs). Those genes play a crucial role in regulating lipolysis mediated by the cAMP signaling pathway and may be contributing to a higher PUFA fatty acid content in beef. For the three comparisons: C1 vs. C2, C1 vs. C3, and C2 vs. C3, the identified lncRNAs, including genic and intergenic (lincRNA were associated with genes affecting immune response, energy metabolism, lipid and FA metabolism, whose seem to play an essential role in the physiological processes related to meat quality. These findings provide new insights to better understand the biological mechanisms involved in gene regulation of FA composition in beef. This could be valuable for further investigation regarding interaction between lncRNAs and mRNAs and how these interactions may affect meat quality.

KEYWORD: Genic lncRNAs, lincRNA, Nellore cattle, RNA-Seq.

CAPÍTULO 2 - LNCRNAS ASSOCIADO AO PERFIL DE ÁCIDOS GRAXOS DO MÚSCULO ESQUELÉTICO DE BOVINOS DE CORTE

RESUMO- O objetivo deste estudo foi identificar RNAs longos não codificantes (lncRNAs) diferencialmente expressos no tecido muscular de bovinos Nellore agrupados de acordo com seu perfil de ácidos graxos. Amostras do músculo *Longissimus thoracis* de 48 touros jovens foram utilizadas para quantificar ácidos graxos (AG) individuais (mirístico, palmítico, esteárico, oleico, linoléico, linoléico conjugado CLA, α-linolênico e os grupos de ácidos graxos saturados (AGS), monoinsaturados (AGM), poliinsaturados (AGP), ômega 3 (ω3), ômega 6 (ω6), razão AGP/AGS e ω6/ω3) e para gerar dados de sequenciamento de RNA para análises transcriptômicas. A análise *K*-means foi utilizada para classificar os 48 animais em três grupos com base nos seus padrões de AG. O C1 apresentou proporções significativamente ($p \leq 0,05$) mais altas de PUFA/SFA, PUFA, ω3, ω6, linoléico e α-linolênico em relação aos grupos C2 e C3. A proporção de MUFA, CLA e oleico no C2 e C3 foi significativamente ($p \leq 0,05$) maior do que no C1, enquanto o C3 teve proporções significativamente ($p \leq 0,05$) mais altas de ω6/ω3, SFA, mirístico, palmítico e esteárico em relação a C1 e C2. As análises DE foram realizadas em três comparações diferentes, C1 vs. C2, C1 vs. C3 e C2 vs. C3 e um total de 25, 28 e 22 DE lncRNAs (*Fold change* > | 2 |, $p <$ 0,01 e taxa de descoberta falsa (FDR) < 0,05) foram encontrados respectivamente. Para comparação de C1 vs. C2, DE novo transcrito "*lncRNA_16456.3*" foi detectado interagindo com os genes *FAM126A* (Família com similaridade de sequência 126 membro A) e *IL6* (Interleucina-6). Esses genes foram enriquecido por ontologia gênica (GO) termosrelacionada a função biológica de "resposta celular à via lipídica". Para a comparação C1 vs. C3, o novo transcrito "lncRNA_13894.1" DE interagiu com o gene *BNIP3* (BCL2/Adenovirus E1B 19 kDa protein-interacting protein 3) e foi enriquecido por termos GO relacionados a função biológica de "diferenciação de células adiposas". Para a comparação C2 vs. C3, o "*lncRNA_16618.6*" interagiu com genes pertencentes a famílias de receptores acoplados à proteína G (GPCRs). Esses genes desempenham um papel crucial na regulação da lipólise mediada pela via de sinalização do cAMP o que pode contribuindo para um perfil de ácidos graxos da carne com maior teor de AGPI. Esses achados fornecem novos perpectivas para melhor entender os mecanismos biológicos envolvidos na regulação gênica da composição de ácidos graxos na carne bovina, o que poderá ser valioso para futuras investigações de interações entre lncRNAs e mRNAs, e como estas podem afetar a qualidade da carne.

Palavra-Chave: Ácidos Graxos, genic lncRNAs, lincRNA, RNA-Seq

1. INTRODUCTION

Consumers' perception of meat quality products has evolved, becoming interested in the nutritional value of food, encompassing factors such as flavor, appearance, tenderness, food safety, health, nutrition, social aspects, and sustainability (TONSOR et al., 2013). Beef meat has a high nutritional value and is a significant source of protein, rich in essential polyunsaturated fatty acids (linoleic and linolenic acid), zinc, B vitamins, and iron, which is often deficient in the human diet. In addition, beef meat fat has a high concentration of monounsaturated fatty acids (MUFA) with a low melting point, which can help reduce bad cholesterol (LDL) concentration in blood circulation (JAKOBSEN et al., 2008).

Fatty acid profile plays a key role in beef meat's quality traits, especially polyunsaturated fatty acids (PUFA), directly affecting sensory characteristics, such as taste, juiciness, and tenderness (FRANK et al., 2016). On the other hand, consumers are becoming more health-conscious and worried about the cholesterol and fats in food, mainly related to the concentration of certain FAs, such as oleic acid, α-linolenic acid, conjugated linoleic acid (CLA), as well as omega-3 (ω3) and omega-6 (ω6), by its impact on human health status and diseases prevention (ALDAI et al., 2006; CALDER, 2004; LEAF et al., 2003; WOOD, 2017). Although improving the FA meat profile can significantly enhance the quality of meat products, thus it is essential to ensure that the content of these FAs is adjusted to support long-term human health.

The amount and type of fatty acids in beef meat depend on different factors, such as breed, nutrition, production system, sex, age, and carcass finishing level (RULE et al., 1997). These factors pose a challenge in determining the deposition and composition of FA, which limits the knowledge of the genetic mechanisms regulating these traits, hindering the genetic progress in producing healthier beef. In tropical production systems, Nellore cattle are a widely used breed and exhibit significant variation in intramuscular fatty acid composition in the *Longissimus thoracis* muscle (LT), which has increased the interest in investigating the molecular mechanisms involved in FA regulating these fatty acids (BERTON et al., 2016, 2022; CESAR et al., 2015; CHIAIA et al., 2017; DE LEMOS et al., 2018; SCHETTINI et al., 2022b). Measuring FA profiles is challenging, as they are regulated by multiple genes and largely affected by environmental factors (LEAL-GUTIÉRREZ & MATEESCU, 2019). Due to their complexity and being expensive to measure on a large scale, traditional selection methods based on phenotype and pedigree become an even greater challenge as they require the animals to be slaughtered, increasing the generation interval and decreasing genetic gain.

The deposition of FA in meat is a complex process involving the regulation of adipose metabolism (e.g., number and size of adipocytes) and the balance between lipogenesis and lipolysis (DEHGHANIAN et al., 2023, MA et al., 2023). This complex regulation involves multiple gene expressions, signal transduction, and network regulation, and new regulatory factors are constantly being identified, such as long non-coding RNAs (lncRNAs) (MA et al., 2023; MUNIZ et al., 2022b; YAN et al., 2021). Different authors have shown that lncRNA plays a crucial role in regulating protein-coding genes at different levels, such as epigenetic, transcriptional, and post-transcriptional regulation (MA et al., 2023; QIAN et al., 2019; ZHANG et al., 2017; PANG et al., 2013). This regulation impacts the FA profile of meat by controlling the development of adipocytes, lipid metabolism, and fat-type conversion. However, there is a lack of information on lncRNA expression patterns, functions, complex gene networks, and molecular determinants related to different intramuscular FA profile deposition in the meat of Nellore cattle. Hence, this study aims to use an RNA-Seq approach to identify differentially expressed lncRNAs from Nellore cattle muscle tissue with different FA profiles, which may provide knowledge on the genetic and molecular mechanisms regulating FA profile to establish strategies for improving beef quality and directional selection.

2. MATERIAL AND METHODS

2.1. Animals, samples collection and fatty acids composition

A total of forty-eight young Nellore bulls, which were offspring of six different sires belonging to the Capivara farm in São Paulo State, Brazil, were used. These animals participated in the Nellore Qualitas (QLT) commercial breeding program. During the growth phase, animals were raised under a grazing production system (*Brachiaria sp.* and *Panicum sp.)* and received mineral supplementation. The animals were finished in feedlots for 90 days, receiving a mixed diet based on corn silage and supplemented with concentrates based on sorghum grain and soybean meal in the proportion of concentrate/silage ratio (50/50 to 70/30). All animals belonged to the same contemporary group and were finished with the same nutritional management.

The animals were slaughtered with an average age of 24 months and body weight of 550 kg in commercial slaughterhouses, following the Brazilian Federal Inspection Service procedures. After 48h postmortem at 0–2 °C, the samples were collected from the *Longissimus thoracis* muscle (12 – 13th ribs of left half carcass) from each animal and stored at −80 °C for posterior FA assessment analyses.

Meat FAs were extracted from the *LT* muscle samples (~100g) according to the method described by Folch et al. (1957). The muscle samples were ground, and the lipids were extracted by homogenizing the sample with a solution of chloroform and methanol in the ratio of 2:1. Then NaCl was added at a concentration of 1.5% to isolate the lipids. The isolated lipids were subjected to methylation, and the resulting methyl esters were formed according to Kramer et al. (1997). The fatty acid composition was quantified using gas chromatography (GC-2010 Plus - Shimadzu AOC 20i auto-injector) equipped with an SP-2560 capillary column (100 m x 0.25 mm diameter with 0.02 mm thickness, Supelco, Bellefonte, PA), as described in Berton et al. (2016). The FA profile was quantified by normalizing the area under the curve of methyl esters using the GS Solution 2.42 software and then expressed as a percentage of the total FA methyl ester. From the identified FA profile, fourteen FAs were selected based on their health importance (seven individuals and seven groups of FAs). The following FAs were determined as:

1 - Saturated Fatty Acids (SFA): Myristic (C14:0), Palmitic (C16:0), Stearic (C18:0) and sum of Saturated Fatty Acid (SFA) [C4:0+ + C6:0 + C8:0 + C10:0 + C11:0 + C12:0 + C13:0 + C14:0 + C15:0 + C16:0 + C17:0 + C18:0 + C21: 0 + C24:0]

2 - Monounsaturated Fatty Acids (MUFA): Oleic (C18:1 cis-9), and sum of MUFA (MUFA) [C16:1 + C17:1 c10 + C18:1 t11 + C15:1 c10 + C20:1 c11 + C24:1 + C22:1 n9 + C18:1c9 + C14:1 + 18:1 n − 7 + C18:1 n9t]

3 - Polyunsaturated Fatty Acids (PUFA): linoleic (C18:2 cis9 cis12 n6); conjugated linoleic acid (CLA) (C18:2 cis9 trans11); alpha-linolenic (C18:3 n3); and sum of PUFA (PUFA) [C18:2 cis9 trans 11 + C18:2 trans10 cis12 + C18:2 n6 + C18:3 n3 + C18:3 n6 + C20:3 n3 c11, c14, c17 + C20:3 n6 c8, c11, c14 + C20:4 n6 + C20:5 n3 + C22:6 n3]

4 - Ratio between PUFA and SFA (PUFA/SFA)

5 - Omega-3 Fatty Acids (ω3): alpha-linolenic (C18:3 n3) and sum of ω3 (C18:3 n3 + C20:3 n3 c11, c14, c17 + C22:6 n3 + C20:5 n3)

6 - Omega-6 Fatty Acids (ω6): linoleic (C18:2 cis9 cis12 n6); conjugated linoleic acid (CLA) (C18:2 cis9 trans11) and sum of ω6 (C18:3 n6 + C20:3 n6 c8, c11, c14 + C18:2 n6 + C20:4 n6)

7 - Ratio between omega-6 and omega-3 (ω6/ω3)

2.2. Animals clustering by amount of fatty acids

The *K*-means method was used to classify 48 animals into three groups by their similarities in FA profiles. These three groups were determined using the gap statistic to compare the total intracluster variation for different values of ***k*** clusters with their expected values under the null reference distribution of the data. Normality for the AF profile was tested using Shapiro–Wilk's normality test (SHAPIRO; WILK, 1965), and followed a normal distribution. The differences between the three groups on the FA profile were compared using the Least-Squares Means package in R (LENTH, 2016) and Tukey's test ($P < 0.05$).

2.3. RNA-Sequencing

Total RNA was isolated from muscle tissue samples (~50 mg) using the RNeasy Lipid Tissue Mini Kit (Qiagen, Valencia, CA, USA) in accordance with the manufacturer's instructions. The extracted RNA's purity was assessed by measuring its absorbance in a NanoDrop 1000 spectrophotometer (Thermo Fisher Scientific). The quality of the total RNA extraction was evaluated using an Agilent 2100 Bioanalyzer, and its concentration and the presence of genomic DNA contamination were quantified using a Qubit® 2, following the procedures outlined in Fonseca et al (2017).

The mRNA paired-end libraries were created from each RNA sample using the Illumina TruSeq mRNA library preparation kit. Sequencing was conducted on the Illumina HiSeq 2500 platform to generate paired-end reads with 2x100 bp.

2.3.1. Quality control and reads alignment

The total RNA extraction and RNA-Sequencing were performed using the methodologies described by Fonseca et al. (2017). For quality control of the reads the following parameters were considered: 1) quality scores, 2) GC-content, 3) N-content, 4) length distributions, 5) duplication, 6) overrepresented sequences, and 7) K-mer content.

The raw reads were processed in two steps using Atropos (v1.1.19) (DIDION et al., 2017), starting with the insert match algorithm using additional parameters: -n 2, -m 1, –op-order GAWCQ, –match-read-wildcards, -O 20, -q 25, –pair-filter any, and –correct-mismatches conservative, followed by the adapter match algorithm for the unprocessed reads that passed by the first step, applying the parameters: -n 2, -m 1, –match-read-wildcards, -O 3, -q 20, and –pair-filter both. Thereafter, the low-quality regions were also trimmed using "PRINSEQ-lite" software (v.0.20.3.; Schmieder e Edwards, 2011) with options: -trim_qual_window: 3, -trim_qual_right: 30,

-min_len: 20, -trim_tail_left 5, -trim_tail_rigth 5, -lc_method dust and -lc_threshold 50. After filtering, HISAT2 (v.2.0.5) (KIM et al., 2015) was used to map trimmed paired-end reads to the bovine genome reference (ARS-UCD1.2 Bos Taurus; http://ftp.ensembl.org/pub/current_fasta/bos_taurus/).

Mapped reads were assembled using Cufflinks program (v2.2.1) using parameters: -min-isoform-fraction 0.20 -small-anchor-fraction 0.08 -max-intron-length 300000 -trim-3-avgcov-thresh 0.05 -overlap-radius 25 -max-bundle-frags 2000000. Then, the matrix with transformed expression values was calculated by logarithm transformation ($\log_{10}$) and normalized using the cuffilinks packages (Cuffmerge/Cuffquant/Cuffnorm pipeline; http://cole-trapnell-lab.github.io/cufflinks/manual/) with default settings. To ensure validity and reliability of any downstream analysis like DE, isoforms with no detectable expression (FPKM value “0” in all samples), or low expression (mean FPKM less than 0.01 in all samples) and transcripts that were shorter than 200 bp were filtered out (LI et al., 2019).

2.3.2. Differential expression analysis of lncrna

LncDIFF is a powerful differential analysis tool for low abundance non-coding RNA expression data and was used to perform differential expression analysis (LI et al., 2019). The package “lncDIFF” was used with its parameter settings: link.function = “log,” simulated.pvalue = FALSE, permutation = 300 (https://CRAN.R-project.org/package=lncDIFF). This package adopts the generalized linear model with zero-inflated exponential quasi-likelihood to estimate group effect on normalized counts and employs the likelihood ratio test in the differentially expressed genes. A pairwise group comparison (C1 vs. C2, C1 vs. C3 and C2 vs. C3) was used. Additionally, a Fold Change (FC) > | 2 |, *p*-value <0.01 and FDR <0.05 were used to filter DE transcripts and then classify the DE lncRNAs.

2.3.3. Identification of lncRNA

Feelnc filter pipeline (WUCHER et al., 2017) was used to identify potential long non-coding RNAs (lncRNAs) from 1,206,35 transcript models.Transcripts shorter than 200 bp, biotype-coding protein, single-exon transcripts, and biexonic transcripts with one exon size shorter than 25 bp were discarded (ETEBARI et al., 2015; GUPTA et al., 2019). After filtering, the FEELnc coding potential module (FEELnc codpot) was used to separate putative long noncoding RNAs (lncRNAs) from protein-coding RNAs by first computing a coding potential core (CPS, ranging from 0 to 1) for each transcript and then computing a CPS cut-off that maximizes both the lncRNA sensitivity and specificity using a tenfold cross-validation according to the input training files. This process helped classify the transcripts as putative lncRNAs or protein-coding RNAs (MUNIZ et al., 2022b). The FEELnc classifier pipeline was also leveraged to classify each lncRNA with respect to its location and orientation compared to its closest annotated protein-coding genes.

The FEELnc classifier module was used for possible function prediction of differentially expressed lncRNAs based on their nearest-neighbor protein-coding genes. The transcripts in this module are categorized in relation to the nearest RNA: Genic or Intergenic (lincRNA), with three subtypes that are the divergent, convergent and same-strand sub-classes, as detailed on the FEELnc website (https://github.com/tderrien/FEELnc). Additional information can be found in the FEElnc GitHub database (https://github.com/tderrien/FEELnc). This classification was employed to separately investigate the results of DE lncRNA within each category. For novel transcript length associated with annotated lncRNAs, genes within 200kb window (100kb upstream (or downstream) of the start (or stop) position of a lncRNA) were annotated using Ensembl Browser (ARS-UCD1.2 assembly; https://useast.ensembl.org/Bos_taurus/Location/). The ClueGO plug-in of the Cytoscape software (PAUL SHANNON et al., 2003) was used to perform the functional analysis on a list of genes associated with lncRNAs identified for each studied comparison.

3. RESULTS

3.1. Phenotypic variation between groups

Based on *K*-means analysis, three clusters with distinct FA profiles were identified (Figure 1) and the descriptive FA content for each cluster are shown in Table 1. Cluster 1 (C1; n=14 young bulls) exhibited the highest content for PUFA/SFA ratio, PUFA, ω6, ω3, linoleic acid and α-linolenic acid. Cluster 2 (C2; n=24) and Cluster 3 (C3; n=10) exhibited the highest contents of MUFA, CLA, and oleic acid. However, C3 had a significantly higher SFA content, including stearic acid, myristic acid, and palmitic acid, compared to C2 and C1 ($p < 0.05$; Table 1 and Figure 1).

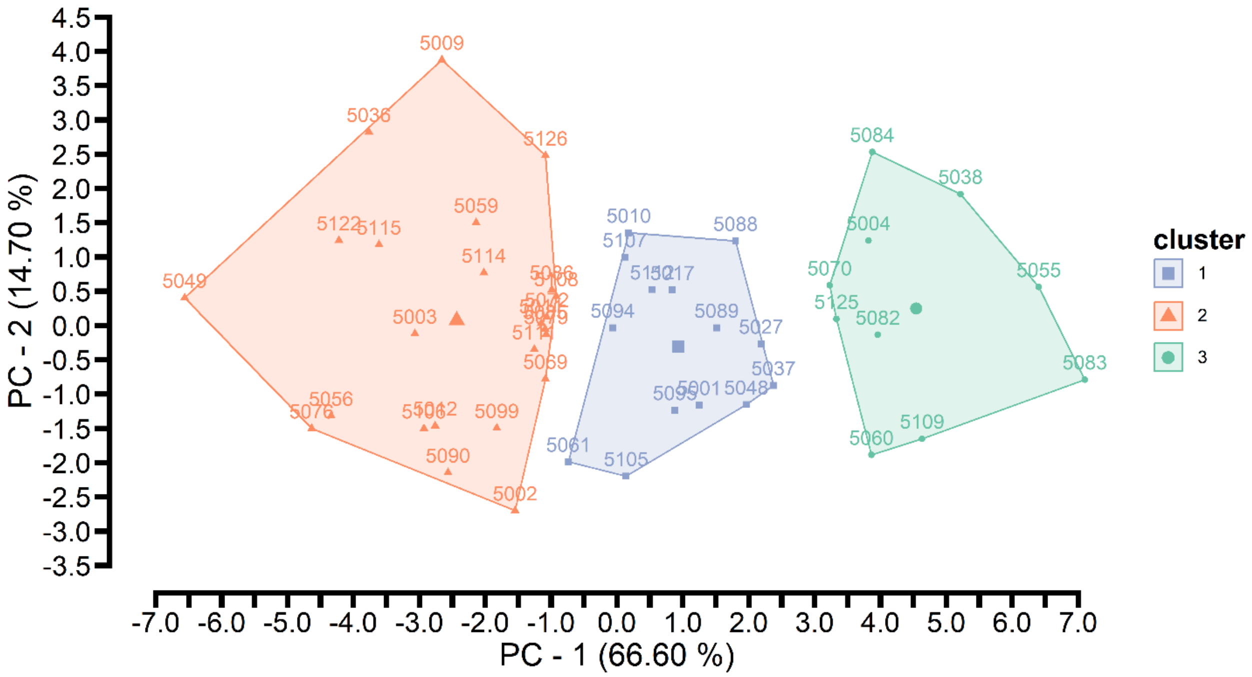

Figure 1. Score plot showed individuals similarities for the fatty acid composition in *Longissimus thoracis* muscle of beef cattle.

Table 1. Average percentage (%) of FAs present in the *Longissimus thoracis* muscle of three clusters of Nellore bulls clustered by their similarities in FA content.

Fatty acids	**Cluster 1 (n= 14)**	**Cluster 2 (n=24)**	**Cluster 3 (n= 10)**	***p*-value**
PUFA/SFA	0.37^{a} ± 0.05	0.24^{b} ± 0.04	0.16^{c} ± 0.03	5.97E-15
PUFA	16.19^{a} ± 2.03	10.80^{b} ± 1.74	7.78^{c} ± 1.68	6.97E-15
ω6	10.19^{a} ± 1.40	6.88^{b} ± 1.33	5.07^{c} ± 1.18	2.09E-12
ω3	5.72^{a} ± 0.87	3.58^{b} ± 0.58	2.33^{c} ± 0.57	6.22E-16
Linolenic	9.37^{a} ± 1.25	6.34^{b} ± 1.22	4.69^{c} ±1.12	1.98E-12
α-Linolenic	0.94^{a} ± 0.13	0.62^{b} ± 0.11	0.46^{c} ± 0.12	1.42E-12
ω6/ω3	1.79^{b} ± 0.19	1.92^{b} ± 0.25	2.18^{a} ± 0.18	1.93E-04
CLA	0.17^{b} ± 0.10	0.27^{a} ± 0.09	0.34^{a} ± 0.12	5.70E-04
Oleic	28.71^{b} ± 2.03	33.18^{a} ± 2.26	33.18^{a} ± 2.26	3.04E-07
MUFA	33.88^{b} ± 2.30	39.06^{a} ± 2.32	39.19^{a} ± 2.95	1.27E-07
SFA	42.91^{b} ± 1.75	43.29^{b} ± 1.46	46.13^{a} ± 2.00	2.48E-05
Stearic	13.34^{b} ± 1.64	13.96^{b} ± 1.43	15.84^{a} ± 1.68	7.06E-04
Miristic	1.54^{c} ± 0.30	2.19^{b} ± 0.26	2.71^{a} ± 0.48	1.23E-10
Palmitic	19.69^{c} ± 1.56	21.98^{b} ± 0.99	23.59^{a} ± 0.97	1.36E-09

Data presented as mean and standard deviation. The concentration of fatty acids was expressed as a percentage of total fatty acid methyl esters. Means sharing different letters between columns were significantly different (p < 0.05) from one another according to Tukey's test.

3.2. Differential expression analysis

3.2.1. Differentially expressed long non-coding RNAs (lncRNA) annotated in the bovine genome reference (ARS.UCD 1.2)

Comparing C1 vs. C2, C2 vs. C3, and C1 vs. C3, a total of 26 long non-coding RNAs were identified (Table 2). For the C1 vs. C2 comparison, one lncRNA was upregulated, while seven lncRNAs were downregulated in animals from the C1 in relation in the C2. Comparing C1 vs. C3, seven lncRNA were upregulated and three lncRNAs were downregulated for C1 agains C3. Pairwise comparison for C2 vs. C3 showed, one lncRNAs upregulated, while seven lncRNAs were downregulated in C2 compared with C3. All these lncRNAs were annotated as novel transcripts in the reference genome (ARS.UCD 1.2), and information about their functionality and roles in literature is scarce.

Table 2. Differentially expressed long non-coding RNAs identified in *Longissimus thoracis* muscle of Nellore cattle with different FA profile.

Feature ID	Position	Length[a]	p-Value	FDR [b]	FC(log2) [c]
Comparison C1 vs. C2					
ENSBTAG00000050891	16:55234389-55238149	341	1.41E-06	9.44E-05	11.17
ENSBTAG00000052746	21:32832866-32857979	647	6.44E-13	1.26E-10	-10.55
ENSBTAG00000054148	25:2533791-2538010	1840	3.25E-11	4.88E-09	-9.91
ENSBTAG00000048982	17:53500021-53509696	447	3.46E-07	2.66E-05	-8.40
ENSBTAG00000048541	10:2284385-2291785	564	6.59E-07	4.76E-05	-7.70
ENSBTAG00000051111	21:33076908-33116941	1347	1.31E-04	5.30E-03	-6.50
ENSBTAG00000054291	17:68750858-68758525	375	1.45E-04	5.78E-03	-11.63
ENSBTAG00000052038	23:50961431-50974711	2044	9.63E-04	2.68E-02	-4.47
Comparison C1 vs. C3					
ENSBTAG00000052922	18:65702277-65710756	1048	1.27E-12	2.27E-10	10.60
ENSBTAG00000052100	18:65720235-65729032	4306	9.27E-10	9.68E-08	6.29
ENSBTAG00000050348	4:49799680-49987158	682	7.83E-07	4.48E-05	11.33
ENSBTAG00000048918	19:50079818-50082870	922	1.47E-04	4.98E-03	3.31
ENSBTAG00000050739	10:30457617-30654451	1039	3.71E-04	1.05E-02	11.41
ENSBTAG00000048899	7:21895844-22018311	1026	3.79E-04	1.07E-02	11.38
ENSBTAG00000052078	25:34334336-34338041	2032	8.26E-04	2.01E-02	4.39
ENSBTAG00000052746	21:32832866-32857979	647	3.14E-12	5.15E-10	-10.63
ENSBTAG00000049466	21:67740943-67754089	791	2.54E-05	1.02E-03	-9.30
ENSBTAG00000048502	9:99053414-99081437	923	8.51E-04	2.06E-02	-3.31
Comparison C2 vs. C3					
ENSBTAG00000050891	16:55234389-55238149	341	1.52E-08	1.17E-06	14.82
ENSBTAG00000051111	21:33076908-33116941	1347	1.39E-14	3.19E-12	-8.79
ENSBTAG00000052922	18:65702277-65710756	1048	5.42E-11	6.97E-09	-8.92
ENSBTAG00000054148	25:2533791-2538010	1840	9.60E-09	7.61E-07	-9.75
ENSBTAG00000052100	18:65720235-65729032	4306	4.75E-08	3.35E-06	-4.92
ENSBTAG00000048544	23:36224038-36465322	1253	1.40E-06	7.11E-05	-10.90
ENSBTAG00000048918	19:50079818-50082870	922	4.39E-05	1.61E-03	-2.99

ENSBTAG00000052078	25:34334336-34338041	2032	3.23E-04	8.82E-03	-4.52

C - Cluster, [a] Transcript Length in bases pair; [b] False Discovery Rate; [c] FC (log2) = Fold change (log2).

3.2.2. Differentially expressed novel transcript length associated with long genic noncoding RNA annotated in the genome reference (ARS.UCD1.2)

C1 vs. C2, 14 novel lncRNA differentially expressed were identified, whereby three were upregulated and eleven were downregulated for animals in C1 group compared with C2 group (Table 3). The DE lncRNAs were related with genes with functions on DNA repair, cellular response to lipid, RNA splicing, phosphorylation, regulation of MAPK cascade and regulation of B cell activation.

Comparing the group C1 vs. C3, 18 novels genic lncRNAs were found to be differentially expressed (Table 3), from those, eight were upregulated and ten were downregulated. The upregulated lncRNAs were associated with response to interleukin-1, central nervous system neuron differentiation, mitochondrion organization, response to hypoxia, and regulation of biosynthetic processes. On the other hand, the downregulated lncRNAs were close to genes associated with response to oxygen levels, cartilage development, mitochondrial transport, and transcription from RNA polymerase II promoter.

In comparing C2 vs. C3, 14 novel lncRNAs were pointed out as DE in the animals of the C2 group compared to the animals of the C3 group (Table 3). Among 14 lncRNAs, five were upregulated lncRNA and were associated with activation of protein kinase activity, response to organic cyclic compound, lipid localization, lipid transport and regulation of cell proliferation. While 9 lncRNAs were downregulated in C2 animals compared to C3 FA profile (Table 3). These were associated with genes with functions in transcription factor binding, cell cortex, B cell proliferation and immune response.

Table 3. Novel transcript length associated with annotated long genic noncoding RNA and differentially expressed in the *Longissimus thoracis* muscle of Nellore cattle with different FA profile.

Feature ID	Positon	Length [a]	*p*-Value	FDR [b]	FC (log2)[c]	lncRNA annotated [d]	Gene Interation [e]
Comparison (C1 vs. C2)							
lncRNA_18429.11	6:42982543-43153637	2562	5.44E-05	2.48E-03	2.48	ENSBTAT00000077875	*ENSBTAG00000033327*
lncRNA_5876.5	16:55234389-55238149	615	1.67E-04	6.49E-03	2.66	ENSBTAT00000081813	*KLHL20, DARS2 ZBTB37, CENPL, RC3H1, SERPINC1*
lncRNA_15786.3	3:58491541-58579144	480	2.14E-04	7.94E-03	13.91	ENSBTAT00000066768	*CCN1, ZNHIT6*
lncRNA_16505.6	4:41775570-41794763	1241	1.38E-12	2.55E-10	-7.55	ENSBTAT00000087015	-
lncRNA_6086.4	16:79121509-79142756	3624	3.81E-12	6.57E-10	-6.54	ENSBTAT00000069212	*ZNF281, KIF14*
lncRNA_5039.5	8:97477285-97504848	5829	5.33E-12	9.08E-10	-4.94	ENSBTAT00000081266	-
lncRNA_18428.3	6:42982543-43153637	692	3.00E-10	4.00E-08	-9.39	ENSBTAT00000077875	*ENSBTAG00000033327*
lncRNA_3567.2	13:37839904-37856732	8398	3.49E-10	4.59E-08	-4.66	ENSBTAT00000085977	*ENSBTAG00000049520, ENSBTAG00000006043, BFSP1, PCSK2 DSTN*
lncRNA_12348.9	23:50961431-50974711	5505	7.12E-10	8.81E-08	-7.44	ENSBTAT00000076672	*MYLK4, WRNIP1*
lncRNA_7981.4	19:8790623-8795920	812	3.46E-07	2.66E-05	-8.40	ENSBTAT00000068745	*VEZF1, SRSF1, DYNLL2, CUEDC1*
lncRNA_19922.3	7:92888299-93014993	1228	1.98E-06	1.28E-04	-9.38	ENSBTAT00000067459	*NR2F1, FAM172A*
lncRNA_10726.5	21:33076908-33116941	1161	1.73E-05	9.05E-04	-4.12	ENSBTAT00000082173	*ENSBTAG00000024311, CSPG4, ODF3L1*
lncRNA_17096.3	4:118297039-118325584	462	2.33E-05	1.17E-03	-9.11	ENSBTAT00000066814	*LMBR1, MNX1, NOM1*
lncRNA_16456.3	4:31551686-31554697	706	1.66E-04	6.47E-03	-11.45	ENSBTAT00000072688	*ENSBTAG00000033806, IL6, TOMM7, FAM126A*
Comparison (C1 vs. C3)							
lncRNA_5876.4	16:55234389-55238149	615	8.24E-14	1.84E-11	8.03	ENSBTAT00000081813	*KLHL20, DARS2, ZBTB37, CENPL, RC3H1, SERPINC1*
lncRNA_6442.5	17:53500021-53509696	6591	3.05E-11	4.14E-09	5.71	ENSBTAT00000074572	*SETD1B, CFAP251, PSMD9, ENSBTAG00000052582, TMEM120B*
lncRNA_6037.8	16:72109237-72128769	2734	6.70E-07	3.90E-05	10.85	ENSBTAT00000067279	*RCOR3, TRAF5*
lncRNA_5415.4	16:1072072-1076246	764	4.89E-06	2.28E-04	5.23	ENSBTAT00000086621	*BTG2, CHI3L1*

lncRNA_13894.1	26:51237158-51241924	3237	5.63E-06	2.59E-04	2.90	ENSBTAT00000073957	*BNIP3*
lncRNA_843.3	1:148950230-148972709	1213	4.96E-05	1.90E-03	3.05	ENSBTAT00000074316	*SIM2*
lncRNA_5415.2	16:1072072-1076246	985	9.50E-04	2.26E-02	3.27	ENSBTAT00000086621	*BTG2, CHI3L1*
lncRNA_12348.11	23:50961431-50974711	7845	1.34E-03	3.01E-02	10.18	ENSBTAT00000076672	*MYLK4, WRNIP1*
lncRNA_16505.6	4:41775570-41794763	1241	6.35E-13	1.21E-10	-8.52	ENSBTAT00000087015	-
lncRNA_20382.10	8:70592566-70718220	1741	3.97E-12	6.38E-10	-10.54	ENSBTAT00000068948	*SLC25A37, NKX3-1, LOXL2, ENTPD4*
lncRNA_18428.3	6:42982543-43153637	692	5.38E-12	8.49E-10	-10.43	ENSBTAT00000077875	*ENSBTAG00000033327*
lncRNA_7981.4	19:8790623-8795920	812	1.49E-09	1.52E-07	-11.27	ENSBTAT00000068745	*VEZF1, SRSF1, DYNLL2, CUEDC1*
lncRNA_19922.3	7:92888299-93014993	1228	5.45E-06	2.51E-04	-10.36	ENSBTAT00000067459	*NR2F1, FAM172A*
lncRNA_10726.5	21:33076908-33116941	1161	1.31E-05	5.59E-04	-4.63	ENSBTAT00000082173	*ENSBTAG00000024311, ODF3L1, CSPG4*
lncRNA_17096.3	4:118297039-118325584	462	2.10E-05	8.64E-04	-9.43	ENSBTAT00000066814	*LMBR1, MNX1, NOM1*
lncRNA_12504	23:36224038-36465322	604	2.41E-04	7.46E-03	-6.70	ENSBTAT00000083528	*SOX4*
lncRNA_20062.10	8:22841637-22873487	2286	3.72E-04	1.06E-02	-2.20	ENSBTAT00000070622	*ENSBTAG00000048891, ENSBTAG00000054099, ENSBTAG00000055152, ENSBTAG00000050194, ENSBTAG00000052859, KLHL9, IFNAG, IFN-TAU*
lncRNA_15244.2	3:12245784-12253963	3500	1.76E-03	3.77E-02	-2.71	ENSBTAT00000072176	*ENSBTAG00000024960 KIRREL1*
Comparison (C2 vs. C3)							
lncRNA_20382.10	8:70592566-70718220	1741	2.96E-16	8.88E-14	10.38	ENSBTAT00000068948	*SLC25A37, NKX3-1, LOXL2, ENTPD4*
lncRNA_5415.4	16:1072072-1076246	764	1.93E-09	1.79E-07	8.12	ENSBTAT00000086621	*BTG2, CHI3L1*
lncRNA_9130.4	19:60774940-60930256	1149	3.50E-09	3.05E-07	3.36	ENSBTAT00000070427	-
lncRNA_18429.11	6:42982543-43153637	2562	2.42E-06	1.17E-04	2.97	ENSBTAT00000077875	*ENSBTAG00000033327*
lncRNA_16618.6	4:55292087-55307803	5601	2.90E-04	8.09E-03	2.61	ENSBTAT00000070495	*GPR85*
lncRNA_6086.4	16:79121509-79142756	3624	2.06E-09	1.89E-07	-8.23	ENSBTAT00000069212	*ZNF281, KIF14*
lncRNA_6442.5	17:53500021-53509696	6591	2.95E-09	2.63E-07	-4.31	ENSBTAT00000074572	*ENSBTAG00000052582, SETD1B, CFAP251, PSMD9, TMEM120B*
lncRNA_5876.4	16:55234389-55238149	615	4.57E-09	3.86E-07	-5.38	ENSBTAT00000081813	*KLHL20, DARS2,*

							ZBTB37, *CENPL*, *RC3H1*, *SERPINC1*
lncRNA_12348.9	23:50961431-50974711	5505	5.18E-09	4.33E-07	-7.90	ENSBTAT00000076672	*MYLK4*, *WRNIP1*
lncRNA_20062.7	8:22841637-22873487	2286	6.91E-07	3.79E-05	-11.23	ENSBTAT00000070622	*ENSBTAG00000048891*, *ENSBTAG00000054099*, *ENSBTAG00000055152*, *ENSBTAG00000050194*, *ENSBTAG00000052859*, *IFN-TAU*, *KLHL9*, *IFNAG*
lncRNA_6037.8	16:72109237-72128769	2734	8.69E-07	4.65E-05	-9.89	ENSBTAT00000067279	*RCOR3*, *TRAF5*
lncRNA_3567.2	13:37839904-37856732	8398	1.01E-04	3.36E-03	-3.17	ENSBTAT00000085977	*ENSBTAG00000049520*, *ENSBTAG00000006043*, *DSTN*, *BFSP*, *PCSK2*
lncRNA_11324.5	22:36444667-36922968	1306	1.55E-04	4.84E-03	-11.06	ENSBTAT00000083930	*ENSBTAG00000019048*
lncRNA_843.3	1:148950230-148972709	1213	3.15E-04	8.64E-03	-2.59	ENSBTAT00000074316	*SIM2*

[a] Transcript Length in basis pair, [b] False Discovery Rate, [c] FC (log2) = Fold change (log2), [d] transcript association = lncRNA annotated in the bovine reference annotation (ARS.UCD1.2), [e] Gene Interaction = Genes associated with annotated lncRNAs within the 200kb window, with 100kb upstream and 100kb downstream, relative to the start and end positions of the gene, respectively.

3.2.3. Differentially expressed novel long intergenic noncoding RNA (LincRNAs)

Comparing C1 vs. C2, a total of 53 novel lincRNAs were identified (Supplementary Table S1, Table 4), with 17 showing upregulation and 36 exhibiting downregulation in C1 animals. These lincRNAs were located mostly in the antisense position of protein-coding (56,60%) transcripts. The upregulated lincRNA (Table 4) was related to genes associated with transcription factor binding, cellular response to lipids, response to hormones, positive regulation of cell proliferation, and mitochondrial transport. Additionally, the downregulated novel lincRNAs (Table 4) were associated with genes related to positive regulation of kinase activity, regulation of B cell activation and DNA repair.

Table 4. Novel long intergenic noncoding RNA differentially expressed in *Longissimus thoracis* muscle of Nellore cattle belong to the C1-fatty acid profile in relation to C2-fatty acid profile (C1 vs. C2)

lncRNA located in sense direction [a]

Feature ID	Positon	Length[b]	*p*-Value	FDR [c]	FC (log2)[d]	mRNA Interaction [e]	Distance [f]	Interaction location [g]
lncRNA_20557.5	8:91054949-91086297	791	6.50E-13	1.26E-10	6.76	ENSBTAT00000079956	14797	upstream
lncRNA_7736.5	18:59685331-59696073	1112	2.60E-09	2.95E-07	6.23	ENSBTAT00000053465	78735	upstream
lncRNA_2305.1	11:49143024-49147255	855	4.03E-08	3.76E-06	3.13	ATOH8-201	16527	upstream
lncRNA_5255.1	15:65502793-65506317	2386	1.01E-04	4.25E-03	2.49	PDHX-201	170	downstream
lncRNA_9933.3	2:126223556-126230654	1190	1.69E-04	6.54E-03	2.04	TRNP1-201	927	downstream
lncRNA_10558.2	21:16690280-16704571	7132	3.04E-04	1.06E-02	3.26	ENSBTAT00000069947	17948	downstream
lncRNA_9517.5	2:52904578-52913931	3169	2.69E-21	1.44E-18	-9.83	ARHGAP15-201	51209	downstream
lncRNA_5265.1	15:65520195-65531625	1885	1.47E-19	6.62E-17	-5.83	PDHX-201	17572	downstream
lncRNA_11848.4	23:14861716-14866193	1681	1.02E-17	3.58E-15	-7.26	UNC5CL-201	85367	downstream
lncRNA_9517.4	2:52904578-52913931	3157	7.74E-17	2.50E-14	-5.91	ARHGAP15-201	51221	downstream
lncRNA_20515.12	8:86704251-86724738	3110	3.62E-10	4.74E-08	-6.88	AUH-203	18194	downstream
lncRNA_19092.2	7:12412390-12426648	2649	2.09E-09	2.40E-07	-3.82	IER2-201	7211	downstream
lncRNA_2526.3	11:82614309-82639458	4315	2.48E-08	2.38E-06	-8.31	DDX1-201	38762	downstream
lncRNA_7736.1	18:59685331-59696073	1111	4.73E-06	2.79E-04	-2.86	ENSBTAT00000053465	78735	upstream
lncRNA_10320.5	20:35647442-35665372	2876	5.10E-05	2.35E-03	-4.52	OSMR-201	75932	upstream
lncRNA_4171.3	13:78409884-78447044	2212	1.67E-04	6.49E-03	-11.44	ENSBTAT00000079695	70437	downstream
lncRNA_13086.2	25:25769133-25823226	880	1.70E-04	6.58E-03	12.97	SBK1-201	27121	upstream
lncRNA_5964.5	16:66395474-66405633	1887	1.81E-04	6.90E-03	-4.48	IVNS1ABP-206	96159	upstream
lncRNA_2540.2	11:86418716-86464641	1033	4.45E-04	1.42E-02	-12.02	E2F6-201	16736	downstream
lncRNA_17103.9	5:5588820-5627943	3571	5.33E-04	1.65E-02	-11.12	PHLDA1-201	12947	upstream
lncRNA_10900.4	21:58589214-58593395	651	6.15E-04	1.86E-02	-2.42	CCDC197-201	10826	upstream
lncRNA_9841.2	2:120677247-120682616	3616	1.36E-03	3.55E-02	-2.02	AK2-201	55	downstream
lncRNA_5964.1	16:66395474-66405633	1811	1.52E-03	3.87E-02	-4.90	IVNS1ABP-206	96159	upstream

lncRNA located in antisense direction [a]

Feature ID	Positon	Length[b]	*p*-Value	FDR [c]	FC(log2)[d]	mRNA Interaction [e]	Distance [f]	Interaction location [g]
lncRNA_5578.3	16:29120343-29123464	2479	7.94E-21	4.11E-18	6.63	H3-3A-201	32484	downstream
lncRNA_8796.3	19:44436479-44443655	1713	2.29E-08	2.21E-06	6.77	CCDC43-201	85	upstream
lncRNA_17393.3	5:43266505-43268113	357	4.92E-08	4.51E-06	14.53	CNOT2-201	110	upstream

lncRNA_5714.2	16:43978464-43985353	3333	6.05E-07	4.40E-05	2.89	SLC25A33-201	31765	upstream
lncRNA_8294.10	19:24908019-24914528	4663	1.52E-04	6.01E-03	2.23	SPNS3-201	22427	upstream
lncRNA_3119.2	12:34735808-34750812	3111	3.20E-04	1.11E-02	2.19	SGCG-201	29925	upstream
lncRNA_18383.11	6:24210504-24526275	3655	3.57E-04	1.20E-02	2.08	DDIT4L-201	360	upstream
lncRNA_8294.4	19:24908019-24914528	4783	5.06E-04	1.58E-02	3.78	SPNS3-201	22307	upstream
lncRNA_15543.1	3:29450215-29462055	2414	1.20E-03	3.20E-02	3.32	HIPK1-201	269	upstream
lncRNA_10184.3	20:10097610-10105934	1436	1.49E-03	3.82E-02	3.20	SMN2-201	44	upstream
lncRNA_13547.2	26:15877179-15880904	1156	1.85E-03	4.55E-02	2.65	NOC3L-202	84	upstream
lncRNA_15009.1	29:49217825-49218972	606	6.03E-19	2.58E-16	-5.78	CD81-201	1014	upstream
lncRNA_20981.1	9:52492142-52495493	706	7.48E-19	3.15E-16	-9.70	MMS22L-202	1636	upstream
lncRNA_17358.2	5:34560404-34576263	1840	2.78E-12	4.89E-10	-10.03	ARID2-202	41974	upstream
lncRNA_15447.1	3:20935933-20944419	2180	2.31E-09	2.64E-07	-10.74	MGC134040-201	6782	upstream
lncRNA_12128.6	1:7104210-7114117	918	1.70E-07	1.41E-05	-6.87	TRIM27-201	88	upstream
lncRNA_20224.2	8:55767546-55825069	1610	2.56E-07	2.05E-05	-10.80	TLE4-204	37635	downstream
lncRNA_280.3	1:69127181-69127765	425	2.91E-07	2.28E-05	-3.26	UMPS-201	102	upstream
lncRNA_10919.2	21:60379945-60383584	1353	1.99E-06	1.29E-04	-3.19	SYNE3-201	92	upstream
lncRNA_18394.1	6:25791826-25795075	1514	7.32E-05	3.21E-03	-4.56	EIF4E-202	86079	downstream
lncRNA_8476.5	19:33209590-33211733	801	1.74E-04	6.68E-03	-13.53	LRRC75A-202	367	downstream
lncRNA_12128.6	23:30046878-30066974	1750	2.54E-04	9.14E-03	-12.48	TRIM27-201	1344	downstream
lncRNA_14743.1	29:37152089-37169227	896	3.20E-04	1.11E-02	-11.32	CCDC86-201	515	upstream
lncRNA_3024.7	12:24728114-24731701	742	3.72E-04	1.24E-02	-12.26	SMAD9-201	180	upstream
lncRNA_6783.6	17:72362986-72382214	4197	7.54E-04	2.19E-02	-6.53	SLC7A4-201	605	upstream
lncRNA_6371.2	17:51604344-51756900	1413	7.54E-04	2.19E-02	-2.42	CCDC92-201	756	upstream
lncRNA_15447.4	3:20935933-20944419	1346	8.08E-04	2.31E-02	-2.26	MGC134040-201	6796	upstream
lncRNA_18711.6	6:102631399-102685256	2552	1.00E-03	2.77E-02	-10.93	ENSBTAT00000008532	565	downstream
lncRNA_11659.21	22:59504587-59508832	2979	1.78E-03	4.40E-02	-5.11	RUVBL1-201	221	upstream
lncRNA_7507.1	18:52318710-52407580	2625	1.97E-03	4.78E-02	-2.97	ZNF180-203	3074	upstream

[a] Direction of transcription of proximal RNA transcripts; [b] Transcript Length in basis pair; [c] FC (log2) = Fold change (log2); [d] False Discovery Rate; [e] transcript interaction = lncRNA gene overlaps with a transcript gene from the bovine reference annotation (ARS.UCD1.2). ; [f] Distance = distance between lincRNA and closest RNA transcript in the bovine reference annotation (ARS.UCD1.2); [g] Interaction location = are defined according to the type of interactions (genic or intergenic), assuming that intergenic lncRNA are not overlapping any RNA transcript (RNA/gene), then it can be further classified in: upstream (the lncRNA is upstream transcribed in head-to-head or tail-to-tail orientation with RNA partner or yet both same orientation) or downstream (the lncRNA is downstream transcribed in head-to-head or tail-to-tail orientation with RNA partner or yet both in same orientation).

In the comparison between C1 and C3, we identified 85 DE lincRNAs (Supplementary Table S2, Table 5), among these, 55 lincRNAs were upregulated whereas 30 were downregulated in C1. It is notable that most of these lincRNAs were located in the antisense direction (65.88%) with respect to their gene interactions. The overexpressed lincRNA showed association with genes related to ATPase and pyrophosphatase activities, mRNA binding, lipid localization and transport, catalytic complexes, fatty acid metabolism, fat cell differentiation, transcription factor binding, and inhibition of macromolecule and RNA biosynthesis. Furthermore, the genes related to RNA biosynthetic process, ATPase complex, transcription factor complex, peptidyl-lysine modification, cartilage development, skeletal system development, and ribonucleoside diphosphate metabolic process were downregulated according to Table 5.

Table 5. Novel long intergenic noncoding RNA differentially expressed in *Longissimus thoracis* muscle of Nellore cattle belong to C1-fatty acid profile in relation to C3-fatty acid profile (C1 vs. C3)

lncRNA located in sense direction [a]								
Feature ID	Positon	Length (pb) [b]	*p*-Value	FDR [c]	FC(log2)[d]	mRNA Interaction [e]	Distance [f]	Interaction location [g]
lncRNA_13486.1	26:9373528-9377974	2490	1.41E-20	1.01E-17	8.10	ATAD1-201	1300	downstream
lncRNA_3864.2	13:58736919-58750531	1453	1.77E-14	4.32E-12	7.13	RBM38-201	7936	downstream
lncRNA_20557.5	8:91054949-91086297	791	3.91E-13	7.73E-11	5.58	ENSBTAT00000079956	14797	upstream
lncRNA_10320.8	20:35647442-35665372	2557	4.37E-09	3.98E-07	9.41	OSMR-201	75932	upstream
lncRNA_5255.1	15:65502793-65506317	2386	3.61E-07	2.26E-05	3.91	PDHX-201	170	downstream
lncRNA_10900.4	21:58589214-58593395	651	3.66E-07	2.28E-05	5.83	CCDC197-201	10826	upstream
lncRNA_10720.6	21:33142026-33155354	5446	3.95E-07	2.44E-05	8.44	ODF3L1-201	12461	downstream
lncRNA_2540.4	11:86418716-86464641	856	1.18E-06	6.39E-05	8.12	E2F6-201	16909	downstream
lncRNA_20515.13	8:86704251-86724738	2977	3.80E-06	1.83E-04	6.22	AUH-203	18207	downstream
lncRNA_3050.2	12:30041465-30044471	2760	1.04E-05	4.54E-04	3.70	MEDAG-201	26845	upstream
lncRNA_10558.1	21:16690280-16704571	7144	6.68E-05	2.49E-03	3.84	ENSBTAT00000069947	17935	downstream
lncRNA_10558.14	21:16690280-16704571	6381	3.34E-04	9.72E-03	12.04	ENSBTAT00000069947	18323	downstream
lncRNA_10558.9	21:16690280-16704571	6833	4.50E-04	1.23E-02	10.39	ENSBTAT00000069947	18000	downstream
lncRNA_10320.7	20:35647442-35665372	2557	6.73E-04	1.70E-02	3.85	OSMR-201	75932	upstream
lncRNA_16508.10	4:43223448-43279119	4572	1.14E-03	2.63E-02	11.50	PHTF2-201	41821	downstream
lncRNA_20515.10	8:86704251-86724738	3120	1.78E-03	3.81E-02	4.97	AUH-203	18184	downstream
lncRNA_9517.4	2:52904578-52913931	3157	1.66E-22	1.76E-19	-7.92	ARHGAP15-201	51221	downstream
lncRNA_5265.1	15:65520195-65531625	1885	1,55E-20	1,08E-17	-6,58	PDHX-201	17572	downstream
lncRNA_1878.2	10:102749910-102772451	4686	2.82E-17	1.09E-14	-6.47	U6-201	8839	downstream
lncRNA_17681.1	5:74669803-74711373	1010	1.74E-11	2.49E-09	-5.55	APOL3-201	11739	upstream
lncRNA_20515.12	8:86704251-86724738	3110	3.79E-10	4.27E-08	-6.90	AUH-203	18194	downstream
lncRNA_19092.2	7:12412390-12426648	2649	1.69E-09	1.69E-07	-4.10	IER2-201	7211	downstream
lncRNA_2526.3	11:82614309-82639458	4315	1.42E-06	7.54E-05	-8.61	DDX1-201	38762	downstream
lncRNA_5964.1	16:66395474-66405633	1811	7.94E-05	2.91E-03	-6.48	IVNS1ABP-206	96159	upstream
lncRNA_5964.6	16:66395474-66405633	1890	2.63E-04	8.01E-03	-4.09	IVNS1ABP-206	96159	upstream
lncRNA_16442.1	4:29193479-29195379	688	3.29E-04	9.59E-03	-2.59	ITGB8-201	77720	downstream
lncRNA_10720.7	21:33142026-33155354	2129	1.69E-03	3.63E-02	-3.79	ODF3L1-201	12368	downstream
lncRNA_7736.1	18:59685331-59696073	1111	1.82E-03	3.87E-02	-2.09	ENSBTAT00000053465	78735	upstream
lncRNA_9841.2	2:120677247-120682616	3616	2.36E-03	4.84E-02	-2.64	AK2-201	55	downstream
lncRNA located in antisense direction [a]								
Feature ID	Positon	Length	*p*-Value	FDR [c]	FC(log2)[d]	mRNA Interaction [e]	Distance [f]	Interaction

		(pb) [b]						location [g]
lncRNA_18738.3	6:108831172-108833800	2254	1.62E-21	1.43E-18	8.41	BOD1L1-201	165	upstream
lncRNA_17511.1	5:56346812-56351225	553	8.71E-18	3.72E-15	9.25	NAB2-201	286	upstream
lncRNA_16747.1	4:76603706-76606680	2756	2.08E-16	7.21E-14	7.95	CCM2-203	239	upstream
lncRNA_18738.2	6:108831172-108833800	2257	6.65E-15	1.71E-12	6.13	BOD1L1-201	162	upstream
lncRNA_18001.6	5:108915063-108924973	5266	2.19E-12	3.76E-10	8.62	CECR2-201	12869	upstream
lncRNA_10184.1	20:10097610-10105934	1477	2.29E-11	3.18E-09	7.74	SMN2-201	62	upstream
lncRNA_10586.5	21:21519283-21537669	2678	8.21E-11	1.03E-08	11.66	ENSBTAT00000031184	2866	downstream
lncRNA_6258.4	17:18453124-18474698	914	3.88E-10	4.36E-08	8.57	ELF2-201	147	upstream
lncRNA_17577.2	5:62725526-62731661	1158	3.38E-09	3.16E-07	4.51	TMPO-201	12982	downstream
lncRNA_8796.1	19:44436479-44443655	1717	7.06E-09	6.25E-07	9.87	CCDC43-201	76	upstream
lncRNA_15455.1	3:20557127-20560106	1103	7.88E-09	6.89E-07	10.62	VPS45-201	139	upstream
lncRNA_2042.4	11:10233550-10238310	936	1.14E-08	9.67E-07	8.13	DCTN1-201	2229	upstream
lncRNA_5578.3	16:29120343-29123464	2479	2.08E-07	1.37E-05	6.95	H3-3A-201	32484	downstream
lncRNA_11659.1	22:59504587-59508832	3008	7.97E-07	4.54E-05	10.73	RUVBL1-201	189	upstream
lncRNA_12128.7	23:30046878-30066974	1748	1.93E-06	9.93E-05	10.44	TRIM27-201	1344	downstream
lncRNA_11659.14	22:59504587-59508832	3229	2.97E-06	1.47E-04	7.29	RUVBL1-201	187	upstream
lncRNA_18674.6	6:97716507-97750095	1377	3.65E-06	1.77E-04	6.65	THAP9-201	2602	upstream
lncRNA_3855.2	13:58045731-58049015	1933	7.19E-06	3.23E-04	3.17	RAB22A-201	101	upstream
lncRNA_791.1	1:144934585-144937622	2739	7.97E-06	3.55E-04	4.48	ADARB1-201	204	upstream
lncRNA_8476.6	19:33209590-33211733	1021	2.85E-05	1.14E-03	7.20	LRRC75A-202	367	downstream
lncRNA_929.1	1:157196721-157198350	1337	5.73E-05	2.17E-03	4.94	RAB5A-201	151	upstream
lncRNA_11659.17	22:59504587-59508832	3069	9.52E-05	3.42E-03	6.37	RUVBL1-201	188	upstream
lncRNA_2036.1	11:10142822-10146171	2000	1.22E-04	4.23E-03	3.14	LBX2-201	2603	downstream
lncRNA_8294.4	19:24908019-24914528	4783	1.70E-04	5.61E-03	12.95	SPNS3-201	22307	upstream
lncRNA_3433.4	13:17510294-17518504	1169	1.72E-04	5.65E-03	12.94	IL15RA-201	121	upstream
lncRNA_5714.2	16:43978464-43985353	3333	1.97E-04	6.32E-03	2.53	SLC25A33-201	31765	upstream
lncRNA_3024.5	12:24728114-24731701	896	2.16E-04	6.82E-03	12.14	SMAD9-201	165	upstream
lncRNA_20692.2	8:111823798-111834775	3395	2.38E-04	7.38E-03	2.49	MYT1L-201	86892	upstream
lncRNA_8476.12	19:33209590-33211733	1027	2.57E-04	7.88E-03	12.76	LRRC75A-202	367	downstream
lncRNA_17511.6	5:56346812-56351225	562	2.62E-04	7.99E-03	12.74	NAB2-201	1804	upstream
lncRNA_18001.15	5:108915063-108924973	4906	2.85E-04	8.57E-03	12.62	CECR2-201	12876	upstream
lncRNA_6258.1	17:18453124-18474698	801	3.24E-04	9.48E-03	12.45	ELF2-201	1432	upstream
lncRNA_18001.17	5:108915063-108924973	5263	4.86E-04	1.31E-02	3.22	CECR2-201	12878	upstream
lncRNA_1840.2	10:92511630-92522957	1195	7.11E-04	1.78E-02	3.83	GTF2A1-201	161	upstream
lncRNA_11659.3	22:59504587-59508832	3272	8.38E-04	2.03E-02	11.46	RUVBL1-201	188	upstream

lncRNA_15403.1	3:19689039-19726184	1166	1.28E-03	2.90E-02	11.14	MLLT11-201	410	downstream
lncRNA_18674.5	6:97716507-97750095	1465	1.77E-03	3.79E-02	9.31	THAP9-201	1096	upstream
lncRNA_17653.3	5:73720268-73724709	3062	2.17E-03	4.50E-02	2.30	RASD2-201	24336	upstream
lncRNA_18001.14	5:108915063-108924973	5266	2.24E-03	4.62E-02	3.19	CECR2-201	12869	upstream
lncRNA_20041.2	8:16267188-16287915	1056	1.77E-19	1.01E-16	-10.37	LINGO2-201	11117	upstream
lncRNA_20981.1	9:52492142-52495493	706	2.65E-19	1.45E-16	-10.80	MMS22L-202	1636	upstream
lncRNA_17194.2	5:26047734-26049634	869	2.23E-12	3.81E-10	- 9.12	HOXC10-201	369	upstream
lncRNA_20224.2	8:55767546-55825069	1610	3.43E-08	2.67E-06	-13.89	TLE4-204	37635	downstream
lncRNA_38.1	1:7104210-7114117	918	3.84E-07	2.38E-05	-8.67	CCT8-201	88	upstream
lncRNA_15447.4	3:20935933-20944419	1346	1.87E-05	7.75E-04	-3.37	MGC134040-201	6796	upstream
lncRNA_2832.1	11:104798248-104801001	2140	4.33E-05	1.68E-03	-2.47	BRD3-201	3413	downstream
lncRNA_12128.6	23:30046878-30066974	1750	7.19E-05	2.66E-03	-14.61	TRIM27-201	1344	downstream
lncRNA_14743.1	29:37152089-37169227	896	1.01E-04	3.58E-03	-13.40	CCDC86-201	515	upstream
lncRNA_3024.7	12:24728114-24731701	742	1.53E-04	5.12E-03	-14.73	SMAD9-201	180	upstream
lncRNA_6802.5	18:1343804-1405315	1285	1.41E-04	4.79E-03	12.72	VAC14-202	6224	upstream
lncRNA_6783.6	17:72362986-72382214	4197	6.04E-04	1.56E-02	-7.45	SLC7A4-201	605	upstream
lncRNA_18394.1	6:25791826-25795075	1514	8.06E-04	1.97E-02	-4.31	EIF4E-202	86079	downstream
lncRNA_3863.4	13:58694491-58705765	984	1.12E-03	2.60E-02	-2.57	CTCFL-201	11892	downstream
lncRNA_8796.4	19:44436479-44443655	1699	1.61E-03	3.50E-02	-2.61	CCDC43-201	98	upstream
lncRNA_18001.20	5:108915063-108924973	5271	1.87E-03	3.95E-02	-4.23	CECR2-201	12869	upstream
lncRNA_10586.3	21:21519283-21537669	5164	2.28E-03	4.70E-02	-4.16	ENSBTAT00000031184	2866	downstream

[a] Direction of transcription of proximal RNA transcripts; [b] Transcript Length in basis pai; [c] FC (log2) = Fold change (log2); [d] False Discovery Rate; [e] transcript interaction = lncRNA gene overlaps with a transcript gene from the bovine reference annotation (ARS.UCD1.2); [f] Distance = distance between lincRNA and closest RNA transcript in the bovine reference annotation (ARS.UCD1.2); [g] Interaction location = are defined according to the type of interactions (genic or intergenic), assuming that intergenic lncRNA are not overlapping any RNA transcript (RNA/gene), then it can be further classified in: upstream (the lncRNA is upstream transcribed in head-to-head or tail-to-tail orientation with RNA partner or yet both same orientation) or downstream (the lncRNA is downstream transcribed in head-to-head or tail-to-tail orientation with RNA partner or yet both in same orientation).

Fifty-six DE lincRNA were identified in the C2 vs. C3 comparison, with 9 upregulated and 47 downregulated (Supplementary Table S3; Table 6) in C2. The majority of these lincRNA were situated in the antisense direction (67.85%) with respect to their gene interactions. The upregulated DE lincRNAs were associated with transcription factor binding, nuclear body, and the negative regulation of cell proliferation. Meanwhile, the DE downregulated lincRNAs (Table 6) were linked to lipid localization, transport, import into the cell, positive regulation of cytoskeleton organization, receptor metabolic process, actin cytoskeleton, transcription factor complex, and the regulation of cellular component size.

Table 6. Novel long intergenic noncoding RNA differentially expressed in *Longissimus thoracis* muscle of Nellore cattle belong to C2-fatty acid profile in relation to C3-fatty acid profile (C2 vs. C3).

lncRNA located in sense direction [a]								
Feature ID	Positon	Length [b]	p-Value	FDR [c]	FC(log2)[d]	mRNA Interaction [e]	Distance [f]	Interaction location [g]
lncRNA_7736.5	18:59685331-59696073	1112	8.56E-11	1.05E-08	6.16	ENSBTAT00000053465	78735	upstream
lncRNA_9933.3	2:126223556-126230654	1190	5.22E-09	4.35E-07	3.61	TRNP1-201	927	downstream
lncRNA_10558.2	21:16690280-16704571	7132	2.29E-07	1.41E-05	4.98	ENSBTAT00000069947	17948	downstream
lncRNA_2305.1	11:49143024-49147255	855	2.07E-06	1.01E-04	2.73	ATOH8-201	16527	upstream
lncRNA_13486.1	26:9373528-9377974	2490	5.24E-22	4.27E-19	-8.31	ATAD1-201	1300	downstream
lncRNA_9517.5	2:52904578-52913931	3169	2.16E-19	1.17E-16	-9.76	ARHGAP15-201	51209	downstream
lncRNA_3864.2	13:58736919-58750531	1453	5.77E-16	1.65E-13	-7.39	RBM38-201	7936	downstream
lncRNA_10900.4	21:58589214-58593395	651	7.42E-11	9.24E-09	-8.25	CCDC197-201	10826	upstream
lncRNA_17681.1	5:74669803-74711373	1010	6.50E-10	6.72E-08	-4.77	APOL3-201	11739	upstream
lncRNA_11848.4	23:14861716-14866193	1681	3.04E-09	2.70E-07	-6.31	UNC5CL-201	85367	downstream
lncRNA_10320.8	20:35647442-35665372	2557	5.36E-09	4.46E-07	-9.86	OSMR-201	75932	upstream
lncRNA_20515.13	8:86704251-86724738	2977	8.68E-08	5.83E-06	-7.23	AUH-203	18207	downstream
lncRNA_10720.6	21:33142026-33155354	5446	9.51E-08	6.34E-06	-8.51	ODF3L1-201	12461	downstream
lncRNA_5964.5	16:66395474-66405633	1887	4.68E-07	2.70E-05	-12.51	IVNS1ABP-206	96159	upstream
lncRNA_2540.4	11:86418716-86464641	856	4.46E-06	2.01E-04	-7.85	E2F6-201	16909	downstream
lncRNA_3470.1	13:23312186-23369059	4082	1.02E-04	3.38E-03	-2.32	ENSBTAT00000067334	45318	downstream
lncRNA_20515.10	8:86704251-86724738	3120	1.89E-04	5.67E-03	-5.74	AUH-203	18184	downstream
lncRNA_10558.14	21:16690280-16704571	6381	3.30E-04	8.99E-03	-11.93	ENSBTAT00000069947	18323	downstream
lncRNA located in antisense direction [a]								
Feature ID	Positon	Length (pb) [b]	p-Value	FDR [c]	FC(log2)[d]	mRNA Interaction [e]	Distance [f]	Interaction location [g]
lncRNA_20041.2	8:16267188-16287915	1056	2.05E-09	1.89E-07	4.57	LINGO2-201	11117	upstream
lncRNA_17393.3	5:43266505-43268113	357	4.00E-08	2.85E-06	14.89	CNOT2-201	110	upstream
lncRNA_5578.2	16:29120343-29123464	2479	3.23E-06	1.51E-04	2.96	H3-3A-201	32484	downstream
lncRNA_2417.4	11:68352230-68367299	1440	8.63E-06	3.68E-04	4.14	PCBP1-201	44166	upstream
lncRNA_8294.10	19:24908019-24914528	4663	3.12E-04	8.59E-03	2.87	SPNS3-201	22427	upstream
lncRNA_7978.2	19:8080110-8091884	3121	3.53E-04	9.51E-03	13.23	MSI2-205	451	upstream
lncRNA_18738.2	6:108831172-108833800	2257	1.33E-20	8.89E-18	-7.61	BOD1L1-201	162	upstream
lncRNA_17511.1	5:56346812-56351225	553	1.86E-19	1.03E-16	-9.49	NAB2-201	286	upstream
lncRNA_16747.1	4:76603706-76606680	2756	1.13E-17	4.34E-15	-8.66	CCM2-203	239	upstream

lncRNA_15009.1	29:49217825-49218972	606	2.22E-13	4.30E-11	-6.76	CD81-201	1014	upstream
lncRNA_17194.2	5:26047734-26049634	869	3.11E-13	5.92E-11	-9.73	HOXC10-201	369	upstream
lncRNA_10184.1	20:10097610-10105934	1477	1.88E-11	2.67E-09	-7.02	SMN2-201	62	upstream
lncRNA_2042.4	11:10233550-10238310	936	6.55E-11	8.23E-09	-8.15	DCTN1-201	2229	upstream
lncRNA_10586.5	21:21519283-21537669	2678	7.73E-10	7.86E-08	-9.83	ENSBTAT00000031184	2866	downstream
lncRNA_15455.1	3:20557127-20560106	1103	4.17E-09	3.56E-07	-9.59	VPS45-201	139	upstream
lncRNA_3433.2	13:17510294-17518504	1039	1.80E-08	1.36E-06	-3.38	IL15RA-201	109	upstream
lncRNA_8796.1	19:44436479-44443655	1717	1.16E-07	7.59E-06	-8.42	CCDC43-201	76	upstream
lncRNA_17577.2	5:62725526-62731661	1158	3.57E-07	2.12E-05	-3.59	TMPO-201	12982	downstream
lncRNA_3855.2	13:58045731-58049015	1933	9.01E-07	4.80E-05	-3.24	RAB22A-201	101	upstream
lncRNA_17358.2	5:34560404-34576263	1840	1.26E-06	6.49E-05	-9.96	ARID2-202	41974	upstream
lncRNA_11659.14	22:59504587-59508832	3229	1.62E-06	8.15E-05	-7.35	RUVBL1-201	187	upstream
lncRNA_11659.1	22:59504587-59508832	3008	2.17E-06	1.05E-04	-10.27	RUVBL1-201	189	upstream
lncRNA_12128.7	23:30046878-30066974	1748	3.26E-06	1.53E-04	-9.98	TRIM27-201	1344	downstream
lncRNA_18674.6	6:97716507-97750095	1377	2.35E-05	9.10E-04	-5.76	THAP9-201	2602	upstream
lncRNA_10919.2	21:60379945-60383584	1353	3.69E-05	1.38E-03	-6.04	SYNE3-201	92	upstream
lncRNA_791.1	1:144934585-144937622	2739	4.16E-05	1.53E-03	-3.66	ADARB1-201	204	upstream
lncRNA_11659.17	22:59504587-59508832	3069	4.95E-05	1.79E-03	-8.28	RUVBL1-201	188	upstream
lncRNA_2036.1	11:10142822-10146171	2000	7.48E-05	2.56E-03	-3.11	LBX2-201	2603	downstream
lncRNA_3024.5	12:24728114-24731701	896	1.02E-04	3.37E-03	-11.63	SMAD9-201	165	upstream
lncRNA_929.1	1:157196721-157198350	1337	1.03E-04	3.39E-03	-4.11	RAB5A-201	151	upstream
lncRNA_3433.4	13:17510294-17518504	1169	1.13E-04	3.68E-03	-14.02	IL15RA-201	121	upstream
lncRNA_8476.14	19:33209590-33211733	514	1.24E-04	4.00E-03	-4.73	LRRC75A-202	367	downstream
lncRNA_6802.5	18:1343804-1405315	1285	1.70E-04	5.23E-03	-12.54	VAC14-202	6224	upstream
lncRNA_1840.2	10:92511630-92522957	1195	1.85E-04	5.58E-03	-3.74	GTF2A1-201	161	upstream
lncRNA_18001.17	5:108915063-108924973	5263	2.73E-04	7.70E-03	-2.95	CECR2-201	12878	upstream
lncRNA_6258.1	17:18453124-18474698	801	2.95E-04	8.20E-03	-11.43	ELF2-201	1432	upstream
lncRNA_10210.6	20:12278005-12356729	2124	3.10E-04	8.53E-03	-12.26	CD180-201	61290	upstream
lncRNA_280.3	1:69127181-69127765	425	3.50E-04	9.45E-03	-3.12	UMPS-201	102	upstream

[a] Direction of transcription of proximal RNA transcripts; [b] Transcript Length in basis pair; [c] FC (log2) = Fold change (log2); [d] False Discovery Rate; [e] transcript interaction = lncRNA gene overlaps with a transcript gene from the bovine reference annotation (ARS.UCD1.2); [f] Distance = distance between lincRNA and closest RNA transcript in the bovine reference annotation (ARS.UCD1.2); [g] Interaction location = are defined according to the type of interactions (genic or intergenic), assuming that intergenic lncRNA are not overlapping any RNA transcript (RNA/gene), then it can be further classified in: upstream (the lncRNA is upstream transcribed in head-to-head or tail-to-tail orientation with RNA partner or yet both same orientation) or downstream (the lncRNA is downstream transcribed in head-to-head or tail-to-tail orientation with RNA partner or yet both in same orientation).

3.3. Functional Analyses

The genes associated with differentially expressed lncRNAs were functionally classified into molecular function, biological process, and cellular component categories. This classification was done for three cluster comparisons, namely C1 vs. C2, C1 vs. C3, and C2 vs. C3. For the C1 vs. C2 comparison, 41 significant GO terms (p-value < 0.05), were identified, which included 4 molecular functions (MF), 32 biological processes (BP), and 5 cellular components (CC) terms (Table 7). Similarly, for the C1 vs C3 comparison, 145 significant GO terms were identified, which included 27 molecular functions (MF), 98 biological processes (BP), and 20 cellular components (CC) terms (Table 8). For the C2 vs C3 comparison, 61 significant GO terms were identified, which included 1 molecular function (MF), 47 biological processes (BP), and 13 cellular components (CC) terms (Table 9).

Tabela 7. Gene Ontology (GO; Biological Process, Cellular Component, and Molecular Function terms) associated with genes interacting with differentially expressed lncRNAs in *Longissimus thoracis* muscle of Nellore cattle with two different fatty acid profiles (C1 vs. C2)

GO:ID	GOTerm	Ontology Source	*p*-value	Nr. Genes *	Genes
GO:0022411	cellular component disassembly	GO_BiologicalProcess	0.00	5	*ARID2, DSTN, RUVBL1, SMN2, TOMM7*
GO:0048545	response to steroid hormone	GO_BiologicalProcess	0.01	4	*CNOT2, EIF4E, IL6, NR2F1*
GO:0008284	positive regulation of cell proliferation	GO_BiologicalProcess	0.01	7	*CD81, CYR61, HIPK1, IL6, KIF14, OSMR, SLC25A33*
GO:0014070	response to organic cyclic compound	GO_BiologicalProcess	0.01	6	*CNOT2, DDX1, EIF4E, IL6, NR2F1, SMAD9*
GO:0050731	positive regulation of peptidyl-tyrosine phosphorylation	GO_BiologicalProcess	0.01	3	*CD81, CSPG4, IL6*
GO:0006403	RNA localization	GO_BiologicalProcess	0.02	3	*RUVBL1, SRSF1, ZNHIT6*
GO:0071383	cellular response to steroid hormone stimulus	GO_BiologicalProcess	0.03	3	*CNOT2, EIF4E, NR2F1*
GO:0009725	response to hormone	GO_BiologicalProcess	0.03	5	*CNOT2, EIF4E, IL6, NR2F1, SLC25A33*
GO:0032147	activation of protein kinase activity	GO_BiologicalProcess	0.04	3	*CD81, CSPG4, KIF14*
GO:0045860	positive regulation of protein kinase activity	GO_BiologicalProcess	0.04	4	*CD81, CSPG4, CYR61, KIF14*
GO:0043410	positive regulation of MAPK cascade	GO_BiologicalProcess	0.04	4	*CD81, CSPG4, IL6, UNC5CL*
GO:0071396	cellular response to lipid	GO_BiologicalProcess	0.04	4	*CNOT2, EIF4E, IL6, NR2F1*
GO:0044255	cellular lipid metabolic process	GO_BiologicalProcess	0.04	4	*AUH, CD81, LOC530653*
GO:0071407	cellular response to organic cyclic compound	GO_BiologicalProcess	0.05	4	*CNOT2, EIF4E, NR2F1, SMAD9*
GO:0001558	regulation of cell growth	GO_BiologicalProcess	0.05	3	*CYR61, KIF14, SLC25A33*
GO:0006281	DNA repair	GO_BiologicalProcess	0.05	4	*DDX1, MMS22L, RUVBL1, WRNIP1*
GO:0006839	mitochondrial transport	GO_BiologicalProcess	0.05	3	*RUVBL1, SLC25A33, TOMM7*
GO:0050864	regulation of B cell activation	GO_BiologicalProcess	0.05	2	*CD81, IL6*
GO:0008380	RNA splicing	GO_BiologicalProcess	0.05	3	*DDX1, SMN2, SRSF1*
GO:0033674	positive regulation of kinase activity	GO_BiologicalProcess	0.05	4	*CD81, CSPG4, CYR61, KIF14*
GO:0036464	cytoplasmic ribonucleoprotein	GO_CellularComponent	0.00	4	*CNOT2, DDX1, EIF4E, SMN2*

	granule				
GO:0016604	nuclear body	GO_CellularComponent	0.02	6	*DDX1*, *HIPK1*, *KLHL20*, *NOC3L*, *SMN2*, *SRSF1*
GO:0008134	transcription factor binding	GO_MolecularFunction	0.01	6	*ATOH8*, *CNOT2*, *EIF4E*, *RUVBL1*, *TLE4*, *ZNHIT6*
GO:0004386	helicase activity	GO_MolecularFunction	0.01	3	*DDX1*, *RUVBL1*, *WRNIP1*
GO:0016887	ATPase activity	GO_MolecularFunction	0.01	5	*DDX1*, *DYNLL2*, *KIF14*, *RUVBL1*, *WRNIP1*

*Nr. Genes : Number of genes.

Tabela 8. Gene Ontology (GO; Biological Process, Cellular Component, and Molecular Function terms) were associated genes with interacting with differentially expressed lncRNAs in *Longissimus thoracis* muscle of Nellore cattle with two different fatty acid profiles (C1 vs. C3)

GO:ID	GOTerm	Ontology Source	*p*-value	Nr. Genes*	Genes
GO:0006366	transcription from RNA polymerase II promoter	GO_BiologicalProcess	0.00	18	*BRD3*, *CTCFL*, *E2F6*, *ELF2*, *GTF2A1*, *HOXC10*, *IER2*, *MNX1*, *NKX3-1*, *NR2F1*, *RCOR3*, *RUVBL1*, *SIM2*, *SMAD9*, *SOX4*, *TLE4*, *TRIM27*, *VEZF1*
GO:0021953	central nervous system neuron differentiation	GO_BiologicalProcess	0.00	5	*ADARB1*, *BTG2*, *HOXC10*, *MNX1*, *SOX4*
GO:0032774	RNA biosynthetic process	GO_BiologicalProcess	0.00	27	*BRD3*, *CTCFL*, *DDX1*, *E2F6*, *ELF2*, *GTF2A1*, *HOXC10*, *IER2*, *LOC508131*, *LOXL2*, *MLLT11*, *MNX1*, *MYT1L*, *NAB2*, *NKX3-1*, *NR2F1*, *RCOR3*, *RUVBL1*, *SIM2*, *SLC25A33*, *SMAD9*, *SMN2*, *SOX4*, *TLE4*, *TRAF5*, *TRIM27*, *VEZF1*
GO:0001501	skeletal system development	GO_BiologicalProcess	0.01	6	*HOXC10*, *ITGB8*, *LOXL2*, *NAB2*, *SMAD9*, *SOX4*
GO:0000959	mitochondrial RNA metabolic process	GO_BiologicalProcess	0.01	2	*DARS2*, *SLC25A33*
GO:0006839	mitochondrial transport	GO_BiologicalProcess	0.02	4	*BNIP3*, *RUVBL1*, *SLC25A33*, *SLC25A37*
GO:2000113	negative regulation of cellular macromolecule biosynthetic	GO_BiologicalProcess	0.02	11	*BTG2*, *E2F6*, *EIF4E*, *LOXL2*, *NAB2*, *NKX3-1*, *NR2F1*, *RCOR3*, *SIM2*, *TLE4*, *TRIM27*

	process				
GO:0010822	positive regulation of mitochondrion organization	GO_BiologicalProcess	0.02	3	*BNIP3*, *MLLT11*, *RUVBL1*
GO:0001666	response to hypoxia	GO_BiologicalProcess	0.03	3	*BNIP3*, *LOXL2*, *NKX3-1*
GO:0010638	positive regulation of organelle organization	GO_BiologicalProcess	0.03	6	*BNIP3*, *DCTN1*, *KIRREL1*, *MLLT11*, *RUVBL1*, *TRIM27*
GO:0021954	central nervous system neuron development	GO_BiologicalProcess	0.03	2	*ADARB1*, *BTG2*
GO:0006869	lipid transport	GO_BiologicalProcess	0.04	3	*APOL3*, *NKX3-1*, *SPNS3*
GO:0051495	positive regulation of cytoskeleton organization	GO_BiologicalProcess	0.04	3	*DCTN1*, *KIRREL1*, *TRIM27*
GO:0070482	response to oxygen levels	GO_BiologicalProcess	0.04	3	*BNIP3*, *LOXL2*, *NKX3-1*
GO:0010821	regulation of mitochondrion organization	GO_BiologicalProcess	0.04	3	*BNIP3*, *MLLT11*, *RUVBL1*
GO:0032271	regulation of protein polymerization	GO_BiologicalProcess	0.04	3	*DCTN1*, *KIRREL1*, *TRIM27*
GO:0032535	regulation of cellular component size	GO_BiologicalProcess	0.04	4	*KIRREL1*, *RAB22A*, *RAB5A*, *TRIM27*
GO:0060070	canonical Wnt signaling pathway	GO_BiologicalProcess	0.05	2	*RAB5A*, *SOX4*
GO:0010876	lipid localization	GO_BiologicalProcess	0.05	3	*APOL3*, *NKX3-1*, *SPNS3*
GO:0070555	response to interleukin-1	GO_BiologicalProcess	0.05	2	*CHI3L1*, *NKX3-1*
GO:0008380	RNA splicing	GO_BiologicalProcess	0.05	4	*DDX1*, *RBM38*, *SMN2*, *SRSF1*
GO:0018205	peptidyl-lysine modification	GO_BiologicalProcess	0.05	4	*CTCFL*, *RUVBL1*, *SETD1B*, *SOX4*
GO:0045444	fat cell differentiation	GO_BiologicalProcess	0.05	3	*BNIP3*, *MEDAG*, *TMEM120B*
GO:0071383	cellular response to steroid hormone stimulus	GO_BiologicalProcess	0.05	3	*EIF4E*, *NKX3-1*, *NR2F1*
GO:0006631	fatty acid metabolic process	GO_BiologicalProcess	0.05	3	*AUH*
GO:0030904	retromer complex	GO_CellularComponent	0.00	2	*DCTN1*, *TRIM27*
GO:0010494	cytoplasmic stress granule	GO_CellularComponent	0.01	2	*DDX1*, *EIF4E*
GO:0034708	methyltransferase complex	GO_CellularComponent	0.01	3	*E2F6*, *RUVBL1*, *SETD1B*
GO:0045335	phagocytic vesicle	GO_CellularComponent	0.02	2	*RAB22A*, *RAB5A*
GO:0015629	actin cytoskeleton	GO_CellularComponent	0.03	5	*DCTN1*, *DYNLL2*, *IVNS1ABP*, *RAB22A*, *RAB5A*
GO:1990234	transferase complex	GO_CellularComponent	0.04	7	*E2F6*, *GTF2A1*, *HOXC10*, *KLHL20*, *RUVBL1*, *SETD1B*, *VAC14*
GO:0035770	ribonucleoprotein granule	GO_CellularComponent	0.04	3	*DDX1*, *EIF4E*, *SMN2*

GO:0003677	DNA binding	GO_MolecularFunction	0.00	20	*CTCFL, DDX1, E2F6, ELF2, GTF2A1, HOXC10, IER2, LBX2, LOC107132564, MNX1, NKX3-1, NR2F1, RCOR3, SIM2, SMAD9, SOX4, THAP9, TMPO, VEZF1, WRNIP1*
GO:1990837	sequence-specific double-stranded DNA binding	GO_MolecularFunction	0.01	8	*CTCFL, E2F6, HOXC10, IER2, NKX3-1, NR2F1, SOX4, VEZF1*
GO:0001067	regulatory region nucleic acid binding	GO_MolecularFunction	0.01	9	*CTCFL, E2F6, HOXC10, IER2, NKX3-1, NR2F1, RCOR3, SOX4, VEZF1*
GO:0003729	mRNA binding	GO_MolecularFunction	0.05	3	*AUH, RBM38, SRSF1*

*Nr. Genes: Number of genes.

Tabela 9. GO Biological Process, GO Cellular Component, and GO Molecular Function terms were associated with genes interacting with differentially expressed lncRNAs in *Longissimus thoracis* muscle of Nellore cattle with two different fatty acid profiles (C2 vs. C3)

GO:ID	Term	Ontology Source	*p*-value	Nr. Genes*	Genes
GO:0042100	B cell proliferation	GO_BiologicalProcess	0.00	4	*CD180, CD81, IFN-TAU, IFNAG*
GO:0006898	receptor-mediated endocytosis	GO_BiologicalProcess	0.01	4	*ATAD1, CD81, LOXL2, RAB5A*
GO:0016197	endosomal transport	GO_BiologicalProcess	0.01	4	*DCTN1, KLHL20, RAB5A, TRIM27*
GO:0032147	activation of protein kinase activity	GO_BiologicalProcess	0.01	4	*CD81, CHI3L1, KIF14, NKX3-1*
GO:0042113	B cell activation	GO_BiologicalProcess	0.01	4	*CD180, CD81, IFN-TAU, IFNAG*
GO:0046651	lymphocyte proliferation	GO_BiologicalProcess	0.01	4	*CD180, CD81, IFN-TAU, IFNAG*
GO:0043549	regulation of kinase activity	GO_BiologicalProcess	0.01	7	*ADARB1, CD81, CHI3L1, KIF14, NKX3-1, TRIM27, VAC14*
GO:0043112	receptor metabolic process	GO_BiologicalProcess	0.01	3	*ATAD1, CD81, RAB5A*
GO:0070661	leukocyte proliferation	GO_BiologicalProcess	0.02	4	*CD180, CD81, IFN-TAU, IFNAG*
GO:0021953	central nervous system neuron differentiation	GO_BiologicalProcess	0.02	3	*ADARB1, BTG2, HOXC10*
GO:0032535	regulation of cellular component size	GO_BiologicalProcess	0.02	4	*DSTN, RAB22A, RAB5A, TRIM27*
GO:0002285	lymphocyte activation involved in immune response	GO_BiologicalProcess	0.02	3	*CD180, IFN-TAU, IFNAG*
GO:0051495	positive regulation of cytoskeleton organization	GO_BiologicalProcess	0.03	3	*DCTN1, DSTN, TRIM27*
GO:0008285	negative regulation of cell	GO_BiologicalProcess	0.04	5	*ADARB1, ARID2, ATOH8, BTG2, NKX3-1*

	proliferation				
GO:0006869	lipid transport	GO_BiologicalProcess	0.04	3	*APOL3*, *NKX3-1*, *SPNS3*
GO:0002366	leukocyte activation involved in immune response	GO_BiologicalProcess	0.04	3	*CD180*, *IFN-TAU*, *IFNAG*
GO:0002263	cell activation involved in immune response	GO_BiologicalProcess	0.04	3	*CD180*, *IFN-TAU*, *IFNAG*
GO:0010638	positive regulation of organelle organization	GO_BiologicalProcess	0.05	5	*CNOT2*, *DCTN1*, *DSTN*, *RUVBL1*, *TRIM27*
GO:0033674	positive regulation of kinase activity	GO_BiologicalProcess	0.05	4	*CD81*, *CHI3L1*, *KIF14*, *NKX3-1*
GO:0010876	lipid localization	GO_BiologicalProcess	0.05	3	*APOL3*, *NKX3-1*, *SPNS3*
GO:0014070	response to organic cyclic compound	GO_BiologicalProcess	0.05	5	*CNOT2*, *IFN-TAU*, *IFNAG*, *NKX3-1*, *SMAD9*
GO:0002250	adaptive immune response	GO_BiologicalProcess	0.05	3	*IFN-TAU*, *IFNAG*, *TRIM27*
GO:0034440	lipid oxidation	GO_BiologicalProcess	0.05	1	*AUH*
GO:1990234	transferase complex	GO_CellularComponent	0.01	8	*E2F6*, *GTF2A1*, *HOXC10*, *KLHL20*, *KLHL9*, *RUVBL1*, *SETD1B*, *VAC14*
GO:0015629	actin cytoskeleton	GO_CellularComponent	0.02	5	*DCTN1*, *DSTN*, *IVNS1ABP*, *RAB22A*, *RAB5A*
GO:0035770	ribonucleoprotein granule	GO_CellularComponent	0.03	3	*CNOT2*, *PCBP1*, *SMN2*
GO:0005938	cell cortex	GO_CellularComponent	0.04	3	*BFSP1*, *DCTN1*, *DSTN*
GO:0008134	transcription factor binding	GO_MolecularFunction	0.00	7	*ATOH8*, *CNOT2*, *GTF2A1*, *NAB2*, *NKX3-1*, *RCOR3*, *RUVBL1*

*Nr. Genes: Number of genes.

4. DISCUSSION

The fatty acid profile of meat contributes to its sensory properties and plays a significant role in determining the quality of the final product from a health perspective. Therefore, it is essential to select animals with the genetic potential to produce healthier beef by increasing the levels of CLA and maintaining the optimal ratio of PUFA to SFA and ω6 to ω3. Our study identified significant genetic differences between animals (p-value < 0.05), which were classified into three groups based on their FA profile (Figure 1). The C1 cluster showed higher levels of PUFA/SFA, PUFA, ω6, ω3, linolenic, and α-linolenic compared with C2 and C3, which have helpful activities in human metabolism, making C1 a potential group for animal selection. The C2 cluster exhibited intermediate fatty acid profile and cluster C3 showed a lower PUFA/SFA ratio and higher levels of SFA, MUFA (including OA), and CLA compared to the other groups. The simultaneous presence of elevated SFA, MUFA and CLA in cluster C3 may be related to activities of the Δ9 desaturase enzyme. This enzyme converts SFA into MUFA and transvaccenic acid (MUFA) into its corresponding conjugated linoleic acid (CLA), contributing to them being metabolically interconnected and mutually dependent (MIERLITA et al., 2011).

The genome produces numerous lncRNAs, which are transcripts with more than 200 nucleotides and do not encode protein-coding gene. Studies have indicated that the structure of lncRNA is one of the most critical factors regulating different biological processes at the epigenetic, transcriptional, and translational levels (DURAN et al., 2019; DHINGRA et al., 2005). LncRNAs can be classified into different categories depending on their location, structure, and function. In this study, we categorized lncRNAs based on their genomic location as genic lncRNAs (Table 3) and intergenic lncRNAs (lincRNA) (Table 4 and 5). Genic lncRNAs are long non-coding RNAs that overlap coding proteins and may intersect gene exons, introns, or an entire gene, respectively (DURAN et al., 2019). LincRNA is defined by the absence of direct overlap with intergenic noncoding RNA and protein-coding genes. They exhibit distinct attributes setting them apart from mRNA-coding genes and serve functions such as chromatin and genome architecture remodeling, transcriptional regulation and RNA stabilization. (ZHANG et al., 2019).

In this study, 25 DE genic lncRNAs were found to be annotated in the bovine genome reference (ARS.UCD 1.2), 46 DE novel transcript was associated with annotated lncRNA and 194 DE novel lincRNA were identified and associated with FA content in beef cattle. These lncRNAs are important in regulating the expression of genes related to FA profile deposition in meat by affecting FA biosynthesis pathway and lipid metabolism. However, the specific functions of these lncRNAs still need to be further explored.

4.1. DE lncRNAs in C1 vs. C2

Among the DE genic lncRNAs found to the C1 vs. C2 comparison, we can highlight the lncRNA_16456.3, downregulated in C1 in relation to C2 and interacting with the genes *IL6* (Interleukin-6) and *FAM126A* (Family with sequence similarity 126 member A) (Table 3). Although the function of *FAM126A* is unclear, some studies have reported it as important for lipid metabolism and lipid homeostasis (BASKIN et al., 2016; WALLNER et al., 2014; YU et al., 2021). The *FAM126A* gene encodes the Hyccin protein, which is essential for function of protein complex, known as the phosphatidylinositol-5-phosphate synthesis complex (PI5P4K). This complex may interfere with phospholipid synthesis (BASKIN et al., 2016). Phospholipids (PLs) are essential structural lipids in cell membranes, and their fatty acid composition plays a critical role in determining membrane properties and functions (CATALÁ, 2012). Intramuscular fat consists mainly of triglycerides (TG) and PLs (DANNENBERGER et al., 2007). In general, the PL fraction is rich in PUFA due to the preferential incorporation of PUFA into PL associated with cell membranes, while SFA and MUFA are mainly deposited in the TG fraction (ALFAIA et al., 2009; DINH et al., 2021; INSAUSTI et al., 2008). In this regard, the phospholipid content in intramuscular fat can influence the composition of fatty acids in meat. Thus, DE lncRNA_16456.3 may be interfering in phospholipid synthesis through interaction with the *FAM126A* gene, contributing to a profile with lower PUFA content in C2.

Moreover, Interleukin 6 (*IL6*) is a pro-inflammatory adipokine produced by adipose tissue, skeletal muscle, and macrophages (SANTOS et al., 2018). The *IL6* was related to the biological process GO terms such as cellular response to lipid (GO:0071396) and it plays a role in immune response, regulates lipid homeostasis, fatty acid oxidation, lipolysis in adipocytes, skeletal muscle tissue, and hepatocytes (EDER et al., 2009; MAKKI et al., 2013). According to *in vitro* studies, palmitic acid can stimulate the transcription of *IL6* in different biological tissues (AJUWON et al.,2005; SHIRASUNA et al., 2016; ZHOU et al., 2018). The inflammatory response triggered by palmitic acid is mediated by *IL6*, which is responsible for the upregulation of *IL6* (WEIGERT et al., 2004). A study conducted on pigs, found that *IL6* polymorphism exhibited a significant association with PUFA levels (POTHAKAM et al., 2021). *IL6* in swine is located near the QTL regions associated with PUFA, CLA, and SFA content (IQBAL et al., 2015;

PARK et al., 2017; UEMOTO et al., 2012; WON et al., 2018; ZHANG et al., 2016). Additionally, it has been reported that *IL6* deficient mice may be associated with obesity, increased accumulation of intramuscular lipids, and altered SFA composition. (CHABOWSKI et al., 2008; RUDLING et al., 2002). These studies showed that the *IL6* gene may have a pleiotropic effect on the composition of fatty acids. It is associated with the concentration of both SFA, MUFA and PUFA. Our results showed *lncRNA_13894.1* was downregulated for high PUFA, ω3, ω6 and low SFA, MUFA, CLA, and oleic acid; C1), suggesting that its negative regulation and associated with *IL6* may contribute to a genetic profile with lower PUFA and higher SFA, MUFA, CLA an oleic acid.

Still comparing C1 vs. C2, *lncRNA_15786.3* interacted with the *CCN1* gene (Cellular communication network factor 1) and was upregulated compared with C1 cluster (Table 3). *CCN1* encodes a protein associated with the extracellular matrix that is essential regulating cellular processes such as proliferation, migration, and cell adhesion (GRZESZKIEWICZ et al., 2002). A study showed that *CCN1* regulates the lipogenic gene *FASN* (Fatty acid synthase), crucial for fatty acid synthesis, contributing to lipid formation and fat accumulation in cells (MENENDEZ et al., 2016). In a study of SNP in Chinese Holstein dairy cattle, homozygous genotypes for *FASN* were associated with higher levels of PUFAs in milk (LI et al., 2016). Zhang et al. (2008) identified SNPs associated with lower levels of myristic acid, palmitic acid, and total SFA in the *Longissimus dorsi* muscle of American Angus bulls. These findings support our results, in which C1 showed significant differences in FA content, such as higher levels of PUFA and lower levels of SFA, myristic acid, and palmitic acid compared to the FA profile of the C2 cluster. This suggests that animals in C1 would have a genetic profile associated with a healthy and desirable FA composition, characterized by higher PUFA and lower SFA content. In this context, *lncRNA_15786.3* may influence the concentration of these fatty acids through its interaction with the *CCN1* gene, contributing to a healthy fatty acid profile.

For C1 vs. C2 comparison, 17 DE long intergenic RNA (lincRNA) were upregulated and 36 were downregulated in C1 in relation to C2. The *lncRNA_18383.11* was associated with the *DDIT4L* (DNA Damage Inducible Transcript 4 Like) gene (Table 3). This *DDIT4L* gene, also known as *REDD2* (Regulated in Development and DNA Damage Responses 2) acts as an inhibitor of the mTOR signaling pathway, responsible for regulating cell growth and proliferation, and for been involved in the stress response (CARON; et al., 2015; DRUMMOND et al., 2009). Zhao et al. (2019) observed that decrease mTORC1 signaling pathway activity triggered increased FA oxidation in mice. Oxidation of fatty acids takes place within the mitochondria to produce cellular energy. Liu et al. (2020) reported that diets with a high level of PUFAs (ω3/ω6) were able to inhibit the activity of the mTORC1 signaling pathway, reducing the cellular response to growth stimulus and consequently preventing metabolic syndromes such as weight loss, insulin resistance, inflammation and mitochondrial dysfunction in mice. In this study, C1 showed a significantly higher difference in PUFAs, including ω6, ω3 linoleic and alpha-linolenic, with lower values for palmitic acid and myristic acid than C2. Our results support these findings and suggest that the interaction between *lncRNA_18383.11* and the *DDIT4L* gene may affecting concentration of PUFA in C1, influencing FA oxidation by inhibiting of the mTORC signaling pathway. When PUFAs are oxidized, there is a reduction in the supply of free fatty acids for the synthesis of pro-inflammatory lipids, such as SFA (CALDER, 2008; ROCHA et al., 2016; OPPEDISANO et al., 2020). In this sense, *lncRNA_18383.11* may contribute to a healthier beef fatty acid profile with higher PUFA and lower SFA.

4.2. DE lncRNAs in C1 vs. C3

For the C1 vs. C3 comparison, the lncRNAs *lncRNA_13894.1* and *lncRNA_12504* interacted with *BNIP3* (BCL2/Adenovirus E1B 19 kDa protein-interacting protein 3) and *SOX4* (SRY-Box transcription factor 4), respectively (Table 3). These genes were enriched by GO biological function terms related to mitochondrial transport (GO:0006839), fat cell differentiation (GO:0045444), and canonical Wnt signaling pathway (GO:0060070) (Table S2). The *lncRNA_12504* was downregulated (Table 3) in animals from the C1 compared with C3. In this study, C1 showed significant differences in the content of total PUFAs, including linoleic acid, α-linolenic acid, ω3, ω6, and PUFA/SFA ratio, compared to the fatty acid profile of C3, while the content of SFA, stearic acid, myristic acid, palmitic acid was significantly lower in C3. The Wnt pathay enriched the *SOX4* gene which is an important developmental transcription factor that regulates stem cell characteristics, neuronal differentiation (POTZNER et al., 2007) and osteoblast development (NISSEN-MEYER et al., 2007). *SOX4* plays a role in fat development that remains to be clarified (HE et al., 2023). A recent study investigated the role of *SOX4* protein in forming white adipocytes in mice under obese conditions (HE et al., 2023). These authors demonstrated that *SOX4* can control white adipose tissue hyperplasia by inhibiting preadipocyte determination. Adipose tissue hyperplasia can also be induced by a high rate of SFA (MACDOUGALD et al., 2002). Despite the above authors' promising results, there are still questions about the exact role of

SOX4 in fat development in cattle. In this study, C3 had a higher SFA content compared to C2, which suggests that *lncRNA_12504* can interact with the *SOX4* gene and influence the control of hyperplasia in adipose tissue. However, more studies are needed to understand better these interactions and the role of *SOX4* and how they can affect the fatty acid profile of beef in Nellore cattle. The *BNIP3* encodes a protein in the outer mitochondrial membrane, where it functions in mitophagy and mitochondrial dynamics (GLICK et al., 2012). This gene acts in different metabolic pathways, such as lipid metabolism (GLICK et al., 2012) glycolysis (Gang et al., 2015) and mitochondrial bioenergetics (RIKKA et al., 2011). The *BNIP3* gene directly interacts with acetyl-CoA acyltransferase 2 (ACAA2), an enzyme involved in lipid metabolism that facilitates the final stage of β-oxidation within mitochondria (CAO et al., 2008; GLICK et al., 2012). The *ACAA2* enzyme plays a crucial role in malonyl-CoA formation, essential for elongating fatty acid chains (EATON et al., 1996). Studies on single nucleotide polymorphisms in sheep showed that the *ACAA2* gene was associated with the ω6/ω3 ratio in milk (SYMEOU et al., 2020). Our study showed that C1 has high levels of ω6, ω3, linoleic, alpha-linolenic and ω6/ω3 in relation to C3 and found *lncRNA_13894.1* interacted with the *BNIP3* gene upregulated in animals from C1, suggesting the relationship of the *lncRNA_13894.1* in the FA determination, contributing to PUFA concentration in bovine muscle tissue.

4.3. DE lncRNAs in C2 vs. C3

For the C2 vs. C3 comparison, the *lncRNA_16618.6* and *lncRNA_11324.5* interact with *GPR85* (G Protein-Coupled Receptor 85) and *ENSBTAG00000019048,* respectively. The *lncRNA_16618.6* was upregulated in animals from the C2 in relation to C3 and interacted with *GPR85*. The *GPR85* is a gene involved in G protein-coupled receptors (GPCRs). This protein superfamily (GPCRs) has a wide chemical diversity of possible ligands, including nucleosides and nucleotides, peptide and protein hormones, lipids, and eicosanoids (KLABUNDE; HESSLER, 2002). Studies have shown that GPCRs play an essential role in binding extracellular FAs, including small, medium, and long-chain FAs (BRISCOE et al., 2003; BROWN et al., 2003; ITOH et al., 2003; WANG et al., 2006). Additionally, G proteins can regulate the cAMP-mediated signaling pathway (JIA et al., 2022; KANO et al., 2019; PACE et al., 1991). In the context of lipid metabolism, the cAMP/PKA pathway may play an important role in the regulation of lipolysis, which is the breakdown of triglycerides into fatty acids (FAs) and glycerol to provide energy for cells (SAMMETH et al., 2008). Lim et al. (2013) reported that oleic acid stimulates the cAMP/PKA pathway and activates the SIRT1-PGC1α transcriptional complex to modulate FA oxidation rates and, consequently, is vital for maintaining energy homeostasis in the body. Madsen et al. (2008) conducted an in vitro and in vivo study and observed that cAMP/PKA signaling is fundamental in regulating the adipogenic effect of n-6 PUFAs in 3T3-L1 cells. Additionally, the anti-inflammatory effect of docosahexaenoic acid (22:6 n-3, DHA) on different cells is mediated through the cAMP/PKA signaling pathway, as reported by (MORSHED et al., 2023; PARK et al., 2016). In this study, the C2-fatty acid profile exhibited significant differences in PUFA, ω3 and ω6 content compared to the C3-fatty acid profile, indicating that this lncRNA *lncRNA_16618.6* and its interaction with *GPR85* may influence the concentration of ω6 and ω3 fatty acids, thereby contributing to a beef fatty acid profile with higher PUFA levels in C2. The *lncRNA_11324.5* was downregulated in animals from the C2 in relation to C3 and interacted with *ENSBTAG00000019048.* The *ENSBTAG00000019048* (Mediator complex subunit 28 - MED28), also known as magicin, is expressed in numerous cell lines and tissues (WIEDERHOLD et al., 2004). *MED28* has been shown to regulate smooth muscle cell differentiation negatively (BEYER et al., 2007). This gene has been identified as associated with IMF content and carcass traits in cattle in GWAS studies (ANTON et al., 2018; NIU et al., 2021). However, there are no reports of the role of *MED28* in lipid metabolism. In the present study, C3 showed significant differences in the total SFA content, including myristic, palmitic and stearic acids in relation to C2. IMF content in bovine is characterized by its abundant SFA, especially palmitic acid and stearic acid (SCHEEDER et al., 2001). These findings suggest that the interaction between *lncRNA_11324.5* and *MED28* may be acting for a fatty acid profile with higher levels of SFA, as shown in C3. However, further studies are needed to better understand these interactions, and the role of *MED28* and how they might affect the beef fatty acid profile in Nellore cattle.

4.4. DE lncRNAs between comparisons

The novel transcript lengths, *lncRNA_19922.3* and *lncRNA_17096.3,* were associated with *ENSBTAT00000067459* and *ENSBTAT00000066814* lncRNAs, and interacted with *NR2F1* (COUP transcription factor 1) and *MNX1* (Motor neuron and pancreas homeobox 1) genes, respectively. These transcripts were downregulated in C1 vs. C2 and C1 vs. C3 comparisons (Table 3). For the C1 vs. C2 comparison, *NR2F1* was enriched by GO biological function terms related to cellular lipid response (GO: 0071396), while for the C1 vs. C3 comparison, this gene was enriched by GO biological function terms related to cellular response to steroid hormone stimulus (GO:0071383). The *NR2F1* gene (also known as nuclear receptor 2F1, or COUP-TF1) is a sterol-binding transcription factor involved in the regulation of synthesis and transport of triglycerides in enterocytes and associated to lipid, cholesterol, and carbohydrate metabolism (BERKENSTAM at al., 2005; DAI et al., 2010; ZHANG et al., 2004). Researchers have validated *NR2F1* as a candidate gene for IMF deposition using multi-omic data in *Longissimus dorsi* and *gluteus medius* tissues of pigs (GONZÁLEZ-PRENDES et al., 2019; WANG et al., 2021). In a genomic association study in pigs conducted by Pena et al. (2019), the *NR2F1* gene was identified in genomic regions that regulate a relevant percentage of genetic variance for the composition of fatty acids, being these palmitoleic, oleic acid and MUFA. In this study, the C3 showed significant differences in the total content of SFAs, including myristic, palmitic, stearic acid, and oleic acid compared to the C1 and C2 fatty acid profiles. MUFA and oleic contents were very similar between C2 and C3, differing significantly from C1 with small values for MUFA and oleic contents. These results suggest that animals in the C2 would have a genetic profile associated with more balanced MUFA and SFA contents.

The *MNX1* gene regulates the development of motor neurons and pancreatic beta cells, playing a crucial role in the proper formation of these tissues during embryonic development (LEOTTA et al., 2014). Although the function of this gene in cattle remains unclear, Zhang et al. (2016) demonstrated that *MNX1* enhances lipid synthesis by stimulating the expression of *SREBP1* gene and fatty acid synthesis in humans. SNP studies indicated that the bovine *SREBP1* polymorphism is associated with proportions of SFA, palmitic acid, stearic acid, and triglycerides in the fat composition in Simmental and Korean Hanwoo bulls (XU et al., 2013; BHUIYAN et al. 2009). De Smet et al. (2004) observed that SFA and MUFA are mainly deposited in the triglycerides fraction. In this context, the interaction of the novel transcripts *lncRNA_19922.3* and *lncRNA_17096.3* with potential genes such as *NR2F1* and *MNX1*, suggest an important role in the regulation of elements associated with the high content of MUFA and SFA, including individual FAs such as palmitic and stearic fatty acids as observed in C3 and C2.

The *lncRNA_5415.4* associated with the *BTG2* (BTG Anti-Proliferation Factor 2) gene was upregulated in C1 vs. C3 and C2 vs. C3 comparisons (Table 3). C1 and C2 presented significant differences in the contents of ω3 and ω6, in addition to linoleic and alpha-linolenic in relation to C3. Omegas 3 and 6 play a significant role in reducing coronary heart disease, regulating the immune system, maintaining cerebral activities, as well as aiding visual and cognitive development (DYALL et al., 2010; GALANO et al., 2018; WALL et al., 2010). For C1 vs. C3 comparison, *BTG2* was enriched by GO biological function terms related with negative regulation of macromolecule biosynthetic process (GO:0010558) and central nervous system neuron differentiation (GO:0021953) (Table 8). While for C2 vs. C3 this gene was enriched by GO biological function terms related with negative regulation of cell proliferation (GO:0008285) and central nervous system neuron differentiation (GO:0006839) (Table 9). *BTG2* gene encodes a multifunctional protein capable of inhibiting cell proliferation, inducing apoptosis and modulating gene expression. This gene is induced by the *TP53* gene, leading to the cessation of cellular proliferation (DURIEZ et al., 2002). Scherma (2017) investigated the effects of ω6 and ω3 FAs on submandibular gland tumorigenesis in mice and, reported that ω3 rich diet significantly reduced the number and size of tumors compared to the control diet. In addition, the ω3 rich diet increased the expression of the *TP53* gene, which is involved in apoptosis and tumor suppression. Several studies have suggested ω3 and ω6 as biologically effective classes of lipids for the treatment of cancer and their potential influence on the regulation of several pathways and genes responsible for tumor suppressor action (ANDRADE-VIEIRA et al., 2013; KATO et al., 2002; LEINEWEBER et al., 2020; MORSHED et al., 2023; XIA et al., 2006). Our data suggest that *lncRNA_5415.4* may participate in cell proliferation regulation and be involved in programmed cell death under the influence of ω3 and ω6 fatty acids.

Additionally, we can highlight the *lncRNA_20062.7*, which interacts with the genes *IFN-TAU* (Interferon Tau), *IFNAG* (Interferon-gamma), *IFNA5* (Interferon Alpha 5), and *IFNW1* (Interferon Omega 1) and was downregulated in the C1 vs. C3 and C2 vs. C3 comparisons (Table 3). These genes were enriched by GO biological function terms related to cell activation involved in immune response (GO:0002263), leukocyte activation involved in immune response (GO:0002366), lymphocyte activation involved in immune response (GO:0002285), adaptive immune response (GO:0002250), B cell activation (GO:0042113) in C2 vs. C3 comparison (Table 9). These genes belong to the interferon superfamily, are cytokines and play a fundamental role in the immune response (MUÑOZ-CARRILLO et al., 2019), regulate the production of inflammatory cytokines and control cell growth and proliferation (SILVEIRA et al., 2021). Furthermore, they may play a role in adipogenesis, lipid accumulation, and fatty acid metabolism in mammalian muscle tissue (Mekchay et al., 2022). In vitro studies with Huh-7 cells have shown that oleic acid and palmitic acid increase the expression of interferon-stimulated genes and NF-κβ-dependent pro-inflammatory genes (TSE et al., 2015). While high levels of ω3 PUFA block gene expression pathways related to interferon in mouse (BENNINGHOFF et al., 2019), which corroborates with our findings. C3 presented a profile with a higher oleic and palmitic concentration of acid and lower ω3 compared with other clusters (C1 and C2). In this sense, *lncRNA_20062* can interact with interferons in response to the higher content of these fatty acids in meat.

Some lincRNAs (*lncRNA_18394.1* and *lncRNA_2526.3*) were downregulated in C1 vs. C2 and C1 vs. C3 comparisons (Table 3) and were associated with the *EIF4E* (Eukaryotic Translation Initiation Factor 4E) and *DDX1* (DEAD-Box Helicase 1) genes. These genes were enriched by GO biological function terms related to RNA splicing (GO:0008380) and cellular response to lipid (GO:0071396) for C1 vs. C2. Whereas for the C1 vs. C3 comparison, these genes were enriched by GO biological function terms related to negative regulation of macromolecule biosynthetic process (GO:0010558) and cytoplasmic stress granule (GO:0010494) (Table 8). The *EIF4E* (eukaryotic initiation factor 4E) gene encodes an RNA-binding protein that plays a crucial role in regulation of translation and protein synthesis in eukaryotic cells (SCHEPER; PROUD, 2002). *EIF4E* can regulate several metabolic pathways, including lipid metabolism. Studies in mice have shown that *EIF4E* is a key regulator of lipid homeostasis and energy metabolism in the liver and adipose tissue, through the synthesis and oxidation of FAs (CONN et al., 2021). Furthermore, in bovine mammary epithelial cells, it was observed that *EIF4E* is an important regulator in the synthesis of milk fatty acids through the mTOR/eIF4E signaling pathway (ZHAO et al., 2021). *DDX1* gene is a member of the DEAD box family of RNA helicases and involved in many of biological processes, DNA repair, microRNA processing, tRNA maturation and mRNA transport (IWASAKI et al., 2016). Li et al (2018) observed that exposure to saturated FAs such as palmitic acid, decreases in insulin production in pancreatic beta cells, which may contribute to the development of insulin resistance and diabetes. Additionally, they found that the molecular mechanism underlying this reduction in insulin production has been attributed to the interaction of the DDX1 protein with the untranslated region of insulin mRNA, which inhibits of its translation. It is recognized that SFA contributes to the development of insulin resistance (DEER et al., 2015; DENHEZ et al., 2020; LAWRENCE, 2021; LI et al., 2020; ROUMANS et al., 2020). In this study, the content of total SFA, including palmitic acid, myristic acid and stearic acid, MUFA, CLA and oleic acid were significantly higher in C3 and C2 compared to C1, which may be influenced by the regulation of the lincRNAs *lncRNA_18394.*1 and *lncRNA_2526.3* for a genetic profile with higher MUFA and SFA.

The lincRNAs, *lncRNA_17681.1* and *lncRNA_17194.2* were downregulated in C1 vs. C3 and C2 vs. C3 comparison, respectively, and were associated with the *APOL3* (Apolipoprotein L3) and *HOXC10* (Homeobox C10) genes (Table 5 and 6). The *APOL3*, is a member of the apolipoprotein L protein family (APOL-I to VI) and associated with the transport/recycling of cholesterol and sphingolipids in skeletal muscle and other human tissues (MADHAVAN et al., 2011). Furthermore, specific roles of the *APOL3* protein in the immune system and inducing cellular apoptosis when overexpressed in cancer cells, suggest that *APOL3* may inhibit cell proliferation (LIU et al., 2005; OKABE et al., 2007). In beef cattle this gene has been associated with IMF deposition and identified as a highly duplicated gene in the genome (BICKHART et al., 2012; POLETI et al., 2018). The *HOXC10* gene, a member of the HOX gene family, plays crucial roles in mammalian physiological processes such as limb regeneration (CARLSON et al., 2001) and differentiation of lumbar motor neurons (HOSTIKKA et al., 2009). *HOXC10* is also associated with angiogenesis (TAN et al., 2018), fat metabolism (KATO et al., 2021; MA et al., 2020) and sexual regulation (YATSU et al., 2016). Kang et al (2017) studied the transcriptome of fat-tailed sheep and reported the *HOXC10* as a candidate gene for adipose deposition. In humans, evidence suggests that the *HOXC10* may play an important role in the development of obesity, adverse fat distribution, and subsequent changes in whole-body metabolism and function (BRUNE et al., 2016). In this study, C3 showed significant differences in the total SFA content, including myristic, palmitic, stearic and oleic acid compared to the C1 and C2 fatty acid profile, while the MUFA and oleic acid contents were similar between C2 and C3, and differed significantly from C1 which interestingly had small values for MUFA, oleic content, SFAs. In this sense, lincRNAs (*lncRNA_17681.1* and *lncRNA_17194.2*) associated with *APOL3* and *HOXC10* genes can regulate a genetic profile with higher SFA and MUFA content, presented in C3.

The DE lincRNA, *lncRNA_17393.3,* was upregulated in C1 vs. C2 and C2 vs. C3 comparisons (Table 3) and interacted with the *CNOT2* (CCR4-NOT Transcription Complex Subunit 2) gene. In both comparisons, *CNOT2* was enriched by GO biological function terms related to response to organic cyclic compound (GO:0014070), response to hormone (GO:0009725) and cellular response to lipid (GO:0071396) (Table 7 and 9). The *CNOT2* gene encodes the synthesis of the CCR4-NOT2 protein involved in the regulation of gene expression, acting in mRNA degradation, transcription, protein synthesis and cell cycle control (Liu et al. 1997; Ito et al., 2011). The heterozygous intragenic deletion of *CNOT2* in humans displayed disordered phenotypes including learning disabilities, developmental delays, and hypothyroidism (Alesi et al., 2019). The CCR4-NOT2 protein has been associated with several cellular processes, immune response and lipid synthesis (LIU et al., 2019; SOHN et al., 2015). Sohn et al. (2015)

investigated the role of the CCR4-NOT2 gene in adipocyte differentiation and lipogenesis in 3T3-L1 cells. The authors reported that overexpression of the *CCR4-NOT2* gene promotes adipocyte differentiation and lipogenesis by upregulating transcription factors such as *PPARγ* and *CEBPα*. In addition, the presence of ω3 PUFA in cells promoted increased mRNA expression of transcription factors *PPARγ* and *CEBPα* that act in the adipogenesis process (ANTRACO et al., 2021; ROSEN et al., 2006). In this study, C1 showed significant differences in total PUFA/SFA, PUFA, including ω3, ω6, linoleic and *α*-linoleic compared to the C2 fatty acid profile, and these differ significantly from C3 which interestingly had small values for the same fatty acids.

In all comparisons C1 vs. C2, C1 vs. C3, and C2 vs. C3, the lincRNAs (*lncRNA_10184.1 lncRNA_10184.3; lncRNA_11659.1, lncRNA_11659.3, lncRNA_11659.14, lncRNA_11659.17, lncRNA_11659.21; lncRNA_20515.10, lncRNA_20515.12, lncRNA_20515.13; lncRNA_12128.6 lncRNA_12128.7; lncRNA_3024.5, lncRNA_3024.7*) were DE and associated with the *SMN2* (Survival Of Motor Neuron 2, Centromeric), *RUVBL1 (*RuvB Like ATPase 1*), AUH* (AU RNA Binding Methylglutaconyl-CoA Hydratase), *TRIM27* (Tripartite Motif Containing 27*),* and *SMAD9* (SMAD Family Member 9) genes, respectively (Table 4, 5, and 6). These genes were enriched by GO biological function terms related to RNA splicing, RNA localization, adaptive immune response, regulation of protein polymerization, response to organic cyclic compound, response to organic cyclic compound. Among these genes, only the *AUH* gene was associated with GO biological function terms related to lipid metabolism, such as cellular lipid metabolic process, FA metabolic process, and lipid oxidation. *AUH* is a gene that encodes an RNA-binding protein specific for AU and has intrinsic enoyl-CoA hydratase activity, an enzyme involved in the degradation of fatty acids (regardless of its degree of saturation) (NAKAGAWA et al., 1995). Thus, *AUH* is essential in maintaining energy homeostasis and regulating metabolism. As the function of *AUH* in cattle has not been studied yet, it is worth investigating the mechanism by which the interaction between lincRNAs and *AUH* may contribute to the fatty acid profile investigation in beef future studies.

4.5. Enrichment analyses

Overall, enrichment analyses revealed gene ontology (GO) terms related to immune response in the C2 vs. C3 comparison. These GO terms include cell activation involved in immune response (GO:0002263), adaptive immune response (GO:0002250), leukocyte activation involved in immune response (GO:0002366), lymphocyte activation involved in immune response

(GO:0002285), and B cell activation (GO:0042113). Studies have shown that SFAs can promote inflammation in adipose tissue through various mechanisms. SFAs activate pattern recognition receptors (PRRs) and toll-like receptor 4 (TLR4), leading to the production of pro-inflammatory cytokines, including tumor necrosis factor-alpha (TNF-α) and interleukin-6 (IL6) (CHEN; NUÑEZ, 2010; FESSLER; RUDEL; BROWN, 2009; ROCHA et al., 2016). Moreover, SFAs induce endoplasmic reticulum stress and activate inflammatory signaling pathways, such as nuclear factor-kappa B (NF-κB), further exacerbating adipose tissue inflammation (LEE et al., 2001). These events triggered to accelerate inflammation in adipose tissue (SUGANAMI et al., 2007). These studies support that the composition of fatty acids, particularly SFAs, plays a crucial role in modulating inflammatory responses and immune activation in adipose tissue. In this study, the C2 and C3 profiles, which exhibit a high content of SFA compared to the C1, may be contributing to the enrichment of immune system-associated gene ontology terms.

The present study has identified several differentially expressed genic and intergenic lncRNA. However, further in-depth investigations are required to better understand the molecular mechanisms through which these lncRNAs regulate fatty acid metabolism, to more accurately discern their impact on Nellore cattle beef quality traits.

5. CONCLUSION

This study identified 38 novel genic lncRNAs and 194 intergenic lncRNAs, highlighted *lncRNA_16456.3, lncRNA_13894.1, lncRNA_16618.6, lncRNA_11325.5, lncRNA_5415.4, lncRNA_18383.11* and *lncRNA_17393.3* that were interacting with genes essential in regulating fatty acid composition for higher PUFA, ω3, ω6 e lower SFA content contributing to healthier meat. These genes play an essential role in adipogenesis (*CCN1*), fatty acids binding *(GPR85),* muscle cell differentiation *(MED28),* β-oxidation (*BNIP3),* lipogenesis (*CNOT2*) and regulation of cell proliferation (*BTG2 e DDIT4L).* These findings provide valuable information about the intricate network of lncRNAs and their gene interactions affecting the fatty acid regulation in beef cattle and expand our knowledge about regulatory elements (e.g., lncRNAs) associated with a relevance feature for meat quality traits. Therefore, further investigations are warranted to elucidate the molecular mechanisms underlying the regulation and control of fatty acid composition in beef cattle.

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7. MATERIAL SUPPLEMENTAR

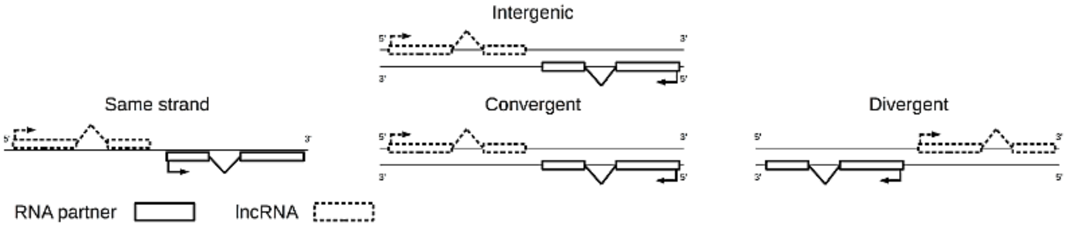

Figure S1. Illustration of the classification for sbtypes according to intergenic location. Divergent: the lncRNA is transcribed in head to orientation with RNA transcripts. Convergent: the lncRNA is oriented in tail to tail with orientation with RNA trancripts. Same_strand: the lncRNA is transcribed in the same orientation with RNA transcripts. Fonte: Wucher et al., 2017.

Table S1. Subtypes defined according to orientation of the proximal RNA transcripts interactions in C1 vs. C2

lncRNA located in sense direction [a]		
Feature ID	**Positon**	**Subtype [b]**
lncRNA_20557.5	8:91054949-91086297	same_strand
lncRNA_7736.5	18:59685331-59696073	same_strand
lncRNA_2305.1	11:49143024-49147255	same_strand
lncRNA_5255.1	15:65502793-65506317	same_strand
lncRNA_9933.3	2:126223556-126230654	same_strand
lncRNA_10558.2	21:16690280-16704571	same_strand
lncRNA_9517.5	2:52904578-52913931	same_strand
lncRNA_5265.1	15:65520195-65531625	same_strand
lncRNA_11848.4	23:14861716-14866193	same_strand
lncRNA_9517.4	2:52904578-52913931	same_strand
lncRNA_20515.12	8:86704251-86724738	same_strand
lncRNA_19092.2	7:12412390-12426648	same_strand
lncRNA_2526.3	11:82614309-82639458	same_strand
lncRNA_7736.1	18:59685331-59696073	same_strand
lncRNA_10320.5	20:35647442-35665372	same_strand
lncRNA_4171.3	13:78409884-78447044	convergent
lncRNA_13086.2	25:25769133-25823226	same_strand
lncRNA_5964.5	16:66395474-66405633	same_strand
lncRNA_2540.2	11:86418716-86464641	same_strand
lncRNA_17103.9	5:5588820-5627943	same_strand
lncRNA_10900.4	21:58589214-58593395	same_strand
lncRNA_9841.2	2:120677247-120682616	same_strand
lncRNA_5964.1	16:66395474-66405633	same_strand
lncRNA located in antisense direction [a]		
Feature ID	**Positon**	**Subtype [b]**
lncRNA_5578.3	16:29120343-29123464	convergent
lncRNA_8796.3	19:44436479-44443655	divergent
lncRNA_17393.3	5:43266505-43268113	divergent
lncRNA_5714.2	16:43978464-43985353	divergent

lncRNA_8294.10	19:24908019-24914528	divergent
lncRNA_3119.2	12:34735808-34750812	divergent
lncRNA_18383.11	6:24210504-24526275	divergent
lncRNA_8294.4	19:24908019-24914528	divergent
lncRNA_15543.1	3:29450215-29462055	divergent
lncRNA_10184.3	20:10097610-10105934	divergent
lncRNA_13547.2	26:15877179-15880904	divergent
lncRNA_15009.1	29:49217825-49218972	divergent
lncRNA_20981.1	9:52492142-52495493	divergent
lncRNA_17358.2	5:34560404-34576263	divergent
lncRNA_15447.1	3:20935933-20944419	divergent
lncRNA_12128.6	1:7104210-7114117	divergent
lncRNA_20224.2	8:55767546-55825069	convergent
lncRNA_280.3	1:69127181-69127765	divergent
lncRNA_10919.2	21:60379945-60383584	divergent
lncRNA_18394.1	6:25791826-25795075	convergent
lncRNA_8476.5	19:33209590-33211733	divergent
lncRNA_12128.6	23:30046878-30066974	convergent
lncRNA_14743.1	29:37152089-37169227	divergent
lncRNA_3024.7	12:24728114-24731701	divergent
lncRNA_6783.6	17:72362986-72382214	divergent
lncRNA_6371.2	17:51604344-51756900	divergent
lncRNA_15447.4	3:20935933-20944419	divergent
lncRNA_18711.6	6:102631399-102685256	convergent
lncRNA_11659.21	22:59504587-59508832	divergent
lncRNA_7507.1	18:52318710-52407580	divergent

[a] Direction of transcription of proximal RNA transcripts; [b] Orientation of transcription of proximal RNA transcripts.

Table S2. Subtypes defined according to orientation of the proximal RNA transcripts interactions in C1 vs. C3

lncRNA located in sense direction [a]		
Feature ID	**Positon**	**Subtype** [b]
lncRNA_13486.1	26:9373528-9377974	same_strand
lncRNA_3864.2	13:58736919-58750531	same_strand
lncRNA_20557.5	8:91054949-91086297	same_strand
lncRNA_10320.8	20:35647442-35665372	same_strand
lncRNA_5255.1	15:65502793-65506317	same_strand
lncRNA_10900.4	21:58589214-58593395	same_strand
lncRNA_10720.6	21:33142026-33155354	same_strand
lncRNA_2540.4	11:86418716-86464641	same_strand
lncRNA_20515.13	8:86704251-86724738	same_strand
lncRNA_3050.2	12:30041465-30044471	same_strand
lncRNA_10558.1	21:16690280-16704571	same_strand
lncRNA_10558.14	21:16690280-16704571	same_strand
lncRNA_10558.9	21:16690280-16704571	same_strand
lncRNA_10320.7	20:35647442-35665372	same_strand
lncRNA_16508.10	4:43223448-43279119	same_strand
lncRNA_20515.10	8:86704251-86724738	same_strand
lncRNA_9517.4	2:52904578-52913931	same_strand
lncRNA_5265.1	15:65520195-65531625	same_strand

lncRNA_1878.2	10:102749910-102772451	same_strand
lncRNA_17681.1	5:74669803-74711373	same_strand
lncRNA_20515.12	8:86704251-86724738	same_strand
lncRNA_19092.2	7:12412390-12426648	same_strand
lncRNA_2526.3	11:82614309-82639458	same_strand
lncRNA_5964.1	16:66395474-66405633	same_strand
lncRNA_5964.6	16:66395474-66405633	same_strand
lncRNA_16442.1	4:29193479-29195379	same_strand
lncRNA_10720.7	21:33142026-33155354	same_strand
lncRNA_7736.1	18:59685331-59696073	same_strand
lncRNA_9841.2	2:120677247-120682616	same_strand
lncRNA located in antisense direction [a]		
Feature ID	**Positon**	**Subtype [b]**
lncRNA_18738.3	6:108831172-108833800	divergent
lncRNA_17511.1	5:56346812-56351225	divergent
lncRNA_16747.1	4:76603706-76606680	divergent
lncRNA_18738.2	6:108831172-108833800	divergent
lncRNA_18001.6	5:108915063-108924973	convergent
lncRNA_10184.1	20:10097610-10105934	convergent
lncRNA_10586.5	21:21519283-21537669	convergent
lncRNA_6258.4	17:18453124-18474698	divergent
lncRNA_17577.2	5:62725526-62731661	convergent
lncRNA_8796.1	19:44436479-44443655	divergent
lncRNA_15455.1	3:20557127-20560106	divergent
lncRNA_2042.4	11:10233550-10238310	divergent
lncRNA_5578.3	16:29120343-29123464	convergent
lncRNA_11659.1	22:59504587-59508832	divergent
lncRNA_12128.7	23:30046878-30066974	divergent
lncRNA_11659.14	22:59504587-59508832	divergent
lncRNA_18674.6	6:97716507-97750095	divergent
lncRNA_3855.2	13:58045731-58049015	convergent
lncRNA_791.1	1:144934585-144937622	divergent
lncRNA_8476.6	19:33209590-33211733	divergent
lncRNA_929.1	1:157196721-157198350	divergent
lncRNA_11659.17	22:59504587-59508832	divergent
lncRNA_2036.1	11:10142822-10146171	convergent
lncRNA_8294.4	19:24908019-24914528	divergent
lncRNA_3433.4	13:17510294-17518504	divergent
lncRNA_5714.2	16:43978464-43985353	divergent
lncRNA_3024.5	12:24728114-24731701	divergent
lncRNA_20692.2	8:111823798-111834775	divergent
lncRNA_8476.12	19:33209590-33211733	convergent
lncRNA_17511.6	5:56346812-56351225	divergent
lncRNA_18001.15	5:108915063-108924973	divergent
lncRNA_6258.1	17:18453124-18474698	divergent
lncRNA_18001.17	5:108915063-108924973	divergent
lncRNA_1840.2	10:92511630-92522957	divergent
lncRNA_11659.3	22:59504587-59508832	divergent
lncRNA_15403.1	3:19689039-19726184	convergent
lncRNA_18674.5	6:97716507-97750095	divergent
lncRNA_17653.3	5:73720268-73724709	divergent
lncRNA_18001.14	5:108915063-108924973	divergent
lncRNA_20041.2	8:16267188-16287915	divergent

lncRNA_20981.1	9:52492142-52495493	divergent
lncRNA_17194.2	5:26047734-26049634	divergent
lncRNA_20224.2	8:55767546-55825069	convergent
lncRNA_38.1	1:7104210-7114117	divergent
lncRNA_15447.4	3:20935933-20944419	divergent
lncRNA_2832.1	11:104798248-104801001	divergent
lncRNA_12128.6	23:30046878-30066974	convergent
lncRNA_14743.1	29:37152089-37169227	divergent
lncRNA_3024.7	12:24728114-24731701	divergent
lncRNA_6802.5	18:1343804-1405315	divergent
lncRNA_6783.6	17:72362986-72382214	divergent
lncRNA_18394.1	6:25791826-25795075	convergent
lncRNA_3863.4	13:58694491-58705765	divergent
lncRNA_8796.4	19:44436479-44443655	divergent
lncRNA_18001.20	5:108915063-108924973	divergent
lncRNA_10586.3	21:21519283-21537669	convergent

[a] Direction of transcription of proximal RNA transcripts; [b] Orientation of transcription of proximal RNA transcripts.

Table S3. Subtypes defined according to orientation of the proximal RNA transcripts interactions in C2 vs. C3

lncRNA located in sense direction [a]		
Feature ID	**Positon**	**Subtype [b]**
lncRNA_7736.5	18:59685331-59696073	same_strand
lncRNA_9933.3	2:126223556-126230654	same_strand
lncRNA_10558.2	21:16690280-16704571	same_strand
lncRNA_2305.1	11:49143024-49147255	same_strand
lncRNA_13486.1	26:9373528-9377974	same_strand
lncRNA_9517.5	2:52904578-52913931	same_strand
lncRNA_3864.2	13:58736919-58750531	same_strand
lncRNA_10900.4	21:58589214-58593395	same_strand
lncRNA_17681.1	5:74669803-74711373	same_strand
lncRNA_11848.4	23:14861716-14866193	same_strand
lncRNA_10320.8	20:35647442-35665372	same_strand
lncRNA_20515.13	8:86704251-86724738	same_strand
lncRNA_10720.6	21:33142026-33155354	same_strand
lncRNA_5964.5	16:66395474-66405633	same_strand
lncRNA_2540.4	11:86418716-86464641	same_strand
lncRNA_3470.1	13:23312186-23369059	same_strand
lncRNA_20515.10	8:86704251-86724738	same_strand
lncRNA_10558.14	21:16690280-16704571	same_strand
lncRNA located in antisense direction [a]		
Feature ID	**Positon**	**Subtype**
lncRNA_20041.2	8:16267188-16287915	divergent
lncRNA_17393.3	5:43266505-43268113	divergent
lncRNA_5578.2	16:29120343-29123464	convergent
lncRNA_2417.4	11:68352230-68367299	divergent
lncRNA_8294.10	19:24908019-24914528	divergent
lncRNA_7978.2	19:8080110-8091884	divergent
lncRNA_18738.2	6:108831172-108833800	divergent
lncRNA_17511.1	5:56346812-56351225	divergent
lncRNA_16747.1	4:76603706-76606680	divergent

lncRNA_15009.1	29:49217825-49218972	divergent
lncRNA_17194.2	5:26047734-26049634	divergent
lncRNA_10184.1	20:10097610-10105934	divergent
lncRNA_2042.4	11:10233550-10238310	divergent
lncRNA_10586.5	21:21519283-21537669	convergent
lncRNA_15455.1	3:20557127-20560106	divergent
lncRNA_3433.2	13:17510294-17518504	divergent
lncRNA_8796.1	19:44436479-44443655	divergent
lncRNA_17577.2	5:62725526-62731661	convergent
lncRNA_3855.2	13:58045731-58049015	divergent
lncRNA_17358.2	5:34560404-34576263	divergent
lncRNA_11659.14	22:59504587-59508832	divergent
lncRNA_11659.1	22:59504587-59508832	divergent
lncRNA_12128.7	23:30046878-30066974	convergent
lncRNA_18674.6	6:97716507-97750095	divergent
lncRNA_10919.2	21:60379945-60383584	divergent
lncRNA_791.1	1:144934585-144937622	divergent
lncRNA_11659.17	22:59504587-59508832	divergent
lncRNA_2036.1	11:10142822-10146171	convergent
lncRNA_3024.5	12:24728114-24731701	divergent
lncRNA_929.1	1:157196721-157198350	divergent
lncRNA_3433.4	13:17510294-17518504	divergent
lncRNA_8476.14	19:33209590-33211733	convergent
lncRNA_6802.5	18:1343804-1405315	divergent
lncRNA_1840.2	10:92511630-92522957	divergent
lncRNA_18001.17	5:108915063-108924973	divergent
lncRNA_6258.1	17:18453124-18474698	divergent
lncRNA_10210.6	20:12278005-12356729	divergent
lncRNA_280.3	1:69127181-69127765	divergent

[a] Direction of transcription of proximal RNA transcripts; [b] Orientation of transcription of proximal RNA transcripts.

CHAPTER 3 - DIFFERENTIALLY EXPRESSED MESSENGER MRNA ISOFORMS IN BEEF CATTLE SKELETAL MUSCLE WITH DIFFERENT FATTY ACID PROFILES

ABSTRACT - The aim of this study was to identify differentially expressed mRNA isoforms in muscle tissue of Nellore cattle clustered according to their intramuscular fatty acid profile. *Longissimus thoracis* muscle samples from 48 young bulls were used to quantify fatty acids (FA) and generate RNA-Sequencing data for transcriptomic analyses. K-means analysis was used to group the 48 animals into three clusters based on their FA patterns (seven individuals and seven groups). The C1 presented significantly ($p \leq 0.05$) higher PUFA/SFA, PUFA, ω3, ω6, linoleic and α-linolenic ratios. The proportion of MUFA, CLA and oleic in the C2 and C3 were significantly ($p \leq 0.05$) higher than that in C1, while C3 had significantly ($p \leq 0.05$) higher proportions of ω6/ω3, SFA, myristic, palmitic and stearic. Differential expression (DE) analyzes were performed on three different comparisons, C1 vs. C2, C1 vs. C3 and C2 vs. C3. A total of 62, 26 and 30 transcripts were differentially expressed (DE) for the C1 vs. C2, C1 vs. C3 and C2 vs. C3, respectively. In the C1 vs. C2 comparison, we highlighted the mRNA isoforms *C7-203, ADD1-204* and *OXT-201*, which act on glycogen and lipid metabolism and in the regulation of key genes involved in fatty acid synthesis, contributing to higher PUFA content profile. In the C1 vs. C3 comparison, the mRNA isoforms *RBM3-202* and *TRAG1-202* play an important role in muscle development, adipogenesis and concentration of PUFA and MUFA, respectively. In the C1 vs. C2 and C2 vs. C3 comparisons, the downregulation of THRSP-201, FABP4-201, and CIDEC_mRNA1 isoforms, associated with lipogenesis, fatty acid transport and downregulation of lipolysis, contribute to higher levels of PUFA and lower levels of MUFA and SFA. The results suggest that the identified mRNA isoforms may be used as potential candidate genomic regions to select animals with potential to improve meat fatty acid profile.

Keywords: Beef cattle**,** Fatty acids, transcriptomic, RNA-Seq

CAPÍTULO 3 - ISOFORMAS DE MRNA MENSAGEIRO DIFERENCIALMENTE EXPRESSAS NO MÚSCULO ESQUELÉTICO DE BOVINOS COM DIFERENTES PERFIS DE ÁCIDOS GRAXOS

RESUMO - O objetivo deste estudo foi identificar isoformas de mRNA diferencialmente expressos no tecido muscular de bovinos Nelore agrupados de acordo com seu perfil de ácidos graxos. Amostras do músculo *Longissimus thoracis* de 48 touros jovens foram utilizadas para quantificar ácidos graxos (AG) e gerar dados de sequenciamento de RNA para análises transcriptômicas. A análise K-means foi usada para agrupar os 48 animais em três clusters com base em seus padrões de AG (sete indivíduais e sete grupos. O cluster C1 apresentou proporções significativamente ($p \leq 0{,}05$) maiores de AGP/AGS, AGP, ω3, ω6, proporções de ácido linoléico e α-linolênico. A proporção de AGM, CLA e ácido oleico nos clusters C2 e C3 foi significativamente ($p \leq 0{,}05$) maior do que C1, enquanto o cluster C3 teve proporções significativamente ($p \leq 0{,}05$) maiores de ω6/ω3, AGS, mirístico, palmítico e esteárico em relação aos clusters C1 e C2. Análises de expressão diferencial (DE) foram realizadas em três compações diferentes, C1 vs. C2, C1 vs. C3 e C2 vs. C3. Um total de 62, 26 e 30 mRNA idoformas DE (fold change > | 2 | and p-value <0.01) para as comparações C1 vs. C2, C1 vs. C3 e C2 vs. C3 eram DE, respectivamente. A comparação C1 vs. C2 destacou as mRNA isoformas *C7-203*, *ADD1-204* and *OXT-201* que atuam no metabolismo do glicogênio e de lipídios e regulação de genes-chaves envolvidos na síntese de ácidos graxos contribuindo para um perfil com maior conteúdo de AGPI. A comparação C1 vs. C3 as isoformas de mRNAs e *RBM3-202* and *TRAG1-202* atuam desenvolvimento muscular, adipogênese e contribuem para um perfil genético com maior concentração de AGP e AGM, respectivamente. As Comparações C1 vs. C2 e C2 vs. C3 mostraram que expressão negativa das isoformas *THRSP-201, FABP4-201* e *CIDEC_mRNA1* associadas lipogênese, transporte de ácidos graxos e regulação negativa lipólise contribuíram para um perfil com maior AGP e menor AGM e AGS. Os resultados sugerem que as isoformas de mRNA identificadas podem ser utilizadas como potenciais regiões genômicas candidatas para selecionar animais com o potencial para melhorar o perfil de ácidos graxos da carne.

Palavra-Chave: Ácidos Graxos, Bovinos de corte, Isoformas de mRNA, RNA-Seq.

1. INTRODUCTION

Beef fatty acid (FA) composition has been a major concern for producers and consumers due to its significant role in meat flavor and human health. For example, it is known that oleic acid contributes to the flavor of beef (LEE et al., 2017), polyunsaturated fatty acids such as ω3 and ω6 provide protection against autoimmune diseases and cancer (LUU et al., 2018), while saturated fatty acids such as myristic acid and palmitic acid contribute to the susceptibility to cardiovascular disease and inflammatory conditions (EILANDER et al., 2015; ZONG et al., 2016). FA composition present in beef is quite variable and complex due to the large number of genes and related metabolic pathways, which are shaped by genetic and environmental factors. Identifying and selecting animals with genetic advantages for a healthier fatty acid profile with higher PUFA and MUFA and lower SFA can improve beef nutritional and sensory attributes, which could positively benefit beef production and human consumption.

Alternative splicing of messenger RNAs (mRNAs) produces multiple transcripts and protein isoforms that may have similar or quite different functions in mammalian tissues (BARASH et al., 2010; PARK et al., 2018a; REYES et al., 2018). Next-generation sequencing applied to tissue transcriptomes (RNA-Seq) is capable of accurately reveal the entire transcriptome with high sensitivity and detecting wide variations in expression. RNA-Seq can quantify the expression of functional candidate genes and allows to identify important molecular mechanisms, creating variations in pathways that result in different tissue phenotypes. The technology has also been used to identify new mRNA isoforms in cattle associated with meat tenderness, color, and marbling in Nellore cattle (MUNIZ et al., 2020, 2021, 2022a).

Transcript diversity of the bovine muscle has been poorly characterized for the fatty acid profile so far, particularly for Nellore cattle. The majority of studies developed have compared the transcriptome of cattle with distinct phenotypic attributes have focused on global differences in gene expression, rather than identifying the specific transcripts that are differentially expressed (DE) (BERTON et al., 2016, 2022; OLIVIERI et al., 2021; SCHETTINI et al., 2022a; TIZIOTO et al., 2015). In this context, the main hypothesis of the present study is that these different fatty acid profile show different expression patterns. Therefore, the aim of this study was to identify novel mRNA isoforms differentially expressed in *Longissimus thoracis* tissues of

Nellore cattle among bull groups with different fatty acid profile, which may help to identify biological mechanisms and key mRNAs associated with beef fatty acid.

2. MATERIAL AND METHODS

2.1. Data collection

The samples collected belonged to 48 Nellore young bulls, sons of six sires, belonging to the Nellore Qualitas program, from the Capivara farm, located in the state of São Paulo, Brazil. The animals were raised on pasture and subsequently kept in feedlots for a period of 90 days. Animals were slaughtered at an average age of 24 months and with a live weight of 550 kg. Following a 48-hour post-mortem period, samples were retrieved from the *Longissimus thoracis* (LT) muscle, positioned between the 12th and 13th ribs of each animal (left half carcass) and preserved at -80°C in airtight plastic bags for RNA extraction and fatty acids measurement analysis, as detailed in Berton et al. (2016).

Meat FAs were extracted from intramuscular fat (IMF) of the LT muscle using the method described by (FOLCH e LEES, 1957). Muscle samples (~100g) were homogenized with a chloroform and methanol solution (2:1) for lipid extraction. The lipids were isolated by adding 1.5% NaCl and subsequently methylated. The methyl esters were formed according to Kramer et al., (1997). Fatty acid methyl esters (FAME) were analyzed in a Shimadzu GC-2010 Plus gas chromatograph equipped with a Shimadzu AOC-20i auto-injector. The FAs were quantified by normalizing the area under the curve of methyl esters using GS Software Solutions (version 2.42). The FA contents were expressed as a percentage of the total FA methyl ester quantified, as detailed in Berton et al. (2016).

Based on the identified acids, 14 FAs (seven individuals and seven groups of FAs) were selected due to their importance in human health were determined (Figure 1). More details in chapter 2.

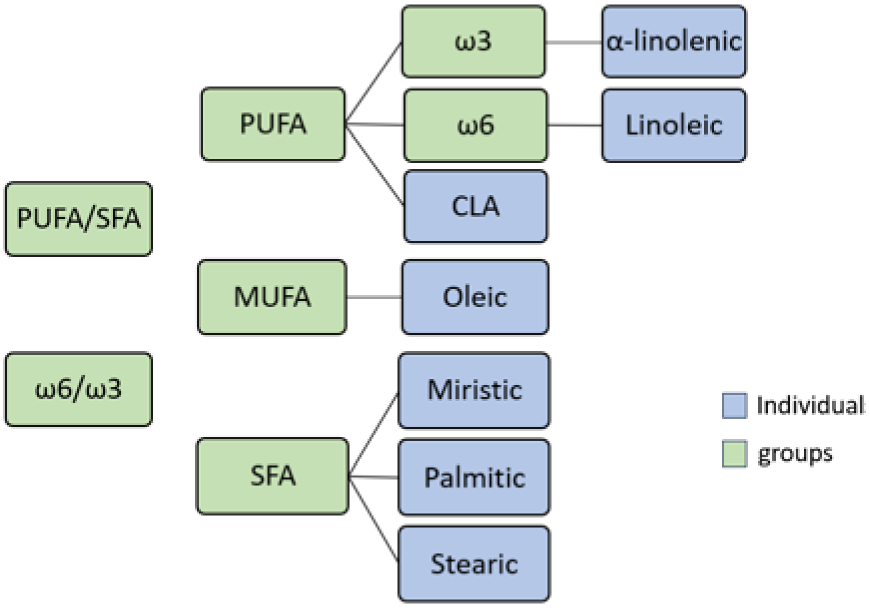

Figura 1. Ther hierarchy of the 14 FAs (seven individual FAs and seven groups of FAs) were selected.

The k-means method was used to classify 48 animals into three clusters by their FA profiles and ANOVA and Tukey test was applied to determine if there were statistically significant differences between the clusters. For more details see the previous chapter.

Based on k-means analysis was identified three distinct clusters, each exhibiting unique fatty acid (FA) profiles, as described in the previous chapter. The average FA content for each cluster can be found in Table 1. Cluster 1 (C1; n=14 bulls' sample) was significantly higher for PUFA/SFA ratio, PUFA, ω6, ω3, linoleic acid, and α-linolenic acid. Cluster 2 (C2; n=24) and Cluster 3 (C3; n=10) was significantly higher for MUFA, CLA, and oleic acid compared with C1. However, SFA, including stearic acid, myristic acid, and palmitic acid, were significantly higher in C3 compared to the other groups ($P < 0.05$; Table 2). For ω6/ω3 ratio, MUFA, SFA and stearic acid, clusters C1 and C2 showed no significant difference between them.

2.2. RNA Sequencing analysis

Total RNA was extracted from muscle samples (~50 mg) using the RNeasy Lipid Tissue Mini Kit (Qiagen, Valencia, CA, USA) according to the manufacturer's recommendations. The purity of the extracted RNA was analyzed by reading the absorbance in a NanoDrop 1000 spectrophotometer (Thermo Fisher Scientific). The quality of the extracted total RNA was evaluated in an Agilent 2100 Bioanalyzer and its concentration and contamination with genomic DNA were quantified in a Qubit® 2, as described in Fonseca et al. (2017) work.

mRNA paired-end libraries were generated from each of the RNA samples using Illumina TruSeq mRNA library preparation kit. Sequencing was performed on the HiSeq 2500 platform (Illumina) to generate paired-and reads with 2x100 bp.

2.3. RNA-seq quantification

2.3.1. Quality Control and alignment of reads

FastQC (v.0.11.4) was utilized to assess the quality of RNA-Seq reads, including parameters like quality scores, GC content, N content, length distributions, duplication, overrepresented sequences, and K-mer content (ANDREWS et al., 2010). The raw reads were processed in two steps using Atropos (v1.1.19) (DIDION et al., 2017), starting with the insert match algorithm using additional parameters: -n 2, -m 1, –op-order GAWCQ, –match-read-wildcards, -O 20, -q 25, –pair-filter any, end –correct-mismatches conservative, followed by the adapter match algorithm for the unprocessed reads that passed by the first step, applying the parameters: -n 2, -m 1, –match-read-wildcards, -O 3, -q 20, and –pair-filter both. After “PRINSEQ-lite” software (v.0.20.3.; Schmieder e Edwards, 2011) was used to trim3'-and sequences dynamic pruning, in which were trimmed 3’-end with quality average setting up to three bases (--trim_qual_window: 3) and less than 30 (--trim_qual_right: 30). Parameters were used to ensure minimum size of the output reads (--min_len: 20) and remove all poly-A/T tails with a minimum pre-requisite of 5 continuous As or Ts in the 5′ (--trim_tail_left: 5), or 3′ (--trim_tail_rigth: 5) end of the contigs. Low complexity transcripts (lc_method: dust) bigger than 50 score (lc_threshold: 50) also were removed. After filtering, HISAT2 (v.2.0.5) software (KIM et al., 2015) was used to map paired-end reads for bovine genome (ARS-UCD1.2Bos Taurus) deposited in Ensembl (http://ftp.ensembl.org/pub/current_fasta/bos_taurus/). Mapped reads were then assembled by the Cufflinks program (v2.2.1) using parameters: -min-isoform-fraction 0.20 -small-anchor-fraction 0.08 -max-intron-length 300000 -trim-3-avgcov-thresh 0.05 -overlap-radius 25 -max-bundle-frags 2000000. The individual transcript assemblies were merged into a master transcriptome using Cuffmerge, which was subsequently utilized by Cuffquant and Cuffnorm to generate normalized expression in FPKM and count tables for each cluster, employing the default settings.

2.3.2. Differential expression analysis of mRNA isoforms between fatty acids profile

Differentially expressed transcripts among the three fatty acid profiles were detected using the Cufflinks/Cuffdiff pipeline http://cole-trapnell-lab.github.io/cufflinks/manual/) with default settings (TRAPNELL et al., 2012). A two-group experiment were used to compare the three fatty acid profiles (C1 vs. C2, C1 vs. C3 and C2 vs. C3). Next, the fold change (FC) > | 2 | and p-value <0.01 was used to as threshold to filter differentially expressed transcripts identified in the C1 vs. C2, C1 vs. C3 and C2 vs. C3 comparisons.

2.4. Functional enrichment analysis and gene annotation

Functional annotation was performed through the Cytoscape (SHANNON et al., 2003) plug-in of the ClueGO software (Bindea *et al.*, 2009) considering threshold of p-value ≤ 0.05. The analyses were done based on mRNA isoforms and an annotated genes list. The Gene ontology (GO) terms associated with the three main GO categories such as biological processes, molecular function and cellular component were analyzed. ClueGO performed a single cluster analysis for each cluster comparison. The ClueGO network was created with kappa statistics and reflected relationships between terms based on the similarity of their associated genes. The functionally grouped network with terms such as nodes was linked based on its kappa score level (k-score≥0.5).

To annotate new mRNA isoforms of unknown genes, we obtained the coding sequences from the Genome Data Viewer tool provided by the National Center for Biotechnology Information (NCBI) database (https://www.ncbi.nlm.nih.gov/genome/gdv/browser/genome/?id=GCF_002263795.1P). Afterwards, we employed the "Nucleotide BLAST" method, which is accessible through the Basic Local Alignment Search Tool (BLAST; https://blast.ncbi.nlm.nih.gov/Blast.cgi.) platform. The purpose of this approach was to identify similarities between the nucleotide sequences of the newly discovered mRNA isoforms and the nucleotide collection (nt) available in the database. The nt comprises sequence information sourced from GenBank, EMBL, DDBJ, PDB, and RefSeq. However, it omits EST, STS, GSS, WGS, TSA, phase 0, 1, and 2 HTGS sequences, as well as sequences exceeding 100 Mb in length. This database is designed to eliminate redundancy, wherein identical sequences are merged into a solitary entry, preserving accessions, GIs, titles, and taxonomy details for each individual entry.

3. RESULTS

3.1. Statistics of alignment

The average of paired-end reads per sample after filtering was approximately 28.2 million reads. Of the trimmed reads, 86% wereuniquely mapped to the bovine reference genome ARS-UCD1.2 Bos Taurus. The overall alignment rate was 91.95% (approximately 25.9 million reads mapped in pairs) with sequencing coverage of 45× (coverage for all transcripts of all samples). From the three transcripts expression comparison analyzes performed, an average of 31,058 genes and 106,560 transcripts were expressed in the LT muscle of Nellore cattle. Out of the total number of transcripts being expressed in LT muscle, a total of 65, 26, and 33 mRNA isoforms were differentially expressed (DE; p-value < 0.01 and FC > |2|) for the C1 vs. C2, C1 vs. C3 and C2 vs. C3 comparisons, respectively.

3.2. Differentially expressed mRNA isoforms annotated in ARS.UCD1.2 genome reference

For the C1 vs. C2, 43 up-regulated transcripts were found considering C1 compared to C2 (Table 2). These transcripts were related with genes associated to ubiquitin-protein ligase, e.g., *UBE2V1* (Ubiquitin Conjugating Enzyme E2 V1), *ASB8* (Ankyrin Repeat and *SOCS* Box Containing 8), *ITCH* (Itchy E3 Ubiquitin Protein Ligase) and *BTBD2* (BTB Domain Containing 2). In addition, a few genes related to lipid metabolism as *BMP6* (Bone Morphogenetic Protein 6), *CYB5B* (Cytochrome B5 Type B), *BBS4* (Bardet-Biedl Syndrome 4), *LMO4* (LIM Domain Only 4) were found (Table 2). Three annotated mRNA isoforms (*ACTC1-201*, *EGR1-201*, *CCL1-201*) associated with the *ACTC1* (Actin Alpha Cardiac Muscle 1), *EGR1* (Early Growth Response 1), and *CCL1* (C-C Motif Chemokine Ligand 1)) were down-regulated in animals from the C1 compared to C2 (Table 2) and were associated to actin and chemokines encoder and growth factors.

For the C1 vs. C3, three annotated mRNA isoforms (*RBM3-202*, *HBB-201* and *NREP-201*) associated with the *RBM3* (RNA Binding Motif Protein 3), *HBB* (Hemoglobin Subunit Beta) and *NREP* (Neuronal Regeneration Related Protein) genes were up-regulated in C1 in relation to C3 and associated to RNA splicing factor, globin and myogenesis. Out of these, nine annotated mRNA isoforms (*TSPO-202*, *TRARG1-201, THRSP-201, FABP4-201, EMX2-201, PMF1-201, FBLN7-201*, *CCDC3-201* and *MT1A-201*) were down-regulated in animals from the C1 in relation to C3 and associated with regulation of lipid and fatty acid metabolism, transport and signaling and cell proliferation.

For the C2 vs. C3, 18 mRNA isoforms were differentially expressed in relation to C3 (Table 2). Out of those, 17 were downregulated, which were related to lipid and fatty acids metabolism (*THRSP-201*, *FABP4-201*, *AIMP1-201*), cell proliferation (*POP5-202* and *PPP4C-202*), chaperone (PIFF-201). Only *MSTN-201* (Table 2) was upregulated in this analysis, which is associated to myosin encoder.

Tabela 2. Differentially expressed mRNA isoforms identified in *Longissimus thoracis* muscle of Nellore cattle with different fatty acids profile.

Cluster 1 vs. Cluster 2						
Gene ID	**Transcript ID**	**Transcript name**	**Length (bp)**	**Position**	***p*-value**	**Log2 (FC) [1]**
ENSBTAG00000002904	ENSBTAT00000003771.5	ZNF787-201	1718	18:63496808-63512692	5,00E-04	982.71
ENSBTAG00000016895	ENSBTAT00000079943.1	NIPA2-205	1349	2:1044597-1064844	3,65E-03	959.98
ENSBTAG00000052681	ENSBTAT00000067953.1	SMIM10L1-201	207	5:98568748-98571841	5,00E-05	909.34
ENSBTAG00000049062	ENSBTAT00000078807.1	-	273	17:72990819-72995082	1,05E-03	907.76
ENSBTAG00000021071	ENSBTAT00000028059.6	TRIM8-202	1859	26:23265724-23279452	1,70E-03	869.26
ENSBTAG00000009643	ENSBTAT00000068832.1	ZBTB25-202	1265	10:76612258-76648488	3,85E-03	867.38
ENSBTAG00000005146	ENSBTAT00000076472.1	-	921	23:28525462-28527983	6,55E-03	798.58
ENSBTAG00000019234	ENSBTAT00000025614.6	BMP6-201	1491	23:47476519-47663412	1,55E-03	724.50
ENSBTAG00000012658	ENSBTAT00000076338.1	TMA16-203	567	6:2104629-2141956	8,35E-03	643.10
ENSBTAG00000005385	ENSBTAT00000007083.4	POP5-202	1418	17:62851493-62854683	3,10E-03	537.68
ENSBTAG00000002412	ENSBTAT00000003129.5	CYB5B-202	435	18:36548064-36572990	3,20E-03	505.82
ENSBTAG00000011766	ENSBTAT00000066856.1	C7-203	2451	20:33535886-33593157	9,30E-03	461.00
ENSBTAG00000011096	ENSBTAT00000068753.1	ERGIC2-202	1026	5:80232307-80278857	6,95E-03	366.96
ENSBTAG00000006213	ENSBTAT00000008157.3	-	561	14:56697845-56698406	2,25E-03	328.05
ENSBTAG00000027316	ENSBTAT00000078205.1	UBE2V1-202	456	13:78114944-78143441	6,90E-03	314.42
ENSBTAG00000030608	ENSBTAT00000065152.2	-	372	11:98883498-98889714	8,20E-03	245.50
ENSBTAG00000013493	ENSBTAT00000066357.1	BNIP2-202	1297	10:50557149-50614948	8,25E-03	238.18
ENSBTAG00000016290	ENSBTAT00000021671.5	MOB1B-202	648	6:86250300-86308361	7,55E-03	237.21
ENSBTAG00000020026	ENSBTAT00000072579.1	C3H1orf43-203	946	3:16300762-16309171	3,55E-03	234.60
ENSBTAG00000038126	ENSBTAT00000068851.1	WIPF2-204	1168	19:40490707-40546357	1,10E-03	219.88
ENSBTAG00000007614	ENSBTAT00000069146.1	BBS4-202	1594	10:19486142-19532111	3,65E-03	216.68
ENSBTAG00000010492	ENSBTAT00000068822.1	VPS4B-203	1326	24:61648830-61686320	2,20E-03	211.28
ENSBTAG00000015434	ENSBTAT00000073890.1	DSTN-203	816	13:37954687-37980471	8,40E-03	204.48
ENSBTAG00000030784	ENSBTAT00000068638.1	APPL2-202	1968	5:68701355-68753242	7,15E-03	202.73
ENSBTAG00000011126	ENSBTAT00000079734.1	STK38-202	1290	23:10417307-10458908	9,50E-04	169.90
ENSBTAG00000009410	ENSBTAT00000030290.3	TBRG1-202	1436	29:28100115-28111910	1,20E-03	162.47
ENSBTAG00000014294	ENSBTAT00000079733.1	ST7L-202	1575	3:30702702-30885081	2,60E-03	154.58
ENSBTAG00000000289	ENSBTAT00000068209.1	ASB8-202	2141	5:32135752-32144532	4,00E-04	153.93
ENSBTAG00000004230	ENSBTAT00000076410.1	AIMP1-201	1611	6:18836657-19074173	2,35E-03	152.14

ENSBTAG00000020756	ENSBTAT00000081246.1	GSK3A-201	2469	18:51032201-51042278	1,55E-03	140.83
ENSBTAG00000000308	ENSBTAT00000073616.1	ITCH-202	2556	13:63784805-63885055	6,50E-03	140.18
ENSBTAG00000008026	ENSBTAT00000010554.3	OXT-201	512	13:52113968-52114975	7,30E-03	137.50
ENSBTAG00000012928	ENSBTAT00000085744.1	PPP4C-202	1421	25:26202156-26210410	3,50E-04	135.82
ENSBTAG00000009881	ENSBTAT00000013034.6	ENTPD6-202	1269	13:42738785-42757351	3,75E-03	135.33
ENSBTAG00000019454	ENSBTAT00000077111.1	ANGEL1-201	696	10:88043281-88065331	1,75E-03	130.76
ENSBTAG00000016108	ENSBTAT00000043281.4	BTBD2-201	1383	7:44279980-44302294	3,30E-03	128.01
ENSBTAG00000000305	ENSBTAT00000071074.1	LMO4-202	1353	3:56698580-56715731	2,30E-03	125.05
ENSBTAG00000021128	ENSBTAT00000028152.5	ADD1-204	2383	6:115807624-115902662	6,60E-03	117.05
ENSBTAG00000010778	ENSBTAT00000014270.4	NDUFAF1-202	1394	10:36820289-36837237	7,90E-03	116.17
ENSBTAG00000030164	ENSBTAT00000042547.4	RPL38-201	320	19:57192443-57196471	2,00E-04	114.82
ENSBTAG00000016711	ENSBTAT00000022213.2	PPIF-201	1463	28:34966649-34973641	2,40E-03	106.61
ENSBTAG00000032996	ENSBTAT00000070960.1	P4HA1-203	2198	28:29066316-29167290	1,90E-03	95.95
ENSBTAG00000019299	ENSBTAT00000068612.1	GRHPR-202	987	8:61555132-61594251	2,65E-03	16.96
ENSBTAG00000010069	ENSBTAT00000013284.6	EGR1-201	3655	7:49825960-49830306	5,00E-05	-103.77
ENSBTAG00000008832	ENSBTAT00000047653.2	CCL1-201	1516	19:15782651-15786668	4,00E-04	-117.51
ENSBTAG00000005714	ENSBTAT00000007504.6	ACTC1-201	1385	10:30288636-30294046	4,20E-03	-186.96

Cluster 1 vs. Cluster 3

Gene ID	Transcript ID	Transcript name	Length (bp)	Position	*p*-value	Log2 (FC) 1
ENSBTAG00000011338	ENSBTAT00000015066.4	NREP-201	1943	10:2212690-2245525	7.50E-04	564.53
ENSBTAG00000037644	ENSBTAT00000057323.3	HBB-201	733	15:48362235-48363999	1.00E-04	178.60
ENSBTAG00000025848	ENSBTAT00000024034.4	RBM3-202	1547	X:86690452-86694645	2.20E-03	103.52
ENSBTAG00000054808	ENSBTAT00000069619.1	MT1A-201	1714	18:24023261-24026381	9.25E-03	-10.55
ENSBTAG00000040490	ENSBTAT00000056087.3	CCDC3-201	2599	13:11479615-11570661	2.90E-03	-109.32
ENSBTAG00000009230	ENSBTAT00000012159.6	FBLN7-201	2887	11:245529-303203	8.55E-03	-109.82
ENSBTAG00000009432	ENSBTAT00000012411.4	PMF1-201	1096	3:14553829-14575605	4.90E-03	-166.27
ENSBTAG00000003027	ENSBTAT00000003937.5	EMX2-201	2163	26:37830785-37837033	4.30E-03	-216.37
ENSBTAG00000037526	ENSBTAT00000000079.4	FABP4-201	754	14:44676541-44681059	5.00E-05	-217.64
ENSBTAG00000011666	ENSBTAT00000015490.5	THRSP-201	1398	29:17989040-17994955	4.50E-04	-234.08
ENSBTAG00000019379	ENSBTAT00000025822.5	TRARG1-201	3540	19:21603446-21620221	1.00E-04	-263.98
ENSBTAG00000018073	ENSBTAT00000073517.1	TSPO-202	860	5:113927818-113940490	1.00E-03	-991.42

Cluster 2 vs. Cluster 3

Gene ID	Transcript ID	Transcript	Length (bp)	Position	*p*-value	Log10

		name				**(FC) 1**
ENSBTAG00000011808	ENSBTAT00000015674.5	MSTN-201	2997	2:6278629-6285486	8.00E-03	118.095
ENSBTAG00000023730	ENSBTAT00000000568.4	TUBB3-201	1831	18:14708939-14717352	1.90E-03	-11.13
ENSBTAG00000037526	ENSBTAT00000000079.4	FABP4-201	754	14:44676541-44681059	3.70E-03	-15.40
ENSBTAG00000011666	ENSBTAT00000015490.5	THRSP-201	1398	29:17989040-17994955	5.00E-05	-20.72
ENSBTAG00000016895	ENSBTAT00000079943.1	NIPA2-205	1349	2:1044597-1064844	9.35E-03	-107.01
ENSBTAG00000005385	ENSBTAT00000007083.4	POP5-202	1418	17:62851493-62854683	7.95E-03	-122.47
ENSBTAG00000016711	ENSBTAT00000022213.2	PPIF-201	1463	28:34966649-34973641	1.05E-03	-129.95
ENSBTAG00000012928	ENSBTAT00000085744.1	PPP4C-202	1421	25:26202156-26210410	3.20E-03	-133.28
ENSBTAG00000038126	ENSBTAT00000068851.1	WIPF2-204	1168	19:40490707-40546357	8.55E-03	-137.31
ENSBTAG00000018735	ENSBTAT00000075553.1	C13H20orf27-202	1227	13:51430649-51443752	2.60E-03	-138.60
ENSBTAG00000053048	ENSBTAT00000080180.1	-	195	24:14321376-14321571	4.60E-03	-149.51
ENSBTAG00000000289	ENSBTAT00000068209.1	ASB8-202	2141	5:32135752-32144532	6.30E-03	-150.99
ENSBTAG00000004230	ENSBTAT00000076410.1	AIMP1-201	1611	6:18836657-19074173	6.75E-03	-153.12
ENSBTAG00000003530	ENSBTAT00000074495.1	DDX31-203	2187	11:102659359-102733293	2.15E-03	-22323
ENSBTAG00000019454	ENSBTAT00000077111.1	ANGEL1-201	696	10:88043281-88065331	8.65E-03	-464.46
ENSBTAG00000023831	ENSBTAT00000084764.1	ELP4-204	1261	15:62296510-62553952	7.10E-03	-602.85
ENSBTAG00000049062	ENSBTAT00000078807.1	-	273	17:72990819-72995082	9.50E-03	-779.03
ENSBTAG00000052681	ENSBTAT00000067953.1	SMIM10L1-201	207	5:98568748-98571841	8.40E-03	-845.20

[1] Log2(FC) = Fold change (log2)

3.3. Differentially expressed novel transcript lengths associated with genes annotated in the ARS.UCD1.2 genome reference

Differentially expressed novel transcript lengths of annotated genes in the bovine genome (ARS.UCD1.2) were identified for the three fatty acid profiles comparisons (C1 vs. C2, C1 vs. C3 and C2 vs. C3) Table 3. For the C1 vs. C2, seven novel transcript lengths were up-regulated in C1 compared to C2. These transcripts were associated with genes related to mitochondrial protein import and activity and amyloid e.g., *DNAJC19* (DnaJ Heat Shock Protein Family (Hsp40) Member C19), *MRPS36* (Mitochondrial Ribosomal Protein S36) and *APBB2* (Amyloid Beta Precursor Protein Binding Family B Member 2). In addition, four down-regulated novel transcript lengths were found for this comparison (Table 3), which were associated with genes related to regulation of adipogenesis, such as *CDS1* (CDP-Diacylglycerol Synthase 1), *SERPINE1* (Serpin Family E Member 1) and *FOSB* (FosB Proto-Oncogene, AP-1 Transcription Factor Subunit).

For the C1 vs. C3, four up-regulated novel transcript lengths were found when compared C1 with C3 cluster (Table 3). These transcripts were related to genes associated with cystoskeletal protein and insulin-like growth factor binding protein and transmembrane domain proteins, e.g. *MYO1F* (Myosin IF) and *IGFBP5* (Insulin Like Growth Factor Binding Protein 5). While nine down-regulated novel transcripts length were found for this comparison (Table 3), those transcripts were associated with genes related to fat specific protein, adiponectin, matrix-remodeling-associated protein, homeostasis and deubiquitinating enzyme, e.g. *CIDEC* (Cell Death Inducing DFFA Like Effector C), *ADIPOQ* (Adiponectin, C1Q and Collagen Domain Containing), *MXRA5* (Matrix Remodeling Associated 5), *THBS1* (Thrombospondin 1) and *OTUD1* (OTU Deubiquitinase 1).

For the C2 vs. C3, two up-regulated novel transcript lengths were found when compared C2 with C3 cluster (Table 3). These transcripts were related to genes associated with Ca2+ mobilization (*ZNF593*: Zinc Finger Protein 593 2) and transcription regulation by RNA polymerase II (*BANK1*: B Cell Scaffold Protein with Ankyrin Repeats 1). Five down-regulated novel transcript length were related to muscle growth (KY: Kyphoscoliosis Peptidase), lysosome localization (ENSBTAG00000025283: KXD1, KxDL motif containing 1), mitochondria (UQCC3: Ubiquinol-Citocromo C Redutase Complexo Fator de Montagem 3), fat specific protein (CIDEC) and homeostasis (THBS1) (Table 3).

Table 3. Differentially expressed novel transcript length associated with associated with annotated genes in the *Longissimus thoracis* muscle of Nellore cattle with different fatty acids profile.

Cluster 1 vs. Cluster 2						
Transcript ID	**Gene ID**	**Gene name**	**Length (bp)**	**Position**	***p*-value**	**Log2(FC)**[1]
TMC4_mRNA1	ENSBTAG00000020062	TMC4	276	18:63227988-63238070	1.00E-04	586.67
TTLL1_mRNA1	ENSBTAG00000012030	TTLL1	1277	5:113827071-113903956	9.60E-03	461.12
CABCOCO_mRNA1	ENSBTAG00000010967	CABCOCO	1116	28:17588045-17759708	3.30E-03	430.07
APBB2_mRNA1	ENSBTAG00000027569	APBB2	4519	6:59711757-60095904	1.50E-03	364.97
UQCC3_mRNA1	ENSBTAG00000010470	UQCC3	1565	29:41001415-41003100	4.00E-04	158.23
DNAJC19_mRNA1	ENSBTAG00000023513	DNAJC19	1023	1:86035423-86041190	2.95E-03	151.85
MRPS36_mRNA1	ENSBTAG00000011045	MRPS36	1832	20:10466110-10475928	2.75E-03	128.75
CDS1_mRNA1	ENSBTAG00000045787	CDS1	4422	6:99411737-99491688	2.45E-03	-12.89
FOSB_mRNA1	ENSBTAG00000008182	FOSB	7113	18:53073568-53081311	6.40E-03	-16.16
SERPINE1_mRNA1	ENSBTAG00000014465	SERPINE1	3216	25:35596137-35617193	4.50E-04	-105.97
HMGN4_mRNA1	ENSBTAG00000031747	HMGN4	437	23:31562751-31573268	8.30E-03	-813.25
Cluster 1 vs. Cluster 3						
Transcript ID	**Gene ID**	**Gene name**	**Length (bp)**	**Position**	***p*-value**	**Log2 (FC)**[1]
TDRD7_mRNA1	ENSBTAG00000003719	TDRD7	1543	8:62612937-62715815	3.40E-03	147.49
IGFBP5_mRNA1	ENSBTAG00000054218	IGFBP5	5028	2:104656175-104680014	3.55E-03	112.12
MYO1F_mRNA1	ENSBTAG00000007661	MYO1F	9209	7:17119428-17157724	9.00E-04	111.28
FZD8_mRNA1	ENSBTAG00000052989	FZD8	4586	13:26783496-26788082	3.50E-04	103.25
THBS1_mRNA1	ENSBTAG00000002006	THBS1	5772	10:35209572-35225348	6.50E-04	-100.87
OTUD1_mRNA1	ENSBTAG00000047752	OTUD1	4207	13:24387530-24392096	7.00E-04	-103.12
ALCAM_mRNA1	ENSBTAG00000000088	ALCAM	4899	1:49930774-50162039	4.25E-03	-106.29
KLHL30_mRNA1	ENSBTAG00000053361	KLHL30	6637	3:117472265-117488380	3.10E-03	-106.62
GLI4_mRNA1	ENSBTAG00000003606	GLI4	1458	14:1323798-1335465	8.35E-03	-109.09
MXRA5_mRNA1	ENSBTAG00000022150	MXRA5	10175	X:138515673-138541754	5.70E-03	-154.43

ADIPOQ_mRNA1	ENSBTAG00000052213	ADIPOQ	5174	1:80411744-80427813	5.00E-04	-205.52
CIDEC_mRNA1	ENSBTAG00000007969	CIDEC	1183	22:16864533-16873831	2.20E-03	-317.74
SERF2_mRNA1	ENSBTAG00000047694	SERF2	2964	21:55410997-55421853	2.35E-03	-609.99
Cluster 2 vs. Cluster 3						
Transcript ID	**Gene ID**	**Gene name**	**Length (bp)**	**Position**	***p*-value**	**Log2 (FC)** [1]
ZNF593_mRNA1	ENSBTAG00000009562	ZNF593	390	2:126929532-126930613	8.95E-03	430.51
BANK1_mRNA1	ENSBTAG00000015297	BANK1	1476	6:22694864-23016141	1.45E-03	139.24
ENSBTAG00000025283_mRNA1	ENSBTAG00000025283	-	1324	18:14929519-14930843	4.75E-03	-14.06
KY_mRNA1	ENSBTAG00000051206	KY	5517	1:134699503-134745978	6.85E-03	-120.22
UQCC3_mRNA1	ENSBTAG00000010470	UQCC3	1565	29:41001415-41003100	2.35E-03	-152.98
CIDEC_mRNA1	ENSBTAG00000007969	CIDEC	1183	22:16864533-16873831	6.80E-03	-305.76
TMC4_mRNA1	ENSBTAG00000020062	TMC4	276	18:63227988-63238070	2.30E-03	-539.68

[1] Log2(FC) = Fold change (log2).

3.4. Annotation of novel differentially expressed transcript lengths associated with non-annotated genes

Novel transcript lengths associated with non-annotated genes are shown in Table 4. A total of 7, 1 and 4 novel transcripts associated with non-annotated genes were identified in the C1 vs. C2, C1 vs. C3 and C2 vs. C3 comparisons, respectively. Furthermore, it was noted that these transcripts exhibited lengths ranging from 266 to 6680 bp. The newly identified mRNA isoforms were subjected to sequence comparison against the nucleotide collection using the BLAST tool (NCBI, 2023). The degree of similarity observed between them ranged from 96.32% to 100% in three comparisons, representing the highest level of identity for each transcript when aligned to segments of the same subject sequence.

In C2 vs. C3, the novel differentially expressed transcripts *26759_novelmRNA* were downregulated in C2 compared to C3. This novel mRNA presented 100% of similarity with ADRB2 (Bos taurus adrenoceptor beta 2) mRNA. The *ADRB2* (Adrenoceptor Beta 2) gene encodes receptor which is a member of the G protein-coupled receptor superfamily and is involved in the regulation of energy expenditure and lipolysis in adipose tissue.

Table 4. Novel differentially expressed transcripts (*p*-value < 0.01; FC > | 2 |) associated with unannotated genes in the *Longissimus thoracis* muscle of Nellore cattle with different fatty acids profile.

Cluster 1 vs. Cluster 2

mRNA ID[1]	mRNA Length (bp)	Position	Log2 (FC)[2]	*p*-value	Locus Description*	Cover[3] (%)	E-value	Identity[4] (%)	Pred. gene Accession[5]	Protein Association
4456_novelmRNA	1041	12:65399858-65401320	602.01	0.0086	Bos taurus strain mammals genome assembly, chromosome: 12	100	0	100	OX344701.1	*PAOX*
29662_novelmRNA	1419	9:103333517-103342412	311.46	0.0074	Bos taurus genome assembly, chromosome: 25	3	4,00E-119	96.32	LR962881.1	*TM9SF5*
17100_novelmRNA	496	23:24712271-24712767	305.89	0.0026	Bos taurus genome assembly, chromosome: 23	100	0	99.81	LR962754.1	-
23525_novelmRNA	1066	4:59577806-59578872	253.19	0.0061	Bos taurus genome assembly, chromosome: Y	9	1e-27	90.48	LR962769.1	-
15338_novelmRNA	358	21:27228902-27229260	127.35	0.0020	Bos gaurus x Bos taurus genome assembly, chromosome: 21	100	0	100	OX258975.1	*NSA2*
1195_novelmRNA	266	1:6042883-6043149	102.71	0.0098	Bos taurus strain mammals genome assembly, chromosome: 29	98	1e-127	98.11	OX344718.1	-
2615_novelmRNA	613	10:86487878-86488491	-12.01	0.0079	Bos mutus isolate yakQH1 chromosome 10	79	0	98.78	CP027078.1	-

Cluster 1 vs. Cluster 3

mRNA ID[1]	mRNA Length (bp)	Position	Log2 (FC)[2]	*p*-value	Locus Description*	Cover[3] (%)	E-value	Identity[4] (%)	Pred. gene Accession[5]	Protein Association
11742_novelmRNA	6680	19:57313997-57323226	-122.44	0.0020	Bos taurus genome assembly, chromosome: 19	100	0	99.23	LR962749.1	-

Cluster 2 vs. Cluster 3

mRNA ID[1]	mRNA Length (bp)	Position	Log2 (FC)[2]	*p*-value	Locus Description*	Cover[3] (%)	E-value	Identity[4] (%)	Pred. gene Accession [5]	Protein Association

15338_novelmRNA	358	21:27228902-27229260	-125.99	0.0056	Bos gaurus x Bos taurus genome assembly, chromosome: 21	100	0	100	OX258975.1	*NSA2*
26759_novelmRNA	1896	7:60232675-60271234	-358.83	0.0079	Bos taurus adrenoceptor beta 2 (ADRB2), mRNA	5	0	100	NM_174231.1	*ADRB2*
14776_novelmRNA	1353	21:60379945-60383584	-622.96	0.0074	Bos taurus genome assembly, chromosome: 21	100	0	99.78	LR962877.1	-
2548_novelmRNA	527	10:20961295-20961822	-401.34	0.0026	Bos taurus transmembrane 9 superfamily member 1 (TM9SF1), transcript variant X3, mRNA	100	0	100	XM_024997743.2	*TM9SF1*

[1] mRNA ID = code given for new transcripts

[2] Log2(FC) = Fold change (log2).

[3] Cover = the percent of the query length that is included in the aligned segments.

[4] Identify = the highest percent identity for a set of aligned segments to the same subject sequence.

[5] Accession number of predicted gene.

*Locus description is the best match in BLAST using GenBank and RefSeq database.

3.5. Overlapping mRNA isoforms abundance identified among three comparisons (C1 vs. C2, C1 vs. C3 and C2 vs. C3) of three FA profiles

Comparing the results obtained in the three comparisons (C1 vs. C2, C1 vs. C3 and C2 vs. C3; Figure 3) were observed a connectivity of gene and biological function related to mechanism associated to FA. Unique genes were identified in each differential expression analyses; however, 14 genes were common identified in at least two comparisons, which were mRNA directly related to FA. Among these, were highlight genes between C1 vs. C3 and C2 vs. C3 comparisons (n=3) (Table 2 and 3), the mRNAs isoforms *THRSP-201*, *FABP4-201* and novel transcript length of annotated gene *CIDEC_mRNA1* were downregulated considering the C1 and C2 in relation to C3.

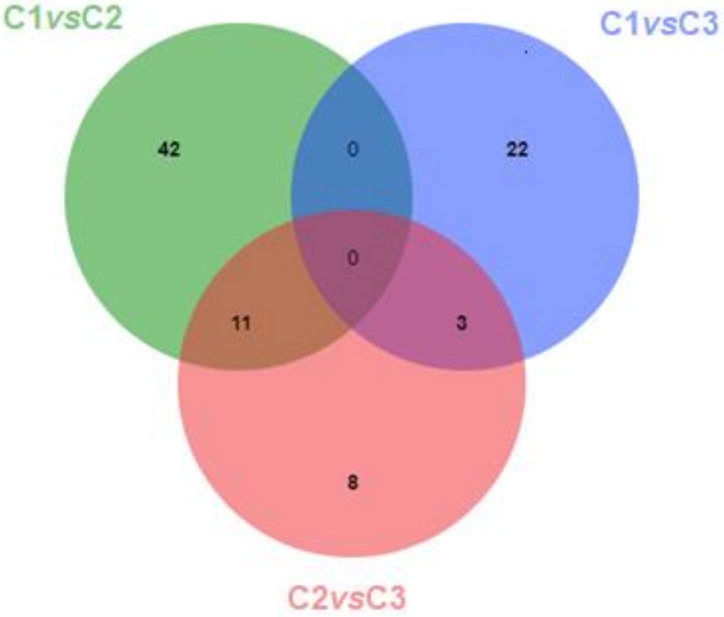

Figure 3. Venn diagram analyses showed differentially expressed mRNA isoforms commonly identified among three different fatty acid profile in beef cattle comparison (Cluster 1 (C1), Cluster 2 (C2) and cluster 3 (C3).

3.6. Functional enrichment analysis

For functional analysis, a list of genes related to the differentially expressed (DE) mRNAs isoforms, identified for the three comparisons (C1 vs. C2, C1 vs. C3, and C2 vs. C3) was used to perform functional enrichment analysis, as shown in Supplementary Tables S1, S2, and S3, respectively. For the C1 vs. C2, 102 significant GO terms (p-value < 0.05), including 5 to molecular function (MF), 28 biological process (BP), and 9 cellular component (CC) terms were identified (Supplementary Table S1 e Figure 4). The most significant biological process terms were associated with regulation of leukotriene production involved in inflammatory response (GO:0035490), arachidonic acid metabolite production involved in inflammatory response (GO:0002538), regulation of lipopolysaccharide-mediated signaling pathway (GO:0031664), interleukin-1 (GO:0032612) and interleukin-8 (GO:0032637).

For the C1 vs. C3, 80 significant GO terms, including 13 molecular function (MF), 60 biological process (BP), and 8cellular component (CC) terms were identified (Supplementary Table S2, Figure 5). The most significant biological process terms were associated with muscle cell proliferation (GO:0033002), regulation of lipid localization (GO:1905952), positive regulation of hemostasis (GO:1900048), oxygen binding (GO:001982), fatty acid binding (GO:000550) and interleukin-12 production (GO:0032615).

For the C2 vs. C3, 50 significant GO terms were found, being 6 molecular function (MF), 30 biological process (BP), and 14 cellular component (CC) terms, (Supplementary Table S3 e Figure 6). The most significant biological process terms were associated with regulation of cellular response to insulin stimulus (GO:1900076), muscle adaptation (GO:0043500), muscle cell cellular homeostasis (GO:0046716) and fatty acid binding (GO:000550).

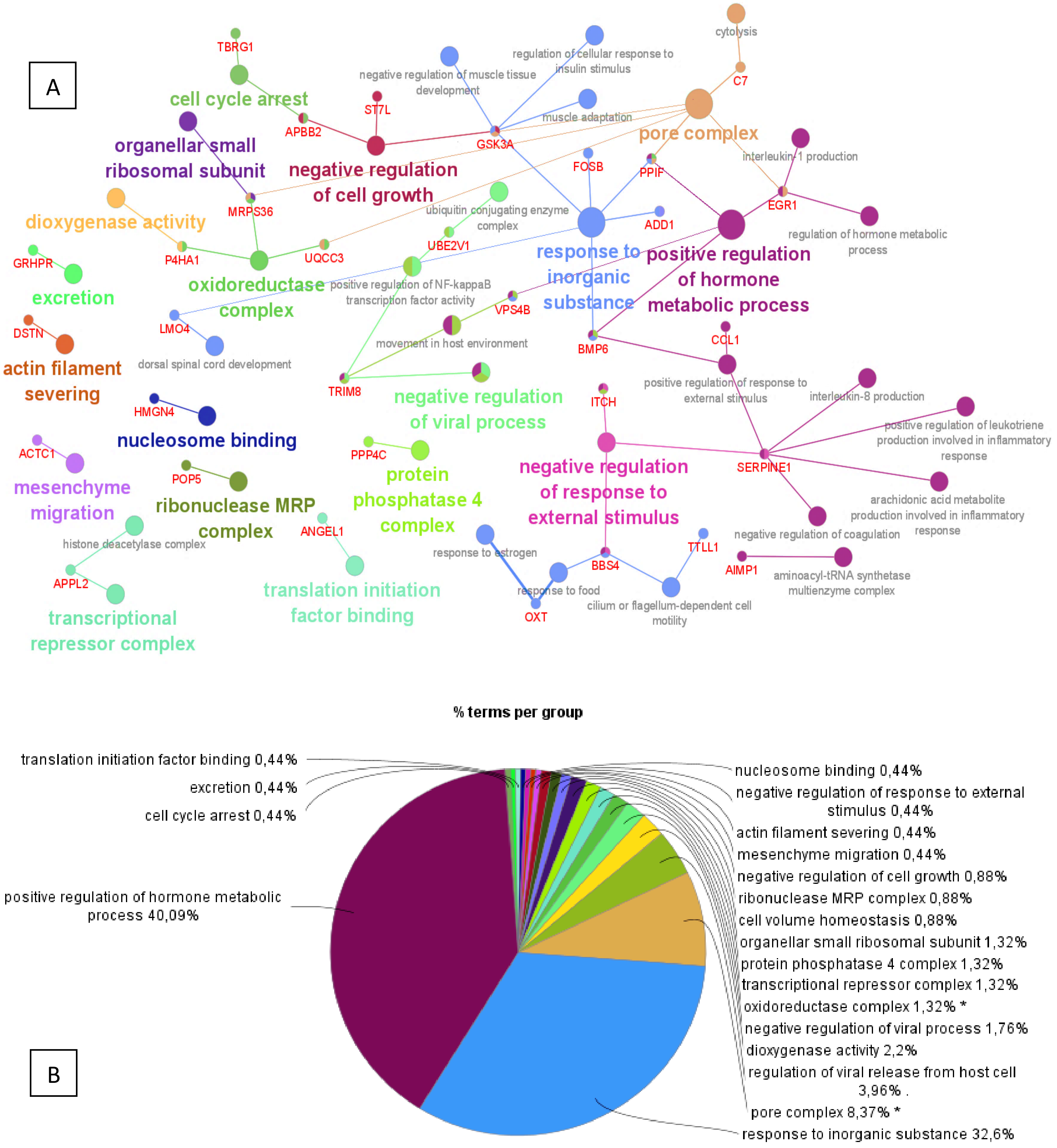

Figure 4. Functional enrichment analysis for genes associated with differentially expressed mRNAs identified in the C1 vs. C2 comparasion . (A) Network view for GO terms corresponding to C1 vs. C2 genes. (B) Functional groups represented in a piechart, the proportion of each group is calculated based on the number of the terms included in the group.

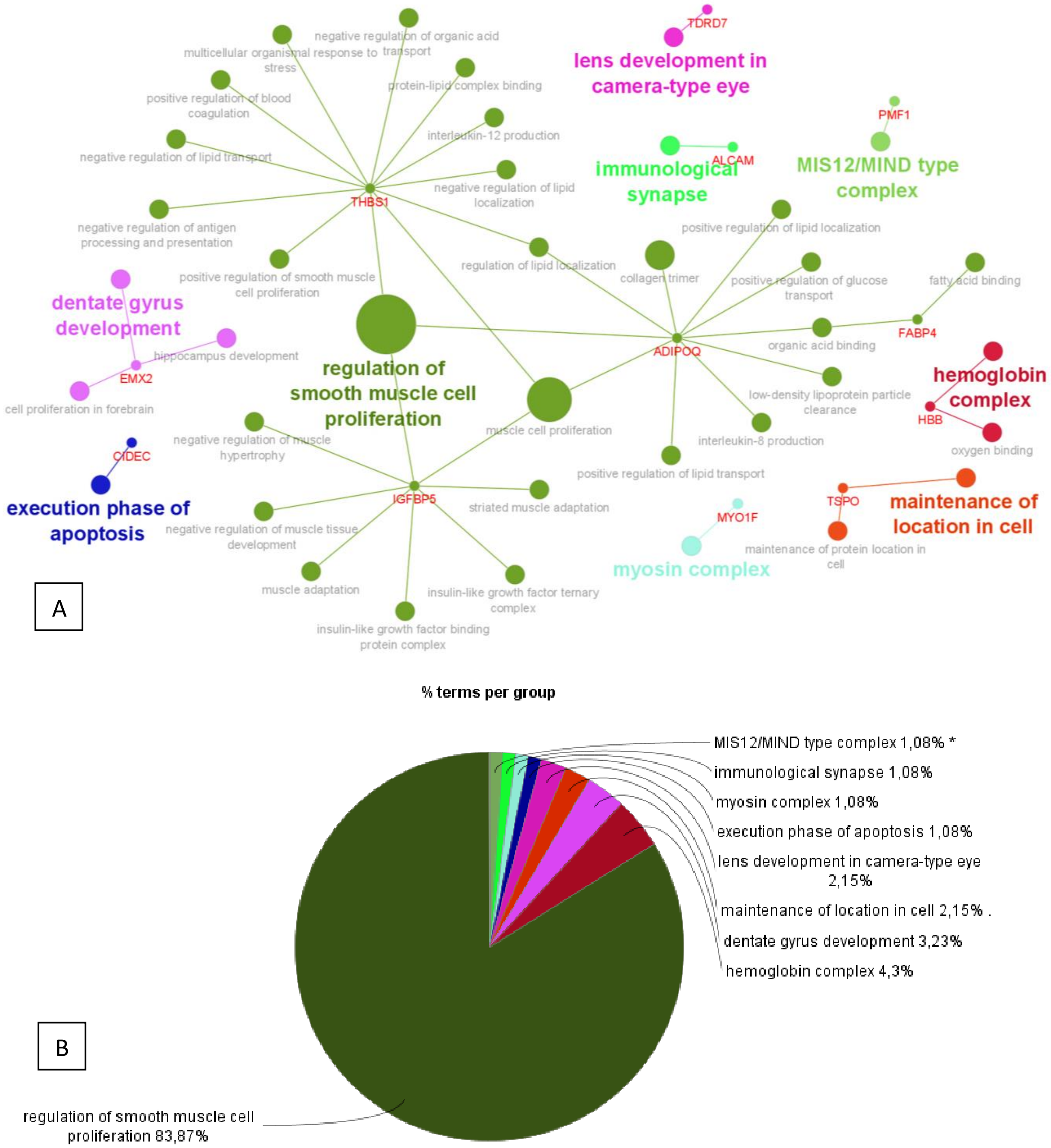

Figure 5. Functional enrichment analysis for genes associated with differentially expressed mRNAs identified in the C1 vs. C3 comparasion. (A) Network view for GO terms corresponding

to C1 vs. C3 genes. (B) Functional groups represented in a piechart, the proportion of each group is calculated based on the number of the terms included in the group.

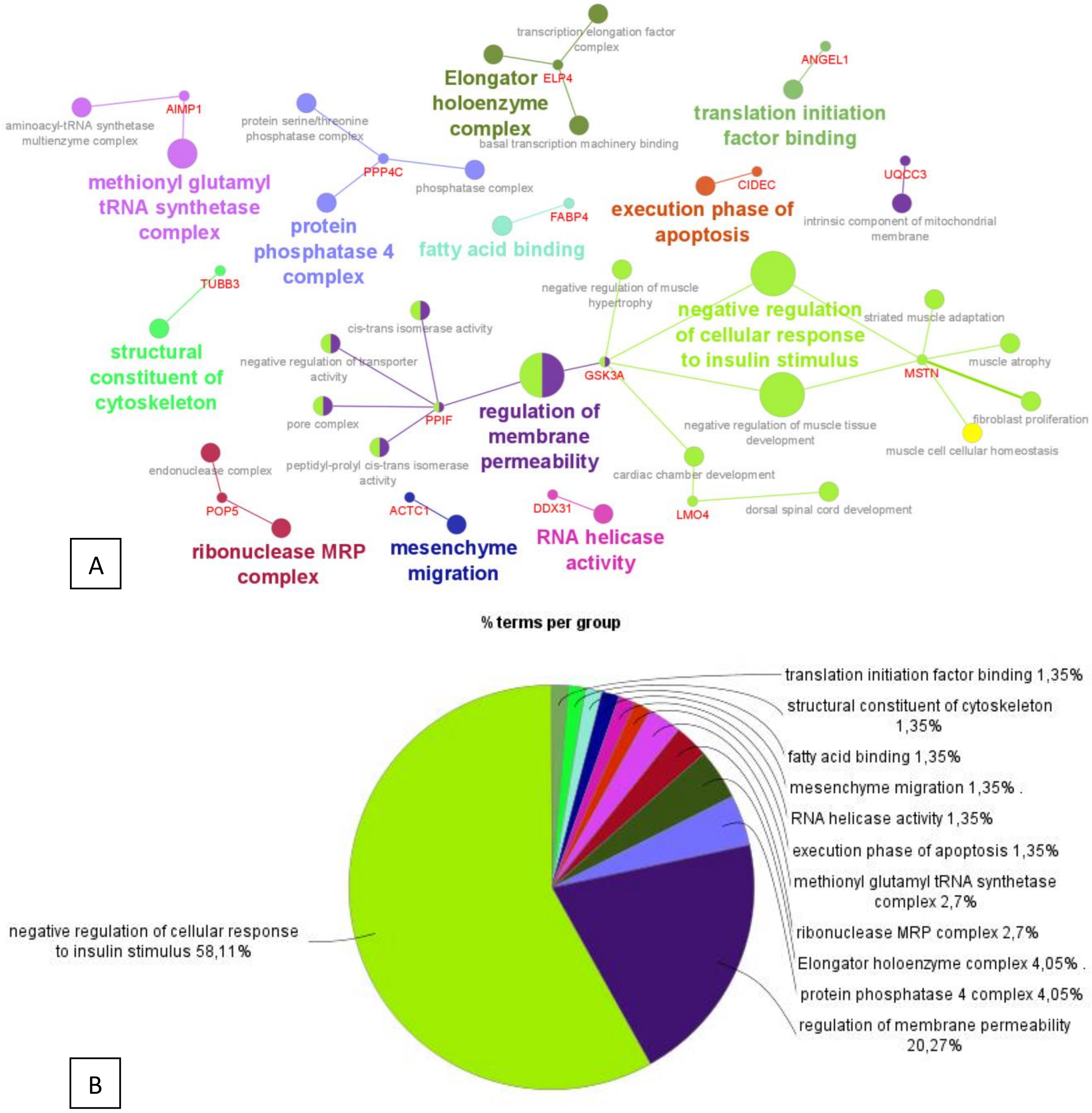

Figure 6. Functional enrichment analysis for genes associated with differentially expressed mRNAs identified in the C2 vs. C3 comparasion. (A) Network view for GO terms corresponding to C2 vs. C3 genes. (B) Functional groups represented in a piechart, the proportion of each group is calculated based on the number of the terms included in the group.

4. DISCUSSION

Gene expression analysis has proven to be a promising strategy for identifying candidate genes associated with economically important traits (BERTON et al., 2016; CESAR et al., 2015; SCHETTINI et al., 2022a). The RNA-Seq, a precise and reliable technique for transcriptomic analyses and has been efficient in quantifying mRNA isoforms (MADSEN et al., 2015; MUNIZ et al., 2021) was used to compare the FA clusters among them (C1 vs. C2, C1 vs. C3, and C2 vs. C3).

For the C1 vs. C2 comparison, the *C7-203* (complement component C7), *BBS4-202* (bardet-biedl syndrome 4), *OXT-201* (oxytocin-neurophysin 1), *ACTC1-201* (actin alpha cardiac muscle 1) and *ADD1-204* (adducin 1) were DE in the LT muscle of animals in the C1 compared to C2. The C7 gene is related to the membrane attack complex and plays a crucial role in the innate immune response by assisting in inflammatory responses against infections (Würzner, 2003). The ω3 acids exert their influence on the activation of inflammatory cellular processes, spanning from signal transduction to protein expression (GRIMM et al., 2002). Our study showed that C1 has high levels of ω6, ω3, linoleic and alpha-linolenic in relation to C2, and found a isoform of this gene (*C7-203*) upregulated in animals from C1, suggesting a relationship between *C7-203* isoform and alpha-linolenic acid concentration in Nelore beef, which also corroborates with ROVADOSCKI et al. (2018) that reported the association of C7 gene with increase in alpha-linolenic acid (ω3) deposition in sheep.

The *BBS4* gene modulates the expression of molecular markers of proliferation and adipocyte differentiation, also playing a role in fatty acid profile, lipolysis, and fat accumulation (AKSANOV et al., 2014; PRIETO-ECHAGÜE et al., 2020). A GWAS study in American Angus Cattle has associated the SNP of this gene with long-chain fatty acids and medium-chain fatty acids (DAWOOD et al., 2021). Our results corroborate with these authors and suggests that upregulation of *BBS4-204* may be directly associated with high PUFA concentration.

The *OXT* gene is responsible for encoding oxytocin, which has multiple functions such lipid storage, energy expenditure, and processes like as lipolysis and secretory activity (SAUSVILLE et al., 1985; Fonseca-Alaniz *et al*., 2006). Fonseca et al., (2020) reported that *OXT* gene was upregulated in low-marbling Nellore beef cattle. Zhang et al., (2017) observed that animals with more PUFA concentration, lower intramuscular fat presented a higher rate of metabolism for lipolysis in Yunling and Chinese Simmental breeds. In this work, we found that

OXT-201 isoform is upregulated in the group with high PUFA concentration agreeing with these previous results.

The *ACTC1* gene is related to the development and muscle growth and meat quality traits in cattle (BERTOLA et al., 2008; BONGIORNI et al., 2016). Schettini et al. (2022) studying the transcriptome profile in Nellore cattle according to the fatty acid content, found the *ACTC1* was downregulated in the high polyunsaturated fatty acid (PUFA) group, our studies corroborated with these authors, where we found *ACTC1-201* isoform downregulated in C1 animals which has high PUFA concentration.

The *ADD1* gene, also called *SREBP1*, regulates the expression of several key genes involved in fatty acid and triglyceride metabolism in adipocytes of mouse and humans (BROWN et al., 1997; HUA et al., 1993). This agrees with results from Shimano (2017) and Hishikawa et al., (2020) indicating that this gene controls the expression of genes encoding enzymes involved in de novo fatty acid synthesis and PUFA production.

The *APBB2* gene encodes a protein that interacts with the cytoplasmic domains of the beta-amyloid precursor protein (A4). Both A4 and the amyloid precursor protein are present in adipocytes (LEE et al., 2008) increasing the release of free fatty acids and pro-inflammatory adipokines (WAN et al., 2015). Additionally, studies have indicated that the A4 polymer exhibits moderate binding affinity to the stearic acid (AVDULOV et al., 1997). The *APBB2* gene was indicated as a potential candidate for stearic acid content in muscle and adipose tissue in pigs (LEE et al., 2018; SHIMANO et al., 2017). In this study, we observed that *APBB2* was upregulation for C1 compared with C2 FA profile; however, no significant differences were found between the groups regarding stearic acid content. Further investigations are necessary to better understanding of the regulatory mechanisms underlying *APBB2* and its potential impact on the fatty acid profile.

The ubiquitin ligases *TRIM8* (tripartite motif containing 8), *ITCH* (itchy e3 ubiquitin protein ligase), and *BTBD2* (BTB domain containing 2), along with the conjugating enzyme *UBE2V1* (ubiquitin conjugating enzyme e2 v1), play a key role in the regulation of protein degradation (ATTAIX et al., 2005). *ITCH* gene is known for its role in immunity and inflammation and involved in lipid metabolism (MARINO et al., 2014; STÖHR et al., 2015), while positive regulation of the *UBE2V1* gene associated with increased levels of ω3 fatty acids in humans (VEDIN et al., 2012). In this study, *UBE2V1* and *TRIM8* gene was related to GO term such as positive regulation of NF-kappaB transcription factor activity (GO:0051092), the NF-kappaB is essential in regulating the immune response and inflammation (Grigoriadis et al., 1996). The ITCH gene, by positively regulating the activity of NF-kappaB, enhances the immune response and the activation of genes associated with inflammation (Ajibade et al., 2013; Liu et al., 2017; Saito et al., 2022). Isoforms *TRIM8-202, ITCH -202, BTBD2-201* and *UBE2V1-202* were upregulated considering the C1, cluster with higher PUFA, ω6, ω3, linoleic and alpha-linolenic content. Our results corroborate with studies suggesting a relationship between ω3 and alpha-linolenic fatty acids and regulation of NF-κB signaling pathway (Brown et al., 2020; Feng et al., 2021; Zhao et al., 2005). However, it is important to note that research on the specific role of these ubiquitin ligases in fatty acids metabolism is still ongoing.

In the C1 vs. C2 comparison, the *EGR1-201* (early growth response 1), *FOSB_mRNA1* (AP-1 transcription factor subunit), and *SERPINE1_mRNA1* (serpin family E member 1) mRNA isoforms were downregulated in C1, which presented low miristic acid, palmitic acid and MUFA content, in relation to C2. There are key genes involved in the regulation of lipid metabolism (HUANG et al., 2017). High amount of SFA was related to postprandial upregulation of genes associated with pro-inflammatory pathways in peripheral blood mononuclear cells in comparison with MUFA or PUFA (CALDER et al., 2011; CAO et al., 2008; SILVEIRA et al., 2008).The *EGR1* gene may influence adipocyte functions by improving insulin resistance in the body through the regulation of the PI3K/Akt and Erk/MAPK signaling balance, as well as the expression of pro-inflammatory adipokines such as tumor necrosis factor-α (TNF-α) and *IL6* in adipose tissue (YU et al., 2011). Additionally, *SERPINE1* is a pro-inflammatory protein, expression of which is increased in adipose tissue of obese animals and is used as a marker of adipose tissue inflammation (WEISBERG et al., 2003). In our findings, *SERPINE1* gene was associated with gene ontology (GO) terms related to the immune system, such as the production of molecular mediator involved in inflammatory response (GO:0002532) and interleukin-8 production (GO:0032637). These findings justify the downregulation of *SERPINE1_mRNA1* and *EGR1-201* isoforms for low SFA. Furthermore, multiple studies indicate that the induction and expression of the *AP-1* (*FOSB*) transcription factor play a crucial role in adipocyte differentiation in murine models (KVEIBORG et al., 2004; LUTHER et al., 2011, 2014). A transcriptomic study in Wagyu cattle, the FOSB gene was up-regulated in subcutaneous adipose tissue, and associated with lipid metabolism and adipogenesis in beef cattle (HUANG et al., 2017). These findings may suggest the involvement of the *FOSB_ mRNA1* isoform in the FA concentration.

For the C1 vs. C3 comparison, *RBM3-202* (RNA binding motif protein 3) mRNA isoform was upregulated in the LT muscle of animals in C1 cluster whose have high PUFA, ω6, ω3, linoleic and alpha-linolenic content (Cluster 1). The upregulation of these mRNA isoforms may contribute to high PUFA content. RBM3 is an RNA-binding and cold-shock protein expressed in muscle and associated with prolonged lifespan and maintenance of protein synthesis. RNA-binding proteins are known to regulate subsets of genes involved in RNA processing, lipid, beta-oxidation of fatty acids and mitochondrial metabolism (DEY et al., 2023). Dey et al., (2023) reported that *RBM3* may cause increased fatty acid mobilization that correlates with decreased triglyceride levels. Among these fatty acids we can highlight n-3 PUFA, several studies have associated those with decrease of triglycerides in humans (COVINGTON et al., 2004; TRUCHIS et al., 2007; WANG et al., 2023; BOSOMWORTH, 2008).

For the C1 vs. C3 comparison, mRNA isoforms of *TRARG1-202* (trafficking regulator of *GLUT4-1 (SLC2A4)*) and *ADIPOQ_mRNA1* (adiponectin, C1Q and collagen domain containing) were downregulated in C1 in relation C3. The C1 presented high ω3 and, ω6 and low MUFA content, these results corroborated with studies showing an association between *TRARG1* gene and FA concentration. In cattle, *TRARG1* gene regulates fat development through cell proliferation and adipocyte differentiation (ZHANG et al., 2022). Valdés-Hernández et al., (2023) evaluated the FA profile in porcine muscle and observed a positive correlation between *TRARG1* gene and MUFA content (VALDÉS-HERNÁNDEZ et al., 2023). The *TRARG1* gene is involved in adipose tissue glucose uptake in bovine (KEOGH et al., 2023). The *ADIPOQ_mRNA1* participate directly in the metabolic pathways related to FAs production and in FAs oxidation and beta-oxidation (TANG et al., 2018). In this study, *ADIPOQ_mRNA1* was enriched in the regulation of lipid localization (GO:1905952), positive regulation of lipid transport (GO:0032370). Our results corroborate a previous study reported by Berton et al. (2016) observed that the *ADIPOQ_mRNA1* gene was downregulated in animals with high ω3 and ω6 content, as well were found in ours results.

Common mRNA isoforms were found for the comparisons C1 vs. C2 and C2 vs. C3 (*THRSP-201*, *CIDEC_mRNA1* and *FABP4_mRNA1*). The mRNA isoform of *THRSP-201* (thyroid hormone responsive protein) was downregulated in the group with had higher ω3, ω6 and low MUFA, oleic acid, SFA content (C1 vs. C3); and low SFA (C2 vs. C3). The *THRSP* gene encodes a protein S14 (ZHU et al., 2005). S14 is expressed exclusively in lipogenic tissues and is correlated with the lipogenic rate (JUMP et al., 1985). A SNP identification study performed in

Korean cattle, observed that polymorphism in THRSP gene were associated with high MUFA content (OH et al., 2014). Saatchi et al. (2013), in a SNP study reported that *THRSP* is a candidate gene for stearic, miristic, palmitic and oleic fatty acids in Angus. In this study, we observed that the THRSP-201 mRNA isoform was downregulated in groups with a higher concentration of PUFA and MUFA (C1 vs. C3; FC= -234.0) and (C2 vs. C3; FC = -20.7), suggesting that the suppression of THRSP-201 is directly related with an increasing concentration of total PUFA, ω3, ω6 and decreasing in total MUFA, oleic acid and SFA content.

The mRNA isoforms of *CIDEC_mRNA1* (Cell Death Activator CIDE-3) and *FABP4-201* (fatty acid binding protein 4) were downregulated in C1 and C2 in relation to C3, those cluster had higher PUFA, ω3, ω6 and low MUFA, oleic acid, SFA content (C1 vs. C3); and higher PUFA, ω3, ω6 and low SFA (C2 vs. C3). The *CIDEC* is a novel lipid droplet-associated protein and contributes to the accumulation of triacylglycerol, expressed in adipose tissue and involved in lipid metabolism (inhibition of lipolysis, regulation of lipid storage and motility of lipid droplet) (SU et al., 2002; YU et al., 2003; MARTINS et al., 2020). A study observed that ω3 lessens the expression of *CIDEC*, reducing adipogenesis in adipocyte cells (Martins et al., 2020). Zhang et al., (2021) in study gene expression reported that *CIDEC* had positive correlation with the proportion of oleic acid and long-chain fatty acids in bovine mammary gland. In this sense, the negative regulation of the mRNA isoform of *CIDEC_mRNA1* for both comparisons (C1 and C2) are associated with a healthier genetic profile. The FABP4 is a protein predominantly expressed in adipocytes, where it regulates lipolysis and fatty acids storage and is an essential mediator of inflammation (FURUHASHI et al., 2015; FLORESTA et l., 2022) and was enriched in the fatty acid binding (GO:0005504) in this study. In macrophages, an increase in the inflammatory response induced by palmitic acid was observed, leading to an increase in the expression of *FABP4* (Korbercki et al., 2019). In Nellore cattle, the *FABP4* gene it was downregulated in animals with high for ω3 and ω6 concentration (BERTON et al., 2016). We detected variability in *FABP4-201* isoform expression levels among both C1 vs. C3 (-217.6 FC) and C2 vs. C3 (-15.4 FC) (Table 2), suggesting negative gene expression contributes to higher concentration of total PUFA, ω3 and ω6 and lower palmitic acid, MUFA and oleic acid content.

The enrichment analyses revealed gene ontology (GO) terms related to insulin in the C2 vs. C3 comparison, where 58,11% of the identified GO terms were associated with negative regulation of cellular response to insulin stimulus (GO:1900077) (Figure 6). The SFA can inhibit the activation of insulin receptor substrate-1, protein kinase B or phosphatidylinisitol-3-kinase in adipocytes, causing reduced insulin sensitivity of muscle cells, stimulating the secretion of inflammatory cytokines (KENNED et al., 2009). Studies have shown that SFAs can promote insulin resistance (VERAS et al., 2023; DEER et al., 2015; MIN et al., 2018; YANG et al., 2015; Robertson et al., 2002), corroborating our results. Our results corroborated with these studies, showing that animals in C2 and C3 exhibit a high SFA, including stearic acid, myristic acid and palmitic acid in relation to C1, and the genes identified in their comparison were related to negative regulation of cellular response to insulin stimulus GO terms.

In summary, some genes identified in this study corroborate previous research that investigated the fatty acid profile (*ADIPOQ*, *FABP4* and *ACTC1*) and their correlation with traits such as intramuscular fat and marbling in cattle (Zhang et al., 2017; Fonseca et al., 2020). Furthermore, were identified mRNA isoforms of genes (*RBM3, TRARG1, TRIM8, BTBD2, UBE2V1, ITCH,* and *SERPINE1*) associated with lipid metabolism, oxidation, ubiquitin, immunity, and inflammation that were unexplored in cattle regarding the fatty acid profile were identified.

5. CONCLUSION

This study identified mRNA isoforms of genes that acts on to the modulation of beef FA profile in the *Longissimus thoracis* of Nellore cattle finished in feedlot. Among these mRNA were highlighted, *C7-203, OXT-201, ADD1-204, RBM3-202* and *UBE2V1_mRNA1* as potential regulatory mRNAs of PUFA, ω3 and alpha-linolenic; *THRSP-201, ERG1-201* and *FABP4-201* as potential regulatory mRNAs for MUFA, SFA and palmitic acid. New mRNA isoforms associated with the content of ω3 (*CIDEC_mRNA1* and *UBE2V1_mRNA1*), MUFA (*ADIPQO_mRNA1*) and SFA (*SERPINE1_mRNA1*) were also identified. These mRNA isoforms were associated with biological mechanisms involved in immune response, inflammation, lipolysis, oxidation, fatty acid binding and adipogenesis. Our findings make a valuable contribution to the identification of potential biomarkers for complex and economically important traits. They highlight reliable genomic regions for future studies of genetic markers and can help in the selection of animals with a better profile of bovine fatty acids for human health.

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7. SUPPLEMENTARY MATERIAL

Table S1. GO Biological Process, GO Cellular Component, and GO Molecular Function terms associated with genes related to the differentially expressed mRNAs isoforms in *Longissimus thoracis* muscle of Nellore cattle with two different fatty acid profiles (C1 *vs.* C2).

GO:ID	**GO Term**	**Ontology Source**	***p*-value**	**Nr. Genes ***	**Associated Genes Found**
GO:0032352	positive regulation of hormone metabolic process	GO_BiologicalProcess	0,00	2	*BMP6, EGR1*
GO:0010035	response to inorganic substance	GO_BiologicalProcess	0,00	5	*ADD1, BMP6, FOSB, GSK3A, PPIF*
GO:0052192	movement in environment of other organism involved in symbiotic interaction	GO_BiologicalProcess	0,00	2	*TRIM8, VPS4B*
GO:0035490	regulation of leukotriene production involved in inflammatory response	GO_BiologicalProcess	0,00	1	*SERPINE1*
GO:0035491	positive regulation of leukotriene production involved in inflammatory response	GO_BiologicalProcess	0,00	1	*SERPINE1*
GO:0032350	regulation of hormone metabolic process	GO_BiologicalProcess	0,00	2	*BMP6, EGR1*
GO:0007098	centrosome cycle	GO_BiologicalProcess	0,00	3	*BBS4, BNIP2, VPS4B*
GO:0030308	negative regulation of cell growth	GO_BiologicalProcess	0,00	3	*APBB2, GSK3A, ST7L*
GO:1901724	positive regulation of cell proliferation involved in kidney development	GO_BiologicalProcess	0,01	1	*EGR1*
GO:0090559	regulation of membrane permeability	GO_BiologicalProcess	0,01	2	*GSK3A, PPIF*
GO:0002538	arachidonic acid metabolite production involved in inflammatory response	GO_BiologicalProcess	0,01	1	*SERPINE1*
GO:0042446	hormone biosynthetic process	GO_BiologicalProcess	0,01	2	*BMP6, EGR1*
GO:1902117	positive regulation of organelle assembly	GO_BiologicalProcess	0,01	2	*BBS4, VPS4B*
GO:0002249	lymphocyte anergy	GO_BiologicalProcess	0,01	1	*ITCH*
GO:0072110	glomerular mesangial cell proliferation	GO_BiologicalProcess	0,01	1	*EGR1*
GO:0045926	negative regulation of growth	GO_BiologicalProcess	0,01	3	*APBB2, GSK3A, ST7L*
GO:0060296	regulation of cilium beat frequency involved in ciliary motility	GO_BiologicalProcess	0,02	1	*BBS4*

GO:1901030	positive regulation of mitochondrial outer membrane permeabilization involved in apoptotic signaling pathway	GO_BiologicalProcess	0,02	1	*GSK3A*
GO:0051918	negative regulation of fibrinolysis	GO_BiologicalProcess	0,02	1	*SERPINE1*
GO:0034754	cellular hormone metabolic process	GO_BiologicalProcess	0,02	2	*BMP6, EGR1*
GO:1901722	regulation of cell proliferation involved in kidney development	GO_BiologicalProcess	0,02	1	*EGR1*
GO:0035845	photoreceptor cell outer segment organization	GO_BiologicalProcess	0,02	1	*BBS4*
GO:0060586	multicellular organismal iron ion homeostasis	GO_BiologicalProcess	0,02	1	*BMP6*
GO:0032103	positive regulation of response to external stimulus	GO_BiologicalProcess	0,02	3	*BMP6, CCL1, SERPINE1*
GO:0032102	negative regulation of response to external stimulus	GO_BiologicalProcess	0,02	3	*BBS4, ITCH, SERPINE1*
GO:0051014	actin filament severing	GO_BiologicalProcess	0,02	1	*DSTN*
GO:0002517	T cell tolerance induction	GO_BiologicalProcess	0,02	1	*ITCH*
GO:0031666	positive regulation of lipopolysaccharide-mediated signaling pathway	GO_BiologicalProcess	0,02	1	*BMP6*
GO:0002532	production of molecular mediator involved in inflammatory response	GO_BiologicalProcess	0,03	1	*SERPINE1*
GO:0001935	endothelial cell proliferation	GO_BiologicalProcess	0,03	2	*AIMP1, BMP6*
GO:0014741	negative regulation of muscle hypertrophy	GO_BiologicalProcess	0,03	1	*GSK3A*
GO:0051092	positive regulation of NF-kappaB transcription factor activity	GO_BiologicalProcess	0,03	2	*TRIM8, UBE2V1*
GO:0002687	positive regulation of leukocyte migration	GO_BiologicalProcess	0,03	2	*CCL1, SERPINE1*
GO:0051701	interaction with host	GO_BiologicalProcess	0,04	2	*TRIM8, VPS4B*
GO:0050921	positive regulation of chemotaxis	GO_BiologicalProcess	0,04	2	*CCL1, SERPINE1*
GO:1900182	positive regulation of protein localization to nucleus	GO_BiologicalProcess	0,04	2	*BMP6, TRIM8*
GO:0007050	cell cycle arrest	GO_BiologicalProcess	0,04	2	*APBB2, TBRG1*
GO:0010829	negative regulation of glucose transport	GO_BiologicalProcess	0,04	1	*GSK3A*
GO:0031664	regulation of lipopolysaccharide-mediated signaling pathway	GO_BiologicalProcess	0,04	1	*BMP6*
GO:0008217	regulation of blood pressure	GO_BiologicalProcess	0,05	2	*BBS4, GSK3A*

GO:0006884	cell volume homeostasis	GO_BiologicalProcess	0,05	1	*ADD1*
GO:0046621	negative regulation of organ growth	GO_BiologicalProcess	0,05	1	*GSK3A*
GO:1901862	negative regulation of muscle tissue development	GO_BiologicalProcess	0,05	1	*GSK3A*
GO:1904018	positive regulation of vasculature development	GO_BiologicalProcess	0,05	2	*EGR1*, *SERPINE1*
GO:0043901	negative regulation of multi-organism process	GO_BiologicalProcess	0,05	2	*ITCH*, *TRIM8*
GO:1900048	positive regulation of hemostasis	GO_BiologicalProcess	0,05	1	*SERPINE1*
GO:0030194	positive regulation of blood coagulation	GO_BiologicalProcess	0,05	1	*SERPINE1*
GO:0048515	spermatid differentiation	GO_BiologicalProcess	0,05	2	*BBS4*, *TTLL1*
GO:0050820	positive regulation of coagulation	GO_BiologicalProcess	0,05	1	*SERPINE1*
GO:0032612	interleukin-1 production	GO_BiologicalProcess	0,05	1	*EGR1*
GO:0035148	tube formation	GO_BiologicalProcess	0,05	2	*BBS4*, *LMO4*
GO:0032637	interleukin-8 production	GO_BiologicalProcess	0,05	1	*SERPINE1*
GO:1990204	oxidoreductase complex	GO_CellularComponent	0,00	3	*MRPS36*, *P4HA1*, *UQCC3*
GO:0098798	mitochondrial protein complex	GO_CellularComponent	0,01	3	*MRPS36*, *PPIF*, *UQCC3*
GO:0045239	tricarboxylic acid cycle enzyme complex	GO_CellularComponent	0,02	1	*MRPS36*
GO:0031371	ubiquitin conjugating enzyme complex	GO_CellularComponent	0,02	1	*UBE2V1*
GO:1990763	arrestin family protein binding	GO_MolecularFunction	0,02	1	*ITCH*
GO:0031543	peptidyl-proline dioxygenase activity	GO_MolecularFunction	0,03	1	*P4HA1*
GO:1990841	promoter-specific chromatin binding	GO_MolecularFunction	0,05	1	*EGR1*
GO:0031369	translation initiation factor binding	GO_MolecularFunction	0,05	1	*ANGEL1*

*Nr. Genes : Number of genes

Table S2. GO Biological Process, GO Cellular Component, and GO Molecular Function terms were associated genes related to the differentially expressed mRNAs isoforms in *Longissimus thoracis* muscle of Nellore cattle with two different fatty acid profiles (C1 vs. C3).

ID	**Term**	**Ontology Source**	***p*-value**	**Nr. Genes ***	**Associated Genes Found**
GO:0033002	muscle cell proliferation	GO_BiologicalProcess	0,00	3	*ADIPOQ*, *IGFBP5*, *THBS1*

GO:0048662	negative regulation of smooth muscle cell proliferation	GO_BiologicalProcess	0,00	2	*ADIPOQ, IGFBP5*
GO:2000146	negative regulation of cell motility	GO_BiologicalProcess	0,00	3	*ADIPOQ, IGFBP5, THBS1*
GO:0051271	negative regulation of cellular component movement	GO_BiologicalProcess	0,00	3	*ADIPOQ, IGFBP5, THBS1*
GO:1905952	regulation of lipid localization	GO_BiologicalProcess	0,00	2	*ADIPOQ, THBS1*
GO:0014745	negative regulation of muscle adaptation	GO_BiologicalProcess	0,01	1	*IGFBP5*
GO:0032891	negative regulation of organic acid transport	GO_BiologicalProcess	0,01	1	*THBS1*
GO:0002577	regulation of antigen processing and presentation	GO_BiologicalProcess	0,01	1	*THBS1*
GO:0014741	negative regulation of muscle hypertrophy	GO_BiologicalProcess	0,01	1	*IGFBP5*
GO:0044342	type B pancreatic cell proliferation	GO_BiologicalProcess	0,01	1	*IGFBP5*
GO:0034383	low-density lipoprotein particle clearance	GO_BiologicalProcess	0,01	1	*ADIPOQ*
GO:0021542	dentate gyrus development	GO_BiologicalProcess	0,01	1	*EMX2*
GO:0032369	negative regulation of lipid transport	GO_BiologicalProcess	0,02	1	*THBS1*
GO:0002089	lens morphogenesis in camera-type eye	GO_BiologicalProcess	0,02	1	*TDRD7*
GO:0021846	cell proliferation in forebrain	GO_BiologicalProcess	0,02	1	*EMX2*
GO:0031069	hair follicle morphogenesis	GO_BiologicalProcess	0,02	1	*IGFBP5*
GO:1903846	positive regulation of cellular response to transforming growth factor beta stimulus	GO_BiologicalProcess	0,02	1	*THBS1*
GO:0034381	plasma lipoprotein particle clearance	GO_BiologicalProcess	0,02	1	*ADIPOQ*
GO:0014888	striated muscle adaptation	GO_BiologicalProcess	0,03	1	*IGFBP5*
GO:0045912	negative regulation of carbohydrate metabolic process	GO_BiologicalProcess	0,03	1	*ADIPOQ*
GO:0048661	positive regulation of smooth muscle cell proliferation	GO_BiologicalProcess	0,03	1	*THBS1*
GO:0090317	negative regulation of intracellular protein transport	GO_BiologicalProcess	0,03	1	*ADIPOQ*
GO:1901862	negative regulation of muscle tissue development	GO_BiologicalProcess	0,04	1	*IGFBP5*
GO:0010828	positive regulation of glucose transport	GO_BiologicalProcess	0,04	1	*ADIPOQ*
GO:1900047	negative regulation of hemostasis	GO_BiologicalProcess	0,04	1	*THBS1*
GO:0060761	negative regulation of response to cytokine stimulus	GO_BiologicalProcess	0,04	1	*ADIPOQ*
GO:0032615	interleukin-12 production	GO_BiologicalProcess	0,04	1	*THBS1*
GO:0043502	regulation of muscle adaptation	GO_BiologicalProcess	0,04	1	*IGFBP5*
GO:0032370	positive regulation of lipid transport	GO_BiologicalProcess	0,04	1	*ADIPOQ*
GO:0032507	maintenance of protein location in cell	GO_BiologicalProcess	0,05	1	*TSPO*

GO:1905954	positive regulation of lipid localization	GO_BiologicalProcess	0,05	1	*ADIPOQ*
GO:0032637	interleukin-8 production	GO_BiologicalProcess	0,05	1	*ADIPOQ*
GO:0016942	insulin-like growth factor binding protein complex	GO_CellularComponent	0,01	1	*IGFBP5*
GO:0001772	immunological synapse	GO_CellularComponent	0,03	1	*ALCAM*
GO:0005581	collagen trimer	GO_CellularComponent	0,07	1	*ADIPOQ*
GO:0019838	growth factor binding	GO_MolecularFunction	0,01	2	*IGFBP5, THBS1*
GO:0043177	organic acid binding	GO_MolecularFunction	0,01	2	*ADIPOQ, FABP4*
GO:0019825	oxygen binding	GO_MolecularFunction	0,01	1	*HBB*
GO:0005504	fatty acid binding	GO_MolecularFunction	0,02	1	*FABP4*
GO:0140104	molecular carrier activity	GO_MolecularFunction	0,03	1	*HBB*
GO:0001786	phosphatidylserine binding	GO_MolecularFunction	0,03	1	*THBS1*

*Nr. Genes: Number of genes

Table S3. GO Biological Process, GO Cellular Component, and GO Molecular Function terms were associated genes related to the differentially expressed mRNAs isoforms in *Longissimus thoracis* muscle of Nellore cattle with two different fatty acid profiles (C2 vs. C3).

GO:ID	GO Term	Ontology Source	*p*-value	Nr. Genes *	Associated Genes Found
GO:1900077	negative regulation of cellular response to insulin stimulus	GO_BiologicalProcess	0,00	2	*GSK3A, MSTN*
GO:1901862	negative regulation of muscle tissue development	GO_BiologicalProcess	0,00	2	*GSK3A, MSTN*
GO:0090559	regulation of membrane permeability	GO_BiologicalProcess	0,00	2	*GSK3A, PPIF*
GO:1900076	regulation of cellular response to insulin stimulus	GO_BiologicalProcess	0,00	2	*GSK3A, MSTN*
GO:0043500	muscle adaptation	GO_BiologicalProcess	0,00	2	*GSK3A, MSTN*
GO:0048640	negative regulation of developmental growth	GO_BiologicalProcess	0,00	2	*GSK3A, MSTN*
GO:1901861	regulation of muscle tissue development	GO_BiologicalProcess	0,01	2	*GSK3A, MSTN*
GO:0090131	mesenchyme migration	GO_BiologicalProcess	0,01	1	*ACTC1*
GO:1901030	positive regulation of mitochondrial outer membrane permeabilization involved in apoptotic signaling pathway	GO_BiologicalProcess	0,01	1	*GSK3A*
GO:0014889	muscle atrophy	GO_BiologicalProcess	0,01	1	*MSTN*
GO:0048630	skeletal muscle tissue growth	GO_BiologicalProcess	0,01	1	*MSTN*
GO:0003205	cardiac chamber development	GO_BiologicalProcess	0,01	2	*GSK3A, LMO4*
GO:0014741	negative regulation of muscle hypertrophy	GO_BiologicalProcess	0,01	1	*GSK3A*

GO:1905208	negative regulation of cardiocyte differentiation	GO_BiologicalProcess	0,01	1	*GSK3A*
GO:0046685	response to arsenic-containing substance	GO_BiologicalProcess	0,01	1	*PPIF*
GO:0010829	negative regulation of glucose transport	GO_BiologicalProcess	0,02	1	*GSK3A*
GO:0002931	response to ischemia	GO_BiologicalProcess	0,02	1	*PPIF*
GO:0046716	muscle cell cellular homeostasis	GO_BiologicalProcess	0,02	1	*MSTN*
GO:0046621	negative regulation of organ growth	GO_BiologicalProcess	0,02	1	*GSK3A*
GO:0021516	dorsal spinal cord development	GO_BiologicalProcess	0,02	1	*LMO4*
GO:0048147	negative regulation of fibroblast proliferation	GO_BiologicalProcess	0,02	1	*MSTN*
GO:0014888	striated muscle adaptation	GO_BiologicalProcess	0,03	1	*MSTN*
GO:0070265	necrotic cell death	GO_BiologicalProcess	0,04	1	*PPIF*
GO:0055017	cardiac muscle tissue growth	GO_BiologicalProcess	0,04	1	*GSK3A*
GO:0021517	ventral spinal cord development	GO_BiologicalProcess	0,05	1	*LMO4*
GO:0043502	regulation of muscle adaptation	GO_BiologicalProcess	0,05	1	*GSK3A*
GO:0060419	heart growth	GO_BiologicalProcess	0,05	1	*GSK3A*
GO:1903524	positive regulation of blood circulation	GO_BiologicalProcess	0,05	1	*GSK3A*
GO:0032410	negative regulation of transporter activity	GO_BiologicalProcess	0,05	1	*PPIF*
GO:0030289	protein phosphatase 4 complex	GO_CellularComponent	0,00	1	*PPP4C*
GO:0000172	ribonuclease MRP complex	GO_CellularComponent	0,01	1	*POP5*
GO:0098800	inner mitochondrial membrane protein complex	GO_CellularComponent	0,01	2	*PPIF, UQCC3*
GO:0005504	fatty acid binding	GO_MolecularFunction	0,02	1	*FABP4*
GO:0031369	translation initiation factor binding	GO_MolecularFunction	0,03	1	*ANGEL1*
GO:0001098	basal transcription machinery binding	GO_MolecularFunction	0,04	1	*ELP4*
GO:0003755	peptidyl-prolyl cis-trans isomerase activity	GO_MolecularFunction	0,04	1	*PPIF*
GO:0051018	protein kinase A binding	GO_MolecularFunction	0,04	1	*GSK3A*
GO:0016859	cis-trans isomerase activity	GO_MolecularFunction	0,05	1	*PPIF*

*Nr. Genes: Number of genes.