

**UNIVERSIDADE ESTADUAL PAULISTA - UNESP
CÂMPUS DE JABOTICABAL**

**GLICERINA BRUTA NA ADAPTAÇÃO E DESEMPENHO DE
OVINOS CONFINADOS**

**Márco Túlio Costa Almeida
Zootecnista**

2018

**UNIVERSIDADE ESTADUAL PAULISTA - UNESP
CAMPUS DE JABOTICABAL**

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OVINOS CONFINADOS**

Márco Túlio Costa Almeida

Orientador: Profa. Dra. Jane Maria Bertocco Ezequiel

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Tese apresentada à Faculdade de Ciências Agrárias e Veterinárias – Unesp, Câmpus de Jaboticabal, como parte das exigências para a obtenção do título de Doutor em Zootecnia

A447g Almeida, Márco Túlio Costa
Glicerina bruta na adaptação e desempenho de ovinos confinados / Márco Túlio Costa Almeida. -- Jaboticabal, 2018
x, 95 p. : il. ; 29 cm

Tese (doutorado) - Universidade Estadual Paulista, Faculdade de Ciências Agrárias e Veterinárias, 2018

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Bibliografia

1. Cordeiros. 2. Desempenho. 3. Glicerina bruta. 4. Metabolismo. 5. Papilas ruminais. 6. Ovinos. I. Título. II. Jaboticabal-Faculdade de Ciências Agrárias e Veterinárias.

CDU 636.03:636.3

Ficha catalográfica elaborada pela Seção Técnica de Aquisição e Tratamento da Informação –
Diretoria Técnica de Biblioteca e Documentação - UNESP, Câmpus de Jaboticabal.



UNIVERSIDADE ESTADUAL PAULISTA

Câmpus de Jaboticabal



CERTIFICADO DE APROVAÇÃO


TÍTULO DA TESE: GLICERINA BRUTA NA ADAPTAÇÃO E DESEMPENHO DE OVINOS CONFINADOS

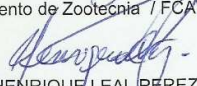
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AGRADECIMENTOS

Primeiramente a **Deus**, que em toda minha caminhada não me deixou um só momento desamparado, por não me deixar fraquejar, por sempre estar ao meu lado e por colocar no meu caminho pessoas amigas e generosas.

Aos meus pais **Júlio Cesar de Almeida** e **Cláudia Costa de Almeida** pelo amor, carinho, ensinamentos e compreensão ao longo das minhas conquistas e pelo exemplo de pessoa que são.

Às minhas irmãs **Juliana** e **Eryka** pelo amor, carinho e apoio.

Aos meus avós, **Vô Cláudio “Migué”** (*in memoriam*), **Vó Tereza**, **Vô Dito** (*in memoriam*) e **Vó Rosa** (*in memoriam*) pelo amor, carinho e incentivo aos estudos.

À toda minha família pelo amor e amizade.

À minha querida e adorável namorada **Josimari (Raxinha)** pela ajuda durante a condução do experimento e análises laboratoriais, pelo incentivo, carinho, companheirismo, apoio, ensinamentos, paciência, alegria e amor.

À **Universidade Estadual Paulista “Júlio de Mesquita Filho”, Faculdade de Medicina e Veterinária e Zootecnia**, pela minha formação profissional.

Ao **Programa de Pós-graduação em Zootecnia** da Faculdade de Ciências Agrárias e Veterinária da UNESP, Câmpus de Jaboticabal pela oportunidade de aprimoramento profissional.

À Professora **Jane Maria Bertocco Ezequiel**, pela orientação, pela confiança, pelos ensinamentos, e principalmente pela amizade e carinho a mim dedicados.

A todos **professores da UNESP** Câmpus Botucatu e Câmpus Jaboticabal, pelos ensinamentos.

Aos **Doutores** Mauro Dal Secco de Oliveira, Henrique Leal Perez, André Pastori D’Aurea e Robson Sfaciotti Barducci por aceitarem participar da banca examinadora.

À **Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP)** pela concessão das bolsas de estudos, Processo nº 2015/04595-4 e Processo BEPE nº 2016/07572-8.

À empresa **Caramuru Alimentos Ltda** pelo fornecimento da casca de soja e glicerina bruta.

A todos os amigos e companheiros da **Unidade Animal de Estudos Digestivos e Metabólicos** pela amizade, ajuda, incentivos e alegrias.

Aos prestativos **estagiários** que passaram pela Unidade Animal e pelo Laboratório de Ingredientes e Gases Poluentes (LIGAP).

Aos amigos da República **Mata Bixera**, que me acolheram e deixaram fazer parte desta família, e pelos bons momentos de descontração vividos juntos.

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

Muito Obrigado!

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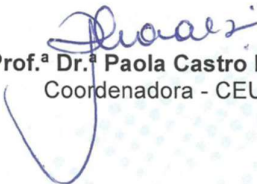


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CERTIFICADO

Certificamos que o Protocolo nº 06329/14 do trabalho de pesquisa intitulado “**Cinética do glicerol em ovinos**”, sob a responsabilidade da Prof.^a Dr.^a Jane Maria Bertocco Ezequiel está de acordo com os Princípios Éticos na Experimentação Animal adotado pelo Conselho Nacional de Controle de Experimentação Animal (CONCEA) e foi aprovado "Ad-referendum" pela COMISSÃO DE ÉTICA NO USO DE ANIMAIS (CEUA), em 10 de abril de 2014.

Jaboticabal, 10 de abril de 2014.



Prof.ª Dr.ª Paola Castro Moraes
Coordenadora - CEUA

GLICERINA BRUTA NA ADAPTAÇÃO E DESEMPENHO DE OVINOS CONFINADOS

RESUMO - A glicerina bruta tem sido utilizada como ingrediente energético na alimentação dos ruminantes, e devido a sua baixa capacidade de acidificação do ambiente ruminal, a partir de produtos da sua fermentação, pode ser utilizada para evitar distúrbios metabólicos decorrentes de sistemas de confinamento com alto concentrado. Neste sentido, experimentos foram conduzidos com o objetivo de avaliar a inclusão crescente de glicerina bruta (83% de glicerol) em substituição total ou parcial ao milho das dietas para ovinos em confinamento. No experimento 1, foram distribuídos aleatoriamente em baias individuais cinquenta e cinco cordeiros mestiços de 3 meses de idade (Santa Inês × Dorper, $21,7 \pm 2,7$ kg de peso corporal), dispostos em um delineamento de blocos ao acaso. Foram utilizadas quatro dietas experimentais contendo 0, 10, 20 ou 30% MS de glicerina bruta. Para as avaliações, três animais foram abatidos no final do período de pré-adaptação (d0), doze no final do período de adaptação (d14) e o restante ($n = 40$) quando atingiram ~ 35 kg PC. Foram avaliados o comportamento alimentar, o desempenho em confinamento, as características de carcaça e carne, os componentes comestíveis da não-carcaça e os compartimentos do estômago e fígado dos animais. Independentemente da inclusão de glicerina bruta, foram observadas diferenças significativas entre os períodos do confinamento para as variáveis peso inicial e final, GMPD e CMS. A inclusão de mais de 10% de glicerina bruta nas dietas aumentou os dias de confinamento e diminuiu o CMS e o GMPD dos animais. A inclusão também aumentou o número de mastigações e o tempo gasto mastigando por bolo de alimentação. Não houve efeitos da inclusão de glicerina bruta sobre o pH e a cor do músculo *Longissimus* a 45 min ou 24 h após o abate, bem como sobre as outras características de carcaça e componentes comestíveis não-carcaça. A inclusão de glicerina bruta reduziu a gordura perirrenal, porém sem efeito prejudicial em outros locais de deposição de gordura. Todos os compartimentos do estômago, número de papilas do rúmen e índice mitótico foram maiores para o período final do confinamento. Os tratamentos com glicerina bruta apresentaram maiores pesos de rúmen quando comparados ao tratamento controle no período final do confinamento. No segundo experimento, oito carneiros Santa Inês × Dorper ($64,5 \pm 8,5$ kg de PC) portadores de cânulas ruminais foram distribuídos em um quadrado latino duplo 4×4 . Foram avaliados os parâmetros ruminais dos animais, tais como o pH, concentrações de N-NH₃, AGCC e degradabilidade *in situ* das dietas, bem como a produção *in vitro* de gases de efeito estufa e digestibilidade *in vitro* das dietas. As dietas experimentais continham 0, 10, 20 ou 30% de glicerina bruta. A inclusão de glicerina bruta nas dietas tendeu a promover um efeito quadrático no CMS, com maiores valores observados para tratamentos com 10 e 20% de glicerina bruta. A glicerina bruta tendeu a aumentar o pH ruminal e o N-NH₃, mas reduziu linearmente a concentração molar total de AGCC, acético, butírico, isobutírico e isovalérico. A inclusão de glicerina bruta nas dietas diminuiu linearmente a produção total de gases e CO₂ (mL/g degradada) e tendeu a reduzir a produção de CH₄ (mL/g degradada). Um aumento linear da fração solúvel em água ("a") das dietas foi observado com a inclusão crescente de glicerina bruta. A fração insolúvel e potencialmente

degradável ("b") de MS e FDN das dietas foi linearmente reduzida e aumentada, respectivamente. A degradação potencial e efetiva das dietas foi linearmente aumentada com a crescente inclusão do coproduto. Os tratamentos aumentaram linearmente a digestibilidade *in vitro* da MS e reduziram linearmente a digestibilidade da FDN das dietas. Em conclusão, a inclusão crescente de até 30% de glicerina bruta em dietas para cordeiros mestiços não comprometeu a eficiência alimentar, os compartimentos do estômago e as medidas das papilas do rúmen em ambos os períodos do confinamento. No entanto, a inclusão de 10% de glicerina bruta parece ser a estratégia mais interessante para o período de acabamento, promovendo o maior desempenho animal. A substituição de grãos de milho por glicerina bruta (até 30% MS) altera os parâmetros de fermentação do rúmen, diminui a produção AGCC, e a produção *in vitro* de gás total e CH₄. Além disso, a degradação potencial e efetiva, bem como a digestibilidade *in vitro* das dietas foram melhoradas com a inclusão de glicerina bruta. Como nenhuma manifestação clínica resultante de acidose ruminal (como abscesso hepático, ruminite e lesões na mucosa ruminal) foi observada, concluímos que todas as dietas foram eficazes na adaptação dos animais.

Palavras-chave: cordeiros, desempenho, glicerina bruta, metabolismo, papilas ruminais, ovinos

ADAPTATION AND PERFORMANCE OF FEEDLOT LAMBS FED CRUDE GLYCERIN

ABSTRACT - Crude glycerin has been used as a source of energy in ruminant diets, however, due to low capacity ruminal acidifying from products of their fermentation, it can be used to prevent ruminal metabolic problems. Two trials were conducted to evaluate the effects of crude glycerin (83% of glycerol) in diets of feedlot as ingredient able to reduce nutritional metabolic disorders in total or partial replacement of corn. In experiment 1, fifty-five 3-month-old crossbred lambs (Santa Inês × Dorper, 21.7 ± 2.7 kg bodyweight) were randomly allocated in individual pens indoors, assigned to a complete randomized block design and fed with four experimental diets, containing 0, 10, 20 or 30% crude glycerin. Three animals were slaughtered at the end of the pre-adaptation period (d0), twelve at the end of the adaptation period (d14), and the remaining (n= 40) when they reached ~35 kg BW. The feed intake, feeding behaviour, growth performance, carcass and meat traits, edible non-carcass components, stomach compartments and liver were evaluated. Regardless of the inclusion of crude glycerin, significant differences among feedlot periods were observed for the initial and final BW, shrunk final BW, ADG and DMI. The inclusion of more than 10% of crude glycerin in the diets increased days on feed and decreased DM intake and average daily gain. Crude glycerin increased number of chews and the time spent chewing per feed bolus. There were no effects of crude glycerin on pH and colour of *Longissimus* muscle at 45 min or 24 h after slaughter, as well as on other carcass and edible non-carcass characteristics. The addition of crude glycerin reduced perirenal fat without detrimental effect on others fat deposition sites. All stomach compartments, number of rumen papillae and mitotic index were higher for the finishing period. Crude glycerin treatments showed greater rumen weights when compared to control treatment in the finishing period. In the second trial, eight ruminally-cannulated male Santa Inês × Dorper sheep (64.5 ± 8.5 kg bodyweight) were distributed in a replicated 4 × 4 Latin square design. The ruminal parameters, such as pH, NH₃-N and volatile fatty acids concentrations, *in situ* degradability, as well as *in vitro* greenhouse gas production and *in vitro* digestibility were evaluated. The experimental diets contained 0, 10, 20 or 30% of crude glycerin. The inclusion of crude glycerin in the diets tended to promote a quadratic effect in DMI, with greater values observed for treatments with 10 and 20% of crude glycerin. Crude glycerin tended to increase the ruminal pH and NH₃-N, but linearly reduced the total molar concentration of VFA, acetic, butyric, isobutyric and isovaleric acids. The inclusion of crude glycerin in the diets linearly decreased the *in vitro* total gas and CO₂ production (mL/g degraded) and tended to reduce CH₄ (mL/g degraded). A linear increase of soluble fraction in water (“a”) of the diets were observed with the increasing inclusion of crude glycerin. The insoluble but potentially degradable fraction (“b”) of DM and NDF of the diets were linearly decreased and increased, respectively. The potential and effective ruminal degradation of the diets were markedly and linearly increased with the increasing inclusion of the by-product. Treatments linearly increased *in vitro* DM digestibility of diets and linearly reduced NDF digestibility. In conclusion, the increasing inclusion of up to 30% of crude glycerin in diets for crossbred lambs did not compromise the feed efficiency, stomach compartments and rumen papillae measurements in both periods of the feedlot.

However, the inclusion of 10% of crude glycerin seems to be the most interesting strategy for the finishing period, promoting the greatest animal performance. The replacement of corn cracked grain by crude glycerin (up to 30% DM) changes rumen fermentation parameters, decreasing VFA production, *in vitro* total gas production and CH₄. Additionally, the potential and effective degradation as well as *in vitro* DM digestibility of diets are improved while fiber digestibility is impaired. As no clinical manifestations resulted from ruminal acidosis (such as liver abscess, ruminitis, and lesions in the ruminal mucosa) were observed, we concluded that all diets were effective in the animals' adaptation.

Keywords: glycerin, lambs, metabolism, performance, ruminal papillae, sheep

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CAPÍTULO 1 – Considerações gerais

1. Introdução

Os ruminantes possuem como característica adaptativa principal o aproveitamento de carboidratos (CHO) fibrosos das dietas. Os CHO quando ingeridos sofrem hidrólise por enzimas e são convertidos, por fermentação microbiana ruminal, em ácidos graxos de cadeia curta (AGCC). A absorção epitelial dos AGCC, quando eficiente, contribui em pelo menos 65 – 75% do fornecimento total de energia metabolizável (BERGMAN, 1990; KOZLOSKI, 2016).

Contrariando essa peculiaridade dos ruminantes, a fim de atender à crescente demanda do mercado da carne, práticas alimentares como a utilização de dietas altamente fermentáveis (baixo teor de fibra) em sistemas intensivos vêm sendo utilizadas para alcançar altos níveis de produção em menor escala de tempo. Essas dietas exigem menores custos operacionais, menor área para produção e armazenamento, além de serem mais eficientes biologicamente (BARIONI et al., 2017). Segundo Burgi (1996), na década de 90 nutricionistas utilizavam de 50 a 80% de ingredientes volumosos na matéria seca (MS) total das dietas, e atualmente, 81,8% dos técnicos recomendam e utilizam entre 71 a 90% de ingredientes concentrados, sendo em sua maioria os grãos de milho (OLIVEIRA; MILLEN, 2014).

Contudo, as dietas de alto concentrado possuem alto poder de fermentação no rúmen e tendem a aumentar a taxa de produção de AGCC, reduzindo o pH do meio. Este fato pode afetar negativamente a fermentação microbiana, a função do epitélio ruminal, e conseqüentemente, a saúde e produtividade do animal (GÄBEL; ASCHENBACH; MÜLLER, 2002; NOCEK, 1997). A redução no pH ruminal quando muito drástica resulta em um estado agudo de acidose no rúmen com sinais clínicos evidentes, tais como diarreia, timpanismo, abscessos hepáticos, laminite, ruminite e lesões na mucosa ruminal (NOCEK, 1997; OWENS et al., 1998). Os estados menos graves (acidose ruminal subaguda) são muitas vezes difíceis de detectar, porém são altamente prevalentes em sistemas de produção intensiva (KRAUSE; OETZEL, 2006) e têm conseqüências econômicas associadas à diminuição da ingestão de MS e menor desempenho dos animais (STONE, 2004).

A acidose ruminal é intensificada pela fermentação diferenciada dos CHO não estruturais via rota do acrilato ao invés da via do succinato, gerando em maior escala o ácido láctico, o qual possui maior poder de acidificação do meio quando comparado aos outros AGCC (KOZLOSKI, 2016; OWENS; BASALAN, 2016). Segundo Penner et al. (2009), o equilíbrio entre a produção e remoção de ácido pelo rúmen se dá por absorção, neutralização e passagem. Portanto, proporcionar tempo suficiente para a adaptação do epitélio ruminal e dos microrganismos à dieta tem sido recomendado como uma estratégia para reduzir o risco a acidose. De acordo com Schwartzkopf-Geinswein et al., (2003), são necessários de 10 a 14 dias para que o animal se recupere das mudanças metabólicas ocorridas após uma rápida e extensa fermentação ocasionadas por dietas de alto concentrado.

O período de adaptação à dieta é o momento de preparo do ambiente ruminal para o recebimento de grande quantidade de CHO altamente fermentáveis, sendo considerado um momento crítico, pois práticas realizadas neste período podem influenciar positiva ou negativamente o desempenho final dos animais, em que períodos longos podem aumentar o custo do ganho de peso, e períodos curtos podem não proporcionar adaptação necessária. Neste sentido, recomenda-se pelo menos um período de 14 dias de adaptação às dietas, utilizando o sistema de escadas com aumento gradual no teor de concentrado (PARRA, 2011; BARDUCCI et al., 2012; WATANABE et al., 2015).

A utilização de ingredientes que favoreçam a fermentação ruminal, a fim de alterar a fermentação pela rota do ácido láctico, evitando a acidose ruminal, também se torna uma prática interessante quando se trata de pecuária intensiva. A glicerina bruta é um ingrediente promissor, e sua inclusão em substituição a ingredientes energéticos convencionais, como o milho grão, poderia promover benefícios a adaptação dos animais e à redução da acidose em confinamento, principalmente por ser rapidamente absorvida pelas papilas ruminais e/ou ser fermentada a ácido propiônico e butírico por via alternativa (via do succinato), não gerando o ácido láctico, podendo assim beneficiar o desenvolvimento do rúmen melhorando a eficiência alimentar dos animais (KREHBIEL, 2008; ABUGHAZALEH; ABO EL-NOR; IBRAHIM, 2011; OMAZIC et al., 2015).

No entanto, a inclusão adequada de glicerina bruta no período de adaptação e a sua conseqüente continuação no período de terminação do confinamento ainda são desconhecidos. Neste sentido, experimentos de desempenho e metabolismo foram conduzidos com o objetivo de avaliar a inclusão crescente de glicerina bruta (até 30% MS) em dietas para ovinos em confinamento como ingrediente capaz de minimizar distúrbios metabólicos nutricionais em substituição total ou parcial ao milho das dietas.

2. Revisão de Literatura

2.1. Glicerina, coproduto da produção do biodiesel

A glicerina, também conhecida como glicerol ou 1, 2, 3 propanotriol, é o principal coproduto da produção do biodiesel. Aproximadamente 10% do volume da matéria prima (óleo ou gordura) adicionada inicialmente ao processo produtivo são convertidos em glicerina (DASARI et al., 2005).

A glicerina é um produto viscoso, resultante, entre outros, do processo de transformação de um triglicerídeo em ésteres de ácidos graxos (biodiesel) a partir de uma reação de transesterificação, na presença de um catalisador (ácido, básico ou enzimático) e de um álcool de cadeia curta, podendo ser metanol ou etanol (CORDEIRO et al., 2011).

A composição da glicerina pode variar conforme o tipo de processamento e em relação ao seu teor de glicerol e impurezas (metanol, sais, sabões e ácidos graxos). Segundo Hippen, Defrain e Linke (2008), a glicerina pode ser classificada em três diferentes graus de pureza: Baixa pureza - obtida logo após a separação do biodiesel, a qual contém baixos teores de glicerol (400 a 700 g/kg de glicerina) e elevados níveis de catalisadores, álcool, água, ácidos graxos e sabões; Média pureza - é a glicerina bruta após sofrer tratamento ácido, seguido de remoção dos ácidos graxos e sabões, possui normalmente 750 a 900 g de glicerol/kg de glicerina, sendo o restante formado por água, sais e metanol, e Alta pureza - após sofrer bi destilação a vácuo e tratamento com absorventes, contendo mais de 990g de glicerol/kg de glicerina.

Com os incentivos governamentais concedidos a cadeia do biodiesel, houve um enorme crescimento na produção deste biocombustível, o que, por sua vez, também aumentou a disponibilidade de seus coprodutos, tais como os farelos e principalmente a glicerina bruta (PELLEGRIN et al., 2012). Esse grande excedente muitas vezes ficava estocado nas indústrias ou era descartado de forma incorreta na natureza. Uma das alternativas para evitar essa destinação incorreta da glicerina é a sua utilização, na forma bruta (média pureza), na alimentação animal.

A glicerina bruta já foi estudada como ingrediente energético na alimentação de animais nos anos 1950 e 1960 (JOHNS, 1953; GARTON; LOUGH; VIOQUE, 1961). Contudo, devido ao aumento na disponibilidade e ao custo relativamente baixo, tornou-se novamente o foco dos estudos como fonte energética alternativa em dietas para ruminantes (DONKIN et al., 2009; EZEQUIEL et al., 2015; VAN CLEEF et al., 2015; FÁVARO et al., 2016; ALMEIDA et al., 2017).

2.2. Utilização da glicerina na alimentação animal

No Brasil, a utilização de glicerina bruta na nutrição animal foi aprovada pela Instrução Normativa N° 42 do Ministério da Agricultura, Pecuária e Abastecimento (MAPA), de 16 de dezembro de 2010, em que os parâmetros mínimos para uso são: teor mínimo de 800 g de glicerol/kg, menos de 150 ppm de metanol/kg e máximo de 120 g de umidade/kg.

A glicerina apresenta características energéticas semelhantes ao grão de milho e a comumente utilizada na alimentação animal é a de média pureza, apresentando em média 810 g de glicerol, 45 g de cinzas, 0,3 g de metanol, 0,1 g de proteína bruta, 3 g de extrato etéreo, 120 g de umidade, 10 g de sódio, 80 mg de potássio, 35 mg de cálcio, 16 mg de magnésio, 240 mg de fósforo e 3656 cal de energia bruta por kg de produto (TECPAR, 2010; FUNDAÇÃO-ABC, 2010; LANA, 2010).

Durante a última década grandes avanços foram alcançados com a utilização de glicerina bruta. Seu uso já é comprovado para animais não ruminantes (SIMON; BERGNER; SCHWABE, 1996; CERRATE et al., 2006; GROESBECK et al., 2008; LAMMERS et al., 2008) e ruminantes (CARVALHO et al., 2015; EZEQUIEL et al.,

2015; VAN CLEEF et al., 2015; FAVARO et al., 2016; PASCHOALOTO et al., 2016; SCARPINO-VAN CLEEF et al., 2016), com resultados promissores.

Especificamente em ovinos, estudos recentes demonstraram que inclusões de 15 e 20 % de glicerina bruta com altas concentrações de glicerol (mais de 780 g/kg de glicerina) podem ser utilizadas sem efeito prejudicial sobre a ingestão, desempenho e características de carcaça dos animais (GUNN et al., 2010a, GUNN et al., 2010b; GOMES et al. 2011; REGO et al., 2015). No entanto, quando a glicerina bruta apresentou baixo teor de glicerol (menos de 400 g/kg) e altos teores de impurezas (isto é, metanol, NaCl), mesmo quando em baixas inclusões (120 g/kg de DM) reduziu o desempenho dos animais em confinamento (LAGE et al. 2010), provavelmente devido ao baixo aproveitamento do glicerol e efeito tóxico do metanol. Neste sentido, para evitar o confundimento e interpretações equivocadas, se torna imprescindível o conhecimento da origem e composição nutricional da glicerina bruta a ser utilizada.

2.3. Fermentação ruminal e metabolismo da glicerina

O ingrediente glicerina, na alimentação de ruminantes, é classificado como uma fonte energética de grande assimilação pela microbiota ruminal e com ampla metabolização no fígado (ABO EL-NOR et al., 2010).

Segundo Krehbiel (2008), os possíveis destinos da glicerina em ruminantes são: passagem direta (13%), fermentação ruminal (44%) e absorção direta (43%). De acordo com Johns (1953), após duas horas de incubação ruminal menos de 80% da quantidade inicial da glicerina é encontrada no fluido ruminal, e após quatro horas cerca de 50% desaparece, e o desaparecimento total ocorre após 24 horas.

No rúmen, a glicerina é rapidamente utilizada pelos microrganismos para formação de AGCC, e em um prazo de 4 a 6 horas desaparece quase que totalmente (FERRARO et al., 2009; MACH et al., 2009; ABO EL-NOR et al., 2010, ABUGHAZALEH et al., 2011). Com a fermentação ruminal da glicerina bruta observa-se uma alteração na produção de AGCC em favor do ácido propiônico (DONKIN, 2008; VAN CLEEF et al., 2015). No entanto, também pode ocorrer aumento nas concentrações de ácido butírico e valérico (ABUGHAZALEH et al.,

2011; SAN VITO et al., 2016), e, quando em altas inclusões (45% MS), podem reduzir a concentração de acetato, pela redução nas populações das bactérias fermentadoras de CHO estruturais, como a *Butyrivibrio fibrisovens* e *Selenomonas ruminantium* (ABUGHAZALEH et al., 2011).

Segundo Valadares Filho e Pina (2006), o ácido propiônico formado pela fermentação microbiana da glicerina bruta pode ser absorvido pelo epitélio ruminal de forma passiva, ou seja, sem gasto de energia e ser transportado via porta para o fígado, sendo a rota metabólica usual o ciclo de Krebs, onde o Succinil-CoA, após reações bioquímicas origina o oxaloacetato que pode ser utilizado para formar glicose pela via gliconeogênica.

A absorção direta do glicerol, que escapa do metabolismo microbiano, através do epitélio do rúmen acontece de forma passiva e precisa passar pelas proteínas integrais de membrana. Essas proteínas são classificadas em dois grandes subgrupos funcionais: aquaporinas (altamente específicas para conduzir água) e aquagliceroporinas (especializadas no transporte de glicerol, FROGER et al., 2001). As aquagliceroporinas AQP3, AQP7 e AQP9 são descritas como uma classe de canais de água permeáveis ao glicerol. A AQP3 é encontrada nas células epidermais, nos olhos, rins, estômago, baço e eritrócito (FROGER et al., 2001; MACDOUGALD; BURANT, 2005) e age como um canal de glicerol, mantendo a hidratação, a elasticidade e funcionamento dos órgãos. A AQP7 é encontrada em abundância no tecido adiposo e atua como um canal de glicerol para os adipócitos (HIBUSE et al., 2005). A AQP9 é específica do fígado e funciona como porta de entrada no hepatócito, regulando a entrada do glicerol na célula para ser utilizada como substrato gliconeogênico durante o jejum (FROGER et al., 2001).

O glicerol então absorvido é transportado via porta para ser metabolizado no fígado, onde com ação da enzima glicerol-quinase ocorre fosforilação do glicerol + ATP a glicerol-3-fosfato + ADP ao nível de triose fosfato (LEHNINGER, 2006), sendo então destinados a formação de triacilgliceróis, fosfolipídios ou glicose, em conjunto com ácidos graxos livres (NELSON; LEHNINGER; COX, 2008; MOTTA, 2009), podendo entrar na via da gliconeogênese, ou ser oxidado para a produção de energia via glicólise (BRISSEON et al., 2001). A fosforilação do glicerol é um passo inicial na síntese de glicose, triglicerídeos ou oxidação completa a CO₂. A glicerol-

quinase é encontrada no fígado e nos rins, mas também no cérebro, adipócitos e músculos esquelético e cardíaco (RAHIB et al., 2009). Entretanto, a remoção hepática do glicerol presente na veia porta pode ser baixa. Segundo Krehbiel (2008), há relatos de aumento na concentração plasmática de glicerol em resposta a infusão do composto no rúmen sem ser observado aumento simultâneo na concentração de glicose plasmática.

A maioria do glicerol no organismo é encontrada na forma de triglicerídeos no tecido adiposo e é liberado para a corrente sanguínea por lipases durante a lipólise, e é normalmente assumido como sendo um substrato gliconeogênico em animais ruminantes. Krehbiel (2008) e Lage et al. (2010), relataram que a melhora na conversão alimentar obtida com a inclusão de glicerina na dieta, provavelmente é devida a melhora no status metabólico dos animais, proporcionado pelo maior aporte energético de origem gliconeogênica suprido pelo glicerol absorvido no rúmen ou intestino grosso, ou mesmo pela fermentação ruminal do glicerol a propionato.

2.4. Fermentação ruminal a ácido propiônico

O ácido propiônico é o ácido graxo volátil de cadeia curta que mais contribui para a síntese de glicose no ruminante, sendo de fundamental importância, servindo como fonte de energia para o animal (SWENSON; REECE, 1996).

O principal precursor de ácido propiônico no rúmen são os CHO não estruturais, em especial o amido. Após ingerido, os produtos da degradação dos CHO (polissacarídeos, oligossacarídeos, dissacarídeos ou monossacarídeos) são absorvidos pelas bactérias ruminais e utilizados para a produção de proteína microbiana e/ou AGCC (KOZLOSKI, 2016). Muitas espécies de bactérias são fermentadoras de amido e são classificadas como amilolíticas, sendo as principais espécies as *Bacteroides amylophilus*, *Butyrivibrio fibrisolvens*, *Bacteroides ruminicola*, *Selenomona lactylitica*, *Streptococcus bovis*, *Prevotella ruminicola*, *Eubacterium ruminantium*, *Ruminobacter amylophilus*, *Ruminococcus bromii*, *Succinimonas amylolytica* e *Lactobacillus sp.* (CHURCH, 1979; KOTARSKI et al., 1992).

O piruvato é o intermediário comum do catabolismo dos CHO pelas bactérias ruminais. A partir do piruvato, no entanto, várias rotas diferentes podem ser utilizadas até a formação de AGCC. A ramificação das rotas metabólicas no processo fermentativo ruminal permite maior flexibilidade e maior capacidade de adaptação das bactérias às variações do ambiente ruminal.

A fermentação do amido pode ser realizada por duas rotas, pela via do acrilato ou succinato. O que vai influenciar a rota é o próprio ambiente ruminal, sendo o pH o grande responsável. Geralmente, a fermentação se dá pela via do acrilato, onde 5% dos ácidos produzidos são ácido lático. Esta via é utilizada por algumas bactérias por não produzir ATP e também por algumas bactérias não conseguirem descarboxilar o succinato, reduzindo assim o lactato (VAN SOEST, 1994, KOZLOSKI, 2016).

O alto consumo de amido pelo animal pode gerar acúmulo de ácido propiônico no rúmen, gerando quedas bruscas no pH ruminal pelo excesso de fermentação. Com essa redução, há também o acúmulo de glicose no líquido ruminal. A presença de glicose e o baixo pH podem favorecer o crescimento de *Streptococcus bovis* e *Lactobacillus sp.*, os quais promovem a conversão de glicose/piruvato em ácido lático, o que afeta negativamente o pH ruminal. O ácido lático pode ser absorvido e convertido à glicose no fígado. Porém, a sua absorção é mais lenta que a dos outros AGCC, sendo então acumulado no rúmen, e como o ácido lático tem maior constante de dissociação, cerca de 10 vezes mais que os AGCC, ele é considerado um ácido mais forte e tem maior influência negativa sobre o pH ruminal, gerando assim um quadro de acidose láctica ruminal (KOZLOSKI, 2016).

A taxa de absorção dos ácidos depende de alguns fatores, como a sua concentração no rúmen, poder tamponante (pKa) e o tamanho da molécula (HOOVER; MILLER, 1991). A absorção de ácidos ionizados é benéfica à manutenção do pH ruminal, pois libera para o meio bicarbonato, contudo, quando o pH ruminal encontra-se em declínio e se aproxima do pKa dos ácidos, ocorre a maior absorção dos ácidos na forma não ionizada, reduzindo o pH ruminal devido ao não tamponamento do conteúdo ruminal (BERGMAN, 1990; ENEMARK; JORGENSEN; ENEMARK, 2002)

2.5. Fermentação ruminal *versus* adaptação

Na maioria das vezes, todos os animais que chegam ao confinamento são oriundos de pastagens e muitas vezes nunca receberam alimentos concentrados. Esses animais passam por várias mudanças fisiológicas à medida que são ambientados ao sistema, necessitando assim de um período para se adaptar ao manejo de trato, estrutura social na baia e adaptação aos novos alimentos.

O período de adaptação à nova dieta é o momento de preparo do ambiente ruminal para o recebimento de grande quantidade de CHO não estruturais, como o amido por exemplo. Este período é considerado crítico e merece atenção especial, pois as práticas realizadas neste período podem influenciar positiva ou negativamente o desempenho final dos animais, em que períodos longos podem aumentar o custo do ganho de peso, e períodos curtos podem não proporcionar adaptação necessária. Neste sentido, recomenda-se pelo menos um período de 14 dias de adaptação às dietas, utilizando o sistema de escadas com aumento gradual no teor de concentrado (PARRA, 2011; BARDUCCI et al., 2012; WATANABE et al., 2015). De acordo com Schwartzkopf-Geinswein et al., (2003), são necessários de 10 a 14 dias para que o animal se recupere das mudanças metabólicas ocorridas após uma rápida e extensa fermentação ocasionada por dietas de alto concentrado.

As dietas de confinamento muitas vezes são altamente fermentáveis no rúmen e tendem a aumentar a taxa de produção de AGCC, fato este que pode reduzir drasticamente o pH ruminal. Neste sentido, realizar adaptação adequada é imprescindível para minimizar o risco de acidose metabólica (PENNER et al., 2007), a qual pode causar lesões no epitélio ruminal diminuindo sua motilidade e capacidade absorptiva (DILorenzo et al., 2008), bem como os distúrbios associados a ela, como os abscessos hepáticos (NAGARAJA e LECHTENBERG, 2007) e laminite (NOCEK, 1997).

O perfil de AGCC produzido durante a fermentação altera a morfologia do epitélio ruminal e pode gerar papilas metabolicamente ativas ou inativas (GÁLFI, GABEL e MARTENS, 1993; PAULINO et al., 2010). O epitélio ruminal exerce funções de absorção, metabolismo e proteção (SAKATA; TAMATE, 1979; GÁLFI;

NEOGRADI; SAKATA, 1991), e as papilas têm papel importante na absorção e remoção dos AGCC do rúmen, e são projeções da mucosa para o lúmen do rúmen podendo ter diferentes tamanhos e formatos (HENRIKSON, 1970). As papilas são responsáveis pelo aumento da área absorptiva e tem seu desenvolvimento estimulado pelos AGCC (BANKS, 1991). Segundo Costa et al. (2008), o ácido butírico, propiônico e láctico são os principais responsáveis pela proliferação celular da camada basal do epitélio ruminal, e o propiônico responsável pelo crescimento de papilas metabolicamente ativas.

De acordo com Jones, Hunt e King (2000), com a proliferação celular do epitélio ruminal há aumento na velocidade de absorção dos AGCC e assim maior estabilização do pH ruminal. Porém, quando a proliferação for proporcionalmente maior que a descamação, haverá aumento no número de camadas de células no epitélio, gerando hiperplasia das células. A paraqueratose ruminal é prevalente em sistemas de confinamento com alto concentrado, e é resultante da indução da alta taxa de proliferação e migração celular sem tempo suficiente para completa diferenciação celular (TAMATE e KIKUCHI, 1978; GOODLAD, 1981). Quando a hiperplasia do epitélio é caracterizada por aumento na espessura da camada córnea ocorre a hiperqueratose ruminal, também devido a elevada capacidade de absorção de AGCC no epitélio (JENSEN et al., 1954).

2.6. Problemas metabólicos gerados por dietas de alto concentrado

A acidose é um problema comum em sistemas intensivos de produção animal. De acordo com Oliveira e Millen (2014), a acidose é um dos principais problemas de saúde encontrado nos confinamentos do Brasil (34,4% dos problemas), ficando atrás somente dos problemas respiratórios (40,6%). Segundo Stock, Klopfenstein e Shain (1983), animais que recebem dietas com alto concentrado são expostos à acidose ruminal pelo menos uma vez durante o confinamento, isto porque estas dietas fornecem substratos para todas as bactérias ruminais, inclusive para as oportunistas como a *Streptococcus bovis* e *Lactobacillus* sp. (RUSSELL e HINO 1985). Estas bactérias produzem ácido láctico, que é um ácido forte (pKa: 3,86) com alto poder de acidificação ruminal, sendo o principal

responsável pela acidose ruminal em confinamentos, enquanto que o propionato e butirato são considerados ácidos fracos pois possuem pKa mais alto, 4,87 e 4,82, respectivamente (DUNLOP, 1972).

Quando o pH ruminal encontra-se abaixo de 5,0 muitas bactérias têm seu crescimento reduzido, e apenas as bactérias das espécies *S. bovis* e *Lactobacillus* sp. continuam seu crescimento com a produção de ácido láctico. Estas bactérias produzem D e L- lactato, que são absorvidos pela parede do rúmen e chegam à corrente sanguínea. O L- lactato é metabolizado mais rapidamente que o isômero D, sendo o acúmulo deste último o responsável pela acidose metabólica (BOLTON; PASS, 1988).

A redução do pH ruminal também gera danos a mucosa ruminal, pois favorecem a entrada de microrganismos bacterianos patogênicos. Estes microrganismos invadem a parede do rúmen e iniciam sua colonização, gerando o problema conhecido como rumenite. A rumenite é uma lesão na parede ruminal devido às inflamações no epitélio (NAGARAJA; CHENGAPPA, 1998). As rumenites geram descamações e danos às papilas, redução na taxa de absorção, liberam endotoxinas e histamina para o meio ruminal (MULLENAX; KEELER; ALLISON, 1966; AHRENS, 1967), além de serem passagem para as bactérias oportunistas, como a *Fusobacterium necrophorum*, que podem chegar ao fígado, se colonizar e gerar abscessos (NAGARAJA e LECHTENBERG, 2007), comprometendo a função e condenação do fígado nos frigoríficos.

As condenações de fígado apresentam perdas econômicas diretas para a indústria frigorífica e indiretas para o produtor, pois os animais com os órgãos comprometidos não têm o mesmo desempenho produtivo dos sadios (SOUZA et al., 2007; KALE et al., 2011, SOUZA et al., 2017). De acordo com Vechiato et al., 2011, os abscessos hepáticos podem reduzir aproximadamente 11% do potencial produtivo do animal, pois causam diminuição no metabolismo de nutrientes. No Brasil, a frequência de condenações hepáticas está em torno de 12% (SOUZA et al., 2007, SILVA et al., 2013, SOUZA et al., 2017), porém dependendo da região pode chegar em até 40% (VIEIRA et al., 2011).

Os abscessos hepáticos são inflamações purulentas delimitadas em cápsulas de tecido conjuntivo e podem ocorrer em todas as faixas etárias, sexo ou raça.

Porém, são comumente encontrados em animais de pecuária intensiva, principalmente nos alimentados com dietas de alto concentrado. Este fato é devido às práticas errôneas de manejo alimentar, em que não se respeita o período de adaptação do animal à dieta, deixando-o vulnerável à acidose ruminal (NAGARAJA; CHENGAPPA, 1998).

Algumas estratégias nutricionais podem ser utilizadas para prevenir ou controlar estes efeitos adversos relacionados às dietas de alto concentrado, dentre elas podemos citar o uso de ingredientes que proporcionam a modulação no consumo do animal. Essa modulação pode vir de ingredientes que elevam a produção de propionato no rúmen, devido ao efeito hipofágico gerado por este ácido, ou seja, pela sensação de saciedade (ALLEN, BRADFORD e OBA, 2009).

Recentes trabalhos têm demonstrado que a glicerina tem um padrão de fermentação ruminal diferenciado, pois além de ser rapidamente absorvida pelas papilas ruminais pode ser fermentada a ácido propiônico e butírico por via alternativa (via do succinato), não gerando o ácido lático (VAN CLEEF et al., 2015, TRABUE et al., 2007, WANG et al., 2009). Por evitar fermentações indevidas e ter baixo poder acidificante, a glicerina bruta poderia favorecer o desenvolvimento papilar dando maiores condições para absorção de AGCC. A glicerina bruta é um ingrediente promissor, e sua inclusão em substituição a ingredientes energéticos convencionais, como o milho grão, poderia promover benefícios à redução da acidose e demais problemas associados à queda brusca no pH ruminal em confinamento, fato que melhoraria a eficiência alimentar dos animais.

2.7. Objetivos

O trabalho teve como objetivo geral avaliar diferentes níveis de inclusão de glicerina bruta, em substituição total ou parcial ao milho das dietas, no período de adaptação e terminação de cordeiros em confinamento, como ingrediente redutor de distúrbios metabólicos nutricionais e melhorador da eficiência alimentar.

O objetivo do primeiro estudo foi avaliar os efeitos da inclusão crescente de glicerina bruta (até 30% MS) em dietas para cordeiros mestiços (Santa Inês × Dorper) confinados sobre o comportamento alimentar, desempenho em

confinamento, características de carcaça e carne, componentes comestíveis da não-carcaça, morfometria das papilas ruminais e os compartimentos do estômago e fígado dos animais.

O segundo estudo teve como objetivo avaliar os efeitos da inclusão crescente de glicerina bruta (até 30% MS) em dietas para ovinos mestiços (Santa Inês × Dorper) confinados sobre os parâmetros ruminais dos animais, tais como o pH, concentrações de N-NH₃, AGCC e degradabilidade *in situ* das dietas, bem como a produção *in vitro* de gases de efeito estufa e digestibilidade *in vitro* das dietas.

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CAPÍTULO 2

O capítulo a seguir está redigido em formato de artigo científico conforme normas da revista “**Animal Production Science**”, exceto a posição das tabelas. Publicado em 22 de fevereiro de 2017 sob identificação DOI: doi.org/10.1071/AN16628.

CAPÍTULO 2 – Effects of high concentrations of crude glycerin in diets for feedlot lambs: feeding behaviour, growth performance, carcass and non-carcass traits

ABSTRACT - The effects of high concentrations of crude glycerin were investigated in diets for feedlot lambs. Forty crossbred (Santa Ines × Dorper) uncastrated male lambs (21.7 ± 2.7 kg bodyweight) were assigned to a complete randomised block and subjected to four experimental diets containing 0, 100, 200, or 300 g crude glycerin/kg DM. Animals were weighed at 14-day intervals and were harvested when they reached ~35 kg bodyweight. The feed intake, feeding behaviour, growth performance, carcass and meat traits, and edible non-carcass components were evaluated. The inclusion of more than 100 g/kg crude glycerin in the diets increased days on feed and decreased DM intake and average daily gain. Crude glycerin increased number of chews and the time spent chewing per feed bolus. There were no effects of crude glycerin on pH and colour of *Longissimus* muscle at 45 min or 24 h after slaughter, as well as on other carcass and edible non-carcass characteristics. The addition of crude glycerin reduced perirenal fat without detrimental effect on others fat deposition sites. In conclusion, the addition of up to 100 g/kg DM in diets for crossbred finishing lambs seems to be the most interesting strategy, as it promotes greatest animal performance. However, the inclusion of up to 300 g/kg DM of the by-product could be possible depending on glycerin market price and the structure of feedlot operation, with favourable economic results despite high inclusions reflect in greater number of days on feed.

Additional keywords: by-product, glycerol, sheep.

Introduction

The increasing worldwide concern over global warming and the imminent depletion of petroleum reserves have led researchers to search for alternatives to fossil fuels. In this scenario, biodiesel appears as the main substitute for this energetic matrix, which in addition to being a renewable energy source, has lower power pollution (Lima *et al.* 2014). The government incentives given to biodiesel agribusiness has led to a huge growth in the production of this biofuel, which in turn also increases the availability of its by-products such as cakes, meals, and especially crude glycerin.

Approximately 10% of the total volume of biodiesel produced becomes crude glycerin (Dasari *et al.* 2005; Johnson and Taconi 2007). This by-product has been studied as an animal feed ingredient since the 1950s and 1960s (Johns 1953; Garton *et al.* 1961). Due to the increased availability and relatively low cost, it became again the focus of studies as an alternative energy source in diets for ruminants (Donkin *et al.* 2009; Ezequiel *et al.* 2015; Van Cleef *et al.* 2015; Fávoro *et al.* 2016) with promising results.

During the last decade, great advancements have been achieved with crude glycerin utilisation. Recent studies with ovine species has demonstrated that high concentrations of this by-product (more than 780 g/kg of glycerol) can be used without detrimental effect on feed intake, performance and carcass traits (Gunn *et al.* 2010a, 2010b; Gomes *et al.* 2011; Rego *et al.* 2015). However, when crude glycerin has low content of glycerol (less than 400 g/kg) and high impurity contents (i.e. methanol, NaCl), even fed at low levels (120 g/kg DM) can decrease feedlot performance (Lage *et al.* 2010). Nevertheless, until now there has been no data reported on the replacement of corn grain with high concentrations of crude glycerin for feedlot lambs as well as its effects on animal performance. We hypothesised that crude glycerin could provide an alternative to totally replace corn grain, without affecting animal health or performance.

Thus, the objective of this study was to evaluate the effects of high concentrations of crude glycerin (up to 300 g/kg DM basis) in diets for crossbred feedlot lambs, on feeding behaviour, feedlot performance and carcass and non-carcass traits.

Materials and methods

The experiment was conducted at the Laboratory of Ingredients and Greenhouse Gases of the Department of Animal Science from São Paulo State University (FCAV/Unesp), Jaboticabal, Brazil. The experimental procedures were approved by the Animal Welfare and Ethics Commission from São Paulo State University (Protocol 06329/14).

Animals, diets and experimental design

Forty crossbred (Santa Ines × Dorper) uncastrated male lambs (21.7 ± 2.7 kg bodyweight (BW) and ~90 days old) were sorted by initial weight and assigned to a complete randomised block design and subjected to four experimental diets containing 0, 100, 200, or 300 g crude glycerin/kg DM (Table 1). The animals were housed in individual pens (1.2 m²) indoors, with individual feed bunks and collective waterers. Upon arrival, animals received exclusively corn silage for 7 days, and then they were submitted to a 14-day adaptation period, with three step-up diets containing increasing levels of concentrate (20%, 40% and 60%).

The experimental diets were formulated to be isonitrogenous (177 g/kg crude protein (CP) DM) and isocaloric (11,3 MJ/kg DM) to supply the minimum requirements of a 20–30-kg lamb with moderate growth for daily gain of 250 g, according to National Research Council (2007). The dietary treatments were labelled as: G0 (Control treatment, containing no crude glycerin), G10 (containing 100 g crude glycerin/kg of ration DM), G20 (containing 200 g crude glycerin/kg of ration DM) and G30 (containing 300 g crude glycerin/kg of ration DM). The crude glycerin replaced totally the corn grain in the G30 treatment. The crude glycerin was obtained from a commercial soybean oil and meal production plant, and contained 830.0 glycerol, 950.0 DM, 11.0 CP, 60.0 salts, and less than 0.1 methanol g/kg.

Table 1. Composition of experimental diets

Control, control diet; G10, inclusion of 100 g crude glycerin/kg DM; G20, inclusion of 200 g crude glycerin/kg DM; G30, inclusion of 300 g crude glycerin/kg DM

Item	Treatments			
	Control	G10	G20	G30
<i>Ingredients (g/kg)</i>				
Corn silage	400	400	400	400
Corn cracked grain	300	200	100	00
Soybean hulls	78	72	63	45
Soybean meal	206	210	216	231
Urea	06	09	11	13
Crude glycerin	00	100	200	300
Mineral-vitamin premix ^A	05	05	05	05
Limestone	05	05	05	05
Dicalcium phosphate	00	00	00	02
<i>Calculated chemical composition</i>				
DM (g/kg)	658	661	664	666
CP (g/kg DM)	177	177	177	177
ME (MJ/kg DM) ^B	11.7	11.7	11.3	11.3
Ether extract (g/kg DM)	30	27	23	20
aNDF (g/kg DM) ^C	348	330	311	287
ADF (g/kg DM)	192	185	177	165
Ca (g/kg DM)	05	05	05	05
P (g/kg DM)	03	03	03	03

^AComposition per kg: P (75 g), Ca (223 g), S (10 g), Zn (3 g), Na (60 g), Co (20 mg), I (40 mg), Se (24 mg), F (750 mg), Mg (5 g), Mn (1,8 g), Fe (402 mg), Vit A (312.500 UI), Vit D (50.000 UI), Vit E (437 UI).

^BMetabolisable energy (MJ/kg DM) was calculated from NRC, 2007 model.

^CNDF assayed with a heat stable amylase and expressed inclusive of residual ash.

Feed intake and growth performance

The concentrate and corn silage were weighed separately daily and mixed with crude glycerin at the moment of feed delivery (0800 hours and 1600 hours), feeding animals 50% of total mixed ration in each meal. Before morning feeding, 10% of orts of each animal were sampled in order to monitor DM intake. The feed

efficiency was calculated as the ratio between average daily gain (ADG, kg) and daily DM intake (DMI, kg). To evaluate growth performance, animals were weighed upon arrival, at the end of the adaptation period, and every 14 days until slaughter.

Chemical analyses

Samples of feed and orts were composited and at the end of the experiment, dried in a forced-air oven at 55°C for 72 h, and ground using a sieve with mesh size of 1 mm (AOAC 1998; method 934.01). The DM concentration was determined by drying the material in an oven at 105°C for 24 h (AOAC 1995; method 930.15), and ash content was obtained by sample incineration in a muffle furnace at 600°C for 3 h (AOAC 1990; method 942.05) to determine the organic matter. Nitrogen concentration was determined using the micro-Kjeldahl method (AOAC 1998; method 988.05), and CP content was estimated multiplying N content by 6.25. The ether extract (EE) content was determined by extraction with petroleum ether in a Soxhlet apparatus for 4 h (AOAC 1990; method 930.15). The acid detergent fibre (ADF) and neutral detergent fibre (NDF) contents were estimated according to recommendations of Van Soest and Wine (1967), using a heat-stable α -amylase, without sodium sulfite, and expressed inclusive of residual ash.

Slaughter, carcass evaluation, and sample collection

When the animals reached ~35 kg BW, they were submitted to a 16-h fasting period and bodyweights were recorded to determine the slaughter bodyweight. Animals were transported to São Paulo State University Goat Unit's experimental abattoir and were stunned by brain concussion, using a non-penetrating bolt pistol.

Bleeding was performed by severing the carotid arteries and jugular veins immediately after stunning. After evisceration, carcasses were weighed to determine the hot carcass weight and hot carcass yield, and then refrigerated at ~4°C for 24 h. After the post mortem chill period, the cold carcass weight and cold carcass yield were determined. A cross-section cut was made between the 12th and 13th ribs of left half carcass, exposing the *Longissimus* muscle to measure back fat thickness, which were taken at 3/4 of the length ventrally over the *Longissimus* muscle (Greiner *et al.* 2003). *Longissimus* muscle area was measured using the equation $(A/2*B/2)*\pi$,

according to Silva Sobrinho (1999), where 'A' is the maximum length of the muscle and 'B' is the maximum depth of the muscle (cm).

Total edible non-carcass components yield (TENCC) was calculated as the sum of weights of blood, tongue, lungs + trachea, liver + gall bladder, heart, kidneys, gastrointestinal tract (reticulum, rumen, omasum, abomasum and intestines), and abdominal and kidney fats. Total yield of usable products was calculated as the sum of hot carcass weight and TENCC.

Meat pH and colour

The initial pH was measured between the 12th and 13th ribs in the centre of the *Longissimus* muscle, ~45 min after slaughter, using a digital penetration pH meter model 205 (Testo Inc., Sparta, NJ, USA). After refrigeration of the carcasses (24 h, at 4°C), the ultimate pH was measured at the same site. Evaluation of the meat colour was performed as described by Houben *et al.* (2000), using a Minolta CR-400 colourimeter (Konica Minolta, Osaka, Japan), calibrated before the readings with standards white and black. The lightness (L*), redness (a*), and yellowness (b*) indexes were assessed using the CIE L* a* b* colour system (CIE International Commission on Illumination 2004). Thirty min before the readings, a cut was made on the muscle to expose the myoglobin to oxygen. Three readings were performed on each sample, and the averages were used in the statistical analysis.

Feeding behaviour

The feeding behaviour was evaluated using the methodology described by van Cleef *et al.* (2016) and Bürger *et al.* (2000). The animals were evaluated for three times during the feedlot period (beginning [Day 5], middle [Day 20] and end [Day 35]). The behaviour activities were recorded on intervals of 5 min during 12 h (0800 hours to 2000 hours) by six trained observers. The variables evaluated were: interaction with feed bunk (when animal positioned the head towards the feed bunk, without specifying whether ingested, smelled or played with the feed); interaction with water (when animal positioned the head towards the waterer, without specifying whether ingested, or played with the water); stand still (when the animal was with four feet in contact with the pen's floor without moving the body); stand ruminating; laid

(when the animal was in lateral recumbent position); laid ruminating; stereotypes (when the animal was chewing pen's wood, biting, repetitively licking or repetitively butting), and other activities. Time (expressed in minutes) spent in each activity was calculated by the number of observations recorded multiplied by 5.

The feeding behaviour efficiencies of DM and NDF were calculated as: DM intake divided by time spent eating, and NDF intake divided by time spent eating, respectively. Rumination efficiencies of DM and NDF were calculated as: DM intake divided by time spent ruminating, and NDF intake divided by time spent ruminating, respectively. Number of feed boli (when the feed was masticated and swallowed in the shape of a bolus) was obtained dividing time spent ruminating by the time spent ruminating one feed bolus. Time spent chewing each bolus was also recorded, as well as the number chews per bolus and total time chewing.

Statistical analyses

Data on performance, carcass and non-carcass traits and meat characteristics were analysed as a randomised complete block design with animals being blocked according to initial bodyweight. Four treatments (levels of crude glycerin) were used with 10 replications each. Data were analysed using the MIXED procedure of SAS, version 9.2. The treatments were considered as fixed effects and blocks as a random effect. The linear and quadratic effects of crude glycerin were tested, as well as the contrast Control treatment × crude glycerin treatments. Feeding behaviour data were analysed as repeated-measures using the same procedure of SAS, and day of observation and interaction diets × day were included in the model. The covariance structure with the best fit (smallest Akaike's value) was the unstructured. Treatment means were computed with the LSMEANS option and significance was defined as $P < 0.05$ and trends as $0.05 \leq P \leq 0.10$.

Results

Feed intake and growth performance

The increasing inclusion of crude glycerin in the diets linearly increased days on feed ($P = 0.02$, Table 2), with a tendency of quadratic effect ($P = 0.09$). A quadratic effect in DMI ($P = 0.04$), with greater intake for the animals fed treatment

G10 was observed. The by-product also decreased ADG of animals (Linear, $P = 0.003$, Quadratic, $P = 0.07$). When all treatments with crude glycerin were compared with Controls, there was a tendency of reduction in ADG ($P = 0.09$). There was also a tendency of reduction in feed efficiency (Linear, $P = 0.07$) with crude glycerin inclusion. Body conformation of animals fed G20 tended to be greater when compared with other treatments (Quadratic, $P = 0.06$).

Table 2. Effects of crude glycerin intake on performance of feedlot lambs

Control, control diet; G10, inclusion of 100 g crude glycerin/kg DM; G20, inclusion of 200 g crude glycerin/kg DM; G30, inclusion of 300 g crude glycerin/kg DM

Item	Treatments				s.e.m	P-value		
	Control	G10	G20	G30		Linear	Quad	0 ×
No. of lambs	10	10	10	10	–	–	–	–
Days on feed	42	41	44	52	2.00	0.02	0.09	0.11
	<i>BW (kg)</i>							
Initial	21.9	21.5	22.1	21.0	0.67	0.45	0.62	0.61
Final	35.0	35.1	34.9	34.7	0.29	0.32	0.67	0.65
Shrunk final	33.5	34.0	33.7	33.1	0.34	0.31	0.19	0.74
DMI (kg/day)	1.119	1.169	1.075	1.007	0.03	0.002	0.04	0.29
ADG (kg)	0.319	0.332	0.293	0.262	0.01	0.0003	0.07	0.09
G : F (kg/kg)	0.286	0.286	0.272	0.261	0.01	0.07	0.59	0.30
Body	3.4	3.5	3.6	3.1	0.14	0.11	0.06	0.75
Body condition score ^B	3.4	3.5	3.4	3.2	0.12	0.13	0.19	0.65

^AEffect of Control diet containing no crude glycerin vs all diets containing crude glycerin. ^BScoring range = 1 to 5.

Feeding behaviour

There was no interaction of the day of observation × treatment ($P > 0.05$). The increasing inclusion of crude glycerin promoted quadratic effect on DMI during the 12-h period of observation ($P = 0.009$, Table 3), with greater intake observed in animals fed treatment G10. There was a linear decrease in NDF intake (NDFI, $P < 0.0001$) during the observation period and when comparing treatments with crude glycerin with Controls, regarding the concentration of inclusion, the by-product

inclusion decreased NDFI ($P < 0.0001$). Crude glycerin also altered the number of feed boli and the time spent chewing each bolus (Quadratic, $P < 0.05$), with greater values observed in animals fed G10. There was tendency of reduction in rumination efficiency of NDF with crude glycerin inclusion in the diets (Linear, $P = 0.07$). The other variables evaluated were unaffected by experimental treatments.

Table 3. Effects of crude glycerin intake on feeding behaviour of feedlot lambs

Control, control diet; G10, inclusion of 100 g crude glycerin/kg DM; G20, inclusion of 200 g crude glycerin/kg DM; G30, inclusion of 300 g crude glycerin/kg DM

Item	Treatments				s.e.m	P-value		
	Control	G10	G20	G30		Linear	Quad	0 × G ^A
DMI 12 h (g) ^B	553.8	591.7	542.2	505.3	13.49	0.003	0.009	0.63
NDFI 12 h (g) ^C	185.9	184.9	159.6	139.7	4.11	<0.0001	0.13	<0.0001
	<i>Behaviour (min)</i>							
IF ^D	148.9	144.9	130.6	129.5	12.01	0.19	0.90	0.32
IWE ^E	9.4	8.4	7.6	13.5	2.62	0.33	0.20	0.88
RS ^F	3.2	3.2	10.3	5.0	2.31	0.23	0.26	0.27
RL ^G	167.6	156.6	172.9	154.0	13.52	0.69	0.77	0.68
Rtotal ^H	170.8	160.8	183.3	159.0	13.73	0.85	0.63	0.83
SS ^I	125.9	133.4	131.6	138.5	13.47	0.56	0.98	0.58
LD ^J	245.1	242.6	229.9	248.0	20.79	0.97	0.62	0.84
ES ^K	16.9	27.9	34.5	25.0	8.27	0.41	0.22	0.21
OA ^L	3.0	3.0	2.5	6.5	1.81	0.22	0.28	0.63
	<i>Chew activity</i>							
NCB ^M	75.8	87.2	83.4	71.5	3.71	0.32	0.004	0.27
TCB ^N	38.8	44.2	43.4	37.1	2.01	0.51	0.006	0.25
FE ^O	4.0	4.5	4.6	4.0	0.41	0.91	0.18	0.39
RE _{DM} ^P	3.5	3.8	3.42	3.3	0.36	0.62	0.54	0.89
RE _{aNDF} ^Q	1.2	1.2	1.0	0.9	0.11	0.07	0.58	0.34
TCT ^R	319.7	304.7	313.8	288.5	17.4	0.29	0.77	0.39
NB ^S	270.5	221.7	263.7	260.5	24.87	0.92	0.36	0.45

^AEffect of Control diet containing no crude glycerin versus all diets containing crude glycerin. ^BDry matter intake during 12-h observation period. ^CNeutral detergent fibre intake during 12-h observation period. ^DInteraction with feed bunk. ^EInteraction with waterer. ^FRuminating stand. ^GRuminating laid.

^HTotal time ruminating. ^IStanding still. ^JLaid. ^KEsteriotypes. ^LOther activities. ^MNumber of chews per feed bolus. ^NTime spent chewing each bolus (seconds). ^OFeeding efficiency (g MS/h). ^PRuminating efficiency of DM (g MS/h). ^QRuminating efficiency of NDF (g FDN/h). ^RTotal time chewing (min/12h). ^SNumber of feed boli (no./12h).

Carcass and meat traits

The increasing inclusion of crude glycerin in the diets did not influence carcass characteristics of feedlot lambs ($P > 0.05$, Table 4), except for the variable fat cover score, which tended to linearly decrease ($P = 0.06$).

Table 4. Effects of crude glycerin intake on carcass and meat traits of feedlot lambs

Item	Treatments				s.e.m	P-value		
	Control	G10	G20	G30		Linear	Quad	0×G ^A
Empty bodyweight (kg)	29.7	30.0	30.1	29.9	0.32	0.55	0.49	0.40
Hot carcass weight (kg)	16.4	16.7	16.4	16.4	0.20	0.94	0.41	0.49
Hot dressing percentage	48.9	49.2	48.8	48.8	0.65	0.49	0.58	0.70
Cold carcass weight (kg)	15.8	16.2	15.9	16.0	0.19	0.91	0.40	0.37
Cold dressing	47.3	47.7	47.2	48.3	0.57	0.34	0.55	0.52
Chilling loss (g/kg)	32.0	29.0	31.0	28.0	0.29	0.49	0.98	0.43
<i>Longissimus</i> muscle	14.2	15.2	14.5	14.8	0.46	0.54	0.42	0.20
12th-rib fat thickness	4.5	4.1	4.3	3.6	0.37	0.13	0.64	0.26
Conformation score ^B	4.0	4.0	3.9	3.8	0.11	0.22	0.69	0.57
Fat cover score ^B	4.1	4.0	4.1	3.8	0.08	0.06	0.30	0.24
pH ^{45 min}	6.51	6.52	6.47	6.45	0.04	0.24	0.73	0.55
pH ^{24 h}	5.61	5.68	5.65	5.63	0.05	0.88	0.40	0.45
		<i>Colour^{45 min}</i>						
Luminosity (L*)	28.2	28.6	28.1	28.2	0.50	0.85	0.82	0.88
Redness (a*)	13.8	14.9	14.2	13.5	0.54	0.28	0.18	0.73
Yellowness (b*)	2.4	2.6	2.4	2.4	0.14	0.90	0.68	0.73
		<i>Colour^{24 h}</i>						
Luminosity (L*)	32.3	33.1	34.6	32.0	0.92	0.89	0.19	0.41
Redness (a*)	13.5	13.7	13.4	13.4	0.67	0.89	0.91	0.98
Yellowness (b*)	4.6	4.6	4.1	4.0	0.43	0.29	0.85	0.54

^AEffect of Control diet containing no crude glycerin versus all diets containing crude glycerin. ^BScoring range = 1 to 5.

Edible non-carcass components and total usable products

Perirenal fat tended to present a quadratic effect of crude glycerin inclusion ($P = 0.06$, Table 5). When compared with animals fed Control, treatments containing crude glycerin, regardless of the concentration, promoted less perirenal fat ($P = 0.02$). None of the other edible non-carcass components or total usable products were affected by increasing concentrations of crude glycerin in the diets.

Table 5. Effects of crude glycerin intake on edible non-carcass components and total usable products of feedlot lambs

Control, control diet; G10, inclusion of 100 g crude glycerin/kg DM; G20, inclusion of 200 g crude glycerin/kg DM; G30, inclusion of 300 g crude glycerin/kg DM

Item	Treatments				s.e.m	<i>P</i> -value		
	Control	G10	G20	G30		Linear	Quad	0 × G
	<i>ENCC^B yield (kg)</i>							
Oesophagus	0.050	0.052	0.047	0.049	0.003	0.54	0.96	0.78
Tongue	0.078	0.078	0.074	0.059	0.006	0.15	0.33	0.30
Blood	1.454	1.483	1.443	1.373	0.047	0.19	0.31	0.70
Spleen	0.065	0.068	0.071	0.065	0.004	0.87	0.28	0.54
Liver	0.667	0.721	0.689	0.674	0.021	0.93	0.11	0.25
Heart	0.159	0.161	0.156	0.155	0.005	0.47	0.84	0.73
GI tract	2.364	2.201	2.401	2.617	0.134	0.12	0.18	0.77
Kidneys	0.112	0.118	0.128	0.108	0.009	0.95	0.14	0.56
Pancreas	0.049	0.050	0.036	0.036	0.007	0.12	0.95	0.34
Perirenal fat	0.419	0.328	0.339	0.354	0.027	0.14	0.06	0.02
Mesenteric fat	0.372	0.355	0.346	0.389	0.034	0.78	0.40	0.84
Omental fat	0.626	0.526	0.593	0.616	0.066	0.89	0.36	0.54
Testicles	0.320	0.297	0.330	0.324	0.021	0.63	0.69	0.91
TENCC ^C (kg)	6.736	6.436	6.662	6.819	0.160	0.51	0.17	0.61
TUP ^D (kg)	23.11	23.16	23.09	23.27	0.237	0.70	0.81	0.81

^AEffect of Control diet containing no crude glycerin versus all diets containing crude glycerin. ^BEdible non-carcass components. ^CTotal edible non-carcass components. ^DTotal usable products = TENCC + HCW (hot carcass weight).

Discussion

Feed intake and growth performance

The increase in days on feed of animals fed increasing concentrations of crude glycerin can be explained by the lesser DMI and, consequently, lesser ADG of animals fed the by-product (Table 2). Reductions in DMI with increasing inclusion of crude glycerin may be related to the digestion of fibre fractions of diets. Deleterious effects with the inclusion of crude glycerin have been reported, mainly by the reduced activity of cellulolytic bacteria (Roger *et al.* 1992; Paggi *et al.* 2004). Fact that changes ruminal fermentation, with consequently a reduction in the concentration of acetic acid (Shin *et al.* 2012; van Cleef *et al.* 2014, 2015). Thus, the low digestibility of the fibre leads to a lower rate of passage of feed through the rumen, thereby reducing the DMI.

Thus, the animals fed crude glycerin tended to be less efficient than animals fed the Control diet. Control treatment was 9% more efficient than treatment containing 300 g crude glycerin/kg DM. Similarly, Gunn *et al.* (2010a), working with sheep, and van Cleef *et al.* (2014), working with beef cattle, evaluated the inclusion of 200 and 300 g crude glycerin/kg DM, respectively, and did not observe alterations in feed efficiency. However, other researchers reported that inclusions of crude glycerin at up to 120 g/kg DM improved feed efficiency in finishing beef cattle (Pyatt *et al.* 2007; Parsons *et al.* 2009).

Nevertheless, despite the reductions observed in DMI and ADG of animals in the present study, the observed ADG was greater than that recommended by the NRC (2007), based on the DMI observed in this study. On average, for all treatments, the ADG was 0.302 kg/day. According to NRC (2007), for weight gains of 0.200 to 0.300 kg/day, animals with BW between 20 and 30 kg should consume an average of 1.0 kg DM/day, and in the present study the observed cumulative DMI was of 1.1 kg/day.

Feeding behaviour

The greatest DMI (12-h observation period), time and number of chews per feed bolus observed for the animal fed G10 (Table 3) could be explained by the good acceptability of the by-products by the animals. Because crude glycerin is highly palatable and has a sweet taste, it can increase the ingestion of feed by the animals (Farias *et al.* 2012). However, recent studies have shown decreases in DMI when animals are fed high concentrations of crude glycerin (Parsons *et al.* 2009; Ezequiel *et al.* 2015). In the present study, the greatest inclusions of the by-product (200 and 300 g/kg DM) led to lower DMI during the behaviour observation period, as well as number of feed boli ingested, and time to chew each feed bolus. As crude glycerin changed the physical form of diet, it became easier to be chewed. According to Krehbiel (2008) the high ingestion of glycerol can improve the animals' metabolic status due to the increased energy intake from glucose with increased propionate and reduced acetate : propionate ratio in the rumen, satiating the animal in terms of energy.

The linear reduction on intake of NDF during the 12-h observation period with the inclusion of increasing concentrations of crude glycerin is probably due to the decreasing concentrations of fibre in the diets (Table 1). As the crude glycerin was included in the diets, there was a substitution of ingredients that had more structural carbohydrates in its composition, a fact that reduced the final content of fibre in the diets, thus reducing the consumption of NDF.

Carcass and meat traits

The lack of differences in carcass characteristics (Table 4) can be explained by the uniformity of the animals in the beginning of feedlot period and the similar slaughter weight (average 33.6 kg). It shows that the inclusion of crude glycerin by itself does not affect any of those characteristics, despite the increase in days on feed observed with the increasing inclusion of the by-product (Table 2).

The homogeneity of the animals at the day of slaughter is also evidenced by the similar values of conformation and fat cover score, with average values of 3.9 and 4.0 respectively (scale of 1 to 5), showing good quality of the final product in terms of

carcass conformation and fat cover. Consequently, the inclusion of crude glycerin also did not affect dressing percentage of animals.

Despite the decreasing linear trend in the fat cover score, no differences were observed in 12th-rib fat thickness. Similar results were reported by Carvalho *et al.* (2015), which evaluated the inclusion of crude glycerin at up to 300 g/kg DM in diets for Santa Ines lambs and did not observe differences in 12th-rib fat thickness, but observed average values much lower than those found in the present study (2.2 vs 4.1 mm). High subcutaneous fat thickness values are considered important for preserving the quality of carcasses, because it serves as a protective cover avoiding the burning of the meat during freezing, as the sudden drop in temperature can cause shortening of muscle fibres in the early development of *rigor mortis*, affecting meat tenderness (Marsh *et al.* 1978).

The increasing inclusion of crude glycerin also did not affect the *Longissimus* muscle area, and the average value observed in the present study was 14.7 cm². Similar results were observed by Carvalho *et al.* (2015) and Gomes *et al.* (2011), which added 300 and 450 g crude glycerin/kg in lambs' diets, respectively.

The pH value of the muscle after slaughter is one of the most important factors that affect meat quality, because it can influence tenderness, colour and water-holding capacity. In the first hours after slaughter there is the transformation of muscle in meat, involving the depletion of muscle glycogen stores with the production and accumulation of lactic acid. This process triggers *rigor mortis*, which in sheep carcasses occurs when the pH is between 5.6 and 5.8 for the *Longissimus* muscle (Koochmaraie *et al.* 1991; Oliveira *et al.* 2004). In this study, the pH of *Longissimus* muscle 24 h after slaughter was, on average, 5.6. It can be inferred from these low values that no DFD meat (dry, firm and dark muscle) was observed, proving also by the high L* values found 24 h after slaughter, reflecting on high values of a* and b*. According to Souza *et al.* (2004) ovine meat usually presents values from 30.6 to 38.0 for L*, 12.3 to 18.0 for a*, and 3.3 to 5.7 for b*. The average values observed in the present study for each of these variables were 33.0, 13.5 and 4.3, respectively.

Edible non-carcass components and total usable products

The reduction of more than 20% in the perirenal fat with inclusion of crude glycerin in the diets can be due to the lesser DMI of those animals, resulting in less energy intake. As the perirenal fat is the first fat tissue to be deposited in animal body (Oliveira *et al.* 2011), this result was expected, however, no effects were observed of crude glycerin inclusion on mesenteric or omental fat tissue deposits.

Nonetheless, among treatments with crude glycerin, the G10 showed lower values of perirenal fat even though it was the treatment with the greatest DMI. This can be explained by the energy source of the diet. According Kamalzadeh *et al.* (1998), the organs and viscera have different growth rates and are influenced mainly by the chemical composition of the diet and energy level. Thus, despite the G20 and G30 treatments have lower DMI, crude glycerin intake was higher compared with the G10 (0117 vs 0215 and 0117 vs 0302 kg/day DM, respectively). As the fermentation of glycerin crude rumen increases propionate and decreases acetate, reducing the ratio C2 : C3 in the rumen (van Cleef *et al.* 2015), it may have resulted in increased availability of energy in the form of glucose, which favoured lipogenesis and consequently the greater deposition of perirenal fat.

The lack of effect of treatments on non-carcass edible components indicates that crude glycerin could be an alternative energy source in the diets of feedlot lambs as it does not affect the production of marketable animal products.

Conclusions

The addition of up to 100 g crude glycerin/kg DM in diets for crossbred finishing lambs seems to be the most interesting strategy, as it promotes greatest animal performance. However, the inclusion of up to 300 g/kg DM of the by-product could be possible depending on glycerin market price and the structure of feedlot operation, with favourable economic results despite high inclusions reflect in greater number of days on feed.

Acknowledgements

Authors thank São Paulo Research Foundation (FAPESP) for the scholarships to first (grant#2015/04595–4) and last author (grant#2013/18118–8), and Caramuru Alimentos SA for providing part of the ingredients used in this research.

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CAPÍTULO 3

O capítulo a seguir está redigido em formato de artigo científico conforme normas da revista “**Small Ruminant Research**”.

CAPÍTULO 3 – Performance, rumen and liver variables of lambs fed with high inclusions of crude glycerin in adaptation and finishing period of feedlot

ABSTRACT - Crude glycerin is the main by-product of biodiesel industry and has great potential for reducing the feed costs in ruminant feedlot systems without affecting animal health and performance, mainly as a replacement for corn grain. The objective of this study was to evaluate the effects of increasing inclusions of crude glycerin (up to 30%) in diets for crossbred feedlot lambs in two different periods (adaptation and finishing) on the animal performance, rumen morphometric and liver variables. Fifty-five 3-month-old crossbred lambs were randomly allocated in individual pens indoors, assigned to a complete randomized block design and fed with four experimental diets, containing 0, 10, 20 or 30% crude glycerin. Three animals were slaughtered at the end of the pre-adaptation period (d0), twelve at the end of the adaptation period (d14), and the remaining (n= 40) when they reached approximately 35 kg BW. After slaughtered, the animal performance, stomach compartments and liver were collected and evaluated. Regardless of the inclusion of crude glycerin, significant differences among feedlot periods were observed for the initial and final BW, shrunk final BW, ADG and DMI ($P < 0.05$). Animals fed with 10% of crude glycerin had a higher DMI in the finishing period ($P_{\text{quad}} = 0.04$). The increasing inclusion of crude glycerin linearly decreased the ADG in the finishing period of feedlot ($P = 0.003$). All stomach compartments, number of rumen papillae and mitotic index were higher for the finishing period ($P < 0.05$). Crude glycerin treatments showed greater rumen weights when compared to control treatment ($G0 \times Gly$, $P = 0.01$) in the finishing period. The increasing inclusion of up to 30% of crude glycerin/kg DM in diets for crossbred lambs did not compromise the feed efficiency, stomach compartments and rumen papillae measurements in both periods of the feedlot. However, the inclusion of 10% of crude glycerin/kg DM seems to be the most interesting strategy for the finishing period, promoting the greatest animal performance. As no clinical manifestations resulted from ruminal acidosis (such as liver abscess, ruminitis, and lesions in the ruminal mucosa) were observed, we concluded that all diets were effective in the animals' adaptation.

Keywords: by-product, glycerol, papillae, sheep

1. Introduction

Sheep production in Brazil has been continuously increasing in the past years. The number of heads increased about 6% over a period of five years (2010-2015), rising from 17.4 to 18.4 million animals (IBGE, 2015). Except for the South region of Brazil, due to its suitable soil and climate conditions to sheep grazing systems (Sarmiento, 2017), most of the Country faces a massive seasonality of forage production induced by the well-defined and extended dry season (Pezzopane et al., 2017).

To overcome this problem, many farmers have adopted the feedlot as alternative production system (Vieira et al., 2013). However, to achieve production efficiency, this system requires diets with high inclusion of concentrate. Brazilian nutritionists have recommended more than 60% of concentrate in finishing diets, with the corn grain as the primary ingredient (Oliveira and Millen, 2014), due to its high-energy content (Alves and Cargnelutti Filho, 2017).

On the other hand, abrupt changes in diets, as well as the high inclusion of starch-rich cereal grains, adversely affects the animal performance in the two first weeks of the feedlot, mainly due to ruminal acidosis (Nagaraja and Titgemeyer, 2007; McCann et al., 2016). Changes in feeding behavior with reduced feed intake (Missio et al., 2010) and clinical manifestations such as laminitis, liver abscesses, ruminitis, and inappropriate ruminal fermentation (Dirksen, 1985, Resende Júnior et al., 2006, Steele et al., 2009) are prevalent when animals are adapted for less than 14 days, or when they do not receive a correct adaptation to the diets (Brown et al., 2006).

The rumen epithelium has several physiological functions, such as the absorption and metabolism of nutrients and microbial by-products. The rumen papillae increase the surface area for the absorption and microbial attachment to the rumen wall, playing a significant role in animals' feed intake and weight gain (Galfi et al., 1991, Kern et al., 2016). Although concentrate-rich diets may stimulate the development of ruminal papillae more than roughage-rich diets (Rickard and Ternouth, 1965; Stobo et al., 1966), lesions in the papillae caused by acidosis may compromise the animal performance.

Crude glycerin has been evaluated as a macro-ingredient, and it is considered safe for use as animal feed (FDA, 2006, 21 C.F.R.582.1320), with potential to reduce feed costs in ruminant production systems, without causing negative effects on animal performance, when replaces corn grain (Ezequiel et al., 2015; van Cleef et al., 2015; Almeida et al., 2017).

This by-product can prevent metabolic disorders, avoiding severe reduction of the ruminal pH and the development of rumen acidosis, due to its fermentation profile. The larger portion of crude glycerin (~43%) is rapidly absorbed by rumen papillae, whereas 25 to 45% are fermented to butyrate and propionate by alternative fermentative pathway (via succinate), and do not generate lactic acid, benefiting the rumen development and improving the feed efficiency (Krehbiel, 2008; Omazic et al., 2015).

However, the adequate levels of crude glycerin in the adaptation period and the consequences for the finishing period are still unknown. We hypothesized that variation in rumen papillae morphology with the increasing inclusion of crude glycerin may affect lambs' performance in all periods of the feedlot. Thus, the objective of this study was to evaluate the effects of increasing inclusions of crude glycerin (up to 30%, DM basis) in diets for crossbred feedlot lambs in two different periods (adaptation and finishing), on the feedlot performance and rumen morphometric variables.

2. Materials and methods

The Institutional Animal Care and Use Committee of the São Paulo State University approved all experimental protocols adopted in the current study (approval number: 06329/14).

2.1. Animals, diets and feeding procedure

The present study was carried out at the Animal Unit of Digestive and Metabolic Studies in the Department of Animal Science from São Paulo State University, Jaboticabal campus, Brazil. Fifty-five 3-month-old crossbred (Santa Inês × Dorper) male lambs (17.8 ± 0.9 kg BW) were randomly allocated in individual pens

(1.2 m²) indoors, and assigned to a complete randomized block design (by initial BW).

Within 24 h of arrival, the animals were vaccinated and dewormed. During the seven subsequent days, animals were submitted to a pre-adaptation period, when they were fed *ad libitum* a standard forage-based diet, composed of corn silage (95%), soybean hulls, soybean meal, urea and minerals (Table 1). This period was crucial to standardize the animal feeding and ruminal microflora before starting the experimental period. After pre-adaptation period three random lambs were slaughtered (d0) to serve as the initial baseline for comparisons with the future results. The remaining animals (n=52, 17.9 ± 0.5 kg BW) were fed with four experimental diets containing increasing inclusions of crude glycerin for a 14-d adaptation period. It was used three step-up diets containing increasing levels of concentrate (diet 1= 20%, diet 2= 40% and finishing diet= 60%, Table 1 and 2).

After the adaptation period (d14), three lambs from each treatment (n=12) were randomly selected and slaughtered to evaluate the effect of crude glycerin during this period. The remaining animals (n=40, 21.7 ± 2.7 kg BW) continued being fed their respective experimental diets until reached approximately 35 kg BW, and then they were harvested. The total period of trial lasted, on average, 59 d.

Experimental diets were formulated to be isonitrogenous (% CP) and isocaloric (Mcal/kg EM), according to recommendations of NRC (2007), and were offered to the lambs *ad libitum*. Diets were composed of corn silage (roughage source), and corn cracked grain, soybean hulls, soybean meal, urea, crude glycerin (except the control diet), mineral-vitamin premix, limestone, and dicalcium phosphate, as concentrate. Treatments were labeled as: G0 (control treatment, without crude glycerin), G10 (10% of crude glycerin, DM basis), G20 (20% of crude glycerin, DM basis) and G30 (30% of crude glycerin, DM basis). The crude glycerin was obtained from a commercial soybean oil and meal production plant.

Table 1. Ingredients and chemical composition of experimental diets.

Item	Pre-adaptation diet	Adaptation diet 1 ^a				Adaptation diet 2 ^a			
		G0	G10	G20	G30	G0	G10	G20	G30
Ingredients (% DM)									
Corn silage	95	80	80	80	80	60	60	60	60
Corn cracked grain	0.0	15	10	5	0	30	20	10	0
Soybean hulls	1.5	1.0	1.0	1.1	1.1	3.6	3.2	2.8	2.2
Soybean meal	1.9	2.4	2.2	1.8	1.6	5.0	5.0	5.0	5.2
Urea	0.5	0.7	0.9	1.1	1.3	0.4	0.7	1.0	1.3
Crude glycerin ^b	0.0	0.0	5	10	15	0	10	20	30
Mineral-vitamin premix ^c	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.4	0.4
Limestone	0.4	0.3	0.3	0.3	0.3	0.4	0.3	0.4	0.4
Dicalcium phosphate	0.2	0.1	0.2	0.3	0.3	0.2	0.3	0.4	0.4
Chemical composition									
DM, %	33.4	42.2	42.3	42.5	42.6	53.9	54.2	54.5	54.8
CP, % of DM	9.4	10.3	10.3	10.3	10.3	10.9	10.9	10.9	10.9
ME, Mcal/kg DM ^d	2.3	2.4	2.4	2.4	2.4	2.6	2.6	2.6	2.6
EE, % of DM	3.1	3.2	3.0	2.8	2.7	3.3	2.9	2.6	2.2
aNDF, % of DM ^e	52.0	45.8	45.1	44.4	43.7	39.8	38.0	36.3	34.5
ADF, % of DM	33.5	28.7	28.5	28.3	28.1	24.1	23.5	22.9	22.2
Ca, % of DM	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
P, % of DM	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3

^a Experimental diets: G0, control diet; G10, inclusion of 10% crude glycerin; G20, inclusion of 20% crude glycerin; G30, inclusion of 30% crude glycerin.

^b Composition per kg: glycerol (830.0 g), DM (950.0 g), CP (11.0 g), salts (60.0 g) and methanol (less than 0.1 g).

^c Composition per kg: P (75 g), Ca (223 g), S (10 g), Zn (3 g), Na (60 g), Co (20 mg), I (40 mg), Se (24 mg), F (750 mg), Mg (5 g), Mn (1,8 g), Fe (402 mg), Vit A (312.500 UI), Vit D (50.000 UI), Vit E (437 UI).

^d Metabolizable energy (Mcal/kg DM) was calculated using NRC (2007).

^e NDF assayed using heat stable α -amylase and expressed inclusive of residual ash.

Table 2. Ingredients and chemical composition of experimental diets.

Item	Finishing diets ^a			
	G0	G10	G20	G30
Ingredients (% DM)				
Com silage	40	40	40	40
Corn cracked grain	30	20	10	0
Soybean hulls	7.8	7.2	6.3	4.5
Soybean meal	20.6	21.0	21.6	23.1
Urea	0.6	0.9	1.1	1.3
Crude glycerin ^b	0	10	20	30
Mineral-vitamin premix ^c	0.5	0.5	0.5	0.5
Limestone	0.5	0.5	0.5	0.5
Dicalcium phosphate	0	0	0	0.2
Chemical composition				
DM, %	65.8	66.1	66.4	66.6
CP, % DM	17.7	17.7	17.7	17.7
ME, Mcal/kg DM ^d	2.8	2.8	2.7	2.7
EE, % DM	3.0	2.7	2.3	2.0
aNDF, % DM ^e	34.8	33.0	31.1	28.7
ADF, % DM	19.2	18.5	17.7	16.5
Ca, % DM	0.5	0.5	0.5	0.5
P, % DM	0.3	0.3	0.3	0.3

^a Experimental diets: G0, control diet; G10, inclusion of 10% crude glycerin; G20, inclusion of 20% crude glycerin; G30, inclusion of 30% crude glycerin.

^b Composition per kg: glycerol (830.0 g), DM (950.0 g), CP (11.0 g), salts (60.0 g) and methanol (less than 0.1 g).

^c Composition per kg: P (75 g), Ca (223 g), S (10 g), Zn (3 g), Na (60 g), Co (20 mg), I (40 mg), Se (24 mg), F (750 mg), Mg (5 g), Mn (1,8 g), Fe (402 mg), Vit A (312.500 UI), Vit D (50.000 UI), Vit E (437 UI).

^d Metabolizable energy (Mcal/kg DM) was calculated using NRC (2007).

^e NDF assayed using heat stable α -amylase and expressed inclusive of residual ash.

Lambs were fed twice daily (0800 and 1600 hours) and had *ad libitum* access to water. The concentrate and corn silage were weighed separately daily and mixed with crude glycerin at the moment of feed delivery, feeding animals 50% of total

mixed ration in each meal. The dry matter intake (DMI, kg) were recorded daily and determined by the weighing feed delivered and orts, collecting a composite sample and, posteriorly, determining its dry matter. To evaluate the growth performance, the animals were weighed upon arrival, at the beginning and at the end of the adaptation period and every 14 d until slaughter, and then the average daily gain (ADG) was calculated.

2.2. Slaughter, sample collection, rumenitis and liver abscess

Three animals (17.5 ± 1.9 kg BW) were randomly selected and slaughtered at the end of pre-adaptation period (d0), twelve (21.4 ± 0.8 kg BW) at the end of adaptation period (d14), and the remaining ones (n= 40) when they reached approximately 35 kg BW.

All the animals were subjected to a 16-h fasting period and body weights were recorded to determine the shrunk final BW. The lambs were stunned by brain concussion, using a non-penetrating bolt pistol. Bleeding was performed by severing the carotid arteries and jugular veins immediately after stunning. After evisceration, rumen, reticulum, omasum and abomasum were emptied, washed and weighed. The rumen received a score according to the incidence of lesions using a scale of 0 (no lesions noted) to 10 (severe lesions and abnormalities), as described by Bigham and McManus (1975). At slaughter, each liver was examined and scored regarding of the size and number of abscesses (A- = 1, A = 2 and A+ = 3), according to Brink et al. (1990). Edible livers without abnormality were labeled as normal and received the grade 0.

2.3. Rumen papillae evaluations

Two fragments (1 cm^2) of each rumen were collected from the cranial region of the ventral sac. One fragment was immediately placed into a phosphate buffer solution (0.1 M, pH 7.4) and cooled for macroscopic analysis. The other fraction was fixed with Bouin solution for 24 h and kept in alcohol solution (70°), until histological routine processing in paraffin (Daniel et al., 2006; Resende Junior et al., 2006).

The macroscopic morphological variables evaluated at rumen wall were: the number of papillae per cm^2 of wall (NOP); average papillae area (APA); total

absorptive surface area per cm² of wall (ASA) and papillae area (PA), expressed as a percentage of ASA. The NOP was manually counted by 3 independent trained evaluators, and 10 papillae were randomly sectioned at the base of each fragment, scanned and the APA was measured using the UTHSCSA Image Tool software (The University of Texas – Health Science Center, San Antonio, TX, USA). The rumen wall ASA (cm²) and PA (% of ASA) were calculated as follows: $ASA = 1 + (NOP * APA) - (NOP * 0.002)$ and $PA = (NOP * APA) / (ASA) * 100$, where the number 1 represents the 1-cm² fragment collected and 0.002 is the estimated basal area of papillae.

The other fragment (1 cm²) was microscopically evaluated for the proportion of epithelial basal cells undergoing mitosis. Three separate papillae of each sample (animal) were stained with hematoxylin and eosin, embedded in paraffin wax and sectioned (Odongo et al., 2006). The mitotic index (MI) was calculated by dividing the number of cells showing mitotic figures by the total number of nuclei on the epithelium basal layer.

2.4. Chemical analyses

Feed (pooled within treatment) and orts (pooled within animal) samples of each experimental period (pre-adaptation, adaptation and finishing) were analyzed for dry matter (DM; method 930.15), crude protein (CP; method 988.05) and ether extract (EE; method 920.39), using standard procedures of AOAC (1990). The neutral detergent fiber (NDF) and acid detergent fiber (ADF) contents were estimated according to Van Soest and Wine (1967), using a heat-stable α -amylase, without sodium sulfite, and expressed inclusive of residual ash.

2.5. Statistical analysis

All data recorded were processed as a randomized complete block design with animals being blocked according to initial BW. Data were analyzed using the MIXED procedure of SAS (version 9.4), including the CONTRAST option. The models included the main effects of treatments (G0, G10, G20 and G30), periods (adaptation and finishing) and the treatment \times period interaction (TR \times PR). When the TR \times PR effect was significant, orthogonal contrasts were used to assess the linear and quadratic effects of crude glycerin, as well as the contrast control treatment \times crude

glycerin treatments (G0 × Gly). The covariance structure with the best fit (smallest Akaike's value) was the unstructured (UN). Treatment means were computed with the LSMEANS option and significance was defined as $P < 0.05$ and trends as $0.05 \leq P \leq 0.10$.

3. Results

3.1. Animal Performance

Regardless of the inclusion of crude glycerin, significant differences among feedlot periods were observed for initial and final BW, shrunk final BW, ADG and DMI ($P < 0.05$), with higher values observed in the finishing period (Table 3). A tendency to a treatment effect (TR, $P = 0.08$) for the shrunk final BW was observed. There was an effect of crude glycerin inclusion for the DMI ($P = 0.04$) and ADG ($P = 0.02$), with significant effects in the finishing period (TR × PR effect, $P = 0.0260$ and $P = 0.0060$, respectively). A quadratic effect in DMI ($P = 0.0432$), with higher intake for the animals fed with 10% of crude glycerin, was observed in this period (Figure 1). The increasing inclusion of crude glycerin also linearly decreased the ADG in this period ($P = 0.0034$, Figure 2), with a tendency for a quadratic effect ($P = 0.0696$). No significant effects of TR, PR and TR × PR ($P > 0.05$) were observed for feed efficiency.

Table 3. Effects of crude glycerin on intake and performance of feedlot lambs.

Item	Day 0 (n=3)	Adaptation ^a (n=13)				Finishing ^a (n=10)				SEM	Contrast ^b , <i>P</i> -value		
		G0	G10	G20	G30	G0	G10	G20	G30		TR	PR	TR×PR
Body weight (kg)													
Initial	17.5	18.0	17.7	17.9	17.9	21.7	21.3	22.2	21.5	0.42	0.54	<0.01	NS
Final	17.5	21.4	21.6	21.5	20.9	34.7	35.2	34.8	34.7	0.88	0.12	<0.01	NS
Shrunk final	16.8	20.0	20.0	19.7	19.5	33.2	34.0	33.8	33.1	0.89	0.08	<0.01	NS
DMI (kg/dia) ^c	-	0.99	1.00	0.92	0.83	1.12	1.17	1.08	1.01	0.03	0.04	0.01	F
ADG (kg/dia) ^d	-	0.24	0.28	0.26	0.22	0.31	0.33	0.29	0.26	0.01	0.02	0.01	F
Feed : Gain	-	4.07	3.65	3.67	3.92	3.70	3.56	3.79	3.72	0.07	0.57	0.44	NS
Gain : Feed	-	0.25	0.28	0.28	0.26	0.28	0.28	0.27	0.26	0.01	0.51	0.77	NS

^a G0 = control diet; G10 = inclusion of 10% crude glycerin; G20 = inclusion of 20% crude glycerin; G30 = inclusion of 30% crude glycerin.

^b TR= effect of treatments; PR = effect of periods; TR × PR = interaction treatments × periods: A = adaptation period; F = finishing period; NS = not significant.

^c DMI = dry matter intake.

^d ADG = average daily gain.

Contrast: DMI (TR × PR), *P* = 0.0260 (F).

Contrast: ADG (TR × PR), *P* = 0.0060 (F).

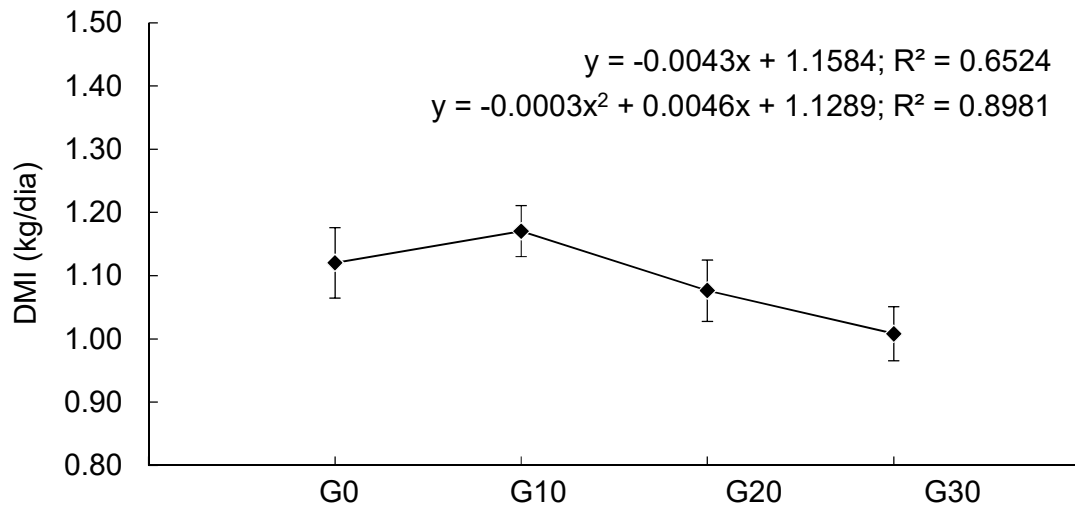


Figure 1. Interaction of TR × PR (Finishing period, $P = 0.0260$, SEM = 0.0288) for dry matter intake (DMI) of feedlot lambs fed increasing concentrations of crude glycerin. Contrast: P linear = 0.0025, P quadratic = 0.0432.

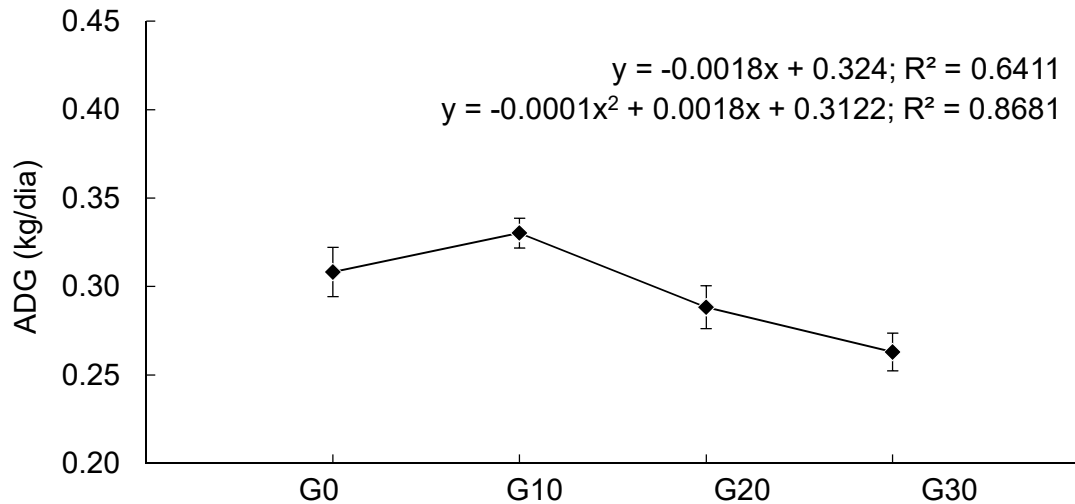


Figure 2. Interaction of TR × PR (Finishing period, $P = 0.0060$, SEM = 0.0072) for average daily gain (ADG) of feedlot lambs fed increasing concentrations of crude glycerin. Contrast: P linear = 0.0034, P quadratic = 0.0696.

3.2. Stomach compartments, rumenitis score and liver abscess incidence

Significant differences among feedlot periods were observed for all stomach compartments (rumen, reticulum, omasum and abomasum), with the highest weights observed for the animals slaughtered after the finishing period ($P < 0.05$, Table 4). There was effect of treatment (TR, $P = 0.01$) and significant interaction of TR \times PR (Finishing period, $P = 0.0054$) for rumen weight (Figure 3). In the finishing period, crude glycerin treatments showed greater rumen weights when compared to control treatment (G0 \times Gly, $P = 0.0110$). A tendency for a treatment effect (TR, $P = 0.06$) and a significant interaction of TR \times PR ($P = 0.0399$) were observed for omasum weight after the finishing period, with a tendency for a quadratic effect ($P = 0.0658$, Figure 4). A tendency for an interaction of TR \times PR for abomasum weight was observed after the finishing period ($P = 0.0640$). The increasing inclusion of crude glycerin did not compromise the rumen and liver of the animals, showing a tendency for TR and PR effect ($P = 0.09$) on rumenitis scores.

Table 4. Effects of crude glycerin on relative size of stomach compartments, ruminitis score and liver abscess incidence of feedlot lambs.

Item	Day 0 (n=3)	Adaptation ^a (n=3)				Finishing ^a (n=10)				SEM	Contrast ^b , <i>P</i> -value		
		G0	G10	G20	G30	G0	G10	G20	G30		TR	PR	TR×PR
Stomach compartments													
RUM (kg) ^c	0.43	0.48	0.50	0.52	0.45	0.63	0.71	0.65	0.68	0.01	0.01	<0.01	F
RET (kg) ^d	0.09	0.07	0.09	0.09	0.08	0.11	0.12	0.11	0.10	0.01	0.21	<0.01	NS
OMA (kg) ^e	0.05	0.06	0.06	0.06	0.05	0.08	0.09	0.08	0.08	0.01	0.06	<0.01	F
ABO (kg) ^f	0.12	0.11	0.11	0.11	0.10	0.14	0.17	0.16	0.14	0.01	0.11	<0.01	F
RUM score ^g	0.00	0.48	0.90	0.33	0.37	0.60	1.06	0.70	0.80	0.07	0.09	0.09	NS
ABS incidence ^h	0.00	0.50	0.33	0.00	0.00	0.00	0.22	0.00	0.00	0.04	0.10	0.11	NS

^a G0 = control diet; G10 = inclusion of 10% crude glycerin; G20 = inclusion of 20% crude glycerin; G30 = inclusion of 30% crude glycerin. ^b TR= effect of treatments; PR = effect of periods; TR × PR = interaction treatments × periods: A = adaptation period; F = finishing period; NS = not significant. ^c RUM = rumen. ^d RET = reticulum. ^e OMA = omasum. ^f ABO = abomasum. ^g RUM score = ruminitis score. ^h ABS incidence = liver abscess incidence. Contrast: RUM (TR × PR), *P* = 0.0054 (F). Contrast: OMA (TR × PR), *P* = 0.0399 (F). Contrast: ABO (TR × PR), *P* = 0.0640 (F).

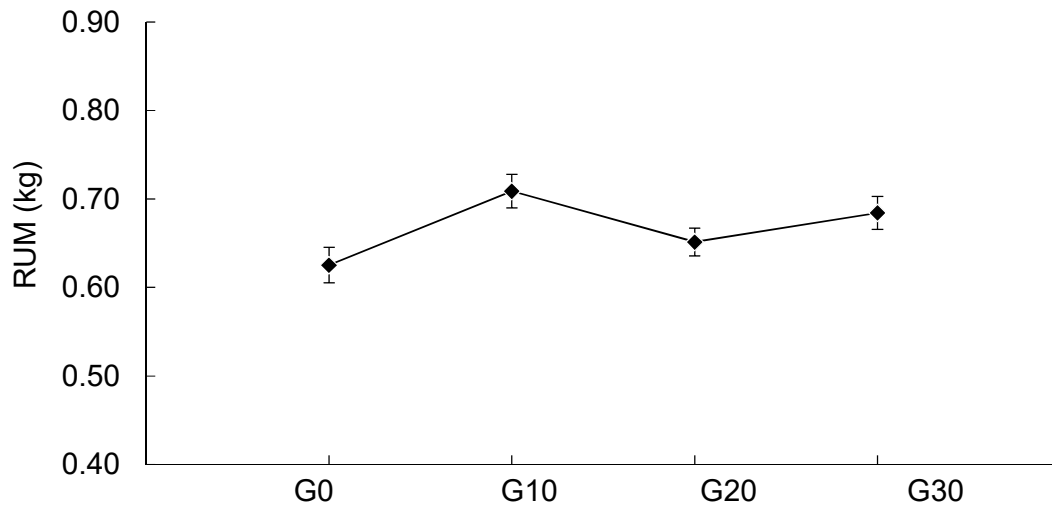


Figure 3. Interaction of TR × PR (Finishing period, $P = 0.0054$, SEM = 0.0152) for Rumen weight (RUM) of feedlot lambs fed crude glycerin. Contrast: G0 × Gly, $P = 0.0110$.

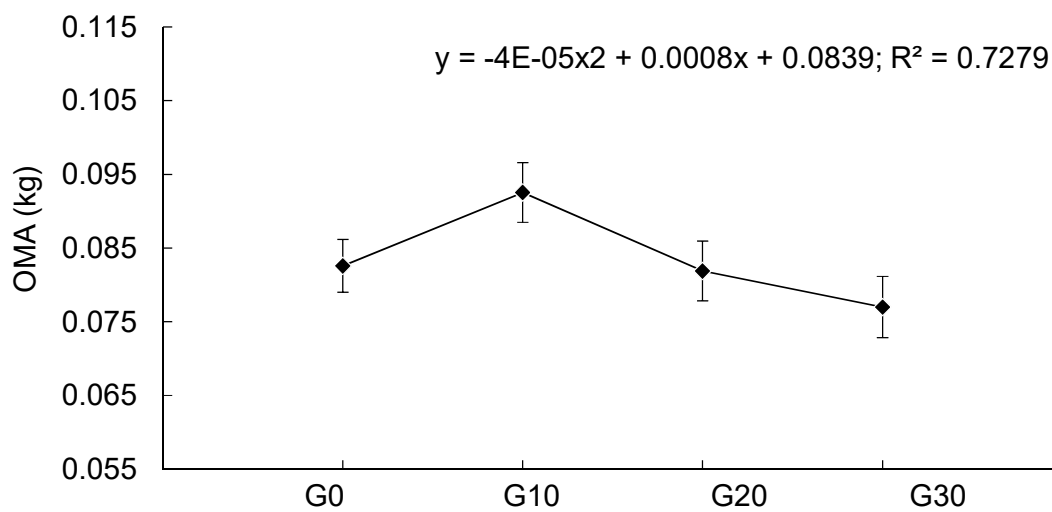


Figure 4. Interaction of TR × PR (Finishing period, $P = 0.0399$, SEM = 0.0021) for omasum weight (OMA) of feedlot lambs fed crude glycerin. Contrast: P quadratic = 0.0658.

3.3. Rumen papillae measurements

Significant differences among feedlot periods were observed for the number of papillae (NOP, $P = 0.02$) and mitotic index (MIT, $P = 0.01$), with the highest values observed for the finishing period (Table 5). A tendency for an interaction of TR \times PR for the number of papillae was observed in the adaptation period ($P = 0.0959$).

Table 5. Effects of crude glycerin on papillae measurements of feedlot lambs.

Item	Day 0 (n=3)	Adaptation ^a (n=3)				Finishing ^a (n=10)				SEM	Contrast ^b , <i>P</i> -value		
		G0	G10	G20	G30	G0	G10	G20	G30		TR	PR	TR \times PR
Papillae measurements													
NOP (n) ^c	74.0	53.6	60.7	56.3	44.3	58.8	57.4	64.7	59.0	1.20	0.15	0.02	A
APA (cm ²) ^d	0.33	0.69	0.58	0.51	0.63	0.62	0.58	0.59	0.74	0.03	0.29	0.63	NS
ASA (cm ²) ^e	46.5	38.7	36.4	29.2	29.1	36.7	35.3	38.2	44.0	1.64	0.68	0.19	NS
PA (% of ASA) ^f	95.6	97.9	97.6	96.9	96.8	97.4	97.2	97.3	97.8	0.12	0.54	0.60	NS
MIT index ^g	0.48	0.86	0.83	0.89	0.92	0.94	1.04	1.02	1.06	0.02	0.42	0.01	NS

^a G0 = control diet; G10 = inclusion of 10% crude glycerin; G20 = inclusion of 20% crude glycerin; G30 = inclusion of 30% crude glycerin. ^b TR= effect of treatments; PR = effect of periods; TR \times PR = interaction treatments \times periods: A = adaptation period; F = finishing period; NS = not significant. ^c NOP = number of papillae. ^d APA = average papillae area. ^e ASA = absorptive surface area. ^f PA = papillae area (% of ASA). ^g MIT index = mitotic index (% of basal cells). Contrast: NOP (TR \times PR), $P = 0.0959$ (A).

4. Discussion

The lambs started in the feedlot with similar BW (17.8 ± 1.7 kg) and were subjected to a pre-adaptation period of 7 days to standardize rumen conditions before being assigned to a complete randomized block design (by initial BW) for future comparisons. We believe that no variable analyzed in this study was directly influenced by the individual animal status since little or no evidence was observed assuming that the animals used were homogeneous at the beginning of the experiment.

4.1. Animal performance

The significant differences for initial and final BW, shrunk final BW, ADG and DMI observed among periods of feedlot can be explained by the normal curve of growth and the consequent time in each period of feedlot until the slaughter. Therefore, the longer the stay in confinement, the higher the body weight.

The tendency observed for the shrunk final BW with the inclusion of crude glycerin can be due to the great variations among treatments regarding of the loss of weight during the solid-fast period (16 h), with average of $4.01 \pm 2.64\%$ in the beginning of the feedlot (d0), $7.26 \pm 1.38\%$ in the adaptation period and $3.80 \pm 2.07\%$ in the finishing period. The lowest losses were observed for the treatments G10 (3.41%) and G20 (2.87%). It can be basically explained by the greater water intake of those animals during fasting period and also due to bigger stomach compartments of those animals (7.14% bigger than others). However, despite the variations, the rate of fasting loss is according to the data reported by previous studies (Thompson et al., 1987; Osório et al., 1996).

The reduction of DMI when crude glycerin was included at more than 10% can be related to the inhibition of the growth and cellulolytic ruminal bacteria activity due to negative effects on the adhesion and enzymatic activities (Roger et al., 1992; El-Nor et al., 2010). This inhibition may be directly related to reductions in fiber digestion, passage rate and feed intake, as already evidenced by previous studies with increasing inclusion of crude glycerin in lambs' diets (Gunn et al., 2010; Lage et al., 2014; Barros et al., 2015). In the present study, the inclusion of 10% of crude glycerin stimulated the

consumption and improved the performance of the animals, regardless of the period of feedlot. It was probably due to the improvement in ruminal fermentation, to the better use of feed and also to passage rate increase. Those animals had, respectively, 9.5 and 9.3% greater weight gain compared to other treatments during adaptation and finishing periods, thus confirming its greater acceptance by the animals.

These facts support the possible deleterious effect of high inclusions of crude glycerin with consequent reduction in DMI. Although it is known that crude glycerin improves the palatability by its sweet-tasting characteristic (Farias et al., 2012; Chanjula, 2017). It also promotes changes in the physical form of the diets, which may increase the passage rate due to its liquid and viscous form. However, despite these differences mentioned above, the increasing inclusion of crude glycerin did not impair the feed efficiency in all feedlot periods, reflecting only in greater days on feed (Linear, $P = 0.02$, data not shown). In the present study, DMI explained 99% of the animals' daily weight gain variation (Fig. 5).

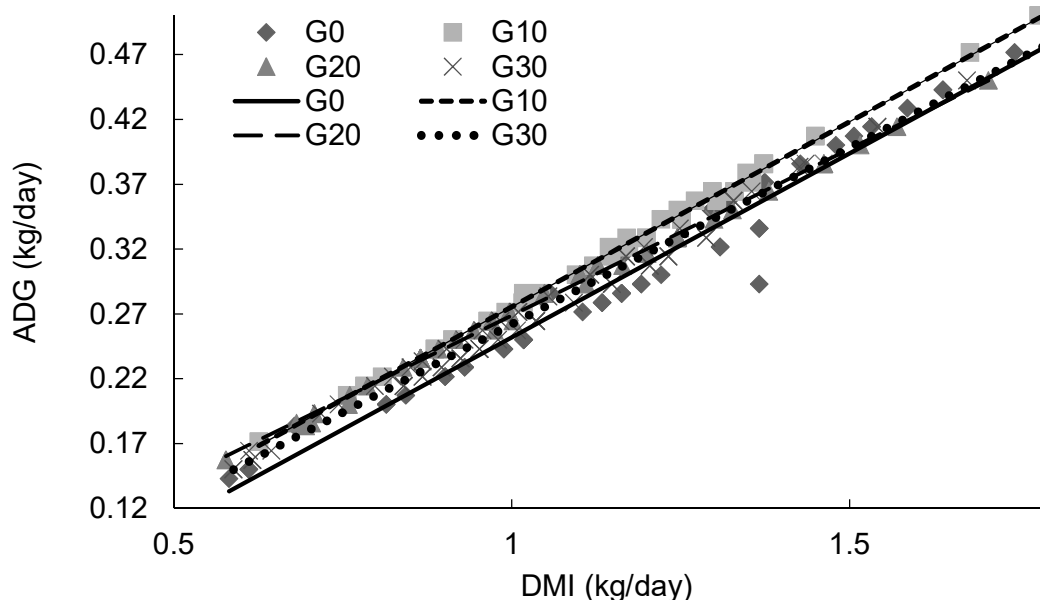


Figure 5. Relationship between dry matter intake (DMI) and average daily gain (ADG) from feedlot lambs fed with crude glycerin during the total experimental period. Linear regression equations: G0, $ADG \text{ (kg/day)} = 0.2836 \times DMI \text{ (kg/day)} - 0.0317$, $R^2 = 0.9665$;

Considering the crude glycerin as a low-cost by-product, the greater days on feed promoted by its greater inclusion could be worth it due to the lower cost of the final diet, and may even financially benefit the producer, as already reported by previous studies (Barros et al., 2015; Rego et al., 2015)

4.2. Stomach compartments and rumen papillae measurements

The increasing body weight of the animals during feedlot also reflected in greater weight of stomach compartments (rumen, reticulum, omasum and abomasum), thus justifying the observed difference among periods. However, the inclusion of crude glycerin resulted in larger rumen sizes in the finishing period, being 8% heavier compared with the control treatment (G0 x Gly). Probably, the crude glycerin may have stimulated rumen growth due to its fermentative characteristic, mainly by increasing the total rumen VFA (DeFrain et al., 2004; Trabue et al., 2007) with greater molar concentrations of rumen propionate (van Cleef Et al., 2015) and butyrate (San Vito et al., 2016). The presence of VFA in the ruminal lumen promotes the growth and proliferation of the papillae, with increased absorption area, improving the removal capacity of these acids as well as providing greater energy absorption for the animal (Sander et al., 1959; Costa et al., 2008; Gorka et al., 2009). However, as the effect of the crude glycerin inclusion on the area of papillae absorption was not observed, it is assumed that the greater weight of rumen may be due to the greater proliferation of the papillae, thus generating a greater rumen wall surface.

The mitotic index and number of papillae of the ruminal epithelium increased considerably during the finishing period, and the greater mitotic index is indicative of cell proliferation and represents the cells in renewal cycle. This index can be stimulated by butyric (Sakata and Tamate, 1977a,b) and propionic acid (Sakata and Tamate, 1979), however the butyric acid is far more effective (Sutton et al., 1963; Kauffold et al., 1977). Because most of the crude glycerin is converted to propionate and this is a stimulator of insulin release *in vivo* (Sakata et al., 1980), it is possible that insulin could be a mediator in the stimulation of mitosis in the ruminal epithelium. According to Goodlad (1981), ruminal

epithelial mitotic index has been shown to be stimulated by intravenous insulin infusions.

These results represent aspects of the adaptation physiological process and can indicate that adaptation was correctly conducted. However, the number of cells in the epithelium can also represent a disturb in the rates of cell division and turnover, causing pathological conditions such as hyperkeratosis, parakeratosis or ruminitis (Fell and Weekes, 1975). The weight of the rumen epithelium has a clear and major impact on the digestive capabilities and supply of substrates to the growing ruminant (Baldwin et al., 2004). Thus, considering that the rumen consists of a nonglandular mucosa and represents the fermentative chamber where most of the digestion happens by the joint action of the microorganisms, it can be assumed that the volumetric capacity of the rumen determines the quantity of feed intake and that the greater development of the muscular layer also can be associated to a greater necessity of ruminal motility (Membrive, 2016). In this sense, the best performance of animals fed G10 is justified by their higher DMI, possible better fermentation conditions and greater energy supply provided by this diet.

According to Membrive (2016), the VFA are the main products formed in the fermentative digestion and represents the greatest energy source for ruminants (50-70%). In addition, the higher consumption may provide an increased number of microorganisms, being an important protein source for improved animal performance. Nevertheless, the VFA production is the main mechanism of ruminal pH reduction. When it drastically happens and rumen epithelium is not fully developed to absorb these products, the consequence is the ruminal acidification. The ruminal acidosis usually occurs during the first weeks of feedlot, and it is due to abrupt changes in the diets with great impact in DMI and, in more severe cases, causes animal death (Nagaraja and Titgemeyer, 2007; McCann et al., 2016; Millen et al., 2016). As no clinical manifestations resulted from ruminal acidosis (such as laminitis, liver abscess, ruminitis and lesions in the ruminal mucosa) were observed, we concluded that all diets were effective in adapting the animals.

The observed tendencies for higher ruminitis score for G10 in both periods can be justified by the higher DMI and greater formation of VFA. However, the scores observed were very low, considering the scale from 1 to

10, not being able to compromise the performance of the animals. According to Owens et al. (1998), the high incidence of ruminitis can compromise the absorption of nutrients and consequently reduce animal performance. In addition, a greater formation of butyrate could also justify this tendency, because the excess of acidity can cause abnormal growth and agglomeration of papillae, causing a keratinizing effect (Costa et al., 2008). A little or no evidence of liver abscess indicates that there was no disorder started by acidosis, such as ruminitis. In addition, ruminal epithelium may be the first step in the development of future abscesses, and some ruminal microorganisms like *Fusobacterium necrophorum* can migrate through blood portal system and cause liver abscesses (Nagaraja and Chengappa 1998; Millen et al., 2016).

The initial (d0) and final data of stomach compartments show that changes among them were still happening. At the beginning of feedlot (d0), rumen and abomasum represented approximately 62.3% and 17.4% of stomach compartments, respectively, reaching 66.1% and 14.8% at the end of finishing period. According to Membrive (2016), the rumen epithelium reaches the complete development at approximately 2–3 months of age, and it determines the absorption capacity of the rumen. Thus, a better initial stimulus for increased rumen and papillae growth would reflect in higher ingestion capacity and higher animal performance, as observed in this study for animals fed G10 (they were confined when were 3 months old).

The greater DMI may also justify the higher omasum weight and the tendency to higher abomasum for those animals (G10) during the finishing period. In addition, the greater passage of substrates may also have stimulated the growth of these organs.

5. Conclusions

The increasing inclusion of up to 30% of crude glycerin/kg DM in diets for crossbred lambs did not compromise the feed efficiency, stomach compartments, and rumen papillae measurements in both periods of the feedlot. However, the inclusion of 10% of crude glycerin/kg DM seems to be the most interesting strategy for the finishing period, as it promotes greatest animal performance. As no clinical manifestations resulted from ruminal acidosis, such

as liver abscess, ruminitis, and lesions in the ruminal mucosa were observed, we concluded that all diets were effective in adapting the animals.

Depending on crude glycerin market price, this by-product could provide favorable economic results, mainly in the adaptation period. And despite the greater days on feed promoted by its greater inclusion in the finishing period, it could be worth it due to the lower cost of the final diet.

Conflict of interest

The authors declare no known conflict of interest.

Acknowledgment

The authors thank São Paulo Research Foundation (FAPESP) for the scholarships (grant#2015/04595-4 and grant#2013/18118-8), and Caramuru Alimentos SA for providing part of the ingredients used in this research.

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CAPÍTULO 4

O capítulo a seguir está redigido em formato de artigo científico conforme normas da revista "**Small Ruminant Research**". Publicado em 27 de dezembro de 2017 sob identificação DOI: doi.org/10.1016/j.smallrumres.2017.12.011.

CAPÍTULO 4 – Effects of partial or total replacement of corn cracked grain with high concentrations of crude glycerin on rumen metabolism of crossbred sheep

ABSTRACT - Crude glycerin, a by-product of the biodiesel industry, has been used as a strategic ingredient in intensive ruminant production systems, mainly in substitution of starch-rich ingredients. The present study was performed to evaluate the effects of the inclusion of up to 30% of crude glycerin in diets for feedlot sheep, on ruminal parameters, such as pH, NH₃-N and volatile fatty acids concentrations, *in situ* degradability, as well as *in vitro* greenhouse gas production and *in vitro* digestibility. Eight ruminally-cannulated male Santa Inês × Dorper sheep (64.5 ± 8.5 kg) were distributed in a replicated 4 × 4 Latin square design. The experimental diets contained 0, 10, 20 or 30% of crude glycerin and were labeled as G0, G10, G20 and G30, respectively. The crude glycerin totally replaced the corn cracked grain in treatment G30. The inclusion of crude glycerin in the diets tended to promote a quadratic effect in DMI, with greater values observed for treatments G10 and G20. Crude glycerin tended to increase the ruminal pH and NH₃-N, but linearly reduced the total molar concentration of VFA, acetic, butyric, isobutyric and isovaleric acids. The inclusion of crude glycerin in the diets linearly decreased the *in vitro* total gas and CO₂ production (mL/g degraded) and tended to reduce CH₄ (mL/g degraded). A linear increase of soluble fraction in water (“a”) of the diets were observed with the increasing inclusion of crude glycerin. The insoluble but potentially degradable fraction (“b”) of DM and NDF of the diets were linearly decreased and increased, respectively. The potential and effective ruminal degradation of the diets were markedly and linearly increased with the increasing inclusion of the by-product. Treatments linearly increased *in vitro* DM digestibility of diets and linearly reduced NDF digestibility. The replacement of corn cracked grain by crude glycerin (up to 30% DM) changes rumen fermentation parameters, decreasing VFA production, *in vitro* total gas production and CH₄. Additionally, the potential and effective degradation as well as *in vitro* DM digestibility of diets are improved while fiber digestibility is impaired.

Keywords: by-product, glycerol, greenhouse gas, metabolism, sheep

1. Introduction

The use of agroindustry by-products as animal feed is becoming increasingly common throughout the world. The high price of conventional ingredients, such as corn and soybean, has led meat producers to look for alternative ingredients, which usually have a lower purchase cost, but require further understanding of their nutritional value and acceptability (van Cleef et al., 2014).

Crude glycerin, a by-product of the biodiesel industry, is a new well-documented ingredient, which has been used as a strategic ingredient in intensive ruminant production systems, mainly in substitution of starch-rich ingredients, such as corn (Donkin et al., 2009; Carvalho et al., 2015; Almeida et al., 2017; van Cleef et al., 2017). The current annual production of biodiesel in the world is around 34.5 billion liters OECD/FAO (2016). The Brazil is the second largest biodiesel producer in the world, with 3.8 billion liters, in 2016 (United States lead with 5.5 billion liters), generating around 420 million liters of crude glycerin in 2016 (ANP, 2017), that could be used to feed livestock.

This by-product is mainly composed of glycerol, which is an energetic compound of great assimilation by rumen microorganisms and with extensive metabolism in the liver (Abo El-Nor et al., 2010). In the rumen, glycerol is rapidly metabolized by microorganisms to form short chain fatty acids, mainly propionate and butyrate (Donkin, 2008; AbuGhazaleh et al., 2011). The glycerol disappears almost entirely from the rumen in the first 24 hours (Trabue et al., 2007), but can also be directly absorbed by the epithelium of the digestive system and act as a gluconeogenic substrate in the liver (Krehbiel, 2008).

The fermentation of glycerin may promote better stability to the rumen environment compared with starch-rich ingredients, mainly by reducing lactic acidosis as a consequence of the increase in the population of lactate-consuming bacteria and undue fermentation (Krueger et al., 2010). However, they may have a detrimental effect on the growth of structural carbohydrate fermenting bacteria (Roger et al., 1992; AbuGhazaleh et al., 2011), resulting in reduced fiber digestibility and methane production (Shin et al., 2012; van Cleef et al., 2015).

Nonetheless, recent studies have shown that the inclusion of high levels of crude glycerin does not impair the consumption, performance or carcass characteristics of feedlot sheep (Gunn et al., 2010a; Gunn et al., 2010b; Gomes et al., 2011). Therefore, it is essential to study high inclusions of crude glycerin in total substitution to corn, with evaluations of rumen fermentation characteristics, to establish an adequate level for a healthier rumen environment.

Therefore, the objective of this study was to evaluate the effects of the inclusion of up to 30% of crude glycerin (on DM basis) in diets for feedlot crossbred sheep, on ruminal parameters, such as pH, NH₃-N, VFA concentrations, *in situ* DM and NDF degradabilities, as well as *in vitro* greenhouse gas production and DM and nutrients *in vitro* digestibility.

2. Materials and methods

The study was conducted at the Animal Unit of Digestive and Metabolic Studies from the Department of Animal Science of São Paulo State University (Unesp), Jaboticabal, São Paulo, Brazil. The São Paulo State University Institutional Animal Care and Use Committee approved all experimental protocols adopted in the current study (approval number: 06329/14).

2.1. Animals, diets and experimental design

Eight ruminally-cannulated crossbred uncastrated male Santa Inês × Dorper lambs (64.5 ± 8.5 kg and approximately 18 months old) were distributed in a replicated 4 × 4 Latin square arrangement of treatments, according to initial body weight. The animals were housed in individual semi-roofed, concrete-surfaced pens (2.5 m²), with individual feed bunks and waterers, and received the experimental diets for 21-d periods, including 14 d of adaptation, followed by 7 d of sample collection.

The experimental diets contained 0, 10, 20 or 30% of crude glycerin (on DM basis) and were formulated to be isonitrogenous (17.7% CP/DM) and isoenergetic (2.7 - 2.8 Kcal ME/kg DM) to supply the requirements of a 20–30 kg lamb with moderate growth for daily gains of 200 g, according to NRC (2007), and with a roughage:concentrate ratio of 40:60. The dietary treatments were labeled as: G0 (control treatment, containing no crude glycerin), G10

(containing 10% crude glycerin in diet DM), G20 (containing 20% crude glycerin in diet DM), and G30 (containing 30% crude glycerin in diet DM). The crude glycerin totally replaced the corn cracked grain in treatment G30 (Table 1).

Table 1. Ingredient and chemical composition of experimental diets.

Item	Treatments ^b			
	G0	G10	G20	G30
Ingredient composition (%)				
Corn silage	40.0	40.0	40.0	40.0
Corn cracked grain	30.0	20.0	10.0	0.0
Soybean hulls	7.8	7.2	6.3	4.5
Soybean meal	20.6	21.0	21.6	23.1
Urea	0.6	0.9	1.1	1.3
Crude glycerin	0.0	10.0	20.0	30.0
Mineral/vitamin premix ^a	0.5	0.5	0.5	0.5
Limestone	0.5	0.5	0.5	0.5
Bicalcium phosphate	0.0	0.0	0.0	0.2
Nutrient composition				
DM, %	65.8	66.1	66.4	66.6
CP, %	17.7	17.7	17.7	17.7
ME, Mcal/kg	2.8	2.8	2.7	2.7
EE, %	3.0	2.7	2.3	2.0
aNDF, %	34.8	33.0	31.1	28.7
ADF, %	19.2	18.5	17.7	16.5
Ca, %	0.5	0.5	0.5	0.5
P, %	0.3	0.3	0.3	0.3

^a Composition per kg: P (75g), Ca (223g), S (10g), Zn (3g), Na (60g), Co (20mg), I (40mg), Se (24mg), F (750mg), Mg (5g), Mn (1.8g), Fe (402mg), Vit A (312,500 UI), Vit D (50,000 UI), Vit E (437 UI).

^b G0 = Diet without crude glycerin, G10 = Diet with 10% crude glycerin, G20 = Diet with 20% crude glycerin, G30 = Diet with 30% crude glycerin.

The crude glycerin used in this trial contained 95% DM, 83% glycerol, 1.1% CP, 6% salts, 4.8% other compounds and less than 0.01% methanol. The concentrate and corn silage were weighed and mixed with crude glycerin at the moment of feeding (0700 and 1900 h), delivering 50% of total mixed ration in each meal. Before subsequent feeding, orts were weighed and approximately 10% of each animal were sampled to determine DM to adjust feed delivery and to monitor daily dry matter intake.

2.2. Dry matter intake, rumen pH, ammonia nitrogen, and VFA profiles

The concentrate and corn silage were weighed and mixed with crude glycerin at the moment of feeding, delivering 50% of total in each meal. Before subsequent feeding, samples of orts of each animal were collected to monitor dry matter daily intake.

Rumen fluid samples were collected on d 15 of each experimental period, at 0, 2, 4, 6, 8, 10, and 12 h after feeding to measure pH, and evaluate ammonia nitrogen ($\text{NH}_3\text{-N}$) and volatile fatty acids (VFA) concentrations. Approximately 500 g of ruminal contents of each animal were collected from the dorsal and ventral rumen sites, and strained through four layers of cheesecloth to separate liquid and solid phases. The pH was measured immediately after rumen fluid sampling using a digital pH meter (model Digimed DM-20; Digicrom Analítica Ltda, São Paulo, SP Brazil), and $\text{NH}_3\text{-N}$ concentrations was determined using a micro-Kjeldhal apparatus (model TE-0364; Tecnal Equip. para Laboratórios, Piracicaba, SP, Brazil), with 5 mL of KOH 2N, and a distillation flux of 2 mL/min. Samples were centrifuged at $3,000 \times g$ for 20 min, and the supernatant was used to determine $\text{NH}_3\text{-N}$. The distilled sample was dropped in 10 mL boric acid solution (2%), and then titrated with HCl 0.005N.

Approximately 2.0 mL of rumen fluid was centrifuged twice ($12,000 \times g$ for 15 min at 4°C (Sorvall Superspeed RC2-B, Newton, CT, USA) with formic acid 98–100% (Merck KGaA). After centrifugation, approximately 0.5 mL of supernatant was transferred to chromatographic vials. The concentration of VFA was determined by injecting 0.5 μL of sample in a gas chromatograph (TRACE 1300, Thermo Scientific, MA, USA) equipped with a HP-FFAP capillary column (19091F-112; 25 m; 0.320 mm; 0.50 μm ; J&W Agilent Technologies Inc.; Palo Alto, CA, USA). The carrier gas was helium at a flow rate of 1 mL/min.

The oven temperature programme was 1 min at 60°C, followed by an increase to 200°C at a rate of 5°C/min. The injector temperature was 270°C, and the detector temperature was 300°C. The sample was injected into a split/splitless system (split ratio 1:10). The calibration curve was made using chromatographic standards (Chem Service, West Chester, PA, USA) of acetic acid (99.5%; CAS 64-19-97), propionic acid (99%; CAS 79-09-4), isobutyric acid (99%; CAS 79-31-2), butyric acid (98.7%; CAS 107-92-6), isovaleric acid (99%; CAS 503-74-2), and valeric acid (99%; CAS 109-52-4).

2.3. Gas measurements

At d 19 of each experimental period, approximately 500 g of ruminal content were collected from each animal, strained through eight layers of cheesecloth, placed into pre-heated thermos (39°C), and transported to São Paulo State University Ingredients and Pollutant Gases Laboratory. The strained ruminal fluid was gassed with O₂-free gas, and mixed with McDougall's buffer, in a ratio of 1:2. Substrates consisted of the same TMR finishing diets G0, G10, G20, and G30 from previous trial. The diets were ground through a 1-mm mesh screen, and 200 mg were used in each penicillin-type 100-mL glass flask. Fifteen fermentation flasks of each substrate received 20 mL of McDougall's buffer and 20 mL of rumen fluid and six blank flasks were prepared without any target substrate. The flasks were purged with O₂-free gas, capped with rubber seals, and immediately placed in a 39°C pre-warmed orbital shaker (SL 222, Solab, Piracicaba, SP, Brazil), equipped with a polystyrene foam structure to hold bottles during the shaking procedure. The incubation was held for 24 h and, at the end, the flasks were chilled in an ice bath to cease microbial activity. The head-space gas pressure was measured using a digital pressure meter equipped with a pressure transducer (Theodorou et al., 1994). Gas pressure was transformed in gas volume using a methodology adapted from Bueno et al. (2005).

The fermentative gasses were sampled from the flasks after 24 h of incubation to determine concentrations of CO₂ and CH₄, using a gas chromatograph (Trace GC Ultra™, Thermo Scientific, San Jose, CA USA). The GC was equipped with a Porapak column and molecular sieve. The oven

temperature was set to 70°C, and the injector temperature used was 110°C. The carrier gas used was argon, with 25 mL/min flow.

After the 24-h incubation period, the samples' residues were centrifuged (500 × g, 10 min), washed three times with deionized H₂O and dried (105°C for 16 h) to calculate DM disappearance.

2.4. *In situ* ruminal degradability

In situ ruminal degradability of DM and NDF was determined using the methodology proposed by Orskov and McDonald (1979). Samples of diets were pre-dried (55°C for 72 h), ground (silage = 5 mm and concentrate = 2 mm), and 2 g of total mixed ration were placed into nylon bags (5 × 10 cm, 50µm, Ankom Technology, Macedon, NY). Bags were heat-sealed and reserved until incubation. Each sample was incubated in two replicates along with a blank bag for each incubation time in the rumen of cannulated sheep. The bags were placed into a nylon mesh bag (20 × 30 cm) tied with rubber band and pre-soaked in tap water. The incubation times used in this trial were 0, 3, 6, 12, 24, 48, 72 and 96 h. The incubation was started with time "96 h" and was finished with time "0 h", to remove all bags at the same time at the end of the trial. After the incubation, the bags were removed from the mesh bag and dipped in ice-cold water to cease ruminal microorganisms' activity. Then, bags were rinsed and manually washed until the water becomes clear, and were dried at a forced-air oven (55°C for 72 h). After being weighed, the bags were opened, the samples were ground in micro mill (1-mm sieve), and analyzed for DM (AOAC, 2005; method 967.03) and NDF (Goering and Van Soest, 1970).

The DM and NDF degradation kinetics were fitted into the equation: $D = a + b(1 - e^{-ct})$, where "D" = ruminal degradability at time "t", "a" = the soluble fraction in water, "b" = the insoluble and potentially degradable fraction, "c" = the degradation rate of fraction "b" per hour, and "t" = the time of incubation. The *in situ* effective degradability (ED) of DM and NDF was estimated by the equation: $ED = a + b \times [Kd / (Kd + Kp)]$, where "a", "b" = the degradation constants described previously, "Kd" = constant rate of degradation of fraction "b", and "Kp" = the passage rate from the rumen. Degradation constants were estimated using the NLIN procedure of SAS (Version 9.4).

2.5. *In vitro* total tract digestibility

In vitro digestibility of DM and NDF was assessed using the methodology proposed by Holden (1999). On d 21 of each experimental period, approximately 1 kg of ruminal content were collected from each animal, strained through eight layers of cheesecloth, placed into pre-heated thermos (39°C), and transported to São Paulo State University Ingredients and Pollutant Gases Laboratory. The ruminal fluid from animals from the same treatment were mixed prior to incubation.

Ankom Daisy^{II} fermenter (ANKOM Technology Corp., Macedon, NY) was used to evaluate *in vitro* digestibility of DM and NDF. Ankom F57 filter bags (n = 25; 24 with samples and 1 blank; ANKOM Technology Corp., Macedon, NY) were filled with substrates (1-mm ground; 0.5 g), heat-sealed and placed into fermentation jars. A solution composed of 400 mL of rumen fluid, 1330 mL of buffer A (10.0 g/L KH₂PO₄, 0.5 g/L MgSO₄.7H₂O, 0.5 g/L NaCl, 0.1 g/L CaCl₂.2H₂O and 0.5 g/L urea) and 266 mL of buffer B (15.0 g/L Na₂CO₃ and 1.0 g/L Na₂S.9H₂O) was prepared and placed into fermentation jars. The containers were purged with CO₂, and placed into the pre-heated (39°C) Daisy^{II} fermenter. After 48-h incubation, 40 mL of 6 N HCl and 8 g of pepsin (1:10,000) were added to each digestion jar, and incubated for another 24-h period. The filter bags containing substrates' residues were rinsed and manually washed and dried. Substrates and residues were evaluated for DM (AOAC, 2005; method 967.03) and NDF (Goering and Van Soest, 1970) contents, to calculate DM and NDF *in vitro* digestibilities.

2.6. Statistical analysis

Data were analyzed as a replicated 4 × 4 Latin square design using PROC MIXED of SAS version 9.4 (SAS Institute Inc., Cary, NC). The fixed effect consisted of treatment, and random effects consisted of sheep and period. The statistical model for the trial was as follows: $Y_{ijkl} = \mu + \alpha_i + \beta_j + \gamma_{k(j)} + \lambda_{l(j)} + \alpha\beta_{(ij)} + \varepsilon_{ijkl}$, where Y_{ijkl} = value observed in the plot for treatment i, Latin square j, period k, and cow l; μ = overall mean; α_i = effect of treatment i; β_j = effect of Latin square j; $\gamma_{k(j)}$ = effect of period k within Latin square j; $\lambda_{l(j)}$ = effect of cow l within Latin square j; $\alpha\beta_{(ij)}$ = effect of the interaction treatment i and Latin square j; and ε_{ijkl} = residual error.

Data of pH, NH₃-N, and VFA were considered as repeated measures. Several covariance structures were tested and the best one was chosen for each variable, based on Akaike information criterion. The degrees of freedom and tests were adjusted using the option KR. Treatment effects were tested using the following contrasts: (1) linear effect of crude glycerin concentration, (2) quadratic effect of crude glycerin level, and (3) control treatment × glycerin treatments (effect of the addition of two concentrations of crude glycerin combined *versus* treatment without addition of the byproduct). Values were reported as least squares means and associated standard errors, and significance was defined as $P < 0.05$ and trends as $0.05 \leq P \leq 0.10$.

3. Results

3.1. Dry matter intake, rumen pH, ammonia nitrogen, and VFA profiles

The inclusion of crude glycerin in the diets promoted a tendency for a quadratic effect in DMI ($P = 0.09$), with greater values observed for treatments with 10 and 20% of the by-product (Table 2).

There was no interaction of time of sampling × treatment for the variables pH, NH₃-N and VFA ($P > 0.05$). Thus, only the main effects of treatment and time of sampling were evaluated. The ruminal pH tended to increase ($P = 0.07$) with the increasing inclusion of crude glycerin in the diets. There was also a tendency for a quadratic effect on the concentration of NH₃-N ($P = 0.06$), with the lowest values observed for treatments with 10 and 20% of the by-product (Table 2).

The increasing inclusion of crude glycerin linearly reduced the total molar concentration of VFA ($P < 0.0001$), as well as the molar concentrations of acetic ($P < 0.0001$), butyric ($P = 0.0004$), isobutyric ($P = 0.0007$) and isovaleric ($P = 0.003$) acids (Table 2). No alterations on propionic and valeric acid were observed ($P > 0.10$), however the ratio C2:C3 was linearly reduced when the crude glycerin was included ($P < 0.0001$).

Table 2. Dry matter intake and rumen parameters of sheep fed increasing concentrations of crude glycerin.

Item (mM/L)	Treatments ^a					Contrast, <i>P</i> -value ^b		
	G0	G10	G20	G30	SE	L	Q	0 × G
DMI, g	1237	1375	1337	1205	135	0.69	0.09	0.46
pH	5.97	6.07	6.13	6.16	0.1	0.07	0.68	0.09
NH ₃ -N, mg/dL	11.0	9.6	10.2	11.9	0.9	0.34	0.06	0.63
Total	40.1	37.2	27.6	23.8	6.1	<0.0001	0.86	0.001
Acetic (C2)	25.9	22.4	14.3	11.5	3.8	<0.0001	0.98	0.0005
Propionic (C3)	8.6	9.0	8.8	8.2	1.7	0.61	0.44	0.94
Butyric	4.3	4.0	3.3	2.9	0.61	0.0004	0.89	0.006
Isobutyric	0.26	0.22	0.17	0.15	0.05	0.0007	0.35	0.002
Valeric	0.47	0.49	0.52	0.57	0.13	0.21	0.88	0.37
Isovaleric	0.73	0.65	0.56	0.56	0.11	0.003	0.36	0.006
C2:C3	2.8	2.5	1.7	1.5	0.18	<0.0001	0.81	<0.0001

^a G0 = Diet without crude glycerin, G10 = Diet with 10% crude glycerin, G20 = Diet with 20% crude glycerin, G30 = Diet with 30% crude glycerin.

^b L = Linear, Q = Quadratic, 0 × G = Treatment without crude glycerin × glycerin treatments.

3.2. Gas measurements

The increasing inclusion of crude glycerin linearly decreased the total gas and CO₂ production (mL/g degraded, *P* = 0.01, Table 3). When both treatments with crude glycerin were compared with G0, a tendency for decreased CO₂ (mL/g degraded) production was observed (*P* = 0.09), with the lowest value for G20 treatment. There were tendencies for linear reduction of CO₂ (mL/g) and CH₄ (mL/g degraded) production (*P* = 0.07 and *P* = 0.09, respectively) with increasing inclusion of crude glycerin in the diets.

Table 3. Gas production and quality of in vitro cultures containing increasing concentrations of crude glycerin.

Item*	Treatments ^a					Contrast, <i>P</i> -value ^b		
	G0	G10	G20	G30	SE	L	Q	0×G
Total, mL/g	155.5	153.8	151.1	137.9	8.9	0.13	0.48	0.40
CH ₄ , mL/g	34.4	34.0	34.0	32.5	4.1	0.55	0.82	0.71
CO ₂ , mL/g	114.4	113.2	110.7	99.6	6.5	0.07	0.39	0.32
Total, mL/gd	267.8	258.2	250.8	220.2	14.0	0.01	0.44	0.11
CH ₄ , mL/gd	59.7	57.7	57.0	52.2	7.0	0.09	0.71	0.36
CO ₂ , mL/gd	198.1	192.0	185.7	160.1	11.0	0.007	0.31	0.09

^a G0 = Diet without crude glycerin, G10 = Diet with 10% crude glycerin, G20 = Diet with 20% crude glycerin, G30 = Diet with 30% crude glycerin. ^b L = Linear, Q = Quadratic, 0×G = Treatment without crude glycerin × glycerin treatments.

*mL/g = production in mL per gram, mL/gd = gas production in mL per gram degraded.

3.3. *In situ* ruminal degradability

The increasing inclusion of crude glycerin resulted in a linear increase of soluble fraction (“a”) of DM of the diets ($P < 0.0001$, Table 4). Concomitantly, a linear decrease of insoluble but *potentially* degradable fraction (“b”) of DM of the diets ($P < 0.0001$) was observed with increasing concentrations of crude glycerin.

The DM degradation rate of the diets was not influenced by the inclusion of crude glycerin in the diets. However, the potential degradation ($P < 0.0001$) and the effective degradation, considering a passage rate of 2%/h ($P < 0.0001$), 5%/h ($P < 0.0001$) and 8%/h ($P < 0.0001$) were markedly and linearly increased.

The fraction “a” of NDF of the diets was linearly increased ($P < 0.0001$), while the fraction “b” was linearly increased ($P < 0.01$), ranging from 42.7% in control treatment to 52% in treatment with 30% crude glycerin (Table 4).

The NDF degradation rate was decreased in treatments containing crude glycerin compared with control treatment (0 × G, $P = 0.01$). There was a tendency for increase of the potential degradation of NDF ($P = 0.06$), although

the effective degradation rates (2, 5 e 8%/h) were not altered by the inclusion of crude glycerin ($P > 0.10$).

Table 4. *In situ* degradation parameters of DM and NDF of diets containing increasing concentrations of crude glycerin in sheep.

Item ^a	Treatments ^b					Contrast, <i>P</i> -value ^c		
	G0	G10	G20	G30	SE	L	Q	0 × G
Dry matter								
a, %	34.6	38.3	48.2	58.9	2.0	<0.0001	0.18	<0.0001
c, %	17.7	14.1	12.4	11.7	1.9	0.0001	0.12	0.0002
b, %	47.4	47.6	39.3	29.4	2.3	<0.0001	0.13	0.002
kd, %/h	3.5	3.1	3.0	3.2	0.4	0.48	0.27	0.25
DP, %	80.0	82.6	84.5	86.5	2.4	0.0001	0.75	0.0008
DE2, %	64.7	66.6	71.1	76.9	2.4	<0.0001	0.13	0.0001
DE5, %	54.0	56.3	62.6	70.5	2.3	<0.0001	0.17	<0.0001
DE8, %	48.9	51.5	58.8	67.6	2.1	<0.0001	0.16	<0.0001
Neutral detergent fiber								
a, %	14.3	14.7	15.2	15.8	0.07	<0.0001	0.23	<0.0001
c, %	43.0	34.9	32.6	32.3	6.0	0.004	0.12	0.002
b, %	42.7	50.4	52.3	52.0	6.0	0.01	0.11	0.004
kd, %/h	3.6	2.5	2.5	2.6	0.5	0.05	0.09	0.010
DP, %	54.5	59.3	61.4	60.4	6.5	0.10	0.27	0.061
DE2, %	40.7	42.4	43.7	43.3	4.7	0.36	0.66	0.35
DE5, %	31.4	31.6	32.4	32.5	3.4	0.62	0.99	0.73
DE8, %	27.1	26.9	27.6	27.8	2.6	0.64	0.88	0.82

^a a = soluble, b = insoluble but potentially degradable, c = undegradable, kd = fermentation rate, DP = potential degradability, DE2, 5, 8 = effective degradability for passage rates of 2, 5 e 8%/h.

^b G0 = Diet without crude glycerin, G10 = Diet with 10% crude glycerin, G20 = Diet with 20% crude glycerin, G30 = Diet with 30% crude glycerin.

^c L = Linear, Q = Quadratic, 0 × G = Treatment without crude glycerin × glycerin. treatments.

3.4. *In vitro* total tract digestibility

The increasing inclusion of crude glycerin linearly increased the *in vitro* DM digestibility of diets ($P = 0.01$, Figure 1), and linearly reduced NDF digestibility ($P = 0.008$).

When all treatments with crude glycerin were evaluated against the control one ($0 \times G$), the same trend was observed for DM and NDF ($P = 0.007$ and $P = 0.006$, respectively). In average, DM and NDF digestibility's in treatments with crude glycerin were, respectively, 81.7 and 37.2%, against 73.6 and 51.5% for control treatment. Additionally, no effect of treatments was observed for *in vitro* digestibility of OM or CP ($P > 0.10$).

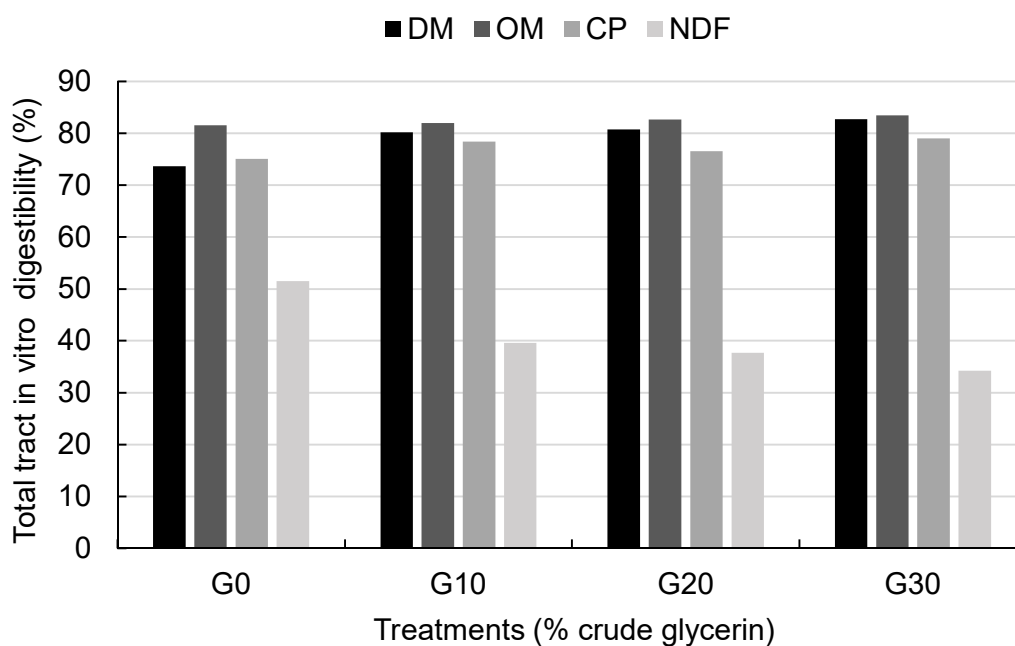


Figure 1. *In vitro* total tract digestibility of experimental diets in sheep fed increasing concentrations of crude glycerin. *In vitro* dry matter digestibility (IVDMD), SE = 0.5, $P_{\text{linear}} = 0.01$, $P_{0 \times G} = 0.007$; *In vitro* organic matter digestibility (IVOMD), SE = 1.2; *In vitro* crude protein digestibility (IVCPD), SE = 3.2; *In vitro* neutral detergent fiber digestibility (IVNDFD), SE = 3.8, $P_{\text{linear}} = 0.008$, $P_{0 \times G} = 0.006$; G0 = Diet without crude glycerin, G10 = Diet with 10% crude glycerin, G20 = Diet with 20% crude glycerin, G30 = Diet with 30% crude glycerin.

4. Discussion

4.1. Dry matter intake, rumen pH, ammonia nitrogen, and VFA profiles

High concentrations of crude glycerin change the physical form of the diets, which it becomes more viscous due to crude glycerin high viscosity (approximately 8.5 cs, Thompson and He, 2006). At some point, it could help aggregation of particles and facilitate the feed intake. However, in practice, inclusions of more than 20% of crude glycerin, especially in tropical conditions, do not appear to be a good strategy because the by-product becomes more liquid, resulting in accumulation of the ingredient in the bottom of the feed bunk.

The quadratic effect observed for DMI agrees with the study conducted by Parsons et al. (2009), who fed beef heifers 0, 2, 4, 8, 12, or 16% crude glycerin and observed increased DMI for intermediate levels of crude glycerin and a decrease when 16% of the by-product were added. However, Chanjula et al. (2014) feeding goats up to 20% crude glycerin, and Gomes et al. (2011), feeding lambs up to 30% crude glycerin did not find any difference in DMI of experimental animals. On the other hand, in some studies a drop in DMI is observed, even using low concentrations of crude glycerin for beef cattle and goats (Schneider, 2010; Chanjula et al., 2016). This discrepancy in DMI is probably due to differences in crude glycerin used, as well as the roughage:concentrate ratio, amount of dietary starch and fiber and the tolerance and acceptability of the ingredient by each animal species.

The tendency of linearly increased ruminal pH observed in present trial agrees with Polizel et al. (2013), who fed lambs up to 20% crude glycerin in high concentrate diets and also observed a linear increase in rumen pH values. The increase occurred probably due to the linear decrease observed in total VFA concentrations (40.1 in G0 and 23.8 mM/L in G30). However, in most of findings reported from studies using high concentrations of crude glycerin (up to 20%), authors did not observe alterations of rumen pH values (Chanjula et al., 2014; Favaro et al., 2015). Low ruminal pH may decrease DMI, fiber digestibility, and microbial yield and thus decrease animal production (Allen et al., 1996).

Although the tendency for a quadratic effect of rumen NH₃-N concentrations, only the treatment containing 10% crude glycerin (9.6 mg/dL) did not reach the minimum value considered for the maximization of ruminal

digestion which is 10 mg/dL, according to Leng (1990). Previous studies did not report differences in rumen NH₃-N concentrations, when 10.8 (Abo El-Nor et al., 2010) or 21% (Avila et al., 2011) crude glycerin were used in *in vitro* trials. The NH₃-N concentrations may be also related to the increased level of urea, which was greater with the increasing inclusion of crude glycerin in the diets (Table 1).

The effects of crude glycerin inclusion on total and individual VFA are significant. Although no changes in molar concentrations of propionic and valeric acids were observed, the proportion of both VFA in relation to total VFA was notably altered (from 21.4% to 34.4% for propionic acid and from 1.2 to 2.4% for valeric acid). Total VFA decrease was also observed by Polizel et al. (2013), but it was not observed by Ramos and Kerley (2012), feeding continuous fermenters, nor by Chanjula et al. (2014), working with goats.

Although the absolute yield of propionate was not affected by the increasing inclusion of crude glycerin, a linear increase in the proportion of propionate was observed. The change in the relative proportion of propionate was, probably, due to fermentation characteristics of glycerin, which is fermented, mainly by bacteria of genus *Selenomonas* and it is mostly used by animals in the first 4 or 6 h after ingestion (Ferraro et al., 2009; Mach et al., 2009). On the other hand, the linear decrease in acetic acid concentration contributed to the linear decrease in C2:C3 ratio, and was due, mainly, to the deleterious effect of crude glycerin on rumen fibrolytic microorganisms, as described by Abo El-Nor et al. (2010) and confirmed by the decrease in fiber fraction *in vitro* total tract digestibility observed in the present study. Previous studies, using even low concentrations of crude glycerin fed to dairy cows reported commensurable results (Boyd et al., 2013).

4.2. Gas measurements

The linear reduction of total gas (mL/g degraded) and CO₂ production (mL/g and mL/g degraded) observed with increasing inclusion of crude glycerin is probably due to the lower ruminal fermentation and VFA formation of glycerin treatments (Table 2). According to Owens and Basalan (2016), the VFA formation is directly associated with the amount of gas production in the rumen, as feed fermentation results in VFA, as well as CO₂ and H₂.

Previous studies have shown that the total gas production, resulted from crude glycerin inclusion in the diets, is reduced mainly by the reduction of ruminal CO₂ (Lee et al., 2011; van Cleef et al., 2015). Homem Junior et al. (2017) evaluated *in vitro* gas production of different feed ingredients and observed that crude glycerin promoted less gas compared with corn grain, citrus pulp, soybean hulls and corn silage, mainly by the lowest ruminal CO₂ production. In the present trial, the production of CO₂ (mL/g degraded) were 3.1 (G10), 6.3 (G20), and 19.2% (G30) lesser than the treatment without crude glycerin, reflecting in lesser values of total gas production for glycerin treatments.

The tendency for a linear reduction of CH₄ production (mL/g degraded) observed can be due to a detrimental effect of crude glycerin on the growth of structural carbohydrate fermenting bacteria (AbuGhazaleh et al., 2011), resulting in reduced fiber digestibility and CH₄ production, when the by-product is fed at up to 10 or 30%, respectively to dairy or beef cattle (Shin et al., 2012; van Cleef et al., 2015). And the linear reduction of molar concentration of acetic acid in the rumen, observed in this trial, support that hypothesis (Table 2).

4.3. *In situ* ruminal degradability

The increase observed in fraction “a” of dry matter of the diets could be explained by the high solubility of crude glycerin, as reported by Donkin (2008). Thus, increasing a highly soluble substrate, it is expected to increase soluble fraction of DM of total diet, and consequently, to decrease, proportionally, its undegradable fraction. In addition, with the inclusion of crude glycerin in the diets, the concentration of acid detergent fiber (ADF) of experimental diets linearly decreased, ranging from 18.2% in control treatment (without crude glycerin addition), and 15.5% for G30. This decrease was due to the removal of corn cracked grain (4.5% ADF) and of soybean hulls (50% ADF), with the concomitant addition of crude glycerin, which does not contain any fiber in its composition.

Previous study evaluating degradation of OM and NDF reported no differences, when crude glycerin was added at up to 12% in diets for lambs *in vitro* (Peripolli et al., 2014). However, even at low concentration crude glycerin seems affect *in situ* ruminal degradation parameters of steers. Wang et al.

(2009), reported a quadratic effect on the degradation rate of fraction “b” of DM and NDF with increasing concentration of crude glycerin supplementation for steers.

In the present study, the linear increase of fraction “a” of NDF was probably due to the increasing contribution, in percentage, of soybean meal in the glycerin diets, which presents a NDF solubility of around 32% (Valadares Filho et al., 2017).

The tendency of increased potential degradation and the lack of effects in effective degradation are against the premise that crude glycerin promotes a negative effect on the digestibility and ruminal degradability of the fibrous fraction of the diets and ingredients, when the animals were fed crude glycerin as energy source (Abo El-Nor et al., 2010; D'Aurea, 2010; Schneider, 2010; van Cleef, 2012; van Cleef et al., 2014), and it might be also that the high solubility of the crude glycerin compensates for the decrease in fiber degradation, which ends up with higher total degradability of the diet.

4.4. *In vitro* total tract digestibility

The linear increase in IVDMD obtained in this trial disagrees with results reported by Abo El-Nor et al. (2010), which fed *in vitro* fermenters up to 10% crude glycerin and did not observe any difference in this variable. Chanjula et al. (2014) feeding up to 20% crude glycerin to goats, also did not observe effects on DM digestibility. However, most of results in literature are discrepant due to the differences in feed ingredients, diets, and methods of analysis used.

Wang et al. (2009) fed steers low concentrations of glycerol (up to 3.3%) and observed a linear increase of OM and CP total tract digestibilities. These authors suggest that glycerol modulates the digestive microorganisms or enzymes in a dose-dependent manner, promoting changes in ruminal environment and, consequently, the digestibility of nutrients.

The decreased NDF *in vitro* total tract digestibility observed in glycerin treatments is consistent with results reported previously when beef cattle were fed crude glycerin at up to 30% (van Cleef et al., 2014), and even at low concentrations, as demonstrated by Parsons and Drouillard (2010), which included up to 4% in diets for beef heifers and observed linear reduction of fiber digestibility.

The same effect was observed when dairy cows were fed up to 15% glycerin (Donkin et al., 2009; Shin et al., 2012). Glycerin inclusion reduces the number of microorganisms involved in fiber digestion (Roger et al., 1992), promoting a reduced acetic acid concentration in the rumen, as observed in present trial. However, the mechanisms involved are not completely clarified, and are probably related to changes in rumen osmolarity, the physical protection of the feed particles, or the competition or preference for another substrate by microorganisms, and in this case the glycerol (van Cleef et al., 2014).

5. Conclusions

The replacement of corn cracked grain by crude glycerin (up to 30% DM) changes rumen fermentation parameters, decreasing VFA production, *in vitro* total gas production and CH₄. Additionally, the potential and effective degradation as well as the *in vitro* DM digestibility of diets are improved, while fiber digestibility is impaired.

Conflicts of interest

The authors declare no known conflict of interest.

Acknowledgment

Authors thank the undergraduate students and staff from Animal Unit of Digestive and Metabolic Studies from the Department of Animal Science of São Paulo State University for animal care, Caramuru Alimentos Inc. for the donation of part of ingredients used in this research, and Fundação de Amparo à Pesquisa do Estado de São Paulo for the scholarships provided (Grant numbers: 2013/18118-8 and 2015/04595-4).

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CAPÍTULO 5 – Implicações

O Brasil é um grande produtor de resíduos, subprodutos e coprodutos agroindustriais, fato que torna o uso destes viáveis economicamente, principalmente durante as épocas de alta nos preços dos ingredientes comumente utilizados na alimentação animal, como o milho e a soja. Neste sentido, avaliar o uso da glicerina bruta, coproduto do biodiesel, em substituição ao milho, principalmente como ingrediente capaz de reduzir distúrbios metabólicos, torna-se interessante.

Os experimentos realizados pela equipe da Professora Dra. Jane Maria Bertocco Ezequiel têm demonstrado que o uso de glicerina bruta em dietas para ruminantes é possível e viável em diversas espécies e categoria animal, não alterando o desempenho dos animais e, por vezes, reduzindo o custo da produção através da redução do consumo sem alterar o ganho de peso dos animais, o que torna este ingrediente muito interessante em períodos de alta nos preços dos grãos. Contudo, para evitar o confundimento e interpretações equivocadas, se torna imprescindível o conhecimento da origem e composição nutricional da glicerina bruta a ser utilizada, lembrando que existe um padrão regulamentado pelo MAPA autorizando a venda deste coproduto somente com altas concentrações de glicerol (maiores que 80%) e baixos níveis de contaminantes.

Neste trabalho, a ideia inicial foi realizar abates periódicos durante as diferentes etapas do período experimental (pré-adaptação, adaptação e terminação dos animais), visando acompanhar *in locu* a morfologia do rúmen associando-a com dados de desempenho e metabolismo ruminal frente à dieta alto concentrado (70%) na presença ou ausência de glicerina bruta. Assim, este estudo permitiria indicar a melhor inclusão ou não deste coproduto em ambos os períodos do confinamento ou somente em um específico.

De acordo com os resultados apresentados neste estudo, a inclusão de 10% MS de glicerina bruta parece ser a estratégia mais interessante para o período de terminação dos animais, pois promoveu maior desempenho em confinamento. Em relação ao período inicial de adaptação dos animais, como não foram observadas nenhuma manifestação clínica resultante de acidose ruminal (como abscesso hepático, ruminite e lesões na mucosa ruminal) para

nenhum dos tratamentos, concluímos que todas as dietas foram eficazes na adaptação dos animais e recomendamos a inclusão de qualquer nível estudado (10, 20 ou 30 % de glicerina bruta na MS da dieta final).

Outro fato interessante deste coproduto é a sua característica aglutinante com ação na redução do pó das dietas, principalmente quando utilizado no momento do preparo do concentrado na fábrica de ração. Porém, baixas inclusões devem ser utilizadas devido a sua forma líquida, pois inclusões acima de 10% podem empaçocar os equipamentos.

Dependendo do preço de mercado de glicerina bruta, este coproduto pode fornecer resultados econômicos favoráveis, principalmente no período de adaptação. E, apesar do maior tempo de permanência observado para as maiores inclusões, poderia valer a pena economicamente devido ao menor custo da dieta final.