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LENISE FREITAS MUELLER DA SILVEIRA

Analysis of adipogenesis related genes and intramuscular fat composition in Angus x Nelore cattle of different gender status

Pirassununga
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Corrected version

Doctoral thesis presented to the Faculdade de Zootecnia e Engenharia de Alimentos, Universidade de São Paulo, as part of the requirements to obtain the title of Doctor of Science.

Concentration area: Animal Quality and Productivity

Advisor: Prof[a]. Dr[a]. Angélica Simone Cravo Pereira

Co-advisor: Dr[a]. Mirele Daiana Poleti

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DEDICO

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Ao meu esposo Juliano, pelo seu amor incondicional, apoio e encorajamento.

Aos meus pais Alcides e Cleonice, irmãos, cunhados (as) e sobrinhas.

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(Cecília Sfalsin)

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“Todas as vitórias ocultam uma abdicação”.

(Simone de Beauvoir)

BIOGRAFIA

Sou Lenise Freitas Mueller da Silveira, filha de Cleonice Freitas Mueller e Alcides Luiz Mueller, irmã de Joice e Michel Freitas Mueller, nasci em 10 de julho de 1987 em Rio Pardo, Rio Grande do Sul, Brasil. Enquanto criança, sempre amei os animais e sonhava em ser médica veterinária. Apesar de não ter nascido e crescido em ambiente rural, tinha avós e tios com propriedades rurais, as quais visitava aos finais de semana. Quando criança e adolescente, nunca tive interesse e nem conhecimento pela área de produção animal, tinha somente animais de pequeno porte em casa, porém, admirava animais de grande porte, com adoração por bovinos, ovinos e equinos. O interesse pela produção animal, área escolhida para execução do mestrado e doutorado, veio mais tarde, durante a faculdade e estágios. Sou casada com Juliano Coelho da Silveira, biólogo e professor universitário, desde setembro de 2015 e mãe da Cléo Mueller da Silveira, minha inspiração de vida.

Sou médica veterinária, formada em 2013 pela Universidade Luterana do Brasil – ULBRA, localizada na cidade de Canoas, RS, Brasil. Aproveitei muito bem a faculdade, sem reprovações e me dediquei ao curso sem medir esforços para aprender e expandir meus conhecimentos. No início do curso realizei estágios em diversas áreas da veterinária, como clínica de pequenos e grandes animais, diagnóstico por imagem, microbiologia de alimentos em laboratório, atendimento clínico e odontológico em projetos de extensão com equinos e caninos, além de campanhas de vacinação e castração de cães e gatos. Também era monitora de algumas disciplinas do curso de veterinária e tinha participação ativa em eventos e congressos. No início da graduação, em 2007, conheci meu esposo, que estava de mudança para o Colorado, nos EUA, reencontrando-o em 2011, quando me mudei para o mesmo local que ele. No meio do curso, surgiu o interesse pela área de higiene e inspeção de produtos de origem animal e então iniciei estágios em frigoríficos para conhecer o sistema de controle de qualidade em empresas processadoras de alimentos.

Após conhecer alguns programas de segurança alimentar e qualidade da carne, descobri meu interesse pela área de pesquisa e realizei estágio no Instituto de Pesquisas Veterinárias Desidério Finamor (IPVDF), em Eldorado do Sul, RS, auxiliando em procedimentos e pesquisas com frangos e ovos no Laboratório de Saúde das Aves. Posteriormente, estagiei no Núcleo de Estudos em Sistemas de Produção de Bovinos de Corte e Cadeia Produtiva (NESPRO) do Programa de Pós-graduação em Zootecnia do Departamento de Zootecnia da Universidade Federal do Rio Grande do Sul – UFRGS em Porto Alegre, RS. Neste núcleo de estudos, aprendi técnicas de produção científica.

Dando continuidade ao meu interesse em realizar pesquisas e aprender inglês, fui em busca de novos aprendizados, conhecimentos e experiências fora do Brasil, iniciando minhas atividades no Center for Meat Safety and Quality, Animal Sciences Department, Colorado State University em Fort Collins, CO, EUA, onde realizei estágio extracurricular e curricular obrigatório de conclusão de curso. Em janeiro de 2013 me formei em medicina veterinária e retornei aos Colorado, onde fui contratada como Research Assistant no mesmo local dos estágios, auxiliando em projetos de pesquisa envolvendo composição nutricional, qualidade, segurança, características sensoriais e comercialização da carne vermelha, além de prestação de serviços para empresas privadas dos EUA. Ainda no Colorado, estagiei na planta frigorífica JBS Swift Beef Company, localizada na cidade de Greeley. Neste mesmo período, aos finais de semana, auxiliava no atendimento clínico de pequenos animais na Highland Veterinary Clinic, em Ault, CO, EUA.

No inicio de 2014, iniciei minhas atividades de prática profissionalizante no Laboratório de Ciência da Carne – LCC /USP do Departamento de Nutrição e Produção Animal, na Faculdade de Medicina Veterinária e Zootecnia – FMVZ da Universidade de São Paulo em Pirassununga, SP. Em julho do mesmo ano, ingressei no mestrado pelo Programa de Pós-graduação em Zootecnia, com ênfase em qualidade e produtividade animal da Faculdade de Zootecnia e Engenharia de Alimentos – FZEA da Universidade de São Paulo, em Pirassununga, SP, concluído em janeiro de 2017. Durante o mestrado, pesquisei a influência da condição sexual de bovinos cruzados Angus x Nelore sobre a qualidade da carne, fui bolsista CAPES, posteriormente contemplada com bolsa da Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP), recebi menção honrosa pelo meu trabalho e o apresentei em congressos nacionais e internacionais. Em 12 de setembro de 2015, em uma noite linda e muito fria, me casei com Juliano, em Porto Alegre, RS, Brasil. Neste mesmo ano, enfrentei junto com minha família, o terceiro diagnóstico de câncer de mama de minha mãe e mesmo com meu casamento tão recente e mestrado em andamento, estive ao lado de minha mãe durante parte do seu tratamento e recuperação.

Em março de 2017 ingressei no doutorado pelo Programa de Pós-graduação em Zootecnia da Faculdade de Zootecnia e Engenharia de Alimentos, Universidade de São Paulo, sendo bolsista CAPES nos primeiros meses e posteriormente contemplada com bolsa da FAPESP. Concluí este grande desafio e conquistei o tão sonhado título de doutora em 10 de setembro de 2021. No doutorado, segui a linha de pesquisa do mestrado, estudando a influência da condição sexual de bovinos cruzados Angus x Nelore, mas desta vez sobre a expressão de genes relacionados à adipogênese e a composição de ácidos graxos em diferentes músculos.

Durante todo o período de pós-graduação fui muito ativa, auxiliando em diversos projetos de pesquisa da equipe, participando de eventos e programas de televisão, ministrando palestras e seminários, organizando simpósios, cumprindo minhas obrigações acadêmicas, prestando serviço no laboratório, além de obter vivência e adquirir experiência de campo ao lidar com sistema de criação de gado de ciclo completo, envolvendo cria, recria e engorda de bovinos no Laboratório de Pesquisa em Gado de Corte – LPGC / USP. Além disso, atuei na indústria frigorífica, realizando pesquisas e trabalhando na melhoria da qualidade da carne. Também neste período fui certificadora de produtos cárneos, prestando serviço para o Programa Carne Angus Certificada, atuando em empresas processadoras de carne no estado de São Paulo e Minas Gerais.

Foi ainda durante o doutorado que grandes acontecimentos marcaram o período, como a terrível pandemia da COVID-19, a qual prejudicou o andamento da minha pesquisa, entre outros episódios tristes no mundo inteiro. Em abril de 2020, no meio da pandemia e na correria com as atividades do doutorado, eu e meu esposo Juliano fomos agraciados com a notícia da gravidez de nossa primeira filha. Desta forma, o acontecimento mais marcante e feliz da minha vida foi em 9 de novembro de 2020, quando nasceu a Cléo, na cidade de Ribeirão Preto, SP. Após o nascimento dela, a vida e as prioridades mudaram, os primeiros meses foram de dedicação exclusiva a ela e passado um tempo, retornei aos poucos com algumas atividades do doutorado, redação da tese e artigos científicos referentes ao meu estudo.

Por fim, tomo a liberdade de dizer que sempre fui intensa em tudo que fiz, especialmente na vida acadêmica, me dedicando e vestindo a camisa de todos os locais que estudei e trabalhei, na maioria das vezes sendo bem tratada e interpretada, em outras situações nem tanto, mas procurando sempre manter um bom relacionamento com as pessoas, tentando deixar uma marca e um legado.

ABSTRACT

SILVEIRA, L. F. M. **Analysis of adipogenesis related genes and intramuscular fat composition in Angus x Nelore cattle of different gender status.** 2021. 108 f. Doctoral Thesis – Faculdade de Zootecnia e Engenharia de Alimentos, Universidade de São Paulo, Pirassununga, 2021.

Cattle growth, muscle adipose tissue and fatty acid composition in different muscles, are significantly affected by gender and influenced by a variety of hormonal factors, affecting the quality of the carcass and the beef. As the adipogenesis is a complex biological process to regulate fat cell metabolism, it can be regulated by hormones and influenced by factors such as different fat deposits, the gender status and diet of cattle. Therefore, it is important to understand the molecular mechanisms involved in adipogenesis in different muscles and gender, since the deposition of fat in these animals is different, to employ new tools to add greater value to meat production. However, the present study evaluated the association of fatty acid (FA) composition and adipogenesis-related genes in the *longissimus* (LO) and *triceps brachii* (TB) muscles of feedlot Angus x Nelore cattle. A total of 150 cattle was confined for 150 days and assigned to three genders, namely, heifers, bulls, and steers, and fed the same diet. Immediately after slaughter, samples of LO and TB muscles were collected for RNA and protein extraction. Twenty-four hours after slaughter, were measured the fat thickness and marbling score between the 12^{th} and 13^{th} ribs and collected samples of the muscles for analyses of total lipids determination and fatty acid composition. The experimental design was completely randomized, with 50 repetitions per treatment, and each animal was considered an experimental unit. The statistical analyses were performed by the proc MIXED SAS® (version 9.3) for the meat quality traits and JMP14 Software (SAS Institute) for the gene expression analyses. There was difference in the carcass traits related to fat deposition ($P \leq 0.05$). The fat thickness and marbling score were higher in heifer carcasses than in carcasses of bulls and steers. Similarly, only gender status affected the content of total lipids ($P \leq 0.05$), where meat from heifers presented increased values compared to steers and bulls. There was a difference in the total saturated FAs (SFAs) by gender and muscle type ($P \leq 0.05$). The meat from bulls had higher levels of SFAs compared to heifers and had no differences in relation to meat from steers. Among muscle types, total SFAs increased in LO muscle compared with TB, independent of gender. The FAs 14:0 and 16:0 were affected by muscle type only, and higher levels were detected in the LO muscle ($P \leq 0.05$). There were differences in gender for total monounsaturated FAs (MUFAs; $P \leq 0.05$). Meat from bulls had lower levels of MUFAs compared with the other genders. The levels of the major FA of total MUFAs, 18:1 n-9c, were higher in the LO muscle than in the TB muscle, independent of gender ($P \leq 0.05$). The total polyunsaturated FAs (PUFAs), total n-3, total n-6, PUFA:SFA ratio, health index and FAs 18:2 n-6c, 20:3 n-3, and 22:4 n-6 were higher in TB than in LO muscle ($P < 0.05$). Otherwise, the atherogenicity and thrombogenicity indexes were higher in the LO muscle ($P \leq 0.05$). Regarding the transcript levels, there was an effect of muscle type on the expression of the *CEBPa* and *LPL* ($P \leq 0.05$) genes, both of which had higher expression in TB muscle. Gender affected the transcript level of *ACC* ($P \leq 0.05$). This gene was increased in bulls, intermediate in steers, and decreased in heifers. Additionally, gender and muscle type interactions were observed for the transcript levels of *FABP3*, *TPM2*, and *TPM3* ($P \leq 0.05$). There was difference for tropomyosin (TPM) protein abundance between muscles only in bulls ($P \leq 0.05$), where the greater TPM abundance was in the TB. Additionally, cattle gender status affected the TPM abundance evaluated in LO and TB muscles ($P \leq 0.05$). The LO muscle of steers showed higher TPM abundance than bulls and heifers, while the TB muscle of bulls showed higher TPM

abundance than the other genders. The cattle gender status modulated the transcripts levels of *ACC* gene, content of total lipids, total SFAs, total MUFAs, and carcass traits, while the muscle type affected the transcripts of *CEBPa* and *LPL* genes, total SFAs, total PUFAs, total n-3 and n-6, besides important FAs, showing a gender and muscle specific effect. In addition, a modulation of TPM in formation and accumulation of lipids was observed, related to the greater subcutaneous fat thickness and marbling score in heifers and steers, and a modulation of this same protein in muscle development, indicated by the lower fat deposition in the carcass of bulls. Furthermore, there is a different lipid profile within each muscle, where the TB muscle is more favorable to human health than the LO, and beef from heifers stands out than the other genders for presenting better carcass traits and FA composition more favorable to human health.

Keywords: Fatty acid synthesis. Crossbred. Gene expression. *Longissimus*. *Triceps brachii*.

RESUMO

SILVEIRA, L. F. M. **Análise da expressão de genes associados à adipogênese e composição da gordura intramuscular em bovinos Angus X Nelore de diferentes condições sexuais**. 2021. 108 f. Tese (Doutorado) – Faculdade de Zootecnia e Engenharia de Alimentos, Universidade de São Paulo, Pirassununga.

A condição sexual dos bovinos influencia o seu crescimento, assim como a formação do tecido adiposo muscular, a composição de ácidos graxos em diferentes músculos e vias de adipogênese, sendo estas influenciadas por uma variedade de sinais hormonais, o que afeta diretamente as características da carcaça e a qualidade da carne. Desta forma, o presente estudo analisou a influência da condição sexual (CS) dos bovinos sobre a expressão dos genes associados à adipogênese, assim como avaliou o perfil de ácidos graxos dos músculos *longissimus* (LO) e *triceps brachii* (TB) de bovinos Angus x Nelore. Foram confinados 150 bovinos, divididos em três CS (50 fêmeas, 50 machos não castrados e 50 castrados). O delineamento experimental considerado foi inteiramente casualizado, com 50 repetições por grupo e cada animal foi considerado como uma unidade experimental. Os animais foram mantidos nas mesmas condições de manejo, recebendo alimentação *ad libitum* utilizando-se a mesma dieta, composta de 86% de concentrado e 14% de volumoso na matéria seca. Após 150 dias de confinamento, os animais foram abatidos aos 16 meses de idade. Durante o abate, imediatamente após a esfola foram coletadas amostras dos músculos LO e TB para extração de RNA e proteína. Durante a desossa foi avaliada a espessura de gordura subcutânea e o escore de marmorização no músculo *longissimus*, entre a 12ª e 13ª costelas e coletadas amostras dos dois músculos para análise de quantificação de lipídeos totais e composição de ácidos graxos. Os dados estatísticos referentes à qualidade de carcaça e carne foram analisados pelo proc MIXED do programa SAS (versão 9.3) e os referentes aos resultados de expressão gênica pelo programa JMP14® (SAS Institute). A CS afetou as características de carcaça avaliadas ($P \leq 0,05$). A espessura de gordura subcutânea e o escore de marmorização foram maiores na carcaça das fêmeas em comparação às dos machos não castrados e castrados. Houve diferença no conteúdo de lipídeos totais somente entre as CS ($P \leq 0,05$), com maior conteúdo na carne das fêmeas, seguida da carne dos machos castrados e não castrados. O total de ácidos graxos saturados (AGS) foi influenciado pela CS e pelo tipo de músculo ($P \leq 0,05$), sendo que a carne dos bovinos não castrados apresentou maior concentração de AGS do que a carne das fêmeas. O total de AGS no músculo LO foi maior que o TB, independente da CS. Já os ácidos graxos (AG) 14:0 e 16:0 foram afetados apenas pelo tipo de músculo, e concentrações mais elevadas foram detectadas no músculo LO ($P \leq 0,05$). O fator CS afetou o total de AG monoinsaturados (AGMI) ($P \leq 0,05$), sendo a maior concentração observada na carne de fêmeas e castrados do que na carne de não castrados. Já a concentração do 18:1 n-9c (oleico) foi influenciada pelo tipo de músculo, com maior concentração no músculo LO em comparação ao TB ($P \leq 0,05$). Houve efeito do tipo de músculo para o total de AG poli-insaturados (AGPI), total de n-3, total de n-6, proporção AGPI:AGS, os AG individuais 18:2 n-6c, 20:3 n-3, 22:4 n-6 e o índice de saúde. As concentrações foram maiores no TB em comparação ao LO ($P \leq 0,05$). Por outro lado, os índices de aterogenicidade e trombogenicidade foram maiores no músculo LO ($P \leq 0,05$). Com relação aos níveis de transcritos, houve efeito do tipo de músculo na expressão dos genes *CEBPa* e *LPL* ($P \leq 0,05$), ambos com maior expressão no músculo TB. Já a CS influenciou o nível de transcritos do *ACC* ($P \leq 0,05$) e o gene foi mais expresso nas fêmeas em relação aos machos castrados. Foi observada interação entre CS e tipo de músculo para os genes *FABP3*, *TPM2* e *TPM3* ($P \leq 0,05$). Houve diferença na abundância da proteína tropomiosina (TPM) entre os músculos, mas somente dentro da condição sexual machos não castrados ($P \leq 0,05$), com maior abundância no músculo TB em comparação ao LO. Adicionalmente, a CS

afetou a abundância da TPM avaliada nos músculos LO e TB ($P \leq 0,05$). O músculo LO dos machos castrados apresentou maior abundância da TPM do que os machos não castrados e fêmeas, enquanto o TB dos machos não castrados apresentou maior abundância do que as demais CS. A CS modulou os níveis de transcritos do *ACC*, conteúdo de lipídeos totais, total de AGS, total de AGMI e as características de carcaça, enquanto o tipo de músculo afetou os transcritos dos genes *CEBPa* e *LPL*, total de AGS, total de AGPI, total de n-3 e n-6, além de importantes AG, demonstrando efeito especifico da CS e o tipo de músculo. Adicionalmente, foi observada uma modulação da tropomiosina na formação e acúmulo de lipídeos, relacionada a maior espessura de gordura subcutânea e escore de marmorização nas fêmeas e machos castrados, e uma modulação desta mesma proteína no desenvolvimento muscular, apontada pela menor deposição de gordura na carcaça de machos não castrados. Além disso, há um perfil lipídico diferente dentro de cada músculo, onde o TB é mais favorável à saúde humana do que o LO, e a carne bovina de fêmeas se destaca das demais condições sexuais por apresentar melhores características de carcaça e composição de AG mais favorável à saúde humana.

Palavras-chave: Síntese de ácidos graxos. Cruzamento. Expressão gênica. *Longissimus*. *Triceps brachii*.

FIGURES

TABLES

SUMMARY

1 GENERAL INTRODUCTION

Brazil occupies a prominent position in agribusiness, being an important beef producer and global exporter, producing 10.3 million tons of beef in 2020 (ABIEC, 2021). Beef producers are adopting practices to improve meat quality, such as castrating males, crossbreeding, feedlot finishing, and harvesting of heifers with adequate subcutaneous and intramuscular fat deposition. Nelore cattle are known to have greater rusticity and adaptability to the tropical climate and conditions, in addition to having greater resistance to ectoparasites (BIANCHINI *et al*., 2007; FERRAZ; FELÍCIO, 2010). However, there is an increasing occurrence of taurine-origin animals, mainly from synthetic crossings (FAVERO et al., 2019), in Brazilian livestock. In recent decades, beef producers have been using increasingly precocious cattle, with greater carcass development, to produce beef with desirable traits. In summary, Brazilian producers are utilizing crossbreeding between *B. indicus* and *taurus* breeds to improve the lean yield and beef quality (GAMA *et al*., 2013; PEREIRA *et al*., 2015; CARVALHO *et al*., 2016).

Cattle growth, muscle adipose tissue and fatty acid composition are significantly affected by gender (BJORNTORP, 1997; LEE, 2000; MUELLER *et al*., 2019; OWENS *et al*., 1993; VENKATA REDDY *et al.*, 2015) and are influenced by a variety of hormonal factors, affecting the quality of the carcass and the beef (MUELLER *et al*., 2019).

Adipogenesis is a complex developmental process from preadipocytes or mesenchymal stem cells to mature adipocytes, it is essential for fat formation and the metabolism of adipose tissues in mammals (ROMAO *et al*., 2011). Muscle is comprised of adipocytes, fibroblasts, myocytes and other less abundant cell types. Cell differentiation is related to the expression of lineage-specific transcription factors (DU et al., 2013), which are expressed as a transcriptional cascade promoting adipocyte differentiation, leading to the mature adipocyte phenotype (ROMAO *et al*., 2011). The development of adipocytes (AILHAUD; GRIMALDI; NKGREL, 1994; SMAS; SUL, 1995) and the distribution of body fat (BJORNTORP, 1997) are regulated by hormones and influenced by factors such as different fat depots, gender status and diet of the cattle (BJORNTORP, 1997; DE SÁ *et al*., 2017; LEE, 2000; MADRUGA *et al*., 2006; SCOLLAN et *al*., 2006; WEBB; O'NEILL, 2008; WOOD; RICHARDSON, 2004). In several mammal species, physiological observations have indicated that adipose

development is affected by a variety of endocrine hormones and many steroid hormones (DE SÁ *et al*., 2017). Sex hormones and their associated hormonal repertoire can affect muscle and adipocyte cells through different molecular pathways (BJORNTORP, 1997).

As adipogenesis is a complex biological process, it needs to be governed by a vast number of enzymes that act together along with key hormones and metabolites to regulate fat cell metabolism. The differentiation of adipocyte precursors is driven by a cascade of events controlled by transcription regulators and other factors. This entire process is closely regulated at the transcriptional level (MOISÁ *et al*., 2013). Transcription factors such as CEPB, PPAR and Zfp423 are involved in the adipogenesis process (GUPTA *et al*., 2010, 2012; ROSEN *et al*., 2002), as well as the lipogenic enzymes LPL, ACC, and FAS (BONNET *et al*., 2000; SMITH; WITKOWSKI; JOSHI, 2003) and the ACSS1 and SLC16A7 genes, which can act in fatty acid synthesis (BERTON et al., 2016). The ACOX gene is associated with fat deposition and lipid metabolism (JIAO *et al*., 2011; MORAIS *et al*., 2007) and may also be the key regulator of energy metabolism. Additionally, a multigenic family of fatty acid-binding proteins is involved in the intracellular and extracellular transport of lipids, transporting fatty acids in adipose tissue and contributing to muscle energy metabolism (BERTON *et al*., 2016; BIONAZ; THERING; LOOR, 2012; PAS; EVERTS; HAAGSMAN, 2004; VURAL *et al*., 2008). The relationship between marbling score and percent intramuscular fat was described by Jeong *et al*. (2012). In this regard, the tropomyosins (TPMs) are a family of actin-linked proteins found in all tissues and has been indicated as a modulator of the formation of adipose tissue (CHO et al., 2016). Moreover, these authors showed that TPM1, TPM2, and TPM3 were differentially expressed depending on sex and adipose depots and TPMs were positively correlated with marbling score and quality grade. Hence, it is important to investigate these genes in different muscles of different gender status of crossed cattle to understand their role affecting directly and indirectly the carcass and meat traits.

Beef quality is better in heifers than bulls, especially the fatty acid composition (MUELLER *et al*., 2019; SHARAF ELDIN *et al*., 2013). Based on that, the use of different cattle gender status can be an interesting strategy to improve beef quality (MUELLER *et al*., 2019). Furthermore, regarding the lipid composition in muscles, fatty acids can be modified according to the muscle (PURCHAS; ZOU, 2008; RAES *et al*., 2004; TURK; SMITH, 2009), however, the most studies in beef cattle are carried

out with the *longissimus* muscle. Therefore, there is a need to explore other muscle groups, such as *triceps brachii*, which presents metabolic and fat deposition differences when compared to other muscles (HOCQUETTE *et al., 2010;* SORET *et al.,* 2016). Thus, in this context and since there is no research exploring the use of different gender status and muscles of cattle, it is important to understand how gender can influence the expression of genes involved in adipogenesis and fatty acid composition in muscles. However, the goal of this study was to understand the influence of gender status on the expression of adipogenesis-related genes, as well as the association with the fatty acid composition in *longissimus* and *triceps brachii* muscles from feedlot Angus x Nelore cattle.

1.1 Hypothesis

We hypothesized that cattle gender status (heifers, bulls, and steers) influence the expression of adipogenesis-related genes and the fatty acid composition in *longissimus* and *triceps brachii* muscles of feedlot crossbred catlle.

1.2 General objective

The goal of this study was to understand the influence of cattle gender status (heifers, bulls, and steers) on the expression of genes associated with adipogenesis, as well as to evaluate the fatty acid composition in different muscles (*longissimus* and *triceps brachii*) from feedlot crossbred cattle.

1.3 Literature review

1.3.1 Crossbreeding as a strategy in beef cattle in the tropic

The most representative breed of Brazilian beef cattle is the Nelore (*Bos indicus*), which is characterized as a breed adapted to the tropical climate, resistant to parasites, efficient in the conversion of tropical grasses, and with good longevity and fertility (ARBOITTE *et al*., 2004; KOURY FILHO, 2005). On the other hand, the Nelore is a breed that has a genetic predisposition for a low deposition of intramuscular fat and, in general, presents a tougher meat (WHIPPLE *et al*., 1990) than animals of European origin, such as the Angus breed (CROUSE *et al*., 1993; MUCHENJE *et al*., 2009). Feitosa *et al*. (2017) reported that when genetically selecting male Nelore cattle to increase their intramuscular fat in the *longissimus thoracis* muscle, the content of polyunsaturated fatty acids (omega 3 and omega 6) was reduced, and the levels of saturated fatty acids increased, thus changing the nutritional quality of the meat of these cattle.

In this sense, animals with the genetic composition of taurine (*Bos taurus*) are common in regions with a mild climate and they have little resistance to the high temperatures in the tropics and ectoparasites (FERRAZ; FELÍCIO, 2010). However, the meat quality of these animals is superior to that obtained from zebu cattle, as the meat has, among other quality attributes, a high content of intramuscular fat (HUFFMAN *et al*., 1990; PEACOCK *et al*., 1979), associated with greater tenderness.

Therefore, strategies such as the use of a cross between zebu and taurine have been used on a large scale in Brazil, exploring heterosis to create genetic variation as an additive to improve the efficiency of production and the quality of the beef *(CROUSE et al*., 1993). Therefore, crossing zebu and taurine has become an important strategy to improve meat production in different cattle farming systems in our country.

1.3.2 Cattle gender status and fat deposition

Attributes such as the general appearance, color and fat content are traits of meat that are considered by consumers when making purchasing decisions, and for this reason, these traits have been evaluated in many studies (LAGE *et al*., 2012; MAGGIONI *et al*., 2010; VIEIRA *et al*., 2007). Carcass and beef quality is significantly affected by the gender of cattle (GAGAOUA *et al*., 2015; MORAIS DE LIMA JÚNIOR *et al*., 2011; WĘGLARZ, 2010). Regarding the deposition of fat in the carcass, females have greater potential for the

deposition of fat and start this phase at lower weights than steers, and steers start it at a lower weight than bulls (BERG; BUTTERFIELD, 1976). The differences in fat deposition between females and males are caused by the differences in their growth curves (OWENS *et al*., 1995). Carcasses of steers and heifers, in general, have lower performance but higher contents of subcutaneous and intramuscular fat and better quality meat relative to the carcasses of bulls (SEIDEMAN *et al*., 1982; WĘGLARZ, 2010). In addition, the handling of bulls is challenging due to their sexual and aggressive behavior that increases the risks of the animal management team (BONNEAU; ENRIGHT, 1995; JAGO; BASS; MATTHEWS, 1997) and cause susceptibility to pre-slaughter stress, resulting in beef with a high pH, a dark color and less tenderness (ABERLE; FORREST, 2001).

The slaughter age of the females affects the quality traits of the beef. In general, females slaughtered in Brazil are older culls, possibly due to reproductive problems, genetic selection or advanced age, and these, in general, have low carcass yields and their meat is of inferior quality (LIMA *et al*., 2004). However, this scenario of only slaughtering old females in Brazil has been changing, as an increasing trend of slaughtering young females has been observed, with a subcutaneous fat layer on the carcass at least 3 to 6 mm thick, and, consequently, awards given by certified programs to the producers, with a bonus because their meat quality is like or even higher than that of steers.

In addition, the timing of slaughter of cattle is also influenced by gender. Animals of different gender statuses will reach slaughter time at different weights or ages *(OWENS et al*., 1993). As an example, females reach slaughter time earlier and they are lighter than males, castrated or not (PURCHAS, 1991). In a study carried out by Mueller *et al*. (2019), carcass and beef traits were compared among heifers, steers and bulls for crossbred Angus x Nelore with the same feedlot period and the same diet. Heifers showed superior carcass and beef quality traits, such as better marbling scores and subcutaneous fat, in addition to a more favorable fatty acid composition for human health as compared to steers and bulls.

Intramuscular fat is indispensable for meat palatability and flavor, and consequently, it becomes indispensable to obtain superior-quality meat (HAUSMAN *et al*., 2009). According to Lee *et al*. (2009), intramuscular fat is the last to be deposited, and heifers have genes that control the efficiency of fat deposition. According to (TATUM; GRUBER; SCHNEIDER, 2007), females normally outperform males in marbling and carcass quality due to their higher capacity to deposit fat. The same researchers found that cows and heifers had higher marbling scores, while bulls had lower rates than steers. As reported by Moore *et al*. (2012) and Choat *et al*. (2006), the carcasses of heifers had higher scores of

intramuscular fat in comparison with carcasses of steers. Thus, these differences found in the intramuscular fat content may be related to the differentiation of adipocytes, the metabolism of fatty acids and the expression of some important adipogenic genes, such as *PPARG*, *CEBPA*, *FABP4* and *WNT10B*, as mentioned by Del Pino *et al*. (2017).

1.3.3 Adipogenesis

Adipogenesis is the process of cell differentiation from preadipocytes to adipocytes which is essential for fat accumulation and the metabolism of adipose tissue by mammals (QUEIROZ *et al*., 2009). Preadipocytes are derived from adipocyte progenitor cells that arise from various sources in the body (DE SÁ *et al*., 2017). Adipose tissue deposition is attributed to an increase in the number of adipocytes (hyperplasia), volume of the adipocytes (hypertrophy) or a combination of both *(CIANZIO et al*., 1982; *HOOD; ALLEN, 1973;* JO *et al*., 2009). The intensity of the processes of hyperplasia and hypertrophy is influenced by factors such as genotype, gender, age, diet and the deposition of individual adipose tissue (CIANZIO *et al*., 1982; JO *et al*., 2009). Adipogenesis has been one of the most intensively studied cell differentiation models with regard to gene expression. While hormones, miRNAs, cytoskeletal proteins and many other factors can modulate the development of adipocytes, the best understood regulators of adipogenesis are the transcription factors that inhibit or promote this process (DE SÁ *et al*., 2017).

1.3.4 Adipogenesis modulators

Adipogenesis is the main pathway for the development of adipose tissue and, therefore, is influenced by a multiplicity of different factors, such as the different fat deposits, the cattle gender status (BJORNTORP, 1997; DE SÁ *et al*., 2017; LEE *et al*., 2000; MADRUGA *et al*., 2006a) and the diet (DEMIREL *et al*., 2006; SCOLLAN *et al*., 2006; WEBB; O'NEILL, 2008; WOODS; FEARON, 2009 WOOD; RICHARDSON, 2004). Researchers have shown that hormones regulate the development of adipocytes (AILHAUD; GRIMALDI; NKGREL, 1994; SMAS; SUL, 1995) and the distribution of body fat (BJORNTORP, 1997). Hormones are internal factors that are prominent modulators of adipogenesis. In multiple mammal species, physiological observations have indicated that adipose development is affected by several endocrine hormones and many steroid hormones (DE SÁ *et al*., 2017). According to Bjorntorp (1997), testosterone and growth hormone (GH) inhibit lipoprotein lipase (LPL) and markedly stimulate lipolysis.

Testosterone also regulates the mobilization of lipids. The adipose tissue of females also contains an androgen receptor, apparently identical to that of males, as assessed by its specificities and affinity determinations (BJORNTORP, 1997). It appears, however, that the effects of testosterone on female adipose tissue may differ from those on male tissues. Complete replacement of the lipolytic machinery after the removal of the ovaries can be achieved with estrogen but not with testosterone (DE PERGOLA *et al*., 1990). In addition, the expression level of the androgen receptor appears to be decreased by estrogen, suggesting that protection against androgenic effects is provided by estrogen (BJORNTORP, 1997).

In summary, in male adipose tissue, testosterone and GH prevent the accumulation of lipids and stimulate lipid mobilization through an androgen receptor. The density of this receptor appears to be regulated by testosterone. This action is probably more pronounced in visceral fat, where the androgen receptor density appears to be greater than in other regions (BENGTSSON *et al*., 1993). On the other hand, in female adipose tissue, the effects of testosterone appear to be different regarding the accumulation of visceral fat. Therefore, it is suggested that there is a direct relationship between the hormonal profile and the formation and lipid composition in cattle of different gender (LEE, 2000). The role of steroidal sex hormones (testosterone and estradiol) in the development and distribution of adipose tissue was investigated by (DIEUDONNE *et al.*, 2000), who studied the effect of these sex steroids on the proliferation and differentiation of preadipocytes in rats, from the deposition of deep to superficial fat. According to these authors, in differentiated preadipocytes that were exposed to sexual steroids, the expression of the main transcription factor responsible for the adipocyte development, *PPARγ*, was increased by estradiol but not by testosterone. Therefore, it is believed that these genes can be expressed differently in different gender status and different muscles of cattle.

The tropomyosin gene family has been found to modulate the formation of adipose tissue (CHO *et al*., 2016). Tropomyosins (TPMs) are a family of actin-linked proteins found in all tissues. Tropomyosins consist of a diverse group of cytoskeletal proteins found in most eukaryotic cells, with distinct isoforms found in muscles (skeletal, cardiac and smooth) and in several nonmuscle cells (DLUGOSZ *et al*., 1984; LIN; LIN, 1986). This family of genes is involved in the transport of GLUT4 to the cell membrane, thus modulating glucose entry (KEE *et al*., 2015). To elucidate whether the properties of the proteins involved in the specific deposition of adipose tissue were sex-dependent, Cho *et al.* (2016) analyzed the protein expression of the intramuscular adipose tissue of Hanwoo

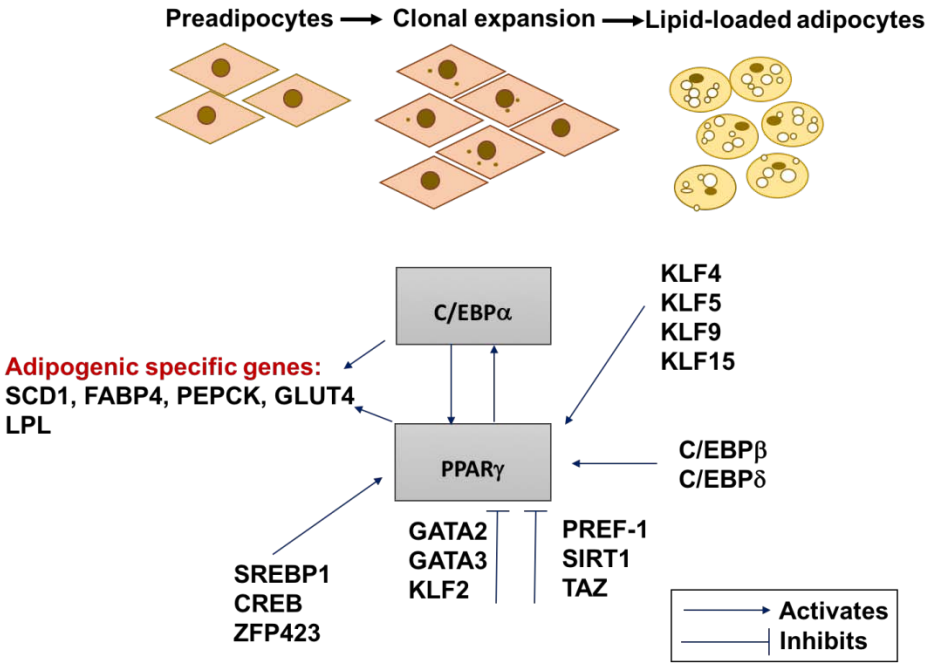
bulls, steers and heifers. They concluded that the levels of transcripts and proteins of the *TPM1*, *TPM2* and *TPM3* genes were differentially expressed depending on the cattle's gender. These transcripts were lower in bulls than in steers and heifers, suggesting that such genes were positively correlated with intramuscular fat deposition and meat quality, in addition are key factors closely associated with muscle development and lipid accumulation in Hanwoo heifers, steers, and bulls.

1.3.5 Transcriptional control of adipocyte development

The differentiation of preadipocytes to adipocytes involves a comprehensive network, including transcription factors responsible for the expression of key proteins that induce the formation of mature adipocytes (FARMER, 2006). Transcription factors are proteins that play an important role in regulating gene expression at the level of transcription, which is the process of transcribing DNA into RNA. In addition to RNA polymerase, the expression of each gene is controlled by many transcription factors that are regulated by physiological and developmental processes. Among the many processes that contribute to controlling gene expression, regulation of transcription is the most common. There are several different families of transcription factors, many classified by the structural features of their DNA-binding domains (DE SÁ *et al*., 2017).

Transcription factors can promote or inhibit the development of adipocytes. Many transcription factors are induced during adipocyte differentiation. In mammalian cells, for example, transcription factors belonging to the C/EBP (*enhancer binding protein*) and PPAR (*peroxisome proliferator-activated receptor*) (DODSON *et al*., 2010; DU; YIN; ZHU, 2010; YAMADA; KAWAKAMI; NAKANISHI, 2009) families are the main modulators of adipocyte development. However, according to Moseti *et al*. (2016), besides the C/EBP*α* expression, PPAR induces the expression of other target genes involved in adipogenesis, including stearoyl CoA desaturase-1 (SCD1), phosphoenol pyruvate carboxykinase (PEPCK), aP2 and Glucose transporter 4 (GLUT4). *The Kruppel-Like Factors* KLF4, KLF5, KLF9 and KLF15 also have been identified and shown to positively regulate adipocyte differentiation (Figure 1).

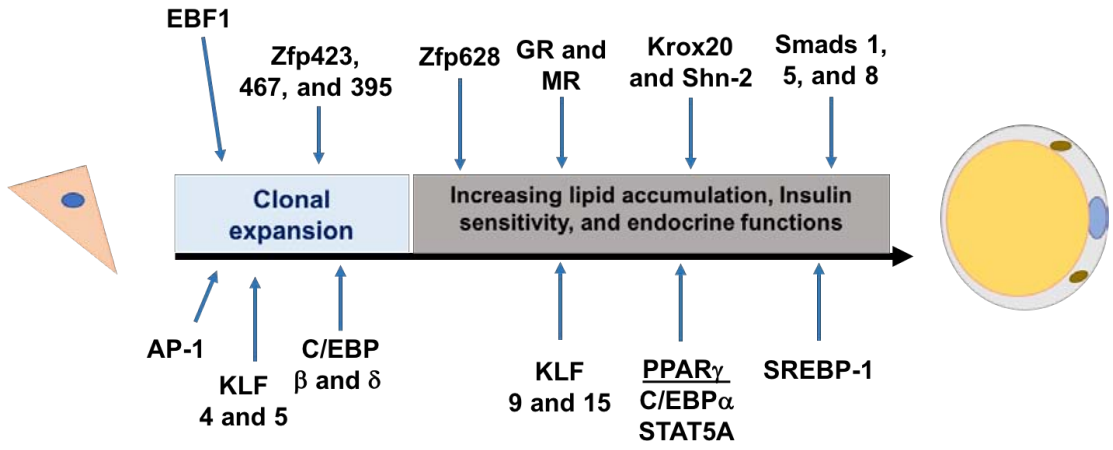
Figure 1 - Molecular regulation of adipogenesis



Adapted from Moseti *et al*. (2016).

During adipogenesis, the transcription factor *CEBPα* is activated and it binds directly to promoters of the *PPARγ* gene, inducing its expression. The expression of *PPARγ* triggers the expression of *CEBPα*, promoting a self-regulating action between these factors, and together, they act by stimulating the differentiation of mesenchymal cells into adipocytes (ROSEN *et al*., 2002). The Kruppel-like family (KLFs), Sterol Regulatory Element-binding Protein 1 (SREBP-1), Cyclic AMP Response Element-binding Protein, and Zinc Finger Protein 423 (Zfp423) are factors that promote adipocyte differentiation *in vitro* and *in vivo* (DE SÁ *et al*., 2017; Figure 2).

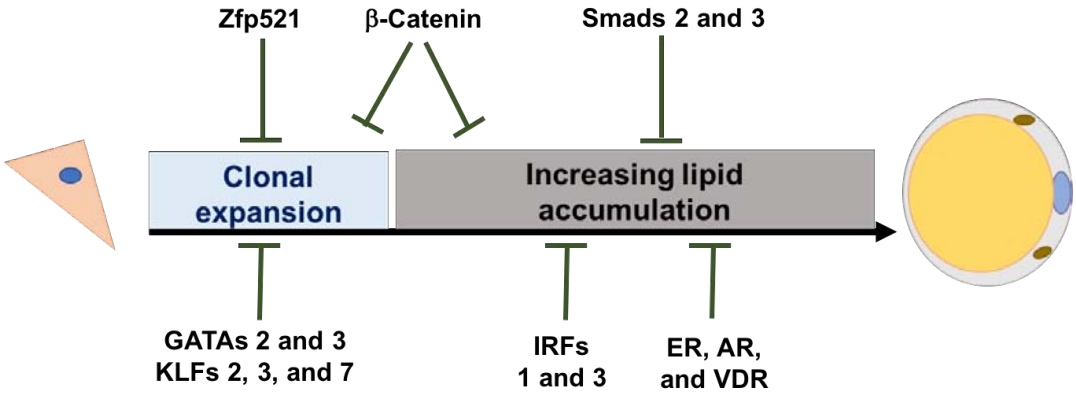
Figure 2 - Transcription factors that promote adipogenesis



Adapted from De Sá *et al*. (2017).

However, according De Sá *et al*. (2017), many specific transcription factors inhibit the development of adipocytes, by decreasing PPAR, C/EBP*α*, and SREBP1 expression, like the Kruppel-like factor 2 (KLF 2), or decreasing only PPAR expression, like GATA binding protein 2 and GATA binding protein 3 (GATA 2 and 3), and the expression of most of these factors decreases with the development of adipocytes (Figure 3).

Figure 3 - Transcription factors that inhibit adipogenesis



Adapted from De Sá *et al*. (2017).

1.3.6 Effects of beef fatty acids on human health and meat quality

Meat is the most important source of animal protein for the human diet, and currently, research has been conducted to improve the quality of beef, attracting consumers and expanding its competition with poultry and pork. Meat quality, in general, is evaluated by

sensory traits; however, other aspects are also relevant in the evaluation of quality, including the fat content and the composition of the fatty acids, mainly polyunsaturated (PUFA) and conjugated linoleic acid (FERNANDES *et al*., 2009).

An important technological issue is related to adipose tissue, which is an important component of the carcass and determines its quality and economic value. Adipose tissue is a very flexible component compared to muscle and bone tissue (BERG; BUTTERFIELD, 1976). Fat also assists in the transport and absorption of fat-soluble vitamins A, D, E and K through the intestine, in addition to playing an important role in the immune response, both in humans and in animals (WEBB; O'NEILL, 2008).

According to Wood and Richardson (2004), the profile of consumers has changed, and there has been a greater concern with the nutritional quality of meat, especially in relation to the composition of fat. In addition to consumer concerns with meat quality, there is also growing concerns with human health in relation to the beef intake, as well as the recommendations by public health agencies of the ingestion of PUFAs and achieving a dietary balance among unsaturated fatty acids (WOOD; RICHARDSON, 2004). However, many consumers do not educate about the quantity and quality of meat fat that is suitable for eating and can provide health benefits. In addition, long-chain fatty acids in adipose or muscle tissue contribute to important aspects of meat quality and are fundamental to its nutritional and sensory values (WEBB; O'NEILL, 2008).

The excessive consumption of fat, mainly saturated, of both animal and vegetable origin, influences the inflammatory process, leading to the appearance of cardiovascular diseases, diabetes and cancer (CALDER *et al*., 2009; MARQUES; VALENTE; ROSA, 2009). The growing interest in developing strategies to manipulate the fatty acid composition of beef is related to the need to produce healthier meat to reduce the association of beef consumption with the occurrence of diseases (WOOD; RICHARDSON, 2004). Therefore, studies are needed to investigate the fat content that can be consumed without damaging health. In this sense, ruminant meat fat has a higher concentration of saturated fatty acids (SFAs) and a lower PUFA:SFA ratio than nonruminant meat, mainly due to the biohydrogenation process of unsaturated fatty acids in the rumen by the action of microorganisms (FRENCH *et al*., 2000).

However, not all SFAs are considered hypercholesterolemic (which increases the levels of bad cholesterol - LDL). The stearic FA (18:0), for example, represents 43% of the total SFA in meat (FREITAS, 2006), and have a neutral effect, as it is turned into oleic acid (C18:1) in the body, not influencing blood cholesterol levels (SINCLAIR, 1993). In

contrast, the most undesirable fatty acid is myristic acid (14:0) (FRENCH *et al*., 2003), a saturated FA which in the study by Mueller *et al*. (2019), represented only 3% of the total FA in beef. Additionally, palmitic FA (16:0) was cited as having the least hypercholesterolemic effect (FRENCH *et al*., 2003).

For monounsaturated fatty acids (MUFAs), higher values of oleic acid in cis form are desirable because they have a hypocholesterolemic action, with the advantage of not reducing HDL cholesterol (good cholesterol), acting in the protection against coronary diseases. These FAs are said to be "good for health". Of all FAs, Mueller *et al*. (2019) observed that oleic acid is the one with the highest concentration in the meat of heifers, steers, and bulls, being the major FA among the total MUFAs.

The proportion of PUFA n6:n3 is particularly beneficial (balanced) in the meat of ruminants. These FAs have several effects on the immune and inflammatory response. PUFAs of the n3 series have suppressive effects, such as inhibition of lymphocyte proliferation, production of antibodies and cytokines, expression of adhesion molecules and the activation of natural killer (NK) cells. PUFAs of the n6 series have both inhibitory and stimulating effects on the immune response (ANDRADE; CARMO, 2006; CALDER *et al*., 2009). The ratio of the daily intake of source foods in FA n6:n3 is of great importance in human health, but the recommendations vary according to some authors and countries. The convergence trend of the proportion between FA n6:n3 is in the range of 4:1 to 5:1 (HEALTH *et al*., 1990; NATIONAL ACADEMIES OF SCIENCES AND MEDICINE, 2016; SCHAEFER, 2002). The essential FAs include the n-3 and n-6 families, which are not biologically synthesized by humans, but they are necessary for biological processes and therefore should be eaten in the human diet.

Conjugated linoleic acid (CLA) is representative of micro components in products of animal origin, which are a mixture of FAs that occur as intermediates in the biohydrogenation of PUFAs (BAUMAN *et al*., 1999). This substance is interesting because it acts as a potent natural anticarcinogen, prevents atherosclerosis and diabetes (RAINER; HEISS, 2004), and acts as a nutrient-sharing agent capable of altering the deposition of fat and muscle. CLA not only prevents but also “attacks” tumor cells already present in the body, reducing previously formed tumors, but it also has positive effects on immune function (COOK *et al*., 1993) and body composition (RAINER; HEISS, 2004). Mueller *et al*. (2019) showed a greater CLA c9,t11 isomer content in beef of heifers than in steer and bull beef of crossbred Angus x Nelore cattle.

Additionally, grass-fed beef has been shown to contain higher quantities of CLA than grain-fed beef (SHANTHA; CRUM; DECKER, 1994). According to Jiang *et al*. (1999), the average intake of the c9,t11 isomer of CLA is 0.16 g/day and Fritsche and Steinhart (1998) suggested that the average CLA c9,t11 isomer intake is 0.35 g/day among women and 0.43 g/day among men, with meat and meat products supplying approximately a quarter of total intake of the c9,t11 isomer. On the other hand, Sanhueza *et al*. (2002) reported that to obtain biological effects, a human being would need to consume approximately 5g of CLA per day. Furthermore, on average, a single serving of nearly 100g of beef from pasture-raised animals provides 1.23 grams of CLA, 25% of the daily requirement for a biological effect (DUGAN *et al*., 1999). In contrast, beef from feedlot cattle provides 0.48 grams in 100g of meat, providing 9.6% of the CLA needed for positive physiological effects (FRENCH *et al*., 2000).

Regarding technological properties and chemical composition, especially the fatty acid composition, the quality of beef is better in heifers than in bulls (MUELLER *et al*., 2019; SHARAF ELDIN *et al*., 2013; VENKATA REDDY *et al*., 2015). According to Panjono et al. (2009), the hormonal status of cattle is directly related to the distribution of fatty acids in muscles, and females have genes more favorable to fat deposition. According to previous studies (DE SMET; RAES; DEMEYER, 2004; WOOD *et al*., 2008), the differences in the fatty acid composition of meat are also associated with the intramuscular fat content due to the higher proportion of membrane phospholipids (high in PUFAs) and lower triacylglycerol (high in SFAs and MUFAs) in leaner animals. Mueller *et al*. (2019) and Barton *et al*. (2011) reported a higher content of MUFAs in heifer meat than in bull meat. Moreover, Ardiyanti *et al*. (2009) observed that in the intramuscular adipose tissue of heifers, the C allele showed a significant increase in the content of oleic acid, MUFAs and unsaturated fatty acids. In addition, the contents of palmitic acid and SFAs were reduced. In a study evaluating the fatty acid composition of beef from crossbred Angus x Nelore cattle, Mueller *et al*. (2019) observed that cattle gender directly influences the fatty acid composition of beef by comparing heifers, steers and bulls. The beef of heifers showed a fatty acid composition more favorable to human health compared to the meat of bulls and steers, due to the lower n6:n3 proportion and the lower fatty acid content of the n6 group, which are considered harmful to health, and higher contents of CLA, MUFAs and oleic acid, considered beneficial to human health.

1.3.7 Relationship of Fatty Acids Composition to Muscles

Regarding the content and composition of fat in foods, this has been a target of concern in human nutrition, since SFAs present in beef are related to the development of cardiovascular diseases and cancers (WOOD; RICHARDSON, 2004). This higher saturation in the meat of ruminants is due to the process of microbial biohydrogenation in the rumen. The production of specific biohydrogenation intermediates in the rumen is an area of increasing interest due to the recognized potential of individual fatty acids to induce specific and potent effects on the metabolism of ruminants and on human health (LOCK; HARVATINE; DRACKLEY, 2006).

According to Purchas and Zou (2008), the composition of FAs can be modified depending on the muscle. These researches observed differences between the *infraspinatus* and *longissimus* muscles for the composition of SFAs, MUFAs and PUFAs, together with the concentrations of some bioactive components (coenzyme Q10, carnosine and taurine) in the meat of steers and bulls. These findings suggested that such differences found in the study seem to vary more between the evaluated muscles than between the groups of animals, either finished on pasture or in feedlots. As a way to better explore this issue, Purchas and Zou (2008) also reported that palmitic and stearic FAs showed differences between the muscles evaluated, whereas *longissimus* presented a higher content of palmitic than *infraspinatus*, while stearic FAs were greater in *infraspinatus*. Regarding MUFAs, a higher content of elaidic and transvaccenic acids (precursor to CLA) were observed in the *infraspinatus* muscle than in the *longissumus* muscle. For PUFAs, *infraspinatus* showed higher concentrations of CLA than *longissimus* muscle. On the other hand, the contents of EPA and DHA were lower in *infraspinatus*, as well as the sum of three fatty acids from the n3 group (EPA + DPA + DHA) compared to *longissimus*. Raes et al. (2004) evaluated the differences in the fatty acid composition between the *longissimus* and *triceps brachii* muscles and found higher contents of SFAs and MUFAs in *longissimus*. For total PUFAs, n3 and n6, the contents were higher in *triceps* muscle. Turk and Smith (2009) found higher levels of MUFAs in the *superficial pectoral* muscle and higher levels of SFAs in the *obliquus abdominis internus* muscle.

Therefore, based on the cited literature, it is suggested that muscles present differences in FA deposition, depending on the amount of connective tissue, types of muscle fibers, as well as metabolism, which varies between them. Thus, the difference between muscles can

infer whether one is more favorable for human health than the other, based on the FA composition.

1.3.8 Relationship of gene expression with muscles

It is well established that the muscles present differences in their types of muscle fiber, proteolytic activity, connective tissue and percentage of intramuscular fat, attributes that are related to the molecular, metabolic, structural and contractile properties of the muscle (MORENO-SÁNCHEZ *et al*., 2010; ODDY *et al*., 2001). These characteristics also have a profound influence on the meat traits (DAI *et al*., 2009). In live animals, the muscle is in dynamic balance between the stage of development, the external nutritional environment and the workload. It is the dynamic nature of the muscle that allows environmental effects to be transferred into intrinsic properties that affect the quality of the meat. In view of this, the identification of differentially expressed genes involved in muscle functionality (contractile, metabolic and structural properties) and their potential relationship with differences in muscle flexibility and meat quality are areas of interest (BERNARD *et al*., 2007).

However, according to the literature, most studies in beef cattle only concern the *longissimus* muscle, which can be a limiting factor since the different anatomical regions and divergent functionalities of the muscles may be related to differences in the accumulation of fat and their metabolism (COSTA *et al*., 2008; HOCQUETTE *et al*., 2010; SMITH *et al*., 2009; SORET *et al*., 2016). This, in turn, could be associated with the differential expression of key genes involved in the deposition and development of intramuscular fat. Therefore, extrapolating these results to other muscle types remains a challenge.

Moreno-Sánchez *et al.* (2010) studied the differential expression of genes related to meat quality traits in different muscles (*psoas major* - PM and *flexor digitorum* - FD). Specifically, the increased expression of the *CRYAB* (Crystallin, alpha B) and *CSRP3* (Cysteine and glycine-rich protein 3) genes in the FD muscle was associated with low sensory scores for tenderness, flavor and juiciness compared to the expression of the same genes in the PM muscle. Among other meat quality properties, differences in the intramuscular fat content have been described between the two muscles already mentioned, FD and PM (DÍAZ *et al*., 2006), with PM showing a higher intramuscular fat content.

However, two genes related to fat (*FABP3* and *CLU*) were highly expressed in the FD muscle in comparison to PM.

Additionally, Del Pino *et al.* (2017) evaluated the possible differences in fat, collagen and protein content, as well as the size distribution of adipocytes, from different muscles to determine whether the expression of some adipogenic genes (*PPARγ, CEBPα, WNT10B* and *FABP4*) was associated with muscle and fat deposition. The muscles selected for the study were the *longissimus thoracis (*LT), *semitendinosus* (ST), *masseter* (MS) and *sternormandibularis* (SM). The criteria for muscle selection was according to its dominant metabolism, being either predominantly glycolytic (LT and ST) (LISTRAT *et al*., 2016) or predominantly oxidative (MS and SM) (TOTLAND; KRYVI, 1991) and their commercial value (TUME; SIKES; SMITH, 2010). The researchers found no differences among the muscles for the expression of the main modulators of adipocyte development, *PPARγ* and *CEBPα*, and for the *WNT10B* gene, known as a blocker of adipogenesis and inhibitor of the IMF deposition. The expression of *FABP4* was higher in the LT muscle than in the MS and SM muscles.

The *FABP4* gene is expressed mainly in adipocytes (GERBENS *et al*., 1998; MATARESE; BERNLOHRS, 1988) and encodes proteins related to the absorption, transport and metabolism of fatty acids (GREGOIRE; SMAS; SUL, 1998), which are involved in the deposition of fat (BARENDSE *et al*., 2009; MICHAL *et al*., 2006; THALLER et *al*., 2003). The reason why *FABP4* expression was higher in the *longissimus* muscle than in the other muscles remains unknown, but it may be related to the involvement of *FABP4* in the increase in long-chain fatty acids and the different partitioning of nutrients between organs and tissues that could occur at different points in the development of animals. However, the accumulation of the *FABP* protein could also be an indicator of the number of adipocytes in muscle tissue (ALBRECHT *et al*., 2017; SOUFFRANT; METGES, 2003) and therefore implies a greater number of adipose cells, although probably of smaller size, in the *longissimus* muscle.

Thus, it is important to elucidate the molecular mechanisms involved in adipogenesis, in different muscles and cattle gender status, since the deposition of fat in these animals is different, in order to apply new tools to add greater value to the meat produced.

References

ABERLE, E. D.; FORREST, J. C. **Principles of meat science**. [s.l.] Kendall Hunt, 2001.

ABIEC. **Beef report: Perfil da Pecuária no Brasil 2021**. Disponível em: <http://abiec.com.br/publicacoes/beef-report-2021/>.

AILHAUD, G.; GRIMALDI, P.; NKGREL, R. Hormonal Regulation of Adipose Differentiation. **Trends Endocrinol Metab**, v. 5, p. 132–136, 1994.

ALBRECHT, E. et al. Triennial growth and development symposium: Factors influencing bovine intramuscular adipose tissue development and cellularity. **Journal of Animal Science**, v. 95, n. 5, p. 2244–2254, 1 maio 2017.

ANDRADE, P. de M. M.; CARMO, M. G. T. Ácidos graxos n-3: um link entre eicosanóides, inflamação e imunidade. **Revista Mn-Metabólica**, v. 8, n. 3, p. 135–143, 2006.

ARBOITTE, M. Z. et al. Composição Física da Carcaça, Qualidade da Carne e Conteúdo de Colesterol no Músculo Longissimus dorsi de Novilhos 5/8 Nelore-3/8 Charolês Terminados em Confinamento e Abatidos em Diferentes Estádios de Maturidade 1 Carcass Physical Composition, Meat Qual. **Revista Brasileira de Zootecnia**, p. 959–968, 2004.

ARDIYANTI, A. et al. Effects of GH gene polymorphism and sex on carcass traits and fatty acid compositions in Japanese Black cattle. **Animal Science Journal**, v. 80, n. 1, p. 62–69, fev. 2009.

ASSOCIATION, A. M. S.; (US), N. C. B. A.; (US), N. P. P. C. **Meat evaluation handbook**. [s.l.] Amer Meat Science Assn, 2013.

BAIK, M. et al. Effects of castration on the adiposity and expression of lipid metabolism genes in various fat depots of Korean cattle. **Livestock Science**, v. 168, p. 168–176, 2014.

BARENDSE, W. et al. A splice site single nucleotide polymorphism of the fatty acid binding protein 4 gene appears to be associated with intramuscular fat deposition in longissimus muscle in Australian cattle. **Animal Genetics**, v. 40, n. 5, p. 770–773, out. 2009.

BARTON, L. et al. Effect of sex and age on bovine muscle and adipose fatty acid composition and stearoyl-CoA desaturase mRNA expression. **Meat Science**, v. 89, n. 4, p. 444–450, dez. 2011.

BAUMAN, D. E. et al. Biosynthesis of conjugated linoleic acid in ruminants. In: Proc. Am. Soc. Anim. Sci, **Anais**...1999.

BENGTSSON, B.-I. et al. **Treatment of Adults with Growth Hormone (GH) Deficiency with Recombinant Human GH*Journal of Clinical Endocrinology and Metabolism Copyright**. [s.l: s.n.].

BERG, R. T. (Roy T.; BUTTERFIELD, R. M. (Rex M. **New concepts of cattle growth**. [s.l.] Sydney University Press, 1976.

BERNARD, C. et al. New indicators of beef sensory quality revealed by expression of specific genes. **Journal of Agricultural and Food Chemistry**, v. 55, n. 13, p. 5229–5237, 27 jun. 2007.

BERNARD, L.; LEROUX, C.; CHILLIARD, Y. Expression and nutritional regulation of lipogenic genes in the ruminant lactating mammary gland. **Bioactive components of milk**, p. 67–108, 2008.

BERTON, M. P. et al. Gene expression profile of intramuscular muscle in Nellore cattle with extreme values of fatty acid. **BMC Genomics**, v. 17, n. 1, 25 nov. 2016.

BIANCHINI, W. et al. Efeito do grupo genético sobre as características de carcaça e maciez da carne fresca e maturada de bovinos superprecoces 1. **Revista Brasileira de Zootecnia**, v. 36, n. 6, p. 2109–2117, 2007. Disponível em: <www.sbz.org.br>.

BIONAZ, M.; THERING, B. J.; LOOR, J. J. Fine metabolic regulation in ruminants via nutrient-gene interactions: Saturated long-chain fatty acids increase expression of genes involved in lipid metabolism and immune response partly through PPAR-α activation. **British Journal of Nutrition**, v. 107, n. 2, p. 179–191, 28 jan. 2012.

BJORNTORP, P. Hormonal control of regional fat distribution. **Human reproduction**, v. 12, n. supplemen, 1997.

BLANCO, M. et al. Performance, carcass and meat quality of young bulls, steers and heifers slaughtered at a common body weight. **Livestock Science**, v. 240, p. 104156, 2020.

BLECHA, I. M. Z. et al. Identification and evaluation of polymorphisms in FABP3 and FABP4 in beef cattle. **Embrapa Gado de Corte-Artigo em periódico indexado (ALICE)**, 2015.

BONG, J. J. et al. Differential expression of genes associated with lipid metabolism in longissimus dorsi of Korean bulls and steers. **Meat science**, v. 91, n. 3, p. 284–293, 2012.

BONNEAU, M.; ENRIGHT, W. J. Immunocastration in cattle and pigs. **Livestock Production Science**, v. 42, p. 193–200, 1995.

BONNET, M. et al. **Nutrient-Gene Expression Lipoprotein Lipase Activity and mRNA Are Up-Regulated by Refeeding in Adipose Tissue and Cardiac Muscle of Sheep 1,2**. [s.l: s.n.]. Disponível em: <https://academic.oup.com/jn/article/130/4/749/4686705>.

BONNET, M. et al. Ontogenesis of muscle and adipose tissues and their interactions in ruminants and other species. **Animal**, v. 4, n. 7, p. 1093–1109, 2010.

BRIGGS, M. A.; PETERSEN, K. S.; KRIS-ETHERTON, P. M. Saturated fatty acids and cardiovascular disease: replacements for saturated fat to reduce cardiovascular risk. In: Healthcare, 2, **Anais**...Multidisciplinary Digital Publishing Institute, 2017.

CALDER, P. C. et al. **Inflammatory disease processes and interactions with nutritionBritish Journal of Nutrition**Cambridge University Press, , 2009. .

CARVALHO, R. M. S. et al. Differences between sexes, muscles and aging times on the quality of meat from Wagyu × Angus cattle finished in feedlot. **Animal Production Science**, v. 58, n. 2, p. 350–357, 2016.

CESAR, A. S. M. et al. Differences in the skeletal muscle transcriptome profile associated with extreme values of fatty acids content. **BMC genomics**, v. 17, n. 1, p. 1–16, 2016.

CHEN, D. et al. Adipogenesis, fibrogenesis and myogenesis related gene expression in longissimus muscle of high and low marbling beef cattle. **Livestock Science**, v. 229, p. 188–193, 2019.

CHO, J. H. et al. Regional differences of proteins expressing in Adipose depots isolated from cows, steers and bulls as identified by a proteomic approach. **Asian-Australasian Journal of Animal Sciences**, v. 29, n. 8, p. 1197–1206, 1 ago. 2016.

CHO, S. et al. Fatty acid profiles and sensory properties of longissimus dorsi, triceps brachii, and semimembranosus muscles from Korean Hanwoo and Australian Angus beef. **Asian-Australasian journal of animal sciences**, v. 18, n. 12, p. 1786–1793, 2005.

CHOAT, W. T. et al. The effects of cattle sex on carcass characteristics and longissimus muscle palatability. **Journal of Animal Science**, v. 84, n. 7, p. 1820–1826, jul. 2006.

CHUNG, K. Y. et al. Lipid characteristics of subcutaneous adipose tissue and M. longissimus thoracis of Angus and Wagyu steers fed to US and Japanese endpoints. **Meat Science**, v. 73, n. 3, p. 432–441, 2006.

CIANZIO, D. S. et al. Adipose tissue growth in cattle representing two frame sizes: Distribution among depots. **Journal of Animal Science**, v. 55, n. 2, p. 305–312, 1982.

COLEMAN, L. W. et al. Carcass characteristics and meat quality of Hereford sired steers born to beef-cross-dairy and Angus breeding cows. **Meat Science**, v. 121, p. 403–408, 2016.

COOK, M. E. et al. Immune modulation by altered nutrient metabolism: nutritional control of immune-induced growth depression. **Poultry science**, v. 72, n. 7, p. 1301–1305, 1993.

COSTA, P. et al. Muscle fiber and fatty acid profiles of Mertolenga-PDO meat. **Meat Science**, v. 78, n. 4, p. 502–512, abr. 2008.

CROUSE, J. D. et al. **Comparisons of Comparisons of Bos indicus Bos indicus and and Bos taurus Bos taurus Inheritance for Inheritance for Carcass Beef Characteristics and Meat Palatability Carcass Beef Characteristics and Meat Palatability Comparisons of Bos indicus and Bos ta**. [s.l: s.n.]. Disponível em: <https://digitalcommons.unl.edu/hruskareports/121>.

DAI, F. et al. **Developmental differences in carcass, meat quality and muscle fibre characteristics between the Landrace and a Chinese native pigSouth African Journal of Animal Science**. [s.l: s.n.]. Disponível em: <http://www.sasas.co.za/sajas.asp>.

DE PERGOLA, G. et al. Testosterone treatment of ovariectomized rats: effects on lipolysis regulation in adipocytes. **European Journal of Endocrinology**, v. 123, n. 1, p. 61–66, 1990.

DE SÁ, P. M. et al. Transcriptional regulation of adipogenesis. **Comprehensive Physiology**, v. 7, n. 2, p. 635–674, 1 abr. 2017.

DE SMET, S.; RAES, K.; DEMEYER, D. **Meat fatty acid composition as affected by fatness and genetic factors: A reviewAnimal Research**, mar. 2004. .

DEL PINO, L. M. et al. Adiposity and adipogenic gene expression in four different muscles in beef cattle. **PLoS ONE**, v. 12, n. 6, 1 jun. 2017.

DEMIREL, G. et al. Fatty acids of lamb meat from two breeds fed different forage: Concentrate ratio. **Meat Science**, v. 72, n. 2, p. 229–235, fev. 2006.

DÍAZ, C. et al. Genetic basis of beef quality differences between muscles in beef cattle: Avileña negra-ibérica, a study case. In: Proceedings of the XVI Congresso de Zootecnia, Castelo Branco (Portugal), **Anais**...2006.

DIEUDONNE, M. N. et al. Opposite effects of androgens and estrogens on adipogenesis in rat preadipocytes: evidence for sex and site-related specificities and possible involvement of insulin-like growth factor 1 receptor and peroxisome proliferator-activated receptorγ 2. **Endocrinology**, v. 141, n. 2, p. 649–656, 2000.

DLUGOSZ, A. A. et al. The relationship between stress fiber-like structures and nascent myofibrils in cultured cardiac myocytes. **The Journal of cell biology**, v. 99, n. 6, p. 2268–2278, 1984.

DODSON, M. V et al. **Lipid metabolism, adipocyte depot physiology and utilization of meat an-imals as experimental models for metabolic researchInt. J. Biol. Sci**. [s.l: s.n.]. Disponível em: <http://www.biolsci.org691>.

DOWHAN, W.; MILEYKOVSKAYA, E.; BOGDANOV, M. Diversity and versatility of lipid–protein interactions revealed by molecular genetic approaches. **Biochimica et Biophysica Acta (BBA)-Biomembranes**, v. 1666, n. 1–2, p. 19–39, 2004.

DRANSFIELD, E. et al. Meat quality and composition of three muscles from French cull cows and young bulls. **Animal Science**, v. 76, n. 3, p. 387–399, 2003.

DU, M. et al. Meat science and muscle Biology Symposium: Manipulating mesenchymal progenitor cell differentiation to optimize performance and carcass value of beef cattle. **Journal of Animal Science**, v. 91, n. 3, p. 1419–1427, mar. 2013.

DU, M.; YIN, J.; ZHU, M. J. **Cellular signaling pathways regulating the initial stage of adipogenesis and marbling of skeletal muscleMeat Science**, set. 2010. .

DUGAN, M. E. R. et al. The effects of feeding conjugated linoleic acid on subsequent pork quality. **Canadian Journal of Animal Science**, v. 79, n. 1, p. 45–51, 1999.

EGUINOA, P. et al. Lipogenic enzyme activities in different adipose depots of Pirenaican and Holstein bulls and heifers taking into account adipocyte size. **Journal of Animal Science**, v. 81, n. 2, p. 432–440, 2003.

ERKKILÄ, A. et al. Dietary fatty acids and cardiovascular disease: an epidemiological approach. **Progress in lipid research**, v. 47, n. 3, p. 172–187, 2008.

FARMER, S. R. **Transcriptional control of adipocyte formationCell Metabolism**, out. 2006. .

FAVERO, R. et al. Crossbreeding applied to systems of beef cattle production to improve performance traits and carcass quality. **Animal**, v. 13, n. 11, p. 2679–2686, 1 nov. 2019.

FEITOSA, F. L. B. et al. Genetic correlation estimates between beef fatty acid profile with meat and carcass traits in Nellore cattle finished in feedlot. **Journal of Applied Genetics**, v. 58, n. 1, p. 123–132, 1 fev. 2017.

FERNANDES, A. R. M. et al. Composição química e perfil de ácidos graxos da carne de bovinos de diferentes condições sexuais recebendo silagem de milho e concentrado ou cana-de-açúcar e concentrado contendo grãos de girassol. **Revista Brasileira de Zootecnia**, v. 38, n. 4, p. 705–712, 2009.

FERRAZ, J. B. S.; FELÍCIO, P. E. de. **Production systems - An example from BrazilMeat Science**, fev. 2010. .

FOLCH, J.; LEES, M.; STANLEY, G. H. S. A simple method for the isolation and purification of total lipides from animal tissues. **Journal of biological chemistry**, v. 226, n. 1, p. 497–509, 1957.

FREITAS, A. K. de. **CARACTERÍSTICAS DA CARCAÇA, DA CARNE E PERFIL DOS ÁCIDOS GRAXOS DE NOVILHOS NELORE INTEIROS OU CASTRADOS EM DUAS IDADES**. 2006. 2006.

FRENCH, P. et al. Fatty acid composition, including conjugated linoleic acid, of intramuscular fat from steers offered grazed grass, grass silage, or concentrate-based diets. **Journal of Animal Science**, v. 78, n. 11, p. 2849–2855, 2000.

FRENCH, P. et al. Fatty acid composition of intra-muscular triacylglycerols of steers fed autumn grass and concentrates. **Livestock Production Science**, v. 81, n. 2–3, p. 307–317, 2003.

FRITSCHE, J.; STEINHART, H. Analysis, occurrence, and physiological properties of trans fatty acids (TFA) with particular emphasis on conjugated linoleic acid isomers (CLA)–a review. **Lipid/Fett**, v. 100, n. 6, p. 190–210, 1998.

GAGAOUA, M. et al. Understanding Early Post-Mortem Biochemical Processes Underlying Meat Color and pH Decline in the Longissimus thoracis Muscle of Young Blond dÁquitaine Bulls Using Protein Biomarkers. **Journal of Agricultural and Food Chemistry**, v. 63, n. 30, p. 6799–6809, 5 ago. 2015.

GAGAOUA, M. et al. Associations among protein biomarkers and pH and color traits in longissimus thoracis and rectus abdominis muscles in protected designation of origin Maine-Anjou cull cows. **Journal of Agricultural and Food Chemistry**, v. 65, n. 17, p. 3569–3580, 2017.

GAMA, L. T. et al. Heterosis for meat quality and fatty acid profiles in crosses among Bos indicus and Bos taurus finished on pasture or grain. **Meat Science**, v. 93, n. 1, p. 98–104, jan. 2013.

GAMARRA, D. et al. Distinct correlations between lipogenic gene expression and fatty acid composition of subcutaneous fat among cattle breeds. **BMC veterinary research**, v. 14, n. 1, p. 1–12, 2018.

GANDEMER, G. Lipids and meat quality: lipolysis, oxidation, maillard reaction and flavour. **Sciences des Aliments (France)**, 1999.

GERBENS, F. et al. **The adipocyte fatty acid-binding protein locus: characterization and association with intramuscular fat content in pigs**. [s.l: s.n.].

GREGOIRE, F. M.; SMAS, C. M.; SUL, S. **Understanding Adipocyte DifferentiationPHYSIOLOGICAL REVIEWS**. [s.l: s.n.].

GUPTA, R. K. et al. Transcriptional control of preadipocyte determination by Zfp423. **Nature**, v. 464, n. 7288, p. 619–623, 25 mar. 2010.

GUPTA, R. K. et al. Zfp423 expression identifies committed preadipocytes and localizes to adipose endothelial and perivascular cells. **Cell Metabolism**, v. 15, n. 2, p. 230–239, 8 fev. 2012.

HARPER, G. S.; PETHICK, D. W. How might marbling begin? **Australian Journal of Experimental Agriculture**, v. 44, n. 7, p. 653–662, 2004.

HAUSMAN, G. J. et al. **Board-invited review: The biology and regulation of preadipocytes and adipocytes in meat animalsJournal of Animal Science**, abr. 2009. .

HEALTH, C. et al. Nutrition Recommendations: The Report of the Scientific Review Committee. 1990.

HOCQUETTE, J.-F. et al. Opportunities for predicting and manipulating beef quality. **Meat science**, v. 92, n. 3, p. 197–209, 2012.

HOCQUETTE, J.-F.; GRAULET, B.; OLIVECRONA, T. Lipoprotein lipase activity and mRNA levels in bovine tissues. **Comparative Biochemistry and Physiology Part B: Biochemistry and Molecular Biology**, v. 121, n. 2, p. 201–212, 1998.

HOCQUETTE, J. F. et al. Intramuscular fat content in meat-producing animals: Development, genetic and nutritional control, and identification of putative markers. **Animal**, v. 4, n. 2, p. 303–319, 2010.

HOOD, R.; ALLEN, C. E. Cellularity of bovine adipose tissue. **Journal of lipid research**, v. 14, n. 6, p. 605–610, 1973.

HUFFMAN, R. D. et al. EFFECTS OF PERCENTAGE BRAHMAN AND ANGUS BREEDING, AGE-SEASON OF FEEDING AND SLAUGHTER END POINT ON FEEDLOT PERFORMANCE AND CARCASS CHARACTERISTICS. **Journal of Animal Science**, v. 68, p. 2243–2252, 1990. Disponível em: <https://academic.oup.com/jas/article-abstract/68/8/2243/4704901>.

HWANG, Y.-H.; JOO, S.-T. Fatty acid profiles of ten muscles from high and low marbled (quality grade 1++ and 2) Hanwoo steers. **Korean journal for food science of animal resources**, v. 36, n. 5, p. 679, 2016.

JAGO, J. G.; BASS, J. J.; MATTHEWS, L. R. **Evaluation of a vaccine to control bull behaviourProceedings of the New Zealand Society of Animal Production**. [s.l: s.n.].

JEONG, J. et al. Expression of fat deposition and fat removal genes is associated with intramuscular fat content in longissimus dorsi muscle of Korean cattle steers. **Journal of animal science**, v. 90, n. 6, p. 2044–2053, 2012.

JIANG, J.; WOLK, A.; VESSBY, B. Relation between the intake of milk fat and the occurrence of conjugated linoleic acid in human adipose tissue. **The American journal of clinical nutrition**, v. 70, n. 1, p. 21–27, 1999.

JIAO, Y. et al. Molecular characterization, polymorphism of the ACOX1 gene and association with ultrasound traits in Bos taurus. **Genetics and Molecular Research**, v. 10, n. 3, p. 1948–1957, 2011.

JO, J. et al. Hypertrophy and/or hyperplasia: dynamics of adipose tissue growth. **PLoS Comput Biol**, v. 5, n. 3, p. e1000324, 2009.

KAROLYI, D. et al. Fatty acid composition of muscle and adipose tissue of beef cattle. **Italian Journal of Animal Science**, v. 8, n. sup3, p. 264–266, 2009.

KEE, A. J. et al. An Actin Filament Population Defined by the Tropomyosin Tpm3.1 Regulates Glucose Uptake. **Traffic**, v. 16, n. 7, p. 691–711, 1 jul. 2015.

KOURY FILHO, W. **Escores visuais e suas relações com características de crescimento em bovinos de corte**. 2005. 2005.

KRAMER, J. K. G. et al. Evaluating acid and base catalysts in the methylation of milk and rumen fatty acids with special emphasis on conjugated dienes and total trans fatty acids. **Lipids**, v. 32, n. 11, p. 1219–1228, 1997.

LADEIRA, M. M. et al. Nutrigenomics and beef quality: A review about lipogenesis. **International journal of molecular sciences**, v. 17, n. 6, p. 918, 2016.

LAGE, J. F. et al. Influence of genetic type and level of concentrate in the finishing diet on carcass and meat quality traits in beef heifers. **Meat Science**, v. 90, n. 3, p. 770–774, mar.

2012.

LEE, H. J. Cellularity of Adipose Tissue Obtained from Different Sex and Growth Stages of Hanwoo Cattle and Sheep. **Asian-Australasian Journal of Animal Sciences**, v. 13, n. 2, p. 155–160, 2000.

LEE, H. J. et al. Cellularity of adipose tissue obtained from different sex and growth stages of Hanwoo cattle and sheep. **Asian-Australasian Journal of Animal Sciences**, v. 13, n. 2, p. 155–160, 2000.

LEE, M. J. Hormonal regulation of adipogenesis. **Comprehensive Physiology**, v. 7, n. 4, p. 1151–1195, 2017.

LEE, M. R. F. et al. A comparison between red clover silage and grass silage feeding on fatty acid composition, meat stability and sensory quality of the M. Longissimus muscle of dairy cull cows. **Meat Science**, v. 81, n. 4, p. 738–744, abr. 2009.

LESEIGNEUR-MEYNIER, A.; GANDEMER, G. Lipid composition of pork muscle in relation to the metabolic type of the fibres. **Meat Science**, v. 29, n. 3, p. 229–241, 1991.

LI, X. Z. et al. Adipogenic/lipogenic gene expression and fatty acid composition in chuck, loin, and round muscles in response to grain feeding of Yanbian Yellow cattle. **Journal of animal science**, v. 96, n. 7, p. 2698–2709, 2018.

LIMA, I. de A. et al. CONDIÇÃO CORPORAL E CARACTERÍSTICAS DE CARCAÇA DE VACAS DE DESCARTE NA REGIÃO DE LAVRAS-MG. **Ciên. agrotec.**, v. 28, n. 3, p. 637–646, 2004.

LIN, J. J.-C.; LIN, J. L. Assembly of different isoforms of actin and tropomyosin into the skeletal tropomyosin-enriched microfilaments during differentiation of muscle cells in vitro. **The Journal of cell biology**, v. 103, n. 6, p. 2173–2183, 1986.

LISTRAT, A. et al. **How muscle structure and composition influence meat and flesh qualityScientific World Journal**Hindawi Limited, , 2016. .

LISTRAT, A. et al. What are the drivers of beef sensory quality using metadata of intramuscular connective tissue, fatty acids and muscle fiber characteristics? **Livestock Science**, v. 240, p. 104209, 2020.

LIU, H. et al. miR-32-5p Regulates Lipid Accumulation in Intramuscular Fat of Erhualian Pigs by Suppressing KLF3. **Lipids**, v. 56, n. 3, p. 279–287, 2021.

LIU, T. et al. Fatty Acid Profile of Muscles from Crossbred Angus-Simmental, Wagyu-Simmental, and Chinese Simmental Cattles. **Food Science of Animal Resources**, v. 40, n. 4, p. 563, 2020.

LOCK, A. L.; HARVATINE, K.; DRACKLEY, J. K. **Concepts of fat and fatty acid digestion in ruminants**. [s.l: s.n.]. Disponível em: <https://www.researchgate.net/publication/266499830>.

LOPEZ-HUERTAS, E. Health effects of oleic acid and long chain omega-3 fatty acids (EPA and DHA) enriched milks. A review of intervention studies. **Pharmacological Research**, v. 61, n. 3, p. 200–207, 2010. Disponível em: <http://dx.doi.org/10.1016/j.phrs.2009.10.007>.

MADRUGA, M. S. et al. Efeito do genótipo e do sexo sobre a composição química e o perfil de ácidos graxos da carne de cordeiros. **Revista Brasileira de Zootecnia**, v. 35, n. 4, p.

1838–1844, 2006.

MAGGIONI, D. et al. Animal performance and meat quality of crossbred young bulls. **Livestock Science**, v. 127, n. 2–3, p. 176–182, fev. 2010.

MARQUES, A. C.; VALENTE, T. B.; ROSA, C. S. da. **Revista de Nutrição Toxin formation during food processing and possible consequences to the human bodyTOXINAS EM ALIMENTOS PROCESSADOS | 283 Rev. Nutr**. [s.l: s.n.].

MARTINS, T. S. et al. Molecular factors underlying the deposition of intramuscular fat and collagen in skeletal muscle of Nellore and Angus cattle. **PLoS One**, v. 10, n. 10, p. e0139943, 2015.

MATARESE, V.; BERNLOHRS, D. A. **THE JOURNAL OF BIOLOGICAL CHEMISTRY Purification of Murine Adipocyte Lipid-binding Protein CHARACTERIZATION AS A FATTY ACID-AND RETINOIC ACID-BINDING PROTEIN*Molecular Biology**. [s.l: s.n.].

MICHAL, J. J. et al. The bovine fatty acid binding protein 4 gene is significantly associated with marbling and subcutaneous fat depth in Wagyu x Limousin F2 crosses. **Animal Genetics**, v. 37, n. 4, p. 400–402, ago. 2006.

MOISÁ, S. J. et al. Central role of the PPARγ gene network in coordinating beef cattle intramuscular adipogenesis in response to weaning age and nutrition. **Gene Regulation and Systems Biology**, v. 2014, n. 8, p. 17–32, 8 jan. 2013.

MOORE, M. C. et al. National Beef Quality Audit–2011: In-plant survey of targeted carcass characteristics related to quality, quantity, value, and marketing of fed steers and heifers. **Journal of Animal Science**, v. 90, n. 13, p. 5143–5151, 2012.

MORAIS DE LIMA JÚNIOR, D. et al. **ALGUNS ASPECTOS QUALITATIVOS DA CARNE BOVINA: UMA REVISÃO [Some qualitative aspects of beef: a review]Acta Veterinaria Brasilica**. [s.l: s.n.].

MORAIS, S. et al. Conserved expression of alternative splicing variants of peroxisomal acyl-CoA oxidase 1 in vertebrates and developmental and nutritional regulation in fish. **Physiological Genomics**, v. 28, n. 3, p. 239–252, 2007.

MORENO-SÁNCHEZ, N. et al. Skeletal muscle specific genes networks in cattle. **Functional and Integrative Genomics**, v. 10, n. 4, p. 609–618, nov. 2010.

MOSETI, D.; REGASSA, A.; KIM, W. K. **Molecular regulation of adipogenesis and potential anti-adipogenic bioactive moleculesInternational Journal of Molecular Sciences**MDPI AG, , 19 jan. 2016. .

MUCHENJE, V. et al. **Some biochemical aspects pertaining to beef eating quality and consumer health: A reviewFood Chemistry**, 15 jan. 2009. .

MUELLER, L. F. et al. Gender status effect on carcass and meat quality traits of feedlot Angus × Nellore cattle. **Animal Science Journal**, v. 90, n. 8, p. 1078–1089, 2019.

NATIONAL ACADEMIES OF SCIENCES AND MEDICINE, E. Nutrient requirements of beef cattle. 2016.

NIAN, Y. et al. Effect of castration and carcass suspension method on the quality and fatty acid profile of beef from male dairy cattle. **Journal of the Science of Food and Agriculture**, v. 98, n. 11, p. 4339–4350, 2018.

ODDY, V. H. et al. Nutritional and developmental effects on the intrinsic properties of muscles as they relate to the eating quality of beef. **Australian Journal of Experimental Agriculture**, v. 41, n. 7, p. 921–942, 2001.

OE, M. et al. Distribution of tropomyosin isoforms in different types of single fibers isolated from bovine skeletal muscles. **Meat science**, v. 118, p. 129–132, 2016.

OURY, M.-P. et al. Relationship between rearing practices and eating quality traits of the muscle rectus abdominis of Charolais heifers. **Livestock Science**, v. 111, n. 3, p. 242–254, 2007.

OWENS, F. N. et al. Factors that Alter the Growth and Development of Ruminants1f2. **Journal of Animal Science**, v. 71, p. 3138–3150, 1993. Disponível em: <http://jas.fass.org/content/71/11/3138>.

OWENS, F. N. et al. **Review of some aspects of growth and development of feedlot cattle.Journal of animal science**, 1995. .

PADUA, J. T. et al. Efeito de métodos de castração e do uso de vermífugos sobre o ganho em peso de bovinos mestiços leiteiros. 2003.

PANJONO et al. Carcass characteristics of Hanwoo (Korean cattle) from different sex conditions, raising altitudes and slaughter seasons. **Livestock Science**, v. 123, n. 2–3, p. 283–287, ago. 2009.

PAS, M. F. W. te.; EVERTS, M. E.; HAAGSMAN, H. P. **Muscle development of livestock animals : physiology, genetics and meat quality**. [s.l.] CABI Pub, 2004.

PEACOCK, F. M. et al. BREED AND HETEROSIS EFFECTS ON CARCASS CHARACTERISTICS OF ANGUS, BRAHMAN, CHAROLAIS AND CROSSBRED STEERS. **Journal of Animal Science**, v. 49, n. 2, 1979. Disponível em: <https://academic.oup.com/jas/article-abstract/49/2/391/4700568>.

PEREIRA, A. S. C. et al. Growth performance, and carcass and meat quality traits in progeny of Poll Nellore, Angus and Brahman sires under tropical conditions. **Animal Production Science**, v. 55, n. 10, p. 1295–1302, 2015.

PICARD, B. et al. Biomarkers of tenderness and intramuscular fat in five muscles from French PDO Maine-Anjou: I-Muscle type effect. In: Proceedings of the 63rd international congress of meat science and technology, **Anais**...Wageningen Academic Publishers Cork, Ireland, 2017.

PICARD, B. et al. Beef tenderness and intramuscular fat proteomic biomarkers: Effect of gender and rearing practices. **Journal of proteomics**, v. 200, p. 1–10, 2019.

PICARD, B.; DURIS, M. P.; JURIE, C. Classification of bovine muscle fibres by different histochemical techniques. **The Histochemical Journal**, v. 30, n. 7, p. 473–477, 1998.

PIGHIN, D. et al. A contribution of beef to human health: a review of the role of the animal production systems. **The Scientific World Journal**, v. 2016, 2016.

PUIG-OLIVERAS, A. et al. Expression-based GWAS identifies variants, gene interactions and key regulators affecting intramuscular fatty acid content and composition in porcine meat. **Scientific reports**, v. 6, n. 1, p. 1–12, 2016.

PURCHAS, R. W. Effect of sex and castration on growth and composition. **Advances in meat research (USA)**, 1991.

PURCHAS, R. W.; KNIGHT, T. W.; BUSBOOM, J. R. The effect of production system and age on concentrations of fatty acids in intramuscular fat of the longissimus and triceps brachii muscles of Angus-cross heifers. **Meat Science**, v. 70, n. 4, p. 597–603, 2005.

PURCHAS, R. W.; ZOU, M. Composition and quality differences between the longissimus and infraspinatus muscles for several groups of pasture-finished cattle. **Meat Science**, v. 80, n. 2, p. 470–479, out. 2008.

QUEIROZ, J. C. F. de et al. Control of adipogenesis by fatty acids. **Arquivos Brasileiros de Endocrinologia & Metabologia**, v. 53, n. 5, p. 582–594, 2009.

RAES, K. et al. Effect of linseed feeding at similar linoleic acid levels on the fatty acid composition of double-muscled Belgian Blue young bulls. **Meat Science**, v. 66, n. 2, p. 307–315, 2004.

RAINER, L.; HEISS, C. J. Conjugated linoleic acid: Health implications and effects on body composition. **Journal of the American Dietetic Association**, v. 104, n. 6, p. 963–968, 2004.

ROMAO, J. M. et al. **MicroRNA regulation in mammalian adipogenesisExperimental Biology and Medicine**, set. 2011. .

ROSEN, E. D. et al. C/EBPα induces adipogenesis through PPARγ: A unified pathway. **Genes and Development**, v. 16, n. 1, p. 22–26, 1 jan. 2002.

SANCHEZ-GURMACHES, J. et al. PTEN loss in the Myf5 lineage redistributes body fat and reveals subsets of white adipocytes that arise from Myf5 precursors. **Cell metabolism**, v. 16, n. 3, p. 348–362, 2012.

SANCHEZ-GURMACHES, J.; GUERTIN, D. A. Adipocytes arise from multiple lineages that are heterogeneously and dynamically distributed. **Nature communications**, v. 5, n. 1, p. 1–13, 2014.

SANGALLI, J. R. et al. Development to term of cloned cattle derived from donor cells treated with valproic acid. **PLoS One**, v. 9, n. 6, p. e101022, 2014.

SANHUEZA, J.; NIETO, S.; VALENZUELA, A. Acido linoleico conjugado: un acido graso con isomeria trans potencialmente beneficioso. **Revista chilena de nutrición**, v. 29, n. 2, p. 98–105, 2002.

SCHAEFER, E. J. Schaefer 2002. **Am J Clin Nutr**, v. 75, p. 191–212, 2002.

SCOLLAN, N. et al. **Innovations in beef production systems that enhance the nutritional and health value of beef lipids and their relationship with meat qualityMeat Science**, set. 2006. .

SCOLLAN, N. D. et al. Enhancing the nutritional and health value of beef lipids and their relationship with meat quality. **Meat Science**, v. 97, n. 3, p. 384–394, 2014.

SEIDEMAN, S. C. et al. UTILIZATION OF THE INTACT MALE FOR RED MEAT PRODUCTION: A REVIEW. **Journal of Animal Science**, v. 55, n. 4, 1982. Disponível em: <http://digitalcommons.unl.edu/usdaarsfacpubhttp://digitalcommons.unl.edu/usdaarsfacpub/764>.

SHANTHA, N. C.; CRUM, A. D.; DECKER, E. A. Evaluation of conjugated linoleic acid concentrations in cooked beef. **Journal of Agricultural and Food Chemistry**, v. 42, n. 8, p. 1757–1760, 1994.

SHARAF ELDIN, I. M. A. et al. **Characteristics of beef from intensively fed western Baggara bulls and heifers: quality attributes and chemical composition وعجالت أبقار ال ي خصائص الجودة والتركيب الكيميائ : بقارة السودانية المسمنة على نظام التغذية المركزة خصائص اللحم في عجول أي إم أي شرف الدينIraqi Journal of Veterinary Sciences**. [s.l: s.n.]. Disponível em: <http://vetmedmosul.org/ijvs>.

SHINGFIELD, K. J.; BONNET, M.; SCOLLAN, N. D. Recent developments in altering the fatty acid composition of ruminant-derived foods. **Animal**, v. 7, n. s1, p. 132–162, 2013.

SINCLAIR, A. J. Dietary fat and cardiovascular disease: the significance of recent developments for the food industry. **Food Australia**, v. 45, n. 5, p. 226–231, 1993.

SMAS, C. M.; SUL, H. S. **Control of adipocyte differentiationBiochem. J**. [s.l: s.n.].

SMITH, S. B. et al. Regulation of fat and fatty acid composition in beef cattle. **Asian-Australasian Journal of Animal Sciences**, v. 22, n. 9, p. 1225–1233, 2009.

SMITH, S. B. et al. Adipogenic gene expression and fatty acid composition in subcutaneous adipose tissue depots of Angus steers between 9 and 16 months of age. **Journal of animal science**, v. 90, n. 8, p. 2505–2514, 2012.

SMITH, S.; WITKOWSKI, A.; JOSHI, A. K. **Structural and functional organization of the animal fatty acid synthaseProgress in Lipid Research**Elsevier Ltd, , 2003. .

SORET, B. et al. Expression of genes involved in adipogenesis and lipid metabolism in subcutaneous adipose tissue and longissimus muscle in low-marbled Pirenaica beef cattle. **Animal**, v. 10, n. 12, p. 2018–2026, 1 dez. 2016.

SOUFFRANT, W.-B.; METGES, C. C. **Progress in Research on Energy and Protein Metabolism**. [s.l.] Wageningen Academic Publishers, 2003. v. 109

TATUM, J. D.; GRUBER, S. L.; SCHNEIDER, B. A. Pre-harvest factors affecting beef tenderness in heifers. **National Cattlemen's Beef Association**, 2007.

TAYE, M. et al. Deciphering signature of selection affecting beef quality traits in Angus cattle. **Genes & genomics**, v. 40, n. 1, p. 63–75, 2018.

THALLER, G. et al. DGAT1, a new positional and functional candidate gene for intramuscular fat deposition in cattle. **Animal genetics**, v. 34, n. 5, p. 354–357, 2003.

TORAL, P. G. et al. Modulating ruminal lipid metabolism to improve the fatty acid composition of meat and milk. Challenges and opportunities. **Animal**, v. 12, n. s2, p. s272–s281, 2018.

TOTLAND, G. K.; KRYVI, H. **Anatomy and Embryology Distribution patterns of muscle fibre types in major muscles of the bull (Bos taurus)Anat Embryol**. [s.l: s.n.].

TROY, D. J.; TIWARI, B. K.; JOO, S.-T. Health implications of beef intramuscular fat consumption. **Korean Journal for Food Science of Animal Resources**, v. 36, n. 5, p. 577, 2016.

TUME, R. K.; SIKES, A. L.; SMITH, S. B. Enriching M. sternomandibularis with α-tocopherol by dietary means does not protect against the lipid oxidation caused by high-pressure processing. **Meat Science**, v. 84, n. 1, p. 66–70, jan. 2010.

TURAN, H.; SÖNMEZ, G.; KAYA, Y. Fatty acid profile and proximate composition of the thornback ray (Raja clavata, L. 1758) from the Sinop coast in the Black Sea. **Journal of**

Fisheries Sciences, v. 1, n. 2, p. 97–103, 2007.

TURK, S. N.; SMITH, S. B. Carcass fatty acid mapping. **Meat Science**, v. 81, n. 4, p. 658–663, abr. 2009.

ULBRICHT, T. L. V; SOUTHGATE, D. A. T. Coronary heart disease: seven dietary factors. **The lancet**, v. 338, n. 8773, p. 985–992, 1991.

UNDERWOOD, K. R. et al. Relationship between kinase phosphorylation, muscle fiber typing, and glycogen accumulation in longissimus muscle of beef cattle with high and low intramuscular fat. **Journal of agricultural and food chemistry**, v. 55, n. 23, p. 9698–9703, 2007.

USDA. Standards for Grades of Carcass Beef. p. 1–16, 2017. Disponível em: <https://www.ams.usda.gov/sites/default/files/media/CarcassBeefStandard.pdf>.

VANNICE, G.; RASMUSSEN, H. Position of the academy of nutrition and dietetics: dietary fatty acids for healthy adults. **Journal of the Academy of Nutrition and Dietetics**, v. 114, n. 1, p. 136–153, 2014.

VENKATA REDDY, B. et al. **Beef quality traits of heifer in comparison with steer, bull and cow at various feeding environmentsAnimal Science Journal**Blackwell Publishing, , 1 jan. 2015. .

VIEIRA, C. et al. Breed and ageing extent on carcass and meat quality of beef from adult steers (oxen). **Livestock Science**, v. 107, n. 1, p. 62–69, mar. 2007.

VURAL, B. et al. Presence of fatty-acid-binding protein 4 expression in human epicardial adipose tissue in metabolic syndrome. **Cardiovascular Pathology**, v. 17, n. 6, p. 392–398, nov. 2008.

WAKIL, S. J.; ABU-ELHEIGA, L. A. Fatty acid metabolism: target for metabolic syndrome. **Journal of lipid research**, v. 50, p. S138–S143, 2009.

WARD, R. E. et al. Relationship between the expression of key lipogenic enzymes, fatty acid composition, and intramuscular fat content of Limousin and Aberdeen Angus cattle. **Livestock Science**, v. 127, n. 1, p. 22–29, 2010.

WEBB, E. C.; O'NEILL, H. A. **The animal fat paradox and meat qualityMeat Science**, set. 2008. .

WĘGLARZ, A. **Quality of beef from semi-intensively fattened heifers and bullsAnimal Science Papers and Reports**. [s.l: s.n.].

WHIPPLE, G. et al. EVALUATION OF ATTRIBUTES THAT AFFECT LONGISSIMUS MUSCLE TENDERNESS IN BOS TAURUS AND BOS /ND/CUS CATTLE192. **Journal of Animal Science**, v. 68, p. 2716–2728, 1990.

WOOD, J. D. et al. Effects of fatty acids on meat quality: a review. **Meat science**, v. 66, n. 1, p. 21–32, 2004.

WOOD, J. D. et al. **Fat deposition, fatty acid composition and meat quality: A reviewMeat Science**, abr. 2008. .

WOOD, J. D.; RICHARDSON, R. I. **Factors affecting flavour in beef A literature review, with recommendations for the British beef industry on how flavour can be controlled**. [s.l: s.n.].

WOODS, V. B.; FEARON, A. M. **Dietary sources of unsaturated fatty acids for animals and their transfer into meat, milk and eggs: A reviewLivestock Science**, jan. 2009. .

YAMADA, T.; KAWAKAMI, S. I.; NAKANISHI, N. Expression of adipogenic transcription factors in adipose tissue of fattening Wagyu and Holstein steers. **Meat Science**, v. 81, n. 1, p. 86–92, jan. 2009.

ZHANG, S. et al. DNA polymorphisms in bovine fatty acid synthase are associated with beef fatty acid composition 1. **Animal genetics**, v. 39, n. 1, p. 62–70, 2008.

2 GENDER STATUS MODULATE THE FATTY ACID COMPOSITION AND TRANSCRIPT LEVELS OF ADIPOGENESIS-RELATED GENES IN DIFFERENT MUSCLES OF ANGUS X NELORE CATTLE

PAPER THAT WILL BE SUBMITTED TO THE LIVESTOCK SCIENCE JOURNAL.

Gender status modulate the fatty acid composition and transcript levels of adipogenesis-related genes in different muscles of Angus x Nelore cattle

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HIGHLIGHTS

- Gender status and muscle type can affect the lipid composition of beef.
- Gender status and muscle type modulate the transcript levels of adipogenesis-related genes.
- Cattle gender status affect the tropomyosin protein abundance in the *triceps brachii* muscle.
- Tropomyosin modulates the accumulation of fat in carcass of heifers and steers.
- *Triceps brachii* muscle is more favorable to human health than the *longissimus.*

ABSTRACT

The study evaluated the influence of cattle gender status on the expression of adipogenesis-related genes, as well as the association with the fatty acid composition in *longissimus* (LO) and *triceps brachii* (TB) muscles from feedlot crossbred cattle. A total of 150 cattle was confined for 150 days and assigned to three genders, namely, heifers, bulls, and steers, and fed the same diet. The total lipids content, fatty acid composition, gene expression analyses, and quantification of tropomyosin were performed in the samples of LO and TB muscles. Only the gender status affected the content of total lipids ($P \leq 0.05$), with the greater values in meat from heifers, followed by steers and bulls. There was a difference in the total saturated FAs (SFAs) by gender and muscle ($P \leq 0.05$), where meat from bulls had higher levels of SFAs compared to heifers and had no differences in relation to meat from steers. Among muscle type, total

SFAs increased in LO muscle compared with TB, independent of gender. The FAs 14:0 and 16:0 were affected by muscle type only, and higher levels were detected in the LO ($P \leq 0.05$). There were differences in gender for total monounsaturated FAs (MUFAs; $P \leq 0.05$). Meat from bulls had lower levels of MUFAs compared with the other genders. The levels of the major FA of total MUFAs, 18:1 n-9c, were higher in the LO muscle than in the TB muscle, independent of gender ($P \leq 0.05$). The total polyunsaturated FAs (PUFAs), total n-3, total n-6, PUFA:SFA ratio, health index and the FAs 18:2 n-6c, 20:3 n-3, and 22:4 n-6 were higher in TB than in LO muscle ($P \leq 0.05$). Otherwise, the atherogenicity and thrombogenicity indexes were higher in the LO muscle ($P \leq 0.05$). Regarding the transcript levels, there was an effect of muscle type on the expression of the *CEBPa* and *LPL* ($P \leq 0.05$) genes, both of which had higher expression in TB muscle. Gender affected the transcript level of *ACC* ($P \leq 0.05$). This gene was increased in bulls, intermediate in steers, and decreased in heifers. Additionally, gender and muscle type interactions were observed for the transcript levels of *FABP3*, *TPM2*, and *TPM3* ($P \leq 0.05$). Muscle type and gender status affected the tropomyosin (TPM) protein abundance ($P \leq 0.05$). However, there was difference for TPM abundance between muscles only in bulls ($P \leq 0.05$), where the greater TPM abundance was in the TB. Regarding gender, the LO muscle of steers showed higher TPM abundance than bulls and heifers, while the TB muscle of bulls showed higher TPM abundance than the other genders. The cattle gender status modulated the transcripts levels of *ACC* gene, content of total lipids, total SFAs, total MUFAs, and carcass traits, while the muscle type affected the transcripts of *CEBPa* and *LPL* genes, total SFAs, total PUFAs, total n-3 and n-6, besides important FAs, showing a gender and muscle specific effect. Additionally, there is a different lipid profile within each muscle, where the TB muscle is more favorable to human health than the LO, and beef from heifers stands out than the other genders for presenting better carcass traits and FA composition more favorable to human health.

Keywords: crossbred, gene expression, fat, *longissimus*, *triceps brachii*, hormonal profile.

2.1 INTRODUCTION

Brazil has a prominent position in agribusiness and is an important global beef producer and exporter, producing 10.3 million tons of beef in 2020 (ABIEC, 2021). Our bovine herd is mostly composed of Nelore cattle, which are animals known to have greater rusticity and adaptability to the local climate and conditions (BIANCHINI *et al*., 2007; FERRAZ; FELÍCIO, 2010). However, there is an increasing use of *Bos taurus taurus* origin animals,

mainly in synthetic crossings (FAVERO *et al*., 2019), in Brazilian livestock, which can be explained by the increase in the number of slaughters of these animals and by the greater demand of meat consumers in recent years. The control of meat quality is a societal issue that concerns all meat sectors. Meat quality is defined by a set of intrinsic and extrinsic factors, where the former corresponds to safety, health, convenience, nutritional and sensorial qualities (PICARD *et al*., 2019). Beef consumers are currently looking for higher-quality beef, including the quantity and quality of fat. Thus, it is important that the beef production chain adapts to this scenario of constant change. As strategies to adapt beef production to the demanding and modern consumer market, different tools have been used to improve the productivity and meat traits. Among these tools to improve the final product are genetic selection (FERRAZ; FELÍCIO, 2010), cattle gender status (GAGAOUA *et al*., 2015; MUELLER *et al*., 2019) and crossbred animals (FAVERO *et al*., 2019; GAMA e *et al*., 2013). Thus, gender status could be a strategy used by the industry to achieve market niches in terms of consumer trends.

Cattle gender status can influence animal growth (OWENS *et al*., 1993), muscle adipose tissue (BJORNTORP, 1997; LEE et al *et al*., 2000) and fatty acid composition (VENKATA REDDY *et al*., 2015; MUELLER *et al*., 2019), which are influenced by a variety of hormonal factors, directly and indirectly affecting the carcass and meat traits (VENKATA REDDY *et al*., 2015).

According some researches (AILHAUD; GRIMALDI; NKGREL, 1994; HARPER; PETHICK, 2004; SMAS; SUL, 1995; BJORNTORP, 1997), hormones regulate the development of adipocytes and the distribution of body fat. Hormones are internal factors that are prominent modulators of adipogenesis. Adipogenesis is the main pathway for the development of adipose tissue and is therefore influenced by a multiplicity of factors, such as the different fat deposits, gender status, and diet of cattle (BJORNTORP, 1997; DE SÁ *et al*., 2017; SCOLLAN *et al*., 2006; WEBB; O'NEILL, 2008).

In several mammals, physiological observations have indicated that adipose development is affected by a variety of endocrine hormones and many steroid hormones (DE SÁ *et al*., 2017). Testosterone, for example, regulates lipid mobilization, inhibits lipoprotein lipase (LPL), and stimulates lipolysis (BJORNTORP, 1997). Thus, sex hormones and the associated hormonal repertoire can affect muscle and adipocyte cells through different molecular pathways.

Transcription factors such as CEPB, PPAR and Zfp423 are involved in the adipogenesis process (ROSEN et al., 2002; GUPTA et al., 2010, 2012), as are the lipogenic enzymes

LPL, ACC, and FAS (BONNET *et al*., 2000; SMITH; WITKOWSKI; JOSHI, 2003) and the ACSS1 and SLC16A7 genes, which can act in fatty acid synthesis (BERTON et al., 2016). The ACOX gene is associated with fat deposition and lipid metabolism (JIAO *et al*., 2011; MORAIS *et al*., 2007) and may also be the key regulator of energy metabolism. Additionally, the multigenic family of fatty acid-binding proteins (FABPs) is involved in the intracellular and extracellular transport of lipids, transporting fatty acids in adipose tissue and contributing to muscle energy metabolism (BERTON *et al*., 2016; BIONAZ; THERING; LOOR, 2012; EVERTS; HAAGSMAN, 2004; VURAL *et al*., 2008). Previous studies have found a relationship between marbling score and percent intramuscular fat (JEONG *et al*., 2012). In this sense, the tropomyosins (TPMs) are a family of actin-linked proteins found in all tissues and has been indicated as a modulator of the formation of adipose tissue (CHO *et al*., 2016). Moreover, these authors demonstrated that TPM1, TPM2, and TPM3 were differentially expressed depending on sex and adipose depots and TPMs were positively correlated with marbling score and quality grade.

Therefore, it is important to investigate these genes in different muscles of male and female crossed cattle to understand their role affecting directly and indirectly the carcass and meat traits.

It is well known that beef quality is better in heifers than bulls, especially the fatty acid composition (MUELLER *et al*., 2019; SHARAF ELDIN *et al*., 2013). Based on that, the use of different gender status can be an interesting strategy to improve beef quality (MUELLER *et al*., 2019). Additionally, regarding the lipid profile in muscles, some researchers reported that the fatty acids can be modified according to the muscle (PURCHAS; ZOU, 2008; *RAES et al*., 2004; TURK; SMITH, 2009) and most studies in beef cattle are carried out with the *longissimus* muscle. Therefore, there is a need to explore other muscles, such as *triceps brachii*, which presents metabolic and fat deposition differences when compared to other muscles (HOCQUETTE *et al*., 2010; SORET *et al*., 2016). However, given the above and since there is no research exploring the use of different gender status and muscles of cattle, it is important to understand how gender can influence the expression of genes associated with adipogenesis and fatty acid composition in muscles. Therefore, the goal of this study was to understand the influence of cattle gender status on the expression of adipogenesis-related genes, as well as the association with the fatty acid composition in *longissimus* and *triceps brachii* muscles from feedlot crossbred cattle.

2.2 MATERIAL AND METHODS

2.2.1 Ethics statement

The animal care and handling procedures were approved (protocol #7675200918) by the Ethics Committee on Animal Use of the Faculty of Animal Science and Food Engineering of the University of Sao Paulo, Pirassununga, SP, Brazil.

2.2.2 Animals and management

The experiment was conducted in the Fazenda Leticia feedlot in Rio Verde de Mato Grosso, MS, Brazil. A total of 150 Angus x Nelore cattle, 11 months old, were distributed into three gender status: 50 heifers, 50 bulls, and 50 steers, presenting initial body weights of 231.8 ± 8.8 kg, 239.6 ± 14.8 kg, and 227.6 ± 12.1 kg, respectively. In the feedlot, animals remained separated by gender (3 pens with 3,500 m^2 each) equipped with a feeding trough and automatic drinkers with buoys. All animals were fed the same diet *ad libitum*, following the adaptation stage for 18 days, and then the concentrate was gradually increased until reaching the finishing diet with 14% forage (*Brachiaria*) and 86% concentrate. The experimental diet was offered three times daily during the experimental period of 150 days, with the aim of daily mean weight gain of 1.6 kg/day and was formulated using RLM® software (Esalq/USP, Piracicaba, Sao Paulo, Brazil), with nutritional demands estimated by the National Research Council (NATIONAL ACADEMIES OF SCIENCES AND MEDICINE, 2016) system (Table 1).

Surgical castration of the steers consisted of completely removing the testicles using the scrotum incision technique according to Padua et al. (2003), 30 days before entering the feedlot, with local anesthesia – 10 mL of 2% lidocaine hydrochloride (Lidovet®, Bravet, RJ, Brazil) to each testis. After surgery, a single dose (2.2 mg/kg of body weight) of the nonsteroidal anti-inflammatory drug was used (Flunixin Meglumine, Banamine®, Merck Animal Health, Madison, NJ, USA).

Table 1 – Composition (% dry matter) of the experimental diet during feedlot period

Ingredient (% DM)	
Ground corn grain	55.0
Liquid sugar cane molasses	17.0
Brachiaria hey	14.0
Corn meal 48%	9.5
Mineral mix[1]	3.0
Urea	0.75
Prote-n[2]	0.75
Nutritional composition (% DM)	
DM (%)	86.2
CP (% of DM)	15.8
TDN	74.1
EE	3.49
Starch	39.5
Non-fibrous Carbohydrate[3]	58.6
NDF	17.9
ADF	9.50
Ca	0.82
P	0.29
K	1.16
Sodium monensin (ppm)	25.0
Fatty acid composition (%)	
14:0	0.121
16:0	16.2
18:0	4.0
18:1 n9 *cis*	23.5
18:2 n6 *cis*	49.5
18:3 n3	0.142
C20:2 n6 *cis* 11,14	0.042
C20:3 n3 *cis* 11, 14, 17	0.208
C22:2 n6 *cis* 13, 16	0.053
C22:5 n3	0.018
Other fatty acids	6.21

Note: Estimated by RLM 3.2® software - ESALQ-USP, SP – Brazil.
Abbreviation: DM, dry matter; CP, Crude Protein; TDN, total digestible nutrients; EE, Ether Extract; NDF, neutral detergent fiber; ADF, acid detergent fiber.
[1]Estimated amount of mineral ingredients in the mixture assuming calcitic chloride, sodium chloride, sulfur, monobicalcium phosphate, magnesium oxide, fruit flavoring, grape seed extract, calcium iodate, sodium monensin, manganese monoxide, zinc oxide, sodium selenite, cobalt sulfate, copper sulfate, iron sulfate, vitamin A, vitamin D3, vitamin E, and vehicle.
[2]Livestock urea, paprika oleoresin and vehicle.
[3]Starch, sucrose and pectin.

2.2.3 Sample collection and carcass measurements

At the end of the experimental period (150 days), animals were separated by gender, transported to the abattoir 200 km away, and harvested after solid food fasting for 16 h at 16 months of age. The final body weights of the cattle at slaughtering were 431.3 ± 26.5 kg, 487.9 ± 25.1 kg, and 452.4 ± 30.9 kg, with average daily gains of 1.31 ± 0.16 kg, 1.62 ± 0.17 kg, and

1.47 ±0.13 kg for heifers, bulls and steers, respectively.

The animals were humanely slaughtered on the same day in a commercial abattoir in Terenos, MS, Brazil, under the Brazilian Federal Inspection Service. Samples of LO and TB muscles were collected for RNA and protein extraction immediately *post mortem*, flash frozen in liquid nitrogen and stored at -80 °C until processed.

Twenty-four hours after slaughter, carcass left halves were sampled between the 12th and 13th ribs to measure fat thickness (mm) with a 6" digital caliper (Amatools®, Model ZAAS Precision, Piracicaba, SP, Brazil). The marbling score was assigned considering the degrees: Traces, Slight, Small, Modest, Moderate, Slightly abundant, and Moderately abundant, following the methodology described by the USDA (1997). The quality grade of the carcasses was performed according USDA (2017) as Standard, Select, Low choice, Choice, and Prime. In addition, the Meat Evaluation Handbook (AMERICAN MEAT SCIENCE ASSOCIATION, 2013) encoded the marbling degrees in TR: 2.0 to 2.99; SL: 3.0 to 3.99; SM: 4.0 to 4.99; MT: 5.0 to 5.99; MD: 6.0 to 6.99; SA: 7.0 to 7.99; MA: 8.0 to 8.99. After removing the *longissimus* (LO) and *triceps brachii* (TB) muscles, samples of each animal were obtained, vacuum packed individually in polyethylene bags (Cryovac®, Charlotte, NC, USA), immediately frozen and kept at -80 °C for the analyses of total lipids and fatty acid composition.

2.2.4 Total lipids and fatty acid composition

For total lipids determination and fatty acid (FA) composition analyses, 48 samples of each muscle (LO and TB) were selected, 16 from each gender (heifers, bulls, and steers) in the LO and 16 from each gender in TB. Sample selection was performed based on the criterion of high and low intramuscular fat deposition (marbling), with values close to the average daily weight gain (ADG) of each animal during the experimental period. A margin of approximately 200g down and up in the ADG of each animal was considered. When selecting by ADG, the effect of weight gain on marbling is neutralized, thus maintaining the effect of cattle gender status, which is the general goal of the experiment.

Samples of the LO and TB muscles of each gender status were previously freeze-dried and kept at -80 °C until to perform the analyses. Lipids were extracted from muscles as described by Folch *et al.* (1957). Aliquots of muscle lipids were methylated separately using base (0.5 N sodium methoxide) and acid (5% methanolic HCl) reagents, according to Kramer *et al.* (1997). The FAs were quantified by gas chromatography (GC-2010 Plus - Shimadzu AOC 20i

autoinjector) with an SP-2560 capillary column (100 m × 0.25 mm diameter, 0.02 mm thick, Supelco, Bellefonte, PA). The initial temperature was 70 °C with an increase (13 °C/min) to 175 °C, which was held for 27 min before a further increase to 215 °C (4 °C/min); the final temperature was maintained for 31 min. Hydrogen (H_2) was used as the carrier gas at 40 cm^3/s. The temperature of the flame ionization detector (FID) was 250 °C, the H_2 flow rate was 40 mL/min, the air flow rate was 400 mL/min (synthetic air), the make-up gas flow rate was 30 mL/min kPa (N_2), and the sampling rate was 40 msec.

The FAs were identified by comparing the retention times of methyl esters in the samples with those of the FA C4-C24 (F.A.M.E. mix, Sigma®), GLC 463 Reference Mixture Nu Check, vaccenic acid (V038-1G, Sigma®), linoleic acid (UC-61M 100 mg), conjugated linoleic acid (CLA) (UC-60 M 100 mg, Sigma®), and tricosanoic acid (Sigma®) standards. 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.

The index related to human health and nutritional quality was calculated according to Zhang et al. (2008) and Ulbricht and Southgate (1991).

Health Index = (MUFA+PUFA)/(4*14:0)+16:0);

Atherogenicity Index = (12:0+(4*14:0)+16:0)/(Σn6+Σn3+ΣMUFA);

Thrombogenicity Index = (14:0+16:0+18:0)/((0,5*ΣMUFA)+(0,5*Σn6)+(3*Σn3)+n3/n6).

2.2.5 Total RNA extraction and cDNA preparation

For RNA extraction and gene expression analysis, ten samples of each muscle (LO and TB) were selected by gender (heifers, bulls, and steers). The criterion established for sample selection was based on the mean values of FAs C16:0, C18:0, C14:1 c9, C15:1 c10, C16:1 c9, C18:1 n9c, C18:2 n6c, C20:2 n6, C20:3 n3, C22:5 n3, as differences found in meat of different gender, in a previous experiment with published results (MUELLER *et al.*, 2019). It is important to highlight that for the selection of the samples in this study, the significant differences between gender for the above cited FAs were also considered. The goal of the criterion used was to select the samples based on the phenotype that provides a more desirable FA composition for human health. In the above-mentioned previous experiment, a more desirable FA composition for human health was observed in the meat of heifers and a less desirable profile in the meat of bulls. Thus, for the relative quantification of previously selected

genes, contrasting groups were selected, specified as follows: (1) ten heifers with the most desirable FA composition for human health; (2) ten bulls with a less desirable FA composition; and (3) ten steers with a FA composition similar to heifers.

Approximately 50 mg of LO and TB muscle samples were individually macerated and homogenized for total RNA extraction. Total RNA was extracted using TRIzol® reagent according to the manufacturer's recommendations (Invitrogen Life Technologies, Carlsbad, CA). The total RNA concentration was quantified using a NanoDrop spectrophotometer (Thermo Scientific, Washington, DE). RNA samples were treated with DNase I (Invitrogen Life Technologies, Carlsbad, CA) prior to reverse transcription reaction. cDNA was produced using 30 ng of total RNA per analyzed gene using a High Capacity kit (Applied Biosystems, Foster City, CA) according to the manufacturer's recommendations.

2.2.6 Gene expression analyses

Transcript abundance was analyzed by QuantStudio 6 Real-Time PCR® (Applied Biosystems, Thermo Fisher Scientific, Waltham, MA, USA) using SYBR Green RT-PCR Master Mix (GoTaq® Promega, Madison, WI, USA) with the following cycle parameters: 95 °C for 2 min, followed by 45 cycles at 95 °C for 15 s and 60 °C for 1 min. The mRNA levels were analyzed by quantitative real-time polymerase chain reaction (qRT-PCR) using gene-specific primers according to published by Berton et al. (2016), Cho et al. (2016), Martins et al. (2015), and Sangalli et al. (2014) (Table 2) with similar efficiency obtained by using different cDNA concentrations (5, 15, 30, and 60 ng/µL). Briefly, in each reaction, 5 µL of SYBR Green RT-PCR Master Mix, 1.5 µL of each forward and reverse primer (0.5uM), and 1 µL of cDNA (30 ng of RNA) were mixed with Milli-Q (ultrapure) water in a final volume of 10 µL. After amplification, a melting curve (0.01 C/s) was used to confirm the purity of the product. The "threshold cycle" (Ct) values were normalized (ΔCt) based on the geometric mean Cts of the values obtained for GAPDH and β–actin. Relative levels of gene expression were calculated and represented as $2^{-\Delta Ct}$.

Table 2 - Genes and their respectively sequences of the primers used in this study

Gene name	Abbreviation	GenBank ID	Primer sequence 5'-3'	
CCAAT enhancer binding protein, alpha	*C/EBPα*	NM_176784.2	Forward	TGCGCAAGAGCCGGGACAAG
			Reverse	ACCAGGGAGCTCTCGGGCAG
Peroxissome proliferator activated-receptor gamma	*PPARg*	NM_181024.2	Forward	GTGGAGCCTGTATCCCCACC
			Reverse	TTTATCCCCACAGACCCGGC
Zinc finger protein	*Zfp423*	NM_001101893.1	Forward	GGATTCCTCCGTGACAGCA
			Reverse	TCGTCCTCATTCCTCTCCTCT
Acetyl-CoA carboxylase	*ACC*	NM_174224.2	Forward	TGAAGAAGCAATGGATGAACC
			Reverse	TTCAGACACGGAGCCAATAA
Fatty acid synthase	*FASN*	NM_001012669.1	Forward	TGCTCATTCACTCGGGCTCC
			Reverse	TTTCGGCTGACCCCACAAGT
Lipoprotein lipase	*LPL*	NM_001075120.1	Forward	CTCAGGACTCCCGAAGACAC
			Reverse	GTTTTGCTGCTGTGGTTGAA
Acyl-CoA oxidase	*ACOX*	NM_001035289.3	Forward	GCTGTCCTAAGGCGTTTGTG
			Reverse	ATGATGCTCCCCTGAAGAAA
Tropomyosin 1	*TPM1*	NM_001013590.2	Forward	GAGGATGCCGACCGCAAGTA
			Reverse	TGCCTTCTGAAAGCTCAGCC
Tropomyosin 2	*TPM2*	NM_001010995.2	Forward	AGCTGGAGCGCTCAGAAGAG
			Reverse	GGGACTTGAGGGCCTGATCC
Tropomyosin 3	*TPM3*	NM_001011674.1	Forward	TTGAGGCTCAGGCGGAGAAG
			Reverse	AGCAAACTCAGCACGGGTCT
Fatty acid binding protein 3	*FABP3*	NM_174313.2	Forward	AGAGACATCACTTGTGCGGG
			Reverse	GGAAGGAGAGGGCAGGTCAT
Fatty acid binding protein 4	*FABP4*	NM_174314.2	Forward	GGATGATAAGATGGTGCTGGA
			Reverse	ATCCCTTGGCTTATGCTCTCT
Fatty acid binding protein 7	*FABP7*	NM_001078162.2	Forward	ATGGTGGAGGCTTTCTGTGCT
			Reverse	CCTAGTGGCAAAGCCCACAC
Glycerol-3-phosphate acyltransferase	*GPAM*	NM_001012282.1	Forward	ACACTGAAGAATGCTCTCCTGCT
			Reverse	ACGGCATGGCATTATTGCTCC
Uncoupling protein 3	*UCP3*	NM_174210.1	Forward	GCCCAACATCACGAGGAATGC
			Reverse	GCAGGGGAAGTTGTCGGTGA
Acyl-CoA synthetase short chain family member 1	*ACSS1*	NM_174746.2	Forward	ACGGGGCTTACAGAACAGAGG
			Reverse	GTCAGCCATGGCGTCCTCA
Solute carrier family 16 member 7	*SLC16A7*	NM_001076336.2	Forward	GAGGACTCGTCCAGGGACATA
			Reverse	TTAACTGCTGGTCTCCGGCAC
Glyceraldehyde-3-phosphate dehydrogenase	*GAPDH*	NM_001034034.2	Forward	AGATAGCCGTAACTTCTGTGC
			Reverse	ACGATGTCCACTTTGCCAG
β-Actin	*ACTB*	NM_173979.3	Forward	CAGCAGATGTGGATCAGCAAGC
			Reverse	AACGCAGCTAACAGTCCGCC

Designed by authors.
Berton et al. (2016); Cho et al. (2016); Martins et al. (2015); Sangalli et al. (2014).

2.2.7 Tropomyosin protein abundance

For protein extraction, samples frozen at -80 °C were macerated and homogenized in 0.3 mL of Ripa buffer (150 mM sodium chloride, 1% Triton X-100, 0.5% sodium deoxycholate, 0.1% SDS, 50 mM Tris - pH 8.0) with addition of 1% protease inhibitor MIX, 1 mM sodium orthovanadate, and 25 mM sodium fluoride. Upon protein extraction samples were centrifuged at 16000 g for 20 min at 4 ° C and then the supernatant was collected. Protein quantification was performed based on a bovine albumin standard curve using Bradford Protein Assay reagent (# 500-0006, Bio Rad®, Hercules, CA), with 2mg/mL of bovine albumin.

For electrophoresis, aliquots with 100 µg of proteins were diluted 1:1 in Laemmli 2x buffer (#161-0737, Bio Rad®, Hercules, CA) plus 1% β mercaptoethanol. Samples were incubated at 95 °C for 5 min, and then applied to the 10% SDS-PAGE gel with a 4% stacking gel. A running buffer containing 25 mM Tris, 192 mM glycine and 0.1% SDS (# 161-0732, Bio Rad®, Hercules, CA) was used and the run was performed at 100 V for 2 hours.

Electrotransference was performed by wet transfer method using the transfer buffer (#161-0734, Bio Rad®, Hercules, CA) and PVDF membrane (#162-0177, Bio Rad®, Hercules, CA). The transfer occurred at 4 °C with magnetic stirring during 2 h at 80 V. To verify the complete protein transfer from gel to PVDF membrane was used the method of membrane staining with Ponceau S. Membranes were blocked in TBS-T (#170-6435, #170-6531, Bio Rad®, Hercules, CA) containing 5% of bovine albumin for 1 h at room temperature, and then washed with TBS-T for 5 min, 3 times. Membranes were incubated with specific primary antibody diluted in TBS-T with 2% of bovine albumin over-night at 4 °C. The primary antibodies used were anti-x-Tubulin mouse antibody (1:2000, T9026, Sigma) and an anti-mouse antibody (1:2000, #70765, Cell Signaling) as endogenous controls, and Tropomyosin (FL-284 - Sc-28543), a rabbit polyclonal antibody raised against amino acids 1-284 representing full length Tropomyosin α of human origin, diluted in 1:2,000.

After incubation with primary antibody, the membranes were washed 3 times with TBS-T (5 min per wash) and were then incubated with the appropriate secondary antibody for 1 h at room temperature with low stirring. Secondary antibodies were diluted in TBS-T with 2% of bovine albumin as follows: The Anti-rabbit secondary antibody (A0545, Sigma®) was diluted in 1:2,000 for tropomyosin and anti-mouse secondary antibody for tubulin was diluted in 1:2,000 (#70765, Cell Signaling). After incubation with secondary antibody the membranes were washed with TBS-T (3 times for 5 min per wash). Bands were visualized using Clarity Western ECL substrate (#170-5060, Bio-Rad®, Hercules, CA). The signals generated from the

bands were captured and quantified by the Image Lab 6.0.1 software using ChemiDoc system (Bio-Rad®, Hercules, CA). To account for potential variation in protein loading, intensities of protein bands (%) were expressed relative to the Tubulin levels as endogenous control.

2.2.8 Statistical analyses

The statistical analyses were performed by the proc MIXED SAS® (version 9.3) for the meat quality traits and JMP14 Software (SAS Institute) for the gene expression analyses. The experimental design was completely randomized, with 50 repetitions per treatment, and each animal was considered an experimental unit. For the carcass trait variables, a linear model was used, including gender status as a fixed effect. Total lipid and FA analyses were analyzed by split-plot design using a general linear mixed model, considering the 3x2 factorial arrangement, including gender status (heifers, bulls, and steers) and muscles (LO and TB) as fixed effects, the random effect of cattle within groups, and their interactions. The effects of gender status on each muscle were compared using Fisher's least significant difference when the interaction between gender and muscle was significant. To assess the effect of gender on the characteristics evaluated, the data were submitted to ANOVA, and when there was a significant effect, the means were compared using the Tukey test. Once the normality of the residuals was obtained, the effect of the covariate percentage of total lipids nested in the gender status was tested in the analysis of fatty acid composition.

Gene expression data were normalized by subtracting the target's "threshold cycle" (Ct) value – the normalizer's Ct value. Afterwards, they were tested for the presence of outliers, normality and homoscedasticity. Initially, the existence of an interaction between gender status and type of muscle was evaluated for the results of gene expression. In the case of an interaction, the LS means Tukey HSD test was applied. In the absence of an interaction, comparison tests between means were used, and Student's t-test was applied for comparison data between two experimental groups (muscles). For analyses containing more than two experimental groups, such as gender status, ANOVA was applied followed by Tukey's test. The graphs were generated with GraphPad Prism 7.0 by plotting the transformed normalized Ct values using the formula $2^{-\Delta Ct}$. The results are plotted as relative levels of transcripts. For the tropomyosin protein abundance, the data were submitted to ANOVA and the means were compared by Tukey's test. All analyses were performed at the $P \leq 0.05$ significance level.

2.3 RESULTS AND DISCUSSION

2.3.1 Subcutaneous fat thickness and marbling score

Since the goal of this study was to understand the changes related to fat tissue, the carcass traits evaluated in the present study are related to fat deposition. The ribeye subcutaneous fat thickness and marbling score (intramuscular fat - IMF) were higher in heifer carcasses than in carcasses of other genders ($P \leq 0.05$; Table 3). These results observed in the current experiment are consistent with previously reported studies involving fat deposition (BLANCO *et al*., 2020; MUELLER *et al*., 2019). This was expected because heifers usually show higher marbling scores and better carcass quality than males due to their greater potential and ability for fat deposition, caused by differences in the growth curves between the animal categories (OWENS *et al*., 1993; TATUM; GRUBER; SCHNEIDER, 2007). IMF is the last fat tissue to be deposited, and heifers have genes that control deposition efficiency (LEE *et al*., 2009). Similarly, some authors reported a greater capacity of heifers to improve carcass quality due to their ability to deposit subcutaneous fat and IMF compared to steers and bulls (CHOAT *et al*., 2006; MUELLER *et al*., 2019).

The appreciation of beef is generally positively associated with IMF content, and a decrease in the IMF content can also reduce beef quality (DRANSFIELD *et al*., 2003). Indeed, a minimum amount of IMF is needed for the expression of beef flavor (WOOD; RICHARDSON, 2004). IMF also plays an important role in beef juiciness, and meat with a high IMF content is always less dry than lean meat (PICARD *et al*., 2019).

According to the classification by quality grade (USDA, 2017), carcasses of steers and heifers were classified as Choice, and bulls as Low Choice, referring to the quality grade. Thus, heifer and steers meat can be a better strategy in the production of high-quality beef, compared to bull’s meat.

Table 3 - Carcass traits (±SEM) related to fat deposition of different gender status Angus × Nelore cattle

Variable	Gender status				
	Heifers	Bulls	Steers	SEM	*P*-value
Fat thickness, mm	8.6[a]	5.1[c]	7.3[b]	0.353	0.0001
Marbling score[1]	6.0[a]	4.6[c]	5.3[b]	0.110	0.0001

Note: Means in the same row followed by different letters are significantly different ($P < 0.01$).
[1]Marbling degrees: Traces (TR) – 2.0 to 2.99; Slight (SL) – 3.0 to 3.99; Small (SM) – 4.0 to 4.99; Modest (MT) – 5.0 to 5.99; Moderate (MD) – 6.0 to 6.99; Slightly abundant (SA) – 7.0 to 7.99; Moderately abundant (MA) – 8.0 to 8.99. Quality grade: Standard (TR), Select (SL), Low choice (SM), Choice (MT and MD), Prime (SA and MA).

2.3.2 Total lipids and fatty acid composition

To investigate the lipid and fatty acid compositions, we performed total lipids and fatty acid composition analyses, respectively. There was no interaction between gender status and type of muscle for the content of total lipids ($P \geq 0.05$). Regarding each factor studied separately, there was no effect of muscle type for this variable ($P \geq 0.05$). On the other hand, as was expected, gender status affected the percentage of total lipids ($P \leq 0.05$; Table 4).

The mean percentage of total lipids was 2.35% in LO and 2.23% in TB muscle, depending of gender and 2.95%, 1.55% and 2.36% in meat from heifers, bulls, and steers, respectively, depending of muscle. These results corroborates with those found for fat thickness and marbling scores, where heifers presented greater results for these traits than steers and bulls. Gamarra *et al*. (2018) reported different total lipids between bulls, heifers, and cows, suggesting a possible effect of sex hormones on enzymatic systems affecting lipid metabolism and consequently the content of fat. In disagreement with our results, Raes *et al*. (2004) showed a clearly higher IMF content in the LO muscle than in the TB muscle.

Differences in muscle metabolism can be explained by the higher content of organelles, particularly mitochondria, of red muscles and the specific phospholipid class composition of mitochondrial membranes compared to other membranes (GANDEMER, 1999; LESEIGNEUR-MEYNIER; GANDEMER, 1991). However, we could not see any differences in total lipids between the muscles evaluated because both had similar metabolism (mixed fast oxidative-glycolytic). Glycolytic and oxidative muscles may contain similar amounts of IMF (DE SMET; RAES; DEMEYER, 2004; GAGAOUA *et al*., 2017; OURY *et al.,* 2007; PICARD *et al*., 2017).

Our results of the content of total lipids agree with those reported in the literature for crossbred cattle of different gender status of between 2-4% of intramuscular fat (LISTRAT *et*

al., 2020; SORET *et al*., 2016), as well as for both muscles evaluated in the present study, although we did not observe any difference among muscles. Since IMF is important for juicy meat (PICARD *et al*., 2019), in European countries, an IMF of 3–4% in beef is required for good sensory appreciation by consumers (BONNET *et al*., 2010; HOCQUETTE *et al*., 2010).

Were identified 78 fatty acids (FA) in the fatty acid composition analysis of beef in the present study. In Table 4 are presented the effects of gender status and muscle type on the principal FA related to human health.

Table 4 - Total lipids, fatty acid composition (% of total fatty acids), and SEM (±) of the *longissimus* and *triceps brachii* muscles of feedlot Angus × Nelore cattle for different gender status

(Continue)

Variable	Gender	Muscle		$\bar{\times}$ gender	*P*-value		
		LO	TB		Gender	Muscle	Gender x Muscle
TL (%)	Heifers	3.24 ± 0.22	2.66 ± 0.22	2.95 ± 0.15	<.0001	0.5031	0.1582
	Bulls	1.59 ± 0.23	1.52 ± 0.23	1.55 ± 0.17			
	Steers	2.32 ± 0.23	2.60 ± 0.23	2.36 ± 0.17			
	$\bar{\times}$ muscle	2.35 ± 0.13	2.23 ± 0.13				
SFA							
14:0	Heifers	2.67 ± 0.11	2.54 ± 0.11	2.60 ± 0.08	0.6629	0.**0193**	0.3059
	Bulls	2.68 ± 0.18	2.66 ± 0.20	2.67 ± 0.18			
	Steers	3.28 ± 0.30	2.95 ± 0.29	3.12 ± 0.29			
	$\bar{\times}$ muscle	2.88 ± 0.17	2.72 ± 0.17				
16:0	Heifers	25.2 ± 0.3	24.5 ± 0.3	24.8 ± 0.26	0.4591	**0.0251**	0.3947
	Bulls	23.7 ± 0.3	23.2 ± 0.4	23.5 ± 0.30			
	Steers	25.6 ± 0.3	24.2 ± 0.3	24.9 ± 0.27			
	$\bar{\times}$ muscle	24.8 ± 0.20	23.9 ± 0.20				
18:0	Heifers	11.7 ± 0.3	11.5 ± 0.3	11.6 ± 0.25	0.119	0.114	0.664
	Bulls	15.5 ± 0.3	15.1 ± 0.4	15.3 ± 0.29			
	Steers	13.1 ± 0.3	12.3 ± 0.3	12.7 ± 0.25			
	$\bar{\times}$ muscle	13.4 ± 0.19	13.0 ± 0.19				
∑SFA	Heifers	41.5 ± 0.5	40.5 ± 0.5	41.0 ± 0.3[b]	**0.043**	**0.003**	0.226
	Bulls	43.7 ± 0.5	42.9 ± 0.6	43.3 ± 0.4[a]			
	Steers	43.6 ± 0.5	41.1 ± 0.5	42.4 ± 0.3[a]			
	$\bar{\times}$ muscle	42.9 ± 0.2	41.5 ± 0.3				
MUFA							
14:1 c9	Heifers	0.721 ± 0.040	0.721 ± 0.038	0.721 ± 0.03	0.434	0.408	0.256
	Bulls	0.686 ± 0.066	0.717 ± 0.075	0.701 ± 0.06			
	Steers	1.05 ± 0.110	0.975 ± 0.109	1.01 ± 0.10			
	$\bar{\times}$ muscle	0.819 ± 0.06	0.804 ± 0.06				

Table 4 - Total lipids, fatty acid composition (% of total fatty acids), and SEM (±) of the *longissimus* and *triceps brachii* muscles of feedlot Angus × Nelore cattle for different gender status

(Continue)

Variable	Gender	Muscle		$\bar{\times}$ gender	*P*-value		
		LO	TB		Gender	Muscle	Gender x Muscle
15:1 c10	Heifers	0.057 ± 0.005 ^{Aa}	0.040 ± 0.004 ^{Ba}	0.049 ± 0.003	0.813	0.205	**0.001**
	Bulls	0.063 ± 0.005 ^{Aa}	0.050 ± 0.006 ^{Ba}	0.057 ± 0.004			
	Steers	0.027 ± 0.004 ^{Bb}	0.043 ± 0.004 ^{Aa}	0.035 ± 0.003			
	$\bar{\times}$ muscle	0.049 ± 0.002	0.044 ± 0.002				
16:1 c9	Heifers	3.25 ± 0.13	3.30 ± 0.13	3.27 ± 0.10	0.249	0.494	0.698
	Bulls	2.55 ± 0.19	2.41 ± 0.22	2.48 ± 0.19			
	Steers	3.30 ± 0.31	3.17 ± 0.30	3.23 ± 0.29			
	$\bar{\times}$ muscle	3.03 ± 0.18	2.96 ± 0.18				
18:1 t9	Heifers	0.119 ± 0.020	0.102 ± 0.019	0.110 ± 0.015	0.973	0.165	0.794
	Bulls	0.223 ± 0.030	0.180 ± 0.180	0.201 ± 0.029			
	Steers	0.170 ± 0.048	0.149 ± 0.149	0.159 ± 0.046			
	$\bar{\times}$ muscle	0.170 ± 0.028	0.143 ± 0.028				
18:1 t11	Heifers	0.982 ± 0.143 ^{Bb}	1.18 ± 0.135 ^{Aa}	1.08 ± 0.10	0.724	0.079	**0.017**
	Bulls	2.24 ± 0.217 ^{Aa}	1.90 ± 0.250 ^{Ba}	2.07 ± 0.21			
	Steers	2.27 ± 0.353 ^{Aa}	1.80 ± 0.349 ^{Ba}	2.03 ± 0.34			
	$\bar{\times}$ muscle	1.83 ± 0.20	1.62 ± 0.20				
18:1 n-9c	Heifers	36.3 ± 0.7	35.0 ± 0.6	35.6 ± 0.5	**0.055**	**0.022**	0.838
	Bulls	28.5 ± 1.1	27.9 ± 1.2	28.2 ± 1.1			
	Steers	32.1 ± 1.8	30.7 ± 1.8	31.4 ± 1.7			
	$\bar{\times}$ muscle	32.3 ± 1.0	31.2 ± 1.0				
18:1 c11	Heifers	0.348 ± 0.020	0.360 ± 0.019	0.354 ± 0.014	0.533	**0.027**	0.239
	Bulls	0.342 ± 0.020	0.426 ± 0.024	0.384 ± 0.016			
	Steers	0.353 ± 0.019	0.417 ± 0.019	0.385 ± 0.014			
	$\bar{\times}$ muscle	0.348 ± 0.010	0.401 ± 0.010				
∑MUFA	Heifers	47.7 ± 1.4	48.3 ± 1.3	48.0 ± 0.9 ^{a}	**0.0155**	0.120	0.383
	Bulls	44.5 ± 1.4	40.7 ± 1.7	42.6 ± 1.1 ^{b}			
	Steers	46.8 ± 1.3	45.5 ± 1.3	46.2 ± 1.0 ^{a}			
	$\bar{\times}$ muscle	46.3 ± 0.7	44.8 ± 0.7				
PUFA							
18:2 n-6c	Heifers	4.57 ± 0.40	5.48 ± 0.39	5.03 ± 0.28	0.728	**0.0002**	0.294
	Bulls	7.69 ± 0.40	8.61 ± 0.49	8.15 ± 0.32			
	Steers	4.20 ± 0.38	6.13 ± 0.39	5.16 ± 0.29			
	$\bar{\times}$ muscle	5.49 ± 0.21	6.74 ± 0.21				
18:3 n-3	Heifers	0.156 ± 0.008	0.154 ± 0.008	0.155 ± 0.006	0.449	0.180	0.384
	Bulls	0.118 ± 0.009	0.100 ± 0.010	0.109 ± 0.007			
	Steers	0.114 ± 0.009	0.121 ± 0.009	0.117 ± 0.007			

Table 4 - Total lipids, fatty acid composition (% of total fatty acids), and SEM (±) of the *longissimus* and *triceps brachii* muscles of feedlot Angus × Nelore cattle for different gender status

(Continue)

Variable	Gender	Muscle		$\bar{\times}$ gender	*P*-value		
		LO	TB		Gender	Muscle	Gender x Muscle
18:3 n-3	$\bar{\times}$ **muscle**	0.129 ± 0.005	0.125 ± 0.005				
20:2-n6	Heifers	0.052 ± 0.019	0.089 ± 0.018	0.070 ± 0.014	0.692	0.738	0.660
	Bulls	0.085 ± 0.029	0.119 ± 0.033	0.102 ± 0.028			
	Steers	0.099 ± 0.047	0.110 ± 0.046	0.105 ± 0.045			
	$\bar{\times}$ **muscle**	0.079 ± 0.027	0.106 ± 0.027				
20:3-n6	Heifers	0.015 ± 0.015	0.005 ± 0.015	0.010 ± 0.011	0.490	0.519	0.632
	Bulls	0.065 ± 0.015	0.021 ± 0.019	0.043 ± 0.012			
	Steers	0.035 ± 0.014	0.009 ± 0.015	0.022 ± 0.011			
	$\bar{\times}$ **muscle**	0.038 ± 0.008	0.012 ± 0.008				
20:3-n3	Heifers	1.25 ± 0.13	1.53 ± 0.12	1.39 ± 0.09	0.908	**0.001**	0.108
	Bulls	1.66 ± 0.13	1.85 ± 0.16	1.75 ± 0.10			
	Steers	1.26 ± 0.12	1.95 ± 0.12	1.61 ± 0.09			
	$\bar{\times}$ **muscle**	1.39 ± 0.07	1.78 ± 0.07				
22:2-n6	Heifers	0.021 ± 0.030	0.092 ± 0.029	0.056 ± 0.021 [a]	**0.042**	0.065	0.555
	Bulls	0.041 ± 0.030	0.089 ± 0.036	0.065 ± 0.024 [a]			
	Steers	0.000 ± 0.028	0.110 ± 0.029	0.055 ± 0.021 [a]			
	$\bar{\times}$ **muscle**	0.021 ± 0.016	0.097 ±0.016				
22:3-n3	Heifers	0.025 ± 0.006	0.017 ± 0.005	0.035 ± 0.004	0.616	0.728	0.612
	Bulls	0.036 ± 0.006	0.027 ± 0.007	0.021 ± 0.005			
	Steers	0.044 ± 0.006	0.026 ± 0.006	0.035 ± 0.005			
	$\bar{\times}$ **muscle**	0.035 ± 0.003	0.026 ± 0.003				
22:4-n6	Heifers	0.170 ± 0.018	0.235 ± 0.018	0.202 ± 0.013	0.733	**0.001**	0.202
	Bulls	0.182 ± 0.018	0.247 ± 0.022	0.214 ± 0.015			
	Steers	0.177 ± 0.017	0.296 ± 0.018	0.237 ± 0.013			
	$\bar{\times}$ **muscle**	0.176 ± 0.010	0.259 ± 0.010				
22:5-n3 EPA	Heifers	0.313 ± 0.041	0.341 ± 0.040	0.327 ± 0.028	0.969	**0.008**	0.745
	Bulls	0.320 ± 0.041	0.352 ± 0.050	0.336 ± 0.033			
	Steers	0.253 ± 0.039	0.334 ± 0.040	0.293 ± 0.029			
	$\bar{\times}$ **muscle**	0.295 ± 0.022	0.342 ± 0.022				
22:6-n3 DHA	Heifers	0.031 ± 0.032	0.031 ± 0.031	0.031 ± 0.022	0.602	0.801	0.701
	Bulls	0.073 ± 0.032	0.027 ± 0.039	0.050 ± 0.026			
	Steers	0.024 ± 0.031	0.035 ± 0.031	0.029 ± 0.023			
	$\bar{\times}$ **muscle**	0.043 ± 0.017	0.031 ± 0.017				
CLA							
c9 t11	Heifers	0.237 ± 0.017	0.210 ± 0.015	0.224 ± 0.013			

Table 4 - Total lipids, fatty acid composition (% of total fatty acids), and SEM (±) of the *longissimus* and *triceps brachii* muscles of feedlot Angus × Nelore cattle for different gender status

(Continue)

Variable	Gender	Muscle		$\bar{\times}$ gender	*P*-value		
		LO	TB		Gender	Muscle	Gender x Muscle
c9 t11	Bulls	0.269 ± 0.027	0.257 ± 0.031	0.263 ± 0.026	0.335	**0.034**	0.818
	Steers	0.367 ± 0.046	0.336 ± 0.045	0.351 ± 0.044			
	$\bar{\times}$ **muscle**	0.291 ± 0.026	0.268 ± 0.026				
t10 c12	Heifers	0.013 ± 0.002	0.006 ± 0.002	0.010 ± 0.001	0.507	0.111	0.882
	Bulls	0.014 ± 0.002	0.008 ± 0.002	0.011 ± 0.001			
	Steers	0.011 ± 0.001	0.005 ± 0.002	0.008 ± 0.001			
	$\bar{\times}$ **muscle**	0.013 ± 0.001	0.006 ± 0.001				
∑PUFA	Heifers	7.01 ± 0.60	8.42 ± 0.58	7.72 ± 0.42	0.916	**0.0002**	0.247
	Bulls	10.9 ± 0.60	11.9 ± 0.73	11.4 ± 0.49			
	Steers	6.74 ± 0.57	9.58 ± 0.59	8.16 ± 0.43			
	$\bar{\times}$ **muscle**	8.22 ± 0.32	9.96 ± 0.32				
∑PUFA n-3	Heifers	1.79 ± 0.12	2.07 ± 0.12	1.93 ± 0.08	0.292	**0.0004**	0.060
	Bulls	2.12 ± 0.12	2.39 ± 0.15	2.25 ± 0.10			
	Steers	1.74 ± 0.11	2.50 ± 0.12	2.12 ± 0.08			
	$\bar{\times}$ **muscle**	1.88 ± 0.06	2.32 ± 0.06				
∑PUFA n-6	Heifers	4.90 ± 0.54	5.97 ± 0.53	5.44 ± 0.38	0.965	**0.001**	0.387
	Bulls	8.34 ± 0.54	9.10 ± 0.66	8.72 ± 0.44			
	Steers	4.52 ± 0.51	6.65 ± 0.53	5.58 ± 0.39			
	$\bar{\times}$ **muscle**	5.92 ± 0.29	7.24 ± 0.29				
PUFA:SFA	Heifers	0.169 ± 0.017	0.208 ± 0.016	0.189 ± 0.012	0.995	**0.0002**	0.256
	Bulls	0.253 ± 0.017	0.281 ± 0.021	0.267 ± 0.014			
	Steers	0.115 ± 0.016	0.234 ± 0.016	0.194 ± 0.012			
	$\bar{\times}$ **muscle**	0.192 ± 0.009	0.241 ± 0.009				
n-6:n-3	Heifers	2.67 ± 0.31	2.87 ± 0.30	2.77 ± 0.21	0.268	0.146	0.693
	Bulls	4.16 ± 0.31	3.76 ± 0.37	3.96 ± 0.25			
	Steers	2.62 ± 0.29	2.63 ± 0.30	2.62 ± 0.22			
	$\bar{\times}$ **muscle**	3.15 ± 0.16	3.09 ± 0.16				
HI	Heifers	1.55 ± 0.09	1.67 ± 0.09	1.61 ± 0.06	0.175	**0.020**	0.558
	Bulls	1.67 ± 0.09	1.63 ± 0.11	1.65 ± 0.07			
	Steers	1.47 ± 0.08	1.64 ± 0.09	1.55 ± 0.06			
	$\bar{\times}$ **muscle**	1.56 ± 0.05	1.64 ± 0.05				
AI	Heifers	0.652 ± 0.022	0.613 ± 0.021	0.633 ± 0.015	0.144	**0.002**	0.286
	Bulls	0.623 ± 0.022	0.629 ± 0.027	0.626 ± 0.018			
	Steers	0.693 ± 0.021	0.626 ± 0.021	0.660 ± 0.016			

Table 4 - Total lipids, fatty acid composition (% of total fatty acids), and SEM (±) of the *longissimus* and *triceps brachii* muscles of feedlot Angus × Nelore cattle for different gender status

(Conclusion)

Variable	Gender	Muscle		$\bar{\times}$ gender	*P*-value		
		LO	TB		Gender	Muscle	Gender x Muscle
AI	$\bar{\times}$ muscle	0.656 ± 0.012	0.623 ± 0.012				
TI	Heifers	1.23 ± 0.03	1.16 ± 0.03	1.20 ± 0.02	0.052	**0.0003**	0.162
	Bulls	1.30 ± 0.03	1.30 ± 0.04	1.30 ± 0.02			
	Steers	1.32 ± 0.03	1.19 ± 0.03	1.26 ± 0.02			
	$\bar{\times}$ muscle	1.29 ± 0.01	1.22 ± 0.01				
Non-identified		0.219	0.228				

Note: Means in the same row followed by different lowercase are significantly different (gender status), by Tukey test ($P < 0.05$).
Means in the same row followed by different uppercase are significantly different (type of muscle), by Tukey test ($P < 0.05$).
Abbreviations: LO: *longissimus*; TB: *triceps brachii*; $\bar{\times}$: mean of muscles; SFA: saturated fatty acids; MUFA: monounsaturated fatty acids; PUFA: polyunsaturated fatty acids; CLA: conjugated linoleic acid; HI: healthy index; AI: atherogenicity index; TI: thrombogenicity index.
TL (%): total lipids, express in 1 g of fat /100g of beef.
∑SFA (10:0; 12:0; 13:0; 14:0; 15:0; 16:0; 17:0; 18:0; 19:0; 20:0; 22:0; 24:0); ∑MUFA (14:1 c9; 15:1 c10; 16:1 t9; 16:1 c7; 16:1 c9; 16:1 c10; 16:1 c11; 16:1 c12 t14; 17:1 c9; 17:1 c10; 17:1 c11; 18:1 t9; 18:1 t10; 18:1 t11; 18:1 t12; 18:1 n-9c; 18:1 c10 t15; 18:1 c11; 18:1 c12; 18:1 c13; 18:1 c14 t16; 19:1 c; 19:1 c9, 15; 20:1 c8; 20:1 c11);
∑PUFA (18:2 n-6t; 18:2 t8, c13; 18:2 c9, t12; 18:2 t9, c12; 18:2 t10, c15; 18:2 n-6c; 18:3 n-6y; 18:3 n-3; CLA c9, t11; CLA c11, t13; CLA t10, c12; 20:2 n-6; 20:3 n-9; 20:3 n-6; 20:3 n-3; 22:2 n-6; 22:3 n-3; 22:4 n-6; 22:4 n-3; 22:5 n-3; 22:6 n-3); ∑n-6 (18:2 n-6t; 18:2 n-6c; 18:3 n-6y; 20:2 n-6; 20:3 n-6; 22:2 n-6; 22:4 n-6); ∑n-3 (18:3 n-3; 20:3 n-3; 22:3 n-3; 22:4 n-3; 22:5 n-3; 22:6 n-3).
HI: (MUFA+PUFA)/(4*14:0)+16:0); AI: (12:0+(4*14:0)+16:0)/(∑n6+∑n3+∑MUFA); TI: (14:0+16:0+18:0)/((0,5*∑MUFA)+(0,5*∑n6)+(3*∑n3)+n3/n6).

No interaction between gender status and muscle type was observed for the total saturated FAs (SFAs; $P \geq 0.05$). Nevertheless, there was an individual effect of gender and muscle on the total SFAs ($P \leq 0.05$). Although Venkata Reddy *et al*. (2015) reported that SFA levels do not vary much among cattle of different gender status, in the current study, meat from bulls (43.3%) had no difference compared to meat from steers (42.4%), and both were different of heifers. Meat from heifers (41.1%) showed lower levels of SFAs compared to other gender. Total SFAs accounted for approximately 40% of all fatty acids in LO and TB among all genders. Similar profiles were also presented in other studies investigating crossbred beef cattle (COLEMAN *et al*., 2016; LIU *et al*., 2020; MUELLER *et al*., 2019).

Regarding the individual SFAs myristic acid (C14:0), palmitic acid (C16:0), and stearic acid (C18:0), which together dominantly comprised more than 90% of the total SFAs, there was no difference among the genders. These results are contrary to those observed by Blanco

et al. (2020) and Mueller *et al*. (2019). The hormonal status of beef cattle is directly related to the fatty acid distribution in muscles (LEE *et al*., 2009). Regarding cattle gender, the differences in beef fatty acid composition are associated with the IMF content due to a higher proportion of membrane phospholipids (high in PUFAs) and lower triacylglycerol (high in SFAs and MUFAs) in leaner animals (DE SMET; RAES; DEMEYER, 2004; WOOD *et al*., 2008). Among muscle types, total SFAs were greater in LO muscle (42.9%) than in TB muscle (41.6%). Purchas *et al*. (2005) observed no differences between these muscles in beef but reported that the pattern of fatty acids differs significantly between the LO and TB muscles, with some of the differences being attributable to the higher IMF levels in the LO muscle. SFAs are recognized as a critical predisposing factor in the development of cardiovascular diseases and are implicated in cancers, obesity, diabetes and other health problems (BRIGGS; PETERSEN; KRIS-ETHERTON, 2017; PIGHIN *et al*., 2016). Therefore, beef from the TB muscle and beef from heifers might have a preferable SFA profile that is more satisfactory for the needs of modern consumers than beef of the LO muscle and the other genders evaluated, with a significantly lower total SFA content. The FAs 14:0 and 16:0 were affected by muscle type only, and higher levels were detected in LO muscle ($P \leq 0.05$) than in TB. On the other hand, there was no effect of gender status or muscle type on the concentration of FA 18:0 ($P \leq 0.05$). It is generally accepted that some SFAs that are commonly found in beef, especially C16:0 and C14:0, raise the total cholesterol and low-density lipoprotein and are thus risk factors for coronary heart disease (ERKKILÄ *et al*., 2008; WEBB; O'NEILL, 2008). Thus, TB muscle, with lower proportions of C16:0 and C14:0, might be more beneficial to human health.

There was no interaction between gender and muscle type for the total monounsaturated FAs (MUFAs; $P \geq 0.05$). However, there was an individual effect of gender on total MUFAs ($P \leq 0.05$). Meat from bulls (42.65%) had lower levels of MUFAs than meat from heifers (48.04%) and steers (46.23%).

According to Blanco et al. (2020), very few studies comparing the fatty acid composition of beef among the gender status under similar conditions of husbandry, especially feeding regime, are available. Concerning the different proportions of the most abundant individual fatty acids (measured as a percentage of the total fatty acids), our results agree with those of Blanco *et al*. (2020) and Mueller *et al*. (2019), who observed that heifer meat had higher levels of MUFAs than steers and bulls, and with Barton *et al*. (2011) and Karolyi *et al*. (2009), who also reported higher levels of MUFAs in heifer meat than in bulls. Nian *et al*. (2018) also observed a higher content of total MUFAs in steer meat than in bulls. The level of MUFAs increases faster than that of PUFAs with increasing fatness (DE SMET; RAES; DEMEYER,

2004). Additionally, the increased amount of marbling in beef is associated with increases in MUFAs (SMITH et al., 2009), and allele C had a significant effect on increasing the contents of MUFAs in the FA composition of the intramuscular adipose tissues of heifers (ARDIYANTI *et al*., 2009). As IMF accumulates, there is a concomitant elevation in the concentration of oleic acid, the major FA in the MUFAs group (CHUNG *et al*., 2006). Since the heifers presented higher marbling score than bulls, we suggest that it can be a possible explanation for the greater concentration of total MUFAs in heifers' meat. Based on the concentration of total MUFAs, meat from heifers has an improved beef FA composition compared to the other gender status, by increasing the percentage of MUFAs in the meat, especially oleic acid. Mueller *et al*. (2019) described a more favorable FA profile for human health in heifers and steers compared to bulls, but Fernandes *et al*. (2009) found no differences among genders.

The differences among the genders of cattle have been associated with their different growth and maturing rates, since sex hormones can influence lipid metabolism (BLANCO *et al*., 2020). The greater rate of fat deposition of heifers during the finishing phase, reflected in their higher IMF content, results in larger adipocytes (EGUINOA *et al*., 2003). This larger size of adipocyte implies a different FA composition, with higher triacylglycerides (high in SFAs and MUFAs) in the inner lipid droplets and a lower proportion of membrane phospholipids (high in PUFAs) (DE SMET; RAES; DEMEYER, 2004). For the individual FAs 14:1 c9, 16:1 c9, 18:1 t9, 18:1 n-9c and 18:1 c11, there was no interaction between gender and muscle type ($P \geq 0.05$). Similarly, 14:1 c9, 16:1 c9 and 18:1 t9 were not affected by gender or muscle type ($P \geq 0.05$). Muscle type affected the level of 18:1 n-9c ($P \leq 0.05$), in which it was higher in the LO muscle (32.35%) than in the TB muscle (31.26%). On the other hand, there was no effect of gender on the concentration of this FA ($P \geq 0.05$). Our findings agree with those of Raes *et al*. (2004), who reported a higher concentration of 18:1 n-9c (oleic acid) in LO than in TB muscle. However, Purchas *et al*. (2005), found that oleic acid was lower in LO muscle. It has been shown that histochemical characterization of the fibers has revealed that the LO muscle contains a higher content of glycolytic fibers than the TB muscle (PICARD; DURIS; JURIE, 1998). Glycolytic muscles contain a lower number of mitochondria and thus have a lower phospholipid content. A lower phospholipid content results in a lower amount of PUFAs and therefore more SFAs and MUFAs, and the major FA of MUFAs is oleic acid. Oleic acid is the most abundant MUFA found in skeletal muscle fat (DOWHAN; MILEYKOVSKAYA; BOGDANOV, 2004), and it is present in membrane phospholipids, triglycerides and cholesterol esters. Human consumption of oleic acid has been associated with low levels of low-density lipoprotein (LDL)

and with a potential increase in high-density lipoprotein (HDL) levels in the blood. Previous studies have reported an important contribution of oleic acid intake to general human health, which could lead to a decrease in cholesterol levels, atherosclerosis risk, and diabetes occurrence and has protective effects against viral infection and cancer development (CESAR *et al*., 2016). Therefore, according to our results for this specific FA, once the LO muscle presents a higher concentration of oleic acid, we can suggest a more favorable profile to human health by the LO muscle.

For the total polyunsaturated FAs (PUFAs), total n-3 and n-6, PUFA:SFA ratio and n-6:n-3 ratio, and for the individual PUFAs evaluated in the present study, no interaction was observed between gender status and muscle type ($P \geq 0.05$). Similarly, gender status as an individual factor did not affect the total PUFAs, total n-3 and n-6, or the PUFA:SFA and n-6:n-3 ratios ($P \geq 0.05$). The result for the total n-3 of the current study corroborates with those observed by Mueller *et al*. (2019), who also found no differences among bulls, heifers and steers for the total n-3, and Gamarra *et al*. (2018) between bulls and heifers. On the other hand, several authors (BARTON *et al*., 2011; BLANCO *et al*., 2020; GAMARRA *et al*., 2018; MUELLER *et al*., 2019) found differences in the total PUFAs, total n-6, and the n-6:n-3 ratio among the gender status. Since the gender status of cattle affects the FA composition (BARTON *et al*., 2011; DE SMET; RAES; DEMEYER, 2004; MUELLER *et al*., 2019; VENKATA REDDY *et al*., 2015; WOOD *et al*., 2008), we expected to find differences between groups for total PUFAs, total n-6, PUFA:SFA ratio, and n-6:n-3 ratio and individual FAs, as noted by Blanco *et al*. (2020) and Mueller *et al*. (2019), studying with bulls, steers and heifers, and Barton *et al*. (2011) with bulls and heifers. The content of phospholipids in the muscle is relatively independent of the total fat content and is related to the deposition of PUFAs in the muscle. Since a difference in IMF was observed between the groups, a difference was expected in the PUFAs among the genders. Thereby, we believe that the absence of these differences in our study can be explained by the different conditions that the cattle were submitted to, such as age, weight, diet and feedlot time, comparing to cited studies.

The muscle type affected the total PUFAs, total n-3, total n-6, and PUFA:SFA ratio, and the levels of 18:2 n-6c, CLA c9 t11, 20:3 n-3, 22:4 n-6, and 22:5-n3 EPA ($P \leq 0.05$) whereas the values were increased in the TB muscle compared to the LO for most of FA and higher in LO than the TB muscle for the level of CLA c9 t11. The highest level of total PUFAs observed in the TB muscle was influenced by the content of 18:2 n-6c, since this is the most representative FA among the PUFAs. The results of the current study for total PUFAs, total n-6, total n-3, 18:2 n-6c, 22:4 n-6, and the PUFA:SFA ratio are in agreement with those found by

Hwang and Joo (2016), Cho *et al*. (2005) and Raes *et al*. (2004), who compared the FA composition of beef in the LO and TB muscles and observed higher concentrations of these FAs in TB muscle. It is important to highlight that there is a lack of studies in the literature related to the FA composition in meat that include more than one muscle, as was done in the present study. The fatty acid composition for individual muscle cuts is needed for researchers to accurately evaluate health and palatability of beef. However, most studies have evaluated the beef fatty acid composition by focusing on the LO muscle, but the FA composition can be modified according to the muscle (PURCHAS; ZOU, 2008; RAES *et al*., 2004; TURK; SMITH, 2009), caused by metabolic and fat deposition differences (HOCQUETTE *et al*., 2010; SORET *et al*., 2016). Muscle lipids are composed of polar lipids, mainly phospholipids located in the cell membranes, and neutral lipids, consisting mainly of triacylglycerols, in adipocytes that are located along muscle fibers and in the interfascicular area. A small amount of triacylglycerols is also present as cytosolic droplets in the muscle fibers (GANDEMER, 1999). The content of phospholipids in the muscle is relatively independent of the total fat content and is particularly rich in PUFAs, whereas triacylglycerols contain much lower amounts of PUFAs (DE SMET; RAES; DEMEYER, 2004). In the current study, there was no difference in total fat (total lipids, %) between muscles; however, there was a difference between the muscles for the total PUFAs. As the content of phospholipids in the muscle is independent of the total fat, we found a difference in the total PUFAs; therefore, this can be a factor that contributed to this difference between the muscles.

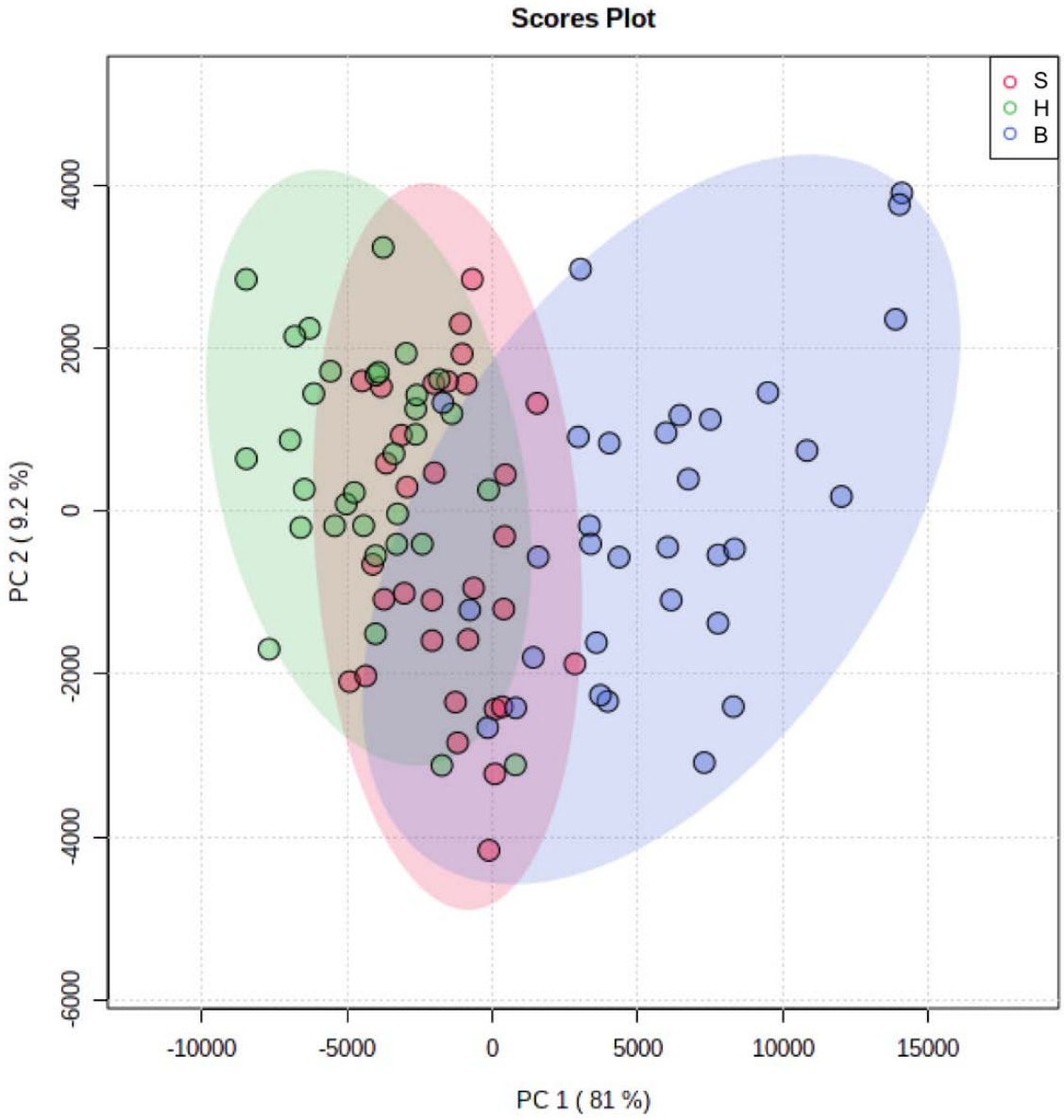
Regarding the PUFA:SFA ratio, the value recommended in the diet should be increased to above 0.4 (WOOD *et al*., 2004) because PUFAs, especially omega-3 series, carry nutritional benefits for human health, playing an important role in the prevention of cardiovascular and inflammatory diseases, neural development, thrombosis, cancer, and improvement in immunity (LOPEZ-HUERTAS, 2010). Although the TB muscle presented a higher value than the LO muscle, none of the muscles showed this ratio. However, according to Wood *et al*. (2004), some meats naturally have a PUFA:SFA ratio of around 0.1.

Muscle type only, affected the health (HI), atherogenicity (AI) and thrombogenicity (TI) indexes ($P \leq 0.05$). The HI was higher in TB, while the AI and TI were lower in TB than in LO muscle. Ulbricht and Southgate (1991) consider that an index such as PUFA:SFA ratio may not be an adequate way to evaluate the nutritional value of fat because some SFAs do not increase plasma cholesterol and it ignores the effects of MUFAs. For this reason, the health (HI), atherogenicity (AI) and thrombogenicity (TI) indexes are becoming increasingly popular for evaluating the nutritional quality of beef fat, indicating whether the food is harmful to human

health and inferring the risks of coronary and cardiovascular diseases, such as atherosclerosis and stroke (HOCQUETTE *et al.*, 2012; TROY; TIWARI; JOO, 2016; VANNICE; RASMUSSEN, 2014). The AI and TI consider the different effects that a single FA might have on human health. Since they indicate the potential to stimulate platelet aggregation, a decrease in the AI and TI shows a greater amount of antiatherogenic FAs present in some oils/fats and, consequently, a greater potential for preventing coronary diseases and better human health (TURAN; SÖNMEZ; KAYA, 2007). The TI considers the FAs C14:0, C16:0 and 18:0 to be thrombogenic, while the AI is calculated by the presence of the C12:0, C14:0 and C16:0 FAs, which promote coronary diseases, and the PUFA n-3 is antithrombogenic and antiatherogenic. The greater level of SFAs in the LO muscle compared to the TB contributed to the increase in these indexes in the LO muscle. The AI and TI observed in the TB muscle are lower than those observed in the LO muscle, however, both muscles presented an AI within the ideal standard recommended by Ulbricht and Southgate (1991) (up to 0.72). On the other hand, for the LO muscle, the value of the TI was slightly above the recommended value, which is up to 1.27, according to the same authors. According to the standard values determined by Ulbricht and Southgate (1991) and based on our results, the type of muscle in the carcass may not represent an important risk or benefit to human health, in terms of health indexes. However, comparing our data of LO and TB muscles, we can suggest some health benefits consuming beef from the region of TB muscle in the carcass.

To understand if the gender status could impact the distribution of the FA, we evaluated the distribution independently of the muscle type. The projection of these fatty acids values on a principal component analysis (PCA) allowed acceptable separation of the three gender classes (Figure 4). To perform this analysis, those FA used to calculate health, atherogenicity and thrombogenicity indexes were considered. The first two principal components (PC) explained around 90% of the fatty acid composition variability, with most variation being explained by the first PC (81%). Thus, according to the PCA, we can conclude that heifers and steers present a distinct FA profile in comparison to bulls.

Figure 4 - Principal component analysis highlighting the distribution of the individuals of each gender class (steers, heifers, and bulls) based on the fatty acids percentages.

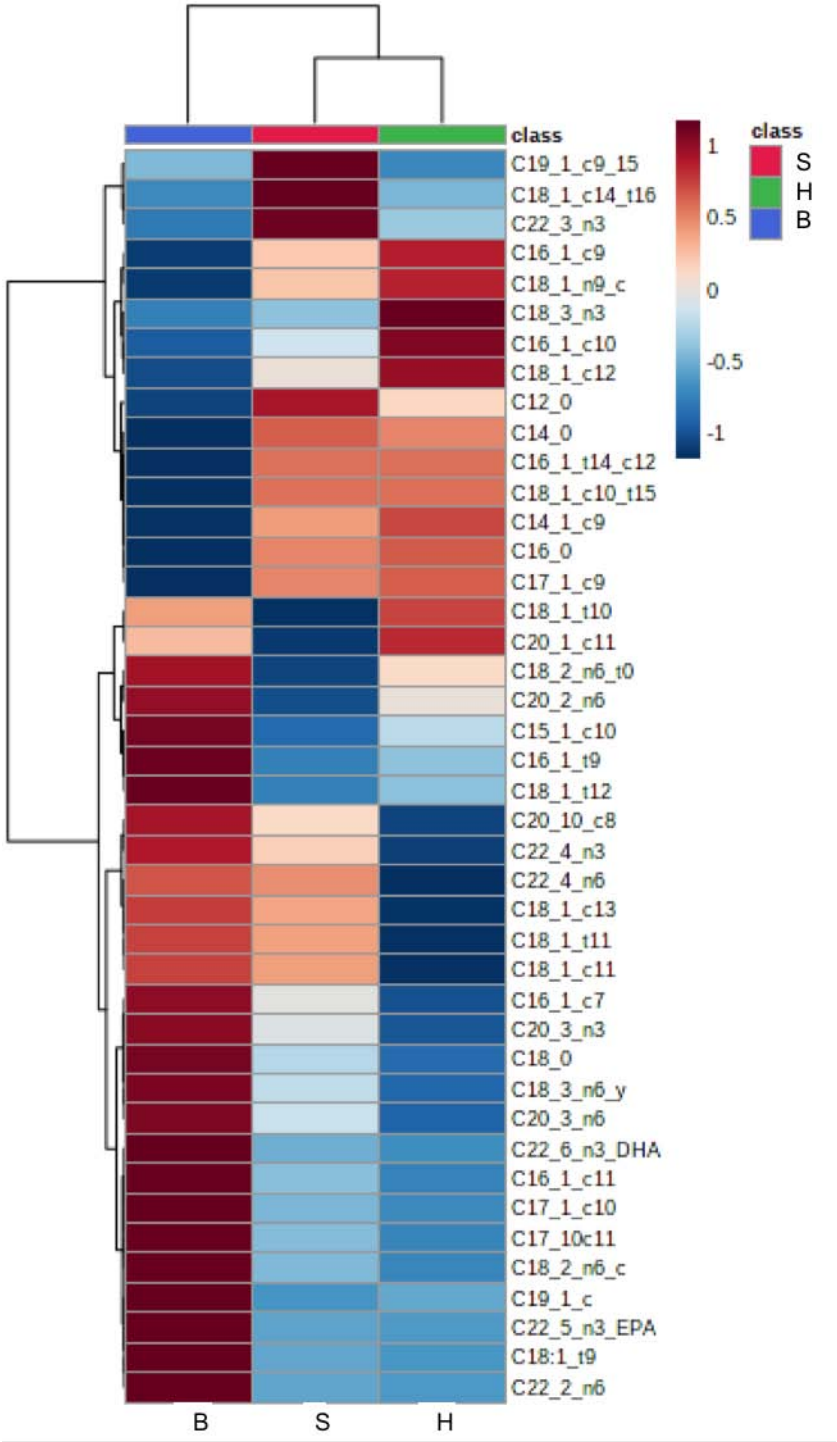


S: Steers; H: Heifers; B: Bulls.

In addition to the PCA distribution plots, the unsupervised heat map analysis demonstrates the heifers and steers are grouped together, compared to bulls in terms of FA percentages (Figure 5). Importantly, individual variation in FA were observed among the three categories. We believe that this individual variation can be a result of different feed efficiency, genetic variations or ruminal environmental as the biohydrogenation, leading to different FA composition. Multiple studies have shown that ruminal lipid metabolism is a key point in determining the content of many desirable FA in ruminant products (SCOLLAN *et al*., 2014; TORAL *et al*., 2018). Rumen microbial fermentation has a further impact on the lipid composition of milk and meat by providing precursors (volatile FA) for *de novo* FA synthesis

in the mammary gland and intramuscular lipid (BERNARD; LEROUX; CHILLIARD, 2008; SHINGFIELD; BONNET; SCOLLAN, 2013).

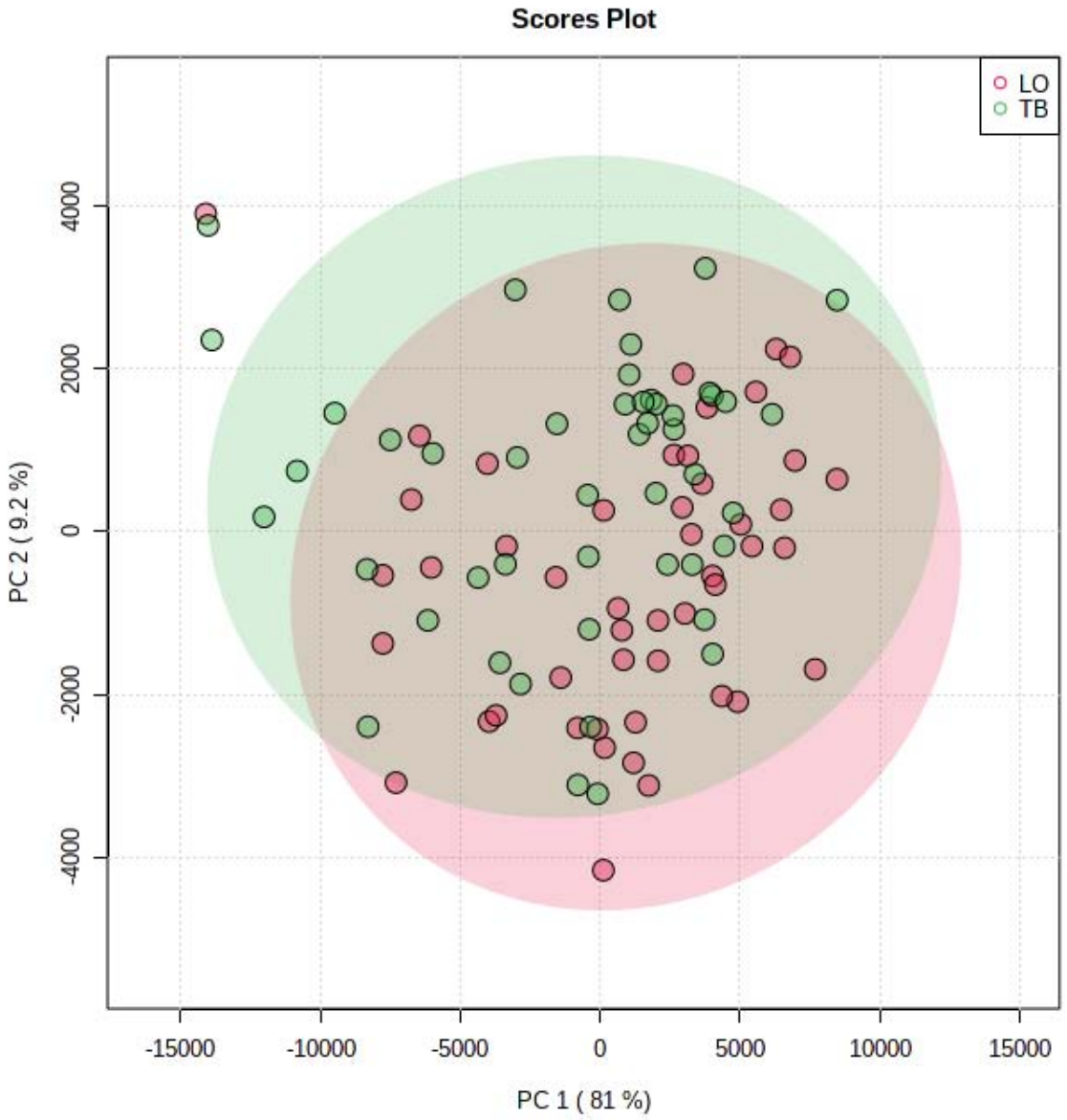
Figure 5 - Heat map comparing the fatty acid composition. The heat map shows de average percentages values for each FA within the gender (steers, heifers, and bulls).



S: Steers; H: Heifers; B: Bulls.

The projection of these fatty acids values on a PCA allowed acceptable separation of the two muscle classes (Figure 6). Importantly, this analysis demonstrates that the FA composition independently of the gender status is similar between TB and LO. Although, is important to highlight that some FA could explain differences between the two muscle independently of the gender status.

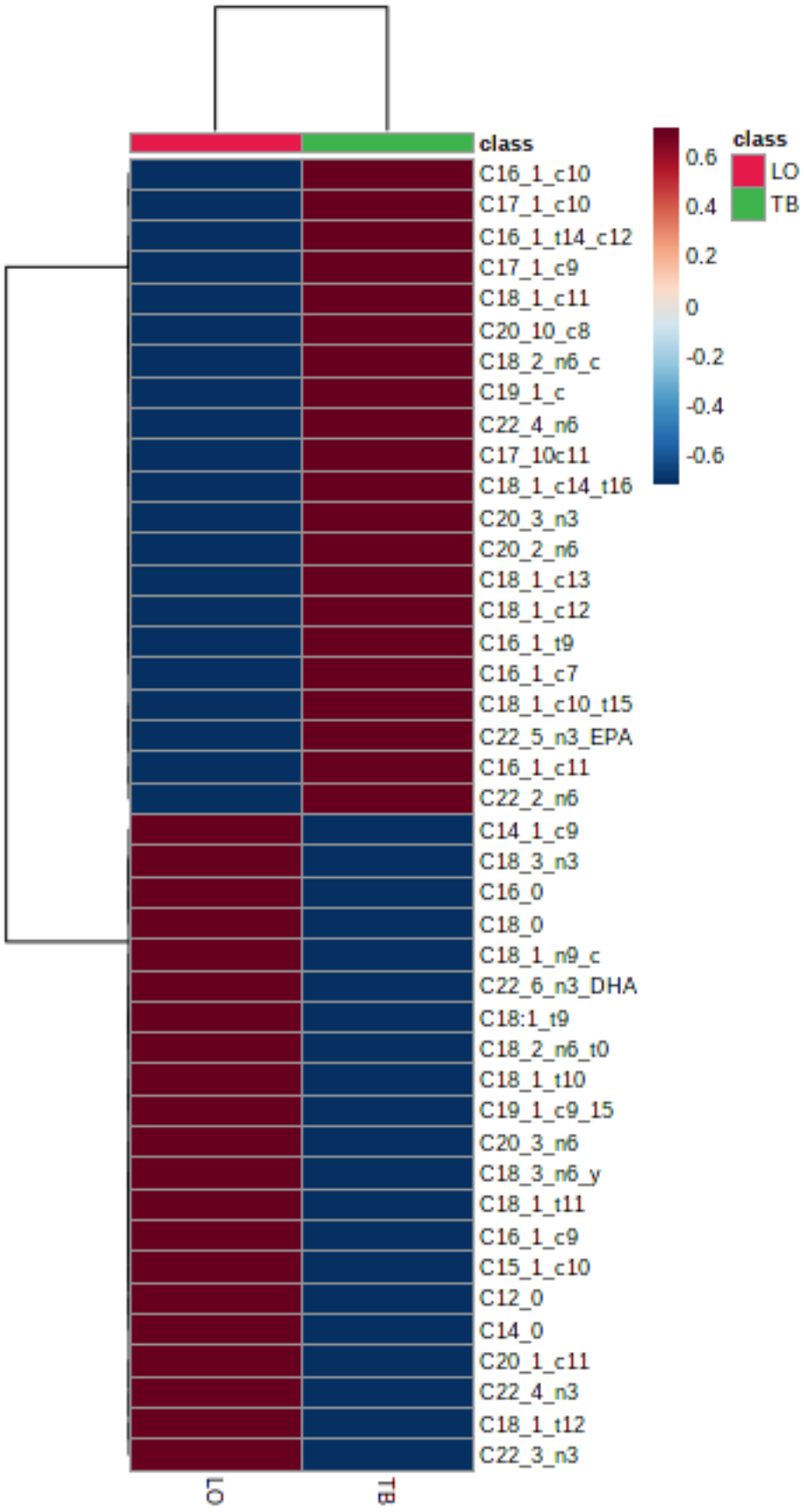
Figure 6 - Principal component analysis highlighting the distribution of the individuals of each muscle class (*longissimus* and *triceps brachii*) based on the fatty acids values.



LO: *longissimus*; TB: *triceps brachii*.

Also, a similar unsupervised heat map analysis demonstrated that most the muscle samples group together suggesting a similar FA composition within the muscle (Figure 7).

Figure 7 - Heat map comparing the fatty acid composition. The heat map shows de average percentages values for each FA within the muscle type (LO and TB).



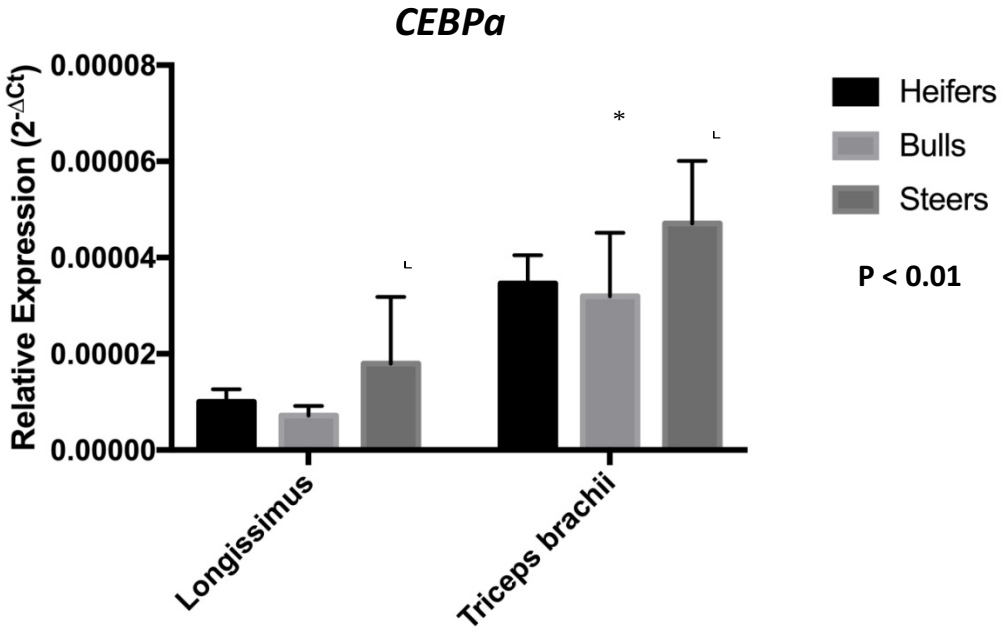
LO: *longissimus*; TB: *triceps brachii*.

As an overall conclusion, these results suggest that the FA composition of LO and TB are different and the gender status is contributing to the observed differences. In addition to that, is important to say that some bulls' samples presented a FA composition similar to the other two gender categories, which could affect FA analysis of the two muscle types. Therefore, genetic associated polymorphisms or physiological events within these males could be contributing to the difference in FA composition. Importantly, according Mueller et al. (2019), the castration of males could approximate the FA composition of these animals to the heifers probably due to acute changes in hormonal profile.

2.3.3 Gene expression

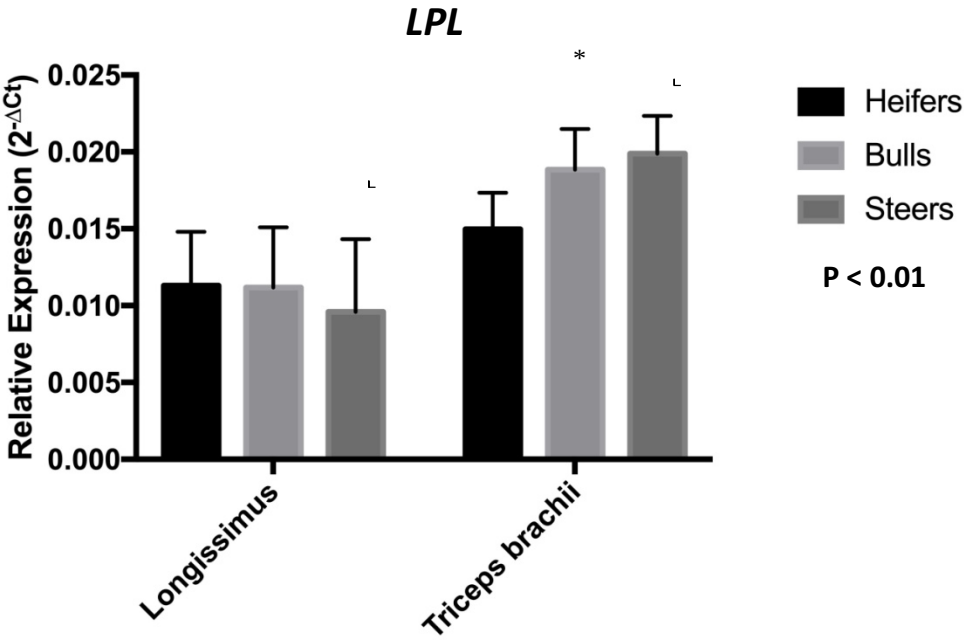
To understand the mechanism regulating different lipid profiles observed in TB and LO as well as according to the gender status, we performed the transcript analyses of adipogenesis related genes in different muscles from the different genders. Regarding the transcript levels, we did not observe an effect of muscle type and gender status on the levels of *PPARg*, *Zfp423*, *ACOX*, *FABP4*, *TPM1*, *UCP3*, *ACSS1* and *SLC16A7* ($P \geq 0.05$). On the other hand, we verified an effect of muscle type on the expression of the *CEBPa* (Figure 8) and *LPL* (Figure 9) genes ($P \leq 0.05$), both of which had higher expression in TB muscle.

Figure 8 - Transcript levels of *CEBPa* in feedlot Angus x Nelore cattle of different gender status and muscle types.



Values are presented as the mean ± standard error of the mean.
* indicates P ≤ 0.05 between groups.

Figure 9 - Transcript levels of *LPL* in feedlot Angus x Nelore cattle of different gender status and muscle types.



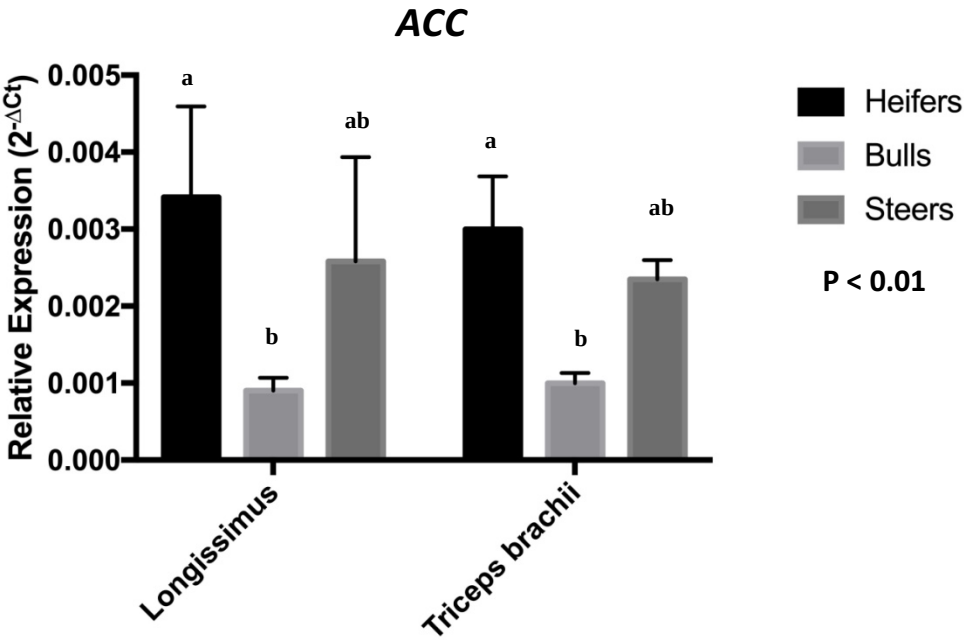
Values are presented as the mean ± standard error of the mean.
* indicates P ≤ 0.05 between groups.

The enzyme LPL is responsible for the hydrolysis of triglycerides from different organs into the bloodstream in the form of free fatty acids and glycerol (HOCQUETTE; GRAULET;

OLIVECRONA, 1998), which are used by muscles for the production and storage of energy by adipose tissue (BONNET *et al*., 2000). Although we did not find differences in the percentage of fat (total lipids) between the muscles, we interpreted that TB intramuscular adipocytes have a greater capacity to accumulate and metabolize fatty acids than adipocytes in the LO muscle. This is consistent with the greater *CEBPa* and *LPL* expression in the TB, which suggests that the adipocytes in the TB have a greater capacity for hydrolysis of circulating triacylglycerols in very-low-density lipoproteins (LI *et al*., 2018). The results suggest that uptake and deposition of fatty acids from the circulation contribute significantly to lipid accumulation in intramuscular adipocytes.

In contrast, gender affected the transcript levels of *ACC* ($P \leq 0.05$). This gene was increased in heifers in comparison to bulls, while there was no difference for steers and bulls or steers and heifers (Figure 10). Bong *et al*. (2012) found no difference between steers and bulls for the levels of the *ACC* gene in the LO muscle, suggesting that the expression of this gene was not changed by castration.

Figure 10 - Transcript levels of *ACC* in feedlot Angus x Nelore cattle of different gender status and muscle types.



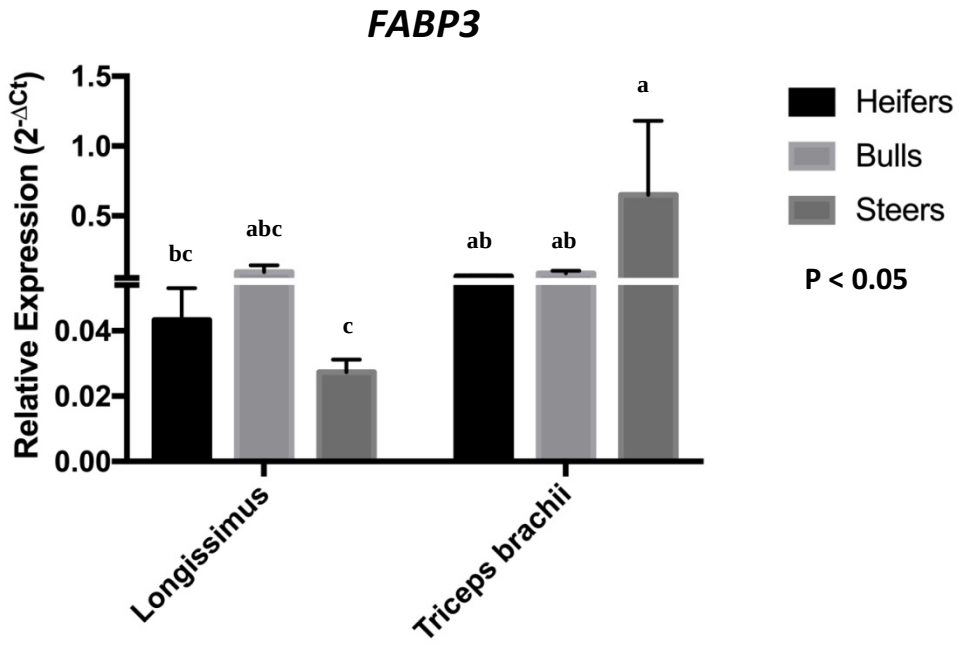
Values are presented as the mean ± standard error of the mean.
Different letters indicate $P \leq 0.05$, between groups.

Acetyl–CoA carboxylase (ACC) is an enzyme that catalyzes the synthesis of malonyl–CoA, a key substrate for adipogenesis (UNDERWOOD *et al*., 2007). It is associated with the

de novo synthesis of lipids and plays important roles in the energy metabolism of fatty acids in the adipose tissue of ruminants (SMITH; WITKOWSKI; JOSHI, 2003; WAKIL; ABU-ELHEIGA, 2009). In our study, heifers had higher expression of the transcript level of ACC than bulls, which suggests that increased adipogenesis contributes to the increased marbling in heifer muscle. According to Ward *et al*. (2010), the IMF content is positively related to the gene expression of *ACC*, which encodes this enzyme responsible for the synthesis of fatty acids. Additionally, Underwood *et al*. (2007) reported that cattle with higher IMF had a higher rate of *ACC* activation, while animals with lower IMF had a higher rate of phosphorylation and inactivation of *ACC*, resulting in a lower level of the active enzyme. Our study and others (BLANCO *et al*., 2020; MUELLER *et al*., 2019) have demonstrated that cattle gender status affects marbling and that heifers present higher marbling scores than bulls and steers. According to Lee *et al*. (2009), heifers had increased expression of genes related to lipogenesis. Since the heifers had higher marbling scores than bulls, it may provide an explanation for the high expression of the transcript levels of this gene in our study.

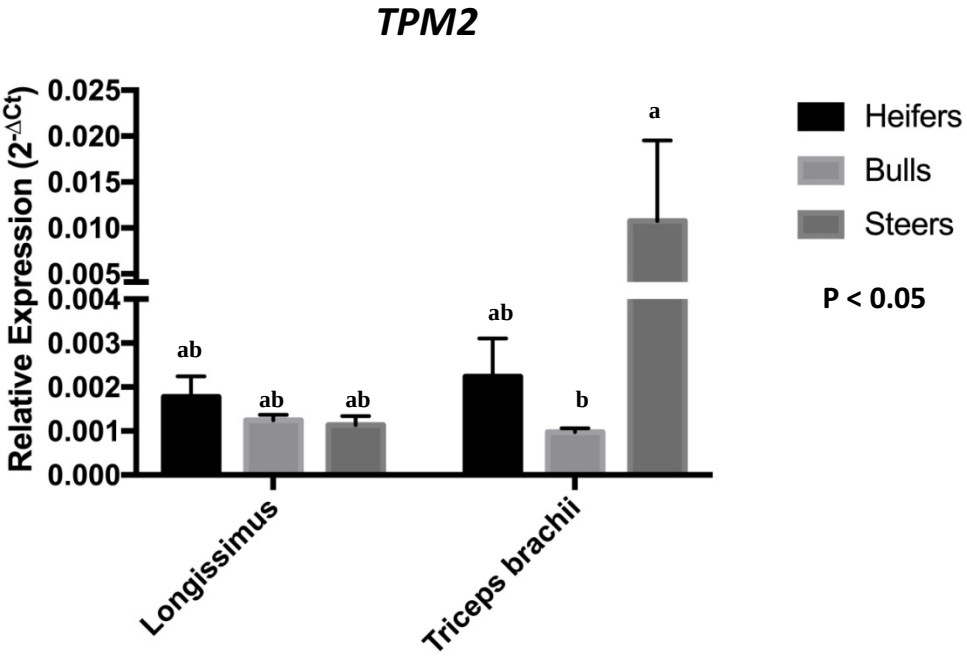
Cattle gender status and muscle type interactions were observed at the transcript level for *FABP3* (Figure 11), *TPM2* (Figure 12), and *TPM3* (Figure 13; $P \leq 0.05$). *FABP3* transcript levels were decreased in the LO of steers compared to the TB in all three genders. Additionally, the levels of *FABP3* transcripts were decreased in the LO from heifers compared to the TB from steers. Similarly, the *TPM2* transcripts were increased in the TB from steers compared to the TB from bulls. On the other hand, *TPM3* was increased in the LO from heifers and bulls, as well as the TB from steers in comparison to steer LO and heifer TB.

Figure 11 - Transcript levels of *FABP3* in feedlot Angus x Nelore cattle of different gender status and muscle types.



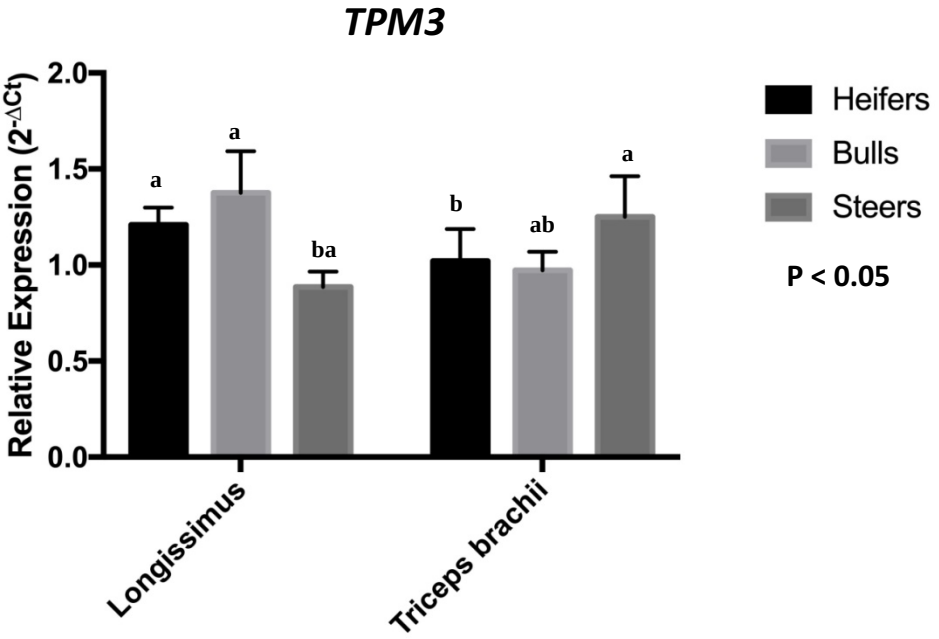
Values are presented as the mean ± standard error of the mean.
Different letters indicate P ≤ 0.05, between groups.

Figure 12 - Transcript levels of *TPM2* in feedlot Angus x Nelore cattle of different gender status and muscle types.



Values are presented as the mean ± standard error of the mean.
Different letters indicate P ≤ 0.05, between groups.

Figure 13 - Transcript levels of *TPM3* in feedlot Angus x Nelore cattle of different gender status and muscle types.



Values are presented as the mean ± standard error of the mean.
Different letters indicate P ≤ 0.05, between groups.

There is a wide range of studies involving the expression of adipogenic genes in different fat depots in the literature (BAIK *et al*., 2014; CHO *et al*., 2016; EGUINOA *et al*., 2003; LIU *et al*., 2021; SMITH *et al*., 2012). However, there is a large deficiency of studies that evaluated the expression of adipogenic genes in different muscles, especially in the LO and TB, as in the current study. Therefore, after evaluating the target transcripts and the composition of fatty acids within each gender, it was possible to show that *ACC*, *TPM2* and *TMP3* were altered, depending on the gender status, as well as the total SFAs, MUFAs and FA 22:2 n-6, suggesting an influence of cattle gender on these genes. Likewise, after evaluating the target transcripts and the composition of fatty acids between the muscle types, our data demonstrated that *CEBPa*, *LPL*, *FABP3* and *TMP3* were altered according to the type of muscle evaluated, as well as the concentrations of FAs 18:2 n-6c, 20:3 n-3, CLA c9 t11, 22:2 n-6, 22:4 n-6, and 22:5 n-3 EPA and total SFAs, PUFAs, n-3 and n-6, suggesting an effect related to muscle organogenesis.

Regarding the effects caused by the gender status of cattle and consequently differential expression of transcripts, the possible associations found for the *ACC*, *TPM2* and *TPM3* genes are highlighted. For the *ACC* gene, there was a decrease in bulls compared to heifers in the LO muscle, suggesting an association and possible explanation for the lower levels of total MUFAs

in bulls compared to heifers. Adipose tissue is a connective tissue derived from multipotent mesenchymal stem cells and is considered to arise from a mesodermal origin along with muscle, cartilage, and bone (LADEIRA *et al*., 2016). Muscle is composed of adipocytes, fibroblasts, myocytes and other less abundant cell types (LEE, 2017). Myogenic and adipogenic cells come from a similar pool of progenitor cells (CHEN *et al*., 2019; DU *et al*., 2013), and approximately 30% of adipose progenitors in all depots derive from the Myf5+ lineage, except in male gonads (SANCHEZ-GURMACHES *et al*., 2012; SANCHEZ-GURMACHES; GUERTIN, 2014). There is a relationship between the formation and differentiation of adipocytes and myogenic factors, and our study demonstrated this mechanism through the observed differences in the expression of the transcription factor *CEBPa*, which is involved in the adipogenesis process, and of the *LPL* gene, which is involved in the synthesis of fatty acids, between the LO and TB muscles, where the expression was greater in the TB muscle. This finding suggests that in addition to the effect of genes on different fat depots, there is a difference in the expression of these genes involved in the synthesis, oxidation and transport of fatty acids within the two different muscle groups. These findings corroborate with some of those observed in the composition of FAs evaluated in the muscles, with a higher concentration in the TB muscle than in the LO muscle. Regarding the effects caused by the type of muscle, in this analysis, it appears that the *TPM3* gene may be involved in the increase in total SFAs in the LO muscle compared to the TB of steers. The same behavior can be observed with the *CEBPa* and *LPL* genes, which may be involved in the increase in FAs 18:2 n-6c, 20:3 n-3, 22:2 n-6, 22:4 n-6, and 22:5 n-3 EPA, total PUFAs, n-3 and n-6 in the TB compared to the LO muscle, regardless of gender.

Since there are no studies in the literature involving the expression of *FABP3*, *TPM2*, and *TPM3* transcript levels comparing two types of beef cattle muscle and different cattle gender status, it is important to highlight the association between the gene expression and the differences found for the FA composition. According to our results, lower levels of *TPM2* transcripts were found in bulls than in steers in the TB, which may be a possible explanation for the lower levels of total MUFAs in bulls than in steers. For the differences found in the total SFAs and the FA 22:2 n-6 between genders, no possible associations were found with the analyzed transcripts, suggesting the involvement of other biological pathways in the modulation of these fatty acids in different cattle gender status. On the other hand, *TPM3* is increased in the LO from heifers and bulls, as well as the TB from steers in comparison to steer LO and heifer TB.

Tropomyosins (TPMs) are a diverse group of cytoskeletal proteins found in most eukaryotic cells, with distinct isoforms found in muscle – skeletal, cardiac, and smooth (OE et *al*., 2016) but are also found in various non-muscle cells (DLUGOSZ et *al*., 1984). According to Cho et al. (2016), the tropomyosin gene family are key factors closely associated with muscle development and modulates the formation of adipose tissue and lipid accumulation . Cho et al. (2016), analyzed the *TPM1*, *TPM2*, and *TPM3* genes in IMF of Hanwoo cows, steers, and bulls and observed that these transcripts were significantly lower in IMF of steers than in that of cows or bulls, being differentially expressed depending on sex and positively correlated with marbling score. Since the transcript is an intermediate product of the protein formation process and we performed the TPM protein abundance, our findings are better explained by the results observed in the TPM protein analysis, discussed below, which corroborate those we found for deposition of subcutaneous fat and marbling score, since we observed a molecular regulation of TPM in adipogenesis in heifers and steers, which had higher subcutaneous fat thickness and marbling score, and even a greater involvement of TPM in muscle development when referring to the lower marbling score and subcutaneous fat thickness of bulls compared to the other genders.

When interpreting the association of *FABP3* expression with the FA levels of 18:2 n-6c, 20:3 n-3, 22:2 n-6, 22:4 n-6, 22:5 n-3 EPA, PUFAs, n-3 and n-6, it is observed that for the gene itself, it is increased in the muscle with influence of the gender, that is, *FABP3* was increased in the TB of steers, in comparison to the LO of steers, and increased in the TB of steers, compared to the LO of heifers. However, when referring to FAs, only the muscle had an effect; that is, these FAs had higher concentrations in the TB than in the LO, without influence from the effect of gender status. The multigenic family of fatty acid-binding proteins (FABP) is involved in the intracellular and extracellular transport of lipids, transporting fatty acids in adipose tissue and contributing to muscle energy metabolism (BERTON *et al*., 2016; BIONAZ; THERING; LOOR, 2012; PAS; EVERTS; HAAGSMAN, 2004; VURAL *et al*., 2008). The *FABP3* gene is described as a gene that affects general carcass (BLECHA *et al*., 2015) and beef quality traits (TAYE *et al*., 2018). Additionally, polymorphisms in the *FABP3* gene have been associated with IMF and fatty acid composition in swine meat (PUIG-OLIVERAS *et al*., 2016), cytosolic fatty acid and lipid binding (BERTON *et al*., 2016), and beef ribeye areas (BLECHA *et al*., 2015). Since *FABP3* is involved in the transport of FAs in adipose tissue and contributes to muscle energy metabolism, it is important to associate this result with the differences found in the composition of FAs, and this association suggests that *FABP3* modulates the transport of

FAs differently between genders; however, the deposition of these FAs in the muscles occurs regardless of gender status.

In general, it was possible in our study to observe some association between the expression of adipogenesis-related genes and the fatty acid composition in different muscles. However, further studies could be performed to validate the association founded in our study, since we did not observe differences in some of the evaluated genes, probably due to the transcript analysis. Also, another important point is the possible effect caused by the different cell types since we performed the analysis in steak samples instead of individual cell types.

2.3.4 Tropomyosin protein abundance

Based on our results of gene expression and due to the role of the *TPM* gene product in muscle development as well as in lipid formation and accumulation (CHO *et al.*, 2016; HARPER; PETHICK, 2004), we investigated TPM1, TPM2 and TPM3 protein levels through the Western blot in LO and TB muscles from heifers, bulls, and steers (Figures 14 and 15).

Figure 14 – Representative Western Blot image showing the tropomyosin protein abundance and the normalizer tubulin in *longissimus* muscle of feedlot Angus x Nelore cattle of different gender status.

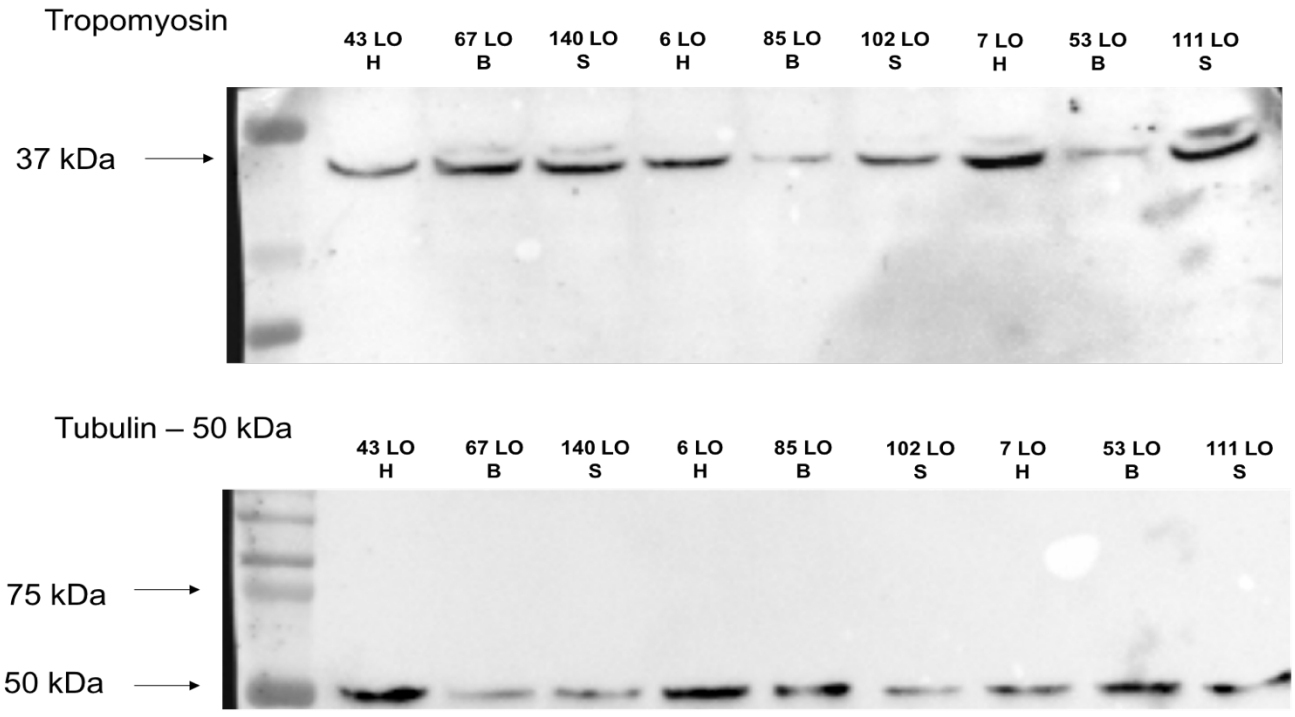
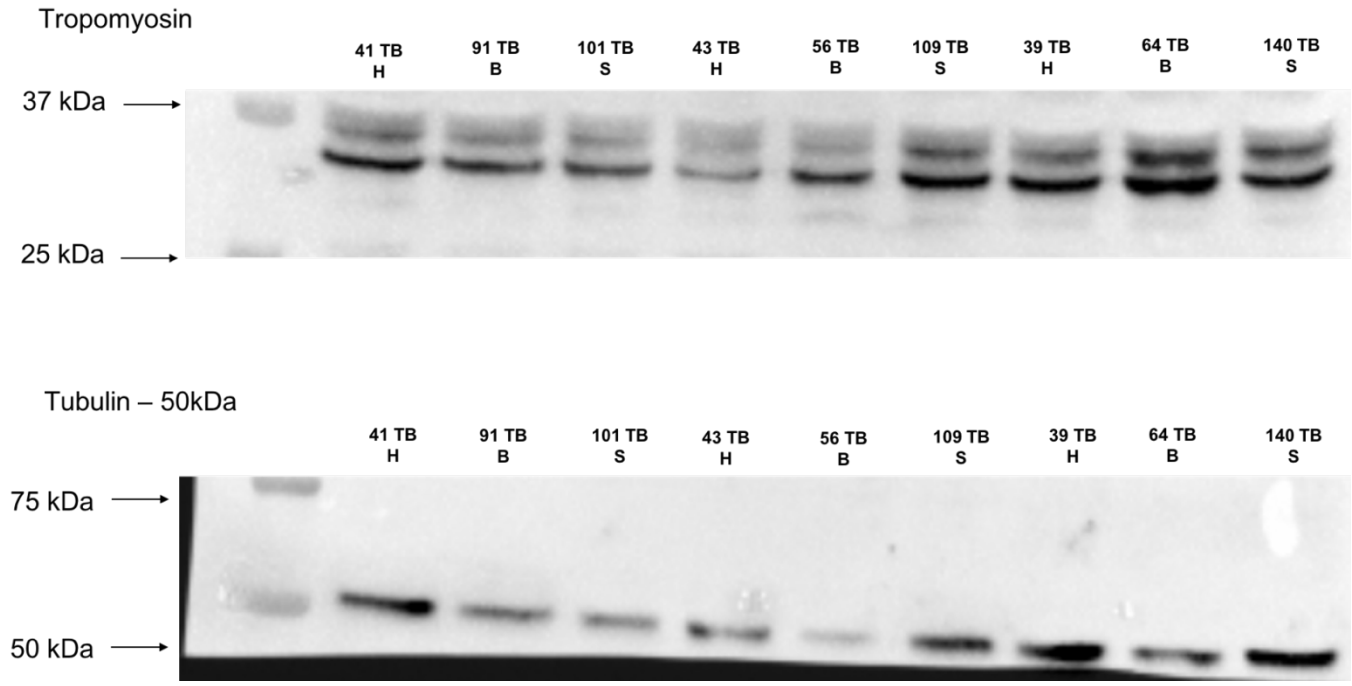
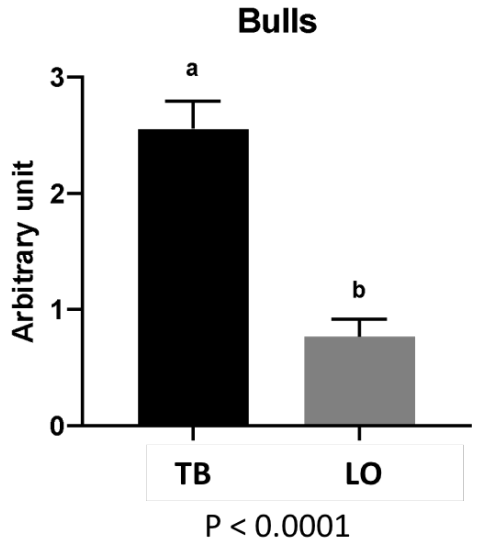


Figure 15 – Representative Western Blot image showing the tropomyosin protein abundance and the normalizer tubulin in *triceps brachii* muscle of feedlot Angus x Nelore cattle of different gender status.



Regarding the muscle type, the results showed that there was difference for TPM protein abundance between LO and TB muscles only in bulls, where the greater TPM abundance was in the TB ($P \leq 0.05$; Figure 16).

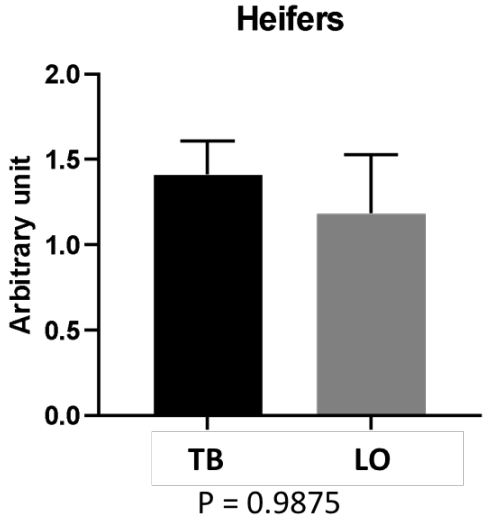
Figure 16 – Quantification of tropomyosin in *longissimus* and *triceps brachii* muscles of feedlot Angus x Nelore bulls.



Values are presented as the mean ± standard error of the mean.
Different letters indicate $P \leq 0.05$ between groups.

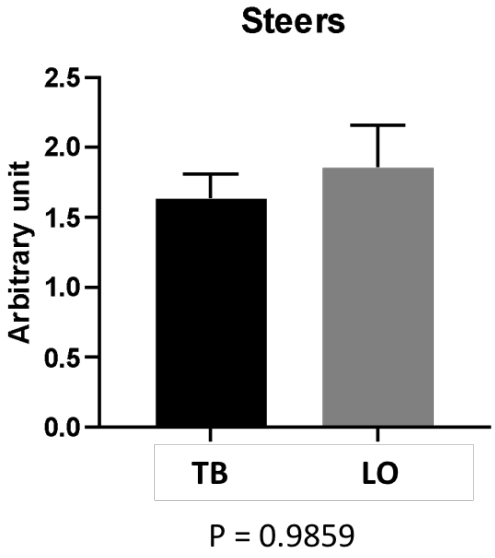
On the other hand, there was no difference for the TPM protein abundance between the muscles for heifers and steers ($P \geq 0.05$; Figures 17 and 18).

Figure 17 – Quantification of tropomyosin in *longissimus* and *triceps brachii* muscles of feedlot Angus x Nelore heifers.



Values are presented as the mean ± standard error of the mean.
Different letters indicate $P \leq 0.05$ between groups.

Figure 18 – Quantification of tropomyosin in *longissimus* and *triceps brachii* muscles of feedlot Angus x Nelore steers.

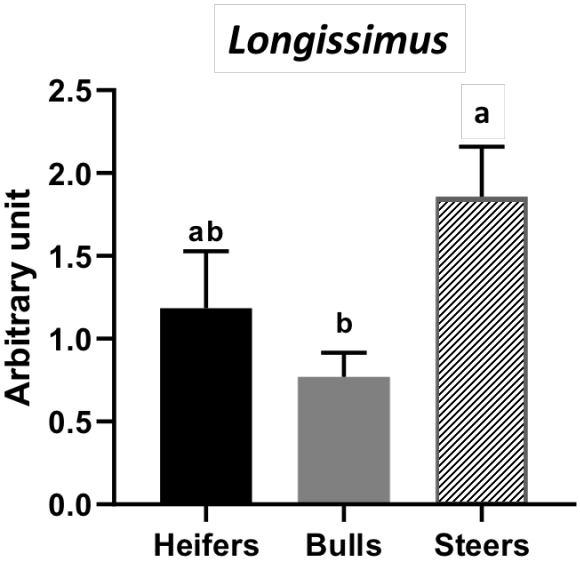


Values are presented as the mean ± standard error of the mean.
Different letters indicate P ≤ 0.05 between groups.

Our results showed that the TB has higher TPM abundance in bulls, suggesting greater activity of this protein for the muscle development in this gender, since this is a protein involved in muscle contraction (OE et *al*., 2016).

Regarding gender, there was difference for TPM protein abundance between the groups evaluated in LO and TB muscles (P ≤ 0.05). The LO muscle of steers showed higher abundance of tropomyosin when compare with bulls and heifers (Figure 19).

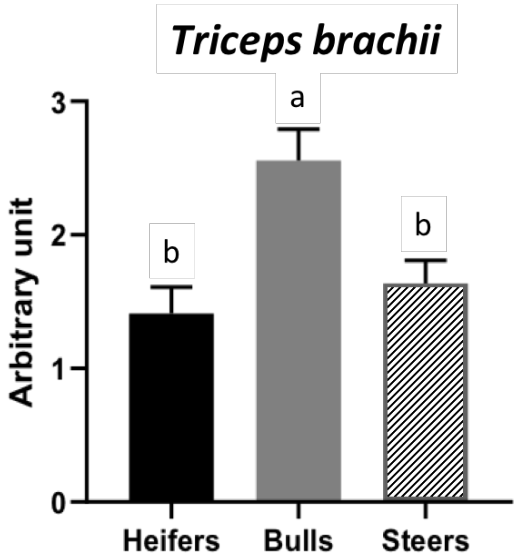
Figure 19 – Quantification of tropomyosin in *longissimus* muscle of feedlot Angus x Nelore cattle of different gender status.



Values are presented as the mean ± standard error of the mean.
Different letters indicate P ≤ 0.05 between groups.

On the other hand, the TB muscle of bulls showed higher abundance of tropomyosin when compare with heifers and steers (Figure 20). This result corroborates with those found for muscle type, where the difference between muscles was only in bulls group, with higher TPM abundance in this gender.

Figure 20 – Quantification of tropomyosin in *triceps brachii* muscle of feedlot Angus x Nelore cattle of different gender status.



Values are presented as the mean ± standard error of the mean.
Different letters indicate P ≤ 0.05 between groups.

Interestingly, relating the transcripts and protein abundance for TPM in the TB it suggests that as the bulls had less RNA it is possible to think that the transcripts were translated and degraded based on the increased levels of proteins. Regarding heifers and steers, we believe that transcripts are blocked and, therefore, accumulated in the cytoplasm of cells until needed. When we relate the TPM result in the LO muscle with the transcripts, there was a greater abundance of TPM in steers and also more RNA, which suggests that the transcription and translation processes are in full activity. It is important to highlight that these results agree with those of Mueller *et al*. (2019), who observed that heifers and steers had a greater marbling score than bulls, which could be explained by higher TPM levels, because of a greater amount of skeletal muscle cells. Cho *et al*. (2016) observed a positive correlation between *TPM* transcripts and protein levels with marbling score in heifers, bulls, and steers. In this sense, our results for

TPM abundance corroborate with those we found for subcutaneous fat thickness and marbling score, since the carcasses of bulls had lower values for these variables when compared to carcasses of heifers and steers. The balance between muscle and fat cells is responsible for the marbling phenotype (HARPER; PETHICK, 2004) and, therefore, the analysis of TPM abundance of beef from bulls confirm the results obtained with other analyzes suggesting a molecular regulation responsible for the lower degree of marbling in bulls when compared to heifers and steers.

When we observed the increased TPM in TB muscle of bulls, we associated its involvement with muscle development, however, when we observed the increased protein in the LO of steers, we inferred an involvement in the fat metabolism as well. Additionally, a recent study demonstrated that TPM3 is an important protein involved in *GLUT4* translocation to the membrane (KEE *et al*., 2015), which could help to explain the increased fat accumulation observed in heifers and steers although it still needed to be tested. In this sense, our study suggests an involvement of TPM with adipose tissue modulation and not only in muscle development. Thus, further investigation could lead to a better comprehension of the role of TPM as a new mechanism that differentiates fat deposition between steers and bulls.

2.4 CONCLUSION

Gender status and muscle type can affect the lipid composition of beef by differential modulation of adipogenesis-related genes from pathways regulating enzymes and proteins involved in synthesis, transport, distribution, degradation and oxidation of long-chain FAs in adipose and muscle cells. The cattle gender status modulated the transcripts levels of *ACC* gene, content of total lipids, total SFAs, total MUFAs, and carcass traits, while the muscle type affected the transcripts of *CEBPa* and *LPL* genes, total SFAs, total PUFAs, total n-3 and n-6, besides important FA, showing a gender and muscle specific effect.

Additionally, the physiological factors associated with gender status can induce molecular changes leading to different lipid profiles within each muscle, where TB muscle is more favorable to human health than LO, and beef from heifers stands out than the other genders for presenting better carcass traits, FA composition more favorable to human health and higher expression of the *ACC* gene, reflecting in greater marbling score.

We suggest that TPM acts as a modulator in the formation and accumulation of adipose tissue in heifers and steers, a result confirmed by the greater deposition of subcutaneous and intramuscular fat in the carcass of these gender compared to the carcass of bulls. Interestingly,

TPM also modulates the muscle development in bulls, since they present greater abundance of this protein, lower subcutaneous fat thickness and marbling score than the other genders.

DECLARATION OF COMPETING INTERESTS

None.

The authors declare no conflicts of interest that could influence or bias this work.

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REFERENCES

ABERLE, E. D.; FORREST, J. C. **Principles of meat science**. [s.l.] Kendall Hunt, 2001.

ABIEC. **Beef report: Perfil da Pecuária no Brasil 2021**. Disponível em: <http://abiec.com.br/publicacoes/beef-report-2021/>.

AILHAUD, G.; GRIMALDI, P.; NKGREL, R. Hormonal Regulation of Adipose Differentiation. **Trends Endocrinol Metab**, v. 5, p. 132–136, 1994.

ALBRECHT, E. et al. Triennial growth and development symposium: Factors influencing bovine intramuscular adipose tissue development and cellularity. **Journal of Animal Science**, v. 95, n. 5, p. 2244–2254, 1 maio 2017.

ANDRADE, P. de M. M.; CARMO, M. G. T. Ácidos graxos n-3: um link entre eicosanóides, inflamação e imunidade. **Revista Mn-Metabólica**, v. 8, n. 3, p. 135–143, 2006.

ARBOITTE, M. Z. et al. Composição Física da Carcaça, Qualidade da Carne e Conteúdo de Colesterol no Músculo Longissimus dorsi de Novilhos 5/8 Nelore-3/8 Charolês Terminados em Confinamento e Abatidos em Diferentes Estádios de Maturidade 1 Carcass Physical Composition, Meat Qual. **Revista Brasileira de Zootecnia**, p. 959–968, 2004.

ARDIYANTI, A. et al. Effects of GH gene polymorphism and sex on carcass traits and fatty acid compositions in Japanese Black cattle. **Animal Science Journal**, v. 80, n. 1, p. 62–69, fev. 2009.

ASSOCIATION, A. M. S.; (US), N. C. B. A.; (US), N. P. P. C. **Meat evaluation handbook**. [s.l.] Amer Meat Science Assn, 2013.

BAIK, M. et al. Effects of castration on the adiposity and expression of lipid metabolism

genes in various fat depots of Korean cattle. **Livestock Science**, v. 168, p. 168–176, 2014.

BARENDSE, W. et al. A splice site single nucleotide polymorphism of the fatty acid binding protein 4 gene appears to be associated with intramuscular fat deposition in longissimus muscle in Australian cattle. **Animal Genetics**, v. 40, n. 5, p. 770–773, out. 2009.

BARTON, L. et al. Effect of sex and age on bovine muscle and adipose fatty acid composition and stearoyl-CoA desaturase mRNA expression. **Meat Science**, v. 89, n. 4, p. 444–450, dez. 2011.

BAUMAN, D. E. et al. Biosynthesis of conjugated linoleic acid in ruminants. In: Proc. Am. Soc. Anim. Sci, **Anais**...1999.

BENGTSSON, B.-I. et al. **Treatment of Adults with Growth Hormone (GH) Deficiency with Recombinant Human GH*Journal of Clinical Endocrinology and Metabolism Copyright**. [s.l: s.n.].

BERG, R. T. (Roy T.; BUTTERFIELD, R. M. (Rex M. **New concepts of cattle growth**. [s.l.] Sydney University Press, 1976.

BERNARD, C. et al. New indicators of beef sensory quality revealed by expression of specific genes. **Journal of Agricultural and Food Chemistry**, v. 55, n. 13, p. 5229–5237, 27 jun. 2007.

BERNARD, L.; LEROUX, C.; CHILLIARD, Y. Expression and nutritional regulation of lipogenic genes in the ruminant lactating mammary gland. **Bioactive components of milk**, p. 67–108, 2008.

BERTON, M. P. et al. Gene expression profile of intramuscular muscle in Nellore cattle with extreme values of fatty acid. **BMC Genomics**, v. 17, n. 1, 25 nov. 2016.

BIANCHINI, W. et al. Efeito do grupo genético sobre as características de carcaça e maciez da carne fresca e maturada de bovinos superprecoces 1. **Revista Brasileira de Zootecnia**, v. 36, n. 6, p. 2109–2117, 2007. Disponível em: <www.sbz.org.br>.

BIONAZ, M.; THERING, B. J.; LOOR, J. J. Fine metabolic regulation in ruminants via nutrient-gene interactions: Saturated long-chain fatty acids increase expression of genes involved in lipid metabolism and immune response partly through PPAR-α activation. **British Journal of Nutrition**, v. 107, n. 2, p. 179–191, 28 jan. 2012.

BJORNTORP, P. Hormonal control of regional fat distribution. **Human reproduction**, v. 12, n. supplemen, 1997.

BLANCO, M. et al. Performance, carcass and meat quality of young bulls, steers and heifers slaughtered at a common body weight. **Livestock Science**, v. 240, p. 104156, 2020.

BLECHA, I. M. Z. et al. Identification and evaluation of polymorphisms in FABP3 and FABP4 in beef cattle. **Embrapa Gado de Corte-Artigo em periódico indexado (ALICE)**, 2015.

BONG, J. J. et al. Differential expression of genes associated with lipid metabolism in longissimus dorsi of Korean bulls and steers. **Meat science**, v. 91, n. 3, p. 284–293, 2012.

BONNEAU, M.; ENRIGHT, W. J. Immunocastration in cattle and pigs. **Livestock Production Science**, v. 42, p. 193–200, 1995.

BONNET, M. et al. **Nutrient-Gene Expression Lipoprotein Lipase Activity and mRNA**

Are Up-Regulated by Refeeding in Adipose Tissue and Cardiac Muscle of Sheep 1,2. [s.l: s.n.]. Disponível em: <https://academic.oup.com/jn/article/130/4/749/4686705>.

BONNET, M. et al. Ontogenesis of muscle and adipose tissues and their interactions in ruminants and other species. **Animal**, v. 4, n. 7, p. 1093–1109, 2010.

BRIGGS, M. A.; PETERSEN, K. S.; KRIS-ETHERTON, P. M. Saturated fatty acids and cardiovascular disease: replacements for saturated fat to reduce cardiovascular risk. In: Healthcare, 2, **Anais**...Multidisciplinary Digital Publishing Institute, 2017.

CALDER, P. C. et al. **Inflammatory disease processes and interactions with nutritionBritish Journal of Nutrition**Cambridge University Press, , 2009. .

CARVALHO, R. M. S. et al. Differences between sexes, muscles and aging times on the quality of meat from Wagyu × Angus cattle finished in feedlot. **Animal Production Science**, v. 58, n. 2, p. 350–357, 2016.

CESAR, A. S. M. et al. Differences in the skeletal muscle transcriptome profile associated with extreme values of fatty acids content. **BMC genomics**, v. 17, n. 1, p. 1–16, 2016.

CHEN, D. et al. Adipogenesis, fibrogenesis and myogenesis related gene expression in longissimus muscle of high and low marbling beef cattle. **Livestock Science**, v. 229, p. 188–193, 2019.

CHO, J. H. et al. Regional differences of proteins expressing in Adipose depots isolated from cows, steers and bulls as identified by a proteomic approach. **Asian-Australasian Journal of Animal Sciences**, v. 29, n. 8, p. 1197–1206, 1 ago. 2016.

CHO, S. et al. Fatty acid profiles and sensory properties of longissimus dorsi, triceps brachii, and semimembranosus muscles from Korean Hanwoo and Australian Angus beef. **Asian-Australasian journal of animal sciences**, v. 18, n. 12, p. 1786–1793, 2005.

CHOAT, W. T. et al. The effects of cattle sex on carcass characteristics and longissimus muscle palatability. **Journal of Animal Science**, v. 84, n. 7, p. 1820–1826, jul. 2006.

CHUNG, K. Y. et al. Lipid characteristics of subcutaneous adipose tissue and M. longissimus thoracis of Angus and Wagyu steers fed to US and Japanese endpoints. **Meat Science**, v. 73, n. 3, p. 432–441, 2006.

CIANZIO, D. S. et al. Adipose tissue growth in cattle representing two frame sizes: Distribution among depots. **Journal of Animal Science**, v. 55, n. 2, p. 305–312, 1982.

COLEMAN, L. W. et al. Carcass characteristics and meat quality of Hereford sired steers born to beef-cross-dairy and Angus breeding cows. **Meat Science**, v. 121, p. 403–408, 2016.

COOK, M. E. et al. Immune modulation by altered nutrient metabolism: nutritional control of immune-induced growth depression. **Poultry science**, v. 72, n. 7, p. 1301–1305, 1993.

COSTA, P. et al. Muscle fiber and fatty acid profiles of Mertolenga-PDO meat. **Meat Science**, v. 78, n. 4, p. 502–512, abr. 2008.

CROUSE, J. D. et al. **Comparisons of Comparisons of Bos indicus Bos indicus and and Bos taurus Bos taurus Inheritance for Inheritance for Carcass Beef Characteristics and Meat Palatability Carcass Beef Characteristics and Meat Palatability Comparisons of Bos indicus and Bos ta**. [s.l: s.n.]. Disponível em: <https://digitalcommons.unl.edu/hruskareports/121>.

DAI, F. et al. **Developmental differences in carcass, meat quality and muscle fibre characteristics between the Landrace and a Chinese native pigSouth African Journal of Animal Science**. [s.l: s.n.]. Disponível em: <http://www.sasas.co.za/sajas.asp>.

DE PERGOLA, G. et al. Testosterone treatment of ovariectomized rats: effects on lipolysis regulation in adipocytes. **European Journal of Endocrinology**, v. 123, n. 1, p. 61–66, 1990.

DE SÁ, P. M. et al. Transcriptional regulation of adipogenesis. **Comprehensive Physiology**, v. 7, n. 2, p. 635–674, 1 abr. 2017.

DE SMET, S.; RAES, K.; DEMEYER, D. **Meat fatty acid composition as affected by fatness and genetic factors: A reviewAnimal Research**, mar. 2004. .

DEL PINO, L. M. et al. Adiposity and adipogenic gene expression in four different muscles in beef cattle. **PLoS ONE**, v. 12, n. 6, 1 jun. 2017.

DEMIREL, G. et al. Fatty acids of lamb meat from two breeds fed different forage: Concentrate ratio. **Meat Science**, v. 72, n. 2, p. 229–235, fev. 2006.

DÍAZ, C. et al. Genetic basis of beef quality differences between muscles in beef cattle: Avileña negra-ibérica, a study case. In: Proceedings of the XVI Congresso de Zootecnia, Castelo Branco (Portugal), **Anais**...2006.

DIEUDONNE, M. N. et al. Opposite effects of androgens and estrogens on adipogenesis in rat preadipocytes: evidence for sex and site-related specificities and possible involvement of insulin-like growth factor 1 receptor and peroxisome proliferator-activated receptorγ 2. **Endocrinology**, v. 141, n. 2, p. 649–656, 2000.

DLUGOSZ, A. A. et al. The relationship between stress fiber-like structures and nascent myofibrils in cultured cardiac myocytes. **The Journal of cell biology**, v. 99, n. 6, p. 2268–2278, 1984.

DODSON, M. V et al. **Lipid metabolism, adipocyte depot physiology and utilization of meat an-imals as experimental models for metabolic researchInt. J. Biol. Sci**. [s.l: s.n.]. Disponível em: <http://www.biolsci.org691>.

DOWHAN, W.; MILEYKOVSKAYA, E.; BOGDANOV, M. Diversity and versatility of lipid–protein interactions revealed by molecular genetic approaches. **Biochimica et Biophysica Acta (BBA)-Biomembranes**, v. 1666, n. 1–2, p. 19–39, 2004.

DRANSFIELD, E. et al. Meat quality and composition of three muscles from French cull cows and young bulls. **Animal Science**, v. 76, n. 3, p. 387–399, 2003.

DU, M. et al. Meat science and muscle Biology Symposium: Manipulating mesenchymal progenitor cell differentiation to optimize performance and carcass value of beef cattle. **Journal of Animal Science**, v. 91, n. 3, p. 1419–1427, mar. 2013.

DU, M.; YIN, J.; ZHU, M. J. **Cellular signaling pathways regulating the initial stage of adipogenesis and marbling of skeletal muscleMeat Science**, set. 2010. .

DUGAN, M. E. R. et al. The effects of feeding conjugated linoleic acid on subsequent pork quality. **Canadian Journal of Animal Science**, v. 79, n. 1, p. 45–51, 1999.

EGUINOA, P. et al. Lipogenic enzyme activities in different adipose depots of Pirenaican and Holstein bulls and heifers taking into account adipocyte size. **Journal of Animal Science**, v. 81, n. 2, p. 432–440, 2003.

ERKKILÄ, A. et al. Dietary fatty acids and cardiovascular disease: an epidemiological approach. **Progress in lipid research**, v. 47, n. 3, p. 172–187, 2008.

FARMER, S. R. **Transcriptional control of adipocyte formationCell Metabolism**, out. 2006. .

FAVERO, R. et al. Crossbreeding applied to systems of beef cattle production to improve performance traits and carcass quality. **Animal**, v. 13, n. 11, p. 2679–2686, 1 nov. 2019.

FEITOSA, F. L. B. et al. Genetic correlation estimates between beef fatty acid profile with meat and carcass traits in Nellore cattle finished in feedlot. **Journal of Applied Genetics**, v. 58, n. 1, p. 123–132, 1 fev. 2017.

FERNANDES, A. R. M. et al. Composição química e perfil de ácidos graxos da carne de bovinos de diferentes condições sexuais recebendo silagem de milho e concentrado ou cana-de-açúcar e concentrado contendo grãos de girassol. **Revista Brasileira de Zootecnia**, v. 38, n. 4, p. 705–712, 2009.

FERRAZ, J. B. S.; FELÍCIO, P. E. de. **Production systems - An example from BrazilMeat Science**, fev. 2010. .

FOLCH, J.; LEES, M.; STANLEY, G. H. S. A simple method for the isolation and purification of total lipides from animal tissues. **Journal of biological chemistry**, v. 226, n. 1, p. 497–509, 1957.

FREITAS, A. K. de. **CARACTERÍSTICAS DA CARCAÇA, DA CARNE E PERFIL DOS ÁCIDOS GRAXOS DE NOVILHOS NELORE INTEIROS OU CASTRADOS EM DUAS IDADES**. 2006. 2006.

FRENCH, P. et al. Fatty acid composition, including conjugated linoleic acid, of intramuscular fat from steers offered grazed grass, grass silage, or concentrate-based diets. **Journal of Animal Science**, v. 78, n. 11, p. 2849–2855, 2000.

FRENCH, P. et al. Fatty acid composition of intra-muscular triacylglycerols of steers fed autumn grass and concentrates. **Livestock Production Science**, v. 81, n. 2–3, p. 307–317, 2003.

FRITSCHE, J.; STEINHART, H. Analysis, occurrence, and physiological properties of trans fatty acids (TFA) with particular emphasis on conjugated linoleic acid isomers (CLA)–a review. **Lipid/Fett**, v. 100, n. 6, p. 190–210, 1998.

GAGAOUA, M. et al. Understanding Early Post-Mortem Biochemical Processes Underlying Meat Color and pH Decline in the Longissimus thoracis Muscle of Young Blond dÁquitaine Bulls Using Protein Biomarkers. **Journal of Agricultural and Food Chemistry**, v. 63, n. 30, p. 6799–6809, 5 ago. 2015.

GAGAOUA, M. et al. Associations among protein biomarkers and pH and color traits in longissimus thoracis and rectus abdominis muscles in protected designation of origin Maine-Anjou cull cows. **Journal of Agricultural and Food Chemistry**, v. 65, n. 17, p. 3569–3580, 2017.

GAMA, L. T. et al. Heterosis for meat quality and fatty acid profiles in crosses among Bos indicus and Bos taurus finished on pasture or grain. **Meat Science**, v. 93, n. 1, p. 98–104, jan. 2013.

GAMARRA, D. et al. Distinct correlations between lipogenic gene expression and fatty acid

composition of subcutaneous fat among cattle breeds. **BMC veterinary research**, v. 14, n. 1, p. 1–12, 2018.

GANDEMER, G. Lipids and meat quality: lipolysis, oxidation, maillard reaction and flavour. **Sciences des Aliments (France)**, 1999.

GERBENS, F. et al. **The adipocyte fatty acid-binding protein locus: characterization and association with intramuscular fat content in pigs**. [s.l: s.n.].

GREGOIRE, F. M.; SMAS, C. M.; SUL, S. **Understanding Adipocyte DifferentiationPHYSIOLOGICAL REVIEWS**. [s.l: s.n.].

GUPTA, R. K. et al. Transcriptional control of preadipocyte determination by Zfp423. **Nature**, v. 464, n. 7288, p. 619–623, 25 mar. 2010.

GUPTA, R. K. et al. Zfp423 expression identifies committed preadipocytes and localizes to adipose endothelial and perivascular cells. **Cell Metabolism**, v. 15, n. 2, p. 230–239, 8 fev. 2012.

HARPER, G. S.; PETHICK, D. W. How might marbling begin? **Australian Journal of Experimental Agriculture**, v. 44, n. 7, p. 653–662, 2004.

HAUSMAN, G. J. et al. **Board-invited review: The biology and regulation of preadipocytes and adipocytes in meat animalsJournal of Animal Science**, abr. 2009. .

HEALTH, C. et al. Nutrition Recommendations: The Report of the Scientific Review Committee. 1990.

HOCQUETTE, J.-F. et al. Opportunities for predicting and manipulating beef quality. **Meat science**, v. 92, n. 3, p. 197–209, 2012.

HOCQUETTE, J.-F.; GRAULET, B.; OLIVECRONA, T. Lipoprotein lipase activity and mRNA levels in bovine tissues. **Comparative Biochemistry and Physiology Part B: Biochemistry and Molecular Biology**, v. 121, n. 2, p. 201–212, 1998.

HOCQUETTE, J. F. et al. Intramuscular fat content in meat-producing animals: Development, genetic and nutritional control, and identification of putative markers. **Animal**, v. 4, n. 2, p. 303–319, 2010.

HOOD, R.; ALLEN, C. E. Cellularity of bovine adipose tissue. **Journal of lipid research**, v. 14, n. 6, p. 605–610, 1973.

HUFFMAN, R. D. et al. EFFECTS OF PERCENTAGE BRAHMAN AND ANGUS BREEDING, AGE-SEASON OF FEEDING AND SLAUGHTER END POINT ON FEEDLOT PERFORMANCE AND CARCASS CHARACTERISTICS. **Journal of Animal Science**, v. 68, p. 2243–2252, 1990. Disponível em: <https://academic.oup.com/jas/article-abstract/68/8/2243/4704901>.

HWANG, Y.-H.; JOO, S.-T. Fatty acid profiles of ten muscles from high and low marbled (quality grade 1++ and 2) Hanwoo steers. **Korean journal for food science of animal resources**, v. 36, n. 5, p. 679, 2016.

JAGO, J. G.; BASS, J. J.; MATTHEWS, L. R. **Evaluation of a vaccine to control bull behaviourProceedings of the New Zealand Society of Animal Production**. [s.l: s.n.].

JEONG, J. et al. Expression of fat deposition and fat removal genes is associated with intramuscular fat content in longissimus dorsi muscle of Korean cattle steers. **Journal of**

animal science, v. 90, n. 6, p. 2044–2053, 2012.

JIANG, J.; WOLK, A.; VESSBY, B. Relation between the intake of milk fat and the occurrence of conjugated linoleic acid in human adipose tissue. **The American journal of clinical nutrition**, v. 70, n. 1, p. 21–27, 1999.

JIAO, Y. et al. Molecular characterization, polymorphism of the ACOX1 gene and association with ultrasound traits in Bos taurus. **Genetics and Molecular Research**, v. 10, n. 3, p. 1948–1957, 2011.

JO, J. et al. Hypertrophy and/or hyperplasia: dynamics of adipose tissue growth. **PLoS Comput Biol**, v. 5, n. 3, p. e1000324, 2009.

KAROLYI, D. et al. Fatty acid composition of muscle and adipose tissue of beef cattle. **Italian Journal of Animal Science**, v. 8, n. sup3, p. 264–266, 2009.

KEE, A. J. et al. An Actin Filament Population Defined by the Tropomyosin Tpm3.1 Regulates Glucose Uptake. **Traffic**, v. 16, n. 7, p. 691–711, 1 jul. 2015.

KOURY FILHO, W. **Escores visuais e suas relações com características de crescimento em bovinos de corte**. 2005. 2005.

KRAMER, J. K. G. et al. Evaluating acid and base catalysts in the methylation of milk and rumen fatty acids with special emphasis on conjugated dienes and total trans fatty acids. **Lipids**, v. 32, n. 11, p. 1219–1228, 1997.

LADEIRA, M. M. et al. Nutrigenomics and beef quality: A review about lipogenesis. **International journal of molecular sciences**, v. 17, n. 6, p. 918, 2016.

LAGE, J. F. et al. Influence of genetic type and level of concentrate in the finishing diet on carcass and meat quality traits in beef heifers. **Meat Science**, v. 90, n. 3, p. 770–774, mar. 2012.

LEE, H. J. Cellularity of Adipose Tissue Obtained from Different Sex and Growth Stages of Hanwoo Cattle and Sheep. **Asian-Australasian Journal of Animal Sciences**, v. 13, n. 2, p. 155–160, 2000.

LEE, H. J. et al. Cellularity of adipose tissue obtained from different sex and growth stages of Hanwoo cattle and sheep. **Asian-Australasian Journal of Animal Sciences**, v. 13, n. 2, p. 155–160, 2000.

LEE, M. J. Hormonal regulation of adipogenesis. **Comprehensive Physiology**, v. 7, n. 4, p. 1151–1195, 2017.

LEE, M. R. F. et al. A comparison between red clover silage and grass silage feeding on fatty acid composition, meat stability and sensory quality of the M. Longissimus muscle of dairy cull cows. **Meat Science**, v. 81, n. 4, p. 738–744, abr. 2009.

LESEIGNEUR-MEYNIER, A.; GANDEMER, G. Lipid composition of pork muscle in relation to the metabolic type of the fibres. **Meat Science**, v. 29, n. 3, p. 229–241, 1991.

LI, X. Z. et al. Adipogenic/lipogenic gene expression and fatty acid composition in chuck, loin, and round muscles in response to grain feeding of Yanbian Yellow cattle. **Journal of animal science**, v. 96, n. 7, p. 2698–2709, 2018.

LIMA, I. de A. et al. CONDIÇÃO CORPORAL E CARACTERÍSTICAS DE CARCAÇA DE VACAS DE DESCARTE NA REGIÃO DE LAVRAS-MG. **Ciên. agrotec.**, v. 28, n. 3, p.

637–646, 2004.

LIN, J. J.-C.; LIN, J. L. Assembly of different isoforms of actin and tropomyosin into the skeletal tropomyosin-enriched microfilaments during differentiation of muscle cells in vitro. **The Journal of cell biology**, v. 103, n. 6, p. 2173–2183, 1986.

LISTRAT, A. et al. **How muscle structure and composition influence meat and flesh qualityScientific World Journal**Hindawi Limited, , 2016. .

LISTRAT, A. et al. What are the drivers of beef sensory quality using metadata of intramuscular connective tissue, fatty acids and muscle fiber characteristics? **Livestock Science**, v. 240, p. 104209, 2020.

LIU, H. et al. miR-32-5p Regulates Lipid Accumulation in Intramuscular Fat of Erhualian Pigs by Suppressing KLF3. **Lipids**, v. 56, n. 3, p. 279–287, 2021.

LIU, T. et al. Fatty Acid Profile of Muscles from Crossbred Angus-Simmental, Wagyu-Simmental, and Chinese Simmental Cattles. **Food Science of Animal Resources**, v. 40, n. 4, p. 563, 2020.

LOCK, A. L.; HARVATINE, K.; DRACKLEY, J. K. **Concepts of fat and fatty acid digestion in ruminants**. [s.l: s.n.]. Disponível em: <https://www.researchgate.net/publication/266499830>.

LOPEZ-HUERTAS, E. Health effects of oleic acid and long chain omega-3 fatty acids (EPA and DHA) enriched milks. A review of intervention studies. **Pharmacological Research**, v. 61, n. 3, p. 200–207, 2010. Disponível em: <http://dx.doi.org/10.1016/j.phrs.2009.10.007>.

MADRUGA, M. S. et al. Efeito do genótipo e do sexo sobre a composição química e o perfil de ácidos graxos da carne de cordeiros. **Revista Brasileira de Zootecnia**, v. 35, n. 4, p. 1838–1844, 2006.

MAGGIONI, D. et al. Animal performance and meat quality of crossbred young bulls. **Livestock Science**, v. 127, n. 2–3, p. 176–182, fev. 2010.

MARQUES, A. C.; VALENTE, T. B.; ROSA, C. S. da. **Revista de Nutrição Toxin formation during food processing and possible consequences to the human bodyTOXINAS EM ALIMENTOS PROCESSADOS | 283 Rev. Nutr**. [s.l: s.n.].

MARTINS, T. S. et al. Molecular factors underlying the deposition of intramuscular fat and collagen in skeletal muscle of Nellore and Angus cattle. **PLoS One**, v. 10, n. 10, p. e0139943, 2015.

MATARESE, V.; BERNLOHRS, D. A. **THE JOURNAL OF BIOLOGICAL CHEMISTRY Purification of Murine Adipocyte Lipid-binding Protein CHARACTERIZATION AS A FATTY ACID-AND RETINOIC ACID-BINDING PROTEIN*Molecular Biology**. [s.l: s.n.].

MICHAL, J. J. et al. The bovine fatty acid binding protein 4 gene is significantly associated with marbling and subcutaneous fat depth in Wagyu x Limousin F2 crosses. **Animal Genetics**, v. 37, n. 4, p. 400–402, ago. 2006.

MOISÁ, S. J. et al. Central role of the PPARγ gene network in coordinating beef cattle intramuscular adipogenesis in response to weaning age and nutrition. **Gene Regulation and Systems Biology**, v. 2014, n. 8, p. 17–32, 8 jan. 2013.

MOORE, M. C. et al. National Beef Quality Audit–2011: In-plant survey of targeted carcass characteristics related to quality, quantity, value, and marketing of fed steers and heifers. **Journal of Animal Science**, v. 90, n. 13, p. 5143–5151, 2012.

MORAIS DE LIMA JÚNIOR, D. et al. **ALGUNS ASPECTOS QUALITATIVOS DA CARNE BOVINA: UMA REVISÃO [Some qualitative aspects of beef: a review]Acta Veterinaria Brasilica**. [s.l: s.n.].

MORAIS, S. et al. Conserved expression of alternative splicing variants of peroxisomal acyl-CoA oxidase 1 in vertebrates and developmental and nutritional regulation in fish. **Physiological Genomics**, v. 28, n. 3, p. 239–252, 2007.

MORENO-SÁNCHEZ, N. et al. Skeletal muscle specific genes networks in cattle. **Functional and Integrative Genomics**, v. 10, n. 4, p. 609–618, nov. 2010.

MOSETI, D.; REGASSA, A.; KIM, W. K. **Molecular regulation of adipogenesis and potential anti-adipogenic bioactive moleculesInternational Journal of Molecular Sciences**MDPI AG, , 19 jan. 2016. .

MUCHENJE, V. et al. **Some biochemical aspects pertaining to beef eating quality and consumer health: A reviewFood Chemistry**, 15 jan. 2009. .

MUELLER, L. F. et al. Gender status effect on carcass and meat quality traits of feedlot Angus × Nellore cattle. **Animal Science Journal**, v. 90, n. 8, p. 1078–1089, 2019.

NATIONAL ACADEMIES OF SCIENCES AND MEDICINE, E. Nutrient requirements of beef cattle. 2016.

NIAN, Y. et al. Effect of castration and carcass suspension method on the quality and fatty acid profile of beef from male dairy cattle. **Journal of the Science of Food and Agriculture**, v. 98, n. 11, p. 4339–4350, 2018.

ODDY, V. H. et al. Nutritional and developmental effects on the intrinsic properties of muscles as they relate to the eating quality of beef. **Australian Journal of Experimental Agriculture**, v. 41, n. 7, p. 921–942, 2001.

OE, M. et al. Distribution of tropomyosin isoforms in different types of single fibers isolated from bovine skeletal muscles. **Meat science**, v. 118, p. 129–132, 2016.

OURY, M.-P. et al. Relationship between rearing practices and eating quality traits of the muscle rectus abdominis of Charolais heifers. **Livestock Science**, v. 111, n. 3, p. 242–254, 2007.

OWENS, F. N. et al. Factors that Alter the Growth and Development of Ruminants1f2. **Journal of Animal Science**, v. 71, p. 3138–3150, 1993. Disponível em: <http://jas.fass.org/content/71/11/3138>.

OWENS, F. N. et al. **Review of some aspects of growth and development of feedlot cattle.Journal of animal science**, 1995. .

PADUA, J. T. et al. Efeito de métodos de castração e do uso de vermífugos sobre o ganho em peso de bovinos mestiços leiteiros. 2003.

PANJONO et al. Carcass characteristics of Hanwoo (Korean cattle) from different sex conditions, raising altitudes and slaughter seasons. **Livestock Science**, v. 123, n. 2–3, p. 283–287, ago. 2009.

PAS, M. F. W. te.; EVERTS, M. E.; HAAGSMAN, H. P. **Muscle development of livestock animals : physiology, genetics and meat quality**. [s.l.] CABI Pub, 2004.

PEACOCK, F. M. et al. BREED AND HETEROSIS EFFECTS ON CARCASS CHARACTERISTICS OF ANGUS, BRAHMAN, CHAROLAIS AND CROSSBRED STEERS. **Journal of Animal Science**, v. 49, n. 2, 1979. Disponível em: <https://academic.oup.com/jas/article-abstract/49/2/391/4700568>.

PEREIRA, A. S. C. et al. Growth performance, and carcass and meat quality traits in progeny of Poll Nellore, Angus and Brahman sires under tropical conditions. **Animal Production Science**, v. 55, n. 10, p. 1295–1302, 2015.

PICARD, B. et al. Biomarkers of tenderness and intramuscular fat in five muscles from French PDO Maine-Anjou: I-Muscle type effect. In: Proceedings of the 63rd international congress of meat science and technology, **Anais**...Wageningen Academic Publishers Cork, Ireland, 2017.

PICARD, B. et al. Beef tenderness and intramuscular fat proteomic biomarkers: Effect of gender and rearing practices. **Journal of proteomics**, v. 200, p. 1–10, 2019.

PICARD, B.; DURIS, M. P.; JURIE, C. Classification of bovine muscle fibres by different histochemical techniques. **The Histochemical Journal**, v. 30, n. 7, p. 473–477, 1998.

PIGHIN, D. et al. A contribution of beef to human health: a review of the role of the animal production systems. **The Scientific World Journal**, v. 2016, 2016.

PUIG-OLIVERAS, A. et al. Expression-based GWAS identifies variants, gene interactions and key regulators affecting intramuscular fatty acid content and composition in porcine meat. **Scientific reports**, v. 6, n. 1, p. 1–12, 2016.

PURCHAS, R. W. Effect of sex and castration on growth and composition. **Advances in meat research (USA)**, 1991.

PURCHAS, R. W.; KNIGHT, T. W.; BUSBOOM, J. R. The effect of production system and age on concentrations of fatty acids in intramuscular fat of the longissimus and triceps brachii muscles of Angus-cross heifers. **Meat Science**, v. 70, n. 4, p. 597–603, 2005.

PURCHAS, R. W.; ZOU, M. Composition and quality differences between the longissimus and infraspinatus muscles for several groups of pasture-finished cattle. **Meat Science**, v. 80, n. 2, p. 470–479, out. 2008.

QUEIROZ, J. C. F. de et al. Control of adipogenesis by fatty acids. **Arquivos Brasileiros de Endocrinologia & Metabologia**, v. 53, n. 5, p. 582–594, 2009.

RAES, K. et al. Effect of linseed feeding at similar linoleic acid levels on the fatty acid composition of double-muscled Belgian Blue young bulls. **Meat Science**, v. 66, n. 2, p. 307–315, 2004.

RAINER, L.; HEISS, C. J. Conjugated linoleic acid: Health implications and effects on body composition. **Journal of the American Dietetic Association**, v. 104, n. 6, p. 963–968, 2004.

ROMAO, J. M. et al. **MicroRNA regulation in mammalian adipogenesisExperimental Biology and Medicine**, set. 2011. .

ROSEN, E. D. et al. C/EBPα induces adipogenesis through PPARγ: A unified pathway. **Genes and Development**, v. 16, n. 1, p. 22–26, 1 jan. 2002.

SANCHEZ-GURMACHES, J. et al. PTEN loss in the Myf5 lineage redistributes body fat and reveals subsets of white adipocytes that arise from Myf5 precursors. **Cell metabolism**, v. 16, n. 3, p. 348–362, 2012.

SANCHEZ-GURMACHES, J.; GUERTIN, D. A. Adipocytes arise from multiple lineages that are heterogeneously and dynamically distributed. **Nature communications**, v. 5, n. 1, p. 1–13, 2014.

SANGALLI, J. R. et al. Development to term of cloned cattle derived from donor cells treated with valproic acid. **PLoS One**, v. 9, n. 6, p. e101022, 2014.

SANHUEZA, J.; NIETO, S.; VALENZUELA, A. Acido linoleico conjugado: un acido graso con isomeria trans potencialmente beneficioso. **Revista chilena de nutrición**, v. 29, n. 2, p. 98–105, 2002.

SCHAEFER, E. J. Schaefer 2002. **Am J Clin Nutr**, v. 75, p. 191–212, 2002.

SCOLLAN, N. et al. **Innovations in beef production systems that enhance the nutritional and health value of beef lipids and their relationship with meat qualityMeat Science**, set. 2006. .

SCOLLAN, N. D. et al. Enhancing the nutritional and health value of beef lipids and their relationship with meat quality. **Meat Science**, v. 97, n. 3, p. 384–394, 2014.

SEIDEMAN, S. C. et al. UTILIZATION OF THE INTACT MALE FOR RED MEAT PRODUCTION: A REVIEW. **Journal of Animal Science**, v. 55, n. 4, 1982. Disponível em: <http://digitalcommons.unl.edu/usdaarsfacpubhttp://digitalcommons.unl.edu/usdaarsfacpub/764>.

SHANTHA, N. C.; CRUM, A. D.; DECKER, E. A. Evaluation of conjugated linoleic acid concentrations in cooked beef. **Journal of Agricultural and Food Chemistry**, v. 42, n. 8, p. 1757–1760, 1994.

SHARAF ELDIN, I. M. A. et al. **Characteristics of beef from intensively fed western Baggara bulls and heifers: quality attributes and chemical composition وعجالت أبقار ال ي خصائص الجودة والتركيب الكيميائ : بقارة السودانية المسمنة على نظام التغذية المركزة خصائص اللحم في عجول أي إم أي شرف الدينIraqi Journal of Veterinary Sciences**. [s.l: s.n.]. Disponível em: <http://vetmedmosul.org/ijvs>.

SHINGFIELD, K. J.; BONNET, M.; SCOLLAN, N. D. Recent developments in altering the fatty acid composition of ruminant-derived foods. **Animal**, v. 7, n. s1, p. 132–162, 2013.

SINCLAIR, A. J. Dietary fat and cardiovascular disease: the significance of recent developments for the food industry. **Food Australia**, v. 45, n. 5, p. 226–231, 1993.

SMAS, C. M.; SUL, H. S. **Control of adipocyte differentiationBiochem. J**. [s.l: s.n.].

SMITH, S. B. et al. Regulation of fat and fatty acid composition in beef cattle. **Asian-Australasian Journal of Animal Sciences**, v. 22, n. 9, p. 1225–1233, 2009.

SMITH, S. B. et al. Adipogenic gene expression and fatty acid composition in subcutaneous adipose tissue depots of Angus steers between 9 and 16 months of age. **Journal of animal science**, v. 90, n. 8, p. 2505–2514, 2012.

SMITH, S.; WITKOWSKI, A.; JOSHI, A. K. **Structural and functional organization of the animal fatty acid synthaseProgress in Lipid Research**Elsevier Ltd, , 2003. .

SORET, B. et al. Expression of genes involved in adipogenesis and lipid metabolism in subcutaneous adipose tissue and longissimus muscle in low-marbled Pirenaica beef cattle. **Animal**, v. 10, n. 12, p. 2018–2026, 1 dez. 2016.

SOUFFRANT, W.-B.; METGES, C. C. **Progress in Research on Energy and Protein Metabolism**. [s.l.] Wageningen Academic Publishers, 2003. v. 109

TATUM, J. D.; GRUBER, S. L.; SCHNEIDER, B. A. Pre-harvest factors affecting beef tenderness in heifers. **National Cattlemen's Beef Association**, 2007.

TAYE, M. et al. Deciphering signature of selection affecting beef quality traits in Angus cattle. **Genes & genomics**, v. 40, n. 1, p. 63–75, 2018.

THALLER, G. et al. DGAT1, a new positional and functional candidate gene for intramuscular fat deposition in cattle. **Animal genetics**, v. 34, n. 5, p. 354–357, 2003.

TORAL, P. G. et al. Modulating ruminal lipid metabolism to improve the fatty acid composition of meat and milk. Challenges and opportunities. **Animal**, v. 12, n. s2, p. s272–s281, 2018.

TOTLAND, G. K.; KRYVI, H. **Anatomy and Embryology Distribution patterns of muscle fibre types in major muscles of the bull (Bos taurus)Anat Embryol**. [s.l: s.n.].

TROY, D. J.; TIWARI, B. K.; JOO, S.-T. Health implications of beef intramuscular fat consumption. **Korean Journal for Food Science of Animal Resources**, v. 36, n. 5, p. 577, 2016.

TUME, R. K.; SIKES, A. L.; SMITH, S. B. Enriching M. sternomandibularis with α-tocopherol by dietary means does not protect against the lipid oxidation caused by high-pressure processing. **Meat Science**, v. 84, n. 1, p. 66–70, jan. 2010.

TURAN, H.; SÖNMEZ, G.; KAYA, Y. Fatty acid profile and proximate composition of the thornback ray (Raja clavata, L. 1758) from the Sinop coast in the Black Sea. **Journal of Fisheries Sciences**, v. 1, n. 2, p. 97–103, 2007.

TURK, S. N.; SMITH, S. B. Carcass fatty acid mapping. **Meat Science**, v. 81, n. 4, p. 658–663, abr. 2009.

ULBRICHT, T. L. V; SOUTHGATE, D. A. T. Coronary heart disease: seven dietary factors. **The lancet**, v. 338, n. 8773, p. 985–992, 1991.

UNDERWOOD, K. R. et al. Relationship between kinase phosphorylation, muscle fiber typing, and glycogen accumulation in longissimus muscle of beef cattle with high and low intramuscular fat. **Journal of agricultural and food chemistry**, v. 55, n. 23, p. 9698–9703, 2007.

USDA. Standards for Grades of Carcass Beef. p. 1–16, 2017. Disponível em: <https://www.ams.usda.gov/sites/default/files/media/CarcassBeefStandard.pdf>.

VANNICE, G.; RASMUSSEN, H. Position of the academy of nutrition and dietetics: dietary fatty acids for healthy adults. **Journal of the Academy of Nutrition and Dietetics**, v. 114, n. 1, p. 136–153, 2014.

VENKATA REDDY, B. et al. **Beef quality traits of heifer in comparison with steer, bull and cow at various feeding environmentsAnimal Science Journal**Blackwell Publishing, , 1 jan. 2015. .

VIEIRA, C. et al. Breed and ageing extent on carcass and meat quality of beef from adult steers (oxen). **Livestock Science**, v. 107, n. 1, p. 62–69, mar. 2007.

VURAL, B. et al. Presence of fatty-acid-binding protein 4 expression in human epicardial adipose tissue in metabolic syndrome. **Cardiovascular Pathology**, v. 17, n. 6, p. 392–398, nov. 2008.

WAKIL, S. J.; ABU-ELHEIGA, L. A. Fatty acid metabolism: target for metabolic syndrome. **Journal of lipid research**, v. 50, p. S138–S143, 2009.

WARD, R. E. et al. Relationship between the expression of key lipogenic enzymes, fatty acid composition, and intramuscular fat content of Limousin and Aberdeen Angus cattle. **Livestock Science**, v. 127, n. 1, p. 22–29, 2010.

WEBB, E. C.; O'NEILL, H. A. **The animal fat paradox and meat qualityMeat Science**, set. 2008. .

WĘGLARZ, A. **Quality of beef from semi-intensively fattened heifers and bullsAnimal Science Papers and Reports**. [s.l: s.n.].

WHIPPLE, G. et al. EVALUATION OF ATTRIBUTES THAT AFFECT LONGISSIMUS MUSCLE TENDERNESS IN BOS TAURUS AND BOS /ND/CUS CATTLE192. **Journal of Animal Science**, v. 68, p. 2716–2728, 1990.

WOOD, J. D. et al. Effects of fatty acids on meat quality: a review. **Meat science**, v. 66, n. 1, p. 21–32, 2004.

WOOD, J. D. et al. **Fat deposition, fatty acid composition and meat quality: A reviewMeat Science**, abr. 2008. .

WOOD, J. D.; RICHARDSON, R. I. **Factors affecting flavour in beef A literature review, with recommendations for the British beef industry on how flavour can be controlled**. [s.l: s.n.].

WOODS, V. B.; FEARON, A. M. **Dietary sources of unsaturated fatty acids for animals and their transfer into meat, milk and eggs: A reviewLivestock Science**, jan. 2009. .

YAMADA, T.; KAWAKAMI, S. I.; NAKANISHI, N. Expression of adipogenic transcription factors in adipose tissue of fattening Wagyu and Holstein steers. **Meat Science**, v. 81, n. 1, p. 86–92, jan. 2009.

ZHANG, S. et al. DNA polymorphisms in bovine fatty acid synthase are associated with beef fatty acid composition 1. **Animal genetics**, v. 39, n. 1, p. 62–70, 2008.

3 FINAL CONSIDERATIONS

In general, cattle gender status modulates differentially expressed genes, which reflects on the lipid profile and, consequently, on carcass traits related to fat deposition. The molecular approach based on the evaluated genes showed that heifers have a molecular regulation aimed at greater accumulation of lipids, consequently reflecting in greater deposition of fat on the carcass, both in the subcutaneous and intramuscular regions and a FA composition with characteristics more favorable to human health than the other gender. Steers, on the other hand, are part of an intermediate group, which, according to the molecular regulation and consequently the lipid profile, presents features that are similar to heifers, but also like the bulls. Bulls present a molecular regulation where the tropomyosin protein abundance revealed to be more associated with muscle development than with fat deposition, reflecting a different lipid profile than heifers and similar to steers at some points, like the expression of the transcript of ACC, the long-chain FAs 22:2n-6, and total SFAs.

Regarding to the meat quality traits, our study suggests that the differences induced by different hormonal conditions are responsible for modulating the formation of adipose tissue in animals influencing the quality, where heifers' meat is superior in terms of fat deposition in the carcass and composition of FA, attending the demand of consumers who are part of a market niche that prefers meat with greater deposition of fat, especially intramuscular (marbling).

Some of the transcripts evaluated between the different muscles were differentially expressed, suggesting that there are differences in the fat deposition process, which reflects on the lipid profile of the muscles, where TB muscle is more favorable to human health than LO.

Additionally, the fat deposition in muscles and the meat fatty acids composition are factors that affect the meat quality and therefore are important for the meat industry.

Finally, for a deeper understanding of the influence of cattle gender status on the molecular mechanisms that regulate adipogenesis, it is important that further studies be performed to validate the association founded in our study, since we did not observe differences in some of the evaluated genes, probably due to the transcript is an intermediate product of protein. Therefore, we design to continue and investigate other protein levels by Western blot to better understand the role of adipogenesis-related genes evaluated in our study, from pathways regulated by enzymes and proteins involved in synthesis, transport, distribution, degradation and oxidation of long-chain FAs in adipose and muscle cells in different muscles regions of the cattle.

4 ATTACHMENTS

4.1 Certificate of approval by Ethics Committee on Animal Use

UNIVERSIDADE DE SÃO PAULO
Faculdade de Zootecnia e Engenharia de Alimentos
Comitê de Ética em Pesquisa da FZEA

CERTIFICADO

Certificamos que a proposta intitulada "Análise da expressão de genes associados à adipogênese e composição da gordura intramuscular em bovinos Angus x Nelore de diferentes condições sexuais", protocolada sob o CEUA nº 7675200918 (ID 001125), sob a responsabilidade de **Angélica Simone Cravo Pereira** *e equipe; Lenise Freitas Mueller da Silveira; Pollyana Leite Matioli Garbossa* - que envolve a produção, manutenção e/ou utilização de animais pertencentes ao filo Chordata, subfilo Vertebrata (exceto o homem), para fins de pesquisa científica ou ensino - está de acordo com os preceitos da Lei 11.794 de 8 de outubro de 2008, com o Decreto 6.899 de 15 de julho de 2009, bem como com as normas editadas pelo Conselho Nacional de Controle da Experimentação Animal (CONCEA), e foi **aprovada** pela Comissão de Ética no Uso de Animais da Faculdade de Zootecnia e Engenharia de Alimentos da Universidade de São Paulo - FZEA/USP (CEUA/FZEA) na reunião de 23/01/2019.

We certify that the proposal "Analysis of adipogenesis related genes and intramuscular fat composition in Angus x Nellore cattle of different gender status", utilizing 150 Bovines (males and females), protocol number CEUA 7675200918 (ID 001125), under the responsibility of **Angélica Simone Cravo Pereira** *and team; Lenise Freitas Mueller da Silveira; Pollyana Leite Matioli Garbossa* - which involves the production, maintenance and/or use of animals belonging to the phylum Chordata, subphylum Vertebrata (except human beings), for scientific research purposes or teaching - is in accordance with Law 11.794 of October 8, 2008, Decree 6899 of July 15, 2009, as well as with the rules issued by the National Council for Control of Animal Experimentation (CONCEA), and was **approved** by the Ethic Committee on Animal Use of the School of Animal Science and Food Engineering - (São Paulo University) (CEUA/FZEA) in the meeting of 01/23/2019.

Finalidade da Proposta: Pesquisa (Acadêmica)

Vigência da Proposta: de 09/2018 a 07/2020 Área: Zootecnia

Origem:	Animais provenientes de estabelecimentos comerciais						
Espécie:	Bovinos	sexo:	Machos e Fêmeas	idade:	11 a 16 meses	N:	150
Linhagem:	F1 Angus x Nelore			Peso:	220 a 500 kg		

Local do experimento: Confinamento: Fazenda Letícia (Grupo Piveta) - Rio Verde de Mato Grosso - MS. Abate e coleta de amostras: Frigorífico Frizelo - Terenos - MS. Desossa e coleta de amostras: VPJ Alimentos - Pirassununga - SP. Análises laboratoriais de qualidade de carne: Laboratório de Ciência da Carne - FMVZ USP (Campus Pirassununga). Análises moleculares da carne: Laboratório de Morfofisiologia Molecular e Desenvolvimento (LMMD) - FZEA USP.

Pirassununga, 21 de maio de 2019

Profa. Dra. Daniele dos Santos Martins
Coordenadora da Comissão de Ética no Uso de Animais
Faculdade de Zootecnia e Engenharia de Alimentos da Universidade de São Paulo - FZEA/USP

Profa. Dra. Cristiane Gonçalves Titto
Vice-Coordenadora da Comissão de Ética no Uso de Animais
Faculdade de Zootecnia e Engenharia de Alimentos da Universidade de São Paulo - FZEA/USP

Av. Duque de Caxias Norte, 225 - CEP 13635-900 Pirassununga/SP - tel: 55 (19) 3565 6759
Horário de atendimento: 2ª a 6ª das 13h30 as 17h : e-mail: cepfzea@usp.br
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4.2 Confirmation of a paper submited to Livestock Science as a Review article

Lenise Freitas Mueller da Silveira <lelemueller@usp.br>

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