UNIVERSIDADE ESTADUAL PAULISTA - UNESP
FACULDADE DE CIÊNCIAS AGRÁRIAS E VETERINÁRIAS
CAMPUS DE JABOTICABAL

QUALIDADE DE CARNE DE BOVINOS SUBMETIDOS A DIFERENTES ESTRATÉGIAS DURANTE A RECRIA E TERMINAÇÃO

Juliana Akamine Torrecilhas
Zootecnista

2020

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Tese apresentada à Faculdade de Ciências Agrárias e Veterinárias – UNESP, Campus de Jaboticabal, como parte das exigências para a obtenção do Título de Doutor em Zootecnia (Nutrição e Produção Animal).

2020

T689q

Torrecilhas, Juliana Akamine

Qualidade de carne de bovinos submetidos a diferentes estrategias durante a recria e terminação / Juliana Akamine Torrecilhas. -- Jaboticabal, 2020

100 f. : il., tabs.

Tese (doutorado) - Universidade Estadual Paulista (Unesp), Faculdade de Ciências Agrárias e Veterinárias, Jaboticabal

Orientadora: Telma Teresinha Berchielli

Coorientador: Elias San Vito

1. Beef cattle. 2. Intensive system. 3. Meat quality. 4. Ruminant. I. Título.

Sistema de geração automática de fichas catalográficas da Unesp. Biblioteca da Faculdade de Ciências Agrárias e Veterinárias, Jaboticabal. Dados fornecidos pelo autor(a).

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CERTIFICADO DE APROVAÇÃO

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Jaboticabal, 05 de maio de 2020

DADOS CURRICULARES DO AUTOR

Juliana Akamine Torrecilhas, filha de José Carlos Torrecilhas e Jucimara Akamine Torrecilhas, nasceu em Maringá, Paraná, no dia 26 de abril de 1988. De 2009 a 2013 desenvolveu o curso de Zootecnia pela Universidade Estadual de Maringá -UEM, onde foi bolsista de iniciação cientifica sobre orientação do prof. Dr. Ivanor Nunes do Prado. Em março de 2014, ingressou no Programa de Pós Graduação em Zootecnia, em nível mestrado, área de concentração Nutrição e Produção Animal, na Universidade Estadual de Maringá sobre orientação do prof. Dr. Ivanor Nunes do Prado, obtendo o título de mestre em fevereiro de 2016. Em fevereiro de 2016, ingressou no Programa de Pós graduação em Zootecnia, em nível doutorado, na Universidade Estadual Paulista – Júlio de Mesquita Filho – Campus de Jaboticabal sobre orientação da Profa. Dra. Telma Teresinha Berchielli. Em março de 2020 submeteu-se à defesa da tese.

"Tenha coragem. Vá em frente. Determinação, coragem e autoconfiança são fatores decisivos para o sucesso. Não importa quais sejam os obstáculos e as dificuldades. Se estamos possuídos de uma inabalável determinação, conseguimos superá-los independentemente das circunstâncias, devemos ser sempre humildes, recatados e despidos de orgulho."

Dalai Lama

"Don't let the noise of others' opinions drown out your own inner voice"

"The only way to do great work is to love what you do."

Steve Jobs

Aos meus pais José Carlos Torrecilhas e Jucimara Akamine Torrecilhas, pelo carinho, pela força, dedicação, amor e contribuição para minha formação, social e pessoal.

Aos meus irmãos, Josiane e Carlos Eduardo, que sempre estiveram ao meu lado.

Aos meus avós, Lídia e José Torrecilhas pelo grande apoio e carinho.

A toda minha família, que mais do que me proporcionaram uma boa infância e formaram os fundamentos do meu caráter.

Dedico.

AGRADECIMENTOS

A Deus pelo dom da vida, por ter me iluminado nas decisões mais difíceis e por ter me guiado ao longo do doutorado.

A Universidade Estadual Paulista (UNESP), a pós graduação em Zootecnia da Unesp e professores do programa que possibilitou a realização deste trabalho.

Ao Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) pela bolsa concedida no início do doutorado. A Fundação de Amparo e Pesquisa do Estado de São Paulo (FAPESP), pela concessão da bolsa de pós graduação (processo 2016/10953-3), e ao projeto auxílio (processo 2015/01147-0).

A minha orientadora Telma Teresinha Berchielli, pela confiança em todo o meu doutorado e pelas oportunidades geradas para minha formação.

Aos meus coorientadores Elias San Vito e Giovani Fiorentini, pelas orientações, conselhos, paciência e toda ajuda.

Aos Prof. Terry Engle e Prof. Dr. Joanis Zervoudakis, por toda amizade, apoio e auxílio durante o estágio de pesquisa no exterior.

Aos amigos, Nomaiaci Andrade, Eulalia Pinheiro, Kênia Alves, Karine Vecchia, Pablo Castagnino, Sergio Pereira, Rayanne Costa, Paloma Gonçalves, Geovany Carvalho, Thaís Brito e Iorrano Cidrini pela amizade, pelo apoio e auxílio nos momentos difíceis, paciência, conselhos e amizade.

Aos que participaram do projeto, Tiago Simioni, Giovane Penasso, Thais Ribeiro, Maria Julia Ganga, Breno Maximo, Erick Escobar, Laís Lima, Paulo Colovate, Vladmir, Alinne Oliveira e Conceição Oliveira por toda ajuda.

Aos técnicos de laboratório da Unesp, João Carlos Campanharo, Claudia Fiorillo e Ana Paula Sader por toda ajuda e auxílio durante o desenrolar das análises.

Ao Prof. Dr. Ivanor Nunes do Prado e Dra. Ana Guerrero, pela amizade adquirida no Mestrado e mantida até os dias atuais, pelos ensinamentos e por terem aceitado participar da minha banca de defesa.

A Dra. Josiane Lage por ter participado do projeto, pelos auxílios durante o doutorado e por ter aceitado participar da minha banca de defesa.

Ao Dr. Fernando Baldi, por ter contribuído na qualificação e aceitado participar da banca de defesa.

Ao grupo de pesquisa da professora Telma Berchielli, Juliana Duarte, Yury Granja e grupo todo pelo ensinamento durante o experimento.

A todos que, direta ou indiretamente, contribuíram para a realização deste trabalho.

SUMÁRIO

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Certificamos que o Protocolo nº 5628/15 do trabalho de pesquisa intitulado "Efeitos de planos nutricionais na recria sobre a fisiologia do crescimento, parâmetros metabólicos e qualidade da carne de tourinhos de diferentes grupos genéticos terminados em pasto ou confinamento", sob a responsabilidade da Profª Drª Telma Teresinha Berchielli está de acordo com os Princípios Éticos na Experimentação Animal adotado pelo Conselho Nacional de Controle de Experimentação Animal (CONCEA) e foi aprovado pela COMISSÃO DE ÉTICA NO USO DE ANIMAIS (CEUA), em reunião ordinária de 01 de abril de 2015.

Jaboticabal, 01 de abril de 2015.

Prof.ª Dr.ª Paola Castro Moraes
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QUALIDADE DA CARNE DE BOVINOS SUBMETIDOS A DIFERENTES ESTRATÉGIAS DURANTE A RECRIA E TERMINAÇÃO

RESUMO – Nosso objetivo foi avaliar o efeito da estratégia de suplementação durante a fase de recria na qualidade da carne de bovinos terminados em pasto mais concentrado ou confinamento. Cento e vinte machos não castrados [10 ± 2 meses de idade e 256,54 ± 28,92 kg de peso vivo (PC) inicial] foram blocados por peso e aleatoriamente designados para os tratamentos em design fatorial 2 × 2, dois suplementos durante a fase de recria: mineral (*ad libitum* – MIN) ou proteína + energia (0,3% PC/ animal/ dia – PRE) e dois sistema de terminação: pasto mais suplementação com concentrado (2% PC/ animal/ dia - PAST); e confinamento (25:75 %; silagem de milho: concentrado - FLOT). Depois de 285 dias os bovinos (503,89 ± 56,61 kg) foram abatidos em frigorifico comercial, a carcaça foi dividida medialmente do esterno e da coluna, resultando em duas metades semelhantes. Amostras (10g) do *Longíssius thoracis* (LT) foram removidas imediatamente depois do abate, entre a 12ª e 13ª costela de cada animal e congeladas (nitrogênio líquido) para: atividade de enzima lipogênica e relativa abundancia de RNAm associado com metabolismo de lipídeos, analisados por qRT-PCR os genes alvos incluíram PPARγ, SREBP1c, SCD1, ACCα LPL, FBP4, CPT2, ACOX e PPARα. Após 24 horas de resfriamento de carcaça, o pH foi mesurado entre a 12ª e 13ª costela do LT. A gordura subcutânea e cinco bifes de 2,54 cm do LT (entre a 9ª a 13ª costela) foram coletados para análises: perfil de ácido graxo, composição química, força de cisalhamento, comprimento de sarcômero, índice de fragmentação miofibrilar, perda de descongelamento, perda de cocção, capacidade retenção de água, cor de gordura, mioglobina, oxidação lipídica e parâmetros de cor que foram avaliados nos dia 1, 4, 7, 10, 14 de exposição ao oxigênio a 0 ± 4°C. O sistema FLOT aumentou (P < 0,050) a espessura de gordura subcutânea (7,01 vs. 3,07 mm), o comprimento de sarcômero (1,59 vs. 1,42 µm) e diminuiu (P < 0,050) a força de cisalhamento da carne (36,29 vs. 43,20 N), enquanto o grupo do PAST mostrou maior (P < 0,050) capacidade retenção de água (68,16 vs. 64,32%), gordura amarela (16,66 vs. 14,30), concentração de mioglobina (4,56 vs. 3,95 mg/g de carne). A carne dos bovinos alimentados com MIN durante a fase de crescimento seguidos pela terminação FLOT mostraram aumento (P < 0,050) da oxidação lipídica comparado aos bovinos do MIN seguidos do sistema PAST (0.35 vs. 0.27 mg/kg de carne). Durante o tempo de armazenamento, a carne dos bovinos alimentados com MIN seguido pelo sistema PAST mostraram menores (P < 0,050) valores de L* comparados com os outros tratamentos. Nos dias, 4 e 7 de display, o grupo FLOT tiveram aumento (P < 0,050) de a* e C* na carne comparado ao PAST. Os animais do sistema PAST mostraram menores (P < 0,050) valores de b* comparado ao FLOT (12,17 *vs.* 13,40, respectivamente). O sistema FLOT aumentou (P < 0,050) o conteúdo de lipídeo na carne do que o PAST (2,72 vs. 1,49%, respectivamente). Os animais alimentados com MIN seguidos da terminação FLOT mostraram um aumento (P < 0,050) dos ácidos C12:0, C14:0, e C16:0, o que levou a um aumento da concentração do total de ácido graxo saturado. Os bovinos terminados no sistema FLOT tiveram maior (P < 0,050) concentração do total de ácido graxo monoinsaturado coincidindo com o aumento (P < 0,050) da expressão do gene SCD1 no musculo em comparação ao sistema PAST.

A carne dos bovinos do sistema FLOT mostrou diminuição (P < 0,050) de C18:2n6, C20:3n6, C20:4n6, C20:5n3, razão n6/n3, e isocitrato desidrogenase em comparação ao sistema PAST. Além disso, os bovinos alimentados com PRE seguidos pela terminação FLOT tiveram maior (P < 0,050) expressão de CPT2 no musculo, enquanto que a suplementação MIN seguido pelo sistema PAST mostraram diminuição (P < 0,050) de SREBP1c, aumento (P < 0,050) da expressão de CPT2 e PPARα no musculo. Em geral, esses resultados sugerirem que os atributos relacionados com a maciez da carne não foram afetados pela fase de crescimento. O sistema PAST diminuiu a maciez e luminosidade da carne, mas esses atributos ainda mostraram dentro dos padrões aceitos na indústria de carne. Além disso, o sistema FLOT aumentou o lipídio intramuscular em comparação com o sistema PAST, mas os animais alimentados com MIN durante a fase de crescimento acompanhado do sistema PAST apresentaram maior expressão de genes relacionados à degradação lipídica e menores genes relacionados à síntese de lipídios.

Palavras – chave: Coloração, Expressão de genes, Marmoreio, Suplementação intensiva

MEAT QUALITY OF BULLS SUBMITTED TO DIFFERENT STRATEGY DURING THE GROWING AND FINISHING PHASE

ABSTRACT – Our objective was to evaluate the supplementation strategy during the growing phase on the meat quality of bulls finished in pasture plus concentrate or feedlot. One hundred and twenty young bulls uncastrated males [10 ± 2 month old and 256.54 ± 28.92 kg of body weight (BW)] were blocked by weight and randomly assigned to treatments in 2 × 2 factorial design, two supplements during the growing phase: mineral (ad libitum - MIN) or Protein + Energy (0.3% BW/ animal/ day - PE) and two finishing system: pasture plus concentrate (2% BW/ animal/ day - PAST) or feedlot (25:75; corn silage: concentrate - FLOT). After 285 days the bulls (503.89 ± 56.61 kg) were slaughtered, the carcasses were divided medially from the sternum and the spine, resulting in two similar halves. The *Longissimus thoracis* (LT) sample (10 g) was removed immediately after slaughtered between 12th and 13th ribs from each animal and frozen (liquid nitrogen) for: lipogenic enzyme activity and relative abundance of mRNA associated with lipid metabolism measured by qRT-PCR, target genes include PPARγ, SREBP1c, SCD1, ACCα, LPL, FBP4, CPT2, ACOX, and PPARα. After 24 h carcass chilling, the pH was measured between the 12th and 13th ribs of the LT. The fat thickness and five steaks of 2.54 cm from LT (between the 9th and 13th ribs) were collected for fatty acid profile, chemical composition, shear force, sarcomere length, myofibrillar fragmentation index, thawing loss, cooking loss, water holding capacity, fat color, myoglobin, lipid oxidation and meat color which was measured on days, 1, 4, 7, 10 and 14 storage time exposed to oxygen at 0 ± 4°C. The FLOT system increased ($P < 0.050$) the fat thickness (7.01 vs. 3.07 mm), sarcomere length (1.59 vs. 1.42 µm), and decreased the meat shear force (36.29 vs. 43.20 N), while the PAST group showed higher ($P < 0.050$) water holding capacity (68.16 vs. 64.32%), yellowness fat (16.66 vs. 14.30), myoglobin concentration (4.56 vs. 3.95 mg/g of meat). The meat of bulls fed with MIN during the growing phase following by FLOT finishing showed increased ($P < 0.050$) of lipid oxidation compared to bulls from MIN following by PAST system (0.35 vs. 0.27 mg/kg of meat). During the storage time, the meat of bulls fed with MIN following by PAST system showed lower ($P < 0.050$) L* value compared to the other treatments. On days, 4 and 7 of the display, the FLOT group had increased ($P < 0.050$) of a* and C* in meat compared to PAST. The bulls from PAST system showed lower ($P < 0.050$) b* value compared to FLOT (12.17 *vs.* 13.40, respectively). The FLOT group had ($P < 0.050$) higher lipid content than PAST (2.72 vs 1.49%, respectively). The bulls fed with MIN following by FLOT system showed an increase ($P < 0.050$) of C12:0, C14:0, and C16:0 which led to greater saturation fatty acid total concentration. The bulls finished in FLOT system had higher ($P < 0.050$) monounsaturated fatty acid total concentration coinciding with the up-regulated ($P < 0.050$) of gene expression of SCD1 compared to PAST system. The meat of bulls from FLOT system showed a decrease ($P < 0.050$) of C18:2n6, C20:3n6, C20:4n6, C20:5n3, n6/n3 ratio and isocitrate dehydrogenase compared to PAST system. In addition, the animals fed with PRE following by FLOT finishing had higher ($P < 0.050$) CPT2 expression, while the MIN supplementation following by PAST

system showed decreased (P < 0.050) of SREBP1c, increased (P < 0.050) CPT2 and PPARα expression. Globally, these results suggested which the attributes relationship with tenderness meat was not affected by the growing phase. The PAST system decreased the tenderness and lightness of meat, but these attributes still showed within the acceptable standards of the meat industry. In addition, the FLOT system increased the intramuscular lipid compared to PAST system, but the animals fed with MIN during the growing phase following by PAST showed greater genes expression related to lipid degradation and lower gene expression related with synthesis of lipids.

Keywords: Colour, Gene expression, Marbling, Intensive supplementation

CAPÍTULO 1 – CONSIDERAÇÕES GERAIS

1. Introdução

O Brasil possui produção de bovinos predominantemente a pasto, a maior parte do território para criação, apresenta característica climática favorável durante a época de verão para produção de gado, devido ao aumento de incidência de chuvas e melhora na quantidade e qualidade de forragem (Moreira et al., 2008). Por outro lado, na época de inverno é caracterizado por limitar o crescimento de forrageiras, que consequentemente fornece menor disponibilidade de nutrientes ao animal, que por sua vez pode comprometer seu desempenho (Detmann et al., 2014).

Para obter eficiência nas respostas produtivas na bovinocultura de corte em regime de pasto, tanto na fase de crescimento quanto na terminação, é fundamental estabelecer desde a idade jovem dos animais, um ambiente adequado e uma estratégia de suplementação alimentar eficiente e bem planejada. A fase de recria é um ponto chave para busca de antecipação da idade de abate, cujo os animais apresentarem uma melhor eficiência biológica quanto a deposição de tecidos, devido apresentarem curva de crescimento mais acentuada (Owens et al., 1993). A deposição de proteína é quatro vezes mais eficiente em comparação a deposição de gordura, uma vez que para a deposição de tecido muscular, aproximadamente 78% de agua é estocada, enquanto que 10% de agua é armazenada para deposição de gordura (Owens et al., 1995).

Dessa forma, a suplementação é uma estratégia de produção que pode ser utilizada para explorar a fase de recria, sendo que o ganho adicional nessa fase é mantido durante a fase de terminação sendo a pasto ou em confinamento, e o tempo necessário para engorda desses animais é reduzido em relação ao uso de sal mineral (Casagrande et al., 2013).

A medida que o animal cresce e se aproxima da maturidade, a intensidade de crescimento muscular é reduzida e a constituição do ganho passa a ser composto pelo crescimento do tecido adiposo (Owens et al., 1993). Assim sendo, na fase de terminação na época de inverno apresenta um entrave devido à escassez de forragem e a baixa oferta de nutrientes para o animal. Por conseguinte, estratégias de dieta com o uso de

alta inclusão de concentrado em relação ao peso corporal animal (1.5 a 2% do peso corporal), seja em sistema de pasto ou confinamento convencional pode reduzir o ciclo de produção e levar a um melhor acabamento de carcaça, aumento do marmoreio da carne, características cada vez mais desejadas pela indústria da carne e mercado consumidor (Carvalho e Smith, 2018).

Dessa forma, a compreensão fisiológica do crescimento animal é de extrema importância, uma vez que a quantidade de cada tecido, a velocidade com que são depositados, e a nutrição animal podem influenciar o ganho de peso animal, na composição do ganho e na qualidade de carne. O objetivo do estudo foi avaliar o efeito da estratégia de suplementação durante a fase de crescimento sobre a qualidade da carne de bovinos jovens terminados em diferentes sistemas de terminação, pasto com suplementação de concentrado (Expresso®) ou em confinamento convencional.

1.1. Suplementação de bovinos durante a fase de recria

A produção de bovinos de corte no Brasil ocorre na maior parte em sistema de produção a pasto, onde é caracterizado por ter duas estações bem definidas, época de verão, que ocorre aumento de chuvas com maior oferta de forragem e de melhor qualidade para o gado de corte, enquanto a época de inverno caracterizado pela diminuição de nutrientes na forragem (Detmann et al., 2014). Assim, é importante fornecer maior disponibilidade de nutrientes neste período, sobretudo de forma constante e com plano nutricional crescente na vida do animal, para maior aproveitamento e benefício na produção de bovinos de corte (Roth et al., 2017).

O crescimento dos tecidos no animal inicia desde a concepção até a fase de terminação (Figura 1). No nascimento os animais apresentam o sistema nervoso já formado, e o tecido ósseo bem desenvolvido, pois estes apresentam crescimento precoce. Até a puberdade, o tecido muscular irá apresentar o maior desenvolvimento, e após esta fase o tecido adiposo possui seu crescimento mais acentuado.

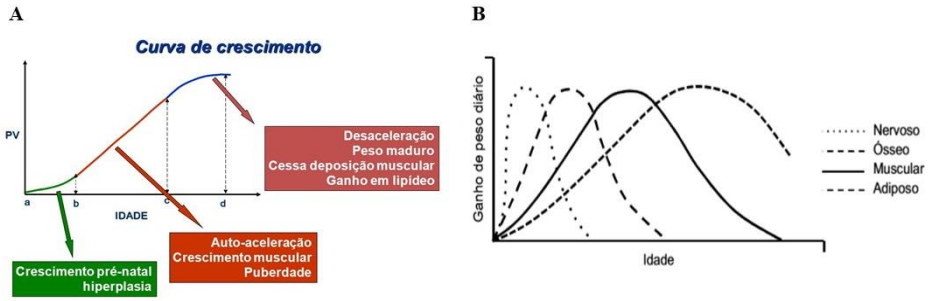

Figura 1. A - Curva representando o crescimento de ruminantes. B - Dinâmica de deposição dos tecidos do corpo do bovino pós-natal. Adaptado de Luchiari Filho (2000).

Dessa forma, a fase de recria, onde o animal apresenta maior crescimento do tecido muscular e necessita de maior exigência de nutrientes, muitas vezes não é suprido apenas pela pastagem. Por conseguinte o animal que apresenta menor taxa de crescimento depois do desmame, pode produzir uma carcaça mais magra e leve na fase de terminação, alterando a quantidade e qualidade da carne produzida (Silva et al., 2017).

Nesse sentido, com o objetivo de alcançar maiores ganhos, a suplementação é uma estratégia para corrigir possíveis deficiências de nutrientes da forragem, aumentando a eficiência de utilização do pasto. Os principais tipos de suplementos são: sal mineral (atender exigência básica de minerais e vitamina dos animais), suplemento proteico (contem cerca de 25 a 50% de PB), que pode ser fornecido a 0,1% do peso corporal animal, e suplemento proteico energético (contem cerca de 18 a 25% de PB) e pode ser fornecido de 0,3 a 0,5% do peso corporal animal.

Embora no período das águas, os animais apresentem melhores desempenhos devido maior quantidade e qualidade da forragem, o uso de suplementação pode ser uma tecnologia que permite maiores benefício durante essa época (Reis et al., 2009). Trabalhos têm mostrado efeito positivo da suplementação proteica (0,1% PC) no desempenho animal, com ganhos médios adicionais de 150 g/dia em comparação a animais suplementados com sal mineral (Roth et al., 2013, Moretti et al., 2013). Oliveira et al. (2016) fornecendo suplementação (0,3% do PC/ animal/ dia) para novilhas (7 meses de idade e 200 kg de PC inicial) durante o período das águas (janeiro a março), encontrou um ganho adicional de 0,214 kg/dia em relação ao suplemento sal mineral.

Entretanto, a resposta no desempenho animal com a suplementação durante a época das águas, pode ser influenciada altura de pastejo, dessa forma faz se necessário o ajuste da composição de suplemento de acordo com a característica da forragem (Reis et al., 2019).

Além disso, estudos com bovinos mantidos em pastagem tropical, tem mostrado estratégias de suplementação na fase de recria e o efeito dessa suplementação na fase de terminação (Roth et al., 2017, Sampaio et al., 2017, Delevatti et al., 2019). De acordo com Roth et al. (2017), trabalhando com diferentes níveis de suplementação em diferentes fases de crescimento de bovinos, observaram que níveis constante de suplementação durante as fases da vida do animal pode fornecer um aporte maior de crescimento, e reduzir os dias de terminação.

O peso corporal durante a terminação tem relação negativa com a fase de crescimento (Drouillard et al., 1991). Animais que tem crescimento restrito na fase de recria quando submetidos a uma terminação com alta taxa de crescimento podem apresentar maior turnover proteico *in vivo* e dessa forma maior maciez da carne (Therkildsen et al. 2011), uma vez que a taxa de crescimento pode afetar a proteólise miofibrilar no *postmortem.* Por outro lado, esses aninais podem produzir carcaça mais leve e magras, o que não é desejado pela indústria da carne.

Silva et al. (2017), trabalhando com três diferentes taxas de crescimentos (0, 0,6 e 1,2 kg ganho médio diário, respectivamente) de bovinos na fase de recria (8,4 meses de idade), e seguidos por uma fase de terminação (1,5 kg ganho médio diário) por 112 dias, observaram que a baixa e média taxa de crescimento reduziram o peso de carcaça quente e a gordura subcutânea em comparação aos que tiveram alta taxa de crescimento. Além disso, Lomas et al. (2009) trabalhando com suplementação de sorgo de bovinos em pastagem, encontram que a suplementação de 1.64 kg / dia, aumentou o marmoreio em relação aos animais não suplementados.

Nesse sentido, a fase de recria é importante para manipulação através da nutrição com objetivo de maximizar a deposição de proteína, consequentemente aumentando a quantidade de tecido muscular na carcaça, reduzindo o tempo durante a fase de terminação, adiantando a deposição de tecido adiposo e melhorando a qualidade da carne. Entretanto, o uso de suplementação de bovinos com proteína e energia na fase

de crescimento só deve ser usada quando na fase subsequente houver um plano nutricional que proporcione alta taxa de ganho de peso (Roth et al., 2017). Quando os animais não são suplementados corretamente, tem seu crescimento e desenvolvimento limitados, comprometendo todo o ciclo de produção até o produto final.

1.2. Fase de terminação de bovinos

A maior parte da produção de carne brasileira é realizado exclusivamente sobre sistema que utiliza o pasto como principal componente da dieta. Entretanto o sistema tradicional da produção a pasto no Brasil é caracterizado pelo baixo desempenho e pouca energia ingerida pelo animal, principalmente no período de terminação, o que eleva o ciclo de produção de bovinos, com médias de 36 meses (Oliveira et al., 2006), além dos animais apresentarem acabamento de carcaça escasso ou ausente (Alves Neto et al., 2018). Dessa forma, a busca pelo aumento de eficiência na produção de carne no Brasil tem direcionado para sistemas que abatem animais jovens, como o uso de confinamento na fase de terminação, que chegou a 12% no ano de 2018 (Anualpec, 2019).

Nesse sentido, a terminação de bovinos em confinamento, principalmente no período seco é uma estratégia, uma vez que permite uso de dietas com maior inclusão de energia na dieta, que proporcionam carcaça com melhor acabamento e espessura de gordura dentro dos padrões exigidos pela indústria frigorifica (mínima de 3 mm de espessura de gordura subcutânea) (Rotta et al., 2009).

No Brasil, o confinamento convencional é caracterizado por períodos de 90 a 120 dias, além do uso de dietas com alto teores de energia, variando de 71 a 90 % de inclusão de concentrado nas dietas de terminação, tendo o milho como principal fonte energética na alimentação desses animais (Oliveira e Millen, 2014).

O uso de altos níveis de concentrado na alimentação de bovinos, fornece maior aporte de glicose no sangue desses animais, uma vez que esse tipo de dieta favorece a produção de propionato no rúmen (principal fonte de glicose para ruminantes através do processo gliconeogênese), em comparação a dietas a base de forragem, assim o aumento da insulina sérica provavelmente levaria a um aumento da captação de glicose

pelos tecidos, contribuindo para desenvolvimento do tecido adiposo. Entretanto o fornecimento de glicose em idades precoces pode promover o maior desenvolvimento de marmoreio mais do que se o fornecimento fossem em estágios posteriores (Smith e Johnson 2016). De acordo com Schoonmaker et al. (2003), estudando com bovinos alimentados com concentrado *ad libitum,* observaram maiores níveis de insulina no sangue e marmoreio na carne, como resultado da fermentação do amido, em comparação aos animais que receberam dieta de foragem *ad libitum* na fase de crescimento.

Nesse sentido, o confinamento é uma estratégia que permite o uso de dietas com alto teor de energia resultando em maior desempenho dos animais, idade de abate mais jovens e com maior grau de acabamento de carcaça (Moretti, 2015). Entretanto alguns entraves como custo com infraestrutura e necessidades de volumoso devem ser levados em consideração. Outra alternativa é a terminação dos bovinos no confinamento Expresso®, que consiste no fornecimento de altos níveis de energia e alta quantidade de concentrado (1.5% a 2% do peso corporal) para os animais no pasto, onde o pasto é a fonte de substrato fibroso para manutenção do ambiente ruminal, o que pode ser uma alternativa já que a terminação a pasto representa menor investimento operacional (Moretti, 2015).

A comparação entre as estratégias de terminação no pasto com alta quantidade de concentrado (Expresso®) e confinamento convencional tem mostrado resultados interessantes. Moretti (2015), comparou a terminação intensiva a pasto e confinamento convencional, com o fornecimento da mesma quantidade de suplementação de energia e observou que os animais na terminação intensiva a pasto apresentaram menor ganho de peso durante esse período, por outro lado tiveram maior rendimento do ganho em relação aos animais do confinamento convencional. Isso ocorreu devido os animais a pasto terem maior seletividade, aumentando a taxa de passagem e diminuindo o conteúdo do trato gastrointestinal.

Uma vez que a proporção de gordura depositada na carcaça está diretamente relacionada com a ingestão de energia, durante a fase de terminação (Byers, 1982), o nível de suplementação é outro ponto que deve ser levado em consideração. Miorin (2018), trabalhando com níveis de suplementação de 0,5% e 2% do peso corporal/dia de

bovinos a pasto durante a fase de terminação, observaram que os animais com maior nível apresentaram espessura de gordura subcutânea dentro dos padrões exigidos pela indústria da carne em comparação aos animais com menor nível (3,5 *vs* 1,8 mm).

Dessa forma o uso de concentrado na dieta de terminação de bovinos, permite maior acabamento de carcaça o que ajuda na conservação e resfriamento da mesma, evitando efeitos negativos causados pelo frio, como perda de água e o encurtamento do sarcômero e escurecimento da carne, e melhorando as características sensoriais do produto final (Biserra et al., 2020). Entretanto há poucos estudos que avaliaram a característica e qualidade de carne de bovinos que terminados em confinamento convencional ou com suplementação intensiva a pasto.

1.3. Desenvolvimento do tecido adiposo

O desenvolvimento inicial do tecido adiposo ocorre por um processo chamado adipogênese, que similar a miogênese, possui seu início de desenvolvimento na fase pré-natal, derivado também de células troncos mesenquimais (Yan et al., 2013). No processo de pré-adipócito, a diferenciação é complexa e é iniciada pela exposição dos pré-adipócitos a muitos estímulos adipogênicos, como hormônios como a insulina, glicocorticoides e IGF-1,e efeitos parácrinos e autócrinos, como ácidos graxos livres (Kirkland et al., 2002).

Os adipócitos são originários de células troncos mesenquimais, na qual se tornam comprometidas com a linhagem adipocitária, essa é a primeira fase de diferenciação da adipogênese chamada de determinação. A segunda fase é a diferenciação terminal na qual os pré adipócitos adquirem característica de adipócitos maduros e são capazes de responderem a hormônios (Queiroz et al., 2009).

Na diferenciação terminal, os fatores de transcrição que regulam a adipogênese incluem os receptores ativados por proliferadores de peroxissoma (PPAR) e as proteínas de ligação a potenciador (C/EBPs) (Yan et al., 2013). As principais isoformas centrais para a adipogêneses são PPARγ e fator de transcrição CCAAT (proteína de ligação ao estimulador alfa C/EBPα) que uma vez ativos se autorregulam para se manterem expressos. Esses fatores de transcrição induzem a transcrição de vários genes-alvos

anteriormente silenciosos, incluindo enzimas e proteínas envolvidas na lipogênese e lipólise (Queiroz et al., 2009).

Diferentemente do crescimento do tecido muscular, o tecido adiposo pode apresentar crescimento através de modificações no número celular (hiperplasia) após nascimento do animal, acontece por diferenciação de pré adipócitos em adipócitos maduro (adipogêneses) (Du et al., 2013). O tecido adiposo pode conter cerca de 15 a 50% de células tronco mesenquimais que são capazes de se dividirem e se diferenciarem (Queiroz et al., 2009).

Na fase de adipócitos maduros a modificações no diâmetro (hipertrofia) ocorre em resposta a ativação de ações metabólicas como a lipogênese e a lipólise, que ocorrem de acordo com a necessidade de liberação ou armazenamento de lipídeos, assim sendo a interação dos nutrientes da dieta e o nível de expressão dos genes envolvidos no metabolismo lipídico podem influenciar na deposição dos ácidos graxos no tecido adiposo (Hiller et al., 2011).

O PPAR possui basicamente três isoformas (α, γ, δ/β), onde sua função está relacionada a especificidade dos tecidos. Em roedores foi encontrada que a isoforma γ está mais expressa no tecido adiposo e atua promovendo a diferenciação dos adipócitos além de poder atuar no armazenamento de lipídeos (caminho da PPAR), enquanto que a isoforma α pode ser mais expressa nos músculos e fígado (Kersten, 2014). O PPAR pode ser regulada por ácidos graxos, dietas ricas em triglicerídeos de cadeia média podem influenciar na adipogênese diminuindo o tecido adiposo através da regulação negativa na expressão do gene PPARγ (Han et al., 2003), enquanto que dietas ricas em ácido graxo de cadeia longa podem estimular a expressão de genes relacionados a adipogênese, aumentando o tecido adiposo (Redonnet et al., 2001). Brown et al. (2003) demostraram que o isômero CLA C18:2 t10 c12 inibe a expressão do gene PPARγ em roedores impedindo o enchimento de adipócitos. A produção desse isômero (C18:2 t10 c12) que ocorre em ruminantes por uma rota alternativa devido à queda do pH ruminal (Figura 2), pode inibir a expressão do SREBP (proteína 1c ligadora do elemento regulado por esteróis) gene importante na adipogênese e lipogênese, e consequentemente pode levar a uma redução na síntese de lipídeos (Obsen et al., 2012).

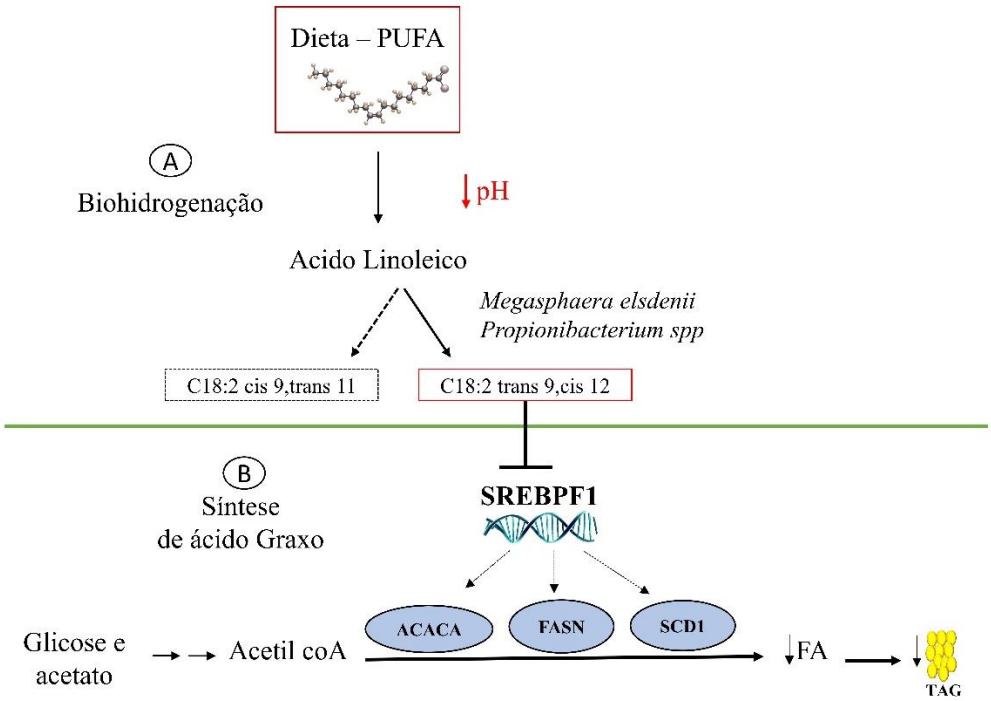

Figura 2. Efeito do pH ruminal na lipogênese no músculo de bovinos. Adaptado de Ladeira et al. (2018).

O fator de transcrição SREBP, possui três formas, os da família 1 (a,b), estão relacionado a genes envolvidos na lipogênese, enquanto que da família 2 regulam a expressão de genes colesterogênicos (Eberlé et al., 2004). Além disso a SREBP controla genes relacionados ao metabolismo lipídico (Clarke, 2001).

Além dos ácidos graxos polinsaturados (PUFA), os hormônios como exemplo a insulina, podem influenciar na regulação da expressão de SREBP.O aumento no nível de insulina pode aumentar a expressão de SREBP, que aumenta a atividade da enzima acetil CoA carboxilase (codificada pelo gene ACAC), e essa enzima está ligada diretamente a lipogênese. Enquanto que PUFA possuem efeito contrário, podem inibir o SREBP1c e consequentemente diminuir a ativação do gene acetil CoA carboxilase (ACAC) (Botolin et al., 2006).

Na síntese *de novo*, primeiramente há ação da acetil CoA carboxilase, enzima importante na deposição de lipídeos, que catalisa a carboxilação irreversível de acetil-CoA para produzir malonil-CoA, e posterior ação da enzima ácido graxo sintetase (FAS),

onde os carbonos são acrescentados de 2 em 2 até formação do ácido graxo saturados (Nelson e Cox, 2015).

As ações metabólicas do tecido adiposo podem ser divididas em lipogênese (síntese de gordura) e lipólise (mobilização de gordura). A síntese de gordura em ruminantes pode ocorrer através da síntese de triglicerídeos (ácidos graxos da dieta) ou através da síntese *de novo* (Rollin et al., 2003) (Figura 3). Os ácidos graxos da corrente sanguínea são derivados da quebra dos triglicerídeos pela enzima lipase lipoproteica (LPL), após a hidrólise, os ácidos graxos conseguem entrar no adipócito e é novamente re-esterificado em triglicerídeo, a enzima pode ser regulada através de nível hormonal e pela necessidade dos tecidos por ácidos graxos (Fielding e Frayn, 1998), dessa forma a LPL atua controlando a divisão de ácidos graxos no tecido adiposo e muscular.

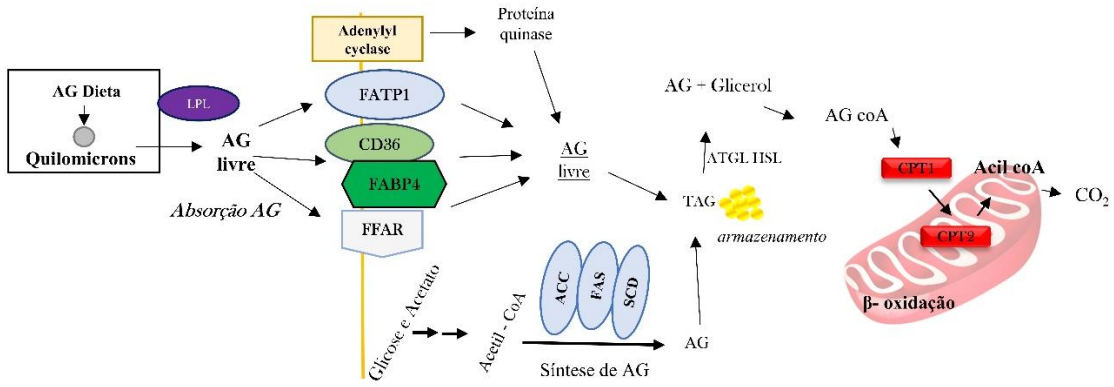

Figura 3. Síntese, absorção e oxidação de ácidos graxos no tecido adiposo de ruminantes. Adaptado de Ladeira et al. (2018).

Após a hidrólise dos triacilgliceróis, os ácidos graxos livres precisam ser transportados para dentro do adipócitos, a proteína de ligação ao ácido graxo (FABP) é responsável por facilitar a entrada do ácido graxo para célula (Jurie et al., 2007). A FABP4, identificada no tecido adiposo, atua na entrada ou saída dos ácidos graxos do tecido adiposo devido a resposta anabólica ou catabólica (Hertzel et al., 2006). Após a entrada do ácido graxo no adipócito, ele pode sofrer ação da enzima estearoil-CoA dessaturase (SCD1), que pode converter ácido graxo saturado em monoinsaturados (MUFA). A enzima SCD1 é a isoforma que pode ser encontrada principalmente no tecido

adiposo, ela permite a introdução de uma dupla ligação no ácido esteárico (C18:0) e palmítico (C17:0) convertendo-os em ácidos oleico (C18:1) e palmitoleico (C17:1), respectivamente (Oliveira, 2013).

Dessa forma, a mudança na expressão da SCD1 reflete no teor de MUFA no tecido adiposo (Matsuhashi et al., 2011). Suplementação com PUFA pode inibir a expressão da enzima que consequentemente reduz a síntese de MUFA. Além disso, a SCD1 também possui um sitio de ligação para SREBP, podendo também ser regulada através da expressão da SREBP (Bénédicte et al., 2006) e ocasionando em mudança na composição dos ácidos graxos.

Smith et al. (2006), também identificou a associação da SCD1 na conversão de vacênico (C:18:1 t 11) para CLA (C18:2 c9 t 11) e observou que o aumento do isômero c18:2 t10 c12 pode influenciar diretamente o perfil de ácidos graxos através da redução da SCD1. Dessa forma um aumento da formação e absorção desse isômero, pode consequentemente diminuir a adipogênese (Palmquist et al., 2005). A enzima SCD1 possui uma maior expressão no tecido subcutâneo do que o intramuscular (Archibeque et al., 2005, Lee et al., 2011), o que que também pode explicar maiores níveis de ácido graxo PUFA no tecido adiposo intramuscular.

A enzima CPT2 (carnitina palmitoil transferase 2), está presente dentro da mitocôndria e participa do processo que antecede a β-oxidação, a CPT1 transporta o ácido graxo ativado (acil CoA) para dentro da mitocôndria, onde se encontra a CPT2 que doa o grupo acila da acil carnitina para uma coenzima A da matriz mitocondrial, liberando a carnitina (Ladeira et al., 2016), catalisa reação entre acil carnitina e CoA para produzir acil-CoA, que é um substrato para a via da β-oxidação.

1.4. Qualidade da carne de bovinos

Qualidade da carne é um conceito amplo, que envolve diversos aspectos inter-relacionados, pode ser influenciada por fatores antes mesmo do nascimento animal até o momento do preparo do produto, dessa forma, os fatores que influenciam a qualidade da carne podem ser divididos como *antemortem* e *postmortem* (Bridi, 2004, Guerrero et al. 2013).

O pH, cor, textura, capacidade de retenção de água, quantidade de gordura são principais atributos de qualidade de carne considerados importante para a indústria da carne. Esses atributos podem ser influenciados por fatores *antemortem* como: idade animal, nutrição, sexo, sistema de produção e manejo, e fatores *postmortem* como: estimulação elétrica e maturação (Bridi, 2004).

O tecido muscular é constituído por um conjunto de feixes de fibras musculares, que por sua vez são formados por miofibrilas, que contém unidades contrácteis chamadas de sarcômeros. O sarcômero é delimitado por duas linhas Z, constituídos por filamentos alternados de proteína, finos e grossos, chamado de miosina e actina, respectivamente (Purslow, 2017). Com a morte do animal, o suprimento de oxigênio é interrompido para as células e devido a homeostase, o metabolismo celular continua em funcionamento até que as reservas de energia esgotem, usando como fonte o ATP e glicogênio (Guo e Greaser, 2017). Após o esgotamento de energia, ocorre a ligação irreversível de actina e miosina, onde há o encurtamento máximo do sarcômero, fase caracterizada como *rigor mortis* (England et al., 2017). Com o ambiente anaeróbico há a conversão de ácido pirúvico para ácido lático, que consequentemente reduz o pH (England et al., 2017).

Após a fase de *rigor mortis* há a fase de resolução do rigor, que é caracterizada pelo amaciamento da carne por ação de enzimas proteolíticas, duas isoformas importantes dessa enzima são a μ-calpaína e m-calpaína, que fazem a degradação da estrutura miofibrilar e influênciam diretamente no amaciamento da carne (Luchiari Filho, 2000). Por outro lado, a calpaína possui também um inibidor, a calpastatina, o aumento da quantidade de calpastatina consequentemente pode diminuir a maciez da carne (Bridi, 2004).

Queda adequada do pH faz com que haja atividade de enzimas relacionada a proteólise, por outro lado resistência na queda do pH devido redução das reservas de glicogênio, que pode ocorrer principalmente por estresse no momento antes do abate pode causar inibição dessa atividade e encurtamento excessivo das fibras, o que torna a carne mais dura e com menor capacidade de retenção de água (Ertbjerg e Puolanne, 2017). Animais mais reativos apresentam maior susceptibilidade ao estresse e

consequentemente menor reserva de glicogênio, o que limita a produção de ácido lático e aumenta a resistência à queda do pH (Apaoblaza et al., 2017).

Muitas vezes os animais que são criados em sistema extensivo podem apresentar alto pH final (Duckett et al., 2007) caracterizada como carne dura, aparência escura e seca, devido uma maior quantidade de água que fica retida no interior das células, fenômeno conhecido como DFD (dark, firm e dry), um dos principais problemas da indústria brasileira de carne (Purslow, 2017). Carne com essa característica pode ser facilmente rejeitado pelo consumidor, devido a coloração escura que o produto apresenta além de ser susceptível a proliferação de microrganismos indesejáveis.

A coloração da carne é um dos principais aspectos de qualidade no momento da compra, principalmente por ser o primeiro atributo a ser avaliado pelo consumidor (Mancini e Hunt, 2005). A diferença da coloração da carne é causada pela quantidade e o estado oxidativo da mioglobina, uma proteína presente no músculo responsável por sua oxigenação (Mancini e Hunt, 2005). A mudança na cor da carne (Figura 2) é causada principalmente pela oxidação, redução ou desoxigenação que ocorre na mioglobina, dessa forma essa proteína pode estar presente de três formas: oximioglobina, metamioglobina e desoximioglobina (Mancini e Hunt, 2005).

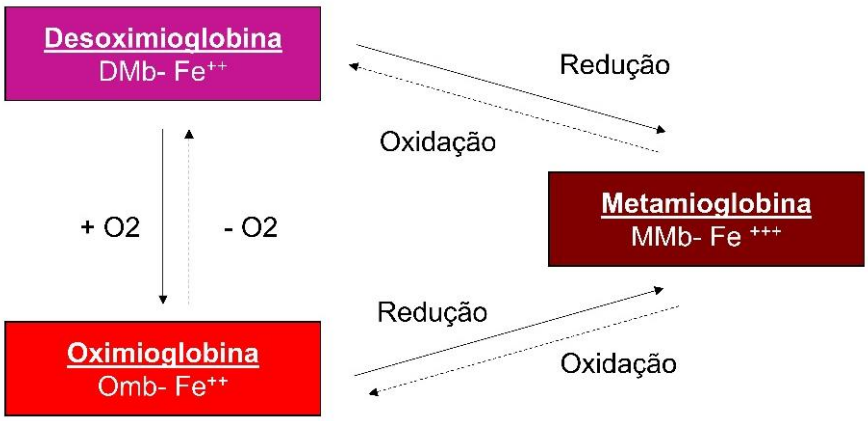

Figura 4. Diagrama da morfologia da mioglobina. Adaptado de Mancini e Hunt (2005).

Fatores como idade, raça, nutrição, sistema de produção e localização anatômica do musculo mostram ter relação com a concentração deste pigmento,

consequentemente possui relação com a coloração da carne (Mancini e Hunt, 2005). Animais de sistema extensivo possuem maior quantidade de mioglobina em comparação a animais de sistema intensivo, isso acontece devido maior atividade física e necessidade de oxigenação dos músculos (Vestergaard et al., 2000). Dessa forma animais terminados exclusivamente em sistema a pasto apresentam carne escura em comparação aos terminados em confinamento, uma vez que coloração escura indicam maiores concentrações de mioglobina (Purslow, 2017).

A maciez da carne é uma característica importante avaliada pelo consumidor, (Ornaghi et al., 2020) e está relacionada ao comprimento de sarcômero (Battaglia et al., 2019) que é influenciado no momento do resfriamento da carcaça pela temperatura e pelo grau de acabamento animal. O nível de ingestão de energia pode afetar a composição da carcaça, principalmente na proporção de gordura. Animais precoce atingem a puberdade mais cedo, a deposição de tecido muscular é reduzida e há o aumento de deposição de tecido adiposo, dessa forma, esses animais apresentam espessura de gordura exigida por alguns países para importação de carne (Prado, 2010). Nesse sentido, o acabamento de carcaça que garanta a deposição de espessura mínima de gordura (3 mm), funciona principalmente como proteção durante o resfriamento da carcaça, evitando escurecimento da carcaça, perdas de água excessiva, encurtamento do sarcômero (cold shortening) e consequentemente diminuição da maciez.

O sistema de terminação está diretamente relacionado com a idade de abate, animais com boa genética que são alimentados com dieta rica em concentrado (energia), possuem uma maior quantidade de gordura subcutânea e intramuscular comparado com animais alimentados com dieta a base de forragem (Rotta et al., 2009b). Dessa forma, o grau ideal de acabamento dos animais ajuda no resfriamento adequado das carcaças, evitando problemas como o escurecimento da carne e o encurtamento excessivo do sarcômero (Joo et al., 2017) enquanto que o marmoreio possui influencia na percepção de maciez e no sabor da carne, devido a composição de ácidos graxos (Nian et al., 2019).

Existe um complexo de sistema interações entre fatores que influenciam na qualidade do produto final, como raça, idade, sexo, nutrição, manejo, entre outros. Dessa forma é fundamental o conhecimento da biologia muscular e dos fatores fisiológicos que

podem afetar a qualidade da carne, para um melhor controle desses aspectos e garantir um produto que agrade o consumidor final.

1.5. Perfil de Ácidos graxos intramuscular

O marmoreio é um fator importante na qualidade da carne, está diretamente relacionado com a palatabilidade (Joo et al., 2017) mas nos últimos anos tem aumentado a preocupação dos consumidores em relação ao consumo de carne, devido a associação da ingestão de carne vermelha com problemas de saúde como doenças cardiovasculares (Islam et al., 2019). A carne bovina é um alimento de elevado valor nutricional, possui proteína de alto valor biológico, é uma fonte rica em minerais e vitaminas, possui um perfil completo de aminoácidos necessários para consumo humano, além disso, possui em sua composição ácidos graxos importantes, por ajudar na prevenção de câncer e doenças cardiovasculares (Wood, 2017).

A carne bovina possui cerca de 2 a 5% de lipídeos (Teixeira et al., 2017, Castagnino et al., 2018), que pode variar de acordo com a nutrição. Os ácidos graxos dos triglicerídeos são classificados de acordo com a presença ou ausência de dupla ligação entre carbonos. Os ácidos que não possuem dupla ligação são chamados de saturados, os que possuem a presença de uma dupla ligação são chamados de monoinsaturados (MUFA) e por fim os poliinsaturados (PUFA) são os que possuem duas ou mais duplas ligações (Willian, 2013).

A gordura intramuscular em sua composição possui cerca de 44 a 45% de ácidos graxos saturados, dos quais o ácido palmítico (C16:0) e o ácido esteárico (C18:0) são os principais constituintes. Já os MUFA, correspondem cerca de 45 a 50%, como exemplo o ácido oleico (C18:1n9), que é um ácido com contribuição importante para a característica de palatabilidade da carne (Frank et al., 2016), e pode reduzir riscos de doenças cardiovasculares pelo aumento do HDL (high-density lipoprotein) em humanos (Gilmore et al., 2011, Gilmore et al., 2013).

Ácidos graxos MUFA em conjunto com os PUFA estão relacionados com redução de níveis do LDL (low-density lipoprotein), através de modificação na composição das membranas celulares e das lipoproteínas, e aumento do HDL e consequentemente a

redução de doenças cardíacas (British Nutrition Foundations, 1994). Os PUFA na carne de ruminantes podem chegar a 5% (Raes et al., 2004), do qual o ácido linolênico (C18:2n-6), linoleico (C18:3n-3) são os principais predominantes desse grupo.

A família ω3, em especial o ácido graxo essencial linolênico, que por alongamento e dessaturação formam os eicosapentanoico (C20:5n-3) e docosahexaenóico (C22:6n-3) (Belda e Pourchet-Campos, 1991), favorecem a diminuição do colesterol LDL no sangue, têm propriedades anti-trombótica e anti-aterogénica, de forma geral possuem efeito protetor no aparecimento de perturbações do ritmo cardíaco e das doenças cardiovasculares (Curi, 2002).

Os ácidos graxos da família ω6 têm como função a proteção contra as doenças cardiovasculares, com uma diminuição do colesterol sanguíneo total. Exercem um importante papel fisiológico como potentes mediadores da inflamação e um efeito benéfico sobre o sistema imune (Pompéia et al., 1999). O ácido linoleico, da família ω6, por alongamento e dessaturação pode originar o diomo-γ-linolênico (C20:3n-6) e o araquidônico (C20:4n-6) (Wood, 2017).

O perfil de ácidos graxos da carne pode ser modificado através da nutrição animal (Calkins e Hodgen, 2007, Rotta et al., 2009, Patino et al., 2015). Os lipídeos das forragens ingeridas pelos ruminantes são constituídos principalmente por galactolípideos e outros glicolipídeos ricos em ácido linolênico. Pelo contrário, os lipídios provenientes de cereais e oleaginosas são constituídos principalmente por triacilgliceróis e ricos em ácido linoleico (Harfoot e Hazlewood, 1997).

Entretanto, os lipídeos ingeridos pelos ruminantes via dieta podem sofrer modificações no rúmen, resultantes de um processo denominado de biohidrogenação ruminal. Esse mecanismo, consiste na adição de hidrogênio por microrganismos do rumem nos ácidos linolênico e linoleico, para desfazer a dupla ligação existente como mecanismo de defesa, convertendo-os em isômeros de CLA (C18:2c9t11 e C18:2t10c12), por outro, a atividade completa leva a geração de ácido esteárico (Kozloski, 2017).

Os sistemas de terminação de bovinos exclusivamente em pastagens ou em confinamento convencional tem efeito direto sobre a modificação do perfil de ácidos graxos na carne (Patino et al., 2015). Animais alimentados em sistema de pastagem

possuem gordura intramuscular com maiores concentrações de CLA, ácido linolênico e isômeros da família ω3 em comparação ao perfil de ácidos graxos de animais terminados em confinamento, que apresentam maior proporção de SFA (Patino et al., 2015).

O perfil de ácidos graxos da carne estão diretamente relacionados a palatabilidade do consumidor (Nian et al., 2019). Embora maiores concentrações de ácidos graxos considerados benéficos a saúde humana (PUFA) sejam encontrados em animais terminados em sistema a pasto, eles podem ter efeito negativo na intensidade de flavour e suculência (Nian et al., 2019). Além disso, os PUFA são mais susceptíveis a oxidação lipídica (López-Bote, 2017) podendo afetar a vida de prateleira do produto. Entretanto, animais terminados a pasto também apresentam maiores concentrações de β caroteno e α tocoferol na carne, antioxidantes naturais encontrados em pastagem, que previne a formação de espécie reativa ao oxigênio e consequentemente aumenta a estabilidade oxidativa da carne (Fruet et al., 2019).

2. Referências

Alves Neto J, Vellini B and Gonçalves P (2018). In **Entendendo o conceito do Boi 777** eds. F Resende, G Siqueira and I Oliveira, 2018. p. 185 -193.

Anualpec 2019. **Anuário da pecuária Brasileira**. São Paulo, Brasil: ANUALPEC.

Apaoblaza A, Strobel P, Ramírez-Reveco A, Jeréz-Timaure N, Monti G and Gallo C (2017). Effect of season, supplementation and fasting on glycolytic potential and activity of AMP-activated protein kinase, glycogen phosphorylase and glycogen debranching enzyme in grass-fed steers as determined in Longissimus lumborum muscle. **Livestock Science** 202, 101-108.

Archibeque S, Lunt D, Gilbert C, Tume R and Smith S (2005). Fatty acid indices of stearoyl-CoA desaturase do not reflect actual stearoyl-CoA desaturase enzyme activities in adipose tissues of beef steers finished with corn-, flaxseed-, or sorghum-based diets. **Journal of Animal Science** 83, 1153-1166.

Battaglia C, Vilella GF, Bernardo AP, Gomes CL, Biase AG, Albertini TZ and Pflanzer SB (2019). Comparison of methods for measuring shear force and sarcomere length and their relationship with sensorial tenderness of longissimus muscle in beef. J**ournal of texture studies**.

Belda M and Pourchet-Campos M (1991). Ácidos graxos essenciais em nutrição: uma visão atualizada. **Ciência e Tecnologia de Alimentos** 11, 5-35.

Bénédicte R, Mullen A, Moloney F, Larondelle Y, Schneider Y-J and Roche HM (2006). Eicosapentaenoic acid and 3, 10 dithia stearic acid inhibit the desaturation of trans-vaccenic acid into cis-9, trans-11-conjugated linoleic acid through different pathways in Caco-2 and T84 cells. **British Journal of Nutrition** 95, 688-695.

Biserra TT, Cecato U, Galbeiro S, Bridi AM, Fernandes HJ, Duarte CFD, Da Silva DR, Vicente JVR and Rogel CP (2020). The effect of different grazing heights on carcass and meat quality characteristics of Nellore cattle fattened in pastures of Convert grass. **Tropical animal health and production** 52, 625-635.

Botolin D, Wang Y, Christian B and Jump DB (2006). Docosahexaneoic acid (22: 6, n-3) regulates rat hepatocyte SREBP-1 nuclear abundance by Erk-and 26S proteasome-dependent pathways. **Journal of Lipid Research** 47, 181-192.

Bridi AM (2004). Qualidade da carne para o mercado internacional. Londrina: Universidade Estadual de Londrina.

Brown JM, Boysen MS, Jensen SS, Morrison RF, Storkson J, Lea-Currie R, Pariza M, Mandrup S and McIntosh MK (2003). Isomer-specific regulation of metabolism and PPARγ signaling by CLA in human preadipocytes. **Journal of Lipid Research** 44, 1287-1300.

Byers FM (1982). Nutritional factors affecting growth of muscle and adipose tissue in ruminants. **In Federation proceedings**, pp. 2562-2566.

Calkins CR and Hodgen JM (2007). A fresh look at meat flavor. **Meat Science** 77, 63-80.

Carvalho VV and Smith SB (2018). Slip points of subcutaneous adipose tissue lipids do not predict beef marbling score or percent intramuscular lipid. **Meat Science** 139, 201-206.

Casagrande DR, Azenha MV, Vieira BR, Resende FDd, Faria MHd, Berchielli TT, Ruggieri AC and Reis RA (2013). Performance and carcass quality of feedlot-or pasture-finished Nellore heifers according to feeding managements in the postweaning phase. **Revista Brasileira de Zootecnia** 42, 899-908.

Castagnino PS, Fiorentini G, Dallantonia EE, San Vito E, Messana JD, Torrecilhas JA, Sobrinho AGS and Berchielli TT (2018). Fatty acid profile and carcass traits of feedlot Nellore cattle fed crude glycerin and virginiamycin. **Meat Science** 140, 51-58.

Clarke SD (2001). Polyunsaturated fatty acid regulation of gene transcription: a molecular mechanism to improve the metabolic syndrome. **The Journal of nutrition** 131, 1129-1132.

Curi R (2002). Entendendo a gordura: os ácidos graxos. In Entendendo a gordura: os ácidos graxos.

Oliveira DM (2013). Expressão de genes envolvidos no metabolismo lipídico no músculo de bovinos de corte alimentados com fontes de lipídeos.

Delevatti LM, Romanzini EP, Koscheck JFW, de Araujo TLdR, Renesto DM, Ferrari AC, Barbero RP, Mulliniks JT and Reis RA (2019). Forage management intensification and

supplementation strategy: Intake and metabolic parameters on beef cattle production. **Animal Feed Science and Technology** 247, 74-82.

Detmann E, Paulino MF, de Campos Valadares Filho S and Huhtanen P (2014). Aspectos nutricionais aplicados a bovinos em pastejo nos trópicos: uma revisão baseada em resultados obtidos no Brasil. Semina: **Ciências Agrárias** 35, 2829-2854.

Drouillard J, Ferrell C, Klopfenstein T and Britton R (1991). Compensatory growth following metabolizable protein or energy restrictions in beef steers. **Journal of animal science** 69, 811-818.

Du M, Huang Y, Das A, Yang Q, Duarte M, Dodson M and Zhu M-J (2013). Meat Science and Muscle Biology Symposium: manipulating mesenchymal progenitor cell differentiation to optimize performance and carcass value of beef cattle. **Journal of animal science** 91, 1419-1427.

Duckett S, Neel J, Sonon Jr R, Fontenot J, Clapham W and Scaglia G (2007). Effects of winter stocker growth rate and finishing system on: II. Ninth–tenth–eleventh-rib composition, muscle color, and palatability. **Journal of animal science** 85, 2691-2698.

Eberlé D, Hegarty B, Bossard P, Ferré P and Foufelle F (2004). SREBP transcription factors: master regulators of lipid homeostasis. **Biochimie** 86, 839-848.

England E, Matarneh S, Scheffler T and Gerrard D (2017). Perimortal muscle metabolism and its effects on meat quality. I**n New aspects of meat quality**, pp. 63-89, Elsevier.

Ertbjerg P and Puolanne E (2017). Muscle structure, sarcomere length and influences on meat quality: A review. **Meat Science** 132, 139-152.

Fielding BA and Frayn KN (1998). Lipoprotein lipase and the disposition of dietary fatty acids. **British Journal of Nutrition** 80, 495-502.

Fruet A, Nörnberg J, Calkins C and De Mello A (2019). Effects of different antioxidants on quality of beef patties from steers fed low-moisture distillers grains. **Meat Science** 154, 119-125.

Gilmore HS, Young FJ, Patterson DC, Wylie ARG, Law RA, Kilpatrick DJ, Elliott CT and Mayne CS (2011). An evaluation of the effect of altering nutrition and nutritional strategies in early lactation on reproductive performance and estrous behavior of high-yielding Holstein-Friesian dairy cows. **Journal of Dairy Science** 94, 3510-3526.

Gilmore LA, Crouse SF, Carbuhn A, Klooster J, Calles JAE, Meade T and Smith SB (2013). Exercise attenuates the increase in plasma monounsaturated fatty acids and high-density lipoprotein cholesterol but not high-density lipoprotein 2b cholesterol caused by high-oleic ground beef in women. **Nutrition Research** 33, 1003-1011.

Guerrero AM, Valero MV, Campo MM, & Sañudo C (2013) Some factors that affect ruminant meat quality: from the farm to the fork. Review. **Acta Scientiarum. Animal Sciences**, 35, 335-347.

Guo W and Greaser M (2017). Muscle Structure, Proteins, and Meat Quality. In New Aspects of Meat Quality, Elsevier, 13-31.

Han J, Hamilton JA, Kirkland JL, Corkey BE and Guo W (2003). Medium-chain oil reduces fat mass and down-regulates expression of adipogenic genes in rats. Obesity Research 11, 734-744.

Harfoot CG and Hazlewood GP (1997). Lipid metabolism in the rumen. In The rumen microbial ecosystem, pp. 382-426, Springer.

Hertzel AV, Smith LA, Berg AH, Cline GW, Shulman GI, Scherer PE and Bernlohr DA (2006). Lipid metabolism and adipokine levels in fatty acid-binding protein null and transgenic mice. **American Journal of Physiology-Endocrinology and Metabolism** 290, E814-E823.

Hiller B, Herdmann A and Nuernberg K 2011. Dietary n-3 fatty acids significantly suppress lipogenesis in bovine muscle and adipose tissue: a functional genomics approach. **Lipids** 46, 557-567.

Islam MA, Amin MN, Siddiqui SA, Hossain MP, Sultana F and Kabir MR (2019). Trans fatty acids and lipid profile: A serious risk factor to cardiovascular disease, cancer and diabetes. Diabetes & Metabolic Syndrome: Clinical Research & Reviews.

Joo S-T, Hwang Y-H and Frank D (2017). Characteristics of Hanwoo cattle and health implications of consuming highly marbled Hanwoo beef. **Meat Science** 132, 45-51.

Jurie C, Picard B, Hocquette JF, Dransfield E, Micol D and Listrat A (2007). Muscle and meat quality characteristics of Holstein and Salers cull cows. **Meat Science** 77, 459-466.

Kersten S (2014). Integrated physiology and systems biology of PPARα. **Molecular metabolism** 3, 354-371.

Kirkland JL, Tchkonia T, Pirtskhalava T, Han J and Karagiannides I (2002). Adipogenesis and aging: does aging make fat go MAD? **Experimental Gerontology** 37, 757-767.

Kozloski GV (2017). Bioquímica dos ruminantes. Fundação de Apoio a Tecnologia e Ciencia-Editora UFSM.

Ladeira MM, Schoonmaker JP, Gionbelli MP, Dias JC, Gionbelli TR, Carvalho JRR and Teixeira PD (2016). Nutrigenomics and beef quality: a review about lipogenesis. I**nternational Journal of Molecular Sciences** 17, 918.

Ladeira M, Schoonmaker J, Swanson K, Duckett S, Gionbelli M, Rodrigues L, & Teixeira P (2018). Nutrigenomics of marbling and fatty acid profile in ruminant meat. **Animal,** 12, 282-294

Lee JH, Yamamoto I, Jeong JS, Nade T, Arai T and Kimura N (2011). Relationship between adipose maturity and fatty acid composition in various adipose tissues of Japanese Black, Holstein and Crossbred (F1) steers. **Animal Science Journal** 82, 689-697.

Lomas L, Moyer J and Milliken G (2009). Effect of energy supplementation of stocker cattle grazing smooth bromegrass pastures on grazing and subsequent finishing performance and carcass traits. **The Professional Animal Scientist** 25, 65-73.

López-Bote C (2017). Chemical and biochemical constitution of muscle. In Lawrie´ s Meat Science, pp. 99-158, Elsevier.

Luchiari Filho A (2000). **Pecuária da carne bovina.** LinBife, São Paulo.

Mancini RA and Hunt MC (2005). Current research in meat color. **Meat Science** 71, 100-121.

Matsuhashi T, Maruyama S, Uemoto Y, Kobayashi N, Mannen H, Abe T, Sakaguchi S and Kobayashi E (2011). Effects of bovine fatty acid synthase, stearoyl-coenzyme A desaturase, sterol regulatory element-binding protein 1, and growth hormone gene polymorphisms on fatty acid composition and carcass traits in Japanese Black cattle. **Journal of Animal Science** 89, 12-22.

Miorin RL (2018). Estratégias de suplementação constante ou crescente na terminação de bovinos de corte em pastagens com adição de enzima fibrolítica.

Moreira FB, Mizubuti IY, do Prado IN, Matsushita M, Matsubara MT and Dognani R (2008). Protein and mineral supplementation for calves grazing a Mombaça pasture during the winter. **Semina: Ciências Agrárias** 29, 203-210.

Moretti MH, Resende FD, Siqueira GR, Roth APTP, Custódio L, Roth MTP, Campos WC, and Ferreira LH (2013). Performance of Nellore young bulls on Marandu grass pasture with protein supplementation. **Revista Brasileira de Zootecnia**, 42, 438-446.

Moretti MH (2015). Estratégias alimentares para a recria e terminação de tourinhos Nelore.

Nian Y, Allen P, Harrison SM, Brunton NP, Prendiville R and Kerry JP (2019). Fatty acid composition of young dairy bull beef as affected by breed type, production treatment, and relationship to sensory characteristics. **Animal Production Science** 59, 1360-1372.

Obsen T, Faergeman NJ, Chung S, Martinez K, Gobern S, Loreau O, Wabitsch M, Mandrup S and McIntosh M (2012). Trans-10, cis-12 conjugated linoleic acid decreases de novo lipid synthesis in human adipocytes. **The Journal of nutritional biochemistry** 23, 580-590.

Oliveira A, Casagrande D, Bertipaglia L, Barbero R, Berchielli T, Ruggieri A, & Reis R (2016). Supplementation for beef cattle on Marandu grass pastures with different herbage allowances. **Animal Production Science**, 56, 123-129.

Oliveira C, & Millen D (2014). Survey of the nutritional recommendations and management practices adopted by feedlot cattle nutritionists in Brazil. **Animal Feed Science and Technology**, 197, 64-75.

Oliveira RL, Barbosa MAA, Ladeira MM, Silva MMP, Ziviani AC, & Bagaldo AR (2006). Nutrição e manejo de bovinos de corte na fase de cria. **Revista Brasileira de Saúde e Produção Animal**, 7.

Ornaghi MG, Guerrero A, Vital ACP, de Souza KA, Passetti RAC, Mottin C, de Araújo Castilho R, Sañudo C and do Prado IN (2020). Improvements in the quality of meat from beef cattle fed natural additives. **Meat Science**, 108059.

Owens FN, Dubeski P and Hanson C (1993). Factors that alter the growth and development of ruminants. **Journal of animal science** 71, 3138-3150.

Owens FN, Gill DR, Secrist DS and Coleman S (1995). Review of some aspects of growth and development of feedlot cattle. **Journal of animal science** 73, 3152-3172.

Palmquist DL, Lock AL, Shingfield KJ and Bauman DE (2005). Biosynthesis of conjugated linoleic acid in ruminants and humans. **Advances in Food and Nutrition Research** 50, 179-217.

Patino H, Medeiros F, Pereira C, Swanson K and McManus C (2015). Productive performance, meat quality and fatty acid profile of steers finished in confinement or supplemented at pasture. **Animal** 9, 966-972.

Prado IN (2010). Produção de bovinos de corte e qualidade da carne. Eduem, Maringá, Paraná, Brasil.

Purslow PP (2017). The structure and growth of muscle. In Lawrie´ s Meat Science, pp. 49-97, Elsevier.

Queiroz JCFd, Alonso-Vale MIC, Curi R and Lima FB (2009). Control of adipogenesis by fatty acids. **Arquivos Brasileiros de Endocrinologia & Metabologia** 53, 582-594.

Raes K, De Smet S and Demeyer D (2004). Effect of dietary fatty acids on incorporation of long chain polyunsaturated fatty acids and conjugated linoleic acid in lamb, beef and pork meat: a review. **Animal Feed Science and Technology** 113, 199-221.

Reis RA, Barbero R, e Delevatti LM (2018). In **Entendendo o conceito do Boi 777** eds. F Resende, G Siqueira and I Oliveira, 2018. p. 135 -193.

Reis RA, Ruggieri AC, Casagrande DR, & Páscoa AG (2009). Suplementação da dieta de bovinos de corte como estratégia do manejo das pastagens. **Revista Brasileira de Zootecnia**, 38, 147-159.

Rollin X, Médale F, Gutieres S, Blanc D and Kaushik SJ (2003). Short-and long-term nutritional modulation of acetyl-CoA carboxylase activity in selected tissues of rainbow trout (Oncorhynchus mykiss). **British Journal of Nutrition** 89, 803-810.

Roth, MTP, Resende FD, Siqueira GR, Fernandes RM, Ferreira LH, Moreira JCA. (2010). Estratégias de suplementação na recria de bovinos de corte manejados em pastagem de capim Tanzânia durante o verão. In: REUNIAO ANUAL DA SOCIEDADE BRASILEIRA DE ZOOTECNIA. **Anais...**Salvador, BA, p. 438 - 446.

Roth M, Resende F, Oliveira I, Fernandes R, Custódio L and Siqueira G (2017). Does supplementation during previous phase influence performance during the growing and finishing phase in Nellore cattle? **Livestock Science** 204, 122-128.

Rotta PP, Prado RM, Prado IN, Valero MV, Visentainer JV and Silva RR (2009). The effects of genetic groups, nutrition, finishing systems and gender of Brazilian cattle on carcass characteristics and beef composition and appearance: a review. **Asian-Australasian Journal of Animal Sciences** 22, 1718-1734.

Sampaio RL, de Resende FD, Reis RA, de Oliveira IM, Custódio L, Fernandes RM, Pazdiora RD and Siqueira GR (2017). The nutritional interrelationship between the growing and finishing phases in crossbred cattle raised in a tropical system. **Tropical animal health and production** 49, 1015-1024.

Schoonmaker J, Cecava M, Faulkner DB, Fluharty F, Zerby H and Loerch S (2003). Effect of source of energy and rate of growth on performance, carcass characteristics, ruminal fermentation, and serum glucose and insulin of early-weaned steers. **Journal of animal science** 81, 843-855.

Silva LH, Paulino PV, Assis GJ, Assis DE, Estrada MM, Silva MC, Silva JC, Martins TS, Valadares Filho SC and Paulino MF(2017). Effect of post-weaning growth rate on carcass traits and meat quality of Nellore cattle. **Meat Science** 123, 192-197.

Smith SB, Lunt DK, Chung KY, Choi CB, Tume RK and Zembayashi M (2006). Adiposity, fatty acid composition, and delta-9 desaturase activity during growth in beef cattle. **Animal Science Journal** 77, 478-486.

Teixeira PD, Oliveira DM, Chizzotti ML, Chalfun-Junior A, Coelho TC, Gionbelli M, Paiva LV, Carvalho JRR and Ladeira MM (2017). Subspecies and diet affect the expression of genes involved in lipid metabolism and chemical composition of muscle in beef cattle. **Meat Science** 133, 110-118.

Therkildsen M, Stolzenbach S and Byrne DV (2011). Sensory profiling of textural properties of meat from dairy cows exposed to a compensatory finishing strategy. **Meat Science** 87, 73-80.

Vestergaard M, Oksbjerg N and Henckel P (2000). Influence of feeding intensity, grazing and finishing feeding on muscle fibre characteristics and meat colour of semitendinosus, longissimus dorsi and supraspinatus muscles of young bulls. **Meat Science** 54, 177-185.

Willian K (2013). Lipids and Lipid Oxidation. The Science of Meat Quality, 147 p.

Wood JD (2017). Meat composition and nutritional value. In Lawrie´ s **Meat Science**, p. 635-659, Elsevier.

Yan X, Zhu M-J, Dodson MV and Du M (2013). Developmental programming of fetal skeletal muscle and adipose tissue development. **Journal of genomics** v.1, 29 p.

CHAPTER 2 - EFFECTS OF THE SUPPLEMENTATION STRATEGY DURING THE GROWING PHASE ON MEAT QUALITY FROM BULLS FINISHED IN DIFFERENT SYSTEM

Abstract

One hundred and twenty young bulls uncastrated males were randomly assigned to treatments in 2 × 2 factorial design, two supplements during the growing phase: mineral (*ad libitum* - MIN) or protein + energy [0.3% body weight (BW)/day; PRE] and two finishing systems: 1) pasture plus concentrate supplementation [2% BW/day (PAST)], and 2) feedlot [corn silage plus concentrate; 25:75% (FLOT)]. The nutritional strategy during the growing phase did not affect ($P > 0.050$) the pH, shear force, sarcomere length, myofibrillar index, and water holding capacity. The bulls fed with MIN during the growing phase and finished in PAST showed lower ($P = 0.011$) lipid oxidation. The FLOT system increased ($P < 0.001$) backfat thickness and improved meat tenderness. Bulls from PAST had increased ($P < 0.050$) yellowness fat, higher myoglobin, less redness meat. Although the meat of bulls finished in PAST showed lower L* values, they had higher stability colour over the display. Globally, supplement strategy during the growing phase did not affect the tenderness attributes, but it modified the colour and lipid stability.

Keywords: Growth rate, *Longissimus* muscle, Meat colour, Tenderness traits

1. Introduction

Beef cattle production in Brazil is characterized mainly by grazing, usually in extensive systems. It has also been linked to decreased backfat thickness (Tatum, Smith, & Carpenter, 1982), which effectively protect the carcass *postmortem* and slows its rate of cooling thus preventing cold-shortening, increasing the tough and decreasing the lightness of meat (Koohmaraie, Seideman, & Crouse, 1988). In this respect, the additional feed supplementation during the growing phase, where the animals have better biological efficiency concerning tissue deposition due to the steeper growth curve (Owens, Dubeski, & Hanson, 1993) can result in a different rate of deposition of animal tissue, which reach the finishing phase with higher body weight and prepared for fat deposition (Roth et al., 2017; Silva et al., 2017).

The use of high concentrate diets at the finishing phase can also shorten the production cycle and improve fat deposition with desired benefits by the meat industry and consumer market (Patino, Medeiros, Pereira, Swanson, & McManus, 2015). Finishing beef cattle in the conventional feedlot is a practice that has recently increased in Brazil over the last few years (Anualpec, 2019), it allows the use of diets rich with concentrate that results in higher animal performance and adequate fat deposition in the carcass. Another alternative for finishing phase is the use of pasture with supplementation (Expresso®), just as in conventional feedlots, it consists of providing high energy levels and high amounts of concentrate supplementation (2% of body weight), where pasture is the source of fibrous substrate to maintain the rumen environment, which may be an alternative since grazing termination represents lower infrastructure costs and operational costs compared to the feedlot.

We hypothesize that protein + energy supplementation during the growing phase would show an increase of backfat thickness and affect the meat quality at the finishing phase compare to animals receiving only mineral supplementation. In addition, the animals finished on intensive system; pasture plus concentrate (2% BW/animal/ day) or feedlot (25:75; corn silage: concentrate), can produce similar meat quality. The objective of this study was to evaluate the effects of the supplement strategies at the growing phase on meat quality of beef cattle finished in pasture plus concentrate or conventional feedlot.

2. Materials and Methods

This experiment was approved by the Ethics and Animal Welfare Committee of the São Paulo University (protocol 5628/15). The research was carried out at the beef cattle facility of São Paulo State University located in Jaboticabal city, São Paulo, Brazil (48°18′58″W, 21°15′22″S).

2.1. Animals source

The experiment period comprised the growing phase (first) and finishing phase (second). The study was conducted during the months of December to September (285 days). One-hundred twenty uncastrated males from three genetic groups: 40 Nellore from the farm in Sao Paulo state, with average 10 ± 2 months old and 264.8 kg body weight (BW), 40 ½ Angus × ½ Nellore from the farm in Sao Paulo state, with average 11 ± 2 months old and 278.0 kg BW, and 40 ½Senepol × ½ Nellore from the farm Goias State, with average 9 ± 2 months old and 226.7 kg BW. Before the experiment, all the bulls were fed with grass and were not creep fed. After weaning all animals remained in the grazing area consisted of Brachiaria grass until the experiment starts.

2.2. Growing phase (first experimental phase)

The experiment was conducted as a randomized block design with two supplements during the growing phase (n = 60): 1) mineral (*ad libitum*; MIN), and 2) protein + energy [0.3% body weight (BW)/day; PRE]. The experimental unit was the animal (60 bulls/treatment). The growing phase was conducted during the summer season in Brazil (December 3rd to May 6th; 155 days). At the beginning of the experiment, the bulls, within each breed group were blocked by BW to one of two treatments supplied during the growing phase. The definition of supplements was based on tropical conditions (Detmann, Paulino, de Campos Valadares Filho, & Huhtanen, 2014), and the composition of the diets is reported in Table 1. The amount of supplement provided was calculated to meet the requirements for an average daily gain of 0.6 kg/d, according to Valadares Filho, Marcondes, Chizzotti, and Paulino (2016). During the growing phase, the bulls were supply once a day (10:00 h). The

grazing area consisted Brachiaria grass (*Urochloa brizantha cv.* "Xaraés") divided into 12 paddocks (approximately 1.8 ha each), 10 bulls/paddock (within each breed group had two supplementation treatments with two replicates each, the experimental design is shown in Figure 1. Each padlock had semicircular drinkers and covered feed troughs (3.0m × 0.5m) with both side access for supplementation. Every 28 d the bulls were weighed, and this BW was used to adjust the amount of supplement. Mineral feed was available to the bulls *ad libitum* and the protein + energy supplement amount was calculated based on BW at the beginning of each experimental period and placed every single day at 10:00 h during the experimental period.

2.3. Finishing phase (second phase experimental)

The finishing phase was conducted during the winter, a dry season (May 7th to September 13th; 120 days). The bulls had a period of adaptation of 20 days based on the "step-up", before the second experimental phase. Following the growing phase (1^{o} experimental phase), the bulls within each supplementation, and each breed were randomly assigned to one of two finishing systems: 1) pasture plus concentrate supplementation (2 % BW/ day; PAST), and 2) feedlot system where bulls received corn silage + concentrate (25:75; FLOT). The experiment was conducted by a 2 x 2 factorial, and design is shown in Figure 1. The finishing diet composition is reported in Table 1. The amount of supplement provided was calculated to meet the requirements for average daily gain 1.5 kg/d according to Valadares Filho et al. (2016). All bulls designated for PAST system were kept in the same paddock that was used during the growing phase. Every 28 d the bulls were weighed, and the BW was used to adjust the amount of concentrate (2% BW/day) for PAST treatment. The bulls from PAST system were fed with concentrate once a day (10:00 h) during the experimental period. The bulls designated for FLOT system remained in individual pens with 12 m^2, partially covered, concrete floors, with feed though and free water access. The FLOT basal diet was 25% of roughage (corn silage) and 75% of concentrate (corn, soybean meal, and premix) on dry matter basis. The bulls were fed twice a day (08:00 and 15:00 h), and the diet amount provided to the bulls was adjusted weekly for refusals of 5%.

2.4. Slaughter procedure and muscle sampling

After 285 days, the bulls with an average of 510.9 kg BW to Nellore, 532.7 kg BW to ½Angus, 466.2 kg BW to ½Senepol were transported to a commercial slaughterhouse (Minerva Foods, Barretos, São Paulo, Brazil) situated 90 km from the experimental area. After a total of 24 hours of feed deprivation (a combination of transport time plus rest period), all bulls were slaughtered following the usual practices of the Brazilian beef industry, according to the Brazilian RIISPOA – Regulation of Industrial and Sanitary Inspection of Animal Products. Afterward, the carcasses were divided medially from the sternum and the spine, resulting in two similar halves. Subsequently, the half-carcasses were washed, identified, and stored in a chilling chamber at 4ºC for 24 h.

After 24 h the pH was subsequently measured between the 12th and 13th ribs of the *Longissimus thoracis* (LT) muscle, using a Mettler 1140 portable digital pH meter (Mettler, Toledo International Inc., Columbus, EUA). After the operation, Four 2.54 cm thick steaks were obtained from each Longissimus muscle sample from the left side of the carcass between the 9th and the 13th ribs, one for the instrumental colour, shear force and sarcomere length measurement, the second for estimations of shelf-life determinations at 1, 4, 7, 10 and 14 days, the third for analysis of myofibrillar fragmentation index and malonaldehyde concentration, and the fourth for myoglobin determination. All samples were stored at -20°C (until analysis) and transported to the Animal Science Laboratory at the São Paulo State University (Jaboticabal).

2.5. Warner – Bratzler shear force (WBSF)

The Warner – Bratzler shear force (WBSF) analyses were determined according to procedures of (AMSA, 1995). The steak sample (2.54 cm) was cooked a pre-heated (180°C) grill (George Foreman GR 30) with its internal temperature monitored by thermocouples. After reaching the internal temperature of 71ºC, the sample was removed from the oven and cooled in a refrigerator for 24 hours at a temperature of 2 to 5ºC. From each steak, six homogeneous cylinders, 1.27 cm in diameter, parallel to the orientation of the muscle fibers, avoiding connective tissue and fat, were using a stainless-steel sampler. The cylindrical samples were sheared perpendicularly to the orientation of the muscular fibers, using shear

force equipment (model CT3 25K – texture analyzer- Brookfield) with Warner-Bratzler blade.

2.6. Myofibril fragmentation index

Myofibril fragmentation indices (MFI) were determined according to Culler, Smith, and Cross (1978). Four grams of minced muscle was homogenized for 30 s in 10 vol (v/w) of a 2°C isolating medium consisting of 100 mM KCl, 20 mM K phosphate, 1 mM EDTA, 1 mM MgCl, and 1 mM sodium azide. The homogenate was centrifuged at 1000×g for 15 min and then the supernatant was decanted. The sediment was then resuspended in 10 vol (v/w) of isolating medium using a stir rod, centrifuged again at 1000×g for 15 min and the supernatant was decanted. The sediment was resuspended in 2.5 vol (v/w) of isolating medium and passed through a polyethylene strainer (18 mesh) to remove connective tissue and debris. An additional 2.5 vol (v/w) was used to facilitate the passage of myofibrils through the strainer. The protein concentration of the myofibril suspension was determined by the biuret method as described by (Gornall, Bardawill, & David, 1949). An aliquot of the myofibril suspension was diluted with an isolating medium to reach a protein concentration of 0.5 ± 0.05 mg/ml. Protein concentration was determined by the biuret method. The diluted myofibril suspension was stirred and poured into a cuvette; absorbance of this suspension was measured immediately at 540 nm. Absorbance was multiplied by 200 to give an MFI for each sample.

2.7. Sarcomere length

The sarcomere length was measured as described by Cross, West, and Dutson (1981). The sample (the remaining 1-day aging time sample from Warner-Bratzler shear force) was incubated in 0.1 M NaHPO4 + 0.2 M Sucrose buffer overnight at 4ºC. After that, the fibers were removed and subjected to Laser diffusion (Thorlabs; model HNL020R, USA). For the calculation the sarcomere length has used the equation below:

$$\mu = \frac{0.6328 \times \mathrm{D} \times \sqrt{\left(\frac{T^2}{D}\right) + 1}}{\mathrm{T}}$$

where:

D = Distance (mm) of the sample to the sheet where the image will be drawn (preferably 100 mm); T = Space (mm) between the diffraction bands; laser wavelength (632.8×10^{-3}).

2.8. Cooking loss, thawing loss, and water holding capacity

To determine thawing losses, the steaks were thawed at 4°C for 24 h. They were weighed, and the thawing losses were calculated as the percentage difference between the fresh and thawed weights (after a period of 24 h at 4°C defrost). To determine cooking losses, the steaks were weighed and wrapped in aluminum foil. Each sample was cooked in a pre-heated grill (George Foreman GR 30) at 200°C until an internal temperature of 72°C was reached, which was monitored using an internal thermocouple (Incoterm, 145 mm, Incoterm LTDA, Brazil). The sample was then removed from the heat and left at ambient temperature to cool. Once the steaks reached 25°C, each steak was weighed, and the cooking losses were calculated as the percentage of difference in weight before and after cooking. The water holding capacity (WHC) was calculated by the difference of weight of a meat sample (approximately 2 grams) before and after subjected to the pressure of 10 kg for five minutes.

2.9. Instrumental fat colour

Five measurements of colour were measured in each fat sample (between 12th and 13th rib of LT) using a portable spectrophotometer MiniScan EZ (model 4500L; Hunter Associates Laboratory, Inc., Reston, Virginia, USA), using D65 illuminant and 10º standard observer, which was calibrated before use. The colour was evaluated for lightness (L*), redness (a*), yellowness (b*), and C* (Chroma) based on the CIE (Commission Internationale de I'Eclairage). The hue angle (h*) (Cañeque et al., 2004; Pflanzer & de Felício, 2011) was calculated as:

$$h^* = tan^{-1}\frac{b^*}{a^*}$$

2.10. Malonaldehyde concentration

The method used to measure lipid oxidation was Thiobarbituric Acid Reactive Substances (Tbars) described by Pikul, Leszczynski, and Kummerow (1989). Five g of meat sample was weighed and 20 mL of trichloroacetic acid (7.5%) added and homogenized for 2 minutes. Samples were filtered and 5 ml of the filtrate was added in test tubes with 5 mL of TBA (0.02M thiobarbituric acid) solution. The tubes were placed in a water bath at 98°C for 40 min. The malonaldehyde concentration was determined by a spectrophotometer with an absorbance of 540 nm.

2.11. Myoglobin

A 1.27 cm thick of LT muscle steak was used to assess myoglobin concentration, which was cut into free cubes of connective tissue and external fat, sprayed on liquid nitrogen. Myoglobin was extracted and quantified following the methods described by Warris (1979) and modified by Hunt, Sørheim, and Slinde (1999). Samples in duplicate of 2.5 g were homogenized in 10 mL of 40 mM potassium phosphate buffer (pH = 6.8). Homogenates were kept on ice for 1 h to allow complete extraction of the pigment before centrifugation (15,000 × g) for 30 min at 4°C. The supernatant (1.5 mL) were filtered in a 4 mL cuvette with 1 mL of 40 mM phosphate buffer and 0.5 mL of sodium hydroxide (10 mg/mL). The absorbance spectrum (400 to 700 nm) were evaluated in each sample using a spectrophotometer (Multiskan^{TM} FC microplate photometer - Thermofisher). It was checked whether the samples were in the reduced state (absorbance peaks between 2 nm at 433 nm), the concentration of the extracted pigment was calculated using the absorbance at 433 nm, the molar coefficient of 114,000 M-1cm-1, the weight molecular weight of myoglobin (16.800) and an appropriate dilution factor.

2.12. Instrumental meat colour

The beef samples were thawed 24 hours in refrigerated before the analysis. The meat samples were packed in polystyrene trays over-wrapped with a retractile film without touching the muscle and displayed in a refrigerated expositor (Klima Expositor Practice,

model 05B0500.1, Venâncio Aires–Rio Grande do Sul, Brazil) at 4 ± 1ºC and fluorescent light (1,200 lx, 12 h), simulating typical Brazilian markets real conditions. The colour was measured at 1, 4, 7, 10, and 14 days. Five measurements were made of colour for each steak using a portable spectrophotometer MiniScan EZ (model 4500L; Hunter Associates Laboratory, Inc., Reston, Virginia, USA), using D65 illuminant and 10º standard observer, which was calibrated before use. The colour was evaluated for lightness (L*), redness (a*), and yellowness (b*) based on the CIE (Commission Internationale de I'Eclairage), and chroma (C*). The hue angle (h*) (Cañeque et al., 2004; Pflanzer & de Felício, 2011) and ΔE values were calculated as:

$$h^* = tan^{-1}\frac{b^*}{a^*}$$

$$\Delta E = [(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2]^{0.5}$$

where ΔL*, Δa*, and Δb* are the derivatives of corresponding parameters.

2.13. Statistical analysis

The experimental design was blocked completely randomized in a 2 × 2 factorial arrangement, two supplementations during the growing phase (MIN or PRE supplementation), and two finishing systems (PAST or FLOT). All data were analysed using PROC MIXED from SAS 9.4 statistical software (SAS Inst. Inc., Cary, North Carolina) using the animal as experimental unit. The treatments and the block (light and heavy) as the fixed effect and the breed as random effects. The mean and standard error of mean were calculated for each variable. When significant main or interaction effects were detected, Tukey's test ($P \leq 0.05$) was used to determine the differences between means. The correlation analysis was performed using the Rstudio software, all data were analysed with Corrplot statistic package with a significant level of 5%.

3. Results

During the growing phase, the animal intake of supplements was 0.100 and 0.910 kg/ day for MIN and PRE, respectively. The average carcass gain and fat thickness (data not are shown) at the end of the growing phase were 0.247 and 0.377 kg/ day and 0.81 and 1.04 mm

for MIN and PRE, respectively, which provides heavier carcasses at the end the growing phase for animals that fed PRE compared to MIN, 181.47 vs 165.40 kg, respectively. In addition, the average intake of concentrate at finishing phase was 1.64, 1.53, 1.60 and 1.56% BW for treatments MIN –FLOT, PRE – FLOT, MIN – PAST, and PRE – PAST, respectively, which provides carcass gain of 1.170, 1.039, 0,957 and 0,897 kg/ day, respectively. The animals fed with MIN during the growing phase showed greater carcass gain at the finishing phase but which was not enough for the animals to have equivalent carcass weight at the slaughter compared to PRE, regardless of the finishing system (284.60 vs 291.01 kg). On the other hand, the animals from FLOT system had greater carcass weight than PAST (296.90 vs 278.71 kg).

3.1. pH, fat thickness, WBSF, sarcomere length, MFI, WHC, thawing loss, and cooking loss

There was no interaction ($P > 0.050$) between the growing phase and finishing system for pH, fat thickness, WBSF, sarcomere length, and MFI, WHC, thawing loss, and cooking loss (Table 2). The bulls from PAST had lower ($P < 0.001$) fat thickness compared to FLOT (3.07 *vs.* 7.01 mm, respectively). In addition, the animals finished in FLOT showed a 16% decreased on WBSF ($P = 0.006$) and 12.23% increased on sarcomere length values ($P < 0.001$) compared to PAST (36.29 *vs.* 43.30 N and 1.59 *vs*. 1.42 µm, respectively). The shear force had a negative correlation ($P < 0.001$, $r = - 0.32$) with sarcomere length, and a positive correlation ($P = 0.002$, $r = 0.29$) was observed between sarcomere length and backfat thickness (Figure 4). The meat from animals finished in FLOT had lower ($P < 0.001$) values for WHC and higher values for thawing loss ($P = 0.010$) compared to PAST system (64.32 *vs.* 68.16%, and 10.03 *vs.* 7.84%, respectively).

3.2. Instrumental fat colour

The fat colour parameters L*, a*, b*, C* and h* (Table 3), there was no interaction ($P > 0.050$) between growing phase and finishing phase. On the other hand, the fat of animals finished in PAST system showed increased for L* ($P < 0.001$), a* ($P = 0.003$), b* ($P < 0.001$), and C* ($P = 0.011$) compared to FLOT system, (70.23 *vs.* 67.23, 5.50 *vs.* 4.49, 16.66 *vs.*

14.30 and 16.00 *vs.* 15.07, respectively). A negative correlation was detected between back fat thickness and parameters fat colour L* (r = - 0.25), a* (r = - 0.31), b* (r = - 0.43), and C*(r = - 0.27), are presented in Figure 4.

3.3. Lipid oxidation, myoglobin, and display

There was no interaction (P > 0.050) between the growing phase and the finishing phase for myoglobin (Table 4). The meat of young bulls from PAST had higher value for myoglobin compared to FLOT (4.56 vs 3.95 mg/g of meat, respectively). In addition, the meat of animals fed with PRE during the growing phase showed higher (P = 0.002) myoglobin values at the end of the finishing phase compared to MIN.

An interaction (P = 0.011) between the growing system and the finishing system was observed for lipid oxidation (Table 4). The meat from bulls fed MIN during the growing phase and finished in PAST system had lower lipid oxidation value compared to meat of bulls fed with MIN and finished in FLOT system (0.272 vs. 0.375 mg malonaldehyde/ kg of meat, respectively), while the bulls fed with PRE following by FLOT, and PRE following by PAST was similar to the others.

For the storage days of meat sample evaluated during the 1, 4, 7, 10 and 14 storage days, no effects of a three-way interaction (P > 0.050) among growing phase, finishing system and days were observed in any colour parameters (L*, a*, b*, C* and h*; Table 5). The bulls fed finished in FLOT, regardless of the treatment during the growing phase showed (P = 0.002) higher L* value compared to bulls fed with MIN or PRE during the growing phase and finished in PAST system (Figure 2). The meat from bulls finished in FLOT showed (P < 0.001) higher L* values on day 10 of storage compared to PAST system (37.41 *vs*. 34.47, respectively; Figure 2). The L* parameter had a negative correlation (P < 0.001; Figure 4) with myoglobin (r = - 0.51) and positive correlation (P < 0.001) with backfat thickness (r = 033), a* (r = 0.37), b* (r = 0.93), C* (r = 0.76) and h* (r = 0.90).

For a* parameter, was observed (P = 0.007) interaction between growing phase and finishing phase, the animals fed with PRE and finished in FLOT showed the highest value of a*, while the meat of bulls fed with PRE following by PAST system showed the lowest value (15.19 vs. 14.11, respectively; Figure 2). However, the interaction was observed to finishing system and days, the steak from bulls finished in FLOT system were significantly more

redness than beef from bulls finished in PAST on day 4 and 7 (17.09 *vs.* 15.90 and 16.38 *vs.* 15.17, respectively; Figure 2)

The finishing system affected ($P < 0.001$) the b* parameters of meat, the animals finished in FLOT showed greater b* value compared to the meat of animals finished in PAST (13.39 *vs.* 12.17, respectively; Figure 2). In addition, the days affect ($P < 0.001$) the b* parameters, yellowness increased from day 1 to 4 of the display, but after there was a decrease to day 14.

An interaction ($P = 0.030$) between the finishing system and days was observed for the C* parameter. The meat of animals finished in FLOT or PAST was similar at 1 and 14 days, on the other hand, the animals finished in FLOT had higher value for C* on 4, 7, and 10 days of storage compared to PAST (22.26 *vs.* 20.54, 21.76 *vs.* 19.89 and 20.59 *vs.* 18.87, respectively; Figure 3). The lowest ($P < 0.001$; Figure 3) h* value was observed to meat of bulls fed with MIN following by PAST system compared to the other treatments, and the h* value increased ($P < 0.001$; Figure 3) with the storage days.

4. Discussion

4.1. pH, WBFS, sarcomere length, MFI, WHC, thawing loss, cooking loss

In the current study, there were no effect of the growing phase affected the meat quality traits (pH, WBSF, sarcomere length, MFI, WHC, thawing loss, or cooking loss). Even the meat pH has not been affected by the growing phase or finishing phase, the means values of pH showed were higher than 5.8, which are considered high for the meat industry (Gallo & Huertas, 2016). This result can be attributed to the use of uncastrated animals in this study, which is characterized by shown a behavior more quickly, resulting in increased stress susceptibility (Gómez et al., 2019). In addition, another factor that can have contributed to a high pH value was the long fasting period before slaughter in this study, since 24 hours fasting periods decrease muscle glycogen reserves at the time of slaughter (Apaoblaza et al., 2017), which can prevent the reduction of pH of meat during *postmortem*.

In addition, animals with a faster growth rate during the finishing phase, due to previously restrict growing phase, may exhibit more *postmortem* myofibril proteolysis and therefore, affect the meat tenderness (Therkildsen, Stolzenbach, & Byrne, 2011). In our study, the

growing phase did not affect the traits relationship with meat tenderness. Similar results were found by Silva et al. (2017), which regardless the growth rate at the growing phase of young bulls, observed no effect on meat tenderness, and attributes this lack of effect to time of the re-alimentation, once that the effect protein turnover by higher growth rate is time-dependent, which becomes equivalent for re-alimentation periods longer than 11 weeks (Therkildsen, 2005).

Our results showed that the FLOT system increased backfat thickness in 128.60 fold compared to PAST (7.01 vs 3.07 mm, respectively). These results can be explained by the energy expenditure by bulls from PAST system, even receiving concentrate supplementation spend more time with activity related to feeding time (Segabinazzi et al., 2014). In addition to the concentrate feed, these bulls need greater displacement, and collection and selection of pasture (source of roughage), which promotes greater energy expenditure than feedlot.

This increase of backfat thickness possibly prevented sarcomere excessive shortening at the chilling of the carcass during the *rigor mortis* (Koohmaraie et al., 1988)where the values observed of sarcomere length were 1.59 vs 1.42 µm to FLOT vs PAST, respectively. The length of sarcomeres after *rigor mortis* plays an important role in steak tenderness (Battaglia et al., 2019) as corroborated by the negative correlation found between sarcomere length and shear force. In this context, animals from FLOT system showed 16 % reduction in shear force values compared to PAST system (36.29 vs 43.20 N, respectively). Tenderness is one of the most important characteristics relating to consumer satisfaction and decreased significantly with increasing animal age due to a decrease in collagen (Moholisa, Hugo, Strydom, & van Heerden, 2017). According to Shackelford, Morgan, Cross, and Savell (1991), all treatments could be rated as “tender” meat (values < 45.11), which might be explained by the early age of the animals in this experiment (2 permanent incisors teeth). In addition, the consumer can detect a noticeable difference between steaks of about 9.81 N (Huffman et al., 1996). Therefore, in this study, the meat of bulls from FLOT or PAST systems probability could have no difference in the tenderness sensorial perception.

The meat of bulls finished in FLOT system that showed higher thawing water loss, on the other hand, lower WHC, which could be associated with the oxidative process in meat. The increase of protein oxidation destabilizes the protein matrix leading to loss of water-binding capacity due to the shrinkage of the inter-filament spaces, thus increase the extracellular

space and decrease the capillary force that holds the water in the inter-filament space, and the meat loses water as exudate (Liu et al., 2010), therefore the meat from bulls finished on grass base are less susceptible to oxidation processes, which led the meat of bulls from PAST system lower exudate loss and greater water holding capacity of the meat.

4.2. Instrumental Fat colour

The growing phase did not affect the fat colour at the end of the finishing phase, on the other hand, the animals finished in PAST system, showed higher L*, a*, b* and C* value compared to FLOT. The backfat thickness was negatively correlated with L*, a*, b*, and C* in this study, suggesting that the increase of fat depth led to a decrease of values fat colour parameter. Yellowness (b*) is an important factor colour parameters of adipose tissue (Dunne, Keane, O'Mara, Monahan, & Moloney, 2004), mainly because a yellow fat can be rejected by the consumer, thus some markets, as Europen and American consumers consider this fat colour less acceptable than white fat (Walker, Warner, & Winfield, 1990). In this context, the difference of b* value possibly occurs due to the presence of pigments such as carotenes found in the grass (Duckett et al., 2007). That way, even though the animals were finished in PAST, the grass intake may have been sufficient to change the concentration of carotenoids in the meat, causing the change in the b* parameters in the meat (Priolo, Micol, & Agabriel, 2001). Besides that, as carotenoids are accumulated in adipocytes, the increase of intramuscular fat may dilute the carotenoids and consequently reduce the yellowness of fat from FLOT system (Moran et al., 2017).

4.3. Lipid oxidation, myoglobin, and instrumental colour

The oxidative stability of meat is the result of the balance between the antioxidant compounds and polyunsaturated fatty acid concentration in meat (Campo et al., 2006). The lower lipid oxidation found in the meat of bulls fed with MIN following by PAST can be attributed to the antioxidants such as β-carotene and α-tocopherol that are found in grass based diet, which could prevent lipid oxidation in meat (Fruet et al., 2018; Humada et al., 2014). All treatments showed MDA values bellow the critical values of 2.0, which considered the limiting threshold for the acceptability of oxidation beef (Campo et al., 2006).

Grazing animals show "dark meat" and difference for meat colour parameters when compared to the intensive system (Bruce, Stark, & Beilken, 2004; Realini, Duckett, Brito, Dalla Rizza, & De Mattos, 2004; Vestergaard et al., 2000). Higher lightness (L*) value observed during the storage period for the FLOT system, regardless of the nutritional strategy (MIN or PRE), suggests a more attractive lightness to the consumer compared to PAST. On the other hand, the lowest lightness value found in meat from MIN following by PAST suggested “darker” meat, possibly have a negative relationship purchasing decisions of consumers (Hedrick 1983). In addition, this group showed mean less than 3 mm, a threshold value that effectively protects the carcass during cooling at the *postmortem* period (Rotta et al., 2009).

Myoglobin was negatively correlated with L* parameter, showing that the increase of myoglobin in the meat of animals from PAST can have resulted in less lightness meat. Although the animals fed with PRE during the growing phase showed higher myoglobin value in meat, the color parameters as L* did not affect only by growing phase. Meat colour is determined not only by the amount and oxidation process of myoglobin but also by the light scattering property of muscle, shorter sarcomere length in ‘dark’ muscles is associated with less light scattering (Hughes, Clarke, Purslow, & Warner, 2017). This was found in this experiment, overall, the bulls finished in PAST system showed lower fat thickness which consequently decreased sarcomere length during chilling, and provided less light scattering and meat with lower L* value. Our results for colour parameters are according to Vestergaard et al. (2000), who reported a "darker" meat colour for animals fed roughage compared to concentrate feed in similar meat pH, they also presented more meat pigmentation but it did not necessarily result in more redness meat.

The a* values were higher in meat from PRE following by FLOT system, which could be explained by an increase in fat thickness. Although in this study was not found a correlation between a* and fat thickness, the L* parameter had a positive correlation with a* and fat thickness, this suggested that the higher fat thickness increased L* value, which also promoted meat with higher a* value. The redness value was similar between treatments on day 1 of the storage period with means of 14.17. The brightness of red beef is associated by consumers to indicate freshness meat. The meat from bulls finished in FLOT were redder

than steak from bulls fed with PAST on days 4 and 7 of storage time (17.09 *vs.* 15.89 and 16.38 *vs.* 15.17, respectively).

The animals finished in PAST showed less variation redness and higher stability during the storage time compared to FLOT meat, which could be associated with greater deposition of natural antioxidants as Vitamin E, α-tocopherol and beta carotene by grass, they can prevent the formation of reactive oxygen species from the oxidation of myoglobin and consequently reduces the formation of metmyoglobin, which leads to meat discoloration (Fruet et al., 2018; Humada et al., 2014). At the end, the storage period (14 d) the a* values were similar between treatments and showed mean of 12.22, which is close to the suggested values as an acceptability threshold for beef redness (O'Sullivan et al., 2002). The value of b* parameter was higher for FLOT than PAST over storage time, which is supported by the fact that is also relationship with intramuscular fat (Duckett et al., 2007), which in our study we observed intramuscular fat increased for animals finished in FLOT compared to PAST system (2.72 vs 1,49 %, respectively, data not shown).

Chroma is an indicator of the saturation of meat colour (Marıa, Villarroel, Sañudo, Olleta, & Gebresenbet, 2003), and is a better parameter to account for the determined importance of redness colour brightness (Holman, van de Ven, Mao, Coombs, & Hopkins, 2017), hence, the decrease of C* can be an indicator of accumulation of metmyoglobin on meat. As expected, considering the finishing system effect on a* values, C* were higher found in meat from FLOT on days 4, 7, and 10 of storage period than PAST. In addition, at 14 days of storage time, the C* index were similar between treatments, however, the values were below 18, which suggest the end of shelf life since the decrease in chroma has been related to the appearance of brown colour in meat rejected by consumers (MacDougall, 1982).

The increase of hue angle is an indication of discoloration (Humada et al., 2014), this suggests greater colour stability in the meat of animals fed with MIN following by PAST, which presented lowest h* values during the display compared to the others treatments, since that they also showed lowest malonaldehyde concentrations.

The colour parameters (L*, a*, b* and C*) showed an increase at the beginning of the first days and a further reduction over the storage time, this is a natural process result of meat oxidation (were induced by increasing availability of oxygen). The decrease of the values of the colour parameters is relationship with the accumulation of larger amount of

metmyoglobin on the meat (Cierach & Niedźwiedź, 2014). In addition, the difference in colour ΔE between the colour of meat from 1 day and 14 days of storage time increased in meat from animals fed with PRE during the growing phase compared to MIN. Although the animals fed with PRE showed higher colour change during the storage time, both supplementation groups showed values above 4, which normally be visible to the average person (according to CIE criteria of classification - *International Commission on Illumination).*

5. Conclusion

Bulls finished in feedlot systems increase the fat thickness and it improves the meat tenderness. On one hand, supplementation with mineral in the growing phase combined with concentrate supplementation in the pasture system during the finishing phase can impair meat colour by increasing dark cutting beef. On the other hand, the pasture system decreased the fat thickness and tenderness in meat without affecting the industry standards of quality.

6. Reference

AMSA. (1995). Research guidelines for cookery, sensory evaluation and instrumental tenderness measurements of fresh meat. In A. M. S. Association (Ed.), *American Meat Science Association* (pp. 48). Savoy, IL: National Livestock and Meat Board.

Apaoblaza, A., Strobel, P., Ramírez-Reveco, A., Jeréz-Timaure, N., Monti, G., & Gallo, C. (2017). Effect of season, supplementation and fasting on glycolytic potential and activity of AMP-activated protein kinase, glycogen phosphorylase and glycogen debranching enzyme in grass-fed steers as determined in Longissimus lumborum muscle. *Livestock Science, 202*, 101-108.

Battaglia, C., Vilella, G. F., Bernardo, A. P., Gomes, C. L., Biase, A. G., Albertini, T. Z., & Pflanzer, S. B. (2019). Comparison of methods for measuring shear force and sarcomere length and their relationship with sensorial tenderness of longissimus muscle in beef. *Journal of texture studies*.

Bruce, H., Stark, J., & Beilken, S. (2004). The effects of finishing diet and postmortem ageing on the eating quality of the M. longissimus thoracis of electrically stimulated Brahman steer carcasses. *Meat Science, 67*(2), 261-268.

Campo, M., Nute, G., Hughes, S., Enser, M., Wood, J., & Richardson, R. (2006). Flavour perception of oxidation in beef. *Meat Science, 72*(2), 303-311.

Cañeque, V., Pérez, C., Velasco, S., Dı, amp, x, . . . De la Fuente, J. (2004). Carcass and meat quality of light lambs using principal component analysis. *Meat Science, 67*(4), 595-605. doi: http://dx.doi.org/10.1016/j.meatsci.2004.01.002

Cierach, M., & Niedźwiedź, J. (2014). Effects of three lighting intensities during display on discolouration of beef semitendinosus muscle. *European Food Research and Technology, 239*(3), 377-383.

Cross, H., West, R., & Dutson, T. (1981). Comparison of methods for measuring sarcomere length in beef semitendinosus muscle. *Meat Science, 5*(4), 261-266.

Culler, R., Smith, G., & Cross, H. (1978). Relationship of myofibril fragmentation index to certain chemical, physical and sensory characteristics of bovine longissimus muscle. *Journal of Food Science, 43*(4), 1177-1180.

Detmann, E., Paulino, M. F., de Campos Valadares Filho, S., & Huhtanen, P. (2014). Nutritional aspects applied to grazing cattle in the tropics: a review based on Brazilian results. *Semina: Ciências Agrárias, 35*(4), 2829-2854.

Duckett, S., Neel, J., Sonon Jr, R., Fontenot, J., Clapham, W., & Scaglia, G. (2007). Effects of winter stocker growth rate and finishing system on: II. Ninth–tenth–eleventh-rib composition, muscle color, and palatability. *Journal of animal science, 85*(10), 2691-2698.

Dunne, P. G., Keane, M. G., O'Mara, F. P., Monahan, F. J., & Moloney, A. P. (2004). Colour of subcutaneous adipose tissue and M. longissimus dorsi of high index dairy and beef × dairy cattle slaughtered at two liveweights as bulls and steers. *Meat Science, 68*(1), 97-106. doi: http://dx.doi.org/10.1016/j.meatsci.2004.02.010

England, E., Matarneh, S., Scheffler, T., & Gerrard, D. (2017). Perimortal muscle metabolism and its effects on meat quality *New aspects of meat quality* (pp. 63-89): Elsevier.

Fruet, A., De Mello, A., Trombetta, F., Stefanello, F., Speroni, C., De Vargas, D., . . . Nörnberg, J. (2018). Oxidative stability of beef from steers finished exclusively with concentrate, supplemented, or on legume-grass pasture. *Meat Science, 145*, 121-126.

Gallo, C., & Huertas, S. (2016). Main animal welfare problems in ruminant livestock during preslaughter operations: a South American view. *Animal, 10*(2), 357-364.

Gornall, A. G., Bardawill, C. J., & David, M. M. (1949). Determination of serum proteins by means of the biuret reaction. *Journal of Biological Chemistry, 177*(2), 751-766.

Holman, B. W., van de Ven, R. J., Mao, Y., Coombs, C. E., & Hopkins, D. L. (2017). Using instrumental (CIE and reflectance) measures to predict consumers' acceptance of beef colour. *Meat Science, 127*, 57-62.

Huffman, K., Miller, M., Hoover, L., Wu, C., Brittin, H., & Ramsey, C. (1996). Effect of beef tenderness on consumer satisfaction with steaks consumed in the home and restaurant. *Journal of animal science, 74*(1), 91-97.

Hughes, J., Clarke, F., Purslow, P., & Warner, R. (2017). High pH in beef longissimus thoracis reduces muscle fibre transverse shrinkage and light scattering which contributes to the dark colour. *Food Research International, 101*, 228-238.

Humada, M., Sañudo, C., & Serrano, E. (2014). Chemical composition, vitamin E content, lipid oxidation, colour and cooking losses in meat from Tudanca bulls finished on semi-extensive or intensive systems and slaughtered at 12 or 14 months. *Meat Science, 96*(2), 908-915.

Hunt, M., Sørheim, O., & Slinde, E. (1999). Color and heat denaturation of myoglobin forms in ground beef. *Journal of Food Science, 64*(5), 847-851.

Koohmaraie, M., Seideman, S., & Crouse, J. (1988). Effect of subcutaneous fat and high temperature conditioning on bovine meat tenderness. *Meat Science, 23*(2), 99-109.

Liu, Z., Xiong, Y. L., & Chen, J. (2010). Protein oxidation enhances hydration but suppresses water-holding capacity in porcine longissimus muscle. *Journal of Agricultural and Food Chemistry, 58*(19), 10697-10704.

MacDougall, D. (1982). Changes in the colour and opacity of meat. *Food Chemistry, 9*(1-2), 75-88.

Marıa, G., Villarroel, M., Sañudo, C., Olleta, J., & Gebresenbet, G. (2003). Effect of transport time and ageing on aspects of beef quality. *Meat Science, 65*(4), 1335-1340.

Moholisa, E., Hugo, A., Strydom, P. E., & van Heerden, I. (2017). The effects of animal age, feeding regime and a dietary beta-agonist on tenderness of three beef muscles. *Journal of the Science of Food and Agriculture, 97*(8), 2375-2381.

Moran, L., O'Sullivan, M. G., Kerry, J. P., Picard, B., McGee, M., O'riordan, E., & Moloney, A. (2017). Effect of a grazing period prior to finishing on a high concentrate diet on meat quality from bulls and steers. *Meat Science, 125*, 76-83.

Muchenje, V., Dzama, K., Chimonyo, M., Strydom, P., Hugo, A., & Raats, J. (2009). Some biochemical aspects pertaining to beef eating quality and consumer health: A review. *Food Chemistry, 112*(2), 279-289.

O'Sullivan, A., O'Sullivan, K., Galvin, K., Moloney, A., Troy, D., & Kerry, J. (2002). Grass silage versus maize silage effects on retail packaged beef quality. *Journal of animal science, 80*(6), 1556-1563.

Owens, F. N., Dubeski, P., & Hanson, C. (1993). Factors that alter the growth and development of ruminants. *Journal of animal science, 71*(11), 3138-3150.

Patino, H., Medeiros, F., Pereira, C., Swanson, K., & McManus, C. (2015). Productive performance, meat quality and fatty acid profile of steers finished in confinement or supplemented at pasture. *Animal, 9*(6), 966-972.

Pflanzer, S. B., & de Felício, P. E. (2011). Moisture and fat content, marbling level and color of boneless rib cut from Nellore steers varying in maturity and fatness. *Meat Science, 87*(1), 7-11. doi: http://dx.doi.org/10.1016/j.meatsci.2010.08.009

Pikul, J., Leszczynski, D. E., & Kummerow, F. A. (1989). Evaluation of three modified TBA methods for measuring lipid oxidation in chicken meat. *Journal of Agricultural and Food Chemistry, 37*(5), 1309-1313.

Priolo, A., Micol, D., & Agabriel, J. (2001). Effects of grass feeding systems on ruminant meat colour and flavour. A review. *Animal Research, 50*(3), 185-200.

Realini, C., Duckett, S., Brito, G., Dalla Rizza, M., & De Mattos, D. (2004). Effect of pasture vs. concentrate feeding with or without antioxidants on carcass characteristics, fatty acid composition, and quality of Uruguayan beef. *Meat Science, 66*(3), 567-577.

Roth, M., Resende, F., Oliveira, I., Fernandes, R., Custódio, L., & Siqueira, G. (2017). Does supplementation during previous phase influence performance during the growing and finishing phase in Nellore cattle? *Livestock Science, 204*, 122-128.

Rotta, P. P., Prado, R. M. d., Prado, I. N. d., Valero, M. V., Visentaine, J. V., & Silva, R. R. (2009). The effects of genetic groups, nutrition, finishing systems and gender of Brazilian cattle on carcass characteristics and beef composition and appearance: a review. *Asian-Australasian Journal of Animal Sciences, 22*(12), 1718-1734.

Savell, J., Cross, H., & Smith, G. (1986). Percentage ether extractable fat and moisture content of beef longissimus muscle as related to USDA marbling score. *Journal of Food Science, 51*(3), 838-839.

Shackelford, S., Morgan, J., Cross, H., & Savell, J. (1991). Identification of threshold levels for Warner-Bratzler shear force in beef top loin steaks. *Journal of Muscle Foods, 2*(4), 289-296.

Silva, L. H., Paulino, P. V., Assis, G. J., Assis, D. E., Estrada, M. M., Silva, M. C., . . . Paulino, M. F. (2017). Effect of post-weaning growth rate on carcass traits and meat quality of Nellore cattle. *Meat Science, 123*, 192-197.

Tatum, J., Smith, G., & Carpenter, Z. (1982). Interrelationships between marbling, subcutaneous fat thickness and cooked beef palatability. *Journal of animal science, 54*(4), 777-784.

Therkildsen, M. (2005). Muscle protein degradation in bull calves with compensatory growth. *Livestock Production Science, 98*(3), 205-218.

Therkildsen, M., Stolzenbach, S., & Byrne, D. V. (2011). Sensory profiling of textural properties of meat from dairy cows exposed to a compensatory finishing strategy. *Meat Science, 87*(1), 73-80.

Valadares Filho, S. d. C., Marcondes, M., Chizzotti, M., & Paulino, P. (2016). Exigências nutricionais de zebuínos puros e cruzados BR-CORTE. *UFV, Suprema Gráfica Ltda: Viçosa, Brazil*.

Vestergaard, M., Therkildsen, M., Henckel, P., Jensen, L., Andersen, H., & Sejrsen, K. (2000). Influence of feeding intensity, grazing and finishing feeding on meat and eating quality of young bulls and the relationship between muscle fibre characteristics, fibre fragmentation and meat tenderness. *Meat Science, 54*(2), 187-195.

Walker, P., Warner, R., & Winfield, C. (1990). *Sources of variation in subcutaneous fat colour of beef carcasses.* Paper presented at the Proceedings of the Australian Society of Animal Production.

Warris, P. (1979). The extraction of haem pigments from fresh meat. *International Journal of Food Science & Technology, 14*(1), 75-80.

Table 1. Composition of the concentrate supplements offered during the growing phase and the feed offered during the finishing phase.

Items	Growing phase		Finishing phase		
	Protein + Energy[1]	*Urochloa brizantha* cv. *Xaraés* (rainny season)	Concentrate[2]	*Urochloa brizantha* cv. *Xaraés* (dry season)	Corn silage
Ingredients, %					
Corn	73.5	-	78.9	-	-
Soybean meal	10.6	-	16.5	-	-
Mineral premix	15.9	-	4.60	-	-
Chemical composition. %					
Dry matter, %	86.0	33.2	89.9	45.8	30.1
Organic matter, %DM	89.2	92.5	91.0	92.3	95.0
Crude protein, %DM	20.5	12.8	16.0	11.3	9.5
Ether extract, %DM	6.3	2.41	6.6	2.3	2.2
Neutral detergent fiber	26.5	57.5	25.1	58.2	33.1

[1]Sodium 80 g/kg; Calcium 153 g/kg; Phosphorus 30 g/kg; Sulfur 30 g/kg; Zinc 1925 mg/kg; Copper 520 mg/kg; Manganese 400 mg/kg; Iodine 30 mg/kg; Cobalt 38 mg/kg; Selenium 10 mg/kg; Monensin 400 mg/kg; NNP 620g /kg.

[2] Sodium 40 g/kg; Calcium 110 g/kg; Phosphorus 20 g/kg; Potassium 40 g/kg; Magnesium 56 g/kg; Sulfur 31 g/kg; Zinc 700 mg/kg; Copper 190 mg/kg; Fluorine 333 g/kg; Manganese 550 mg/kg; Iodine 14 mg/kg; Cobalt 12 mg/kg; Selenium 3.5 mg/kg; Vit A 55000 Ul/kg; Vit D3 7500 Ul/kg; Vit E 750 Ul/kg; Monensin 00 mg/kg; NNP 620g /kg.

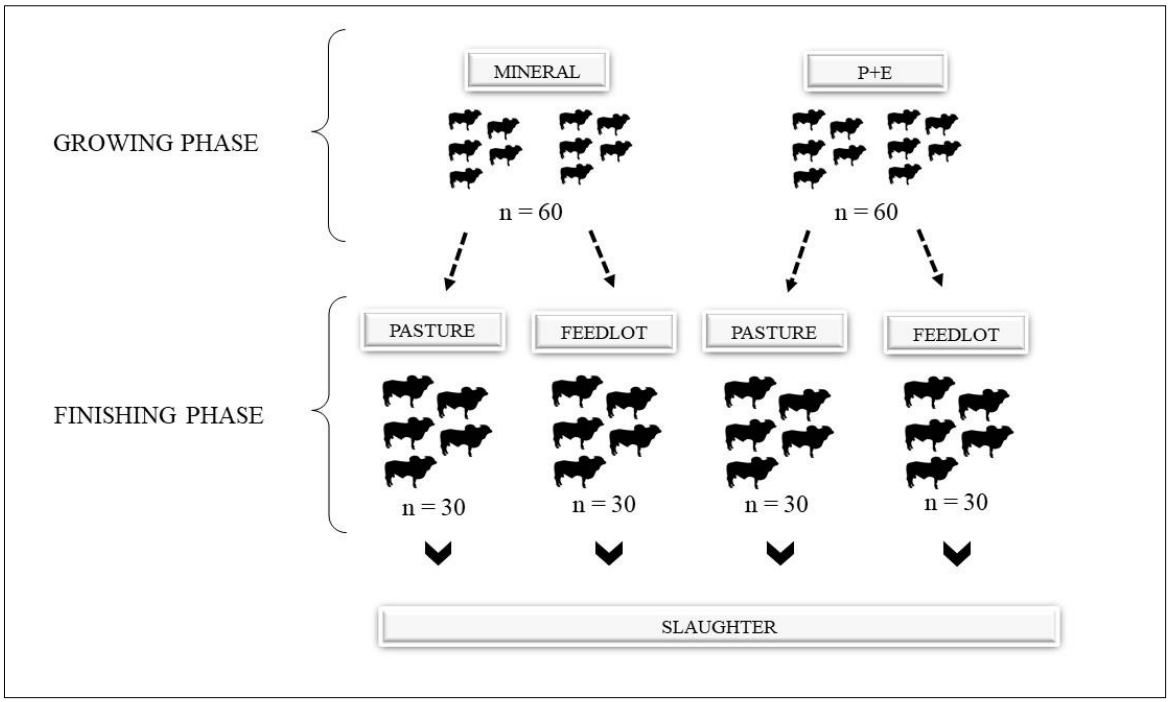

Figure 1. Experimental design.

Mineral (*ad libitum*); Protein + Energy (0.3% BW/day); Feedlot (25:75; corn silage: concentrate); Pasture plus concentrate (2% of BW/day).

Table 2. pH, shear force, sarcomere length, myofibrillar fragmentation index (MFI), water holding capacity (WHC), thawing loss and cooking loss of *Longissimus thoracis* of young bulls supplemented during the growing phase and finished in different system.

Finishing system	FLOT[1]		PAST[2]		SEM[3]	GP[4]	FS[5]	GP×FS
Growing phase	MIN[6]	PRE[7]	MIN[6]	PRE[7]				
pH	6.09	5.96	6.11	5.99	0.089	0.074	0.784	0.975
Fat thickness, mm	6.99	7.04	2.98	3.16	0.272	0.775	<0.001	0.871
Shear Force, N	35.33	37.24	42.05	44.35	3.232	0.395	0.006	0.937
Sarcomere length, µm	1.59	1.60	1.44	1.40	0.037	0.764	<0.001	0.339
MFI	23.87	22.79	21.85	19.30	2.485	0.341	0.150	0.698
WHC, %	64.36	64.27	68.07	68.24	0.903	0.959	<0.001	0.850
Thawing loss, %	9.38	10.69	7.44	8.25	1.089	0.207	0.010	0.763
Cooking loss, %	25.50	26.44	26.74	27.28	1.072	0.365	0.209	0.808

[1]Feedlot (25:75; corn silage: concentrate); [2]Pasture plus concentrate (2% of BW/day);[3]Standard error of mean; [4]Growing phase; [5]Finishing phase;[6]Mineral (*ad libitum*); [7]Protein + Energy (0.3% BW/day).

Table 3. Subcutaneous fat colour parameters of young bulls supplemented during the growing phase and finished in different system.

Finishing system	FLOT[1]		PAST[2]		SEM[3]	GP[4]	FP[5]	GP×FP
Growing phase	MIN[6]	PRE[7]	MIN[6]	PRE[7]				
L*	67.36	67.09	70.00	70.45	0.633	0.845	<0.001	0.460
a*	4.52	4.45	5.63	5.37	0.433	0.613	0.003	0.783
b*	14.36	14.24	16.64	16.68	0.475	0.923	<0.001	0.821
C*	15.10	15.04	16.00	16.00	0.470	0.923	0.011	0.930
h*	72.79	73.12	70.76	71.45	1.310	0.610	0.067	0.857

[1]Feedlot (25:75; corn silage: concentrate); [2]Pasture plus concentrate (2% of BW/day);[3]Standard error of mean; [4]Growing phase; [5]Finishing phase; [6]Mineral (*ad libitum*); [7]Protein + Energy (0.3% BW/day).

Table 4. Myoglobin (MYO) and malonaldehyde (MDA) of *Longissimus thoracis* of young bulls supplemented during the growing phase and finished in different system.

Finishing system	FLOT[1]		PAST[2]		SEM[3]	GP[4]	FS[5]	GP×FS
Growing phase	MIN[6]	PRE[7]	MIN[6]	PRE[7]				
MYO, mg /g of meat	3.72	4.19	4.40	4.73	0.167	0.002	<0.001	0.581
MDA, mg /kg of meat	0.37a	0.28ab	0.27b	0.30ab	0.029	0.175	0.074	0.011

[1]Feedlot (25:75; corn silage: concentrate); [2]Pasture plus concentrate (2% of BW/day); [3]Standard error of mean; [4]Growing phase; [5]Finishing phase; [6]Mineral (*ad libitum*); [7]Protein + Energy (0.3% BW/day).

Table 5. Instrumental colour parameters of *Longissimus thoracis* (14 days of storage time) of young bulls supplemented during the growing phase and finished in different system.

	FS[1]	GP[2]	1	4	7	10	14	SEM[3]	GP	FS	GP×FS	D[4]	GF×D	FS×D	GF×FS×D
L*	FLOT[5]	MIN[7]	36.44	37.56	37.44	38.94	38.10	0.908	0.230	<0.001	0.002	<0.001	0.991	0.947	0.967
		PRE[8]	35.73	36.79	37.39	38.32	37.38								
	PAST[6]	MIN	32.98	33.4	33.88	35.32	33.54								
		PRE	34.06	34.99	35.05	36.27	35.21								
a*	FLOT	MIN	13.84	16.84	16.17	15.05	12.25	0.379	0.732	<0.001	0.007	<0.001	0.604	0.004	0.943
		PRE	14.48	17.33	16.59	15.38	12.18								
	PAST	MIN	14.15	16.24	15.48	14.52	12.53								
		PRE	14.21	15.55	14.87	14.01	11.93								
b*	FLOT	MIN	11.84	14.33	14.27	13.79	12.66	0.389	0.156	<0.001	0.240	<0.001	0.887	0.361	0.953
		PRE	11.99	14.49	14.32	13.77	12.50								
	PAST	MIN	10.48	12.75	12.84	12.32	11.61								
		PRE	11.28	13.04	12.96	12.48	11.93								
C*	FLOT	MIN	18.21	22.11	21.6	20.46	17.54	0.469	0.538	<0.001	0.210	<0.001	0.753	0.030	0.973
		PRE	18.84	22.42	21.93	20.73	17.59								
	PAST	MIN	17.62	20.68	20.12	18.95	17.05								
		PRE	18.12	20.40	19.67	18.78	16.91								
h*	FLOT	MIN	40.28	40.52	41.56	42.71	46.36	0.799	0.091	<0.001	<0.001	<0.001	0.973	0.380	0.998
		PRE	39.46	39.96	40.84	41.79	45.98								
	PAST	MIN	36.35	38.14	39.66	40.33	42.95								
		PRE	38.29	39.94	41.04	41.71	45.19								

[1]Finishing phase; [2]Growing phase; [3]Standard error of mean; [4]Days; [5]Feedlot (25:75; corn silage: concentrate); [6]Pasture plus concentrate (2% of BW/day); [7]Mineral (*ad libitum*); [8]Protein + Energy (0.3% BW/day).

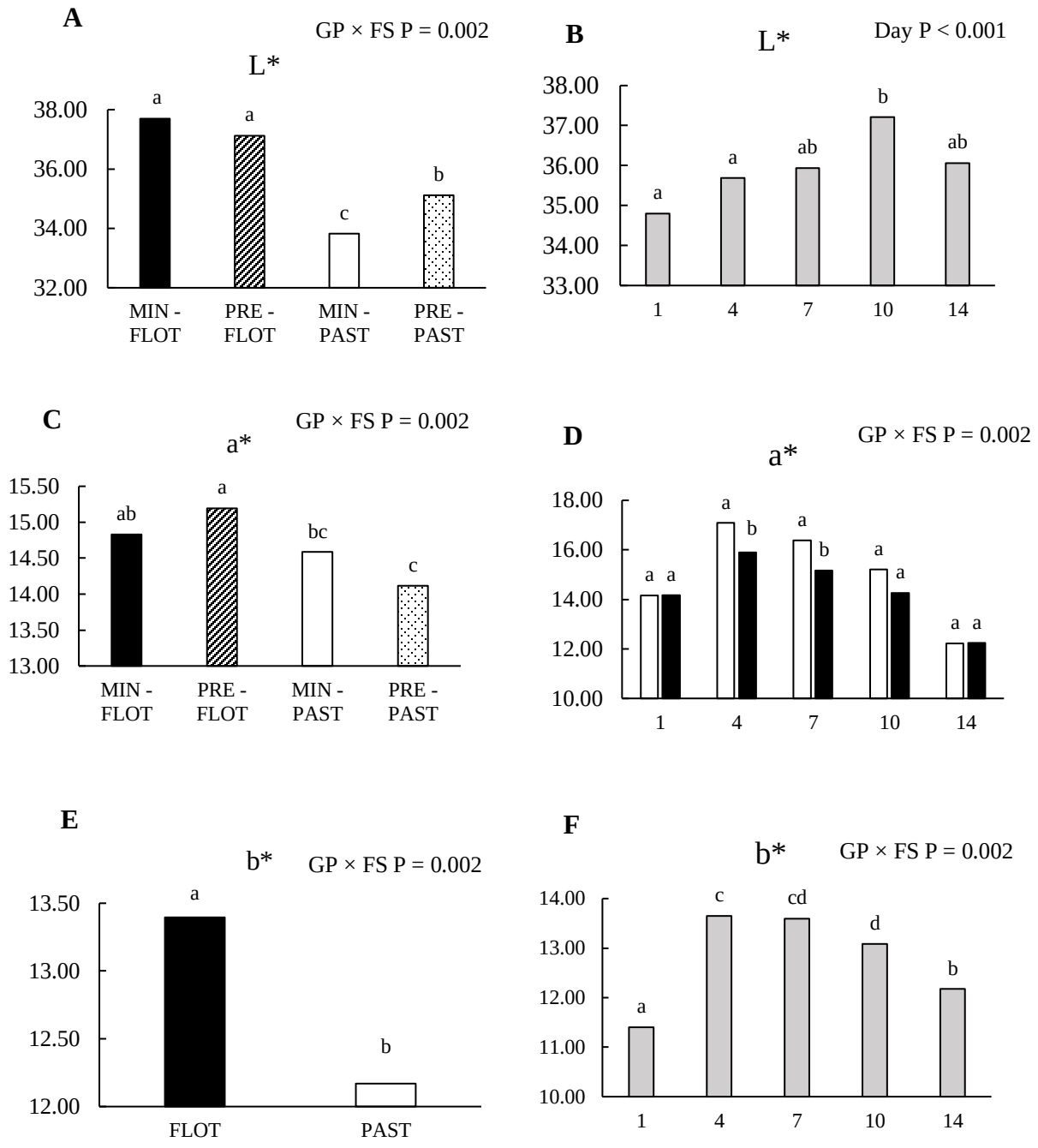

Figure 2. Instrumental colour parameters of of *Longissimus thoracis* (14 days of storage time) of young bulls supplemented during the growing phase and finished in different systems: Lightness (A and B); redness (C and D); yellowness (E and F).

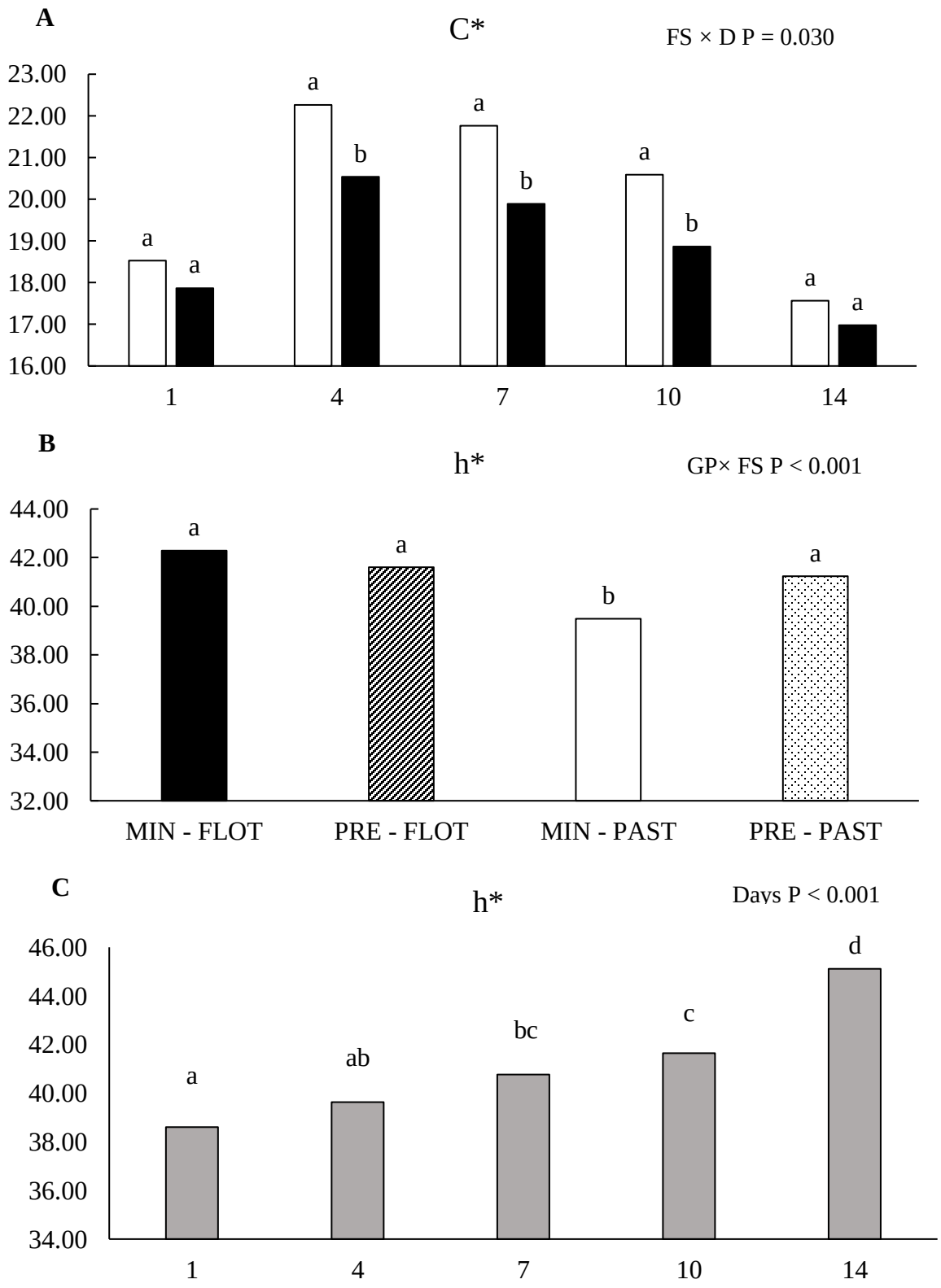

Figure 3. Instrumental colour parameters of *Longissimus thoracis* (14 days of storage time) of young bulls supplemented during the growing phase and finished in different systems: Chroma (A); hue angle (B and C).

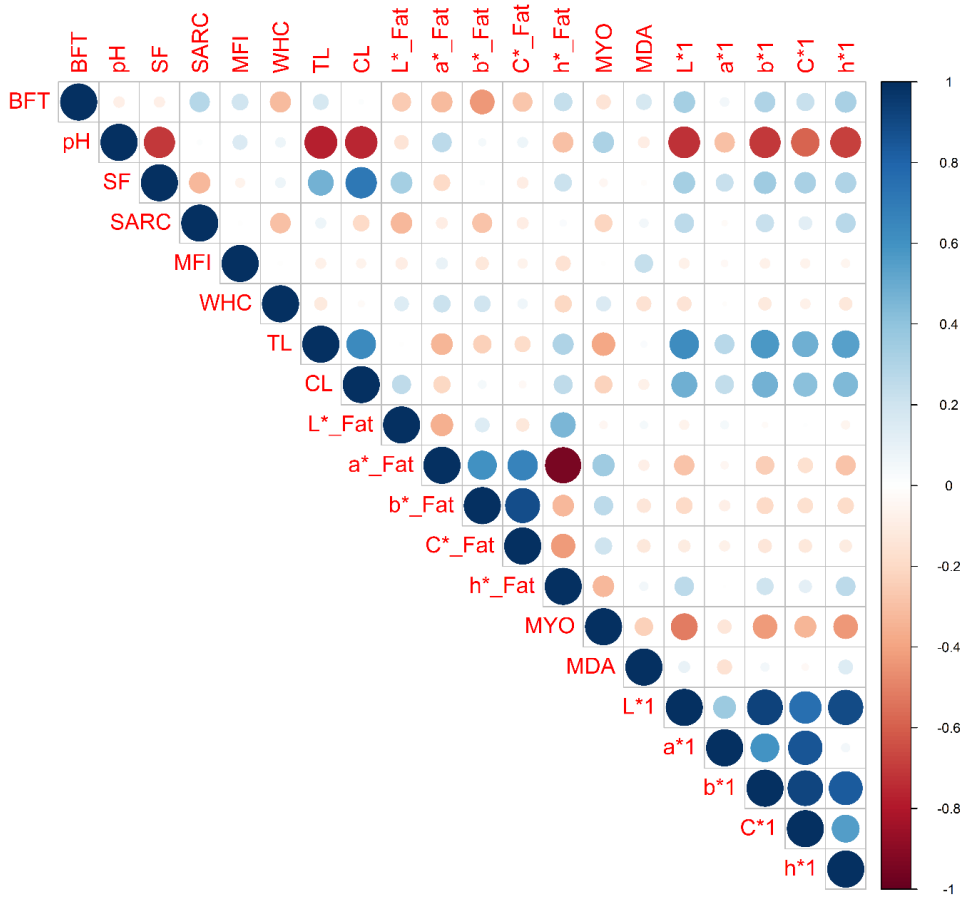

Figure 4. Correlation coefficients between the meat quality variables[a]

[a] Back fat thickness (BFT); shear force (SF); sarcomere length (SARC); myofibrillar fragmentation index (MFI); water holding capacity (WHC); thawing loss (TL); cooking loss (CL); myoglobin (MYO); malonaldehyde (MDA); L* (lightness); a* (redness); b* (yellowness); C* (Chroma); h*(hue angle).

CHAPTER 3. THE EXPRESSION GENE ASSOCIATED IN LIPID METABOLISM OF *LONGISSIMUS THORACIS* FROM BULLS SUPPLEMENTED DURING THE GROWING PHASE AND FINISHED IN DIFFERENT SYSTEMS

Abstract

One hundred and twenty young bulls uncastrated males were randomly assigned to treatments in 2 × 2 factorial design, two supplements during the growing phase: mineral (*ad libitum* - MIN) or Protein + Energy (3g/ kg body weight/ animal/ day - PRE) and two finishing system: pasture plus concentrate supplementation (20g/ kg body weight/animal/ day – PAST), and 2) feedlot system (Concentrate: corn silage; 75:25% - FLOT). The FLOT group had ($P < 0.001$) higher lipid content than PAST group. The animals fed with MIN and finished in FLOT showed an increase ($P < 0.050$) of C12:0, C14:0, and C16:0 which led to greater saturated fatty acid total concentration. The FLOT system had ($P = 0.001$) higher monounsaturated concentration. Although the FLOT showed a decrease ($P \leq 0.030$) of C18:2n6, C20:3n6, C20:4n6, and C20:5n3, similar total polyunsaturated was detected ($P = 0.063$) to PAST and FLOT system. In addition, the n6/n3 ratio was lower ($P < 0.001$) in the meat of animals from FLOT system. The PAST system increased ($P = 0.049$) the isocitrate dehydrogenase. The FLOT group showed higher ($P = 0.005$) SREBP1c, therefore the animals fed with PRE and finished in FLOT had higher ($P = 0.001$) CPT2 level. The animals fed with MIN and finished in PAST showed lower ($P = 0.005$) SREBP1c, greater level of CPT2 ($P = 0.001$) and PPARα ($P = 0.013$). The FLOT system increased the intramuscular lipid compared to PAST system. These results suggested that supplementation strategy during the growing phase following the finishing phase changed the genes expression relationship with lipid metabolism.

Keywords: Cattle, Genes expression, Lipogenesis, Marbling, Fatty acid profile

1. Introduction

Supplementation strategies during the growing phase are intended to increase the efficiency of the grazing system and animal performance, especially as grazing in the tropical system often fails to meet the nutrient supply needs of the animals. The growing phase is a key point in the search for the anticipation of slaughter age, whose animals have better biological efficiency in tissue deposition, due to the steeper growth curve (Owens *et al.*, 1993). However, positive responses are only found in supplementation strategies when the nutrient level is higher or constant in the subsequent phases, which consequently improves animal efficiency and reduces time in the finishing phase (Roth *et al.*, 2017, Sampaio *et al.*, 2017).

Nevertheless, most studies have evaluated the effect of supplementation during the growing phase on the finishing performance of cattle (Roth et al., 2017, Sampaio et al., 2017, Delevatti et al., 2019), there is yet a scarce of knowledge of how these strategies impact meat quality, mainly how they affect intramuscular fat deposition, which is an important meat quality criterion for many countries. Thus, understanding the intramuscular fat deposition is of fundamental importance when the goal is to produce meat with greater intramuscular fat.

Fat deposition occurs in response to activation of metabolic actions such as lipogenesis and lipolysis, which occur according to the need for lipid release or storage, as well as the interaction of dietary nutrients and the level of expression of the genes involved in lipid metabolism (Dong *et al.*, 2014). In this respect, diets glucogenic provides the most of the acetyl units for in vitro lipogenesis in intramuscular fat depot compared to acetogenic diets (Smith *et al.*, 2018). Animals supplementation with concentrated diets may increase the amount of insulin in the blood, which stimulates glucose uptake by tissues, consequently increase the amount of intramuscular lipid (Schoonmaker *et al.*, 2003).

Another important point is the lack of intramuscular lipid content in animals finishing exclusively with grass-based diet (Humada *et al.*, 2014, Ferrinho *et al.*, 2020). In this context, the intensive supplementation with concentrate for bulls on the grass during the finishing phase may promote a higher degree of fat on the carcass. In these aspects, the finishing phase of cattle in pasture with intensive supplementation can be an alternative, once as pasture is the source of fibrous substrate to maintain the rumen environment.

In this respect, this study hypothesizes that the supplement strategy of bulls during the growing phase can affect the lipid content in meat at finishing phase, due to the higher genes expression related with adipogenesis, as well as the pasture system can promote intramuscular lipid content similar to conventional feedlot due to the intensive supplementation during this phase. The objective of this experiment was to evaluate the supplementation strategy during the growing phase, and finishing system (pasture plus concentrate supplementation *vs*. conventional feedlot) on the fatty acid profile, lipogenic enzyme activity, and relative abundance of mRNA associated with lipid metabolism in *Longissimus thoracis* muscle.

2. Materials and Methods

This experiment was approved by the Ethics and Animal Welfare Committee of the São Paulo University (protocol 5628/15). The research was carried out at the beef cattle facility of São Paulo University located in Jaboticabal city, São Paulo, Brazil (48°18′58″W, 21°15′22″S).

2.1. Animals

The experiment period comprised the growing phase (first experimental period) and finishing phase (second experimental period). The study was conducted during the months of December to September (285 days). One-hundred twenty uncastrated males from three genetic groups: 40 Nellore from the farm in Sao Paulo state, with average 10 ± 2 months old and 264.8 kg, 40 ½ Angus × ½ Nellore from the farm in Sao Paulo state, with average 11 ± 2 months old and 278.0 kg, and 40 ½Senepol × ½ Nellore from the farm Goias State, with average 9 ± 2 months old and 226.7 kg. Before the experiment, all the bulls were fed with grass and were not creep fed. After weaning all animals remained in the grazing area consisted of Brachiaria grass until the experiment starts.

2.2. Growing phase (first experimental phase)

The experiment was conducted as a randomized block design with two supplements during the growing phase (n = 60): 1) mineral (*ad libitum*; MIN), and 2) protein + energy [0.3% body

weight (BW)/ animal/ day; PRE]. The experimental unit was the animal (60 bulls/treatment). The growing phase was conducted during the summer season in Brazil (December 3rd to May 6th; 155 days). At the beginning the experiment, the bulls, within each breed group were blocked by BW to one of two treatments supplied during the growing phase. The definition of supplements was based on tropical conditions (Detmann, Paulino, de Campos Valadares Filho, & Huhtanen, 2014), and the composition of the diets is reported in Table 1. The amount of supplement provided was calculated to meet the requirements for an average daily gain of 0.6 kg/d, according to Valadares Filho, Marcondes, Chizzotti, and Paulino (2016). During the growing phase, the bulls were supply once a day (10:00 h). The grazing area consisted Brachiaria grass (*Urochloa brizantha cv.* "Xaraés") divided into 12 paddocks (approximately 1.8 ha each), 10 bulls/paddock (within each breed group had two supplementation treatments with two replicates each, the experimental design is shown in Figure 1. Each padlock had semicircular drinkers and covered feed troughs (3.0m × 0.5m) with both side access for supplementation. Every 28 d the bulls were weighed, and this BW was used to adjust the amount of supplement. Mineral feed was available to the bulls *ad libitum* and the protein + energy supplement amount was calculated based on BW at the beginning of each experimental period and placed every single day at 10:00 h during the experimental period.

2.3. Finishing phase (second phase experimental)

The finishing phase was conducted during the winter, a dry season (May 7th to September 13th; 120 days). The bulls had a period of adaptation of 20 days based on the "step-up", before the second experimental phase. Following the growing phase (1º experimental phase), the bulls within each supplementation, and each breed were randomly assigned to one of two finishing systems: 1) pasture plus concentrate supplementation (2 % BW/ animal/ day; PAST), and 2) feedlot system where bulls received corn silage + concentrate (25:75; FLOT). The experiment was conducted by a 2 x 2 factorial, and design is shown in Figure 1. The finishing diet composition is reported in Table 1. The amount of supplement provided was calculated to meet the requirements for average daily gain 1.5 kg/d according to Valadares Filho et al. (2016). All bulls designated for PAST system were kept in the same paddock that was used during the growing phase. Every 28 d the bulls were weighed, and the BW was used to adjust the amount of concentrate (2% BW/day) for PAST treatment. The bulls from PAST system were fed with concentrate once a day (10:00 h)

during the experimental period. The bulls designated for FLOT system remained in individual pens with 12 m^2, partially covered, concrete floors, with feed though and free water access. The FLOT basal diet was 25% of roughage (corn silage) and 75% of concentrate (corn, soybean meal, and premix) on dry matter basis. The bulls were fed twice a day (08:00 and 15:00 h), and the diet amount provided to the bulls was adjusted weekly for refusals of 5%.

2.4. Slaughter procedure and muscle sampling

After 285 days of the experiment, all bulls (Nellore with an average of 510.86 kg BW, ½ Angus with an average of 532.68 kg BW, and ½ Senepol with 466.24 kg BW) were transported to a commercial slaughterhouse (Minerva Foods, Barretos, São Paulo, Brazil), located 90 km from the experimental area. The bulls were slaughtered following the usual practices of the Brazilian beef industry, according to the Brazilian RIISPOA–Regulation of Industrial and Sanitary Inspection of Animal Products. Afterward, the carcasses were divided medially from the sternum and the spine, resulting in two similar halves. After these procedures, muscle samples were taken from the *Longissimus thoracis* (LT) muscle of the left half-carcass at the 12^{th} to 13^{th} rib height. The muscle samples were frozen and transported in liquid nitrogen and stored at −80°C for gene expression and enzyme analyses. Subsequently, the half-carcasses were washed, identified and stored in a chilling chamber at 4^oC for 24 h. After chilling, the LT muscle samples were collected from the left side of the carcass between the 12^{th} and the 13^{th} ribs and stored at -20°C for meat composition, and fatty acid profile. The sample was transported to the Animal Science Laboratory at the São Paulo State University (Jaboticabal).

2.5. Chemical composition of meat

Lipid, moisture, protein, and content of meat were performed at the Universidade Federal de Lavras. The sample steak was thawed at room temperature, ground and used for chemical composition analyses using the FoodScan Meat Analyser TM^r (FOSS, Hillerod, Denmark) with near-infrared spectrophotometer technology (analyses AOAC method: 2007-04).

2.6. Fatty acid profile of meat and diet

Sample lipids were extracted by the Bligh and Dyer (1959) method. Fifteen grams of meat sample was used for extraction with a chloroform-methanol mixture (2:1 ratio) and later transmethylated (Christie, 1984). A 1 µl aliquot of transmethylated lipid was injected into a gas chromatograph (model-Finnigan Focus GC) with a flame ionization detector and capillary column (CP-Sil 88; Varian Inc®, chemical analysis equipment), 100 m long by 0.25 µm internal diameter and 0.20 µl film thickness). The hydrogen was used as carrier gas at a flow rate of 1.8 ml/min. The temperature program of the oven of the gas chromatograph was: started at 70°C with a standby time of 4 min, then raised to 175°C (13°C/min) with a standby time of 27 min, continued to increase to 215°C (4°C/min) with a standby time of 9 min. and finally, an increase of 7°C/min. up to 230°C for 5 min, totaling 65 min. The injector temperature was 250°C and the detector was 300°C. The identification of fatty acid was performed by comparison of retention times with those obtained with standard sample esters and quantification of the proportion of fatty acid was performed using the Chromquest 4.1 software (Thermo Electron®, Rodano, Italy).

2.7. Lipogenic enzyme activity

Approximately 1.5 g of tissue muscle was cut and placed in 4.5ml of 0.1 M phosphate buffer (K2HPO4, pH 7.4, 25 °C), homogenized and centrifuged 3,000 × g for 15 min at 4 °C, Discard the pellet, centrifuged the supernatant fraction at 15,000 × g for 30 min at 4 °C, The resulting supernatant fractions were utilized for enzyme measurements. NADP-malate dehydrogenase and isocitrate dehydrogenase were measured as described by Schoonmaker *et al.* (2003). All enzyme assays were determined in duplicate using the spectrophotometric absorbance of solutions in cuvettes at 340 nm. Slopes of the linear rates of NADPH production were used to calculate enzyme activities.

2.8. Gene expression analyses

The design of target and reference primers was performed using sequences that are registered and published in the GenBank public data bank, a National Center for Biotechnology

Information platform (Table 3). Primers were designed using OligoPerfect Designer software (Invitrogen, Karlsruhe, Germany) and synthesized (Invitrogen, Carlsbad, CA, USA). Nine target gene were used (PPAR-γ, SREBP-1c, SCD, ACCα, LPL, FABP4, ACOX, CPT2, PPAR-α) and two target reference gene (β-actin and GAPDH), as proposed by Vandesompele *et al.* (2002). The extraction of total RNA from the collected samples was carried out at the Laboratory of Biochemistry and Molecular Biology (LBM) of the Department of Technology of the Faculty of Agrarian Sciences and Veterinary Sciences of Unesp, Jaboticabal. The RNA Extraction was used RNA RNeasy Lipid Tissue Mini Kit (Qiagen, Valencia, CA, EUA). The RNA contamination (260/280 and 260/230) and concentration (ng/ µl) were assessed using a NanoDrop 1000 Spectrophotometer (Thermo Fisher Scientific, Santa Clara, CA, EUA, 2007). The RNA quality was assessed using Agilent 2100 Bioanalyzer (Agilent, Santa Clara, CA, EUA, 2009) and the Agilent RNA 6000 Nano Chip kit (Agilent, Santa Clara, CA, EUA). The cDNA synthesis was performed using the Kit SuperScript III First-Strand Synthesis SuperMix for qRT-PCR (Invitrogen, Carlsbad, CA, EUA) according to the manufacturer's instructions. The equipment used for the qRT-PCR reactions was the 7500 Real-Time PCR (Applied Biosystems, Foster, CA, USA, 2009) with an SYBR green RT-PCR kit from Bio-Rad. The cycling conditions comprised 2 min polymerase activation at 95 °C and 40 cycles at 95 °C for 15 s and 60 °C for 30 s. All PCR efficiencies were above 96%. Relative gene expression of mRNA was calculated according to the detection of $\Delta C_T = C_T$ (target gene) – C_T (average reference genes). The calibration was determined by formula $\Delta\Delta C_T = \Delta C_T$ (sample) – ΔC_T (calibrator), the calibrator used was sample from Mineral-Pasture treatment for each breed evaluated. Relative expression evaluation was performed by the formula $2^{-\Delta\Delta C_T}$.

2.9. Gene set enrichment analysis

The Cluego of the Cytoscape program was used for the analysis of enrichment with the genes studied using the bovine genome UMD 3.1, and then the genes were separated by biological processes within the ontology (GO) gene classification system used to interpret and classify gene sets. An enrichment analysis to visualize non-redundant biological terms for genes was performed using The ClueGo plug-in of the Cytoscape (Bindea *et al.*, 2009), using the bovine genome UMD 3.1 (http://www.ncbi.nlm.nih.gov/genome/?term=bos+taurus), as reference.

2.10. Statistical analysis

The focus of this work was to evaluate the supplementation strategy during the growing phase and finishing system on the meat quality of young bulls. Thus, for a better interpretation of the results, in this study three breeds different were grouped for greater reliability of the results. The experimental design was blocked completely randomized in a 2 × 2 factorial arrangement, two supplementations during the growing phase (MIN or PRE supplementation), and two finishing systems (PAST or FLOT). All data were analysed using the PROC MIXED from SAS 9.4 statistical software (SAS Inst. Inc., Cary, North Carolina) using the animal as experimental unit. The treatments and the block (light and heavy) as the fixed effect and the breed as a random effect. The mean and standard error of mean were calculated for each variable. When significant main or interaction effects were detected, Tukey's test ($P \leq 0.05$) was used to determine the differences between means. The correlation analysis was performed using the Rstudio software, all data were analysed with Corrplot statistic package with significant level of 5%.

3. Results

During the growing phase, the MIN and PRE provided a supplement intake of 0.100 and 0.910 kg/day, respectively. The average carcass gain at the end of the growing phase was 0.247 kg/day for MIN and 0.377 kg/day for PRE, which provides heavier carcasses at the end of the growing phase for animals fed PRE compared to MIN, 181.47 vs 165.40 kg, respectively. In addition, the average intake of supplement at finishing phase was 1.64, 1.53, 1.60 and 1.56% BW for treatments MIN – FLOT, PRE – FLOT, MIN – PAST, and PRE – PAST, respectively, which provides carcass gain of 1.170, 1.039, 0.957 and 0.897 kg/day, respectively. The animals fed with MIN during the growing phase showed greater carcass gain at the finishing phase but which was not enough for the animals to have equivalent carcass weight at the slaughter compared to PRE, regardless of the finishing system (284.60 vs 291.01 kg). On the other hand, the animals from FLOT system had greater carcass weight than PAST (296.90 vs 278.71 kg).

3.1. Meat composition

The meat composition of young bulls is reported in Table 3. The finishing system affected the lipid ($P < 0.001$) and moisture ($P < 0.001$). The meat of animals finished in FLOT showed 82.22% more lipid concentration in meat than animal finished in PAST (2.72 *vs.* 1.49%, respectively). On the other hand, the animals from FLOT showed significatively lower moisture value compared to PAST (72.55 *vs.* 73.74%, respectively). A negative correlation ($P < 0.050$, $r = - 0.80$) between lipid and moisture content was detected by correlation analysis (Figure 5).

3.2. Fatty acid profile

The fatty acid profile is shown in Table 4. An interaction ($P < 0.050$) was observed between growing phase and finishing system for C12:0 (lauric), C14:0 (myristic), C16:0 (palmitic). The meat of animals from MIN following by FLOT showed higher values for lauric fatty acid compared to PRE following by FLOT, while PAST system regardless of supplementation showed similar to the others. In addition, the MIN following by FLOT group showed higher values for myristic and palmitic fatty acid compared to the others. The saturated fatty acid (SFA) total showed higher values to MIN following by FLOT compared to MIN following by PAST and PRE following by PAST, while PRE following by FLOT was similar to the others (1169.20, 768.35, 831.82 and 941.27 mg/100g meat, respectively). A positive correlation ($P < 0.050$) was observed for all saturated fatty acids (Figure 5).

An interaction ($P < 0.050$) was observed between the growing phase and finishing system for C14:1 (myristoleic). The meat of animals fed with MIN following FLOT system had the highest value than the other. In addition, the FLOT increased the C16:1 (palmitoleic), C17:1 (heptadecenoic) and C18:1n9c (oleic) concentration compared to PAST (63.96 vs 49.11, 10.19 vs 8.6 and 856.42 vs 663.68 mg/100g meat, respectively). These results promoted 28.55% increased ($P = 0.001$) monounsaturated fatty acid (MUFA) total in FLOT group than PAST, where values found were 949.81 and 738.83 mg/100g meat, respectively.

The C18:2c9t11 and C18:2t10c12 fatty acid did not affect ($P > 0.050$) by the growing phase or finishing system. However, the FLOT system had higher ($P < 0.050$) C20:2n6 (Eicosadienoic) and C22:6n3 (docosahexaenoic - DHA) concentration than PAST, the value observed were, 1.72

vs 1.51 and 0.78 vs 0.56, respectively. On the other hand, the PAST group increased ($P < 0.050$) C18:2n6c (linoleic) compared to FLOT system (128.98 vs 105.14 mg/100g meat) and increased the isomers, C20:3n6 (eicosatrienoic), C20:4n6 (arachidonic) and C20:5n3 (eicosapentaenoic) which showed values of 5.35 vs 4.28, 26.61 vs 19.31, and 2.72 vs 2.48 mg/100g meat, respectively. The polyunsaturated (PUFA) total had mean of 215.65 mg/100g meat and it was similar ($P = 0.063$) between the treatments.

The n3 total did not affect ($P > 0.050$) by treatments, and the animals had means of 11.04 mg/100g meat). However, the FLOT system had lower n6 total concentration compared to PAST (130.26 vs 162.30 mg/100g meat) and this provided lower n6/n3 ration in FLOT group (12.07 vs 14.20 mg/100g meat, respectively).

3.3. Lipogenic enzyme activity

The growing phase or finishing phase did not modify ($P > 0.050$) the NADP-Malate dehydrogenase (Table 5). However, the muscle of animals finished in PAST showed ($P = 0.049$) higher isocitrate dehydrogenase concentration compared to FLOT (3363.39 vs 2869.04 nmol/min, respectively: Table 5).

3.4. Relative expression of genes relationship with lipid metabolism

The relative expression of genes relationship with lipid metabolism is shown in Table 6. An interaction ($P < 0.050$; Figure 2) among growing phase and finishing system were observed to the expression of peroxisome proliferator-activated receptor γ (PPAR γ), the muscle of animals fed with MIN following by FLOT and PRE following by PAST had higher PPARγ expression compared to muscle from animals fed MIN following by PAST. A positive correlation ($P < 0.050$, $r = 0.54$; Figure 5) was found between PPARγ and sterol regulatory element-binding protein 1c (SREBP1c).

A positive correlation ($P = 0.002$, $r = 0.34$; Figure 5) was detected between SREBP1c and lipid content. The SREBP1c was affected by the finishing system, when the animals were finished in FLOT system, the expression of SREBP1c and stearoyl CoA desaturase (SCD1) were 18.65 and 1.35 times higher than the animals finished in PAST, respectively. In addition, the PRE

supplementation during the growing phase showed (P = 0.001; Figure 2) greater SCD1 expression in the muscle than MIN at the end of the finishing phase.

The expression level of acetyl CoA carboxylase alfa (ACCα), lipoprotein lipase (LPL), and acyl CoA oxidase 1 (ACOX) did not affect (P > 0.050) by growing phase or finishing phase. On the other hand, the expression of the fatty acid-binding protein 4 (FABP4) gene was greater (P < 0.010; Figure 3) in LT muscle of animals finished in FLOT system. The FABP4 had a positive correlation with SREBP1c and PPARγ (P < 0.008, r = 0.30 and r = 0.45, respectively, Figure 5).

An interaction (P < 0.010; Figure 4) between the growing phase and finishing system for the gene relationship with lipid degradation. The gene that encodes carnitine palmitoyltransferase 2 (CPT2) was observed higher expression in muscle of animals fed with PRE following by FLOT and MIN following by PAST than PRE following by PAST, while the MIN following by FLOT was similar to the others treatments. In addition, the PPARα was found a decrease of 50% in the muscle of animals fed with MIN following by FLOT system compared to the MIN following by PAST. The PPARα had a negative correlation (P = 0.012, r = - 0.28) between lipid content (Figure 5).

4. Discussion

Our study hypothesized that the supplementation strategy with PRE during the growing phase affect the lipid content of bulls measured at the end finishing phase, due to greater energy supply which increased the growth rate in these animals. The strategy of feed during the growth phase did not affect the composition of meat of bulls measured at the finishing phase, mainly the lipid content how was expected, this can be explained by hot carcass weight (HCW), which also did not affect by growing phase since the HCW can influence the marbling score in meat (Lancaster *et al.*, 2014). Another explanation may be related to the possibility of these animals fed with MIN during the growing phase, may have diluted the effect of the growth phase on the finishing phase, since that these animals were finished with intensive supplementation during 120 days.

In addition, it was expected that the bulls finished in PAST could have intramuscular fat similar to the bulls finished in FLOT, which is not found in this study, the higher lipid content in meat was found in animals finished in FLOT than PAST with intensive supplementation, since the

concentrate intake was similar between finishing system (15.8 g/kg BW/animal/ day), possibly the corn silage can have provided plus energy, compared to the grass and it higher metabolizable energy intake by animals from FLOT compared to PAST (11.32 vs 10.93 MJ/kg DM; data not shown), which may result in more fat content in meat. In addition, the bulls from pasture system, even receiving concentrate supplementation spend more time with activity related with feeding time (Segabinazzi et al., 2014), since that, in addition to the concentrate feed, these bulls need greater displacement, and collection and selection of pasture (source of roughage), which promotes greater energy expenditure than feedlot. This is also confirmed by the higher carcass weight of the animals in the FLOT system than PAST (297.49 vs 277.79 kg). According to Scollan *et al.* (2014), meat with 2 to 3% intramuscular fat content is considered "low fat" meat, which might be due to the presence of Nellore genes that can result in a low fat deposition (Teixeira *et al.*, 2017). In addition, due to higher fat content, moisture values for FLOT group were the lowest when compared to PAST system, supporting these results was observed a negative correlation between lipid and moisture content.

Lipogenesis in animals occurs by lipid absorbed from the diet, and the *de novo* synthesis of fatty acid (Dong et al., 2014). In this context, the higher concentration of total SFA acid was observed in the meat from bulls fed with MIN following by FLOT system, this was the result of increased lauric, myristic, and palmitic found in this group. The increased of this fatty acid in meat from bulls fed MIN following by FLOT since that corn silage diet showed lower total SFA compared to pasture composition, this suggests that these increased can be by higher *de novo* synthesis of fatty acid, and higher enzyme activity of fatty acid synthase (not measured in this study), which possibly increased isomers of saturated fatty acids (Laliotis *et al.*, 2010), which is a key enzyme in the lipogenic pathway that catalyzes the reactions of the fatty acid biosynthesis, conversion of acetyl-CoA and malonyl-CoA to palmitic acid. This is supporting a strong positive correlation between all saturated fatty acids (Figure 5).

In addition, the isocitrate dehydrogenase plays a crucial role in lipid metabolism, which catalyzes the conversion of oxidative decarboxylation of isocitrate to α-ketoglutarate with the production of NADPH (Ingle *et al.*, 1972) for fatty acid in *de novo* synthesis, this enzyme showed lower level in the meat of animals from PAST than FLOT, this suggested which the FLOT system can have provided higher energy, since that this enzyme can be reduced by increasing energy level (Lee *et al.*, 2020).

The same interaction was also found for myristoleic, which was higher in meat of bulls fed MIN following by FLOT system. However, the increase of MUFA total in feedlot group was due to an increase of palmitoleic, heptadecenoic, and oleic in meat these animals. These results agree to Patino *et al.* (2015), who worked with crossbreed steers, found a higher total concentration of MUFA acid in the meat of animals from feedlot system compared to meat of animals finished in pasture with supplementation, the same was observed in our study. The increase in monounsaturated acid, as oleic acid is related to meat palatability (Frank *et al.*, 2016), where oleic fatty acid represents the largest amount of monounsaturated acid in meat (Smith *et al.*, 2006), which agrees with our results regardless of the treatments.

High concentrations of oleic and palmitoleic acid were observed in the meat of animals from FLOT, these results are due to the activity of the SCD1 enzyme, which was also seen higher values of the concentration of this gene in FLOT group, a key enzyme which catalyzes the desaturation of a range of fatty acyl-CoA substrates, mainly palmitoyl and stearoyl and resulting on palmitoleic and oleic, respectively (Wood *et al.*, 2008). According to Smith *et al.* (2009), adipose tissue accumulates monounsaturated fatty acids coincides with an increase of SCD1 gene expression (Δ9 desaturase enzyme).

The two most common PUFA are linoleic and α-linolenic acid (18:3 n-3). Animals finished exclusively feed with grass or grass plus supplement show greater α-linolenic fatty acid in meat (Fruet *et al.*, 2016). Although an increase of α-linolenic fatty acid in the meat of animals finished in PAST was expected, since α-linolenic levels were higher in grass roughage than corn silage, the animals from this study showed similar concentration between treatments.

In addition, the corn silage had higher linoleic concentration than grass, however, the animals from PAST system showed a higher concentration of this acid in meat than FLOT (128.98 vs 105.14 mg/ 100 g of meat). These results suggest that higher among linoleic diet in FLOT system may have been following by higher activity ruminal biohydrogenation of this fatty acid (Wood *et al.*, 2008a) in these animals. In our study, linoleic acid was the dominant PUFA in meat regardless of the treatment, due linoleic is the main PUFA in plants that are used in animal diets (Wood and Enser, 2017).

Conjugated linoleic acid (Smith *et al.*) are formed in the rumen as intermediates in the biohydrogenation of long-chain unsaturated fatty acids (Kepler and Tove, 1967). French *et al.* (2000) showed that decreasing grain amount in the diet increased the CLA concentration in the

intramuscular fat in meat of crossbred steers. However, this was not found in our study, once that both CLA isomers (c9t11 and t10c12), was similar between treatments. In addition, the γ-linolenic acid (C18:3n6), eicosatrienoic, and arachidonic acid can be produced from linoleic acid by the action of desaturase and elongase enzymes, while the eicosapentaenoic acid and docosahexaenoic acid are producing from linoleic acid (Wood and Enser, 2017). In this context, the increase of the linoleic acid in the meat of bulls from PAST system also coincided with an increase of n6 isomers as arachidonic, eicosatrienoic, and eicosapentaenoic fatty acid. However, the linolenic acid was similar between treatments, an increase of eicosapentaenoic in the meat of bulls from PAST system can be related to higher action of desaturase and elongase in these animals.

The n3 total showed similar among treatment, had means of 11.40 mg/100 g of meat. The finishing of bulls on grass or with high level of roughage decrease n6/n3 ratio (Fruet et al., 2016), which is considered "beneficial" for human health (Wood *et al.*, 2008). Although, the animals from PAST system showed increased n6 total concentration than FLOT, and this provided higher n6/n3 ratio in this group. Thus, the PAST increased the linoleic, and eicosatrienoic and arachidonic, which led to a significant increase in the n6/n3 ratio.

The PPARγ is able to regulate biological processes such as lipid metabolism, on the other hand, and they are more expressed in adipose and participate in the process of adipogenesis and insulin sensitivity (Bionaz *et al.*, 2013). In our study, the PPARγ showed lowest expression level in the meat of bulls fed MIN following by PAST system, which even the intramuscular was similar to PAST group regardless of the supplementation strategy during the growing phase, this group showed numerically lower lipid content (1.44 vs 1.54g 100g of meat, respectively), possibly the MIN supplementation which provided less growth rate following the PAST finishing was not enough to increase the PPARγ.

CLA-C18:2t10c12 can be produced in high concentrate diet, due to the rumen pH reduction that increases the synthesis of C18:2t10c12, which may decrease the relative abundance of the gene responsible to encode sterol regulatory element-binding protein (Obsen *et al.*, 2012, Teixeira *et al.*, 2017), consequently contribute to a reduction in fat deposition. However, this results was not found in this work, our findings showed that the FLOT system increased the SREBP1c, it was not in response to C18:2t10c12 acid since that, it was similar concentration between treatments, it suggests that the mechanisms that regulated SREBP1c expression and fat deposition in our study were other.

The increase of n3 and n6 isomers may have been responsible for decreasing SREBP1c expression, which is the main gene that controlling lipogenesis (Rodríguez-Cruz and Serna, 2017). Therefore, the PAST system provided greater concentrations of eicosatrienoic, arachidonic and eicosapentaenoic in the muscle, which resulted in decreased intramuscular fat concentrations in meat, as corroborated by the negative correlation between SREPB1c and C20:4n6 ($P = 0.009$, $r = -0.30$). In addition, the energy available is a major factor in the gene expression that is related to fat deposition (Yang et al., 2020). Although not measured in this study, the FLOT system may have possibly resulted in higher blood insulin and glucose concentrations, in this respect, the increase of insulin may have increased SREBP1c values, since this gene is associated with two pathway (Figure 3), the insulin signaling and AMPK signaling, according to this, insulin can control the SREBP1c abundance and induces *de novo* lipogenesis (Botolin *et al.*, 2006).

The ACCα is related to the first step fatty acid synthesis, enzyme carboxylation of acetyl CoA into malonyl CoA in response to diet and hormone (Brownsey *et al.*, 2006). Although the ACCα was similar expression between the treatments, possible *de novo* synthesis may have occurred by increased to the other enzyme activity, as fatty acid synthase which is related to fatty acid biosynthesis.

In addition, the SCD1 is associated with the biosynthesis of unsaturated fatty acids and liked with SREBP1c by AMPK and insulin signaling pathways (Figure 6), as expected, considering the finishing system effect the SREBP1c expression, the SCD1 was up-regulated in muscle from FLOT group. This protein participates in the biosynthesis of unsaturated fatty acids, its main function is to introduce a double bond in stearic and palmitic acid converting them into oleic and palmitoleic acids, respectively (Wood *et al.*, 2008, ALJohani *et al.*, 2017). According to our results, in general, higher concentrations of oleic and palmitoleic acids were observed in FLOT group, where higher SCD1 gene expression was also observed. Although an increase of SCD1 in muscle from animals supplemented with PRE during the growing phase, this did not coincide with the effect of the growing phase on palmitoleic or oleic fatty acid in the meat of these animals at the finishing phase.

The transport to adipocytes into the cells is facilitated by the FABP4 (Hertzel *et al.*, 2006). The highest FABP4 expression in muscle from bulls finished in FLOT may have been due to the difference of roughage ether extract level between PAST and FLOT system, thus the highest amount of ether extract in the diet of animals from FLOT possible provided higher ether extract

intake, and may have an increase of FABP4 gene expression in the meat of animals from this system. In addition, the FABP4 had a positive correlation with PPARγ and SREBP1c.

Although PPARα is more expressed in the liver (Bunger *et al.*, 2007), this enzyme is responsible for the peroxisome proliferator-induced responses, including the transcriptional activation of genes involved in fatty acid oxidation (Bionaz *et al.*, 2013). In this way, the PPARα gene was less expressed in meat of bulls fed MIN following by FLOT muscle than MIN following by PAST group, suggesting that bulls fed with MIN (low growth rate) during the growing phase following by finishing in PAST had higher lipid oxidation rate. In addition, was observed a negative correlation between lipid content and PPARα. Another enzyme that is also related to mitochondrial long-chain fatty acid oxidation is the CPT2, in this study higher gene expression was found in the meat of bulls fed PRE following by FLOT and MIN following by PAST.

Fat deposition occurs in response to the activation of metabolic actions such as lipogenesis and lipolysis, which occur according to the need for lipid release or storage, as well as the interaction of dietary nutrients and the level of expression of the gene's relationship in lipid (Teixeira *et al.*). In this respect, even if the hypothesis of the work has not been proven, the supplementation strategy during the growing phase and finishing system affected the gene expression in this study. The treatment PRE following by FLOT showed the greater expression level of genes related to lipid synthesis as SREBP1c, and also had an increase of genes related with β-oxidation (CPT2) which possibly led to similar levels of lipid content to bulls fed MIN following by FLOT system. On the other hand, the PRE supplement during the growing phase following of the PAST system was not enough to increase the intramuscular fat content, since which these bulls had lower CPT2 level but also a decreased of SREPB, which may have prevented the deposition lipid content. In addition, the bulls fed MIN following by PAST showed numerically lower lipid content, which was followed by increased the PPARα, CPT2 (degradation), and decreased SREBP1c, therefore this treatment can have provided a lower energy supply.

5. Conclusion

Our results suggest which, the meat of bulls from pasture system plus concentrate supplementation did not have a higher percentage of n-3 fatty acids and a lower and more desirable n6/n3 ratio, which it did not show the meat with fatty acid profile considered more “beneficial” to human health

than feedlot system. In addition, the feedlot system improved the lipid content in meat and, although the growing phase did not affect the content lipid, the mineral supplementation following the pasture system up-regulated the lipolytic genes and down-regulated the lipogenic genes.

6. Reference

ALJohani AM, Syed DN and Ntambi JM 2017. Insights into stearoyl-CoA desaturase-1 regulation of systemic metabolism. Trends in Endocrinology and Metabolism 28, 831-842.

Bindea G, Mlecnik B, Hackl H, Charoentong P, Tosolini M, Kirilovsky A, Fridman W-H, Pagès F, Trajanoski Z and Galon J 2009. ClueGO: a Cytoscape plug-in to decipher functionally grouped gene ontology and pathway annotation networks. Bioinformatics 25, 1091-1093.

Bionaz M, Chen S, Khan MJ and Loor JJ 2013. Functional role of PPARs in ruminants: potential targets for fine-tuning metabolism during growth and lactation. PPAR research 2013.

Bligh EG and Dyer WJ 1959. A rapid method of total lipid extraction and purification. Canadian Journal of Biochemistry and Physiology 37, 911-917.

Botolin D, Wang Y, Christian B and Jump DB 2006. Docosahexaneoic acid [22: 6, n-3] regulates rat hepatocyte sterol regulatory element binding protein-1 (SREBP-1) nuclear abundance by Erk-and 26S proteasome-dependent pathways. J. Lipid Res 47, 181-192.

Brownsey R, Boone A, Elliott J, Kulpa J and Lee W 2006. Regulation of acetyl-CoA carboxylase. In Portland Press Ltd.

Bunger M, van den Bosch HM, van der Meijde J, Kersten S, Hooiveld GJ and Muller M 2007. Genome-wide analysis of PPARα activation in murine small intestine. Physiological genomics 30, 192-204.

Christie W 1984. Extraction and hydrolysis of lipids and some reactions of their fatty acid components. Handbook of Chromatography, Lipids 1, 33-46.

Delevatti LM, Romanzini EP, Koscheck JFW, de Araujo TLdR, Renesto DM, Ferrari AC, Barbero RP, Mulliniks JT and Reis RA 2019. Forage management intensification and supplementation strategy: Intake and metabolic parameters on beef cattle production. Animal Feed Science and Technology 247, 74-82.

Detmann E, Paulino MF, de Campos Valadares Filho S and Huhtanen P 2014. Nutritional aspects applied to grazing cattle in the tropics: a review based on Brazilian results. Semina: Ciências Agrárias 35, 2829-2854.

Dong G-F, Zou Q, Wang H, Huang F, Liu X-C, Chen L, Yang C-Y and Yang Y-o 2014. Conjugated linoleic acid differentially modulates growth, tissue lipid deposition, and gene expression involved in the lipid metabolism of grass carp. Aquaculture 432, 181-191.

Ferrinho AM, Peripolli E, Banchero G, Pereira ASC, Brito G, La Manna A, Fernandez E, Montossi F, Kluska S and Mueller LF 2020. Effect of growth path on carcass and meat-quality traits of Hereford steers finished on pasture or in feedlot. Animal Production Science 60, 323-332.

Frank D, Ball A, Hughes J, Krishnamurthy R, Piyasiri U, Stark J, Watkins P and Warner R 2016. Sensory and flavor chemistry characteristics of Australian beef: influence of intramuscular fat, feed, and breed. Journal of Agricultural and Food Chemistry 64, 4299-4311.

French P, Stanton C, Lawless F, O'riordan E, Monahan F, Caffrey P and Moloney A 2000. 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 78, 2849-2855.

Fruet APB, Stefanello FS, Júnior AGR, de Souza ANM, Tonetto CJ and Nörnberg JL 2016. Whole grains in the finishing of culled ewes in pasture or feedlot: Performance, carcass characteristics and meat quality. Meat Science 113, 97-103.

Hertzel AV, Smith LA, Berg AH, Cline GW, Shulman GI, Scherer PE and Bernlohr DA 2006. Lipid metabolism and adipokine levels in fatty acid-binding protein null and transgenic mice. American Journal of Physiology-Endocrinology and Metabolism 290, E814-E823.

Humada M, Sañudo C and Serrano E 2014. Chemical composition, vitamin E content, lipid oxidation, colour and cooking losses in meat from Tudanca bulls finished on semi-extensive or intensive systems and slaughtered at 12 or 14 months. Meat Science 96, 908-915.

Ingle DL, Bauman D and Garrigus U 1972. Lipogenesis in the ruminant: in vitro study of tissue sites, carbon source and reducing equivalent generation for fatty acid synthesis. The Journal of nutrition 102, 609-616.

Kepler CR and Tove S 1967. Biohydrogenation of unsaturated fatty acids III. Purification and properties of a linoleate Δ12-cis, Δ11-trans-isomerase from Butyrivibrio fibrisolvens. Journal of Biological Chemistry 242, 5686-5692.

Laliotis G, Bizelis I and Rogdakis E 2010. Comparative approach of the de novo fatty acid synthesis (lipogenesis) between ruminant and non ruminant mammalian species: from biochemical level to the main regulatory lipogenic genes. Current genomics 11, 168-183.

Lancaster P, Krehbiel C and Horn G 2014. Ameta-analysis of effects of nutrition and management during the stocker and backgrounding phase on subsequent finishing performance and carcass characteristics. The Professional Animal Scientist 30, 602-612.

Lee J-H, Go Y, Kim D-Y, Lee SH, Kim O-H, Jeon YH, Kwon TK, Bae J-H, Song D-K and Lee I-K 2020. Isocitrate dehydrogenase 2 protects mice from high-fat diet-induced metabolic stress by limiting oxidative damage to the mitochondria from brown adipose tissue. Experimental & Molecular Medicine 52, 238-252.

Obsen T, Faergeman NJ, Chung S, Martinez K, Gobern S, Loreau O, Wabitsch M, Mandrup S and McIntosh M 2012. Trans-10, cis-12 conjugated linoleic acid decreases de novo lipid synthesis in human adipocytes. The Journal of nutritional biochemistry 23, 580-590.

Owens FN, Dubeski P and Hanson C 1993. Factors that alter the growth and development of ruminants. Journal of animal science 71, 3138-3150.

Patino H, Medeiros F, Pereira C, Swanson K and McManus C 2015. Productive performance, meat quality and fatty acid profile of steers finished in confinement or supplemented at pasture. Animal 9, 966-972.

Rodríguez-Cruz M and Serna DS 2017. Nutrigenomics of ω-3 fatty acids: Regulators of the master transcription factors. Nutrition 41, 90-96.

Roth M, Resende F, Oliveira I, Fernandes R, Custódio L and Siqueira G 2017. Does supplementation during previous phase influence performance during the growing and finishing phase in Nellore cattle? Livestock Science 204, 122-128.

Sampaio RL, de Resende FD, Reis RA, de Oliveira IM, Custódio L, Fernandes RM, Pazdiora RD and Siqueira GR 2017. The nutritional interrelationship between the growing and finishing phases in crossbred cattle raised in a tropical system. Tropical animal health and production 49, 1015-1024.

Schoonmaker J, Cecava M, Faulkner DB, Fluharty F, Zerby H and Loerch S 2003. Effect of source of energy and rate of growth on performance, carcass characteristics, ruminal fermentation, and serum glucose and insulin of early-weaned steers. Journal of animal science 81, 843-855.

Scollan ND, Dannenberger D, Nuernberg K, Richardson I, MacKintosh S, Hocquette J-F and Moloney AP 2014. Enhancing the nutritional and health value of beef lipids and their relationship with meat quality. Meat Science 97, 384-394.

Smith S, Blackmon T, Sawyer J, Miller R, Baber J, Morrill J, Cabral A and Wickersham T 2018. Glucose and acetate metabolism in bovine intramuscular and subcutaneous adipose tissues from steers infused with glucose, propionate, or acetate. Journal of Animal Science 96, 921-929.

Smith SB, Gill CA, Lunt DK and Brooks MA 2009. Regulation of fat and fatty acid composition in beef cattle. Asian-Australasian Journal of Animal Sciences 22, 1225-1233.

Smith SB, Lunt DK, Chung KY, Choi CB, Tume RK and Zembayashi M 2006. Adiposity, fatty acid composition, and delta-9 desaturase activity during growth in beef cattle. Animal Science Journal 77, 478-486.

Teixeira PD, Oliveira DM, Chizzotti ML, Chalfun-Junior A, Coelho TC, Gionbelli M, Paiva LV, Carvalho JRR and Ladeira MM 2017. Subspecies and diet affect the expression of genes involved in lipid metabolism and chemical composition of muscle in beef cattle. Meat Science 133, 110-118.

Valadares Filho SdC, Marcondes M, Chizzotti M and Paulino P 2016. Exigências nutricionais de zebuínos puros e cruzados BR-CORTE. UFV, Suprema Gráfica Ltda: Viçosa, Brazil.

Vandesompele J, De Preter K, Pattyn F, Poppe B, Van Roy N, De Paepe A and Speleman F 2002. Accurate normalization of real-time quantitative RT-PCR data by geometric averaging of multiple internal control genes. Genome Biology 3, research0034. 0031.

Wood J and Enser M 2017. Manipulating the fatty acid composition of meat to improve nutritional value and meat quality. In New Aspects of Meat Quality, pp. 501-535, Elsevier.

Wood JD, Enser M, Fisher AV, Nute GR, Sheard PR, Richardson RI, Hughes SI and Whittington FM 2008. Fat deposition, fatty acid composition and meat quality: A review. Meat Science 78, 343-358.

Table 1. Chemical composition of the experimental diets.

Items	Growing phase		Finishing phase		
	PRE[1]	Pasture	Concentrate[2]	Pasture	Corn silage
Ingredients, g/kg DM					
Corn	735.0	-	789.0	-	-
Soybean meal	106.0	-	165.0	-	-
Mineral premix	159.0	-	46.0	-	-
Chemical composition					
Dry matter	860.0	332.0	899.0	458.0	301.0
Organic matter	892.0	925.0	910.0	923.0	950.0
Crude protein	205.0	128.0	160.0	113.0	95.0
Neutral detergent fiber	265.0	575.0	251.0	582.0	331.0
Ether extract.	63.0	24.1	66.0	23.0	22.0
Fatty acid (g/100 g of total FA)					
Myristic (C14:0)	0.08	1.30	0.08	3.09	0.27
Palmitic (C16:0)	11.20	36.48	11.17	35.29	17.65
Margaric (C17:0)	0.09	0.49	0.09	0.65	0.22
Stearic (C18:0)	3.94	3.60	3.76	4.16	3.46
Arachidic (C20:0)	0.38	0.99	0.38	1.71	0.84
Behenic (C22:0)	0.45	1.28	0.44	2.03	0.44
Lignoceric (C24:0)	0.18	2.12	0.20	3.02	0.78
Palmitoleic (C16:1)	0.12	0.46	0.09	0.42	0.23
Oleic (C18:1n9c)	28.40	4.35	29.86	6.17	34.20
Linoleic (C18:2n6c)	48.64	14.77	47.65	14.06	36.52
α-Linolenic (C18:3n3)	4.62	30.33	4.40	22.56	3.69
SFA	16.30	46.26	16.12	49.93	23.66
MUFA	28.52	4.81	29.95	6.59	34.43
PUFA	53.25	45.10	52.06	36.61	40.21

[1]Sodium 80 g/kg; Calcium 153 g/kg; Phosphorus 30 g/kg; Sulfur 30 g/kg; Zinc 1925 mg/kg; Copper 520 mg/kg; Manganese 400 mg/kg; Iodine 30 mg/kg; Cobalt 38 mg/kg; Selenium 10 mg/kg; Monensin 400 mg/kg; NNP 620g /kg.

[2] Sodium 40 g/kg; Calcium 110 g/kg; Phosphorus 20 g/kg; Potassium 40 g/kg; Magnesium 56 g/kg; Sulfur 31 g/kg; Zinc 700 mg/kg; Copper 190 mg/kg; Fluorine 333 g/kg; Manganese 550 mg/kg; Iodine 14 mg/kg; Cobalt 12 mg/kg; Selenium 3.5 mg/kg; Vit A 55000 Ul/kg; Vit D3 7500 Ul/kg; Vit E 750 Ul/kg; Monensin 500 mg/kg; NNP 620g /kg.

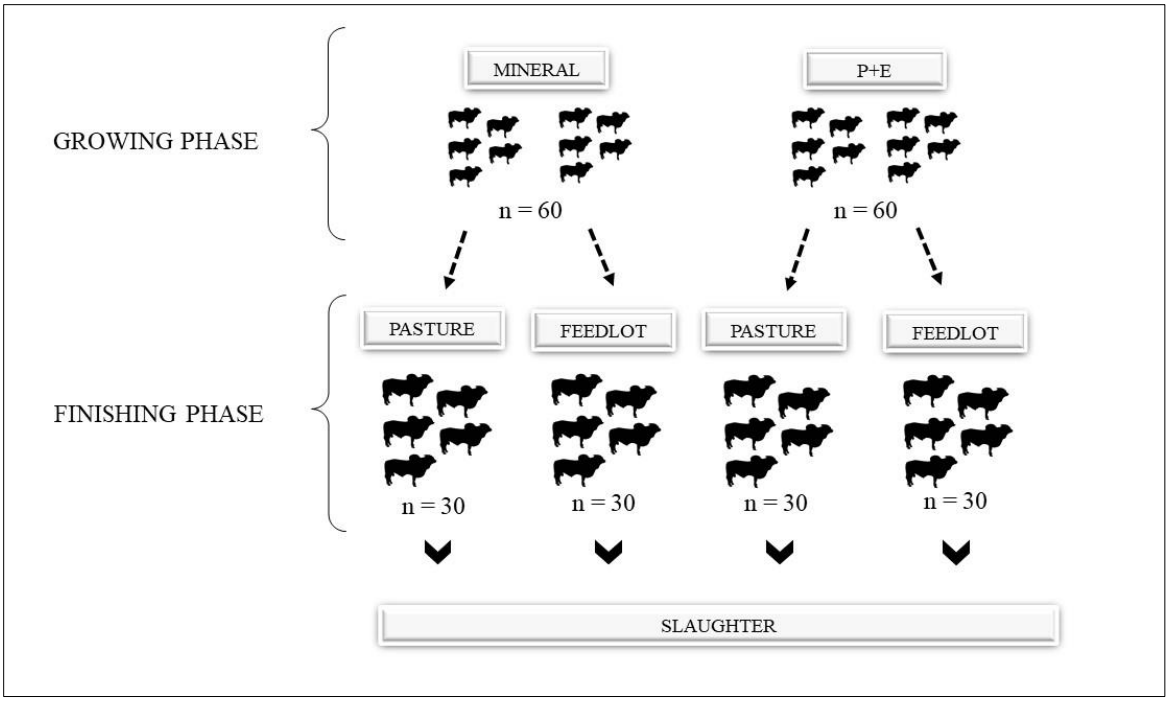

Figure 1. Experimental design
Mineral supplementation (*ad libitum*); Protein + Energy (3g/kg BW/day); Feedlot system (25:75; corn silage: concentrate); Pasture plus concentrate (20g/kg BW/day).

Table 2. Primer sequences used for quantitative RT-PCR analyses

Gene	Target gene (5'-3')	*name*	R2	Efficiency
	Forward (F) and Reverse (R)			
PPARγ	**F:** CGATATCGACCAACTGAACC **R:** AACGGTGATTTGTCTGTCGT	**Peroxisome proliferator-activated receptor gamma**	0.992	90.788
SREBP-1c	**F:** GAGCCACCCTTCAACGAA **R:** TGTCTTCTATGTCGGTCAGCA	**Sterol regulatory element-binding protein-1c**	0.999	100.593
SCD1	**F:** TTATTCCGTTATGCCCTTGG **R:** TTGTCATAAGGGCGGTATCC	**Stearoyl-CoA desaturase**	0.997	94.776
ACCα	**F:** TGAAGAAGCAATGGATGAACC **R:** TTCAGACACGGAGCCAATAA	**Acetyl CoA carboxylase alfa**	0.998	101.32
LPL	**F:** CTCAGGACTCCCGAAGACAC **R:** GTTTTGCTGCTGTGGTTGAA	**Lipoprotein lipase**	0.993	94.257
FABP4	**F:** GGATGATAAGATGGTGCTGGA **R:** ATCCCTTGGCTTATGCTCTCT	**Fatty acid binding protein 4**	0.997	90.259
ACOX	**F:** GCTGTCCTAAGGCGTTTGTG **R:** ATGATGCTCCCCTGAAGAAA	**Acyl-CoA oxidase 1**	0.991	90.993
CPT2	**F:** CATGACTGTCTCTGCCATCC **R:** ATCACTTTTGGCAGGGTTCA	**Carnitine Palmitoyltransferase 2**	0.991	94.577
PPARα	**F:** CAATGGAGATGGTGGACACA **R:** TTGTAGGAAGTCTGCCGAGAG	**Peroxisome proliferator-activated receptor α**	0.994	91.665
β-Actin	**F**: GTCCACCTTCCAGCAGATGT **R**: CAGTCCGCCTAGAAGCATTT	**β-actin**	0.998	93.059
GAPDH	**F**: CGACTTCAACAGCGACACTC **R**: TTGTCGTACCAGGAAATGAGC	**Glyceraldehyde 3 phosphate**	0.994	92.896

Table 3. Lipid, Ash, Protein and Moisture (%) of *Longissimus thoracis* of young bulls supplemented with mineral or protein + energy during the growing phase and finished in different systems.

Finishing system	FLOT[1]		PAST[2]		SEM[5]	GP[6]	FS[7]	GP×FS
Growing phase	MIN[3]	PRE[4]	MIN[3]	PRE[4]				
Lipid	2.63	2.80	1.44	1.54	0.233	0.441	<0.001	0.867
Ash	2.22	2.48	2.65	2.52	0.168	0.622	0.069	0.575
Protein	22.32	22.41	21.99	22.38	0.260	0.229	0.359	0.448
Moisture	72.81	72.28	73.93	73.55	0.392	0.135	<0.001	0.475

[1]Feedlot system (25:75; corn silage: concentrate); [2]Pasture plus concentrate supplementation (20g/kg BW/day); [3]Mineral supplementation (*ad libitum*); [4]Protein + energy (3g/kg BW/day); [5]Standard error of mean; [6] Growing phase; [7]Finishing phase.

Table 4. Fatty acid profile (mg/100g of meat) in the *Longissimus thoracis* of young bulls supplemented with mineral or protein + energy during the growing phase and finished in different systems.

Finishing system	FLOT[1]		PAST[2]		SEM[5]	GP[6]	FS[7]	GP×FS
Growing phase	MIN3	PRE[4]	MNI3	PRE[4]				
C12:0	1.00a	0.64b	0.73ab	0.81ab	0.080	0.151	0.618	0.005
C14:0	69.49a	43.82b	39.80b	42.91b	4.816	0.037	0.002	0.004
C15:0	5.51	4.21	4.83	5.14	0.437	0.355	0.757	0.065
C16:0	668.98a	503.55b	401.07b	421.12b	39.196	0.097	<0.001	0.022
C17:0	21.04	15.65	14.46	15.00	1.442	0.133	0.010	0.051
C18:0	383.55	377.29	305.63	350.84	26.535	0.437	0.059	0.367
C20:0	2.26	2.02	1.69	1.98	0.158	0.845	0.068	0.111
C14:1	15.34a	9.60b	10.48b	10.32b	1.197	0.028	0.071	0.024
C15:1	2.36	1.93	2.07	2.37	0.190	0.976	0.656	0.065
C16:1	73.43	54.49	48.91	49.30	5.050	0.094	0.003	0.058
C17:1	11.39	8.99	8.41	8.31	0.692	0.100	0.006	0.097
C18:1n9c	909.28	803.56	627.41	699.95	57.805	0.932	0.001	0.135
C18:2c9t11	16.42	13.55	16.10	15.91	0.924	0.098	0.173	0.119
C18:2t10c12	39.90	36.97	22.26	38.02	7.741	0.382	0.234	0.261
C18:2n6c	107.45	102.83	130.64	127.32	5.359	0.285	<0.001	0.628
C18:3n3	1.09	1.00	1.11	1.10	0.054	0.261	0.196	0.341
C18:3n6	7.75	6.83	7.98	7.73	0.439	0.176	0.145	0.394
C20:2n6	1.66	1.78	1.53	1.49	0.088	0.622	0.030	0.484
C20:3n6	4.07	4.49	5.59	5.11	0.277	0.680	<0.001	0.168
C20:4n6	17.80	20.82	26.99	26.23	1.146	0.501	<0.001	0.174
C20:5n3	2.33	2.62	2.74	2.70	0.120	0.284	0.030	0.177
C22:6n3	0.82	0.73	0.48	0.65	0.086	0.657	0.017	0.174
SFA	1169.20a	941.27ab	768.35b	831.82b	65.736	0.295	<0.001	0.036
MUFA	1012.51	887.11	699.28	778.39	64.931	0.882	0.001	0.126
PUFA	207.88	208.94	216.51	229.29	8.732	0.582	0.063	0.359
n3	130.26	130.27	164.34	160.26	6.296	0.493	<0.001	0.968
n6	11.12	10.44	11.28	11.33	0.511	0.513	0.214	0.404
n6/n3	11.58	12.56	14.35	14.06	0.495	0.612	<0.001	0.284

[1]Feedlot system (25:75; corn silage: concentrate); [2]Pasture plus concentrate supplementation (20g/kg BW/day); [3]Mineral supplementation (*ad libitum*); [4]Protein + energy (3g/kg BW/day); [5]Standard error of mean; [6] Growing phase; [7]Finishing phase.

Table 5. Enzyme NADP-malate dehydrogenase and isocitrate dehydrogenase in the *Longissimus thoracis* muscle of young bulls fed mineral or protein + energy during the growing phase and finished in different systems.

Finishing system	FLOT[1]		PAST[2]		SEM[5]	GP[6]	FS[5]	GP×FS
Growing phase	MIN[3]	PRE[4]	MIN[3]	PRE[4]				
Isocitrate dehydrogenase, nmol/min	2803.26	2934.82	3518.32	3208.45	324.444	0.720	0.049	0.375
NADP-Malate dehydrogenase, nmol/min	47.17	46.51	48.56	43.61	4.877	0.455	0.839	0.565

[1]Feedlot system (25:75; corn silage: concentrate); [2]Pasture plus concentrate supplementation (20g/kg BW/day); [3]Mineral supplementation (*ad libitum*); [4]Protein + energy (3g/kg BW/day); [5]Standard error of mean; [6] Growing phase; [7]Finishing phase.

Table 6. Relative expression of lipogenic gene in the *Longissimus thoracis* muscle of young bulls fed mineral or protein + energy during the growing phase and finished in different systems.

Finishing system	FLOT[1]		PAST[2]		SEM[5]	GP[6]	FS[7]	GP×FS
Growing phase	MIN[3]	PRE[4]	MIN[3]	PRE[4]				
PPARγ	1.79a	1.47ab	1.08b	1.74a	0.133	0.223	0.122	0.001
SREBP	27.06a	20.15a	1.06b	8.86b	2.168	0.862	<0.001	0.005
SCD1	1.95	3.40	1.04	1.62	0.241	0.001	<0.001	0.169
ACACα	1.22	1.49	1.08	1.18	0.108	0.126	0.057	0.483
LPL	0.86	1.15	1.06	0.89	0.125	0.653	0.843	0.103
FABP4	2.33	3.24	1.06	1.41	0.315	0.058	<0.001	0.402
ACOX	1.01	0.95	1.07	1.06	0.075	0.602	0.278	0.733
CPT2	0.84ab	1.09a	1.05a	0.68b	0.089	0.513	0.279	0.001
PPARα	0.53c	0.66bc	1.06a	0.84ab	0.066	0.510	<0.001	0.013

[1]Feedlot system (25:75; corn silage: concentrate); [2]Pasture plus concentrate supplementation (20g/kg BW/day); [3]Mineral supplementation (*ad libitum*); [4]Protein + energy (3g/kg BW/day); [5]Standard error of mean; [6] Growing phase; [7]Finishing phase.

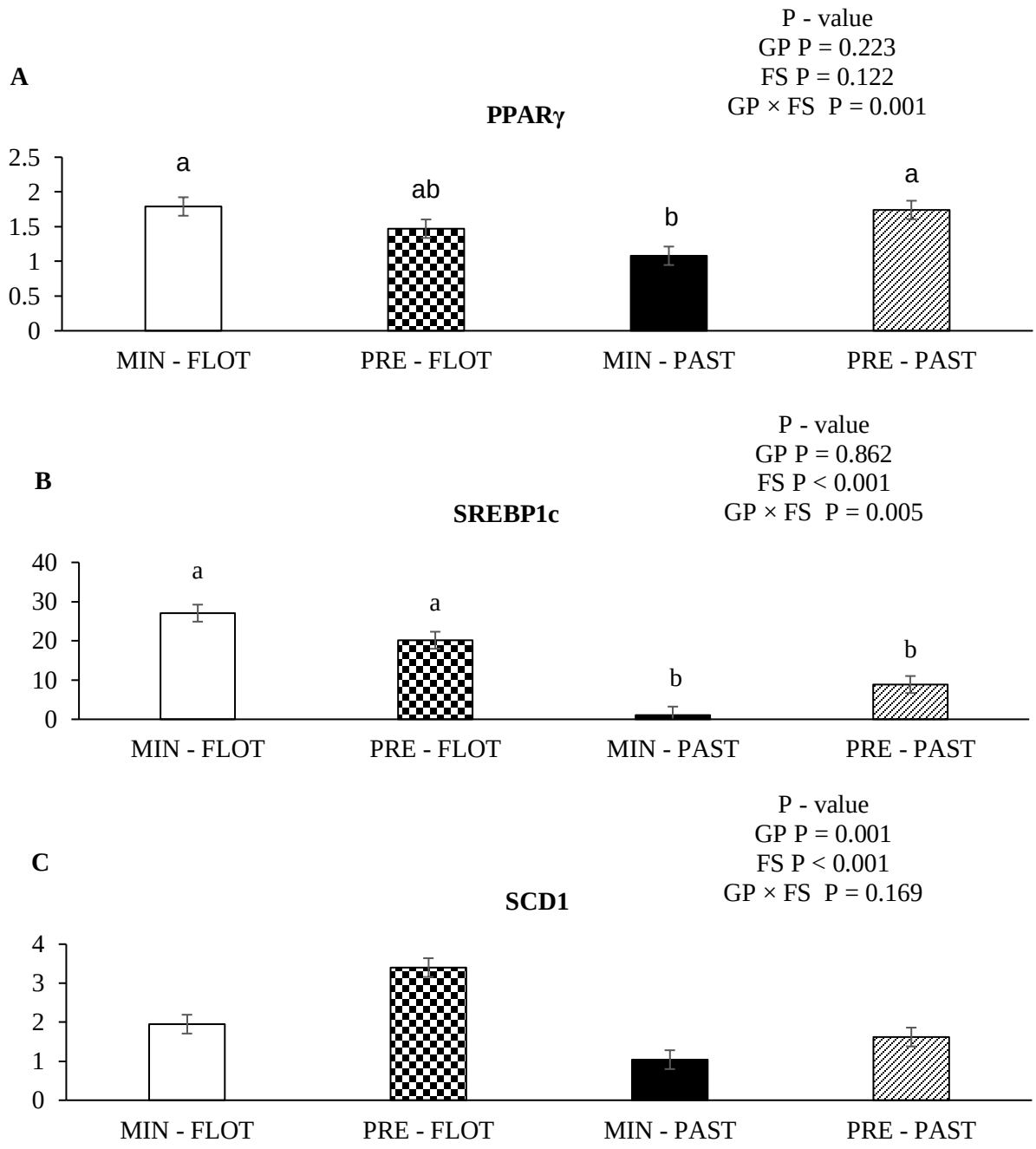

Figure 2. Relative expression of PPARγ (A) , SREBP1c (B) and SCD1 (C) in the *Longissimus thoracis* muscle of young bulls fed MIN (mineral supplementation - *ad libitum*) or PRE (protein + energy - 3 g/kg BW/day) during the growing phase and finished in PAST (20 g/kg BW/day of concentrate) or FLOT (25:75; corn silage: concentrate).
GP = Growing phase; FS = finishing system.

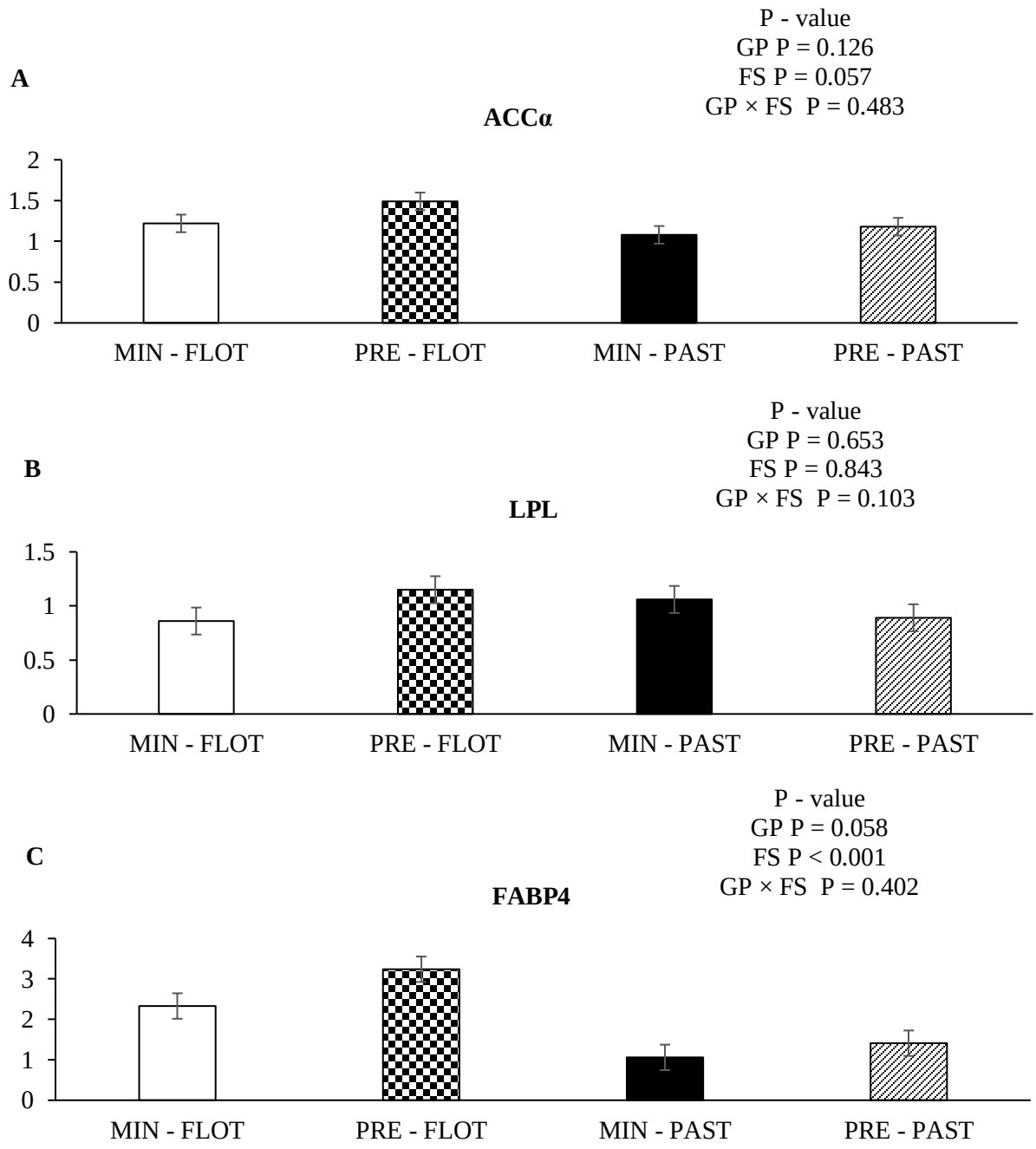

Figure 3. Relative expression of ACCα (A) , LPL (B) and FABP4 (C) in the *Longissimus thoracis* muscle of young bulls fed MIN (mineral supplementation - *ad libitum*) or PRE (protein + energy - 3 g/kg BW/day) during the growing phase and finished in PAST (20 g/kg BW/day of concentrate) or FLOT (25:75; corn silage: concentrate).
GP = Growing phase; FS = finishing system.

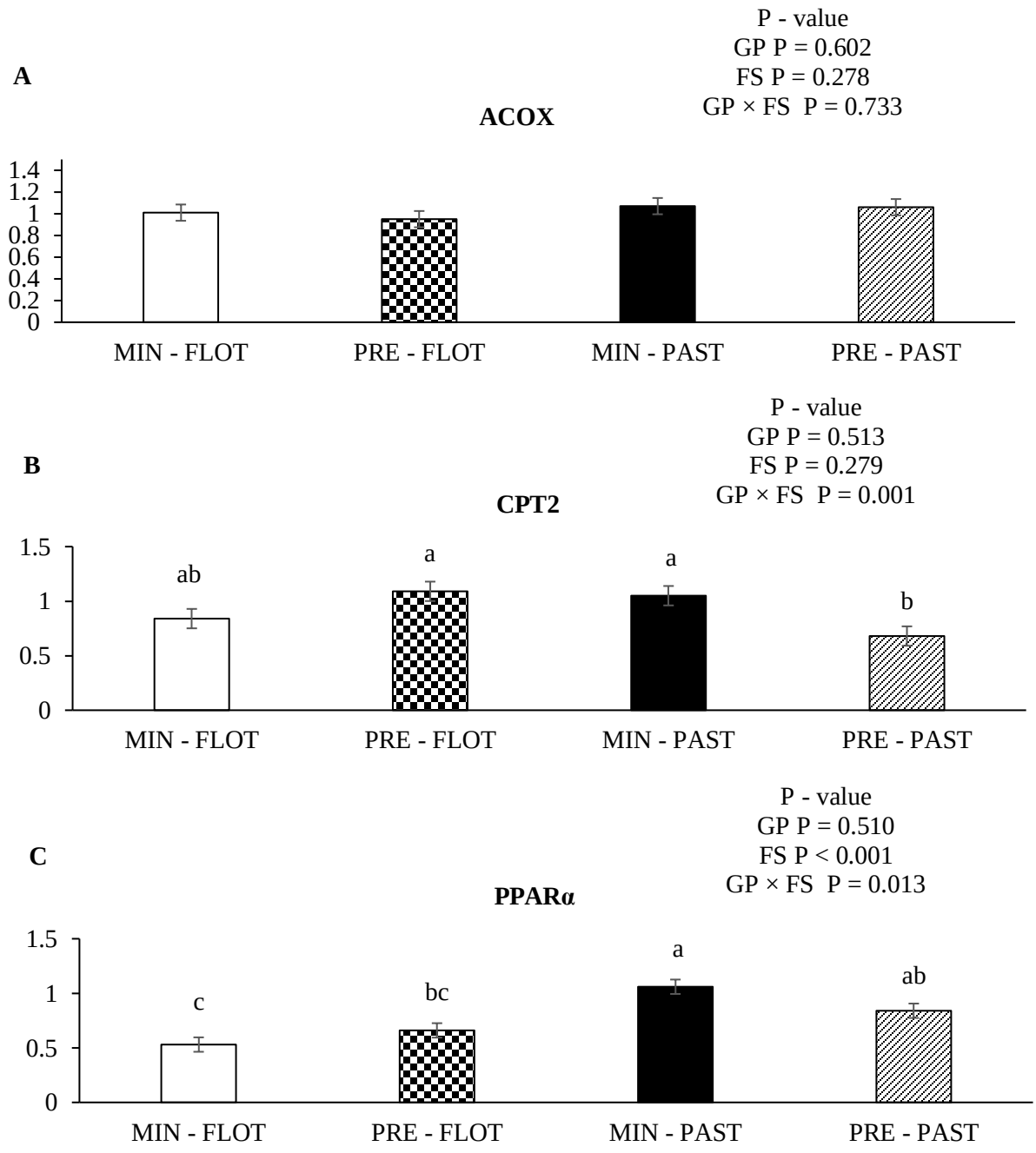

Figure 4. Relative expression of ACOX (A) , CPT2 (B) and PPARα (C) in the *Longissimus thoracis* muscle of young bulls fed MIN (mineral supplementation - *ad libitum*) or PRE (protein + energy - 3 g/kg BW/day) during the growing phase and finished in PAST (20 g/kg BW/day of concentrate) or FLOT (25:75; corn silage: concentrate).
GP = Growing phase; FS = finishing system.

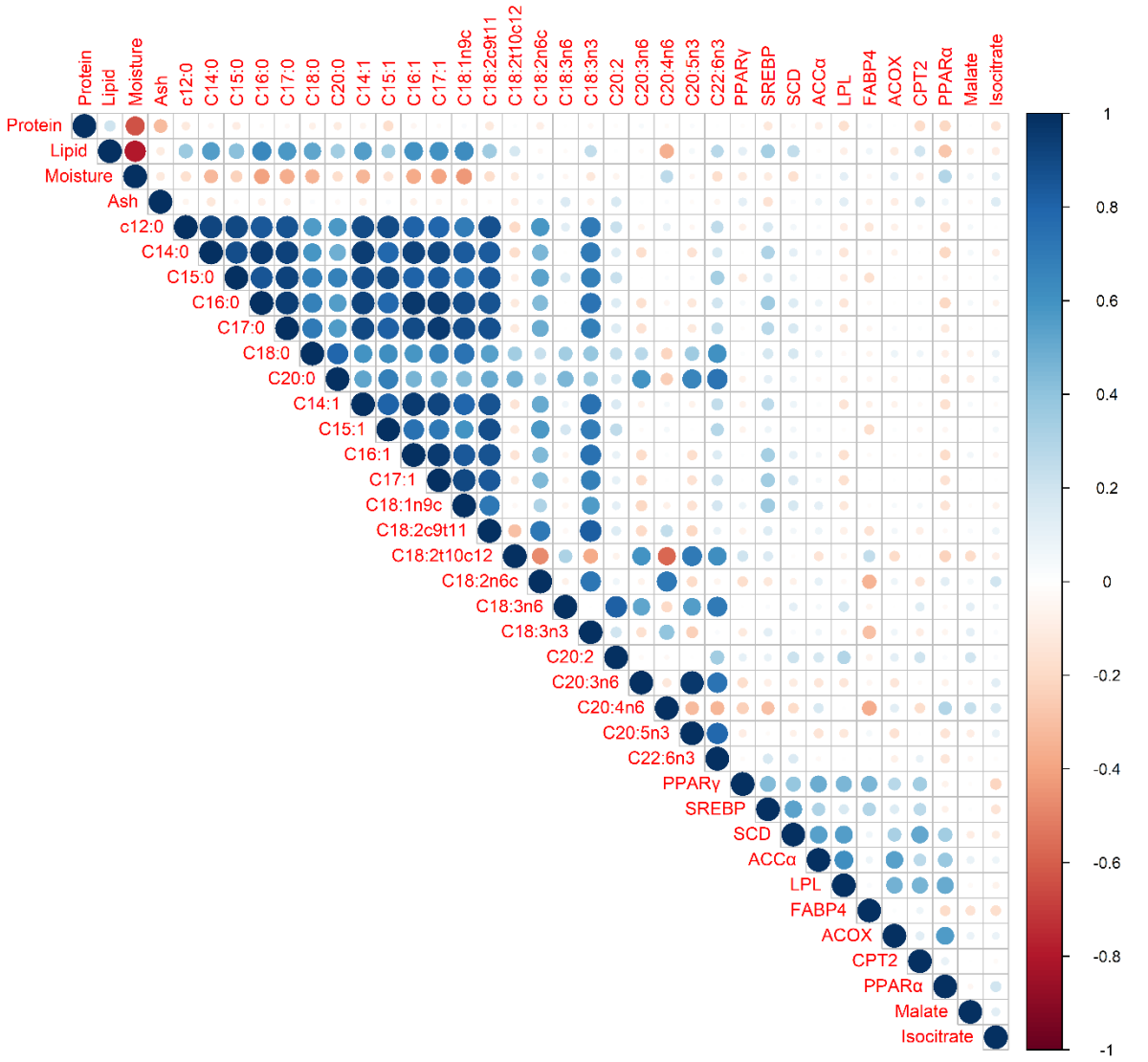

Figure 5. Correlation analysis among chemical composition, enzyme, fatty acid and gene expression of *Longissimus thoracis* muscle of bulls.

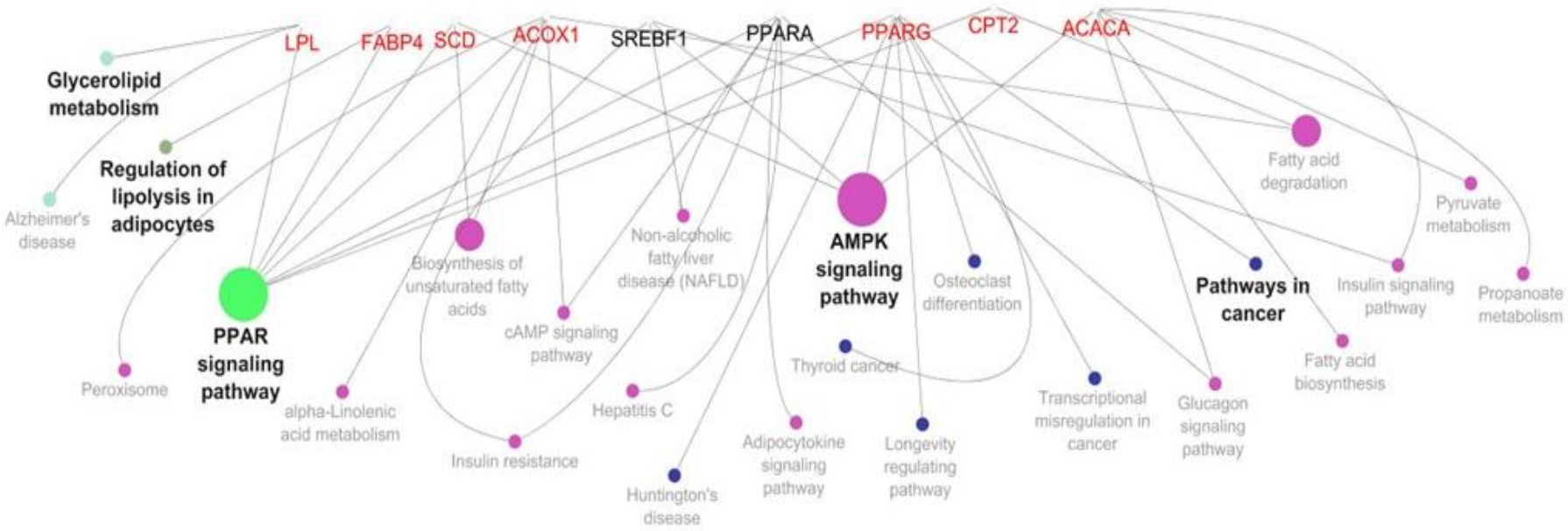

Figure 6. Biological process relationship of lipid metabolism genes