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RESÍDUO DE UVA NA DIETA DE BOVINOS DE CORTE EM CONFINAMENTO:

UMA ALTERNATIVA NUTRICIONAL RENTÁVEL

CHAPECÓ
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Dissertação apresentada como requisito parcial para obtenção do título de mestre em Zootecnia pelo Programa de Pós-Graduação em Zootecnia da Universidade do Estado de Santa Catarina – UDESC.
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“O sucesso nasce do querer, da determinação e persistência em se chegar a um objetivo. Mesmo não atingindo o alvo, quem busca vence obstáculos, no mínimo fará coisas admiráveis.”

(JOSÉ DE ALENCAR)

RESUMO

A estreita diferença entre preço pago ao produtor e os custos de produção é um dos principais desafios enfrentados na bovinocultura. Por este motivo, há necessidade da criação dos animais de forma alternativa, com ênfase em sustentabilidade, a fim de ser mais competitivo, rentável e contínuo. Neste sentido, o objetivo deste estudo foi avaliar os efeitos da inclusão de farelo de bagaço de uva e silagem de bagaço de uva como fontes de fibra dietética alternativas ao farelo de trigo e a casca de soja sobre o desempenho, saúde, qualidade da carne e viabilidade econômica de bovinos de corte em confinamento. Para tanto, foi conduzido um estudo com 24 novilhos cruzados (Charolês x Nelore), divididos em delineamento controlado randomizado pelo peso corporal inicial (248 ± 19.32 kg) em três tratamentos: dieta controle (dieta tradicional de confinamento) dieta GPS (inclusão de 100g/kg na MS de silagem de bagaço de uva na dieta) e dieta GPB (inclusão de 100g/kg na MS de farelo de bagaço de uva na dieta), ambas com proporção 40:60 (volumoso/concentrado). Deste estudo, foram elaborados dois manuscritos, divididos em dados pré-abate (1) e pós-abate (2). No manuscrito 1, podemos verificar que as dietas não influenciaram o consumo de matéria seca. O desempenho e perfil fermentativo ruminal dos animais que receberam a dieta SBU não foram afetados, diferente da dieta FBU, quando aos animais tiveram menor concentrações de acetato, propionato e valerato no rúmen. Os novilhos SBU apresentaram maior contagem de linfócitos, em contrapartida, menores concentrações de ceruloplasmina e haptoglobina. Além disso, os animais SBU apresentaram maior capacidade antioxidante, representada pelo aumento dos PSH sérico, hepático e intestinal, assim como, menores níveis de ROS no fígado; e TBARS no fígado. Tivemos dificuldade de determinar até quanto os efeitos benéficos da dieta FBU sobre a saúde animal, pois apesar da maior resposta antioxidante, esses animais tiveram menor ganho de peso. Por fim, constatamos que o uso de SBU possibilita viabilidade econômica, por proporcionar maior receita sobre os custos de alimentação, efeito não observado pela inclusão da FBU. Para o manuscrito 2, podemos verificar que os novilhos que consumiram as dietas SBU e FBU apresentaram menor peroxidação lipídica (TBARS) e concentrações de ROS na carne. Além disso, no primeiro dia de prateleira, maior atividade da enzima GST foi observado na carne dos animais dos grupos SBU e FBU. A dieta FBU tendeu a uma carne com maior quantidade da soma n-6 ômega. A dieta SBU diminuiu os níveis de ácidos graxos

saturados da carne. Concluímos que a inclusão de SBU na nutrição de novilhos é uma alternativa promissora para reduzir os custos alimentares e melhorar a saúde animal de bovinos em confinamento. Além disso, os tratamentos dietéticos SBU e FBU são uma alternativa promissora para manter os padrões de qualidade da carne durante a vida de prateleira em condições de varejo.

Palavras-chave: Nutrição Animal; Saúde Animal; Vida de Prateleira; Antioxidante.

ABSTRACT

The narrow difference between the price paid to the producer and the respective production costs is one of the main “bottlenecks” faced in cattle farming. For this reason, the need for alternative, sustainable recycling in a rational way in order to be more competitive, profitable and continuous becomes increasingly important. In this sense, the objective of this study was to evaluate whether the inclusion of 100g/kg of grape pomace bran and grape pomace silage as sources of dietary fiber alternative to wheat bran and soybean hulls has positive effects on growth performance, animal health, meat quality and economic viability of steer in confinement. Therefore, a study was conducted with 24 crossbred steers (Charolais x Nellore), divided into a randomized controlled design by initial body weight (248 ± 19.32 kg) in three treatments: control diet (traditional confinement diet) GPS diet (inclusion of 100g /kg in the DM of grape pomace silage in the diet) and GPB diet (inclusion of 100g/kg in the DM of grape pomace bran in the diet). From this study, two manuscripts were prepared, divided into pre-slaughter (1) and post-slaughter (2) data. In manuscript 1, we can verify that the diets did not influence the dry matter intake. The growth performance and ruminal fermentative profile of the animals that received the GPS diet were not affected, unlike the FBU diet, when the animals had lower concentrations of acetate, propionate and valerate in the rumen. SBU steers had higher lymphocyte counts, on the other hand, lower concentrations of ceruloplasmin and haptoglobin. In addition, SBU animals showed greater antioxidant capacity, represented by the increase in serum, hepatic and intestinal PSH, as well as lower levels of ROS in the liver; and TBARS in the liver. It was difficult to determine how much the beneficial effects of the FBU diet on animal health were, because despite the greater antioxidant response, these animals had less weight gain. Finally, we found that the use of SBU makes economic viability possible, as it provides greater revenue on food costs, an effect not observed by the inclusion of FBU. For manuscript 2, we can verify that the steers that consumed the SBU and FBU diets had lower lipid peroxidation (TBARS) and ROS concentrations in the meat. In addition, on the first day of shelf life, greater activity of the GST enzyme was observed in the meat of the animals of the SBU and FBU groups. The FBU diet tended to have meat with a higher amount of the sum n-6 omega. The SBU diet decreased the levels of saturated fatty acids in meat. We conclude that the inclusion of SBU in the nutrition of steers is a promising alternative to reduce feed costs

and improve animal health of cattle in confinement. Furthermore, SBU and FBU dietary treatments are a promising alternative to maintain meat quality standards during shelf life under retail conditions.

Keywords: Animal Nutrition; Animal Health; Shelf Life; Antioxidant.

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LISTA DE ABREVIATURAS E SIGLAS

MS	Matéria Seca
PB	Proteína Bruta
EE	Extrato Etéreo
FDN	Fibra Digestível em Detergente Neutro
FDA	Fibra Digestível em Detergente Ácido
ha	Hectare
NH_3-N	Nitrogênio de Amônia
CH_4	Metano
PUS	Polpa de Uva Seco
AGPI	Ácidos graxos poli-insaturados
GPR	Grape Pomace Residue
GPS	Grape Pomace Silage
GPB	Grape Pomace Bran
DM	Dry Matter
PRAM	Methylene blue reduction test
ME	Metabolizable Energy
GGT	Gamma-glutamyl Transferase
AST	Aspartate Aminotransferase
GST	Glutathione S-transferase
TBARS	Thiobarbituric Acid Reactive Substances
ROS	Reactive Oxygen Species
SCFA	Short-chain Fatty Acid
PUFAs	Polyunsaturated Fatty Acids
WBSF	Warner–Bratzler shear force
WHC	Water-holding capacity
CL	Cooking loss
L*	Lightness, (L*)
a*	Redness
b*	Yellowness
PSH	Protein Thiols

SUMÁRIO

1 INTRODUÇÃO

A produção de resíduos pelas agroindústrias é considerada um problema e um desafio logístico para destinação adequada deste material. O resíduo da produção de vinhos (biomassa secundária da uva), é gerado pela prensa dos sólidos na produção vinho e sucos. Este material é instável, ou seja, altamente perecível nas condições ambientais, os quais devem receber destinação imediata, a fim de, evitar sua deterioração. Isso posto, enfatiza-se o problema logístico deste setor da agroindústria, o que justifica sua subutilização como adubo orgânico.

Concomitantemente a este cenário, existem dificuldades remuneratórias enfrentadas na bovinocultura de corte. A estreita diferença entre preço pago ao produtor e, os respectivos custos de produção que percorrem a muitos anos no setor, ressalta a necessidade da criação sustentável dos animais, por meio da reciclagem, por exemplo de resíduos, a fim de ser mais competitivo, rentável e contínuo. Em particular, destaco os altos custos com a alimentação, em específico os cereais, que abrangem o maior custo diário com animais em confinamento (ABIEC, 2022).

O uso de subprodutos de agroindústrias na formulação de dietas de ruminantes pode ser uma estratégia para reduzir o custo de produção, bem como uma oportunidade e uma ferramenta que possibilita a pecuária sustentável. Fato este possível devido a capacidade dos ruminantes converter insumos não comestíveis em proteína de alto valor (carne e leite) (OLTJEN; BECKETT, 1996). Além da ótima relação custo-benefício, resíduos agroindústrias podem conter em sua composição compostos bioativos (SALAMI et al., 2019), que mostram ter capacidades funcionais ao organismo animal (SANTANA-MÉRIDAS et al. 2012).

A semente e casca de uva (*Vitis vinifera*) além de conter composição nutricional razoável, possui compostos bioativos. Aqui destaco os flavonóides (catequina, epicatequina, procianidinas e antocianinas), ácidos fenólicos e resveratrol presentes nestes materiais. Estes compostos mostraram ter atividades funcionais, capaz de melhorar o desempenho animal e a qualidade da carne, já observados em monogástricos e ruminantes (COSTA et al., 2022; TAYENGWA et al., 2020b).

Em bovinos de corte, esse resíduo tem sido alvo de recentes estudos com várias finalidades, os quais mostram ter efeitos positivos relacionados a vida útil da carne bovina (TAYENGWA et al., 2020a), modulação do perfil de ácidos graxos da carne, sem interferir sobre os aspectos sensoriais (TAYENGWA et al., 2021a). No início de 2021, o primeiro artigo sobre proporção ideal de bagaço de uva seco em dietas para gado confinado foi publicado por Vinyard et al. (2021), que mostrou que nas suas condições experimentais a inclusão ideal é150g/kg. Além disso, a adição de bagaço de uva desidratado possibilita maior consumo de nutrientes, modulação no perfil de fermentação da proteína e consequentemente influência sobre balanço de nitrogênio ruminal (TAYENGWA et al., 2021b).

Neste sentido, embora exista conhecimento sobre os efeitos do resíduo de uva e seus compostos na nutrição animal, os mais recentes trabalhos em bovinos de corte supracitados, possuem resultados exclusivos e circunscritos. Neste contexto, novos ensaios são necessários para validar e aumentar a confiabilidade dos resultados já publicados, bem como, entender novos efeitos e mecanismos envolvidos e propor novas formulações.

Neste estudo, objetivamos avaliar a inclusão do resíduo de uva, dentro de um cenário ainda não explorado pela literatura. Atualmente não há conhecimento dos efeitos do uso do bagaço de resíduo de uva na forma ensilada e desidratada como fontes de fibra dietética alternativas ao farelo de trigo e casca de soja sobre o desempenho de crescimento, carcaça, saúde animal e atributos de qualidade da carne quando incluídos nas dietas de acabamento de novilhos charolês, no Brasil.

Nossa hipótese é que ambos os resíduos (GPS e GPB) podem ser utilizados como fonte de fibra alimentar, pois proporcionam maior saúde animal e desempenho zootécnico. Em particular, acreditamos que o GPB tenha maior aproveitamento pelos animais, pois o resíduo recebeu processamento físico, e assim, efeitos benéficos mais evidentes na saúde e no desempenho, quando comparados aos que recebem o resíduo bruto (GPS).

2 REVISÃO BIBLIOGRÁFICA

2.1 BOVINOCULTURA DE CORTE

A bovinocultura de corte brasileira ao longo dos últimos anos passou importantes e marcantes transformações que colocam hoje a atividade em destaque no cenário nacional e intencional da produção de carne bovina. A evolução da atividade, tange o interesse do produtor na busca da maior eficiência e produtividade do seu rebanho, na migração dos sistemas de produção extensivos para os sistemas de criação intensiva.

O rebanho que há 20 anos atrás não ultrapassava 185 milhões de cabeças, hoje totaliza 224 milhões de cabeças, representando um crescimento anual de 1,05% (IBGE, 2021a). Neste mesmo período, a produção de carne teve um crescimento de taxas ainda maiores (2,93%) (IBGE, 2021a) e junto a isso, houve alteração do perfil dos animais abatidos. A média de peso das carcaças abatidas aumentou em 14,09% (IBGE, 2022). Além disso, o abate de novilhos precoces tornou-se mais casual, neste período houve aumento de 9,97%, que representa acréscimo de 350 mil cabeças abatidas a mais anualmente (IBGE, 2022). Estes valores indicam ganhos representativos na produtividade, e tornaram a carne brasileira mais competitiva, qualificando-a para os mercados nacional e internacional.

Contudo, vale salientar que, ao passo que se intensifica o sistema de criação, concomitantemente aumenta os custos de alimentação, como sugerido pela consultoria Athenagro (ABIEC, 2022) e assim, os desafios sobre a estreita margem de lucro da atividade. Neste sentido, mesmo diante da evolução dos últimos 20 anos da atividade, temos de considerar que a bovinocultura de corte brasileira ainda tem muito a melhorar em eficiência produtiva.

2.2 MANEJO NUTRICIONAL DOS CONFINAMENTOS BRASILEIROS

Reinventar-se nutricionalmente é um dos grandes desafios dos profissionais que trabalham com nutrição de bovinos de corte. O aumento na alimentação confinada demandou e demanda de novas técnicas e metas para otimizar o processo e garantir o sucesso até o produto final. No Brasil, o uso de pesquisas para monitorar o desenvolvimento do agronegócio é uma abordagem

relativamente nova. Nos últimos 15 anos, três pesquisas foram realizadas com nutricionistas de bovinos confinados para fornecer um panorama das práticas nutricionais e de manejo nos confinamentos brasileiros nos anos de 2009 (MILLEN et al., 2009), 2013 (OLIVEIRA; MILLEN, 2014) e 2016 (PINTO; MILLEN, 2018). Os dados dessas pesquisas permitem constatar que o perfil das dietas tem sido modificado de acordo o passar do tempo, e que estas estão cada vez mais densas. Millen et al. (2009) mostram que a média utilizada de volumosos era de 28%, ao passo que a descrita por Pinto e Millen (2018) tinha retraído para 20,6%. Essa redução pode estar relacionada ao aumento do teor energético, que consequentemente depende de maior investimento nutricional.

Vale salientar que, dada a pressão econômica dos custos dos grãos, cerca de 70,6% dos confinamentos fazem uso de algum tipo de coproduto nas dietas de terminação (PINTO; MILLEN, 2018). Segundo estes autores, os três coprodutos mais utilizados é caroço de algodão (36,4%), a polpa cítrica granulada (27,3%), seguida da casca de soja (21,2%) e da casca de algodão, alto teor de óleo (15,1%).

Todavia, para garantir o sucesso na utilização de coprodutos na alimentação animal é necessário bom planejamento, armazenamento adequado e o uso com critério, obedecendo suas franquezas e limitações nutricionais. Além disso, como ponto crucial é necessário a verificação da disponibilidade e sazonalidade do produto (CHAVES et al., 2014).

A região sul, por questões de logística e custo, o caroço de algodão não é muito utilizado, diferente da média nacional relatado por Pinto e Millen (2018). Isso faz com que exista a necessidade de explorar coprodutos regionais, os quais possam viabilizar seu uso, por conta do seu baixo custo. Isto posto, enfatiza o potencial do resíduo de bagaço de uva na região sulina.

2.3 PRODUÇÃO DE UVA NO BRASIL

A viticultura brasileira apresenta particularidades de cultivo, com diferentes características no ciclo de produção, época de colheita, cultivares, tratos culturais, tipo de produto e foco de mercado específicos em cada região (MELLO; MACHADO, 2021). De modo geral, a produção de uva pode ser

destinada a dois destinos: uva para consumo in natura e a uva para o beneficiamento (produção de vinhos e sucos).

O Brasil possui cerca de 75.622 ha de área de cultivo destinados a produção de uva (IBGE, 2021b) e possui produção por safra de 1.748.197 toneladas de uva *in natura* (IBGE, 2021b). Em destaque, a região sul dispões de uma grande área de videiras, sendo que em 2021 esta região foi responsável por produzir cerca de 1.056.985 toneladas, ou seja, 60,46% da produção total nacional. No estado de Santa Catarina, neste mesmo período foi colhido cerca de 59.712 toneladas de uva de 3.911hactares de videiras (IBGE, 2021b)

Com base de dados do IBGE, Mello e Machado (2021) estimaram que em 2020 cerca de 46,72% da produção total de uva foi destinada a processamento, ou seja, se utilizarmos este valor, cerca de 493.823,39 toneladas de uva são destinadas para produção de sucos e vinhos.

2.3.1 Resíduo de Bagaço de Uva (RBU)

O RBU, é gerado após a prensagem dos cachos de uva durante a produção de vinho e suco de uva, que representa em geral cerca de 20% da massa total de uvas processadas. A produção desta biomassa secundária na região se torna bem expressiva, vista sua grande produção, onde podemos estimar torno de 98.764,68 toneladas de resíduo no ano de 2021.

A composição de partículas do RBU pode sofrer alterações por fatores climáticos, genéticos e modo de processamento, mas comumente, possuem em torno de 473g/kg de casca, 277g/kg de talos e 250g/kg de sementes (NERANTZIS; TATARIDIS, 2006) no bagaço *in natura* (Figura 1). De modo geral, este resíduo é caracterizado pela alta concentração de fibras e sua baixa digestibilidade, assim como grande quantidade de extrato etéreo, contudo, podem sofrer alterações de acordo com a mudanças nas participações das partículas (Tabela 1).

Figura 1 - Resíduo de bagaço de uva in natura.

Fonte: Autoria própria (2021)

Tabela 1 - Composição bromatológica da biomassa secundária da uva

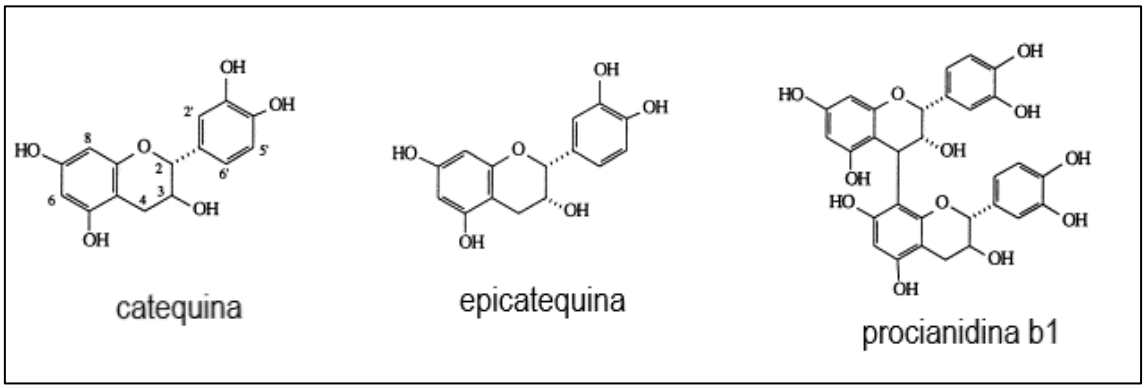
MS (%)	PB (%)	EE (%)	FDN (%)	FDA (%)	Autores
30,59	13,98	8,34	64,07	53,31	Massaro et al. (2021)
29,09	13,36	5,02	53,63	51,15	Flores et al. (2021)
34,10	11,61	7,64	68,82	57,25	Massaro et al. (2020)
41,50	11,70	11,00	62,00	59,40	Autoria própria*
33,82	**12,66**	**8,00**	**62,14**	**55,27**	**Média**

Fonte: Elaborado pelo autor (2022); * Dados não publicados

2.3.2 Compostos fenólicos da uva

Compostos fenólicos são os metabólitos secundários das plantas, usados como agentes de autodefesa (SALTVEIT et al., 2017). Este grupo de moléculas são definidos quimicamente como substâncias que possuem um anel aromático com um ou mais grupos hidroxil, incluindo seus derivados funcionais (SHAHIDI; NACZK, 2004) (Figura 2), podendo ser denominado de polifenóis, quando possuírem mais de um grupo de hidroxil fenólico ligado a um ou mais anéis de benzeno (VERMERRIS; NICHOLSON, 2006).

Figura 2 - Estruturas químicas de alguns polifenóis de bagaço de uva.



Fonte: Adaptado de Lu e Foo, (1999)

Com base nestas estruturas químicas e suas similaridades, os fenólicos naturais são geralmente classificados em quatro grandes classes de polifenóis: ácidos fenólicos, flavonoides, lignanas e estilbenos (SPENCER et al., 2008). No RBU, a composição destes fenólicos varia de acordo a cultivar (MONTEALEGRE et al., 2006), maturidade do ponto de colheita da uva (KENNEDY et al., 2000) e clima (MONTEALEGRE et al., 2006). As hastes das uvas apresentaram um teor de polifenóis totais de 5,8%, destes cerca de 93% foram atribuídos a flavonoides (Markis et al., 2007). As sementes de uva, é a fração do bagaço do resíduo da uva que contém aporte de polifenóis, onde pode chegar a 11,1% (Markis et al., 2007), compostos por ácido gálico e os flavonóis (KAMMERER et al., 2004). As cascas da uva vermelha possuem 3,6% de polifenóis totais, sendo destes 98,9% flavonoides, ao passo que, as cascas da uva branca possuirem 0,97% de polifenóis totais, sendo destes 95,0% flavonoides (MARKIS et al., 2007). Estas substâncias por sua vez, são conhecidas por possuírem atividades funcionais, como (YU; AHMEDNA, 2013).

Os compostos fenólicos uva possuem características anti-inflamatórias (LI et al., 2001 ; COLOMBO et al., 2019 ; RODRÍGUEZ-MORGADO et al., 2015), dada a ação dos seus compostos na inibição da via NF-kB através da ativação do PPAR-gama (MARTINEZ-MICAELO et al., 2015), regulação de quinases de proteína ativada mitogen (WADSWORTH et al., 2001, CHO et al., 2003), regulação do ácido araquidônico (KIM et al., 1998, LUCERI et al., 2002, HOU et al, 2007) ou simplesmente consequência na diminuição de espécies reativas ao oxigênio e sua indução da via inflamatória (SALZANO et al., 2014).

Além disso, estes compostos mostram estimular a sistema imune, na interação sobre os receptores imunológicos (MAGRONE et al, 2020), modulação na produção das citocinas, principalmente do resveratrol na atenuação de interleucina IL-1β e IL-6 e, como resultado, reduzindo a inflamação crônica de baixo grau (SCHWAGER et al, 2017; FOSSATI et al., 1998).

2.4 USO DE RESÍDUO DA UVA COMO INGREDIENTE NA NUTRIÇÃO ANIMAL

Os primeiros relatos na utilização do resíduo de uva na nutrição bovina foram na década de 80 (FAMUYIWA, 1982). Contudo, muitos resultados e informações importantes estão sendo publicadas e investigadas atualmente. Pontos crucias de sua utilização, como níveis de inclusão foram publicados recentemente por Vinyard et al. (2021). Estes autores investigaram a proporção ótima de inclusão do resíduo de uva em dietas para bovinos em terminação sobre os parâmetros de ingestão de nutrientes e sua digestão aparente do trato total, pH e a fermentação ruminal, estimativa da síntese de proteína microbiana, a rota de excreção de N e os metabólitos sanguíneos. Os autores concluem que, as respostas à proporção de resíduo seco de uva dietético foram em sua maioria quadráticas e que nível de inclusão alimentar de até 150g/kg não comprometem o desempenho animal.

Os efeitos negativos no desempenho foram bem marcantes quando Caetano et al. (2019) fizeram altas inclusões do resíduo (30%) e constataram a redução da emissão diárias de CH_4, porém conjuntamente a diminuição do desempenho dos animais. Os autores explicam tal achado devido ao alto teor de taninos condensados e lignina no bagaço de uva, componentes que diminuiu a energia disponível aos animais. Nesse trabalho os autores concluem e ressaltam a importância de novos estudos com inclusões inferiores deste resíduo para verificar possíveis benefícios para ruminantes.

Tayengwa et al. (2021b) publicaram estudos pioneiros na comparação dos efeitos da alimentação com polpa cítrica desidratada ou bagaço de uva desidratado como fontes alternativas de fibra ao farelo de trigo na ingestão e digestibilidade de nutrientes, eficiência de fermentação ruminal, suprimento retenção e eficiência de nitrogênio (N) microbiano. Os autores puderam

constatar que os novilhos alimentados com o bagaço de uva apresentaram maior ingestão de nutrientes, retenção de N e sua eficiência de utilização, assim como, menor digestibilidade aparente dos nutrientes, fornecimento de N microbiano e derivados de purina total em comparação com a dieta de polpa cítrica seca. Assim, concluíram com base nestas descobertas, que o bagaço de uva seco pode ser uma fonte alternativa de fibra alimentar melhor em comparação com o farelo de trigo e a polpa cítrica seca.

Contudo, vale ponderar que para validar a utilização deste resíduo, além de analisar os efeitos sobre os índices zootécnicos é imprescindível verificar os seus efeitos sobre as características de carcaça, assim como, sobre a indicadores da qualidade da carne. De nada adianta produzir de forma eficiente, mas produzir alimentos de menor qualidade.

Tayengwa et al. (2020) observaram efeitos positivos na vida útil da carne bovina durante a exibição no varejo, provenientes da suplementação de 15% de PUS em novilhos angus. No entanto, os autores concluíram que é necessário estudos para averiguar os efeitos da alimentação com PUS como conservantes naturais da dieta, no perfil de ácidos graxos, compostos voláteis e qualidade sensorial descritiva quando incluídos em dietas de acabamento de bovinos de corte.

Na sequência, esses autores (TAYENGWA et al. 2021a) publicaram o primeiro estudo que conduzir uma avaliação conjunta dos efeitos da alimentação com resíduo de bagaço seco sobre os ácidos graxos, compostos voláteis e perfis sensoriais da carne bovina de novilhos Angus. Estes autores averiguaram que, terminação de novilhos com dietas suplementadas com subprodutos vinícolas pode ser uma estratégia viável para aumentar os AGPI individuais e totais e na carne bovina sem comprometer a maioria dos aspectos do perfil sensorial. Porém, estes autores concluem que, pesquisas adicionais seriam necessárias para analisar de forma abrangente o perfil de ácidos graxos do longíssimo torácico e examinar estratégias para mitigar os problemas de maciez de cítricos alimentados com carne bovina e subprodutos vinícolas.

Outros recentes trabalhos em ruminantes, também descrevem efeitos benéficos na utilização do resíduo da uva e seus componentes, dentro de cada objetivo e sistema de criação. Massaro Junior et al. (2021), fizeram inclusões

crescentes do resíduo de uva (10%, 20% e 30%) na dieta de cordeiros, e concluíram que até a dose máxima, que proporciona à produção de carne ovina recursos sustentáveis que atendem às demandas dos consumidores. Também em ovinos, efeitos positivos na qualidade e estabilidade oxidativa da carne sem afetar os atributos sensoriais (FLORES et al., 2021), assim como melhora da saúde do animal quando usado na forma desidrata (MOLOSSE et al, 2021)

Em novilhas em sistema de criação *backgrounding*, a inclusão do resíduo na forma ensilada e desidratado na dieta não afetou o consumo dos animais e diminuiu a excreção de N da urina para as fezes, o que é benéfico do ponto de vista ambiental (REAM et al., 2021). Efeitos similares descritos por Suescun-Ospina et al (2022), que concluíram que substituição parcial de feno misto por resíduo da uva em até 10% MS em dietas ricas e pobres em fibras em condições *in vitro* reduz as concentrações de NH3-N sem efeitos negativos nos parâmetros de fermentação ruminal.

Fora do meio científico, o uso deste resíduo na alimentação de bovinos é algo bem frequente e comum de ser identificado nas regiões produtoras de uva por pecuaristas de corte. O uso empírico deste material é um problema, visto os variados efeitos que ele pode gerar dependendo da situação, identificados nos trabalhos em países vizinhos citados acima. Neste sentido, compreender os efeitos da inclusão do resíduo em bovinos nas realidades do Brasil, é primordial.

3 ARTIGO 1: THE EFFECTS OF THE INCLUSION OF ENSILED AND DEHYDRATED GRAPE POMACE IN BEEF CATTLE DIET: GROWTH PERFORMANCE, HEALTH, AND ECONOMIC VIABILITY

Os resultados desta dissertação são apresentados na forma de artigo, com a seções de acordo com as orientações da Revista Animal Feed Science and Technology.

3.1 ABSTRACT

The present study aimed to evaluate whether the inclusion of 100 g/kg of grape pomace silage (GPS) and grape pomace meal (GPB) as sources of dietary fiber alternative to wheat bran and soybean hulls would improve performance, health, fermentation parameters, and digestibility of feedlot steers in Brazil. Thus, 24 crossbred steers (Charolais x Nellore) with an initial average body weight of 248 kg were used divided in a randomized controlled design (n = 8 steers) into three experimental groups: control group (traditional confinement diet), GPS diet (diet with 100g/kg of grape pomace silage) and GPB group (diet with 100g/kg of grape pomace bran). Diets did not influence dry matter intake, but steer fed the GPB diet had higher starch and ethereal extract (EE) consumption. Steer fed the GPS diet had higher EE consumption. There was lower starch and EE digestibility with the GPS diet. The growth performance and ruminal fermentative profile of the animals that received the GPS diet were not affected, unlike those consuming the GPB diet, which had lower concentrations of acetate, propionate, and valerate in the rumen. The diets influenced animal health, demonstrated by the modulation of the immune and inflammatory responses. The GPS steer showed lymphocyte counts, but lower concentrations of ceruloplasmin and haptoglobin and higher antioxidant capacity, represented by higher serum, hepatic and intestinal protein thiols. GPS steers also showed a decrease in reactive oxygen species (in the liver and serum) and thiobarbituric acid reactive substances (in the liver). It was difficult to determine how significant the benefits of the GPB diet were on health and antioxidant capacity because these animals gained less weight. Finally, GPS improved economic viability, providing more significant revenue from feed costs, an effect not observed with GPB. We conclude that including GPS as an

ingredient in steer diets can reduce feed costs and improve health in feedlot cattle.

Keywords: Ruminal fermentation, Nutrient intake, Byproducts, Animal production.

3.2 INTRODUCTION

Secondary biomass obtained from post-harvest processing of crops into valuable products (JOHNSON; LINKE-HEPP, 2007) is seen as an opportunity by the agri-food industry; this biomass enables sustainable livestock farming because ruminants convert high-value edible protein, meat, and milk (OLTJEN; BECKETT, 1996). In addition to the excellent cost-benefit ratio, agro-industrial residues may contain bioactive (SALAMI et al., 2019), which are shown to have functional capabilities for animals (SANTANA-MÉRIDAS et al., 2012). This study highlights grape pomace residue, the subject of numerous scientific studies in ruminant production in recent years (CAETANO et al., 2019, TAYENGWA et al., 2020a, VINYARD et al., 2021).

GPR is generated after pressing grape bunches while producing wine and grape juice, representing about 20% of the total mass of processed grapes. The particle composition can change due to genetic, climatic factors, and processing methods; however, they commonly contain around 473 g/kg of bark, 277 g/kg of stalks, and 250 g/kg of seeds in the raw residue (NERANTZIS; TATARIDIS, 2006). Grape seeds and skins (pomace components) contain flavonoids (catechin, epicatechin, procyanidins, and anthocyanins), phenolic acids, and resveratrol, which demonstrate beneficial biological and functional activities in animals (YU; AHMEDNA, 2013).

In animal production, the addition of grape residue to the diet made it possible to improve health through increased activity of intestinal digestive enzymes (HUANG et al., 2012), immunological capacity (XIE et al., 2012), and antioxidant activity (ZHAO et al., 2018). Grape residue also improved meat quality and animal growth (TAYENGWA et al., 2020a). Caetano et al. (2019) added 30% of ensiled grape pomace to the diet of Angus steer and found a reduction in daily methane emissions with a decrease in animal performance. Vinyard et al. (2021) investigated the optimal proportion of grape residue in diets for finishing cattle and found that feed inclusion should be 150 g/kg. Pilot studies on the use of dry

grape pomace as an alternative source of fiber to wheat bran on growth performance and meat quality were published by Tayengwa e Mapiye (2018) and Tayengwa et al. (2020); these authors concluded that the substance has sustainable potential.

Although much is known about the effects of grape residue and its components on animal nutrition and its potential effects on productivity, there is a need for tests to understand the mechanisms mediating benefits and (primarily) to determine whether this practice is economically viable. Therefore, the objective of the present study was to determine whether the inclusion of grape residue in ensiled or dehydrated form can replace sources of dietary fiber such as wheat bran and soybean hulls and would positively influence growth performance, health, fermentation parameters, and digestibility. Our hypothesis is that both residues (GPS and GPB) can be used as a source of dietary fiber, as they provide greater animal health and growth performance. In particular, we believe that the GPB has greater use by the animals, because the residue has received physical processing, and thus, more evident beneficial effects on health and performance, when compared to those that receive the raw residue (GPS).

3.3 MATERIALS AND METHODS

The Animal Research Ethics Committee of the Universidade do Estado de Santa Catarina approved the project (protocol number: 4948210322), following the guidelines of CONCEA/Brazil.

3.3.1 GPR

GPR cv. Isabel (*Vitis labrusca*) was collected from an industrial winery located in the municipality of Pinheiro Preto (27º 03' 02" S; 51º 13' 51" W). After pressing and separating the solid fraction from wine production, GPR (3.81% stem, 52.71% bark, 48.5% seeds, based on dry matter [DM]) was immediately stored in a trench-type silo (3.0 X 0.9 X 6.0 m [width, height, and length, respectively]), coated with plastic material on the sides and bottom, compacted with an agricultural tractor, and sealed with a specific tarpaulin for storage of preserved bulky foods (Parcifil®, Sapiranga, Brazil). The GPR was ensiled for 3 months before starting the experiment and was characterized as GPS.

To obtain GPB, GPR was dehydrated by drying two batches of GPR because the rainy weather prevented drying in the sun. The first drying was done in a closed environment, using gas bells, fans, and forced hot air. The second drying was carried out in an open environment, in plastic sheets, and dried in the sun. Both forms of drying were performed at the *Universidade do Estado de Santa Catarina* (27° 06' 17" S 52° 36' 51" W) and lasted about 7 days until reaching values below 10% of humidity to avoid deterioration during storage and to allow grinding in a 4-mm sieve.

3.3.2 Location, facility, and animals

The experiment was carried out in the ruminant sector of the Santa Catarina State University experimental farm (27° 09' 09.2" S 52° 47' 18.8" W). The animals were housed in individual shelters of 15 m² (concrete floor), equipped with automatic drinkers and feeders. The feeding area had a cover where the animals could take shelter from the weather. The shed had a north-south solar orientation, which allowed sunlight exposure.

Twenty-four steers (248 ± 19.32 kg of body weight and 9 months of age) from industrial crosses were used (½ Charolais x ½ Nellore). Before the beginning of the experiment, all animals underwent a sanitary protocol, where they were medicated with injectable Endectocide (Ivomec®, Boehringer Ingelheim), based on 1% ivermectin, according to the manufacturer's instructions.

Before the experiment in the breeding phase, the animals had access to breast milk for up to 7 months, then ad libitum access to protein-energy supplementation (creep-feeding) and *Brachiaria Brizantha Marandú* pasture. After weaning, the animals were given ad libitum access to pasture and received mineral vitamin supplementation. Therefore, the base diet before entering the confinement and the experiment were based on only bulky feeds.

3.3.3 Experimental design, diets, and feeding

The animals were divided into three dietary treatments (eight steer/treatment) in a randomized controlled design to standardize initial body weight between groups, with each steer considered an experimental unit. The

groups were the control diet (traditional confinement diet), the GPS diet (inclusion of 100 g/kg in DM of GPS), and the GPB diet (inclusion of 100 g/kg in DM of grape pomace).

Although GPS and GPB have been used as a substitute for wheat bran and partially for soybean hulls (Table 2), their nutritional contents showed differences (Table 3). That said, this substitution forced small changes in the inclusion of other feeds in the total ration mix to guarantee the same energy and protein density. In particular, the superior participation of soybean meal in alternative diets with dry and wet grape pomace was to compensate for the nitrogen deficit, and corn was used to balance the energy content.

The experimental period started after all the steer passed through 21 adaptation periods with the gradual inclusion of concentrate (performed in a ladder-type protocol) with the following concentrate:forage proportions: 20:80, 40:60, 60:40, in periods 1–7, 7–14, and 14–21 days of the experiment, respectively. Diets were calculated according to the nutritional requirements of the animal category for an estimated average daily gain (DMG) of 1.5 kg of body weight (BR-CORTE, 2016). The daily supply of the diet was divided into two similar meals (08:00h and 18:00h), supplied in the form of a total mixed ration to meet the consumption need at 105%. Clean, fresh water was always available.

3.3.4 Sample and data collection

3.3.4.1 Growth performance and nutrient utilization

The steers were weighed individually at days 1, 21, 71, and 121 of the experiment. All weighing's were performed in the morning, with the animals fasting, using a digital electronic scale (DIGITRON®, ULB-300-90CM). Using the body weight data, it was possible to calculate weight gain (WG) (WG = final body weight [BW] – initial BW), and average daily gain (ADG) [(final BW – initial BW)/number of days)].

During the experiment, all rations offered, and refusals were weighed individually using a digital scale (TODELO®, PRIX PLUS), and refusals were recorded daily throughout the morning before feeding. Feed subsamples and their respective residuals from each animal were collected to determine nutrient

intake, and were frozen at –20 °C. At the end of the experiment, a pool of samples was made, and a homogeneous sample was obtained from these samples by quartering them for chemical analysis.

Between the 105th and 110th day of the experiment, the total feces produced by the steer were collected from the concrete floor immediately after defecation to ensure there was no contamination by urine or any dirt. Subsamples corresponding to 10% of the total daily defecated sample were reserved and frozen at –20 °C. After the collection period, the daily samples of each steer were homogenized and sent for compositional analysis.

3.3.4.2 Collection of blood and rumen fluid

Blood samples were collected on days 1, 21, 71, and 121, through the caudal vein, with the aid of needles and vacuolated tubes, with clot activator (FIRSTLAB®) to obtain serum for biochemical analysis, oxidant levels, and antioxidants and proteinogram. Vacuum tubes with anticoagulant (EDTA K3, FIRSTLAB®) were also used for hematologic analysis. The tubes were kept refrigerated at 10 ºC in an isothermal box until arrival at the laboratory. For serum separation, the tubes will be centrifuged without anticoagulant (7500 RPM for 10 min). The serum was transferred to microtubes, identified, and stored at –20 °C until analysis.

One hundred milliliters of rumen fluid were collected per animal four hours after morning feeding on days 71 and 121 of the experiment, using a 1.5 m x 11 mm (diameter) oro-rumen tube connected to a vacuum pump. Immediately after harvesting, while still on site, pH measurement was performed with a digital pH meter (KASVI®, K39-1420). Immediately after collection, the sedimentation activity time and the functional activity of the ruminal microbiota were analyzed through the PRAM, described by Dirksen (1993). The remaining rumen fluid was then reserved in Falcon® tubes of 15 mL per sample in a cooler. The tubes were frozen at –20 °C for further analysis of volatile fatty acids.

3.3.5 Laboratory analyses

3.3.5.1 Analysis of feed, feed refusals, excreta, and experimental diets

Total ration samples from the three experimental groups, isolated feeds (GPS, grape bran, and wheat bran), and feed refusals during the experimental period and excretions were grouped and analyzed according to the Association of Official Analytical Chemists (1997): DM, method 930.15; crude protein (CP), method 976.05; ash, method 942.05. The concentrations of neutral detergent fiber (NDF), and acid detergent fiber were measured according to Van Soest et al. (1991). Total carbohydrates were calculated as follows: 1000 g/kg – ([ash + CP + EE] g/kg) and non-fibrous carbohydrates 1000 g/kg – ([ash + CP + NDF + EE] g/kg). The associated pectin and sugar content was calculated according to López et al. (2014) by subtracting the starch content (g/kg MS) from the non-fibrous carbohydrate content (g/kg). Starch content was measured according to Silva et al. (2019), and lignin was measured as described by Goering and Van Soest (1970). ME content was expressed as MJ/kg according to CSIRO (2007).

To determine the total phenolic compounds, 0.5g of the experimental diets and residues (GPS and GPB) were weighed in Falcon tubes and 5 mL of solvent (60%) were added to them. The mixture was stirred and kept in a heated bath (96°C) for 20 minutes. Then, they remained for 15 minutes in an ultrasound bath (180 W) and then centrifuged for 15 minutes (4000xg). After that, the supernatant was filtered and the extracts analyzed. For the determination of tannins and flavonoids, it was used the Colorimetry method: Brazilian Compendium of Animal Feed, 2013, method 52.

3.3.5.2 Hematology

The total erythrocyte and leukocyte count, hemoglobin concentration, and hematocrit percentage were performed immediately upon arrival at the laboratory with the aid of an electronic hematologic device (SYSMEX®, KX-21N). White blood cell differentials were calculated as described by Feldman et al. (2000), using blood smears stained with a commercial Panotico rapid kit.

3.3.5.3 Serum biochemistry

We measured serum biochemical variables (total proteins, albumin, glucose, and urea) and hepatic overload parameters (GGT and AST). Globulin levels were calculated as total protein – albumin. All analyses were performed using the semi-automatic Bio-2000 (BioPlus®) and commercial kits (Análise®), according to their respective methodologies.

3.3.5.4 Proteinogram

Sodium dodecyl sulfate-polyacrylamide gel electrophoresis was performed according to Fagliari et al. (1998) using mini-gels (10 x 10 cm). The gels were stained with Coomassie blue and photographed to identify and quantify protein fractions using Labimage 1D software (Loccus Biotechnology). A standard containing fractions with molecular weight between 10 and 250 KD (Kaleidoscope - BIORAD) was used as a reference.

3.3.5.5 Serum oxidative status

GST activity was measured according to Habig et al. (1974) and was expressed as µmol CDNB/min/mg of serum protein. Protein thiols were measured as per Ellman (1959), and the results were expressed as nmol thiols/mg of protein.

The intensity of oxidative reactions was determined using serum lipid peroxidation and measured as TBARS, according to Jentzsch et al. (1996). The reaction was read in a spectrophotometer at 535 nm, and the result was expressed as nmol MDA/Ml. ROS were measured according to Halliwell e Gutteridge (2007) and were expressed as U DCFH/mg protein.

3.3.5.6 Fatty acid profiles of foods

The extraction was carried out as per Bligh and Dyer (1959) with some modifications; 1.5 g of GPS, GPB, and experimental diets, 0.5 mL of water, 5 mL of methanol, and 2.5 mL of chloroform were added to a 15-mL polypropylene tube, and mechanical shaking was performed for 60 min. Then, 2.5 mL of

chloroform and 1.5% Na_2SO_4 solution were added to generate a biphasic system. This mixture was shaken for 2 minutes and centrifuged for 15 minutes at 2000 rpm. Lipids from the chloroform phase were subjected to fatty acid analysis.

Fatty acid methylation was performed by the transesterification method proposed by Hartman and Lago (1973). The extracted lipids were added to 1 mL of 0.4 M KOH methanolic solution in a test tube and shaken in a vortex for 1 min. Samples were placed in a boiling water bath for 10 min. Subsequently, samples were cooled to room temperature, and 3 mL of 1 M H_2SO_4 methanolic solution was added, shaken in a vortex, and maintained in a water bath for 10 min. After cooling, 2 mL of hexane was added and centrifuged at 2000 rpm for 10 min. Finally, hexane with the fatty acid methyl esters (FAME) was subjected to chromatography analysis.

For the FAME determination, we used a gas chromatograph (model TRACE 1310) equipped with a flame ionization detector (Thermo Scientific). One microliter of samples was injected in a splitless injector, operated in 1:20 ratio split mode at 250 °C. Hydrogen was used as carrier gas at a constant flow rate of 1.5 mL/min. FAME separation was performed using an RT 2560 (100 m × 0.25 mm × 0.20 µm film, Restek, USA) chromatography column. The FAME compounds were identified by comparing the experimental retention time with those from a standard (FAME Mix-37, Sigma Aldrich, St. Louis, MO). The results were presented as a percentage of each fatty acid identified in the lipid fraction, considering the chain size equivalent factor of FAME for flame ionization detection and a conversion factor of ester to the respective acid, according to Visentainer and Franco (2006).

3.3.5.7 Determination of short-chain fatty acids

The rumen fluid samples were thawed until they reached 5 °C and then were manually homogenized. Then, 1-mL aliquots of the supernatants from the rumen fluid and silage samples were transferred to polypropylene microtubes (2 mL) which were subsequently centrifuged for 5 min (12300 × *g*). Then, 100 µL of the supernatants were transferred to a new microtube containing 100 µL of formic acid. The mixture was vortexed for 30 seconds and centrifuged again for 3 min. After centrifugation, 50 µL of the supernatant of the mixture was transferred to

250 µL tubes, and 100 µL of the 3-octanol internal standard methanolic solution (665 µg mL^{-1}) was added. The samples were injected into a gas chromatograph equipped with a flame ionization detector (GC-FID; Varian Star 3400) and an autosampler (Varian 8100). One microliter of the extract was injected in 1:10 split mode. The carrier gas used was hydrogen at a constant pressure of 20 psi. The analytes (acetic, propionic, butyric, valeric, and isovaleric acids) were separated on a CP WAX-52CB capillary column (60 m × 0.25 mm; 0.25 µm stationary phase thickness). The validation of the method involved the following parameters: selectivity, linearity, linear range, repeatability, precision, and limits of detection/quantification for acetic, propionic, butyric, valeric, and isovaleric acids. Analytical parameters are shown in Table 4. Linearity was assessed by calculating a regression equation using the least squares method. Sequential dilutions achieved limits of detection and quantification up to signal-to-noise ratios of 3:1 and 6:1, respectively. Precision was assessed by analyzing the repeatability of six replicate samples. Accuracy was determined by recovering known amounts of standard substances added to the samples. The results were expressed in mmol L^{-1} of each SCFA in rumen fluid and as mmol kg^{-1} in silage.

3.3.5.8 Protozoa count

For the total protozoa counts in rumen fluid, a 10-ml subsample of the liquid fraction of the rumen fluid was pipetted and separated without filtering. Then, the sample preparation and counting were performed according to Dehority (1984) using a Sedgewick Raffer chamber, and the results were expressed as the number of protozoa/ml of rumen fluid.

3.3.6 Economic viability

For economic feasibility analysis, revenue over feed costs (RSCA) was calculated according to Buza, Holden, White, and Ishler (2014), modified as follows:

$$RSCA = Total\ Revenue\ (RT) - Total\ Food\ Costs\ (CTA)$$

Where: RT = revenue generated from BW gain (GP x Carcass Yield x Unit Value), CTA = feed cost per diet × DM intake.

3.3.7 Statistical analyses

All data were analyzed using the 'MIXED procedure' of SAS (SAS Inst. Inc., Cary, NC, USA; version 9.4), with Satterthwaite approximation to determine the denominator degrees of freedom for the test of fixed effects. The BW gain, ADG, DM intake, nutrient intake, feed conversion, feed efficiency, apparent digestibility coefficient, rumen sedimentation time, protozoan count, PRAM, and economic viability were tested for fixed effect of treatment using animal (treatment) as a random effect. The data of BW, all blood results, rumen pH, and volatile fatty acids were analyzed as repeated measures and were tested for fixed effects of treatment, day, and treatment × day, using animal (treatment) as random effects. The day 1 results were included as independent covariates. The compound symmetry covariance structure was selected according to the lowest Akaike information criterion. Means were separated using the PDIFF method, and all results were reported as LSMEANS followed by SEM. Significance was defined as $P \leq 0.05$, and a tendency was defined as $P > 0.05$ and ≤ 0.10.

3.4 RESULTS

3.4.1 Nutrient intake and digestibility

Data on nutrient intake and total digestibility are shown in Table 4. The diets only influenced the digestibility and consumption of starch and EE. The steer fed the GPB diet had a higher starch intake than the animals that received the traditional diet (control) ($P = 0.05$), while the animals that received the GPS diet had similar intakes to both groups. The EE intakes were higher in the GPS and GPB diets than in the control diet ($P = 0.01$). Regarding the digestibility data, the steer fed the GPS diet had a lower coefficient of apparent digestibility of starch and EE than the traditional diet ($P < 0.05$).

3.4.2 Growth performance

The zootechnical performance indices are shown in Table 5. The initial BW of the steer in the different diets did not differ ($P > 0.05$). At the end of the experimental period (d 121), the animals on the control and GPS diets had similar and higher body weights than the animals that received the BPG diet ($P < 0.05$),

an effect that starting from day 77 of the experiment. The higher final BW of these groups is supported by the higher mean daily gain and total WG (P = 0.05). The GPS diet provided similar feed conversion (P = 0.0002) and feed efficiency (P = 0.0004) to the control diet. However, this effect was not seen in the BPG group, which had worse efficiency and feed conversion than the control and GPS groups. DM consumption between groups did not differ (P > 0.05).

3.4.3 Hemogram

The hematologic variables are shown in Table 6. There was an effect on two hematologic variables, specifically leukocyte differentiation. The animals that received the diet containing GPS had higher lymphocyte counts than those in the control groups (P = 0.05). For neutrophil counts, there were effects in the interaction treatment x day (P = 0.04) but not for treatment (P > 0.05). After the adaptation period (day 21), the animals in the GPS group showed higher counts of neutrophils than the control animals; a similar effect was observed at the end of the experiment (day 121), where there were higher counts than the control and BPG groups. For the other hematologic variables (erythrocytes, hematocrit, hemoglobin, leukocytes, and eosinophils), the groups did not differ significantly (P > 0.05).

3.4.4 Serum biochemistry

Serum enzyme and metabolism biochemistry of steer are shown in Table 7. Among the biochemical metabolism tests performed, three were influenced by the diet related to protein metabolism. The steer fed the GPS diet had higher concentrations of globulins (P < 0.05) compared to the animals fed the control and GPB diets. As for total proteins, GPS steer showed concentrations similar to GPB animals and higher than animals that received the traditional diet (P = 0.005). Serum urea levels tended to affect the treatment x day interaction (P = 0.10) at the end of the experiment; that is, on day 121, the urea concentration of steer that received the GPS diet was similar to those that received the control diet but higher than the animals that received the GPB diet.

There was a trend of effect (*P* = 0.08) for AST. The steer fed the GPS diet had higher AST activities than those in the control and GPB groups. The diet did not influence the other biochemical variables (albumin, glucose, and GGT).

3.4.5 Ruminal indicators

The rumen indicators are shown in Table 8; the diets influenced the rumen fermentation profile. These effects were not observed in the middle of the experimental period (day 71) but occurred at the end (day 121). Acetic and propionic acid concentrations showed similar behavior across the diets, and the steer fed the GPS diet presented similar values to the control group; these were superior to the GPB group on day 121 (*P* = 0.03). For valeric acid, a trend of effect (*P* = 0.07) was observed between the diets, where GPS steer had a higher concentration than the GPB animals; however, the GPS and GPB groups were similar to the control animals. The acetate:propionate ratio on day 71 tended to be higher in the control diet than in the GPS and GPB diets. The diets did not influence the proportions of butyric and isovaleric acid.

The GPS diet had an effect on PRAM (P = 0.02) and sedimentation time (*P* = 0.08). For PRAM, GPS animal groups showed longer resistance time, while for sedimentation time, it showed a shorter time. The GPB animals had lower total protozoan counts, while the GPS group had similar counts to the control.

3.4.6 Proteinogram

The results of the serum proteinogram are shown in Table 9. Regarding immunity, the residue in the ensiled and dehydrated form in the alternative diets showed modulatory properties. Significant effects on ceruloplasmin concentrations were detected (*P* < 0.05). Steer that consumed the GPS diet had lower concentrations than steer fed the control diet; the animals that received the GPB diet were similar to both groups. Another acute phase protein that was influenced by diets was haptoglobin; at the end of the experimental period (day 121), the alternative diets (GPS and BPB) showed lower concentrations (*P* = 0.004) than the traditional diet (control). An effect was also observed for heavy chain immunoglobulin G levels; that is, after the adaptation period (days 21, 71,

and121), the animals that received dried and ensiled grape pomace in their diets had higher serum concentrations of immunoglobulin G ($P < 0.05$).

3.4.7 Antioxidant status

The oxidant/antioxidant status variables are shown in Table 10. Serum TBARS levels showed effect of the treatment x day interaction (days 21, 71, and 121, $P = 0.001$) and treatment effect ($P = 0.01$). However, only on day 121 was it possible to observe a positive effect of the GPS and GPB diets; that is, there were lower concentrations of TBARS than in the control diet. Serum GST levels also changed on days 21, 71, and 121 ($P = 0.011$), and there was a treatment effect ($P = 0.02$); the animals that received the GPB diet had higher GST activity. The animals that received the GPS diet had higher serum protein thiol levels than the control animals ($P = 0.03$). Effect of treatment x day interaction ($P = 0.05$), but only treatment effect trend ($P = 0.09$) was detected for ROS. On day 71, the GPS animals had lower concentrations of ROS than the other groups.

In the intestine homogenate, indicators of the antioxidant system were stimulated by the GPS and GPB diets. Higher GST activities ($P = 0.01$) were observed in animals that consumed the GPB diets. For the serum protein thiols, the animals that received the GPS and GPB diets had higher concentrations ($P = 0.005$).

In the liver, attenuation of oxidant indicators and increased antioxidants were observed, especially in GPS and GPB animals. For TBARS, the GPS and GPB animals had lower concentrations in the liver ($P = 0.006$). Only the animals that consumed the GPS diet had lower levels of ROS in hepatic homogenates ($P = 0.05$). An effect trend was detected for protein thiols; GPS animals showed a trend toward higher concentration than the control group. The animals in the GPS and GPB groups showed higher activities of GST ($P = 0.05$).

3.4.8 Economic viability

The values of experimental diet costs, total feed costs, and revenue over feed costs are shown in Table 11. The total cost of feeding the steer across diets did not differ ($P > 0.10$). The diets influenced the total revenue and the RSCA (*P*

≤ 0.05), with the highest revenue from steer fed with GPS, followed by the control and GPB diets.

3.5 DISCUSSION

Using GPS as a fiber source to replace traditional cereals did not affect steer performance. The similar DM intake and ADG of the GPS group showed that grape silage did not affect efficiency or feed conversion, a fact explained by the same nutritional composition of the diets, in agreement with Santos et al. (2014). These authors gave increasing inclusions of GPS to 100 g/kg in the diet of dairy cattle and observed no effects on a or milk yield. Similar effects were observed in lambs (MASSARO JUNIOR et al., 2021) and heifers in a backgrounding system (REAM et al., 2021). It is worth mentioning that the success of residue depends on the inclusion level, residue profile, and animal category to which it is supplied. For cattle in confinement, a maximum inclusion of 150 g/kg in the DM of the total diet is recommended (VINYARD et al., 2021).

Nutrient intake was compatible with the DM intake and nutritional balance of the experimental diets. The higher EE consumption of steer that received the GPS and GPB diets was due to the higher lipid intake from the more significant amount of EE in the residue; this phenomenon was observed in lambs (MASSARO JUNIOR et al., 2021) and dairy cattle (SANTOS et al., 2014). Seeds are the fraction of the residue with the highest contribution to EE (YU; AHMEDNA, 2013). The residue used in the present study had a significant fraction of seeds (485 g/kg of residue in DM), which explains the more significant amount of EE from GPS and GPB than the residues in other studies (±159% more than the average described by other researchers [ALBA et al., 2019; TAYENGWA et al., 2021a; FLORES et al., 2021]). According to Bauchart (1993), ruminants have limited activity of pancreatic lipase and bile lipids, which can lead to a decrease in the efficiency of lipid absorption at the intestinal level when consumed in large quantities (> 70 g/kg EE in total DM), resulting in lower EE digestibility. This hypothesis can be discarded, given the low amount of EE in the experimental diets (< 40 g/kg). The seeds in their intact form are not digested and pass through the gastrointestinal tract unchanged. This effect was attenuated in the BPG diet, where the residue was ground, leaving the seeds damaged and exposing their

content. The higher consumption of starch in the animals that received the GPB diet and the intermediate consumption of the animals that received the GPS diet are consequences of the greater inclusion of cornmeal in the diets for the nutritional balance of ME. We believe that the lower starch digestibility in the animals that received the GPS diet is attributed to rumen kinetics because the sedimentation time of the rumen content was shorter for this group. Lower starch digestibility is associated with higher DM intake (although not significantly), and it may favor the passage of starch particles from cornmeal that were large enough to pass through the reticulo-omasal orifice.

Although grape residue and its compounds have been studied for many (FAMUYIWA, 1982), some effects are poorly elucidated in the literature. We hypothesized that both fiber sources would improve productive performance in cattle. However, unlike the works published describing dry grape residue for beef cattle (TAYENGWA et al., 2021a), dairy cattle (MOATE et al., 2020), lambs (ALBA et al., 2019; MOLOSSE et al., 2019; ZHAO et al., 2018), our results were not favorable using grape residue meal. We found significantly different data for this experimental group, even though the residue was processed in the form of silage and bran. Because grape bran was not a suitable food ingredient, we investigated the concentrations of mycotoxins (aflatoxin, fumonisin, T-2 toxin, DON) and the presence/absence of *E. coli* and *Salmonella* from GPB; however, no finding could explain the adverse effects on bovine WG. Nevertheless, it is imperative to report these data without a positive impact, given the working conditions, food preparation method, and its inclusion in the ration, animal category, and diet profile. Because GPB did not provide expected positive results, more detailed investigations are warranted.

Substantial changes in the clinical condition of the steer were observed after the adaptation period, with increases of 103.32% and 69.27% for lymphocytes and neutrophils, respectively, suggesting the presence of an inflammatory process. The inflammatory response is the first defense against harmful stimuli (CECILIANI et al., 2012). The steer that received the GPS diet had the highest counts of these cells but the lowest concentrations of ceruloplasmin and haptoglobin. The increase in these acute phase proteins (PFA, α2 fraction) indicates a broad systemic response when infections and tissue

injuries overwhelm local defenses (GABAY; KUSHNER, 1999); the system seeks to help eliminate the cause of the imbalance and restore homeostasis (CERÓN et al., 2005). This effort can even lead to hyperthermia and increased concentrations of serum cortisol (ECKERSALL, 2000), which are undesirable for production animals. The modulation of the inflammatory response by the GPS diet might be associated with the anti-inflammatory characteristics of phenolic compounds in grapes (LI et al., 2001; COLOMBO et al., 2019; RODRÍGUEZ-MORGADO et al., 2015). These compounds inhibit the NF-kB pathway through PPAR-gamma activation (MARTINEZ-MICAELO et al., 2015), regulation of mitogen-activated protein kinases (WADSWORTH et al., 2001; CHO et al., 2003), regulation of arachidonic acid (KIM et al., 1998; LUCERI et al., 2002; HOU et al., 2007), and decreasing ROS with consequent inhibition of the inflammatory pathway (SALZANO et al., 2014). The anti-inflammatory effect of grape residue has been observed in species such as laying hens (REIS et al., 2019), dairy sheep (ALBA et al., 2019), and suckling lambs (MOLOSSE et al., 2021).

The increase in serum total protein values in cattle that received the GPS diet is a consequence of the increase in the globulin fraction. The diets containing the residue in the ensiled and dehydrated form showed immunomodulatory characteristics, an effect recently observed in ruminants by Engler et al. (2022). These authors added 670 mg of commercial rumen-protected grape extract to the diet of cattle during the vaccination period and observed an improvement in the humoral response. Similar findings were observed in weaned piglets that received 100 mg/kg and 150 mg/kg of grape seed, which showed improvement in humoral and cellular immunity, with increased serum levels of IgG, IgM, C4, and IL-2 (HAO et al., 2015), in sows by the inclusion of 200 ppm or 300 ppm of grape extract (WANG et al., 2019), in lambs (MOLOSSE at al., 2021), and ducks (AO; KIM, 2020). Interestingly, even using the residue in a crude form, in this study, we observed that the inclusion of dry and ensiled grape residue increased the levels of heavy chain immunoglobulins G and fraction ϒ of the PFAs, suggesting stimulation of the humoral system of the animals, and confirming the immune effect of flavonoids (PERCIVAL, 2009). Although we did not analyze markers that explain the mechanism of action of polyphenols on the immune response, we believe that they exert effects on immune receptors (MAGRONE et

al., 2020). However, there is a hypothesis stating that there is modulation in the production of cytokines (primarily resveratrol) in the attenuation of IL-1β and IL-6 and (as a result) reducing low-grade chronic inflammation (SCHWAGER et al., 2017; FOSSATI et al., 2017; FOSSATI et al. al., 1998). In summary, including grape residue in the diet modified the cattle immune system by altering defense cells and their network of proteins that initiate, amplify, sustain, control, and resolve inflammatory reactions.

Oxidative stress results from an imbalance between the generation of oxidant compounds and the action of antioxidant defense systems. This imbalance generates adverse conditions on health and negative impacts on ruminant production efficiency (SORDILLO; AITKEN, 2009). In this study, we measured mediators and components of the oxidative/antioxidant status of the animals and found that the diet has a direct effect on oxidative reactions mediated by the antioxidant stimulation of the residue. Grape residue and its components have a strong antioxidant capacity (YILMAZ; TOLEDO, 2006; NEGRO et al., 2003, BRENES et al., 2016). In agreement with Zhao et al. (2018), who supplemented Dorper lambs with 50 g/kg and 100 gm/kg dried grape pomace and observed an increase in glutathione peroxidase 4, superoxide dismutase and (as a result) a higher total antioxidant capacity. Similar effects have been reported in broilers (GURGON et al., 2021), laying hens (REIS et al., 2019), rabbits (BOUZAIDA et al., 2021), piglets (KAFANTARIS et al., 2018), and dairy sheep (ALBA et al., 2019). Our hypothesis for decreased oxidant compounds and increased antioxidant markers with the use of grape residue is the increase in Nrf2, already observed by Zhao et al. (2018), which in turn has a positive relationship with the production of antioxidant enzymes (ZHU et al., 2005).

Although reduced, changes in the ruminal SCFA profile were detected in steer fed the BPG diet, a subtle modulation observed by Moate et al. (2014) and Vinyard et al. (2021). The findings of this study might be associated with the described effects of tannins on microbial fermentation (VASTA et al., 2019), evident impact on the high inclusion of GPS (300 g/kg in DM) in the diet of beef cattle (CAETANO et al., 2019). However, it is worth noting that several factors influence ruminal fermentation and the consequent molar concentrations of SCFA. The composition of the diet, ingredients (and their respective quality), and

the concomitant use of additives (HORN, 1981) might directly influence the fermentative profile. These findings might explain the small differences in the modulation of SCFA in the rumen with the inclusion of grape residue and condensed tannins in the ruminant diet (VASTA et al., 2019). The inclusion of grape residue has been associated with increased acetate (TAYENGWA et al., 2021a), decreased acetate (MOATE et al., 2014), unchanged propionate (MOATE et al., 2014), and decreased propionate (TAYENGWA et al., 2014). Suescun-Ospina et al. (2022) evaluated the effects of the inclusion of BPG in beef cattle diets with high and low forage content on production and fermentation parameters and observed that the inclusion of 100 g/kg of BPG DM did not generate significant effects on most parameters, while the inclusion of 200 g/kg GPB in DM generated significant negative impacts; these findings appear to be dependent on the roughage-concentrate ratio of the diet. In the present study, given the experimental conditions and the diet profile, the inclusion of 100 g/kg of GPB can negatively influence fermentation, an effect not observed in the animals that received the GPS diet. We believe that the results were not similar for the GPS diet due to the lack of residue processing; therefore, the non-interaction of the components contained in the seed that passed intact through the gastric or intestinal tract did not influence the microbiota and, consequently, the profile. Higher amounts of catechins and procyanidins are found in the seeds (GONZÁLEZ-MANZANO et al., 2004), which show the ability to inhibit the hydrolysis of foliar proteins by the ruminal microflora (TANNER et al., 1994) in addition to apparent affinities with macromolecules such as starch, pectin, and cellulose (WATRELOT et al., 2020), resulting in a decrease in total SCFA from bacterial fermentation. Notably, the lower concentrations of propionate, the primary gluconeogenic precursor in ruminants (BERGMAN, 1990), did not affect serum glucose concentrations in animals fed the BPG diet. Therefore, we cannot rule out that glucose balance by the upregulation of skeletal protein degradation to support hepatic and renal gluconeogenesis (DRACKLEY et al., 2001), partially explaining the lower performance of this experimental group.

Finally, we report that the inclusion of grape residue in the diet of steer in confinement reduces the cost/kg of feed (Table 11). Wineries in southern Brazil provide residues from high-cost cereals free of charge. The higher revenue from

the sale of carcasses and the lower cost per kg of the GPS diet explains the higher revenue over feed costs and, thus, demonstrates the economic viability of the use of GPS in the nutrition of cattle in confinement, as described by Tayengwa et al. (2020). Chikwanha et al. (2019) found higher gross margin returns for the diet with 12.2% grape pomace in the diet of lambs. However, in the present study, we found that, even with the lowest cost/kg of diet, the inclusion of BPG is not economically viable compared to the traditional confinement diet, given the inconsistency in the performance response of the animals during the experimental period.

3.6 CONCLUSION

The replacement of wheat bran and soybean hulls by GPS in the diet of steer stimulated and modulated the antioxidant and immune system, suggesting a positive impact on health. The inclusion of this residue did not affect the ruminal fermentation profile or the productive performance. Interestingly, the effects observed in the inclusion of GPB were not similar or positive in the GPS diet, suggesting its use is unfeasible, which cannot confirm our hypothesis. In conclusion, given the experimental conditions, only the inclusion of GPS as an ingredient in the nutrition of steers is a promising alternative to reduce feed costs and improve health. which cannot confirm our hypothesis. In conclusion, given the experimental conditions, only the inclusion of GPS as an ingredient in the nutrition of steers is a promising alternative to reduce feed costs and improve health.

3.7 TABLES

Table 2 - Proportions of feed ingredients in the experimental diets.

Ingredients, g/kg	**Treatments**		
	Control diet	**GPS diet**	**GPB diet**
Grape pomace silage (GPS)	-	100.0	-
Grape pomace bran (GPB)	-	-	100.0
Corn Silage	400	400.0	400.0
Corn meal	280.3	351.2	352.2
Soybean meal	29.1	48.2	47.5
Soybean hulls	139.8	58.7	59.1
Wheat bran (WB)	110.0	-	-
Mineral[1]	22.0	22.2	22.0
Urea	7.00	7.50	7.00
Sodium bicarbonate	10.0	10.0	10.0
Mycotoxin adsorbent	2.20	2.20	2.20

[1] Calcium (Max.) 220.00 g/kg; Calcium (Min.) 160.00g/kg; Phosphorus (Min.) 40.00g/kg; Magnesium (Min.) 6.00 g/kg; Sodium (Min.) 85.00g/kg; Sulfur (Min.) 12.00g/kg; Cobalt (Min.) 20.00 mg/kg; Copper (Min.) 520.00 mg/kg; Iodine (Min.) 25.00 mg/kg; Manganese (Min.) 650.00 mg/kg; Selenium (Min.) 10.00 mg/kg; Zinc (Min.) 2000.00 mg/kg; Fluoride (Max.) 400.00 mg/kg.

Table 3 - Chemical composition and fatty acid composition of grape pomace silage (GPS), grape pomace bran (GPB), wheat bran (WB), and experimental diets fed to Charolais x Nelore steer.

				Treatment		
Item	**GPS**	**GPB**	**WB**	**Control diet**	**GPS diet**	**GPB diet**
Chemical composition (g/kg)						
Dry matter (DM)	415.0	934.0	847.5	440.7	404.0	475.9
Organic matter (OM)	951.1	968.3	948.4	945.9	950.5	950.7
Ash	48.9	31.7	51.6	54.1	49.5	49.3
Ether extract	112.3	150.9	-	23.3	30.8	34.5
Crude protein	117.0	136.0	148.9	108.3	111.4	111.7
Metabolizable energy (MJ/ kg)	-	-	-	11.2	11.0	10.7
Neutral detergent fibre	620.0	586.0	436.5	381.4	387.2	350.7
Acid detergent fibre	594.0	535.0	139.2	221.0	244.7	218.6
Lignin	334.0	307.0	48.8	27.7	75.3	55.9
Starch	24.6	11.00	233.7	302.7	349.4	366.5
Total carbohydrates	721.8	681.4	799.5	814.3	805.4	804.5
Pectin + sugar	90.8	70.8	129.3	130.6	68.6	87.35
Non-fibrous carbohydrates	101.8	95.4	363.0	432.9	418.2	453.8
Total phenols (mg/kg)	855.0	1710.0	-	223.7	230.8	253.5
Total flavonoids, mg/kg	593.51	1621.35	-	-	-	-
Total tannins, g/kg	6.3	12.6	-	1.4	2.8	3.5
Fatty acid composition[1] (% of total fatty acids)						
C16:0	12.7	10.9	19.3	23.99	19.08	20.60
C18:0	5.8	5.7	2.9	6.82	6.36	7.01
C18:1n9c	21.6	17.9	21.6	25.93	24.16	24.15
C18:2n6c	56.2	61.6	50.5	36.22	43.65	42.35
C18:3n3	1.6	1.0	3.2	2.68	2.65	1.83
Outros[2]	2.1	2.8	2.5	4.35	4.11	4.05

[1] C16:0 (Palmitic), C18:0 (Stearic), C18:1n9c (Oleic), C18:2n6c (Linoleic), C18:3n3 (a-Linolenic).
[2] Others: C12:0, C14:0, C15:0, C16:1, C17:0, C20:0, C20:1n9, C21:0, C20:2, C20:3n3, C20:4n6, C22:0, C24:0, C24:1n9.

Table 4 - Nutrient intake and total digestibility of steer consuming diets containing grape pomace silage and grape pomace bran.

Items	Treatments[1]			SEM	*P* – value
	Control diet	GPS diet	GPB diet		Treat
Nutrient intake (kg/d)					
DM	7.85	8.25	8.07	0.29	0.87
OM	7.29	7.84	7.68	0.12	0.94
CP	0.85	0.95	0.90	0.21	0.60
NDF	3.01	3.16	2.85	0.52	0.82
ADF	1.75	1.99	1.77	0.40	0.76
Ash	0.43	0.41	0.39	0.09	0.55
Starch	2.49[b]	2.98[ab]	3.09[a]	0.06	**0.05**
EE	0.20[b]	0.30[a]	0.27[a]	0.02	**0.01**
NFC	3.57	3.55	3.82	0.10	0.91
Total digestibility (g/kg)					
DM	610	571	598	13.9	0.89
OM	628	592	619	12.4	0.93
CP	487	436	437	39.5	0.26
NDF	583	570	540	33.8	0.48
Ash	287	187	200	51.0	0.40
Starch	939[a]	842[b]	935[a]	10.9	**0.01**
EE	410[a]	267[b]	289[ab]	37.5	**0.01**
NFC	718	689	748	15.1	0.88

[1]Treatments were: control diet (traditional confinement diet), GPS diet (inclusion of 100 g/kg in the DM of grape pomace silage in the diet), and GPB diet (inclusion of 100 g/kg in the DM of grape pomace bran in the diet)[a-b] Within a row, differ ($P \leq 0.05$) or tend to differ ($P \leq 0.10$).

Table 5 - Effects of feeding diets containing grape pomace silage and grape pomace bran on the performance of Charolais steer.

Items	Treatments[1]			SEM	*P* – values	
	Control diet	GPS diet	GPB diet		Treat	Treat × Day
Body weight (BW, kg)					**0.05**	**0.03**
d 0	248	248	248	6.53		
d 21	263	260	254	6.53		
d 71	338[a]	346[a]	317[b]	6.53		
d 121	422[a]	430[a]	391[b]	6.53		
Weight gain (WG, kg)						
d 0 to 21	14.8[a]	12.0[ab]	6.58[b]	2.42	**0.10**	-
d 21 to 71	75.3	85.4	63.2	8.95	0.20	
d 71 to 121	83.5	83.7	73.9	5.43	0.46	-
d 0 to 121	174[a]	181[a]	144[b]	9.69	**0.05**	-
Average daily gain (ADG, kg/day)						-
d 0 to 121	1.43[a]	1.50[a]	1.19[b]	0.08	**0.05**	-
Dry matter intake (DMI, kg/day)	7.85	8.25	8.07	0.35	0.64	-
Feed conversion (DMI/ADG)	5.48[b]	5.50[b]	6.78[a]	0.14	**0.0002**	-
Feed efficiency (ADG/DMI)	0.18[a]	0.18[a]	0.15[b]	0.005	**0.0004**	-

[1]Treatments were: control diet (traditional confinement diet), GPS diet (inclusion of 100 g/kg in the DM of grape pomace silage in the diet,) and GPB diet (inclusion of 100 g/kg in the DM of grape pomace bran in the diet)

[a-b] Within a row, differ ($P \leq 0.05$) or tend to differ ($P \leq 0.10$).

Table 6 - Hemogram of steer consuming diets containing grape pomace silage and grape pomace bran.

(continua)

Items	Treatments[1]			SEM	*P* – values	
	Control diet	GPS diet	GPB diet		Treat	Treat × Day
Erythrocytes (x10^6 µL)					0.60	0.83
d 0	8.85	8.84	8.78	0.30		
d 21	8.43	8.51	8.21	0.30		
d 71	9.35	9.36	8.69	0.30		
d 121	8.57	8.73	8.64	0.30		
Average[2]	8.80	8.86	8.58	0.21		
Hematocrit (%)					0.28	0.58
d 0	40.3	40.2	40.0	1.16		
d 21	39.3	40.4	38.4	1.16		
d 71	49.4	50.4	46.1	1.16		
d 121	36.2	37.2	36.1	1.16		
Average[2]	41.3	42.1	40.1	0.77		
Hemoglobin (g/dL)					0.22	0.44
d 0	9.26	9.26	9.25	0.35		
d 21	8.82	8.93	8.67	0.35		
d 71	15.1	15.6	14.3	0.35		
d 121	13.3	13.9	12.9	0.35		
Average[2]	11.6	11.9	11.3	0.24		
Leukocytes (x10^3 µL)					0.14	0.27
d 0	11.7	9.91	10.5	1.60		
d 21	18.5	22.2	18.7	1.60		
d 71	19.3	19.5	20.1	1.60		
d 121	16.8	21.6	15.4	1.60		
Average[2]	16.6	18.3	16.2	1.08		
Neutrophils (x10^3 µL)					0.20	**0.04**
d 0	3.18	2.49	2.48	0.91		
d 21	4.40^b	6.75^a	5.39^{ab}	0.91		
d 71	6.23	5.15	5.11	0.91		
d 121	6.12^b	9.37^a	4.63^b	0.91		
Average[2]	4.98	5.94	4.40	0.58		

Table 6 - Hemogram of steer consuming diets containing grape pomace silage and grape pomace bran

(continuação)

Items[3]	Treatments[1]			SEM	*P* – values	
	Control diet	GPS diet	GPB diet		Treat	Treat × Day
Lymphocytes (x10^3 µL)					**0.05**	0.92
d 0	7.70	7.85	7.50	1.40		
d 21	12.7	14.5	11.8	1.40		
d 71	11.8	14.4	11.8	1.40		
d 121	9.31	11.2	10.2	1.40		
Average[2]	10.4[b]	12.0[a]	10.3[b]	0.92		
Monocytes (x10^3 µL)					0.77	0.42
d 0	0.30	0.11	0.39	0.17		
d 21	0.87	0.81	1.10	0.17		
d 71	0.62	0.72	0.82	0.17		
d 121	0.76	1.04	0.61	0.17		
Average[2]	0.64	0.67	0.73	0.09		
Eosinophils (x10^3 µL)					0.82	0.47
d 0	0.09	0.05	0.10	0.09		
d 21	0.23	0.39	0.30	0.09		
d 71	0.05	0.08	0.06	0.09		
d 121	0.32	0.17	0.06	0.09		
Average[2]	0.17	0.17	0.13	0.05		

[1]Treatments were: control diet (traditional confinement diet), GPS diet (inclusion of 100 g/kg in the DM of grape pomace silage in the diet), and GPB diet (inclusion of 100 g/kg in the DM of grape pomace bran in the diet).

[2] Treatment mean, referring to the periods, except considered as a covariate.

[a-b]Within a row, differ ($P \leq 0.05$) or tend to differ ($P \leq 0.10$).

Table 7 - Serum biochemistry of steer consuming diets containing grape pomace silage and grape pomace bran.

(continua)

Items[3]	Treatments[1]			SEM	*P* – values	
	Control diet	GPS diet	GPB diet		Treat	Treat × Day
Albumin (g/dL)					0.30	0.25
d 0	2.31	2.50	2.50	0.18		
d 21	2.16	2.23	1.92	0.18		
d 71	2.44	2.19	1.92	0.18		
d 121	2.91	2.49	2.60	0.18		
Average[2]	2.46	2.35	2.23	0.09		
Globulin (g/dL)					**0.0001**	0.25
d 0	5.10	5.49	5.28	0.23		
d 21	5.22	6.16	5.14	0.23		
d 71	3.77	5.01	4.74	0.23		
d 121	4.22	5.07	5.01	0.23		
Average[2]	4.58[b]	5.43[a]	5.04[b]	0.10		
Total protein (g/dL)					**0.005**	0.34
d 0	7.45	7.95	7.76	0.27		
d 21	7.42	8.35	7.04	0.27		
d 71	6.25	6.88	6.64	0.27		
d 121	7.17	7.53	7.60	0.27		
Average[2]	7.07[b]	7.68[a]	7.26[ab]	0.11		
Glucose (mg/dL)					0.96	0.23
d 0	76.9	80.3	74.4	5.07		
d 21	81.5	88.3	83.8	5.07		
d 71	90.2	90.0	87.9	5.07		
d 121	93.2	79.6	93.9	5.07		
Average[2]	85.5	84.5	85.0	2.39		
Urea (mg/dL)					0.88	**0.10**
d 0	16.2	15.7	19.3	1.50		
d 21	17.6	18.0	18.9	1.50		
d 71	10.9[b]	18.3[a]	16.7[a]	1.50		
d 121	17.9[ab]	19.8[a]	14.9[b]	1.50		
Average[2]	18.2	18.0	17.5	0.89		
AST (mg/dL)					**0.08**	0.45
d 0	74.3	73.2	94.9	10.9		
d 21	78.8	85.6	60.7	10.9		
d 71	65.8	84.5	65.3	10.9		
d 121	75.0	74.1	70.3	10.9		
Average[2]	73.5[b]	79.4[a]	70.6[b]	2.93		

Table 7 – Serum biochemistry of steer consuming diets containing grape pomace silage and grape pomace bran.

(continuação)

Items[3]	Treatments[1]			SEM	*P* – values	
	Control diet	GPS diet	GPB diet		Treat	Treat × Day
GGT (mg/dL)					0.55	0.25
d 0	20.3	19.1	20.5	3.01		
d 21	20.2	17.5	15.5	3.01		
d 71	16.7	25.4	22.9	3.01		
d 121	28.7	27.7	22.5	3.01		
Average[2]	21.4	22.4	20.3	1.27		

[1]Treatments were: control diet (traditional confinement diet), GPS diet (inclusion of 100 g/kg in the DM of grape pomace silage in the diet), and GPB diet (inclusion of 100 g/kg in the DM of grape pomace bran in the diet).

[2] Treatment mean, referring to the periods, considered a covariate.

[3] Gamma-glutamyl transferase (GGT) and aspartate aminotransferase (AST).

[a-b]Within a row, differ ($P \leq 0.05$) or tend to differ ($P \leq 0.10$).

Table 8 - Ruminal indicators of steer consuming diets containing grape pomace silage and grape pomace bran.

Items	Treatments[1]			SEM	*P* – values	
	Control diet	GPS diet	GPB diet		Treat	Treat × Day
Rumen pH					0.38	0.51
d 71	6.00	6.14	6.32	0.14		
d 121	6.13	5.95	6.18	0.14		
Average	6.08	6.04	6.25	0.10		
Sedimentation time (min)	30.7[a]	20.4[b]	21.7[b]	3.22	**0.08**	-
Protozoan (× 10^6)	1.77[a]	1.34[ab]	0.72[b]	0.32	**0.10**	-
PRAM (s)	228[b]	341[a]	267[b]	23.4	**0.02**	-
Acetic acid (%)					**0.08**	**0.03**
d 71	56.4	48.0	49.2	3.70		
d 121	77.0[a]	80.7[a]	62.0[b]	3.70		
Average	66.7[a]	64.3[a]	55.6[b]	3.22		
Propionic acid (%)					**0.03**	**0.03**
d 71	11.9	11.8	12.0	1.15		
d 121	21.9[a]	18.9[a]	14.2[b]	1.15		
Average	16.9[a]	15.4[a]	13.1[b]	0.81		
Butyric acid (%)					0.61	0.41
d 71	11.5	9.50	10.3	1.23		
d 121	15.7	16.2	13.9	1.23		
Average	13.6	12.9	12.1	1.00		
Isovaleric acid (%)					0.54	0.74
d 71	1.03	1.06	1.02	0.11		
d 121	1.35	1.36	1.15	0.11		
Average	1.19	1.21	1.08	0.08		
Valeric acid (%)					**0.10**	**0.07**
d 71	1.11	1.00	0.89	0.10		
d 121	1.55[a]	1.96[a]	1.51[a]	0.10		
Average	1.33[ab]	1.48[a]	1.20[b]	0.09		
Acetate:proprionate ratio					0.94	**0.08**
d 71	4.99[a]	4.09[b]	4.07[b]	0.35		
d 121	3.67	4.34	4.37	0.35		
Average	4.33	4.21	4.22	0.27		

[1]Treatments were: control diet (traditional confinement diet), GPS diet (inclusion of 100 g/kg in the DM of grape pomace silage in the diet), and GPB diet (inclusion of 100 g/kg in the DM of grape pomace bran in the diet)

[a-b]Within a row, differ ($P \leq 0.05$) or tend to differ ($P \leq 0.10$).

Table 9 - Proteinogram of steer consuming diets containing grape pomace silage and grape pomace bran.

Items	Treatments[1]			SEM	*P* – values	
	Control diet	GPS diet	GPB diet		Treat	Treat × Day
Ceruloplasmin (g/dL)					**0.04**	0.72
d 0	0.65	0.57	0.63	0.06		
d 21	0.72	0.54	0.56	0.06		
d 71	0.57	0.48	0.58	0.06		
d 121	0.74	0.57	0.56	0.06		
Average[2]	0.67[a]	0.54[b]	0.58[ab]	0.03		
Transferin (g/dL)					0.15	0.32
d 0	0.54	0.51	0.50	0.05		
d 21	0.57	0.68	0.57	0.05		
d 71	0.50	0.51	0.49	0.05		
d 121	0.46	0.33	0.35	0.05		
Average[2]	0.52	0.51	0.48	0.02		
Haptoglobin (g/dL)					0.43	**0.0004**
d 0	0.27	0.35	0.31	0.04		
d 21	0.32[b]	0.31[b]	0.55[a]	0.04		
d 71	0.34	0.33	0.27	0.04		
d 121	0.40[a]	0.19[b]	0.22[b]	0.04		
Average[2]	0.33	0.30	0.34	0.02		
IgG heavy chain (mg/dL)					**<0.0001**	**0.09**
d 0	0.75	0.84	0.80	0.05		
d 21	0.71[b]	0.91[a]	0.88[a]	0.05		
d 71	0.50[b]	0.96[a]	0.85[a]	0.05		
d 121	0.63[b]	0.83[a]	0.88[a]	0.05		
Average[2]	0.65[b]	0.88[a]	0.85[a]	0.02		

[1]Treatments were: control diet (traditional confinement diet), GPS diet (inclusion of 100 g/kg in the DM of grape pomace silage in the diet), and GPB diet (inclusion of 100 g/kg in the DM of grape pomace bran in the diet)

[2] Average of the treatment, referring to the periods, except d0, considered a covariate.

[a-b]Within a row, differ ($P \leq 0.05$) or tend to differ ($P \leq 0.10$).

Table 10 - Antioxidant response of steer consuming diets containing grape pomace silage and grape pomace bran.

(continua)

Items[3]	Treatments[1]			SEM	*P* – values	
	Control diet	GPS diet	GPB diet		Treat	Treat × Day
Serum[2]						
TBARS[3]					**0.01**	**0.001**
d 0	35.8	39.7	34.1	0.91		
d 21	39.1[b]	37.2[b]	46.8[a]	0.80		
d 71	41.2[b]	33.5[c]	56.7[a]	0.81		
d 121	41.8[a]	35.9[c]	39.1[b]	0.82		
Average	40.7[b]	35.5[b]	47.5[a]	0.62		
GST[4]					**0.02**	**0.011**
d 0	154	152	163	5.32		
d 21	196[b]	185[b]	221[a]	4.74		
d 71	165[c]	179[b]	195[a]	4.70		
d 121	149[b]	167[a]	164[ab]	7.71		
Average	170[b]	177[b]	193[a]	3.70		
PSH[5]					**0.03**	**0.08**
d 0	112	117	121	10.8		
d 21	99.2	112	105	10.8		
d 71	135[b]	179[a]	138[b]	10.8		
d 121	151	152	159	10.8		
Average	124[b]	140[a]	131[ab]	3.88		
ROS[6]					**0.09**	**0.05**
d 0	41.6	43.6	39.7	3.06		
d 21	45.6[ab]	44.1[b]	51.8[a]	2.98		
d 71	60.9[a]	50.6[b]	63.7[a]	2.99		
d 121	52.1	50.1	54.7	2.98		
Average	52.8[ab]	48.2[b]	56.7[a]	1.51		
Intestine						
TBARS[3]	13.3	11.2	14.9	1.47	0.62	
GST[4]	656[b]	728[b]	875[a]	32.8	**0.01**	
PSH[5]	1.99[b]	2.72[a]	2.76[a]	0.16	**0.005**	
ROS[6]	421	330	276	34.9	**0.02**	

Table 10 - Antioxidant response of steer consuming diets containing grape pomace silage and grape pomace bran.

(continuação)

Items[3]	**Treatments**[1]				***P - values***	
	Control diet	**GPS diet**	**GPB diet**	SEM	**Treat**	**Treat × Day**
Liver						
TBARS[3]	54.1[a]	33.6[b]	36.5[b]	4.25	**0.006**	
GST[4]	1125[b]	1521[a]	1563[a]	113	**0.05**	
PSH[5]	6.00[b]	7.38[a]	6.50[ab]	0.36	**0.09**	
ROS[6]	962[a]	727[b]	842[ab]	32.0	**0.05**	

[1]Treatments were: control diet (traditional confinement diet), GPS diet (inclusion of 100 g/kg in the DM of grape pomace silage in the diet), and GPB diet (inclusion of 100 g/kg in the DM of grape pomace bran in the diet)

[2] Treatment mean, referring to the periods, except d0, considered a covariate.

[3] Thiobarbituric acid reactive substances (nmol MDA/mL);

[4] Glutathione S-Transferase (µmol CDNB/min/mg protein);

[5] Protein thiols (nmol SH/mg de protein of serum and µmol SH/mg de protein of intestine and liver);

[6] Reactive oxygen species (U DCFH/mg protein)

[a-b]Within a row, differ ($P \leq 0.05$) or tend to differ ($P \leq 0.10$).

Table 11 - The economic viability of steer consuming diets containing grape pomace silage and grape pomace bran

Items	Treatments[1]			SEM	*P* – value
	Control diet	GPS diet	GPB diet		Treat
Price/kg of diet (DM)	0.325	0.305	0.318	-	-
Total costs with feed	191	192	194	9.45	0.90
Total income	366[b]	392[a]	310[c]	21.3	**0.05**
Income over feed costs	174[b]	200[a]	116[c]	14.2	**0.001**

[1]Treatments were: control diet (traditional confinement diet), GPS diet (inclusion of 100 g/kg in the DM of grape pomace silage in the diet), and GPB diet (inclusion of 100 g/kg in the DM of grape pomace bran in the diet)

[a-b]Within a row, differ ($P \leq 0.05$) or tend to differ ($P \leq 0.10$).

4 ARTIGO 2: THE USE OF SECONDARY GRAPE BIOMASS IN BEEF CATTLE NUTRITION, ITS EFFECTS ON MEAT, AND ITS SHELF LIFE

Os resultados desta dissertação são apresentados na forma de artigo, com a seções de acordo com as orientações da Revista Food Chemistry.

4.1 ABSTRACT

We determined whether the inclusion of 100 g/kg dry matter of grape pomace silage (GPS) and grape pomace bran (GPB) as substitutes for other traditional fiber sources in the diet of steers (Charolais x Nellore) would improve carcass characteristics, meat quality and composition, and shelf life. Twenty-four animals (248 ± 19.32 kg of initial body weight) were fed a high concentrate diet for 121 days. Carcass characteristics were measured, and the longissimus dorsi muscle was analyzed for fatty acid (FA) profile and composition. The meat was sliced and stored in air-permeable packages for 10 days. On each sampling day (d 1, 3, 7, and 10), oxidative stability, bacterial load, lipid and protein oxidation, and staining were analyzed. The experimental diets influenced the pH of cold carcasses only. The GPS group had a higher pH than the control. The GPS and GPB groups showed improved oxidant status (i.e., lower lipid peroxidation and concentrations of reactive oxygen species were in the meat of both groups than in control). On the first day of storage, the antioxidant enzyme glutathione S-transferase activity was more significant in the meat of the GPS and GPB groups than in the control. The bacterial loads in the meat were attenuated by GPS inclusion; there were lower total coliform counts and a trend toward lower counts for enterobacteria in the control group. The diets altered the FA profile of the meat; i.e., the GPB diet allowed for a more significant amount of the n-6 omegas in the meat, while the GPS diet showed a tendency for a more significant amount of n-6 9 omegas. Both diets (GPS and GPB) increased the amounts of long-chain FAs. The GPS diet decreased saturated FA levels. We conclude that the dietary treatments GPS and GPB are a promising alternative to maintain meat quality standards throughout in real-world retail conditions. These treatments gave rise to an improvement in the nutritional value of the meat due to the more significant amounts of FAs that improve human health.

Keywords: Animal nutrition, antioxidant, microbiology, residue, physicochemical.

4.2 INTRODUCTION

The shelf life and quality of beef are influenced by oxidative processes, microbiological growth, and color stability; these features are critical for consumer acceptance and predict shelf life. Retail conditions that expose meat to high oxygen concentrations potentiate oxidation (JAKOBSEN; BERTELSEN, 2000), potentially harmful to health (ESTÉVEZ; LUNA, 2017; SOLADOYE et al., 2015).

To prolong shelf life, antioxidants are added to mitigate lipid oxidation, delay the development of unpleasant flavors, and improve color stability. However, some studies showed synthetic antioxidants to be toxic and carcinogenic (ABRAHAM et al., 1986; AHMAD et al., 1995; SARAFIAN et al., 2002; FAINE et al., 2006). Therefore, there is substantial demand for alternative antioxidants, which are standard in the food industry.

The use of secondary biomass obtained from post-harvest processing of grape crops in ruminant nutrition to sustain livestock farming; ruminants convert inedible components into high-value proteins (OLTJEN; BECKETT, 1996). These residues contain substantial numbers of phenolic compounds with functional capabilities (YU; AHMEDNA, 2012). In animal nutrition, grapes in diets were linked to increased microbiological capacity (HASSAN et al., 2019) and oxidative stability (MIELNIK et al., 2006). The proanthocyanidins in grapes (i.e., condensed tannins) influence ruminal biohydrogenation (VASTA et al., 2019), allowing the passage of PUFAs unchanged in the rumen to be absorbed distally, providing meat with better lipid composition and nutritional value (TAYENGWA et al., 2021a).

Food costs have risen for various reasons, and farmers seek alternative ingredients, especially industrial wastes. Therefore, the objective of the present study was to determine whether the inclusion of 100 g/kg of grape residue in ensiled and dehydrated form in the diets for feedlot steers (replacing other traditional fiber sources) would improve meat composition and quality during storage under retail conditions. Our hypothesis is that both residues (GPS and GPB) can improve meat quality aspects, by providing greater meat stability during shelf life. In particular, we believe that the GPB has greater use by the animals,

since the residue received physical processing, and thus, more evident beneficial effects on meat quality, when compared to those receiving the raw residue (GPS).

4.3 MATERIALS AND METHODS

4.3.1 Experiment location

The Animal Research Ethics Committee of the Universidade do Estado de Santa Catarina approved the project (protocol number: 4948210322), following the guidelines of CONCEA/Brasil.

4.3.2 Preparation of grape pomace silage (GPS) and grape pomace brans (GPB)

The GPS in cv. Isabel (*Vitis labrusca*) is composed of 3.81% stem, 52.71% bark, and 48.5% seeds (based on the dry matter). This material was collected in the municipality of Pinheiro Preto (27º 03' 02" S; 51º 13' 51" W) at an industrial winery. The residue was derived from the pressing process and separation of the solid fraction. Soon after being collected, the GPS was destined for storage in a trench-type silo (3.0 X 0.9 X 6.0 m for width, height, and length, respectively), coated with plastic on the sides and bottom, compacted with an agricultural tractor and sealed with specific tarpaulin for storage of preserved bulky foods (Parcifil®, Sapiranga, Brazil). The GPS was ensiled for 3 months before starting the experiment, characterized as grape pomace silage (GPS).

The production of grape pomace bran (GPB) required dehydrating the GPS. For logistical reasons, it was necessary to dry the GPS in two shipments. The first dehydrated batch was carried out in a closed environment, using gas bells and fans to heat the environment and force air circulation. The second batch of GPS was dehydrated in an open environment (i.e., in the sun) on plastic sheets. Both forms of drying were carried out at the Universidade do Estado de Santa Catarina (27° 06' 17" S 52° 36' 51" W) and lasted about 7 days until reaching values below 10% of humidity to avoid deterioration during storage and to allow grinding in a 4-mm sieve.

4.3.3 Meat samples

Were used meat samples from 24 steers slaughtered with an average body weight of 414 kg and 13 months of age) from industrial crosses (½ Charolais x ½ Nellore) according to data previously published by Molosse (2022).

The samples came from animals from three dietary treatments (eight steers/treatment) in a randomized controlled design to standardize initial body weight between groups, considering each steer as an experimental unit. The treatments were fed a control diet (traditional feed diet), GPS diet (inclusion of 100 g/kg in the dry matter (DM) of grape pomace silage in the diet), or GPB diet (inclusion of 100 g/kg in the DM of grape pomace bran in the diet). The animals underwent 121 days of experimentation (21 of diet adaptation and 100 days of the experimental period).

4.3.4 Experimental diets and feed

Diet was defined according to the requirements of the animal category, with an estimated average daily gain of 1.5 kg of body weight (BR-CORTE, 2016). The addition of the by-product (GPS or GPB) based on the total replacement of wheat bran and partial replacement of soybean hulls (Table 12). The rations of the three groups were isoproteic and isonergic.

4.3.5 SAMPLE COLLECTION AND DATA

4.3.5.1 Slaughter procedure, data collection and sampling

The animals were brought to a commercial slaughterhouse in Chapecó, 25 km from the confinement area, 17 hours before slaughter. The transport took about 1 hour. At the slaughterhouse, the animals remained in the covered waiting room for about 16 hours, with food restriction and access to drinking water.

The animals were directed to the containment box and stunned with a captive-bolt pistol. The procedure was according to a municipal animal food inspection seal and was supervised by a veterinarian. The hot carcasses were weighed immediately after the carcass was cleaned on a digital scale. At 30 minutes after slaughter, the pH was measured using a portable digital meter (TEXTO®, 205PH) between the 12th and 13th ribs in the left longissimus dorsi

muscle region. The carcasses were refrigerated 24 hours after slaughter, the pH was measured again, and the longissimus dorsi muscle on the left side was removed and transported in a thermal box to the laboratory for analysis.

Six 2-cm steaks were sectioned from the longissimus dorsi of each sample aseptically with sterile knives in a controlled environment using a bursen burner. Two of these steaks were immediately sent for analysis, one for determining meat color, water-holding capacity, cooking loss, shear force, and FA profile. The second steak was used to analyze shelf life, representing the beginning of the period (day 1). The remaining steaks were stored for further analysis at various shelf-life periods.

4.3.6 Laboratory analysis

4.3.6.1 Water-holding capacity, cooking loss, and shear force

The water-holding capacity was measured using an adaptation of the methodology of Honikel and Hamm (1994). We weighed 0.3 g of ground meat and placed them on filter paper (15 x 15 cm) between two acrylic plates. A weight of 10 kg was placed on top of the sample for 5 minutes. After pressing, the meat was weighed, and water loss was calculated based on the weight difference. The result was expressed as a percentage.

Cooking loss and shear force analyses were performed using 2-cm steaks. Before cooking the meat, the sample was weighed and covered in aluminum foil. Cooking was carried out on a portable grill (Mondial® Due Grill Smart), preheated to 170 °C until reaching an internal temperature of 75 °C (measured using a culinary thermometer inserted in the geometric center of the sample during cooking). After cooking, the samples not wrapped in aluminum foil remained until stabilized; thus, the final weight was obtained according to the methodology adapted from Honikel and Hamm (1994). The percentage of cooking water loss (CL) was calculated as follows: [(weight of raw steak − weight of cooked steak)/weight of raw steak] × 100.

The cooked and cooled samples were cut into subsamples in the fiber's longitudinal direction. Using a digital caliper, the height and width of the subsamples were measured for later calculation of the area. The shear force was

measured on each cuboid perpendicular to the fiber, using a texture analyzer (Stable Micro Systems®, TA-XT plus) coupled to a 1-mm thick Warner-Bratzler V-shaped cutting blade. The test speed was 3.30 mm/s to measure the force required to cross-cut each cylinder, and the values were expressed as Kgf/mm2 of meat.

4.3.7 Shelf life

Meat quality was evaluated at 1, 3, 7, and 10 days of shelf life (a proxy for retail exposure), with day 1 considered 24 hours after slaughter (chilled meat).

Four steaks (2 cm thick per experimental unit) were placed in polystyrene trays coated with a low-density polyethylene film (Supplementary Material 1). The trays were packed, labeled, and randomly placed in a controlled greenhouse, illuminated with white fluorescent light (Blumenau®, five LED Tube lamps, 9W, 6500K, 900 lumens) with forced ventilation and a temperature of 4 ± 0.4 °C. Lighting remained on 12 hours a day, simulating retail display conditions. The wrapped trays were handled daily to minimize differences in the incidence and intensity of light and possible changes in temperature inside the equipment.

4.3.7.1 Microbiology

Before the other analyses, a subsample of 10 g of meat was aseptically separated and weighed in sterile plastic bags for food and homogenized with 90 ml of buffered peptone water on a horizontal orbital shaker table (LOGEN SCIENTIFIC LS2312) for 10 min. From this dilution (10-1), 1 mL was transferred to tubes containing 9 mL of buffered peptone water, obtaining the other serial dilutions of 10-2 and 10-3, used on days 1, 3, and 7 of analysis. For day 10, dilutions of 10-4, 10-5, and 10-6 were used. Total bacterial counts (total mesophils) were performed on standard count agar (SCA), and lactic acid bacteria counts were performed on de Man, Rogosa, and Sharpe agar (MRS) using the pour plate method. The three serial dilutions performed previously were used, where 1 mL of each dilution was inoculated into sterile Petri dishes. Subsequently, the SCA plates were incubated in a bacteriological oven at 37 ± 1 °C for 24 h, and the MRS plates were placed in an anaerobic chamber (Panasonic®, MCO-19AIC UV) in a 5% CO2 atmosphere at 30 ± 1 °C for 72 h.

To count *Escherichia coli*/coliforms and Enterobacteriaceae, 3M Petrifilm™ EC 6414 and EB 6420 plates were used, respectively. Initially, the 10^{-1} dilution was used, and later (day 10), the 10^{-3} dilution was used. The plates were incubated at 37 ± 1 °C for 24 h. Bacterial counts were expressed as logarithms of colony-forming units per gram of meat (log CFU/g meat).

4.3.7.2 Meat color

After 2 minutes of exposure to ambient air, the surface color of the meat was measured using a portable gauging colorimeter (Konica Minolta®, CR-400) with an 8-mm observation aperture and an 11-mm illuminant/observer. Luminosity (L*), redness (a*), and yellowing (b*) were measured at three randomly selected sample points. The values of the parameters were expressed as the mean of three measurements.

4.3.7.3 Oxidant/antioxidant status

From the central area of the steak, 0.5 grams were removed and homogenized in saline solution to analyze the antioxidant/oxidant status. The samples were centrifuged for 10 min at 7500 g. The supernatants were collected and stored in microtubes at –20 ºC until analysis.

Meat glutathione S-transferase (GST) was measured based on Habig et al. (1974) and was expressed as µmol CDNB/min/mg of meat protein. Serum lipid peroxidation was measured based on the amount of thiobarbituric acid reactive substances (TBARS) according to Ohkawa et al. (1978), and the results were expressed as nmol MDA/mL. Determining reactive oxygen species (ROS) in the meat homogenate was based on Halliwell & Gutteridge (2007) and expressed as U DCFH/mg meat protein. According to Ellman (1959), protein thiols were measured, and the results were expressed as nmol thiols/mg of protein.

4.3.7.4 Fatty acid (FA) profile

Some modifications performed extraction from meat and animal feed as per Bligh and Dyer (1959). We added 1.5 g of bovine muscle samples, 0.5 mL of water, 5 mL of methanol, and 2.5 mL of chloroform into 15-mL polypropylene tubes, and mechanical shaking was performed for 60 min. Then, 2.5 mL of

chloroform and Na_2SO_4 1.5% solution were added to promote a biphasic system. This mixture was shaken for 2 minutes and then centrifuged for 15 minutes at 2000 rpm. Lipids obtained from the chloroform phase were subjected to FA analysis.

FA methylation was performed by a transesterification method proposed by Hartman and Lago (1973). We added 1 mL of 0.4 M KOH methanolic solution to the extracted lipids in a test tube and shook the mixture in a vortex for 1 min. Samples were kept in a water bath for 10 min at boiling point. Subsequently, the material was cooled at room temperature, and 3 mL of 1 M H_2SO_4 was added, shaken in a vortex, and maintained in a water bath for 10 min. After cooling, we added 2 mL of hexane and centrifuged at 2000 rpm for 10 min. Finally, hexane with the FA methyl esters (FAMEs) was subjected to chromatography analysis.

For the FAME determination, a gas chromatograph model TRACE 1310 was equipped with a flame ionization detector (Thermo Scientific). One microliter of samples was injected in a splitsplitless injector operated in 1:20 ratio split mode at 250 °C. Hydrogen was used as carrier gas at a constant flow rate of 1.5 mL/min. FAME separation was carried out using an RT 2560 chromatography column (100 m × 0.25 mm × 0.20 μm thickness film, Restek, USA). The FAME compounds were identified by comparing experimental retention times with those of an authentic standard (FAME Mix-37, Sigma Aldrich, St. Louis, MO). The results were expressed as a percentage of each FA identified in the lipid fraction, considering the chain size equivalent factor of FAME for FID and conversion factor of ester to the respective acid, according to Visentainer and Franco (2006).

4.3.8 Statistical analyses

All data were analyzed using the ‘MIXED procedure’ of SAS (SAS Inst. Inc., Cary, NC, USA; version 9.4), with the Satterthwaite approximation to determine the denominator degrees of freedom for the test of fixed effects. The carcass weight, yield, meat pH, shear force, water-holding capacity, CL, and intestinal and liver antioxidant variables were tested for fixed effects of treatment using the animal (treatment) as the random effect. All other variables were analyzed as repeated measures and were tested for fixed effects of treatment, day, and treatment × day, using the animal (treatment) as the random effect. The

compound symmetry covariance structure was selected according to the lowest Akaike information criterion. Means were separated using the PDIFF method, and all results were reported as LSMEANS and standard errors. Significance was defined when $P \leq 0.05$, and tendency when $P > 0.05$ and ≤ 0.10.

4.4 RESULTS

4.4.1 Carcass characteristics

The GPS-fed steers had higher chilled carcass pH than the control animals ($P < 0.05$, Table 14); the animals that received the GPB diet had a pH similar to the steers in the control group. For the other variables (weight, yield, and hot carcass pH), the experimental diets did not have a significant effect ($P > 0.05$)

4.4.2 Physicochemical parameters of meat

The experimental diets did not influence meat luminosity (L*), redness (a*), and yellowing (b*) over various periods of simulated retail exposure ($P > 0.05$, Table 15). There was also no day effect for luminosity ($P = 0.63$) or red color ($P = 0.13$); however, the yellow color of the meat had an effect of the day ($p < 0.001$), smaller on day 1 than the other days (3, 7, and 10) in the three experimental groups. Warner-Bratzler shear force, CL, and water-holding capacity were similar between groups ($P > 0.05$).

The experimental diets influenced the meat's chemical composition ($P < 0.05$). The steers fed the GPB diet had higher crude protein content than the control animals. The DM of the meats was similar between the groups ($P > 0.05$)

4.4.3 Oxidative status of meat

The oxidative/antioxidant status of beef is shown in Table 16. The experimental diets influenced the oxidative indicators. The GPS and GPB groups had lower levels of TBARS and ROS in the meat than the control group ($P < 0.05$). The experimental diets also influenced the antioxidant system indicators. Effects on treatment x day interaction were detected ($P < 0.04$) for GST activity in meat; the animals that received the GPS and GPB diet showed higher activities of the GST enzyme on day 1 than the control group. A trend of effect ($P = 0.07$)

was observed for this variable, indicating more significant GST activity in the steers' meat in the GPS and GPB groups. A day effect ($P < 0.01$) was detected for TBARS, ROS, and GST in all groups, with a linear and increasing trend in the levels of TBARS and ROS. There was a linear and decreasing trend in GST activity. Regarding protein thiols, there were no treatment, treatment x day, or day effects ($P > 0.05$).

4.4.4 Microbiology

The bacterial counts are shown in Table 17. Diets did not influence PCA or MRS counts at various simulated retail exposure periods ($P > 0.05$). There were effects of experimental diets ($P < 0.05$), but not for treatment x day interaction ($P > 0.05$) for total coliforms (TC) and enterobacteria (ENTB). Steers fed the GPS diet had lower TC counts and a tendency toward lower ENTB counts than the control steers; the animals receiving the GPB diet were similar to the control and GPS groups. There was a day effect ($P < 0.001$) for TC, ENTB, and total mesophile counts in the meat of animals from the three experimental groups, with an increasing trend over the days of meat exposed to simulated retail conditions. Likewise, a day effect ($P < 0.001$) was observed for lactic acid concentration, which increased linearly over time.

4.4.5 FA profile

Small modulations were observed by the inclusion of different experimental diets on the lipid profile in fresh meat at 24 h post-slaughter (Table 18). The GPS and GPB diets increased CLFA concentrations when the control diet ($P = 0.05$). The steers fed the GPS diet had lower concentrations of SFA when compared to the control and GPB diets. A trend of effect was observed for individual omega 6 and 9 FAs and their summaries. Steers on the BPG diet tended to have higher amounts of omega 6 when compared to the control group ($P = 0.08$). As for the concentration of omega 9, the animals that received the GPS diet tended to have higher concentrations than the GPB diet ($P = 0.10$). No effects of experimental diets were observed on the amount of total monounsaturated fatty acids (MUFAs) and PUFAs and for the atherogenic index ($P > 0.05$)

4.5 DISCUSSION

The present study is the first to conduct a joint evaluation comparing the inclusion of dehydrated and ensiled grape residue in the diet of Charolais x Nelore steers, focusing on the FA profile, antioxidant activity, microbiology, and chemical-physical profile of fresh beef exposed to simulated shelf life. To evaluate the lipid profile of meat, the study must have similar growth rates between the experimental groups, given that the animal growth rate, through its effect on fat formation, can strongly influence the FA composition (AUROUSSEAU et al., 2004). In this study, the diets provided the same growth rate, with diets with an iso-protein and iso-energetic composition (Table 12).

The lack of effect of diets for meat color (a* L* and b*) is similar to previous findings in the inclusion of pomace residue from grapes in sheep (CHIKWANHA et al., 2019; ZHAO et al., 2018), pomace residue from grape in cattle (TAYENGWA et al., 2020a) and grape seed in sheep (JERÓNIMO et al., 2012). In the present study, it was not possible to observe a day effect for the variables L* and a* that represent inhibition of protein denaturation and conversion of deoxymyoglobin to oxymyoglobin and their effects on the increased indicators of luminosity (WARRIS, 2000) and red coloration (FAUSTMAN; SUMAN, 2017). The addition of ensiled or dehydrated grape residue in the diet of cattle, although showing good antioxidant capacity, did not have the potential to preserve red color until the end of the shelf life, which presumes oxidation of myoglobin or oxymyoglobin to metmyoglobin (FAUSTMAN; SUMAN, 2017). This effect was minimized with higher vitamin E concentrations in sheep meat (GUERRA-RIVAS et al., 2016). It is worth mentioning that, even with no difference in color, the luminosity and redness observed in the present study were in the ideal range (35.3 to 46.3 and <14.5 for L* and a*, respectively) considered acceptable by consumers. of beef (COOPER et al., 2018; HOLMAN et al., 2017).

The inclusion of grape residue in the steers' diets influenced the FA profile in the meat. We associated the highest amounts of LCFA in the meat of steers that received the GPS and GPB diets due to the increased proportion of the long-chain FA C18:2n6c (Table 13). In addition to the differentiated lipid profile of the residue, which directly affects the FA profile of the experimental feed (and, therefore, what the animal consumes), the grape residue has large amounts of

polyphenols, which influence biohydrogenation and PUFA content in meat (VASTA et al., 2019, GUERREIRO et al., 2020). The higher proportions of n-3 and n-6 PUFAs observed in GPS and GPB diets can be attributed to higher levels of polyphenol compounds in the diets that reduce ruminal lipolysis and protect PUFAs from biohydrogenation in the rumen, making them inaccessible to microbes or their enzymes (VAHMANI et al., 2020, VASTA et al., 2019). The investigation for increased MUFA and PUFA content and concomitant declines in SFA content in animal products have been the subject of several studies, given the relationship between dietary FAs and human health (GIVENS, 2005). Diets rich in saturated FAs (SFA) contribute to an increase in the level of LDL-cholesterol, which is positively correlated with diseases of the circulatory system (KRIS-ETHERTON; YU, 1997), in addition to obesity (ZHOU et al., 2020). In contrast, some MUFAs and PUFAs (particularly long-chain n−3 PUFAs) improve human health (SACKS; KATANA, 2002; SIMOPOULOS, 1999). Thus, although the differences in total PUFA concentrations are not significant (except for n-6 and 9), linked to lower SFA concentrations in the GPS group, there is a substantial effect of including the residue in the ensiled and dehydrated form, even with changes and interferences of ruminal biohydrogenation (CHILLIARD et al., 2007). This phenomenon results in higher levels of SFA, compared (for example) with swine meat (ENSER et al., 1996). Steers that consumed BPG had a higher concentration of protein in the meat, an intriguing result that needs to be studied to understand the mechanisms involved and that contributed to this finding of the chemical composition of the meat.

Lipid stability is a dominant determinant of meat quality during shelf life (BUCKLEY et al., 1995) because lipid peroxidation is a primary deterioration mechanism, especially for meat products (KANNER, 1994). In the present study, lipid peroxidation and ROS concentrations were lower in the meat of steers fed the GPS and GPB diets. These findings agree with Tayengwa et al. (2020), who added 150 g/kg of dry grape pomace to the diet of Angus cattle and observed lower lipid oxidation and higher antioxidant activity in meat. Similar effects were observed in lamb meat (CHIKWANHA et al., 2019, GLADINE et al., 2007; GUERRA-RIVAS et al., 2016) and chicken (GOÑÍ et al., 2007). These findings corroborate the literature that describes the antioxidant activities and capacity of

grape phenolics (YILMAZ; TOLEDO, 2006; BRENES et al., 2016). We believe that the effects observed in the meat of these animals are due to the vigorous H+ donor activity (MUCHUWETI et al., 2007), the ability to scavenge free radicals and chelate pro-oxidants (transition metals), and singlet oxygen quenching (OZSOY et al., 2009, CHOE; MIN, 2009).

The antimicrobial and antioxidant potential of grape residue in the meat of animals depends solely on the ingestion of these compounds and their bioavailability, such that there is the accumulation of these compounds or their metabolites in muscle tissues during an animal's life. For polymeric and high molecular weight substances such as condensed tannins, absorption may be limited, and it is unlikely that oligomers larger than trimers can be absorbed in the small intestine in their native form (MANACH et al., 2004). Therefore, we believe the monomer provides the antimicrobial and antioxidant effects, catechins, quercetin, epicatechin, and epicatechin-3-O-gallate (ANDRÉS et al., 2013; CUSHNIE; LAMB, 2005; MOON et al., 2011; KAEWPRACHU et al., 2017). Nevertheless, we cannot rule out the possible biodegradation of polymeric proanthocyanidins, in which they are metabolized into bioavailable compounds with an intact flavonoid ring structure (epicatechin) (Gladiane et al., 2007).

Phenolic compounds (primarily hydroxycinnamic acids, gallic acid, flavonols, flavan-3-ols, and trans-resveratrol) make up the grape and its by-products and provide antimicrobial activity (FRIEDMAN, 2014). This inhibitory potential of microbial proliferation occurs by accumulating these compounds in the lipid bilayer, causing alteration and disarrangement in the membrane, altering and compromising its function. The increase in membrane permeability allows entry into the bacterial cell, exerting inhibitory activity in the cell cytoplasm, leading to lysis and release of intracellular ATP (YUSTE; FUNG, 2003, NAZER et al., 2005). The hydroxyl groups of phenols are primarily responsible for inhibitory activity and can lyse the bacterial cell membrane (LAI; ROY, 2004, XUE et al., 2013). Catechins are highly potent inhibitors of DNA gyrase, vital for DNA transcription and replication and chromosomal segregation of bacteria (KHAN et al., 2018).

Therefore, we associated the lower count of TC and total mesophiles of the GPS diet with the ability of phenolic compounds to inhibit bacterial

proliferation. Our enterobacterial counts are similar to those reported by Viera et al. (2022), who included 50 g/kg DM from the total grape pomace diet in the lambs diet. In contrast, Chikwanha et al. (2019) and Guerra-Rivas et al. (2016) found no effect in sheep meat; however, these authors observed a lower total viable count. Tayengwa et al. (2020) observed lower TC counts with the addition of grape residue, similar to the present study.

Although the GPS diet showed an antibacterial effect on the meat, this effect was insufficient to prolong the shelf life of the meat until the seventh day. According to Normative Instruction 60 (IN60) of microbiological food standards, followed in Brazil, meat from all experimental groups would be outside the acceptable standard on the seventh day of shelf life. On the seventh day, all groups reached a plateau of 5 log CFU/g of meat for total mesophile counts, with the control group numerically superior, followed by the GPB and GPS groups. Although we did not analyze it, we speculate that a possible effect for the GPS diet could be observed on the sixth day, given the bacterial growth behavior and antimicrobial effect on the GPS experimental group. The International Commission on Microbiological Specifications for Food (ICMSF) guidelines suggest a maximum bacterial count limit higher than the IN60 (7 log CFU/g and 5 log CFU/g respectively), which prolongs the shelf life of foods. The microbiology counts did not exceed the values imposed by the ICMSF until the tenth day in the control and GPS groups. Although we did not perform a sensory panel, we considered the meat unacceptable for consumption on the seventh day, given the strong odor. To support our finding, Kim et al. (2018) conducted a sensory panel of meat from raw beef distributed in Korea, and found lower bacterial counts suggested as a critical limit by the ICMSF.

4.6 CONCLUSION

Dietary treatments with the inclusion of GPS and GPB minimized the oxidation of meat fat, as well as improved the nutritional value, due to the greater amount of FA beneficial to human health. In addition, the GPS diet reduced bacterial counts over the course of retail meat exposure. In this sense, we can partly validate our hypothesis; the residues allowed beneficial effects on the

quality of the meat, however the GPB did not allow more avoidant positive effects in relation to the GPS. Therefore, we conclude that the inclusion of these residues replacing other traditional fiber sources in the diet of beef cattle in the finishing phase can serve as an antioxidant source in meat products, denoting a positive effect on meats that are exposed to retail, as well as, allowing a product with nutraceutical properties.

4.7 TABLES

Table 12 - Proportions of feed ingredients in the experimental diets and chemical composition.

Item	GPS	GPB	WB	Treatment		
				Control diet	GPS diet	GPB diet
Ingredients (g/kg)						
Grape pomace silage (GPS)				-	100.0	-
Grape pomace bran (GPB)				-	-	100.0
Corn Silage				400.0	400.0	400.0
Ground Corn				280.3	351.2	352.2
Soybean meal				29.1	48.2	47.5
Soybean hulls				139.8	58.7	59.1
Wheat bran (WB)				110.0	-	-
Mineral[1]				22.0	22.2	22.0
Urea				7.0	7.5	7.0
Sodium bicarbonate				10.0	10.0	10.0
Mycotoxin adsorbent				2.2	2.2	2.2
Chemical composition (g/kg)						
Dry matter (DM)	415.0	934.0	847.5	440.7	404.0	475.9
Organic matter (OM)	951.1	968.3	948.4	945.9	950.5	950.7
Ash	48.9	31.7	51.6	54.1	49.5	49.3
Ether extract	112.3	150.9		23.3	30.8	34.5
Crude protein	117.0	136.0	148.9	108.3	111.4	111.7
TDN[1]						
Neutral detergent fiber	620.0	586.0	436.5	381.4	387.2	350.7
Acid detergent fiber	594.0	535.0	139.2	221.0	244.7	218.6
Lignin	334.0	307.0	48.8	27.7	75.3	55.9
Starch	24.6	11.00	233.7	302.7	349.4	366.5
Total carbohydrates	721.8	681.4	799.5	814.3	805.4	804.5
Pectin + sugar	90.8	70.8	129.3	130.6	68.6	87.35
Non-fibrous carbohydrates (NFC)	101.8	95.4	363.0	432.9	418.2	453.8
Total phenols (mg/kg)	855.0	1710.0	-	223.7	230.8	253.5
Total flavonoids, mg/kg	593.51	1621.35	-	-	-	-
Total tannins, g/kg	6.3	12.6	-	1.4	2.8	3.5

[1] Calcium (Max.) 220.00 g/kg; Calcium (Min.) 160.00 g/kg; Phosphorus (Min.) 40.00 g/kg; Magnesium (Min.) 6.00 g/kg; Sodium (Min.) 85.00 g/kg; Sulfur (Min.) 12.00 g/kg; Cobalt (Min.) 20.00 mg/kg; Copper (Min.) 520.00 mg/kg; Iodine (Min.) 25.00 mg/kg; Manganese (Min.) 650.00 mg/kg; Selenium (Min.) 10.00 mg/kg; Zinc (Min.) 2000.00 mg/kg; Fluoride (Max.) 400.00 mg/kg.

Table 13 - Fatty acid composition of feed ingredients in the experimental diets (% of total fatty acids).

Item[1]	GPS	GPB	WB	Treatment[2]		
				Control diet	GPS diet	GPB diet
C12:0	0.10	0.10	0.10	0.15	0.17	0.13
C14:0	0.3	0.20	0.10	0.23	0.24	0.27
C15:0	-	-	0.10	0.07	0.05	0.06
C16:0	12.7	10.9	19.3	23.99	19.08	20.60
C16:1	0.4	0.6	0.2	0.13	0.19	0.26
C17:0	0.1	0.1	0.2	0.23	0.17	0.17
C18:0	5.8	5.7	2.9	6.82	6.36	7.01
C18:1n9c	21.6	17.9	21.6	25.93	24.16	24.15
C18:2n6c	56.2	61.6	50.6	36.22	43.65	42.35
C20:0	-	-	-	0.78	0.99	0.81
C20:1n9	-	0.1	0.7	0.37	0.27	0.26
C18:3n3	1.6	1.0	3.2	2.68	2.65	1.83
C21:0	-	-	-	0.31	0.11	0.25
C20:2	-	-	0.1	0.30	0.15	0.24
C22:0	0.7	0.8	0.3	0.58	0.71	0.56
C20:3n3	0.1	0.1	0.2	0.17	0.15	0.12
C20:4n6	-	-	-	0.10	0.05	0.05
C24:0	0.4	0.7	0.3	0.70	0.73	0.62
C24:1n9	-	-	0.1	0.23	0.13	0.25

[1] C12:0 (Lauric), C14:0 (Myristic), C15:0 (Pentadecanoic), C16:0 (Palmitic), C16:1 (Palmitoleic), C17:0 (Heptadecanoic), C18:0 (Stearic), C18:1n9c (Oleic), C18:2n6c (Linoleic), C20:0 (Arachidic), C20:1n9 (cis-11-Eicosenoic), C18:3n3 (a-Linolenic), C21:0 (Henicosanoic), C20:2 (cis-11,14-Eicosadienoic), C22:0 (Behenic), C20:3n3 (cis-11,14,17-Eicosatrienoic), C20:4n6 (Arachidonic), C24:0 (Lignoceric), C24:1n9 (Nervonic).

[2] Treatments were: control diet (traditional confinement diet), GPS diet (inclusion of 100 g/kg in the DM of grape pomace silage in the diet), and GPB diet (inclusion of 100 g/kg in the DM of grape pomace bran in the diet)

Table 14 - Effects of diets containing grape pomace silage and grape pomace bran on carcass traits of Charolais x Nelore steers.

Items	Treatments[1]			SEM	*P* – value	
	Control diet	GPS diet	GPB diet		Treat	Treat × Day
Cold carcass weight (kg)	214	218	198	10.2	0.40	-
Dressing (%)	51.7	52.1	50.9	0.67	0.48	-
pH carcass hot	6.50	6.52	6.64	0.05	0.22	-
pH carcass cold	5.30^{b}	5.48^{a}	5.35^{ab}	0.04	**0.05**	-

[1] Treatments were: control diet (traditional confinement diet), GPS diet (inclusion of 100 g/kg in the DM of grape pomace silage in the diet), and GPB diet (inclusion of 100 g/kg in the DM of grape pomace bran in the diet)$^{a-b}$ Within a row, differ (P ≤ 0.05) or tend to differ (P ≤ 0.10).

Table 15 - Physicochemical meat quality of steers consuming diets containing grape pomace silage and grape pomace bran.

Items	Treatments[1]			SEM	*P* – value	
	Control diet	GPS diet	GPB diet		Treat	Treat × Day
Dry matter (g/Kg)	267	283	292	17.0	0.15	-
Crude protein (g/Kg)	234[b]	249[ab]	265[a]	10.0	**0.05**	-
Warner–Bratzler shear force (WBSF) (kgf/cm^2)	7.76	9.00	7.96	0.91	0.57	-
Water-holding capacity (WHC) (g water/g dry matter)	77.0	75.9	75.8	1.60	0.87	-
Cooking loss (CL) (g/Kg)	249	264	258	20.6	0.87	-
Lightness, (L*)					0.35	0.39
d 1	38.1	41.0	40.8	1.49		
d 3	40.9	42.8	42.3	1.49		
d 7	39.6	43.0	43.2	1.49		
d 10	38.2	41.9	39.4	1.49		
Average	39.2	42.2	41.4	1.37		
Redness, (a*)					0.40	0.54
d 1	12.4	13.9	13.6	0.71		
d 3	13.4	15.1	13.7	0.71		
d 7	11.9	13.2	12.0	0.71		
d 10	11.5	11.4	12.0	0.71		
Average	12.3	13.4	12.8	0.54		
Yellowness, (b*)					0.27	0.88
d 1	6.53	7.85	7.57	0.78		
d 3	11.8	12.4	12.2	0.78		
d 7	10.4	12.3	12.6	0.78		
d 10	10.5	12.1	11.8	0.78		
Average	9.79	11.2	11.04	0.60		

[1] Treatments were: control diet (traditional confinement diet), GPS diet (inclusion of 100 g/kg in the DM of grape pomace silage in the diet), and GPB diet (inclusion of 100 g/kg in the DM of grape pomace bran in the diet)

[a-b] Within a row, differ (P ≤ 0.05) or tend to differ (P ≤ 0.10).

Table 16 - Oxidative status of beef maintained under retail exposure conditions of Charolais steers fed grape pomace silage and grape pomace bran.

Items	Treatments[1]			SEM	*P* – values	
	Control diet	GPS diet	GPB diet		Treat	Treat × Day
TBARS[2]					**0.03**	**0.01**
d 1	8.74	8.17	8.29	0.32		
d 3	9.23[a]	8.61[ab]	8.31[b]	0.32		
d 7	12.8[a]	10.5[b]	8.95[b]	0.33		
d 10	14.8[a]	11.1[b]	11.3[b]	0.32		
Average	11.4[a]	9.59[b]	9.21[b]	0.27		
GST[4]					**0.07**	**0.04**
d 1	521[b]	594[a]	586[a]	21.0		
d 3	496[b]	587[a]	556[ab]	36.0		
d 7	457	501	521	38.0		
d 10	369	374	354	37.0		
Average	465[b]	514[a]	504[a]	35.3		
PSH[5]					0.93	0.96
d 1	5.36	6.29	5.95	0.60		
d 3	5.40	5.40	5.66	0.60		
d 7	4.82	4.79	4.78	0.60		
d 10	5.63	5.29	5.57	0.60		
Average	5.30	5.45	5.49	0.37		
ROS[6]					**0.001**	**0.002**
d 1	685[a]	502[b]	512[b]	61.2		
d 3	731[a]	589[b]	573[b]	61.3		
d 7	896[a]	632[b]	590[b]	63.0		
d 10	1056[a]	877[b]	878[b]	64.3		
Average	842[a]	650[b]	638[b]	56.4		

[1] Treatments were: control diet (traditional confinement diet), GPS diet (inclusion of 100 g/kg in the DM of grape pomace silage in the diet), and GPB diet (inclusion of 100 g/kg in the DM of grape pomace bran in the diet)

[2] Thiobarbituric acid reactive substances (nmol MDA/mL);

[4] Glutathione S-Transferase (µmolCDNB/min/mg protein);

[5] Protein thiols (µmol SH/mg of protein);

[6] Reactive oxygen species (U DCFH/mg protein)

[a-b] Within a row, differ ($P \leq 0.05$) or tend to differ ($P \leq 0.10$).

Table 17 - Microbiology of beef kept under retail exposure conditions of Charolais x Nelore steers fed grape pomace silage and white grape pomace.

Items[2]	Treatments[1]			SEM	*P* – values	
	Control diet	GPS diet	GPB diet		Treat	Treat × Day
Total coliforms					**0.04**	0.39
d 1	2.75	1.96	2.43	0.20		
d 3	2.73	2.14	1.88	0.20		
d 7	3.87	3.62	3.79	0.20		
d 10	5.42	4.65	5.29	0.20		
Average	3.69[a]	3.17[b]	3.35[ab]	0.13		
Enterobacterium					**0.10**	0.51
d 1	2.36	2.08	2.21	0.19		
d 3	2.65	1.91	2.32	0.19		
d 7	3.72	3.80	3.85	0.19		
d 10	5.52	5.16	5.52	0.19		
Average	3.56[a]	3.24[b]	3.48[ab]	0.12		
Total mesophiles					0.39	0.58
d 1	4.25	3.87	3.80	0.33		
d 3	4.04	3.77	3.85	0.33		
d 7	5.73	5.28	5.44	0.33		
d 10	6.88	6.31	7.48	0.33		
Average	5.22	4.81	5.14	0.23		
Lactic acid					0.56	0.83
d 1	3.67	3.26	3.27	0.29		
d 3	3.66	3.32	3.49	0.29		
d 7	5.60	5.20	5.06	0.29		
d 10	6.73	6.62	7.08	0.29		
Average	4.91	4.60	4.72	0.23		

[1] Treatments were: control diet (traditional confinement diet), GPS diet (inclusion of 100 g/kg in the DM of grape pomace silage in the diet), and GPB diet (inclusion of 100 g/kg in the DM of grape pomace bran in the diet).

[2] Values expressed as log CFU/g meat.

[a-b] Within a row, differ (P ≤ 0.05) or tend to differ (P ≤ 0.10).

Table 18 - Fatty acid profile of beef kept under retail exposure conditions of Charolais x Nelore steers fed grape pomace silage and white grape pomace.

Items	Treatments[1]			SEM	*P* – values	
	Control diet	GPS diet	GPB diet		Treat	Treat × Day
Intramuscular fat (mg/g muscle)	25.5	25.2	31.0	4.50	0.64	0.60
Σ_AG_CM	39.2	37.9	37.4	0.75	0.25	0.79
Σ_AG_CL	59.9[b]	62.0[a]	62.6[a]	0.80	**0.05**	0.76
Sum fatty acids						
Total SFA	50.9[b]	49.5[a]	50.8[b]	0.40	**0.05**	0.23
Total MUFA	41.5	42.5	40.2	0.95	0.29	0.87
Total PUFA	6.80	7.81	8.95	0.99	0.37	0.38
Ômega 3						
C22:6n3	0.11	0.09	0.10	0.02	0.76	0.70
Σ_n3_PUFA	0.79	0.71	0.74	0.10	0.87	0.55
Ômega 6						
C18:2n6c	4.47	5.15	5.89	0.55	0.28	0.53
C18:3n6	0.02	0.03	0.03	0.007	0.62	0.98
C20:3n6	0.29	0.34	0.42	0.06	0.39	0.52
C20:4n6					**0.07**	**0.08**
d 1	0.89[b]	1.66[a]	1.57[a]	0.35		
Average	1.14[b]	1.48[ab]	1.78[a]	0.19		
Σ_n6_PUFA	5.93[b]	7.00[ab]	8.12[a]	0.68	**0.08**	0.17
Ômega 9						
C18:1n9c	35.5[b]	37.2[a]	35.4[b]	0.64	**0.10**	0.83
C20:1n9	0.13	0.12	0.17	0.03	0.63	0.44
C24:1n9	0.01	0.009	0.01	0.003	0.68	0.81
Σ_n9_PUFA	37.1[ab]	38.6[a]	36.7[b]	0.73	**0.10**	0.77
Health Indexes						
Atherogenic index	10.9	10.4	9.99	0.92	0.78	0.27
Δ9_Desaturase_C18					**0.08**	**0.05**
d 1	70.1[b]	73.3[a]	69.4[b]	0.88		
d 10	71.0	71.1	69.0	0.88		
Average	70.5[ab]	72.2[a]	69.2[b]	0.84		

[1] Treatments were: control diet (traditional confinement diet), GPS diet (inclusion of 100 g/kg in the DM of grape pomace silage in the diet), and GPB diet (inclusion of 100 g/kg in the DM of grape pomace bran in the diet)

[a-b] Within a row, differ ($P \leq 0.05$) or tend to differ ($P \leq 0.10$).

4.8 SUPPLEMENTARY MATERIAL

Figure 3 - Supplementary material 1 - Illustration of steaks placed in polystyrene trays coated with low-density polyethylene film.

5 CONSIDERAÇÕES FINAIS

A utilização alternativa da SBU em substituição ao farelo de trigo e casca de soja na dieta de novilhos estimulou e modulou o sistema antioxidante e imunológico, denotando reflexo positivo na saúde animal dos novilhos. Além disso, a inclusão deste resíduo não afetou o perfil fermentativo ruminal e o desempenho produtivo dos animais e as principais características de carcaça. Sobre os atributos e qualidade da carne, a inclusão dos resíduos minimizou a oxidação da gordura da carne, bem como, possibilitaram uma melhora no valor nutricional, com maior quantidade de AG benéficos a saúde humana. Além disso, a dieta SBU reduziu a contagem bacteriana no decorrer da exposição da carne no varejo. Curiosamente, os efeitos observados na inclusão do farelo de bagaço de uva não foram similares ou positivos ao nível da dieta SBU, inviabilizando a sua utilização, que não confirmam nossa hipótese. Assim sendo, nas condições experimentais do presente estudo, a inclusão da SBU como ingrediente na nutrição de novilhos é uma alternativa promissora para diminuir os custos alimentares e melhorar a saúde animal de bovinos sem afetar o desempenho e, a utilização do resíduo na forma ensilada e desidratada pode ser utilizado como conservantes naturais dos produtos cárneos *in natura*. Neste sentido, os pioneiros resultados do presente estudo efetivam no Brasil grande parte dos efeitos da inclusão da uva já publicados em outros países em bovinos, contudo, em especial, os efeitos da inclusão da uva desidratam trazem forte controversa de sua utilização. Novos ensaios são necessários para avaliar e efetivar os resultados exclusivos deste trabalho.

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ANEXO A – COMPROVANTE DO CEUA

UDESC UNIVERSIDADE DO ESTADO DE SANTA CATARINA | LAGES CENTRO DE CIÊNCIAS AGROVETERINÁRIAS

Universidade do Estado de Santa Catarina
Comissão de Ética no Uso de Animais

CERTIFICADO : EMENDA v21/06/2021

Certificamos que a EMENDA (versão de 21/06/2021) da proposta intitulada "Farinha de resíduo de uva como aditivo na dieta de bovinos em confinamento: efeitos sobre o desempenho, perfil metabólico, saúde animal e qualidade da carne ", CEUA nº 1551091220 (ID 013969), sob a responsabilidade de **Aleksandro Schafer da Silva** - que envolve a produção, manutenção e/ou utilização de animais pertencentes ao filo Chordata, subfilo Vertebrata (exceto o homem), para fins de pesquisa científica ou ensino - está de acordo com os preceitos vigentes para sua apresentação, bem como com as normas editadas pelo Conselho Nacional de Controle da Experimentação Animal (CONCEA), sendo assim **APROVADO** pela Comissão de Ética no Uso de Animais da Universidade do Estado de Santa Catarina (CEUA/UDESC) em 25/06/2021.

Término previsto: 07/2021

Origem:	Animais de proprietários			
Espécie:	Bovinos	sexo: Machos	idade: 8 a 13 meses	Quantidade solicitada: 24
Linhagem:	misto: charoles x nelore		Peso: 250 a 450 kg	

ANIMAIS UTILIZADOS

		Quantidade Aprovada	Quantidade Utilizada
Bovinos	Machos	24	0
Bovinos	Machos	24	0

Lages, 11 de outubro de 2022

José Cristani
Coordenador da Comissão de Ética no Uso de Animais
Universidade do Estado de Santa Catarina

Pedro Volkmer de Castilhos
Vice-Coordenador da Comissão de Ética no Uso de Animais
Universidade do Estado de Santa Catarina

UDESC UNIVERSIDADE DO ESTADO DE SANTA CATARINA | LAGES CENTRO DE CIÊNCIAS AGROVETERINÁRIAS

Universidade do Estado de Santa Catarina
Comissão de Ética no Uso de Animais

CERTIFICADO

Certificamos que a proposta intitulada "Farinha de resíduo de uva como aditivo na dieta de bovinos em confinamento: efeitos sobre o desempenho, perfil metabólico, saúde animal e qualidade da carne ", protocolada sob o CEUA nº 1551091220 (ID 001282), sob a responsabilidade de **Aleksandro Schafer da Silva** - que envolve a produção, manutenção e/ou utilização de animais pertencentes ao filo Chordata, subfilo Vertebrata (exceto o homem), para fins de pesquisa científica ou ensino - está de acordo com os preceitos da Lei 11.794 de 8 de outubro de 2008, com o Decreto 6.899 de 15 de julho de 2009, bem como com as normas editadas pelo Conselho Nacional de Controle da Experimentação Animal (CONCEA), e foi **APROVADA** pela Comissão de Ética no Uso de Animais da Universidade do Estado de Santa Catarina (CEUA/UDESC) na reunião de 16/12/2020.

We certify that the proposal "Grape residue flour as an additive in the diet of feedlot cattle: effects on performance, metabolic profile, animal health and meat quality", utilizing 24 Bovines (24 males), protocol number CEUA 1551091220 (ID 001282), under the responsibility of **Aleksandro Schafer da Silva** - which involves the production, maintenance and/or use of animals belonging to the phylum Chordata, subphylum Vertebrata (except human beings), for scientific research purposes or teaching - is in accordance with Law 11.794 of October 8, 2008, Decree 6899 of july 15, 2009, as well as with the rules issued by the National Council for Control of Animal Experimentation (CONCEA), and was **APPROVED** by the Ethic Committee on Animal Use of the University of Santa Catarina State (CEUA/UDESC) in the meeting of 12/16/2020.

Finalidade da Proposta: Pesquisa (Acadêmica)

Vigência da Proposta: de 02/2021 a 07/2021 Área: Zootecnia

Origem:	Animais provenientes de outros projetos				
Espécie:	Bovinos	sexo: Machos	idade: 11 a 14 meses	N:	24
Linhagem:	Holandês		Peso: 350 a 450 kg		

Lages, 11 de outubro de 2022

José Cristani
Coordenador da Comissão de Ética no Uso de Animais
Universidade do Estado de Santa Catarina

Pedro Volkmer de Castilhos
Vice-Coordenador da Comissão de Ética no Uso de Animais
Universidade do Estado de Santa Catarina