UNIVERSIDADE ESTADUAL PAULISTA JULIO DE MESQUITA FILHO

FACULDADE DE CIÊNCIAS AGRÁRIAS E VETERINÁRIAS – JABOTICABAL

NÍVEIS DE GRÃOS ÚMIDOS DE DESTILARIA DESENGORDURADO EM DIETAS PARA BOVINOS DE CORTE CONFINADOS: DESEMPENHO, CARACTERÍSTICAS DE CARCAÇA E QUALIDADE DE CARNE.

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Zootecnista

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Discente: Mateus Silva Ferreira

Orientador: Prof. Dr. Otávio Rodrigues Machado Neto

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.

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CERTIFICADO DE APROVAÇÃO

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Mateus Silva Ferreira, nascido Presidente Prudente/SP no dia 15 de agosto de 1991. Zootecnista formado pela Universidade Estadual de Maringá, Campus de Maringá/PR, onde obteve o título no ano de 2014. Em 2016, obteve o título de Mestre no programa de Pós-Graduação em Produção Sustentável e Saúde Animal pela Universidade Estadual de Maringá, Campus de Umuarama. Atualmente ocupa o cargo de Assistente Técnico Comercial pela empresa Nutron/Cargill.

“E mesmo que meus passos sejam falsos, mesmo que os meus caminhos sejam errados, mesmo que o meu jeito de levar a vida incomode, eu sei quem sou, e sei pelo que devo lutar. Se você acha que o meu orgulho é grande, é porque nunca viu o tamanho da minha fé!”

Tião Carreiro

Dedico essa conquista aos meus pais, Divino e Helena, aos meus irmãos Mayara e Manoel, meu sobrinho Diego e aos meus avós Divino e Maria do Carmo, com todo amor, respeito e gratidão. Aos meus pais por toda educação, honestidade, e valores ensinados em todos esses anos. Aos meus irmãos por todo o apoio cedido em minha vida. Aos meus avós, por me ensinarem o verdadeiro valor da vida. Sou muito grato e privilegiado por todos vocês em minha vida.

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RESUMO – A oferta de grãos de úmido de destilaria (WDG) para nutrição animal vem crescendo no Brasil, com o aumento da produção de etanol de milho. Assim, este estudo teve como objetivo avaliar os efeitos do WDG de milho desengordurado na dieta de bovinos confinados sobre consumo de matéria seca (CMS), desempenho, características de carcaça, qualidade da carne e expressão de genes envolvidos na lipogênese e metabolismo proteico muscular. Cem machos F1 Angus-Nelore não castrados, com idade entre 20 e 24 meses, com peso corporal inicial médio (PCI) de 369,58 ± 49,17 kg, foram utilizados em um delineamento em blocos casualizado de acordo com o PCI. Os animais foram alimentados com dietas contendo 0, 15, 30 e 45% de WDG desengordurado (4% EE) substituindo o milho e o farelo de soja. Após 129 dias de alimentação, os animais foram abatidos e amostras do músculo *Longissimus thoracis* (LT) foram coletadas para ensaios de biologia molecular e análises físico-química. Genes lipogênicos (SCD1, PPARγ, FASN e CPT2) e genes do metabolismo proteico muscular (eIF4, IGFR1, mTOR, GSK3β e P70S6K) foram analisados por transcrição reversa seguida de reação em cadeia da polimerase em tempo real (RT-PCR). Os dados foram analisados utilizando contrastes polinomiais (linear, quadrático e controle x WDG). A inclusão do subproduto melhorou o CMS e o peso corporal final – PCF ($P < 0,05$). Observou-se uma tendência ($P < 0,10$) para efeito quadrático no CMS e PCF, com maiores valores para animais alimentados com dieta com 30% de WDG. Os animais alimentados com WDG apresentaram maior ($P < 0,05$) peso da carcaça quente (PCQ) e peso de carcaça fria (PCF), e houve efeito quadrático ($P < 0,05$) para a área de olho de lombo (AOL), com maiores valores para os animais alimentados com dieta com 30% de WDG. Além disso, não foram observadas diferenças na espessura da gordura subcutânea (EGS) e no pH da carcaça, enquanto as características da qualidade da carne foram semelhantes ($P > 0,05$) entre os tratamentos (força de cisalhamento, perdas por cozimento, MIF e capacidade de retenção de água). No entanto, a inclusão do WDG levou a uma redução na expressão dos genes lipogênicos (SCD1, PPARγ, FASN e CPT2) e, consequentemente, à quantidade de gordura intramuscular no músculo LT. Além disso, a expressão dos genes eIF4, mTOR e IGFR1 foram menores em animais alimentados com WDG ($P < 0,05$). No geral, a inclusão de WDG desengordurado em dietas de confinamento melhora o desempenho, características de carcaça e reduz a quantidade de gordura intramuscular, sem afetar outras características de qualidade da carne. Embora o aumento dos níveis de WDG desengodurado tenha influenciado o CMS, PCF, pesos de carcaça e AOL (um indicador de deposição muscular) em resposta à alimentação, não produziu alterações conjuntas nos genes do LT relacionados ao metabolismo proteico muscular.

Palavras-Chave: Confinamento, Expressão gênica, Subprodutos de destilaria, Marmoreio, Peso de carcaça

LEVELS OF DE-OILED WET DISTILLERS GRAINS IN THE DIETS OF FEEDLOT CATTLE: PERFORMANCE, CARCASS AND MEAT QUALITY TRAITS

ABSTRACT - The supply of wet distillers grains (WDG) for animal nutrition has been growing in Brazil, with the increase in corn ethanol production. Thus, this study aimed to evaluate the effect of de-oiled corn WDG in the diet of feedlot cattle on dry matter intake (DMI), performance, carcass traits, meat quality and expression of genes involved in the lipogenesis and muscle protein metabolism. One hundred non-castrated F1 Angus-Nellore bulls, aged 20–24 months, with an average initial body weight (IBW) of 369.58 ± 49.17 kg, were used in a randomized complete block design according to IBW. The animals were fed diets containing 0, 15, 30 and 45% de-oiled WDG (4% EE) replacing corn and soybean meal. After 129 days on feed, the animals were slaughtered and *Longissimus thoracis* (LT) muscle samples were collected for molecular biology assays and physical-chemical analysis. Lipogenic genes (SCD1, PPARγ, FASN and CPT2) and muscle protein metabolism genes (eIF4, IGFR1, mTOR, GSK3β and P70S6K) were investigated by real-time reverse transcription polymerase chain reaction (RT-PCR). Data were analyzed using polynomial contrasts (linear, quadratic and control x WDG). The inclusion of by-product improved DMI and final body weight – FBW ($P < 0.05$). A tendency ($P < 0.10$) to a quadratic effect was observed on DMI and FBW, with greater values for animals fed diet with 30% of WDG. The animals fed WDG presented greater ($P < 0.05$) hot carcass weight (HCW) and cold carcass weight (CCW), and there was a quadratic effect ($P < 0.05$) for the ribeye area (REA), with greater values for the animals fed diet with 30% of WDG. Moreover, no differences were observed in backfat thickness (BFT) and carcass pH, while meat quality traits were similar ($P > 0.05$) among treatments (shear force, cooking losses, MFI, and water-holding capacity). However, the inclusion of WDG led to a reduction in the expression of the lipogenic genes (SCD1, PPARγ, FASN and CPT2) and, consequently, the amount of intramuscular fat in the LT muscle. Moreover, the expression of eIF4, mTOR and IGFR1 genes were lower in animals fed WDG ($P < 0.05$). Overall, the inclusion of de-oiled WDG in feedlot diets improves performance, carcass traits and reduces the amount of intramuscular fat, without affect other meat quality traits. Although increased levels of de-oiled WDG influenced DMI, FBW, carcass weights and REA (an indicator of muscle deposition) in response to feeding, it did not produce concerted changes in genes of LT related to muscle protein metabolism.

Keywords: Feedlot cattle, Gene expression, Ethanol by-product, Marbling, Carcass weights

CAPÍTULO 1 – Considerações gerais

1. Introdução

O rebanho brasileiro de bovinos é composto por 238 milhões de animais, sendo que no ano de 2019, foram abatidos 40,65 milhões de bovinos, com produção de 10,22 bilhões de toneladas de carcaça (USDA, 2020). Entretanto, apenas 11% dos animais abatidos no Brasil são originados de confinamento (ANAULPEC, 2018).

O custo com alimentação em animais confinados pode chegar de 70 a 80% do custo total, sendo a fração concentrando com maior participação neste custo (Goes et al., 2013). De acordo com Pinto e Millen (2019), 57,6% dos confinamentos brasileiros utilizam de 81 a 90% de concentrado na dieta, sendo que 81,8% dos confinamentos utilizam mais do que 51% de milho moído nas dietas como principal fonte energética, motivo que explica a grande participação do concentrado no custo dietético.

De acordo com o CEPEA (2020), de novembro de 2019 a outubro de 2020, a preço da saca (60 kg) de milho sofreu um ágio de 44,8%, saindo dos patamares de R$ 44,0 para R$ 64,0. Com esses aumentos expressivos, a inclusão de subprodutos e coprodutos nas dietas de bovinos confinados tem ganhado mais espaço, sendo o caroço de algodão, polpa cítrica e casca de soja, os mais utilizados nas dietas pelos nutricionistas brasileiros (Pinto e Millen, 2018). Porém, grande parte desses subprodutos/coprodutos utilizam o preço do milho como balizador de mercado, o que torna importante estudos que utilizam subprodutos que substituem o milho nas dietas, com o objetivo de reduzir o custo alimentício e melhorar a lucratividade dos negócios.

O grão de destilaria é um subproduto da produção de etanol a partir do milho, o qual pode estar na forma seca (88% MS) ou úmida (32% MS). Com o processo de fermentação do amido presente no milho, alguns compostos nutricionais do grão de destilaria são elevado de 2 a 3 vezes quando comparado ao milho, originando um subproduto com alto teor de proteína (30%), gordura (12%), fibra em detergente neutro (36%) e fósforo (0,9%), tornando-o atrativo para a alimentação animal (Klopfenstein, et al., 2008).

Esse subproduto já tem grande participação nas dietas americanas. De acordo com Samuelson et al. (2016), 97,1% dos confinamentos americanos utilizam grãos de

destilaria na dieta dos bovinos, sendo que o grão úmido é o principal subproduto utilizado nas formulações (58,3%). A inclusão dos grãos de destilaria na dieta de bovinos confinados promove um aumento no ganho de peso diário, eficiência alimentar, peso final, peso de carcaça quente e escore de marmoreio, sem afetar o consumo de matéria seca (Klopfenstein, et al., 2008).

No Brasil, grande parte da produção de etanol é proveniente da cana-de-açúcar, motivo que poucos confinamentos utilizam o subproduto nas dietas. De acordo com a (CONAB, 2020), a estimativa para a safra 20/21 é de uma produção de 30,6 bilhões de litros de álcool, sendo que 27,8 bilhões de litros são provenientes da cana-de-açúcar e o restante de milho. Dos 2,7 bilhões de litros de etanol produzido no Brasil a partir de milho, 94,8% está presente nos estados de Mato Grosso e Goiás.

Mesmo com a pequena participação do milho na produção de etanol, tem-se observado um forte crescimento nessa produção, sendo que em Mato Grosso e Goiás, há uma estimativa de crescimento de 61,5 e 73% na produção, respectivamente (CONAB, 2020). A cada tonelada de milho que é processada e fermentada para a produção de etanol, são gerados 400 litros de etanol, 317 quilogramas de grãos secos de destilaria e 317 quilogramas de CO_2 (Davis, 2001). Com isso, podemos estimar que em 2020 a produção de DDGS ou WDGS no Brasil será de 2,13 ou 6,27 milhões de toneladas, respectivamente.

No Brasil, estudos envolvendo a utilização de subprodutos da usina de etanol de milho para a produção animal são escassos, o que justifica a importância do presente estudo.

Com isso, o objetivo do presente estudo foi avaliar os efeitos da inclusão dos grãos úmidos de destilaria desengordurado nas dietas de bovinos F1 Nelore-Angus sobre o consumo, desempenho, características de carcaça e qualidade de carne.

2. Revisão de Literatura

2.1. Produção de etanol do milho

O Brasil ocupa o segundo lugar no ranking mundial na produção de etanol, totalizando uma produção de 31,25 bilhões de litros, atrás apenas dos Estados Unidos, o maior produtor mundial (USDA, 2020), entretanto, a exportação brasileira de etanol representa 50% no panorama mundial (Andreoli e Souza, 2007). Grande parte do álcool produzido pelo país é proveniente da cana-de-açúcar (91%), diferente da produção americana, onde 95% da produção sucroalcooleira é proveniente do milho.

A produção de etanol a partir do milho vem ganhando destaque no Brasil, com estimativa de produção de 2,7 bilhões de litros na safra 20/21, sendo os estados de Mato Grosso e Goiás com maior produção, totalizando 2,05 e 0,51 bilhões de litros, respectivamente (CONAB, 2020).

Com o processamento de uma tonelada de milho, são produzidos 371 litros de etanol, enquanto com a cana-de-açúcar a produção é de 90 litros (Andreoli e Souza, 2007). De acordo com os mesmos autores, a produção em toneladas por hectare da cana-de-açúcar (90 ton.) é superior comparado ao milho (8,1 ton.), o que torna a cana-de-açúcar mais atrativa. Porém, no período de entressafra da cana-de-açúcar, onde as usinas de etanol *flex* (processam cana-de-açúcar e milho*)* sofrem com a ociosidade de oferta, o milho pode ser uma alternativa viável visando otimizar a produção. Além disso, a produção de etanol a partir do milho tem outra vantagem, por não necessitar de processamento logo após a colheita, como ocorre com a cana-de-açúcar.

O processo de produção de etanol do milho envolve diversas etapas, como a utilização de calor e enzimas específicas que hidrolisam os principais componentes do milho, sendo que o tipo de processo vai determinar a composição química do produto (Tabela 1). A produção de etanol de milho pode ocorrer através de dois processos: moagem a seco e moagem úmida, sendo que primeiro tem como subproduto o grão de destilaria.

Tabela 1. Composição bromatológica de grãos de destilaria oriundos de moagem úmida e a seco

Item[1], %	DDG[2]	WDG[3]
MS	91,0	35,3
PB	28,6	36,6
EE	6,69	9,6
FDN	50,0	42,1[4]
Amido	5,3[5]	5,2
MM	4,68	2,0

[1]Item: MS = matéria seca; PB = proteína bruta; EE = extrato etéreo; FDN = fibra em detergente neutro; MM = matéria mineral.

[2]DDG = grãos de destilaria a seco; adaptado de Corassa et al. (2017).

[3]WDG = grãos de destilaria úmido; adaptado de Kim et al. (2008).

[4]Adaptado de Spiehs et al. (2002).

[5]Adaptado de Kim et al. (2008).

2.1.1. Moagem a seco

A moagem a seco é a primeira etapa do processo de produção de etanol, que no fim, gera um coproduto específico chamado grãos de destilaria úmido ou seco, como mostra na Figura 1. O grão é moído e misturado em água para formar uma pasta com pH entre 5 e 6, onde posteriormente é cozido a uma temperatura de 82 a 95 °C. Após o processo de cozimento, a α-amilase é adicionada para quebrar as moléculas de amido em dextrinas, processo esse chamado de liquefação. Então a biomassa é resfriada à temperatura de 32 °C e levada para tanques de fermentação onde a enzima glicoamilase é adicionada para a sacarificação, processo de conversão da dextrina em açúcares simples. Durante esse procedimento, a levedura (*Saccharomyces cerevisiae)* também é adicionada para produção de etanol e CO_2, sendo que durante essa etapa da fermentação, a fibra e a gordura permanecem intactas. Após o processo de fermentação, ocorre a destilação para separação do etanol e a biomassa úmida. Com a posterior centrifugação da biomassa, obtêm-se a fração sólida como o grão úmido destilaria (proteína e fibra) e a vinhaça fina, onde

óleo encontra-se presente na última. No caso das indústrias que extraem o óleo de milho para a comercialização, ocorre uma nova centrifugação da vinhaça fina, onde o óleo é separado dos solúveis (Figura 1). A fração sólida pode ser comercializada úmida ou passar pelo processo de secagem, dando origem aos grãos de destilaria úmido ou seco, respectivamente. Nos casos onde os solúveis (óleo, proteína e fibra) são adicionados novamente a fração sólida, tem-se como produto final os grãos de destilaria com solúveis (Mumm et al., 2014).

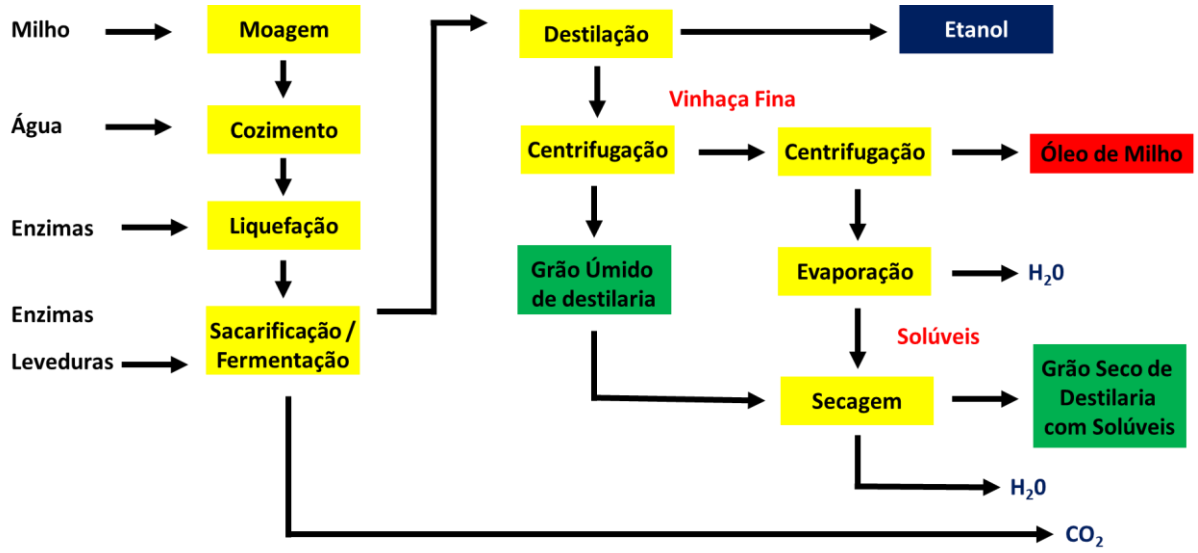

Figura 1. Processo de moagem a seco com extração de óleo para produção de etanol, óleo e grãos de destilaria com ou sem solúveis (Adaptado de Mumm et al., 2014).

2.2. Utilização dos grãos de destilaria na dieta animal

A utilização dos grãos de destilaria na alimentação animal não é recente. Estudos indicam que o uso desse tipo de coproduto tem sido reportado desde o século 19 nos Estados Unidos da América (Henry, 1900). Grãos de destilaria são comumente usados para gado de corte nos Estados Unidos (Klopfenstein et al., 2008). Contudo, no Brasil, os estudos com grãos de destilaria são escassos. Apenas um estudo avaliou a utilização de grãos de destilaria seco (DDG) na dieta de bovinos de corte (Reis et al., 2019).

Grande parte dos estudos mostram que com a utilização dos grãos de destilaria na dieta de bovinos de corte há uma melhora no consumo e o desempenho. Reis et al. (2019) observaram o mesmo comportamento usando 21% de DDGS com base na matéria seca. Entretanto, no Brasil os dados ainda são conflitantes quanto ao nível ideal de coproduto na dieta. Existe estudo que mostra redução no CMS e ganho médio diário quando o DDGS foi incluído acima de 15% (base na MS) na dieta de novilhas (Depenbusch et al., 2009).

A grande concentração de proteína não degradável no rúmen (PNDR) com o uso dos grãos de destilaria pode explicar os motivos pelos quais esse coproduto melhora o desempenho de bovinos de corte. Comparado ao milho, o grãos de destilaria possui 20% a mais de energia (Larson et al., 1993). Dessa forma, a PNDR dos grãos de destilaria perdem menos energia durante a digestão quando comparado à digestão ruminal, usando assim a energia da proteína de forma mais eficiente (Klopfenstein et al., 2008).

Para conhecimento, nenhum estudo no Brasil foi reportado com grãos úmido de destilaria (WDG). Assim como o DDG, o WDG também é uma ótima fonte de proteína e energia para ruminantes. Existem poucas diferenças na composição química do coproduto úmido e seco, entretanto, a mais marcante é o teor de matéria seca menor no WDG (35,3%), comparado aos 88,8% no DDG (Liu, 2011).

Uma melhora linear no ganho de peso, eficiência e peso de carcaça quente foram obtidos quando o WDG foi incluído em até 40% da dieta de bovinos de corte (Klopfenstein et al., 2008). Contudo, os estudos ainda apresentam contradições em relação ao nível ideal de WDG na dieta de bovinos de corte confinados (Luebbe et al., 2012; Vander Pol et al., 2006).

Vander Pol et al. (2006) testaram a inclusão de WDG em até 50% da dieta e observaram aumento do CMS, ganho de peso diário e peso final, melhor eficiência de ganho e peso de carcaça quente quando o WDG foi incluído em 30%. Já Luebbe et al. (2012) apenas observaram efeito deletério da inclusão de WDG no CMS com inclusão máxima de 15%, ao passo que as variáveis de desempenho foram melhoradas até o nível de 60% de inclusão. Com base nos dados da literatura, fica

claro que a utilização de WDG pode ser vantajosa quando observamos o nível de inclusão, possibilitando diminuição dos custos da dieta.

2.3. Utilização de PNDR na dieta animal

A proteína não degradável no rúmen (PNDR) é aquela que passa intacta pelo rúmen e chega ao duodeno. É uma importante fração da proteína metabolizável e seu valor biológico irá depender do perfil de aminoácidos presentes no conteúdo proteico total, sendo um importante caracterizador do valor da proteína bruta da dieta (Westreicher-Kristen et al., 2012). Essa fração ajuda a compor a proteína metabolizável e pode apresentar maior eficiência de utilização durante o metabolismo (Klopfenstein et al., 2008).

Nesse sentido, os grãos de destilaria apresentam ganhos superiores ao milho mesmo apresentando maior concentração de fibra e teoricamente menor energia dietética (Klopfenstein et al., 2008). Esta fonte de proteína será usada com maior eficiência para síntese de proteína corporal e pode também alterar o balanço hormonal (Walli et al., 1993). Contudo, a fonte de proteína não degradável é determinante para o sucesso com o uso dessa fonte proteica, considerando os aminoácidos limitantes para produção de leite ou carne (Santos et al., 1998).

Lalman et al. (1993) não observaram efeito da PNDR no ganho de peso diário de novilhas em confinamento. Estudos avaliando a suplementação de PNDR para suprir as exigências em proteína metabolizável tiveram pequeno ou nenhum efeito no desempenho de animais em terminação (Klemesrud et al., 2000; Ludden et al., 1995; Sindt et al., 1993).

A PNDR têm sido pouco estudada em bovinos de corte, ao passo que em bovinos de leite ela tem sido extensivamente avaliada (Erasmus et al., 1994; Savari et al., 2018; Schwab e Broderick, 2017). Parte disso se deve ao fato de que animais com grande potencial de produção não conseguem atingir os requerimentos em proteína metabolizável apenas com a proteína microbiana. A produção de animais visando produção de carne tem como base em sua dieta carboidratos de alta fermentação (amido) como o principal fornecedor de energia. No entanto, coprodutos

da destilação do milho, apresentam uma característica desejável para produção de bovinos de corte, que é a alta concentração de PNDR. Em termos nutricionais, essa proteína não teria grande participação na composição do ganho, entretanto, os estudos demonstraram que essa fração proteica participa ativamente da composição do ganho como uma fonte extra de aminoácidos que serão usados energeticamente (Klopfenstein et al., 2008).

O turnover muscular representa o balanço entre a proteína sintetizada e degradada, então quando a síntese excede a degradação, ocorre acréscimo proteico e hipertrofia muscular (Schiaffino et al., 2013). Nesse sentido, vários genes estão envolvidos nas rotas de síntese e catabolismo proteico. Os principais genes envolvidos nessas rotas são os genes mTOR e IGFR1 (Schiaffino et al., 2013), enquanto que os genes eIF4, GSK3β e P70S6K podem estar relacionados ao IGF-1. No entanto, apenas o gene IGFR1 foi significativamente relacionado ao IGF-1 (Busato et al., 2016).

2.4. Qualidade de carne, lipogênese e marmoreio

Os consumidores querem se alimentar de uma carne saudável, segura, nutritiva e palatável (Verbeke et al., 2010). Nesse sentido, o conhecimento dos fatores que afetam a qualidade da carne deve ser levado em consideração quando utilizam-se alimentos que podem interferir na qualidade dessa proteína animal.

Existem diversas categorias para avaliar a qualidade da carne desenvolvidas em vários países (Farmer e Farrell, 2018) e levam em consideração múltiplos atributos qualitativos como sexo, idade, ossificação, marmoreio, cor da gordura, cobertura de gordura, textura, maciez, dentre outros (Farmer e Farrell, 2018) e a criação de classificações que ajudam a estabelecer um padrão de qualidade (Aalhus et al., 2014).

A qualidade da carne pode ser influenciada por diversos fatores: sexo (Nogalski et al., 2018), qualidade da dieta (Burggraaf et al., 2020), uso de aditivos na dieta (Ornaghi et al., 2020), (Rabelo et al., 2016) e o uso de coprodutos da indústria do milho (Reis et al., 2019; Roeber et al., 2005).

Reis et al (2019) avaliaram a inclusão de 21% de DDGS na dieta de bovinos de corte e observaram diminuição da umidade e aumento da concentração de proteína no músculo *Longissimus thoracis*, mas não houve efeito na gordura e minerais. No entanto, o perfil de ácidos graxos foi alterado pela adição de DDGS, além do aumento da intensidade da cor amarela. Já o uso de WDG em dietas com alta ou baixa forragem diminuíram a espessura de gordura, o escore de marmoreio e a qualidade da carne (Schoonmaker et al., 2010). Os autores concluíram que a inclusão de WDG não altera o desempenho, no entanto altera o tecido muscular e adiposo de bovinos de corte, principalmente em dietas com baixa inclusão de forragem. Contrariando os achados de Schoonmaker et al. (2010), o estudo de Watson et al. (2014) demonstrou que a inclusão de até 50% de WDG na dieta não influencia o marmoreio, já a espessura de gordura subcutânea foi influenciada negativamente quando mais que 40% de WDG foi incluido na dieta, mostrando que niveis de inclusão acima de 40% pode ter efeito negativo na qualidade da carne de bovinos de corte confinados.

A lipogênese é o processo de formação de triglicerídeos no adipócito através de moléculas de Acetil-CoA e Malonil-CoA, sendo que isso ocorre quando o animal está em homeostase energética. Alguns genes são utilizados como marcadores para a maturação dos adipócitos (GLUT4, LPL e enzimas lipogênicas), adipogênese (PPARy e SREBF-1), tendo grande importância na mensuração do desenvolvimento do tecido adiposo intramuscular (Hocquette, 2010). Sendo assim, alguns genes como o PPARα, PPARy, SREBP-1c, SCD1, LPL, FABP4, FASN, ACOX, CPT2, GPX1 and ACACA estão relacionados à lipogênese, e a expressão desses genes pode determinar a quantidade de gordura intramuscular depositada (Figura 2.)

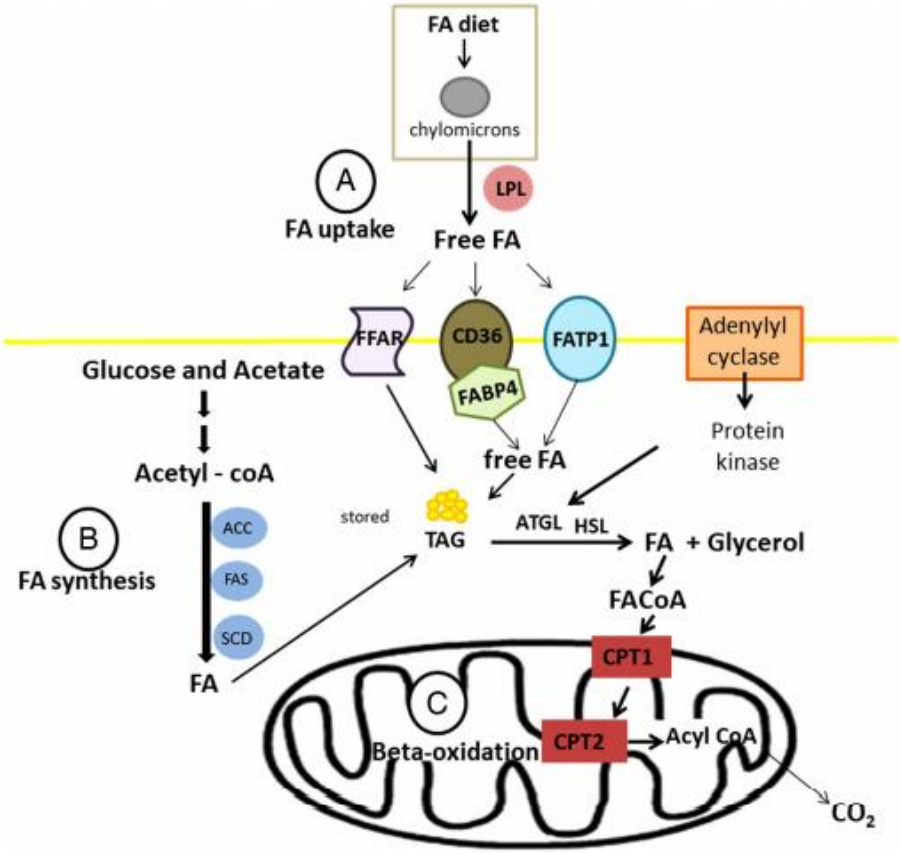

Figura 2. Síntese, absorção e oxidação de ácidos graxos no tecido adiposo dos ruminantes (Ladeira et al., 2018).

A lipoproteína lipase (LPL) tem a função de quebra o TAG em ácidos graxos livres (AGL), no qual o AGL é transportado para o citoplasma do adipócito com o auxílio da enzima FABP4 (Proteína transportadora de ácido graxo). Quando o animal está em homeostase energética, a síntese *de novo* (ácido graxo) ocorre pela ação das enzimas Acetil-CoA carboxilase (encodificada pelo gene ACACA) e a ácido graxo sintase (FAS), dando origem a moléculas de ácidos graxos (Ladeira et al., 2016). A enzima estearoil-CoA dessaturase-1 (Δ-9-dessaturase) tem a função de dessaturação desses ácidos graxos, ou seja, inserir dupla ligação na cadeira de carbono (Ladeira et al., 2018).

Entretanto, existem genes que são indicadores da beta-oxidação como: CPT2 e PPARα. De acordo com Ladeira et al. (2018), no interior da mitocôndria, a enzima

CPT2 (carnitina palmito transferase 2) converte acilcarnitina de cadeira longa em moléculas de acil-CoA, onde elas entram na via de Beta-oxidação para síntese energética.

O uso de grãos de destilaria não afetou a expressão de genes relacionados à adipogênese (Reis et al., 2019). Os genes relacionados à deposição de gordura intramuscular se comportam de maneira similar independente da dieta (Ladeira et al., 2016), no entanto, a expressão desses genes afeta o marmoreio e o perfil de ácidos graxos da carne (Ladeira et al., 2016; Reis et al., 2019).

O marmoreio é um dos principais indicadores de qualidade de carne e está associado intimamente com a palatabilidade e cozimento da carne, tendo influência sobre a decisão de compra do produto (Smith et al., 1988), tendo grande importância econômica e a uma boa variável avaliada pela indústria da carne (Picard et al., 2017). O amido influencia diretamente o marmoreio, sendo que quanto maior a quantidade de amido presente na dieta, maior a produção de propionato no rúmen, e o proprionato é o principal precursor de glicose nos ruminantes. Cerca de 50 a 75% das moléculas de acetil-CoA usadas na lipogênese deriva-se da glicose, mostrando que o marmoreio tende a ser maior em dietas com alto amido (Smith e Grouse, 1984).

Nesse contexto, a inclusão de grãos de destilaria pode diminuir o marmoreio da carne, devido à redução nas concentração de amido na dieta, sendo que o WDG entra como o principal substituto do milho (Schoonmaker et al., 2010). Contudo, o uso de WDG até 50% na dieta de novilhas confinadas não influenciou o escore de marmoreio (Klopfenstein et al., 2008).

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ATESTADO

Atesto que o Projeto "Níveis de grãos de destilaria em dietas para bovinos de corte confinados: desempenho, características de carcaça e qualidade de carne " **Protocolo CEUA 0067/2017** , a ser conduzido por Mateus Silva Ferreira, responsável/orientador Otávio Rodrigues Machado Neto, para fins de pesquisa científica/ensino - encontra-se de acordo com os preceitos da Lei nº 11.794, de 08 de outubro de 2008, do Decreto nº 6.899, de 15 de julho de 2009, e com as normas editadas pelo Conselho Nacional de Controle de Experimentação Animal - CONCEA.

Finalidade	PESQUISA CIENTÍFICA
Vigência do projeto	30/06/2017 a 31/01/2020
Nome Comum / Espécie / Linhagem	BOVINA / BOS TAURUS / F1 Angus-Nelore
Raça	F1 Angus-Nelore
Nº de animais machos	100
Nº de animais fêmeas	0
Nº de animais sexo indefinido	0
Peso médio de animais machos	330 kg
Peso médio de animais fêmeas	0
Peso médio de animais sexo indefinido	0
Idade	1 ano(s) e 6 mes(es) e 0 dia(s).
Procedência	Fazenda - Frigorífico Estrela. Estrela d´Oeste-SP

Projeto de Pesquisa aprovado em reunião da CEUA em 13/04/2017

JOSÉ NICOLAU PRÓSPERO PUOLI FILHO
Presidente da CEUA da FMVZ, UNESP - Campus de Botucatu

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CAPÍTULO 2 - Dry matter intake, performance, carcass traits and expression of genes of muscle protein metabolism in cattle fed increasing levels of de-oiled wet distillers grains[1]

[1]Este capítulo corresponde ao artigo científico publicado na revista **Animal Feed Science and Technology 269:114627, 2020**.

Abstract

The supply of wet distillers grains (WDG) has been growing in Brazil, with the increase in corn ethanol production. Thus, the aim of this study was to evaluate the effect of de-oiled corn WDG in the diet of feedlot cattle on DMI, performance, carcass traits, meat quality and expression of genes involved in the muscle protein metabolism. One hundred non-castrated F1 Angus-Nellore bulls, aged 20–24 months, with average IBW of 369.58 ± 49.17 kg, were used in a randomized complete block design. The animals were fed diets containing 0, 150, 300 and 450 g/kg de-oiled WDG (40 g/kg ofEE) replacing corn and soybean meal. After 129 days on feed, the animals were slaughtered and LT muscle samples were collected for molecular biology assays. Muscle protein metabolism genes such as eIF4, IGFR1, mTOR, GSK3β and P70S6K were investigated by RTqPCR. Data were analyzed using polynomial contrasts (linear, quadratic and control versus WDG). The inclusion of by-product improved DMI and FBW ($P < 0.05$). A tendency ($P < 0.10$) to a quadratic effect was observed on DMI and FBW, with greater values for animals fed diet with 150 and 300 g/kg of WDG. The animals fed WDG presented greater HCW and CCW ($P < 0.05$), and there was a quadratic effect ($P < 0.05$) for the REA, with greater values for the animals fed diet with 300 g/kg of WDG. Moreover, no differences were observed in BFT and carcass pH, and the meat quality traits were similar ($P > 0.05$) among treatments (SF, CL, ILC, MFI, and WHC). However, the inclusion of WDG led to a reduction in the

expression of eIF4, mTOR and IGFR1 genes ($P < 0.05$). Overall, meat quality traits were not affected by nutritional treatments. Although increased levels of de-oiled WDG influenced DMI, FBW, carcass weights and REA (an indicator of muscle deposition) in response to feeding, it did not produce concerted changes in genes of LT related to muscle protein metabolism.

Key-words: Animal nutrition, beef cattle, feedlot, meat quality, molecular biology

List of abbreviations: Activate ribosomal protein S6 kinase (P70S6K), acid detergent fiber (ADF), average daily gain (ADG), backfat thickness (BFT), cancer susceptibility candidate 3 (CASC3), cooking losses (CL), cold carcass weight (CCW), crude protein (CP), dry matter (DM), dry matter intake (DMI), dried distillers grains and solubles (DDGS), ether extract (EE), Eukaryotic translation initiation factor 4E (eIF4), final body weight (FBW), glycogen synthase kinase 3 beta (GSK3β), hot carcass weight (HCW), initial body weight (IBW), insulin-like growth factor receptor 1 (IGFR1), intramuscular lipid content (ILC), *Longissimus thoracis* (LT), mammalian target of rapamycin (mTOR), myofibrillar fragmentation index (MFI), metabolizable protein (MP), neutral detergent fiber (NDF), non-fiber carbohydrates (NFC), quantitative reverse transcription polymerase chain reaction (RT-qPCR), rib eye area (REA), ribosomal protein S6 kinase (P70S6K), rumen degradable protein (RDP), rumen undegradable protein (RUP), shear force (SF), volatile fatty acid (VFA), water holding capacity (WHC), wet distillers grains (WDG), wet distillers grains and solubles (WDGS).

Introduction

The supply of corn by-products has been growing in Brazil with the increase in corn ethanol production. This by-product is of great importance for animal nutrition considering that it contains three times more protein, fat and fiber than corn (Klopfenstein et al., 2008).

Moreover, ethanol industry has been extracting part of the oil from this by-product for biodiesel, making WDG rich in CP (30.5%), NDF (51.5%) and low fat (7.9%) (Jolly-Breithaupt et al., 2018).

In Brazilian industries these by-products have even lower fat content (4%) when compared to the American by-product (7.9%), being less energetic and with a higher CP content (32.5%). These levels allow a greater inclusion of WDG in feedlot cattle diets, although the effects on animal performance are still poorly understood. Therefore, studies are needed to evaluate the intake, performance and carcass traits of animals receiving diets with high de-oiled WDG inclusions.

Unlike the soybean meal, much of WDG protein is not degraded in the rumen, having the abomasum and the small intestine as its main digestive sites (Firkins et al., 1985). According to Stern et al. (2006), diets with low proportion of RUP may impair ruminants' performance and make it difficult for them to achieve high growth rates and weight gain. Another great advantage of the increased intake of RUP is that when supplemented in excess, it can contribute to increased metabolizable protein intake (which does not occur in diets with excess RDP) and can be used as energy source. In addition, excess RUP diets may indirectly contribute to the supply of RDP requirement via urea cycle (Archibeque et al., 2008).

As described by Owens and Basalan (2016), when CP and MP supplies are relatively high, great concentrations of blood urea and great losses of nitrogen through the urinary route may occur, which leads to energy expenditure of protein excretion. However, steers fed up to 50% of distillers grains adapt to excess N intake by increasing activity of urea cycle enzymes, as previously shown (Salim et al., 2016). Another study used steers in finishing diets containing 0% to 45% WDGS and observed that N excretion in urine rose with increasing dietary WDGS and CP, without affecting N retention (Hales et al., 2013). However, more studies are needed regarding the effects of excess CP on the performance of finishing beef cattle.

According to Ponce et al. (2019), diets containing WDG usually have appropriate CP content. The most reported values range from 47 to 63 percent RUP, and there may be RDP deficiency, which could impair microbial protein synthesis in the rumen. In Brazil, most feedlots use flint corn in their finishing diets with coarse grinding (Pinto and Millen, 2019). Due to these factors, RDP requirements may be reduced because of the lower energy supply. However, there are no studies in the literature about the use of diets with a high proportion of de-oiled WDG replacing flint corn.

Nutrients and growth factors may regulate increased protein synthesis of skeletal muscle through the mTOR complex and the activation of this complex depends on signals such as growth factors, amino acids, energy level and oxygen availability, which has the stimulation or inhibition of cell growth as main functions (Brown, 2014). In addition, mTOR can be indirectly stimulated by IGF1 and P70S6K, initiating protein synthesis (Brown, 2014). There are no studies investigating the consequences of the inclusion of de-oiled WDG on the expression of genes related to protein synthesis and muscle mass deposition in cattle. Considering these aspects, we hypothesized that increasing the dietary inclusion of de-oiled WDG would improve DMI, performance, and carcass traits of Angus-Nellore bulls, without impacting beef quality traits. In some regions of Brazil, WDG is a feed with a price lower than corn, allowing nutritionists to use it as an energy source, and its higher inclusion in finishing diets becomes an opportunity to increase the profitability of feedlots.

Therefore, the aim of the present study was to evaluate the effects of increasing levels of de-oiled WDG on the intake, performance, carcass traits, meat quality and expression of genes involved in the muscle protein metabolism of feedlot cattle.

Material and methods

All the procedures performed in the experiment were approved by the Animal Use Ethics Committee (CEUA) of the College of Veterinary Medicine and Animal Science - UNESP (protocol No. 0067/2017).

WDG production and storage

In ethanol industry (SJC Bioenergy, Quirinópolis, Goiás, Brazil), de-oiled WDG was obtained by "Front-End Fractionation" process, in which the corn germ is removed from the grain before fermentation. No condensed distillers solubles were added in the de-oiled WDG. In addition, the contribution of yeasts to the protein content of the de-oiled WDG was not measured. For storage, the byproduct was inoculated with heterofermentative bacteria *Lactobacillus buchneri* and *Lactobacillus plantarum* (FeedtechTM Silage F600, Delaval, Tumba, Sweden) at a concentration of 1 mg/kg of WDG, and were then ensiled in bag silos (5 x 60 m) at a density of approximately 1100 kg natural matter/ m^3.

Facilities and experimental diets

The experiment was carried out at the School of Veterinary Medicine and Animal Science of the São Paulo State University, Botucatu. A total of 100 non-castrated F1 Angus-Nellore bulls with an average IBW of 369.58 ± 49.17 kg, aged 20-24 months, were used. Animals were weighed after 16 hours of water and feed fasting, treated against endo/ectoparasites, rabies, clostridiosis, bovine viral diarrhea, and bovine respiratory disease, and distributed in 2 blocks ("light" and "heavy") according to the IBW. The animals were allocated to collective covered pens (5 x 6 m) with 5 animals each, with concrete floor cast and equipped with bunk and water trough. Then, the pens were randomly allocated among the 4 treatments, totaling 5 replicate pens per treatment, which consisted of the inclusion of de-oiled WDG in the proportions of 0, 150, 300 and 450 g/kg replacing corn and soybean meal (Table 1). Minimum amounts of

potassium chloride and soybean meal were included in diets with de-oiled WDG, therefore, all diets presented 6 g potassium/kg DM.

The composition of the diets was obtained by feed analysis (AOAC, 2005), followed by the procedures of determination of DM (method 976.05), CP (method 976.05, N * 6.25) and ash content (method 942.05). For NDF analysis, samples were treated with alpha amylase at a stable temperature without the addition of sodium sulfite and corrected for ash (aNDFom) (Mertens et al., 2002). The ADFom analysis was measured according to Van Soest et al. (1991) and EE analysis was conducted by the Soxhlet extraction (method 920.39), following previously described procedures (AOAC, 2000). The DM content of WDG used in the experimental diets presented mean values of 320 g/kg as fed, 328 g of CP/kg DM, 589 g of aNDFom/kg DM, 372 g of ADFom/kg DM, 40 g of EE/kg DM, 32 g of ash/kg DM and 11 g NFC/kg DM. The NFC content was estimated by the equation: NFC = 100 - (% CP +% EE +% Ash +% aNDFom).

Concentrations of protein fraction balances, RDP, and RUP from the diet were estimated using NRBC (2016). Data on the chemical composition of the diet and experimental animals were included in the NRBC spreadsheet to obtain estimates for nitrogen balance and Ca: P ratio (Table 1). The observed NEm and NEg values were calculated from performance data using energy requirement equations for maintenance and weight gain according to Zinn and Shen (1998). The expected NEm and NEg values were based on feed analysis, calculated using equations from NRBC (2016) (Table 3).

The animals were submitted to a step-up adaptation protocol, in which they adapted to the facilities and to the experimental diets for 21 days by receiving diets with decreasing ratios of forage (from 32 to 25 to 18 to 11% in 7-d intervals) until reaching the proportion of 890 g of concentrate and 110 g of forage/kg of dietary DM. Animals were fed for 129 days and diets were offered ad libitum twice a day (10 a.m. and 4 p.m.). Feed refusals were collected and

weighed daily to calculate the average daily intake in each pen. One sample of the feed refusals from each pen was collected twice a week and taken to a dry-forced ventilation oven for drying through the method 976.05 (AOAC, 2005) to measure daily dry matter intake. The daily adjustment in dietary supply was based on the intake of the previous day in order to have 5% refusals.

Slaughter and sampling

At the end of the experimental period, the animals were submitted to feed and water fasting for 16 hours and weighed to obtain the FBW, and ADG was calculated using the formula: ADG = (FBW - IBW) / experimental period (129 days). Then the animals were transported to a commercial slaughterhouse (Frigoestrela, Estrela D'oeste, São Paulo, Brazil) where they were stunned by brain concussion using a captive dart gun, followed by bleeding, hide removal and evisceration. Subsequently, the carcasses were divided longitudinally and a sample (approximately 20 g) of the LT muscle was collected from the left half carcass at the 12th rib, which was stored at −80 °C until analysis. The carcasses were weighed to obtain HCW and cooled for 48 h at 2 °C. After cooling, the carcasses were weighed again, obtaining CCW.

Then the left half carcasses were divided between the 12-13th rib for exposure of the LT muscle. Backfat thickness (BFT) was measured with a digital caliper, simulating a 45º angle between the spinous and transverse process of the 12th thoracic vertebrae. At the same point, REA of the muscle was drawn on a transparency with the aid of a permanent pen and measured by ImageJ® software (National Institute of Health, Bethesda, Maryland, United States). The carcass pH (48 h postmortem) was measured at the 12th rib using a Hanna digital device (Model HI 99163, Hanna Instruments, Woonsocket, Rhode Island) with penetration probe.

Post-harvest meat quality analysis

After boning, two beef samples (approximately 2.54 cm thick) were collected between the 11th and 13th rib of the left half-carcass of each animal, vacuum packed with polyethylene packages and kept in refrigerator at 2 °C for 3 days. One steak was used for the measurement of CL and SF. In brief, the samples were weighed to obtain the initial weight and then placed on a rack above a glass baking dish. Subsequently, the thermocouple of a DT-612 digital thermometer (ATP Instrumentation, Ashby-de-la-Zouch, United Kingdom) was inserted into the geometric center of the samples to monitor their internal temperature. The steaks were cooked in an electric oven (Feri90, Venâncio Aires, Rio Grande do Sul, Brazil) equipped with a thermostat to prevent temperature variation. When the internal temperature of the sample reached 40 °C, the steak was turned and further cooked until the internal temperature reached 71 °C, as previously described (Wheeler et al., 1996).

After cooking, the samples were kept at room temperature for 15 minutes, weighed, and cooled at 4 °C for 24 h. The CL were determined as the difference in weight before and after cooking of the sample. After refrigeration, for determination of SF, eight cylinders measuring 1.27 cm in diameter were removed parallel to the muscle fiber with a hollow punch coupled to an industrial drill. The cylinders were sectioned in Brookfield CT-3 Texture Analyzer (AMETEK Brookfield, Middleborough, United States) equipment. The results were presented in kilograms (kg) and replicate measurements per steak were performed to increase results accuracy.

The sample collected between the 11th and 12th rib (stored at -20 °C) was used for the quantification of ILC and for the analysis of MFI, as previously described by Bligh and Dyer (1959) and Culler et al. (1978), respectively. Additionally, the same beef sample was used for the measurement of WHC, by previously described methods (Salim et al., 2019; Verbeken et al., 2005). Briefly, 10 g of LT muscle were centrifuged at 12,000 g for 30 minutes (4 °C) and

the results of WHC were estimated as a percentage of retained-water, using the following equation:

$$WHC = (W2/W1) \times 100$$

here: *W1* = weight sample prior centrifugation; *W2* = sample weight after centrifugation.

Gene expression

Gene expression assays were carried out following previously described procedures (Teixeira et al., 2017). Two animals per pen were randomly selected for the analyzes, totaling 10 replicates per treatment. The design of target primers and references (Table 2) was was performed using sequences recorded and published in the GenBank and National Center for Biotechnology Information databases of the elF4, IGFR1, mTOR, GSK3B and P70S6K genes. Briefly, total RNA was isolated from the frozen muscle tissue using QIAzol reagent (QIAGEN, Valencia, California, United States) and treated with the DNA-free Turbo Kit (Ambion, Austin, Texas, United States) according to the manufacturer's instructions.

To evaluate the integrity of the ribosomal RNA 28S and 18S bands of the extracted samples, the RNA was submitted to 1.0% (w/v) agarose gel electrophoresis stained with GelRed Nucleic Acid Gel Stain and visualized in photocumenter UVITEC Fire Reader XS D77Ls-20 M. Samples were quantified on a NanoDrop Spectrophotometer ND-1000 (Thermo Scientific, Wilmington, Germany) at A260 nm. High-Capacity cDNA Reverse Transcription Kit (Applied Biosystems, Foster City, California, United States) was used for cDNA synthesis according to the manufacturer's indications and the samples were subsequently preserved at -20 °C.

The ABI PRISM 7500 Real-Time PCR Platform (Applied Biosystems, Foster City, California, USA) was used in SYBR Green detection system (Applied Biosystems, Foster City, California, United States) for RT-qPCR analysis. The thermal reaction conditions lasted 2 minutes at 50 °C, 10 minutes at 95 °C followed by 40 cycles of 15 seconds at 95 °C and 1

minute at 60 °C and 15 seconds at 95 °C. For each reaction, 1.0 μL cDNA, 0.3 μL for each primer (forward and reverse) and 5.0 μL of Master Mix SYBR Green were used for a final volume of 10.0 μL / sample in a reaction plate with 96 MicroAmp Optical wells (Applied Biosystems, Foster City, California, United States).

Negative controls and melting curves were included in all analyzes. Data were collected and stored using OS 7500 Fast software (Version 2.1). Real time qPCR analyzes were conducted for each gene using cDNA from 7 biological replicates, with 3 technical replicates per biological replicate. Results were normalized using the threshold cycle (Ct) method for expression of the reference gene β-actin and CASC3. The Ct was determined by the total number of cycles using the comparative Ct method. Validation tests were conducted to demonstrate that the amplification efficiency of the target and reference gene were approximately equivalent. Standard curves were constructed for the genes following the dilutions: 1: 5, 1:25, 1: 125; 1: 625 and 1: 3125. Relative expression levels were calculated according to the method described by Pfaffl, 2001 which is based on the Ct value that was corrected for the amplification efficiency of each primer pair.

Statistical analysis

Data were subjected to a normality analysis using the Shapiro-Wilk's Test procedure of the SAS 9.4 (2011) PROC UNIVARIATE (SAS Institute, Cary, North Caroline, United States). The expression of IGFR1, mTOR, GSK3β and P70S6K genes did not show normal distribution, so they were transformed by PROC RANK of SAS 9.4.

Then, data were analyzed by polynomial contrasts (linear, quadratic and control vs WDG) using the PROC MIXED procedure, where the pen was considered the experimental unit (except for gene expression analysis, in which two animals per pen were randomly

selected), the inclusion of WDG the fixed effect and the block as a random effect, according to model:

$$Yij = \mu + Bi + Wj + eij$$

here: μ = overall mean, *Bi* = random effect of block, *Wj* = WDG levels effect (0, 150, 300 e 450 g/kg DM basis) and *eijl* = experimental error.

The results were reported as Least Square Means (LSMEANS statement). Significant effect was considered when $P \leq 0.05$, and tendencies when $0.05 < P < 0.10$.

Results

Dry matter intake and performance

The inclusion of WDG in animal diets provided greater FBW when compared to the control group (P = 0.04), regardless of the level used. There was a tendency to a quadratic effect for FBW (P = 0.06), showing that the animals fed 300 g/kg of the byproduct had greater FBW at the end of the experiment (Table 3). However, there was no effect on FE, NEm and NEg (P > 0.05). No differences were also observed for observed/expected ratio NEm or NEg (P > 0.05).

Regardless of the inclusion level, there was a tendency (P = 0.06) for a greater ADG when the animals were fed WDG. On average, animals receiving WDG presented 7.7% increase in ADG (1.8 to 1.94 ± 0.09 kg/day) and 3.1% increase in FBW (602 to 620 ± 18.8 kg). Moreover, there was a tendency (P = 0.10) of quadratic effect on ADG according to the levels of de-oiled WDG (Table 3), and animals fed 300 g/kg WDG had greater ADG.

Feed intake (DMI, kg/day and g/kg BW) was affected by the inclusion of WDG in the experimental diets (P = 0.03 and 0.08, respectively). The animals that received a diet containing the byproduct consumed an average of 0.69 kg/day of DM more than the control group. However, there was a tendency to a quadratic effect (P = 0.09) on DMI, and the group of animals fed 150 g/kg WDG had greater intake (11.53 kg/day) (Table 3).

There was a linear increase for aNDFom and CP intake ($P < 0.001$) as WDG was included in the diets (Table 4). However, no difference on intake of net energy for gain was observed when the byproduct was included in the diets ($P > 0.05$).

Carcass and meat quality traits

Animals fed WDG presented greater HCW and CCW ($P < 0.05$) when compared to the control group (Table 5). There was also a tendency to a quadratic effect on HCW and CCW ($P < 0.10$), and the animals fed 300 g/kg of WDG presented heavier carcasses at the end of the experiment. On average, compared to control diet, the inclusion of WDG provided an increase of 10.50 and 10.25 kg for HCW and CCW, respectively.

In addition, as a result of the increase in carcass weights, when WDG was included, there was an increase in REA ($P = 0.043$) when compared to the control group (Table 5). A tendency for quadratic effect was observed in REA ($P = 0.064$) and animals receiving 300 g/kg of WDG showed an increase in this trait (88.40 cm2). Moreover, no differences were observed in BFT and pH ($P > 0.05$). Evaluation of the physical and chemical characteristics of meat quality revealed no significant difference ($P > 0.05$) in SF, CL, ILC, MFI, and WHC regardless of the level of de-oiled WDG (Table 5).

Gene expression

The inclusion of WDG caused a reduction in the expression of eIF4, IGFR1 and mTOR (Fig. 1). On average, the inclusion of WDG reduced ($P < 0.05$) the expression of the eIF4, IGFR1 and mTOR genes by 34.4%, 43.3% and 51.1%, respectively, when compared to the control group.

Discussion

The hypothesis that the inclusion of de-oiled WDG in finishing diets of F1 Angus-Nellore improves feed intake, performance and carcass traits without impacting beef quality was confirmed. Besides, the inclusion of WDG replacing corn drastically reduced (> 23.5%) starch concentration in the experimental diets (Table 1). Consequently, the intake of this nutrient was reduced, which may also have reduced ruminal propionate synthesis. One study showed that ruminal acetate synthesis increased whereas propionate production linearly decreased as WDG were included in the diet of feedlot cattle (Luebbe et al., 2012). However, another study evaluated the effects of diets containing fine-rolled corn DDGS (200 or 400 g/kg DM) on VFA concentration and reported that concentration of butyrate increased, possibly due the fast rate of fermentation of soluble fiber in the DDGS (Keomanivong et al., 2017).

The acetate to propionate ratio and VFA concentration could vary with the substrate type, substrate concentration, and fermentation conditions (Owens and Basalan, 2016). A higher proportion of fermented starch in the rumen generates more propionate, and WDG fiber (greater aNDFom content) increases acetate proportion in the rumen. Thus, in the present study, ruminal fermentation may be affected by diet composition. Greater aNDFom directs acetate production whereas greater starch directs fermentation towards propionate production. However, studying the influence of the grain type and oil concentration of corn DDGS (low = 4.5% versus moderate = 7.9%) on ruminal fermentation, Keomanivong et al. (2019) described no differences among treatments for total VFA concentration after 24 h of incubation.

In the current study, the effects on DMI of animals that receive WDG may be due to the changes in dietary carbohydrate source and, consequently, in the ruminal environment. The diets had 89% concentrate, with 75% corn inclusion in the control diet. Our results suggest that animals could benefit from the increase in dietary aNDFom content (23.64 to 38.65%).

Dry matter intake and performance

Propionate is a precursor of gluconeogenesis in the liver, stimulating hepatic oxidation of acetyl-CoA. On the other hand, the contribution of acetate seems to be insignificant to hepatic oxidation (Allen et al., 2009). According to these authors, in the oxidation process of acetyl-CoA, ATP is released, and it can activate the satiety center in ruminants and inhibit feed intake. This phenomenon could explain the lower DMI of the animals fed control diet when compared to the groups that received diets containing WDG, considering the theory that voluntary DMI is regulated by propionate production for ruminants receiving high energy diets, as proposed by Allen et al. (2009). Thus, VFAs measurement should be considered for future studies to confirm this hypothesis.

The greater DMI of animals receiving WDG in the diet contributed to greater ADG and FBW when compared to the control group. Additionally, according to Firkins et al. (1985), much of the WDG protein is not degradable in rumen (i.e. RUP), being digested mainly in the small intestine. Due to losses in the fermentation process, RUP has greater efficiency in energy synthesis when compared to carbohydrates and proteins that are digested in the rumen environment (Klopfenstein et al., 2008), which partly explains the greater ADG. Considering that there was a greater protein intake for groups receiving de-oiled WDG and that much of this protein is not digested in the rumen, there may have been higher energy synthesis, which also helps explain the greater ADG and FBW of animals that received the byproduct in the diet.

In corn by-products, the CP fraction is influenced by yeast cell growth and, consequently, yeast biomass has been considered to change the aminoacid profile during the ethanol production process, as reviewed by Böttger and Südekum (2018). Considering that yeast biomass is the major component of condensed distillers solubles (absent in de-oiled WDG used in the present study), a reduction in lysine concentration may occur (Liu et al., 2007).

The proportion of total tract CP digestibility may be high for all corn-ethanol byproducts. According to Kelzer et al. (2010), the degree of protein digestibility is higher than 900 g/kg of DM for distillers grains (wet and dry) and high protein DDG (without solubles). Despite ADIN high content in distillers grains, it has been well documented that ADIN concentrations, particularly in concentrate feeds, can provide aminoacids post ruminally. Therefore, this parameter is not an adequate indicator of unavailable protein for corn milling by-products. Kelzer et al. (2010) demonstrated that digestible portion of RUP is high for WDGS (930.1 g/kg), DDGS (919.0 and 921.0 g/kg) and for high protein DDG (977.0 g/kg). In summary, greater RUP in feedlot diets can result in better protein utilization, because when RUP is fed above MP requirements, the excess protein is still absorbed. If the amino acids are not required, the excess of MP is deaminated and the urea is recycled to supply RDP in the rumen. On other hand, when RDP is overfed, no more contributions to MP are observed once the microbial requirements are met.

Moreover, the RUP content of de-oiled WDG used in the current study is probably greater than WDGS used in American studies because there is no condensed distillers solubles. According to Cao et al. (2009), as the inclusion of condensed distillers solubles in the DDG or WDG occurs, the percentage of RUP in the diet decreases.

Estimated nitrogen balance and microbial growth

The estimated rumen nitrogen balance in experimental diets (Table 1) was changed by the inclusion of de-oiled WDG, which may also have contributed to the differences in performance. The estimates obtained in the NRBC (2016), showed that the control diet and the 150 g/kg of de-oiled WDG were deficient in nitrogen, whereas 300 and 450 g/kg WDG showed positive nitrogen balance. According to Boyd et al. (2019) in corn-based diets, the use of 30% WDGS is enough to meet the requirements for MP, although diets may be deficient in RDP.

Furthermore, according to Stalker et al. (2007), supplementation of non-protein nitrogen was not necessary for animals individually fed a 58% corn cob-based diet with 30% DDGS. When dietary supply of MP is greater than the requirements, N is recycled to the rumen, which may provide sufficient RDP when WDG are used for protein supplementation (NRBC, 2016).

The tendency of improvement in the performance observed with the supply of increasing levels of de-oiled WDG can be attributed to the protein energy value, because according to Klopfenstein et al. (2008), the excess protein used as energy is deaminated in the liver, producing ketone bodies to be used as energy, and urea is excreted. It is widely discussed about the energy cost of ureogenesis and that the increase in urea excretion could reduce animal performance, because according to Huntington and Archibeque (1999) between 2.5% to 5% of whole body oxygen consumption was attributable to ureagenesis in the liver. However, if urea nitrogen is derived from amino acid without the involvement of glutamate dehydrogenase, the cost of urea synthesis can be considerably lower (Reynolds et al., 1991a, 1991b). Furthermore, according to Salim et al. (2015) and Wang et al. (2009), tissues can adapt to changes in diet by altering tissue mass or metabolism per gram of tissue.

Carcass and meat quality traits

The carcass weights (HCW and CCW) of the animals were greater in the animals fed de-oiled WDG, which may be associated with the greater DMI, ADG and FBW observed in those animals. Besides the greater efficiency in energy synthesis, increased intake of RUP in cattle diets may improve the dietary amino acid profile, which may explain the higher growth of the animals (Swartz et al., 1991). The diets in which WDG were included may have promoted higher energy efficiency and guaranteed greater amounts of amino acids that are essential for the growth and weight gain in animals (Loy and Lundy, 2019), reflecting in greater carcass weight.

There is little information comparing the use of corn de-oiled WDG as protein source for finishing beef cattle and resulting carcass traits. Our results agree with those of Jolly-Breithaupt et al. (2018) and Stelzleni et al. (2016). In the first study, authors observed increase in carcass weight from cattle receiving 40% MDGS (Modified Distillers Grains with Solubles) when compared to cattle fed corn-based diet, suggesting that cattle fed de-oiled ethanol by-products can present better performance and carcass weight than cattle fed corn-based diets. Stelzleni et al. (2016) used corn DDGS (24.5% DM basis) as a replacement for soybean meal and partial replacement for corn in finishing diets and also reported no impact on carcass traits related to yield or quality.

Our study demonstrated that corn-based de-oiled WDG improve ($P < 0.05$) carcass weight (and consequently REA) without impairing carcass BFT and meat quality traits such as SF, CL, ILC, MFI, and WHC. The results of meat quality traits were similar to those reported by Mello et al. (2012), who evaluated three dietary treatments (0; 150; or 300 g/kg DM WDGS) and fed animals for 167 days, describing similar results on meat tenderness (SF = 3.42; 3.45; and 3.65 ± 0.57 kg, respectively).

Gene expression

GSK3β and P70S6K genes, since part of the ADG and FBW of animals fed WDG could be explained by increased muscle protein synthesis. However, this hypothesis was not confirmed since the inclusion of de-oiled WDG reduced the expression of eIF4, IGFR1, mTOR and did not alter the expression of GSK3β and P70S6K.

Similar molecular events were investigated by gene expression assays in other study using young male Holstein calves (Wang et al., 2014), in which the components involved in insulin pathway (IGFR1), as well as the mTOR signaling pathway were investigated, providing evidence to support the possible physiological effects on protein synthesis. In that study, mTOR

expression decreased in response to different diets and greater nutrient intake throughout the milk-fed and early postweaning phase altered body mass composition (greater carcass and fat mass) partly by changes in molecular profiles associated with glucose and amino acid signaling. There are no studies in the literature evaluating expression of genes of muscle protein metabolism on animals fed with increasing levels of de-oiled WDG. The current study is the first to show that corn de-oiled WDG led to a reduction in the gene expression of eIF4, mTOR and IGFR1 genes. The physiological significance of these changes in specific protein or enzyme activities remains to be determined.

In the current study, the target genes were investigated based on the physiological role in the activation of mTOR, which is related to the phosphorylation of the downstream target proteins such as ribosomal protein S6 kinase 1 (S6K1) and 4E-binding protein 1 (4EBP1), the dissociation of eukaryotic initiation factor 4E·4EBP1, and the active formation of eIF4, leading to enhanced translation process (Ma and Blenis, 2009). Modulation of mTOR pathway by insulin, as well as by other hormones and nutrients, accounts for enhanced protein synthesis (Rhoads et al., 2016), potentially increasing the carcass weights. However, the expression of these genes (levels of mRNA) appears to be unrelated to the animals' ADG, FBW, and HCW, as demonstrated in the present study.

Translational regulation and post-translational modifications play important roles in the determination of phenotype. For future studies, perhaps the use of techniques such as proteomics would help understanding how animal growth may be affected by these nutritional treatments.

Conclusion

The inclusion of de-oiled WDG in finishing diets increases dry matter intake, performance and hot carcass weight of F1 AngusNellore cattle. In addition, it reduces the expression of mTOR, IGFR1 and eLF4 genes.

Increasing the dietary inclusion of de-oiled WDG does not affect meat quality traits. Although increased levels of de-oiled WDG influenced carcass weight and REA (an indicator of muscle deposition) in response to feeding, it did not produce concerted changes in genes of LT muscle.

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Table 1. Composition of the experimental diets

Ingredients and composition	WDG (g/kg DM)			
	0	150	300	450
Ingredients (g/kg of DM)				
Tifton 85	42	42	42	42
Sugarcane bagasse	71	71	71	71
Ground corn	749.2	652.7	520	387.3
Soybean meal	103.6	47.8	29.4	11.0
Wet distillers grains	0	150	300	450
Mineral-vitamin supplement [1]	34.2	34.2	34.2	34.2
Potassium chloride	0	2.3	3.4	4.5
Nutritional Composition (g/kg of DM)	0	150	300	450
Dry Matter (g/kg as fed)	867.8	780.8	694.0	607.2
Crude Protein	128.5	141.9	171.3	200.7
Ruminal Degradable Protein	71.9	57.8	62.2	66.4
Ruminal Undegradable Protein	56.6	84.1	109.1	134.2
Ether Extract	34.2	35.3	35.4	35.5
Neutral Detergent Fiber	162.8	236.4	311.9	387.5
Starch	482.2	417.8	332.4	246.9
Non-fiber Carbohydrates [2]	646.8	560.2	454.2	348.2
Ruminal N balance (g N/day) [3]	-3.0	-11.26	3.63	18.44
Ca: P ratio [3]	2.22	1.85	1.55	1.33

[1]Mineral-vitamin supplement containing: 19.5% Ca; 1.9% S; 1.5% Mg; 4.5% Na; 1.6% P; 1715 ppm Zn; 1285 ppm Mn; 428 ppm Cu; 25 ppm I; 5.7 ppm Se; 8.5 ppm Co; 286 ppm Fe; 86000 UI Vit A; 115000 UI Vit D3; 128000 UI Vit E; 0.39% Urea; 945 ppm of sodium monensin;

[2]Estimated by the equation NFC = 100 - (% CP +% EE +% Ash +% aNDFom)

[3]Estimated by NRBC (2016)

Table 2. Sequences (5′ to 3′) and efficiencies of the primers used in quantitative real-time PCR.

Symbol	Name	Forward (F) and reverse (R)	Access number	Amplicon (bp)	R^2	Efficiency
β-actin	*β-Actin*	F GTCCACCTTCCAGCAGATGT R CAGTCCGCCTAGAAGCATTT	NM_173979.3	90	0.998	100
CASC3	*Cancer susceptibility candidate 3*	F GGACCTCCACCTCAGTTCAA R GTCTTTGCCGTTGTGATGAA	NM_001098069.1	85	0.976	98
eIF4	*Eukaryotic translation initiation factor 4E*	F AAACCACCCCTACTCCGAAT R TGCCCATCTGTTCTGTAAAGG	NM_174310	84	0.989	99
IGFR1	*Insulin like growth factor 1*	F ATGTACTGCGCGCCTCTC R CCTCTACTTGTGTTCTTCAAATG	NM_001077828	85	0.997	99
mTOR	*Mammalian Target of Rapamycin*	F CATGGAAATGGCATCCAAG R GAGTTTGAGGTGAAGCGAGC	XM_015475105.1	90	0.993	94.3
GSK3β	*Glycogen synthase kinase 3β*	F GCCCAGAACCACCTCCTTT R TGCTGCCATCTTTGTCTCTG	NM_001101310.1	83	0.987	96
P70S6K	*Ribosomal protein S6 kinase*	F TTGAACCAAAAATCCGATCC R AGCACCTCTTCCCCAGAAA	AY396564.1	83	0.987	100

Table 3. Intake, performance and net energy for maintenance and gain in F1 Angus-Nellore bulls fed increasing levels of de-oiled WDG.

Item[1]	WDG g/kg DM				SEM [3]	*P-value* [4]		
	0	150	300	450		C vs W	L	Q
Animal Performance [1]								
IBW, kg	369	371	370	369	22.84	0.43	0.74	0.13
DMI, kg/day	10.75	11.53	11.44	11.35	0.33	0.03	0.14	0.09
DMI, g/100 kg BW	2.21	2.35	2.30	2.31	0.06	0.08	0.28	0.22
ADG, kg/day	1.80	1.90	2.01	1.91	0.09	0.06	0.12	0.10
FE, kg/kg	0.17	0.17	0.18	0.17	0.01	0.85	0.57	0.86
FBW, kg	602	617	630	615	18.80	0.04	0.12	0.06
Dietary Energy [2]								
NEm $_{obs.}$, Mcal/kg	2.22	2.18	2.29	2.22	0.07	0.67	0.77	0.88
NEm $_{exp}$, Mcal/kg	1.98	2.04	2.09	2.13	0.06	0.64	1.00	0.84
Observed/expected Ratio NEm	1.12	1.07	1.09	1.04	0.02	0.59	0.79	0.68
NEg $_{obs.}$, Mcal/kg	1.54	1.50	1.60	1.54	0.07	0.67	0.77	0.88
NEg $_{exp.}$, Mcal/kg	1.34	1.39	1.43	1.47	0.05	0.59	0.99	0.75
Observed/expected ratio NEg	1.15	1.08	1.12	1.05	0.01	0.57	0.81	0.74

[1] IBW = initial body weight. DMI = dry matter intake. ADG = average daily gain. FE = feed efficiency and FBW = final body weight.

[2] Observed NEm and NEg.= dietary net energy for maintenance and gain values calculated from performance data using energy requirement equations for maintenance and weight gain according to Zinn and Shen (1998); Expected NEm and NEg. = net energy for maintenance and gain values based on feed analysis, calculated using equations from NRBC (2016).

[3] SEM = Standard Error Mean.

[4] C vs W, control vs WDG; L, linear effect and Q, quadratic effect.

Table 4. Nutrient intake of F1 Angus-Nellore bulls fed increasing levels of de-oiled WDG.

Item [1]	Levels of WDG. g/kg DM				SEM [2]	*P-value* [3]		
	0	150	300	450		C vs W	L	Q
CP_i, kg/day	1.60	1.88	2.20	2.52	0.091	<0.01	<0.01	0.65
CP_i, g/100 kg BW	0.33	0.39	0.46	0.50	0.025	<0.01	<0.01	0.68
$aNDFom_i$, kg/day	1.77	2.75	3.59	4.42	0.136	<0.01	<0.01	0.35
$aNDFom_i$, g/100 kg BW	0.36	0.57	0.74	0.88	0.038	<0.01	<0.01	0.27
$Starch_i$, kg/day	5.21	4.85	3.84	2.84	0.197	<0.01	<0.01	<0.01
$Starch_i$, g/100 kg BW	1.07	1.01	0.79	0.57	0.057	<0.01	<0.01	0.06
NFC_i, kg/day	6.78	6.27	5.01	3.77	0.257	<0.01	<0.01	0.01
NFC_i, g/100 kg BW	1.39	1.31	1.04	0.75	0.075	<0.01	<0.01	0.08
NEg_i, Mcal/day	14.08	14.99	14.64	14.41	0.655	0.126	0.656	0.096

[1]CPi = crude protein intake. $aNDFom_i$ = neutral detergent fibre intake. NFC_i = Non-fibre carbohydrates intake.

[2] SEM = Standard Error Mean.

[3] C vs W, control vs WDG; L, linear effect and Q, quadratic effect.

Table 5. Carcass and meat quality traits of F1 Angus-Nellore bulls fed increasing levels of de-oiled corn wet distiller grain (WDG).

Item [1]	Levels of WDG. g/kg DM				SEM [2]	*P-value* [3]		
	0	150	300	450		C vs W	L	Q
HCW, kg	340.33	348.54	356.10	347.87	10.48	0.04	0.12	0.06
CCW, kg	326.29	334.26	341.61	333.62	10.18	0.08	0.14	0.08
pH (48h)	5.65	5.64	5.66	5.66	0.02	1.00	0.53	0.55
REA, cm^2	84.70	86.62	88.40	86.47	2.46	0.04	0.12	0.06
BFT, mm	7.54	8.24	8.37	7.63	0.56	0.38	0.87	0.19
SF, kg	3.41	3.42	3.46	3.43	0.10	0.88	0.94	0.88
CL, %	20.09	20.15	19.69	21.06	0.74	0.47	0.49	0.44
ILC, %	2.03	1.82	1.93	1.75	0.06	0.25	0.26	0.94
MFI	65.74	65.72	65.45	63.59	0.97	0.72	0.44	0.64
WHC, %	28.02	28.13	27.41	27.74	1.33	0.60	0.40	0.94

[1] HCW = hot carcass weight. CCW = cold carcass weight. REA = ribeye area of *longissimus* muscle - 12-13th. BFT = subcutaneous backfat thickness. SF = shear force (72h postmortem). CL = cooking losses. ILC = intramuscular lipid content. MFI = myofibril fragmentation index. WHC = water-holding capacity.

[2] SEM = Standard Error Mean.

[3] C vs W, control vs WDG; L, linear effect and Q, quadratic effect.

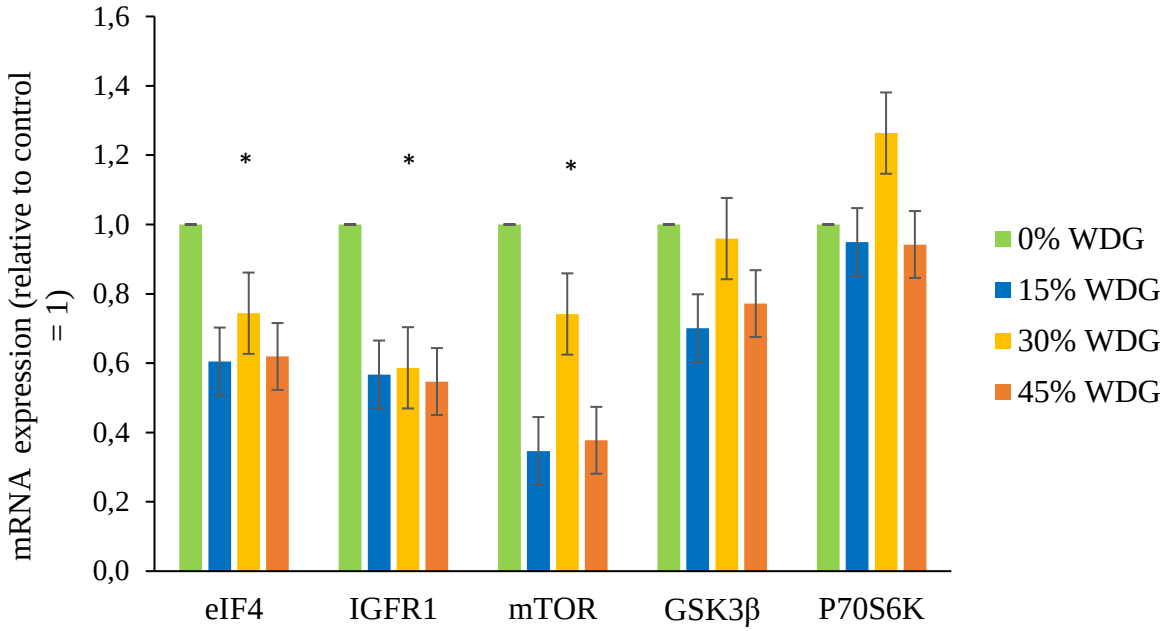

Figure 1. Gene expression of protein metabolism in *Longissimus thoracis* muscle of F1 Angus-Nellore bulls (n = 40) finished in feedlot fed increasing levels of de-oiled corn wet distiller grains (WDG).

* Statistical effect (P<0.05) among treatments control vs WDG.

Capítulo 3 - The inclusion of de-oiled wet distillers grains in feedlot diets reduces the expression of lipogenic genes and fat content in *Longissimus* muscle from F1 Angus-Nellore cattle[1]

[1]Este capítulo corresponde ao artigo científico publicado na revista **PeerJ 7:e7699, 2019.**

Abstract

The inclusion of agro-industry by-products originated from corn ethanol production has increased in animal nutrition in Brazil, reducing formulation costs. In the literature, there is no consensus on how the high inclusion of de-oiled wet distillers grains can affect beef quality and the expression of lipogenic genes in Longissimus muscle. The aim of this study was to evaluate the effects of WDG in the diet of F1 Angus-Nellore cattle on meat quality characteristics, chemical composition and expression of genes involved in lipid metabolism. A hundred F1 Angus-Nellore bulls, with average initial body weight (BW) of 369.5 ± 49 kg were used. The experiment was carried out in a randomized block design, and the animals were divided into two blocks (light and heavy) according to the initial body weight. The animals were fed diets containing levels of 0 (control), 15, 30 and 45% of WDG replacing dry corn and soybean meal. After 129 days of feedlot, the animals were slaughtered and samples of the longissimus thoracis (LT) muscle were collected for quality analyzes such as shear force (3, 10 and 17 aging days), color (luminosity, red, Chroma and Hue), cooking losses, pH and chemical composition (moisture, protein, lipids and ash contents). In addition, the expression of the PPARα, PPARγ , SREBP-1c, SCD1, LPL, FABP4, FASN, ACOX, CPT2, GPX1 and ACACA genes was investigated in the LT muscle by real-time reverse transcription polymerase chain reaction (RT-PCR). Data were analyzed using polynomial contrasts (linear, quadratic and control vs. WDG). There was no interaction ($P > 0.05$) between aging times and the inclusion of WDG in the diets on the meat quality (pH, cooking losses, coloration and tenderness). However, diets with increasing levels of WDG caused a linear reduction ($P = 0.01$) in the intramuscular fat of LT. The lipogenic genes SCD1, PPARγ , FASN and CPT2 were less expressed ($P < 0.05$) in response to the inclusion of WDG. These results suggest that the inclusion of WDG reduced the expression of lipogenic genes and consequently the marbling of LT muscle without affecting tenderness (shear force) and meat color traits.

Keywords: Chemical composition, Meat science, Nutrigenomics, Metabolism, Marbling, Meat science, Meat, Beef

Introduction

Currently, the production of corn ethanol in Brazil has increased along with the availability of by-products originated from this process in feedlot cattle diets. In countries such as the USA, distillers grains have been used in animal nutrition since the 1980s due to the high production of corn ethanol (Firkins, Berger & Fahey, 1985).

In the production of corn ethanol, after starch fermentation, the solid fraction of the remaining by-product tends to present the highest levels of protein (30%), fat (12%) and neutral detergent fiber (NDF = 36%), once the starch consumed in the alcoholic fermentation represents approximately 64% of the corn grain. However, ethanol plants have developed techniques to extract the oil from this by-product, making it rich in high digestibility fiber and rumen non-degradable protein (Jolly-Breithaupt et al., 2018).

Therefore, the replacement of ground dry corn by wet distillers grains leads to a reduction in starch intake, which may affect ruminal production of propionate, which is the main precursor of gluconeogenesis in ruminants. About 50–75% of the acetyl units used in intramuscular lipogenesis are derived from glucose, which might increase marbling in the meat (Smith & Crouse, 1984) when animals are fed diets rich in starch. It may explain the reduction in marbling score of the meat in animals fed WDG (Schoonmaker, Trenkle & Beitz, 2009).

The degree of marbling is one of the most important and definitive characteristics of meat quality since it is closely associated with the palatability of cooked meat (such as tenderness, juiciness and flavor) and influences consumer buying decisions (Smith et al., 1988). According to Picard et al. (2018), marbling is the most economically important and valuable trait for beef the industry and its improvement is a challenge for important markets such as the United States, Japan and Korea. The marbling score can be determined by visual evaluation by trained professionals using patterns of images on the muscular surface (Gerrard, Gao & Tan, 1996). However, visual score of marbling are subjective and may lead to underestimation or overestimation of scores, once some fat deposits (adipocytes) cannot be seen or quantified properly (Cheng et al., 2015; Gerrard, Gao & Tan, 1996). Thus, it is necessary to measure the amount of fat in the muscle and its relationship with the regulatory genes of the lipid metabolism.

Regarding meat tenderness, according to recent studies (Chao et al., 2018; Ribeiro et al., 2019), WDG-fed cattle may present lower shear force values (tender meat) when compared to animals fed ground corn. The literature suggests that this phenomenon is a consequence of the higher ingestion and deposition of unsaturated fatty acids (Ribeiro et al., 2018). According to these authors, when the animals were fed WDG with high fat content, there was a higher concentration of polyunsaturated fatty acids in the membrane of the sarcoplasmic reticulum, which may have anticipated the oxidation process of this membrane and the release of calcium in the muscle. However, more studies are necessary to prove this hypothesis.

In the literature, studies have been used high fat-WDG to evaluate carcass traits and beef quality (Buttrey et al., 2013; Hales, Cole & MacDonald, 2013). However, there are few studies evaluating the inclusion of de-oiled WDG on meat quality of feedlot cattle (Domenech-Pérez et al., 2017; Jolly-Breithaupt et al., 2018). The de-oiled-WDG used in the United States have about 9.2% fat (Jolly-Breithaupt et al., 2018), while industries in Brazil have generated this ingredient with fat content of less than 5%, which may justify the differences when compared to the results reported in the literature. In addition, most of the studies evaluated only the performance, carcass traits and beef quality of cattle (Buttrey et al., 2013; Hales et al., 2013; Domenech-Pérez et al., 2017). Few studies have investigated the molecular mechanisms that regulate these characteristics with WDG in the diet.

Therefore, the aim of this study was to evaluate the effects of the inclusion of increasing levels of de-oiled WDG on the chemical composition, expression of lipogenic genes in the Longissimus muscle and meat quality of feedlot cattle.

Materials and Methods

All the procedures performed in the experiment were approved by the Ethics Committee on Animal Use (CEUA) of the School of Veterinary Medicine and Animal Science - UNESP (protocol no. 0067/2017).

Facilities, management and experimental diets

The experiment was carried out at the School of Veterinary Medicine and Animal Science of the São Paulo State University, Botucatu, São Paulo, Brazil. A hundred F1 Angus-Nellore bulls with average initial body weight of 369.58 ± 49.17 kg were used. The animals were weighed at the beginning of the experiment, treated against endo- and ectoparasites and

distributed in two blocks (denominated ‘‘light’’ and ‘‘heavy’’) according to the initial body weight (iBW). Light animals had an iBW range from 290 to 367 kg (iBW mean = 336.61 ± 18.32 kg), whereas heavy animals had an iBW range from 368 to 495 kg (iBW mean = 415.87 ± 31.10 kg). The animals were allocated to collective covered pens with 5 animals each, with concrete floor cast, equipped with bunk and water trough. Then, the pens were randomly allocated among the four treatments, totaling 25 animals/treatment. The treatments consisted of increasing levels of wet distillers grains, being 0, 15, 30 and 45% inclusion in dietary DM (Table 1). All the WDG used in the experiment were produced by the same ethanol plant (SJC Bioenergia, Quirinópolis, Goiás, Brazil). The animals were fed for 129 days and the diets were given ad libitum twice a day (10 a.m. and 4 p.m.).

Slaughter and collection of samples

The animals were slaughtered at the age 24 months at the end of the experimental period in a commercial slaughterhouse (Frigoestrela, Estrela D’Oeste, São Paulo, Brazil), where they were subjected to fasting for 24 h and free access to water. The animals were desensitized using the brain concussion technique with the aid of a captive dart gun, followed by bleeding, leather removal and evisceration. Subsequently, the carcasses were divided longitudinally and carcass samples (approximately 20 g) were collected from the longissimus thoracis(LT) muscle at the 12th rib in the left half, using RNA stabilizer solution (RNAlater; Sigma-Aldrich, St. Louis, MO, United States) and stored in freezer at −80 ◦C until the beginning of molecular biology analyzes. In the refrigerator, the half-carcasses were refrigerated at 2–4 ◦C for 72 h. After cooling, four steaks (2.54 cm) were collected from the left half of the LT muscle for physical-chemical analyzes. The first steak was collected between the 12th and 13th ribs and the others from the cranial direction of the carcass.

Beef aging and physical-chemical analyzes

The first three steaks were vacuum packed with polyethylene packages and kept in refrigerator at 2 ◦C for 3, 10 and 17 days, respectively. At the end of each aging period, the samples were frozen at −20 ◦C until the physical-chemical analyzes. Subsequently, samples were thawed at 4 ◦C for 24 h and exposed to oxygen for 30 min at 4 ◦C (blooming time). First, the meat pH was measured using a Hanna digital pH meter (Model HI 99163, Hanna Instruments, Woonsocket, RI) with penetration probe.

In the same steak, meat color (L * = luminosity, a * = red intensity, b * = yellow intensity) was measured using the CIELab system of the CR-400 colorimeter (light source A, absorbance angle 10, Y, 0.01 at 160.00% reflectance; Konica Minolta Sensing, Inc., Tokyo, Japan), following the procedures previously described by Baldassini et al. (2017). The colorimeter was calibrated using a standard black and white plate and then three color readings were performed on the surface of the LT muscle sample. An average of the three measurements was generated for each variable (L *, a * and b *). Chroma colorimetric indexes (color saturation) were calculated by the formula [(a∗)2+(b∗)2] 0.5 and the hue angle (H◦) [tang −1(b∗/a∗)], as described by Cañeque et al. (2004).

The samples were placed in a grid over a glass refractory and weighed. Afterwards, a thermocouple was inserted into the geometric center of the samples, coupled to a digital thermometer model DT-612 (ATP Instrumentation, Ashby-de-la-Zouch, UK) to monitor the internal temperature of the samples. The steaks were roasted in a preheated oven (Feri90; Venâcio Aires, RS, Brazil) equipped with a thermostat to avoid temperature variation. When the internal temperature of the steak reached 40 ◦C the sample was turned and remained in the oven until reaching 71 ◦C internal temperature, according to the methodology described by Wheeler et al. (1994). Then, the samples were kept at room temperature for 15 min, weighed and refrigerated at 4 ◦C for 24 h. The cooking loss was determined by the weight difference before and after cooking. The cooking losses were measured from drip and evaporation losses. After cooling, eight cylinders with 1.27 cm diameter were removed from the parallel direction of the muscle fiber using a nozzle coupled to an industrial drill. The cylinders were sectioned in Salter Warner-Bratzler equipment with capacity of 25 kg and speed of 20 cm/minute. The results were presented in Newton (N) and eight replicate measurements per steak were performed to increase results accuracy.

Two animals per pen were randomly selected for chemical composition and gene expression analyzes, totaling 10 replicates per treatment. The analyzes of centesimal composition were performed in FoodScanTM (FOSS, Hillerød, Denmark). The samples were thawed at 4 ◦C for 24 h and the subcutaneous fat was removed from the LT muscle with the aid of a scalpel, then the steak was ground and homogenized for 5 min using a mixer, taking approximately 180 g of sample (Anderson, 2007). Three readings per sample were carried out. Samples were homogenized again and placed in the plate for the next reading. An average was

obtained for the values of moisture, protein, fat and ash and the values were expressed as percentage.

Gene expression

Gene expression tests were performed following procedures previously described (Teixeira et al., 2017). 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 (NCBI) platform (Table 2). Primers were designed using OligoPerfect Designer software (Invitrogen, Karlsruhe, Germany) and synthesized (Invitrogen, Carlsbad, CA, USA). Total RNA was extracted from muscle samples using QIAzol (QIAGEN, Valencia, CA, USA) and treated with DNA-free DNase (Ambion, Austin, TX) according to the manufacturer's instructions. To analyse the 28S and 18S rRNA bands, the total RNA was electrophoresed in a 1.0% (m/v) agarose gel, stained with GelRed nucleic acid gel stain (Biotium, Hayward, CA, USA) and visualized with a E-gel Imager Camera Hood (Life Technologies, Neve Yamin, Israel). The RNA quantity (ng/μL) and quality (260/280 and 260/230) were assessed using a spectrophotometer (DeNovix DS-11 Spectrophotometer) at 260 nm. cDNA synthesis was performed using the HighCapacity cDNA Reverse Transcription Kit (Applied Biosystems, Foster City, CA, USA) according to the manufacturer's instructions, and samples were stored at −20 ◦C.

Real-time qPCR (RT-qPCR) was performed on an Eppendorf Realplex system (Eppendorf, Hamburg, Germany) with a SYBR Green detection system (Applied Biosystems, Foster City, CA, USA). PCRs were incubated in a 96-well plate at 50 C for 2 min, followed by 95 ◦C for 10 min, followed by 40 cycles of 95 ◦C for 15 s and 60 ◦C for 1 min. The RT-qPCR analyses of each studied gene were performed using cDNA from biological replicates, with two technical replicates per biological replicate. Four reference genes were tested, and the best individual gene or combination of endogenous controls was chosen using the web-based tool RefFinder, which selected β-actin and CASC3 genes as more stable for use in muscle gene expression. A validation assay was performed to demonstrate that the amplification efficiencies of the target and reference genes were approximately equivalent. Standard curves were generated for the studied genes with the following dilutions: 1:5, 1:25, 1:125, 1:625 and 1:3125. Relative expression levels were calculated according to the method described by Pfaffl (2001),

which is based on Ct values that are corrected for the amplification efficiency of each primer pair.

Removal of animals and samples

During the experiment, three animals were taken from the study because they presented symptoms of polioencephalomalacia (n = 2) and had their urethras ruptured due to the practice of sodomy (n = 1). During the storage of gene expression samples, four samples had their identifications lost, two from the control group and two from the animals that received 30% WDG. Therefore, eight, 10, eight and 10 samples were used to analyze chemical composition and gene expression for the 0, 15, 30 and 45% treatments, respectively. As the objective of the experiment was to analyze chemical composition and gene expression from the same animals, the chemical composition of the lost samples was not analyzed either.

Statistical analyzes

Data were tested for normality through the Shapiro–Wilk Test, PROC UNIVARIATE procedure of SAS 9.4 (SAS Institute, Cary, NC, USA, 2011). The genes SCD1, PPARγ , SREBP-1c, ACOX, FASN, CPT2, FABP4, GPX1, LPL, PPARα and ACACA did not present normal distribution and were transformed using PROC RANK (SAS 9.4). The chemical composition and gene expression were analyzed using polynomial (linear, quadratic and control vs. WDG) contrasts using the PROC MIXED procedure, in which the inclusion of WDG was considered as fixed effect, the block and the pen as random effect, according to the model:

$$Yijk = \mu + Bi + Aj + Wk + eijk$$

where: μ = overall mean, *Bi* = random effect of block, *Aj* = random effect of pen, Wk = WDG levels effect (0, 15, 30 e 45% DM basis) and eijl = experimental error.

Meat quality data were submitted to Tukey's test using the PROC MIXED procedure, in which the model included the WDG level, aging days and WDG level × days of aging as fixed effect, block and pen as random effect, according to the model:

$$Yijkl = \mu + Bi + Aj + Wk + Dl + WDkl + eijkl$$

where: μ = overall mean, *Bi* = random effect of block, *Aj* = random effect of pen, *Wk* = WDG levels effect (0, 15, 30 e 45% DM basis), *Dl* = efeito dos dias de maturação, *WDkl* = interaction between the level of WDG and aging day and eijkl = experimental error.

In both models, animals were considered as the experimental unit. Significance was considered when $P < 0.05$.

Results

There was no significant difference ($P > 0.05$) for moisture, protein and ash in LT muscle regardless of the level of WDG. However, there was a reduction ($P < 0.05$) in the LT muscle fat content in all treatments (Table 3). On average, this reduction was 15.73% when the animals consumed WDG. It was also observed by polynomial contrasts that the inclusion of WDG reduced linearly ($P = 0.01$) the intramuscular fat content in LT. Then, the lowest intramuscular fat content was observed in the treatment with 45% de-oiled WDG.

The inclusion of WDG in the diets resulted in lower expression of lipogenic genes PPARγ , SCD, FAS and CPT2 ($P < 0.05$). On average, there was a reduction of 83.5%, 79.1%, 67.5% and 47.8% in the expression of the PPARγ , CPT2, SCD1 and FASN genes, respectively (Fig. 1). PPARy gene expression reduced linearly with the increase in WDG levels ($P < 0.01$). Similar results were observed (Fig. 1A) on SCD, FAS and CPT2 genes ($P < 0.05$). The inclusion of WDG reduced the expression of lipogenic genes and, consequently, the amount of intramuscular fat, confirming this study's hypothesis. However, there was no effect ($P > 0.05$) of WDG inclusion levels on meat quality traits such as pH, color (Table 4), cooking loss and tenderness (shear force) measured at three aging times (Table 5). In addition, there was no interaction ($P > 0.05$) between the level of WDG in the diets and aging time. In the present study, these results indicated that the inclusion of WDG in the diet did not affect meat tenderness.

The steaks submitted to aging showed lower pH than the ones that were not ($P < 0.01$). Regardless of the treatment, the average final pH was 5.83, 5.70 and 5.73 for aging days 0, 8 and 16, respectively. The luminosity of samples at aging 16 was 3% higher than in samples at aging 0 ($P < 0.01$). However, there was a lower cooking loss when the meat underwent aging ($P < 0.05$). Also, there was a reduction in the shear force in the steaks submitted to aging ($P < 0.01$). On average, there was a reduction of 3 N at 16 days of aging regardless of the treatment (Fig. 2).

Discussion

Diet effects on intramuscular fat

The hypothesis of the present study was that the inclusion of WDG with low fat content in finishing cattle diets reduces the expression of genes involved in the lipid metabolism of LT muscle and, therefore, affects the deposition of intramuscular fat. The results confirm this hypothesis. In other studies, although applying an intramuscular fat score methodology (Schoonmaker, Trenkle & Beitz, 2010) according to the USDA quality and yield grades, researchers reported reduced marbling in the meat of Angus cattle finished in feedlot with diets containing 40% WDG. In this study, a proportional reduction of 18% in the marbling score (control diet versus 40% WDG) occurred, corroborating the reduction of 23.97% observed in the present study on the meat lipid content, when the animals consumed diets with 45% WDG. In addition, the reduction in intramuscular fat content is not a result of the lower weight gain of the animals, since the inclusion of the by-product promoted better performance (Ferreira et al., 2018). It is possible to infer that the change in ruminal fermentation pattern due to the change in the nutritional profile of the diet (reduction of starch, especially) was responsible for the reduction in intramuscular fat content.

The inclusion of WDG replacing dry corn reduced the starch content of the experimental diets (from 47.63% to 24.63%). When starch is fermented in the rumen, there is a higher production of ruminal propionate, which is the main precursor of hepatic gluconeogenesis in ruminants (Bauman, Mellenberger & Derrig, 1973). According to the same authors, both rumen acetate and glucose synthesized from propionate are the main substrates that form acetyl units in de novo intramuscular synthesis. However, from 50 to 75% of the acetyl-CoA units formed are derived from blood glucose, and ruminal propionate seems to have a direct influence in this process (Smith & Crouse, 1984). The incorporation of acetate into fatty acid formation seems to be greater in subcutaneous adipose tissue than in muscle tissue, and glucose seems to have an inverse effect, being more incorporated into intramuscular tissue when compared to the subcutaneous (Rhoades et al., 2007). These results help to explain the reduction in intramuscular fat when WDG was included in the diets to replace dry corn. However, there are contradictory theories about the participation of glucose as a precursor of the acetyl units in the intramuscular fat in cattle (Nayananjalie et al., 2015).

After the formation of acetyl-CoA units, there is a carboxylation of this molecule that originates malonyl-CoA, through the enzyme acetyl-CoA carboxylase. Then acetyl-CoA and malonyl-CoA molecules bind through multiple enzymatic reactions originating long chain fatty acids, through the enzyme fatty acid synthase, encoded by the FASN gene (Ladeira et al., 2016).

In other words, the inclusion of WDG in the diets linearly reduced FASN gene expression ($P < 0.01$), potentially leading to a lower synthesis of the enzyme fatty acid synthase and lower intramuscular lipogenesis.

Nutrients and lipogenic genes expression

However, there are transcription factors of the PPAR family that regulate the expression of some genes responsible for lipogenesis, with a major role in the control of glucose and lipid regulation (Liu et al., 2014). Among these transcription factors, PPARα seems to activate the genes that are responsible for the oxidation of fatty acids, whereas PPARγ has an antagonistic effect, activating lipogenic and adipogenic genes, being more expressed in obese individuals (Gervois et al., 2000). PPARγ can be regulated by blood glucose concentrations, and it is more expressed when glucose is in high amounts (Liu et al., 2014). This may explain what happened in the present study since the inclusion of WDG in diets may have reduced the amount of blood glucose synthesized through ruminal propionate, reducing the expression of genes that encode PPARγ. Fatty acid synthase has a direct action on PPARγ regulation and adipogenesis, and the lower the synthesis of this enzyme, the lower the expression of PPARγ and, as a consequence, there is a reduction in adipose tissue (Lodhi et al., 2012), which may also have helped in the lower expression of PPARγ .

The transcription factor PPARγ may also play a role in the genes that stimulate fatty acid oxidation, with the CPT2 gene, the gene encoding carnitine palmitotransferase, showing that as PPARγ is silenced, there is a reduction in expression of CPT2 (Sharma et al., 2012). A similar effect of gene expression was observed in the present study, once PPARγ expression was linearly reduced, this effect was also found for the CPT2 gene ($P < 0.01$).

The inclusion of WDG in the diets reduced linearly SCD1 gene expression ($P < 0.01$). This effect may also be associated with the reduction in starch of the diets (Table 1). SCD1 encodes the enzyme stearoyl-CoA desaturase and its main function is the desaturation of fatty acids, transforming saturated into unsaturated (SFA in MUFA in meat). This gene is more expressed in diets with large amounts of starch as it provides higher meat marbling due to de novo synthesis (Graugnard et al., 2009). Therefore, as the ingestion impacts the expression of some key lipogenic genes, such as FASN, this effect reflects on other genes that are important to lipogenesis, such as PPARγ, SCD1 and CPT2, drastically reducing muscle fat and meat marbling. Moreover, Ceciliani et al. (2018) reported that the integration of data derived from

gene expression analyses with those from protein expression studies should be considered to draw a complete and reliable picture of the functions and the activities of adipose tissue due its fundamental importance for the quality of carcasses. Thus, in further nutrigenomic studies protein expression assays should be performed to integrate with data from fat gene expression, dissecting the molecular mechanism involved in intramuscular fat deposition.

De-oiled WDG and beef quality

According to Ribeiro et al. (2018) when animals are fed WDG with a high fat content, there is a higher concentration of polyunsaturated fatty acids in the membrane of the sarcoplasmic reticulum, anticipating the oxidation process of this membrane and the release of calcium in the muscle. These events may accelerate the onset of post-mortem proteolysis, reducing meat toughness. In other words, the inclusion of WDG in animal diets does not affect the tenderness (shear force) during the aging process (Table 5).

Considering that plants tend to extract fat from the by-product, their inclusion in the diets aiming at increasing meat tenderness should not become a common practice in quality meat production systems. Our results suggest that the inclusion of WDG reduced the expression of lipogenic genes and consequently, the marbling of LT muscle, without impairing the characteristics of tenderness (shear force) and meat color. The molecular mechanism associated with marbling and tenderness traits is often investigated in the literature, as reviewed by Picard, Gagaoua & Hollung (2017). According to researchers, molecular biology studies using both proteomics and gene expression approaches led to a substantial increase in knowledge in meat science, confirming the important role of energy metabolism and proteolysis in muscle to meat conversion. Thus, the gene expression assay performed in the present study was also interesting for understanding the mechanisms involved in tenderness (e.g., shear force).

Several studies have already shown improvement in meat tenderness with increasing aging time (Sañudo et al., 2004; Monsón, Sañudo & Sierra, 2005; Cho et al., 2016; Chao et al., 2018). In addition, some studies involving beef aging time have already shown an increase in luminosity (L *) and lower cooking loss with increasing aging time in the LT muscle (Vitale et al., 2014; Cho et al., 2016), corroborating the results obtained in the present study.

Marbling is a trait of great economic importance because, as well as color, it can influence the consumer's purchasing decision. According to Wheeler, Cundiff & Koch (1994),

marbling would explain at most 5% of the variability observed in meat palatability. However, according to this study, there is a large variation in tenderness within each class of marbling score in Bos taurus and Bos indicus animals. This means that it is possible to find meat that is considered tough or tender with different degrees of intramuscular fat. In Zebu animals, for example, researchers found a negative correlation between shear force and marbling score ($r = -0.18$; $P < 0.001$) but found no association between shear force and chemically-extracted intramuscular fat ($r = -0.01$) (Baldassini et al., 2017). Although there was a significant reduction in intramuscular fat content in the present study, the magnitude of this change was not enough to influence meat tenderness by shear force. This was probably because the animals used in this study are non-castrated males, which tend to present meat with low intramuscular fat.

Conclusion

The inclusion of de-oiled WDG promotes significant reduction in meat intramuscular fat content without affecting tenderness. The reduction in intramuscular fat content is a consequence of the lower expression of lipogenic genes. The use of WDG in high proportions and for extended periods, as in this study, might be a problem to obtain high degrees of marbling.

Considering that marbling is not solely determined by gene expression changes, translational regulation and post-translational modifications play important roles in the determination of phenotype. For future studies, perhaps the use of techniques such as proteomics would help in understanding how beef quality may be affected by these nutritional treatments.

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Table 1. Composition of the experimental diets.

Ingredients and diet composition	WDG (%)			
	0	15	30	45
Ingredients (% DM)				
Tifton 85	4.20	4.20	4.20	4.20
Sugarcane bagasse	7.10	7.10	7.10	7.10
Ground corn	74.92	65.27	52.00	38.73
Soybean meal	10.36	4.78	2.94	1.10
Wet distillers grains (WDG)	0	15.00	30.00	45.00
Supplement mineral-vitamin [1]	3.42	3.42	3.42	3.42
Potassium chloride	0	0.23	0.34	0.45
Nutritional composition (% DM)				
Dry matter (% NM)	86.78	78.08	69.40	60.72
Crude Protein	12.85	14.19	17.13	20.07
Ether Extract	3.42	3.53	3.54	3.55
Neutral Detergent Fiber	16.28	23.64	31.19	38.75
Non-fibrous carbohydrates	64.68	56.02	45.42	34.82
Starch	48.22	41.78	33.24	24.69
NEg (MJ/kg DM)	5.44	5.40	5.32	5.28

[1]Supplement mineral-vitamin: 18.5% Ca; 1.9% S; 1.5% Mg; 4.3% Na; 1.6% P; 1714.0 ppm Zn; 1285 ppm Mn; 426.0 ppm Cu; 21 ppm I; 5.7 ppm Se; 8.5 ppm Co; 285.0 ppm Fe; 85700.0 UI Vit A; 11430.0 UI Vit D3; 128.0 UI Vit E; 32.5% Urea; 945.0 ppm of monensin sodium.

Table 2. Sequences (5' to 3') and efficiencies of the primers used in quantitative real-time PCR.

Symbol	Name	Forward (F) and reverse (R)	Access number	Amplicon (bp)	R^2	Efficiency
PPARα	*Peroxisome proliferator-activated receptor α*	F CAATGGAGATGGTGGACACA R TTGTAGGAAGTCTGCCGAGAG	NM_001034036.1	95	0,992	99,2
PPARγ	*Peroxisome proliferator-activated receptor gamma*	F CGACACAAACTGAACACACAGAGT R TCAGCGGGAAGGACTTTATG	NM_181024.2	83	100	99,9
SREBP-1c	*Sterol regulatory element-binding protein-1c*	F GAGCCACCCTTCAACGAA R TGTCTTCTATGTCGGTCAGCA	NM_001113302.1	88	0,985	94,6
SCD1	*Stearoyl-CoA desaturase 1*	F ACCATCACAGCACCTCCTTC R ATTTCAGGGCGGATGTCTTC	NM_173959.4	95	0,999	98
LPL	*Lipoprotein lipase*	F CTCAGGACTCCCGAAGACAC R GTTTTGCTGCTGTGGTTGAA	NM_001075120.1	98	0,99	96,7
FABP4	*Fatty acid binding protein 4*	F GGATGGAAAATCAACCACCA R GTGGCAGTGACACCATTCAT	NM_174314.2	73	0,995	97
FASN	*Fatty acid synthase*	F ATCAACTCTGAGGGGCTGAA R CAACAAAACTGGTGCTCACG	U34794.1	83	0,974	99,5
ACOX	*Acyl-coenzyme A oxidase 1*	F GCTGTCCTAAGGCGTTTGTG R ATGATGCTCCCCTGAAGAAA	BC102761.2	83	0,994	99
CPT2	*Carnitine palmitoyltransferase 2*	F CTATTCCCAAACTTGAAGAC R TTTTCCTGAACTGGCTGTCA	NM_001045889.2	81	0,978	100

GPX1	*Glutathione Peroxidase*	F GGAGATCCTGAATTGCCTGA R CCATTCACCTCGCACTTTTC	NM_174076.3	87	0,991	100
ACACA	*Acetyl CoA carboxylase alfa*	F TGAAGAAGCAATGGATGAACACA R TTCAGACACGGAGCCAATAA	NM_174224.2	88	0,994	100
β-actin	*β-Actin*	F GTCCACCTTCCAGCAGATGT R CAGTCCGCCTAGAAGCATTT	NM_173979.3	90	0,998	100
CASC3	*Cancer susceptibility candidate 3*	F GGACCTCCACCTCAGTTCAA R GTCTTTGCCGTTGTGATGAA	NM_001098069.1	85	0,976	98

Table 3. Chemical composition of the *longissimus thoracis* muscle of F1 Angus-Nellore cattle fed different levels of de-oiled wet distillers grains (WDG).

Item (%)	WDG (% DM)				SEM[1]	*P-value*[2]		
	0	15	30	45		C vs W	L	Q
Moisture	74.41	74.34	74.11	74.49	0.27	0.71	0.99	0.31
Protein	23.20	23.22	23.05	23.58	0.20	0.69	0.24	0.18
Fat	2.67	2.32	2.40	2.03	0.16	0.03	0.01	0.94
Ash	1.14	1.09	1.10	1.12	0.02	0.15	0.62	0.09

[1]Standard Error Mean.

[2]C vs W, control vs WDG; L, linear effect and Q, quadratic effect.

Table 4. Color and pH characteristics of the *longissimus thoracis* muscle at three aging times (3, 10 and 17 postmortem days) of F1 Angus-Nellore cattle (n=100) fed different levels of de-oiled wet distillers grains (WGD).

Item[3]	Aging time (days)												SEM[1]	*P-value*[2]		
	3				10				17					WDG	Day	W x D
WDG (%DM)	0	15	30	45	0	15	30	45	0	15	30	45				
pH	5.81a	5.84a	5.88a	5.77a	5.73bc	5.68bc	5.74bc	5.66bc	5.73c	5.72c	5.74c	5.71c	0.04	0.14	<.01	0.84
L*	37.96a	37.56a	38.11a	37.20a	37.77a	37.31a	36.70a	37.51a	39.15b	38.63b	38.04b	39.28b	0.76	0.87	<.01	0.70
a*	20.30	20.05	20.98	20.48	19.78	20.21	19.91	19.09	20.34	20.12	20.47	20.89	0.56	0.92	0.06	0.48
b*	9.60	9.60	9.85	9.65	9.22	9.54	9.26	9.03	9.10	9.27	9.38	9.48	0.36	0.95	0.08	0.88
Chroma	22.48	22.24	23.41	22.66	21.96	22.57	21.93	21.42	22.42	22.29	23.01	23.37	0.60	0.85	0.05	0.36
Hue	25.26a	25.66a	24.79a	25.05a	24.80ab	25.07ab	24.63ab	24.47ab	23.89b	24.36b	24.37b	24.11b	0.52	0.68	0.01	0.95

[1]Standard Error Mean.

[2]WDG, Effect of the levels of WGD; Day, effect of the aging day; W x D, interaction between WDG and Day.

[3]L*, lightness; a*, redness; b*, yellowness; Chroma, chromaticity or quantify of color; Hue, real color.

Table 5. Cooking loss and shear force of the *longissimus thoracis* muscle at three aging times (3, 10 and 17 postmortem days of F1 Angus-Nellore cattle (n=100) fed de-oiled wet distillers grains (WDG).

Item[3]	Aging time (days)												SEM[1]	*P-value*[2]		
	3				10				17					WDG	Day	W x D
WDG (%DM)	0	15	30	45	0	15	30	45	0	15	30	45				
Evp, %	17.68	17.34	17.02	18.57	17.25	18.33	16.36	17.87	17.56	16.99	16.83	16.78	0.68	0.33	0.27	0.31
DL, %	2.52a	2.80a	2.54a	2.33a	2.00bc	2.29bc	2.25bc	2.15bc	1.98c	2.10c	2.13c	2.03c	0.17	0.24	<.01	0.83
CL, %	20.09a	20.15a	19.69a	21.06a	19.85ab	20.58ab	19.24ab	19.89ab	19.74b	19.18b	18.72b	18.75b	0.74	0.47	0.03	0.73
WBSF, N	30.92a	32.31[a]	32.04a	32.77a	29.04a	32.38a	31.98a	30.74a	28.11b	28.34b	29.41b	30.06b	1.24	0.23	<.01	0.86

[1]Standard Error Mean.

[2]WDG, Effect of the levels of WDG.

[3]Evp, Steak Evaporation Loss. DL, Steak Drip Loss; CL, Cookin Loss; WBSF, Warner Blatzler Shear Force.

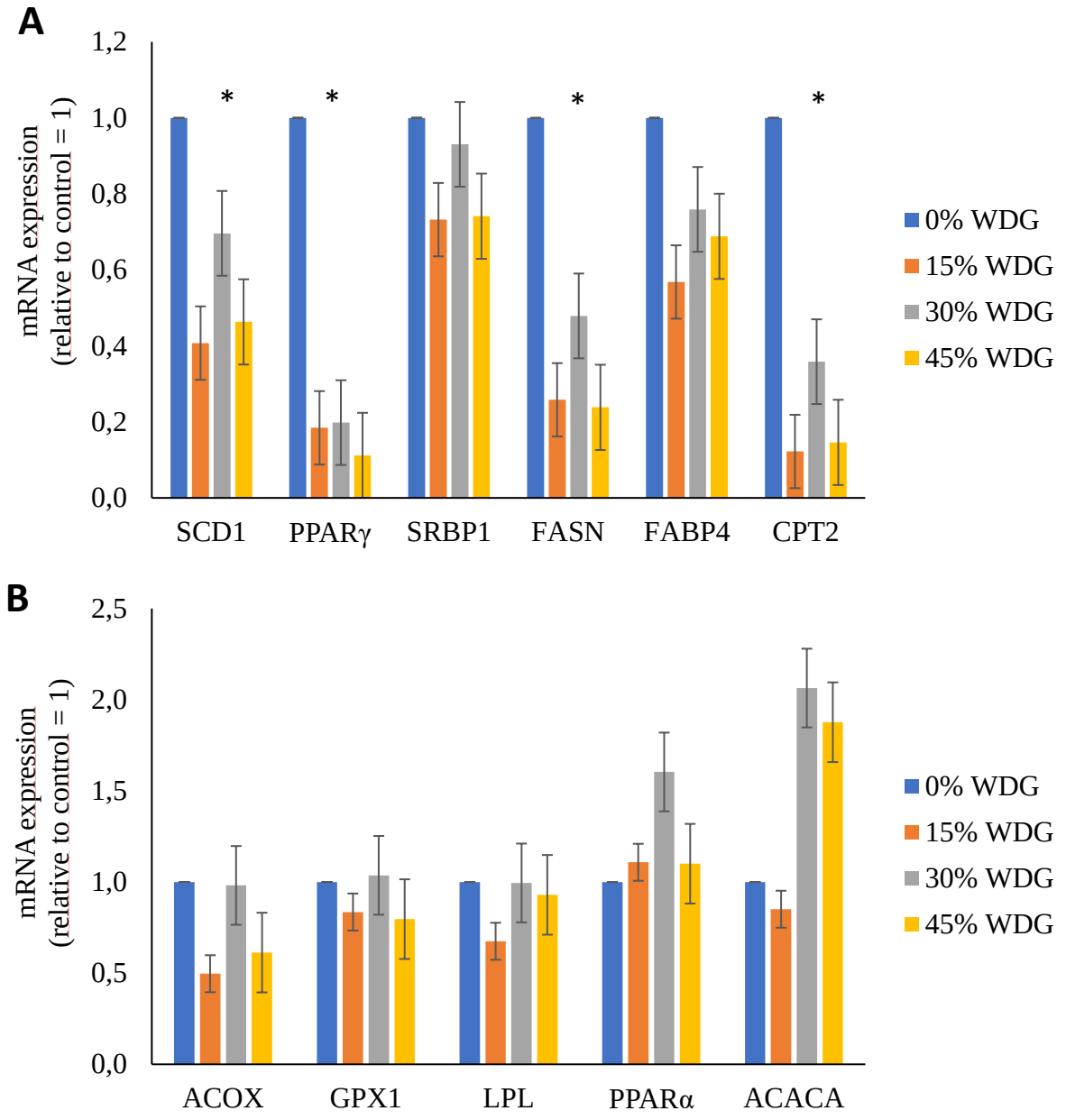

Figura 1. Expression of lipogenic genes in the *longissimus thoracis* muscle of F1 Angus-Nellore finished in feedlot fed different levels of de-oiled wet distillers grains (WDG).
(*) Statistical effect (P<005) between treatments. (A) Genes: Sterol regulatory element-biding protein-1c (SCD1), Peroxisome proliferator-activated receptor gamma (PPARγ), Stearoyl-CoA desaturase 1 (SRBP1), Fatty acid synthase (FASN), Fatty acid binding protein 4 (FABP4), Carnitine palmitoyl transferase 2 (CPT2); (B) Genes: Acyl-coenzyme A oxidase 1 (ACOX), Glutathione Peroxidase (GPX), Lipoprotein lipase (LPL), Peroxisome proliferator-activated receptor α (PPARα), and Acetyl CoA carboxylase alfa (ACACA).

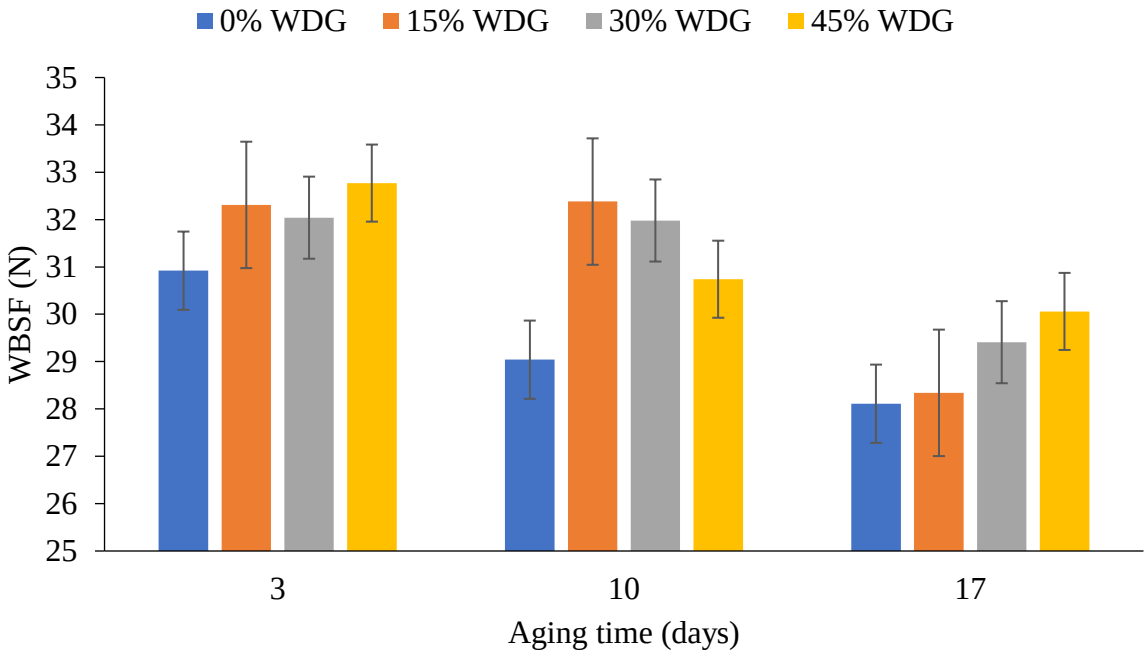

Figura 2. Shear Force (WBSF) of the *longissimus thoracis* muscle at three aging times (3, 10 and 17 days postmortem days) of F1 Angus-Nellore (n=100) finished in feedlot different levels de-oiled wet distillers grains (WGG).

CAPÍTULO 4 – Considerações finais

A utilização dos grãos de destilaria na dieta de bovinos F1 Nelore-Angus melhorou no ganho de peso diário (kg/dia), peso final (kg), peso de carcaça (kg) e área de olho de lombo, sem afetar os padrões de qualidade de carne. Entretanto, alguns nichos de mercado que utilizam do marmoreio da carne como principal atributo voltado a qualidade visual e sensorial do produto, pode ser prejudicado com a utilização do coproduto, visto que no presente trabalho a medida que o grão de destilaria foi incluído nas dietas, houve uma redução na expressão dos genes lipogênicos e na quantidade de gordura intramuscular. Porém, com o aumento na oferta dos grãos de destilaria no Brasil, pesquisas adicionais a essa devem ser realizadas, com o objetivo de testar a inclusão do coproduto em outras raças de bovinos (Zebuínos), outros sistemas de produção (semi-intensivo e extensivo), viabilidade econômica da utilização (distância das plantas de etanol, custo de frete do produto, relação densidade da dieta x custo operacional das plantas de confinamento) e concretizar os níveis ideais de inclusão visando o sistema de produção de bovinos de corte como um todo.