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CENTRO DE CIÊNCIAS AGRÁRIAS, AMBIENTAIS E BIOLÓGICAS

PROGRAMA DE PÓS-GRADUAÇÃO EM CIÊNCIA ANIMAL

CURSO DE MESTRADO

GLICERINA DE MÉDIA PUREZA NA TERMINAÇÃO DE BOVINOS EM CONFINAMENTO

CARLOS EMANUEL EIRAS

CRUZ DAS ALMAS - BAHIA

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Orientadora: Prof. Dr.ª Larissa Pires Barbosa
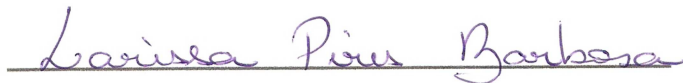
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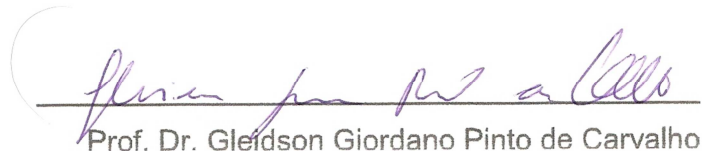COMISSÃO EXAMINADORA DA DEFESA DE DISSERTAÇÃO DE

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CRUZ DAS ALMAS-BAHIA

FEVEREIRO-2013

Veni, vidi, vici

(Júlio César - 47 a.C)

DEDICATÓRIAS

A

Deus, pai de infinita bondade, por nunca me abandonar nos dias bons e, principalmente, nos dias ruins.

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Dedico a vocês essa vitória

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GLICERINA DE MÉDIA PUREZA NA TERMINAÇÃO DE BOVINOS EM CONFINAMENTO

Autor: Carlos Emanuel Eiras
Orientadora: Larissa Pires Barbosa

RESUMO: Objetivou-se avaliar o efeito da substituição do milho pela glicerina de média pureza sobre o desempenho, eficiência alimentar, digestibilidade, comportamento ingestivo, características de carcaça e qualidade de carne de bovinos terminados em confinamento. Foram utilizados 40 bovinos machos não castrados da raça Purunã, com peso corporal médio de 208,8 ± 33,3 kg e 8 ± 0,9 meses de idade. Os tratamentos foram distribuídos em delineamento inteiramente casualizado, sendo os níveis de glicerina testados (0, 6, 12 e 18% da MS) correspondentes a substituição de 18,3; 38,5 e 61,3% do teor de milho da dieta. A silagem de milho foi utilizada como volumoso único na proporção de 53% da dieta e o concentrado formulado a partir de farelo de soja, milho grão moído, glicerina e sal mineral e fornecido na proporção de 47% da dieta. A adição de glicerina na dieta não alterou o desempenho animal e rendimento de carcaça. Entretanto, houve redução na ingestão de MS (% PV), EE, FDN (kg e % PV), CHT, CNF e conversão alimentar. Os coeficientes de digestibilidade aparente aumentaram, com exceção do EE e FDN. As dietas com glicerina alteraram a duração das atividades comportamentais. A frequência de ruminação foi reduzida linearmente, enquanto a frequência de outras atividades apresentou efeito quadrático. Houve redução na duração das frequências de alimentação. Entretanto, a duração das frequências de outras atividades aumentou linearmente. As características físicas de carcaça, marmoreio, textura, coloração, perdas ao descongelamento e à cocção, análise sensorial e composição química do músculo *Longissimus* não foram alteradas. Os ácidos graxos saturados (AGS) e a *n-3:n-6* foram reduzidos. Os ácidos graxos insaturados (AGPI), *n-3, n-6* e AGS:AGPI aumentaram linearmente em função dos níveis de glicerina. A glicerina de média pureza é uma fonte energética alternativa para a substituição parcial da dieta total (18% da MS), principalmente do milho (61,3% da MS), na terminação de bovinos em confinamento.
Palavras-chave: glicerol, ruminante, super precoce.

MEDIUM PURITY GLYCERINE IN THE TOTAL DIET REPLACEMENT OF BULLS FINISHED IN FEEDLOT

Author: Carlos Emanuel Eiras
Orientated by: Larissa Pires Barbosa

ABSTRACT: This work was carried out to study the medium purity glycerin as a corn replacement on animal performance, feed efficiency, apparent digestibility, ingestive behavior, carcass characteristics and meat quality in crossbreed bulls finished in feedlot. Fourty Purunã bulls with 208.8 ± 33.3 kg weight and 8 ± 0.9 months old. The bulls were randomly assigned to 1 of 4 diets containing 0, 6, 12 or 18% glycerine on a DM basis; which represented 18.3; 38.5; and 61.3% of corn replacing. The corn silage (53%) and concentrate mix (soybean meal, cracked corn, glycerin and mineral salt) composed the diets. The corn replacement did not affect the animal performance and carcass characteristics. However, intake of DM (%BW), EE, NDF (kg and %BW), total carbohydrates and feed conversion rates decreased linearly a glycerine levels. The apparent digestibility of nutrients increased linearly with increasing glycerin in the diet, with the exception of EE and NDF. Glycerine changed durations of the bull's behavioral activities. The rumination frequency was reduced linearly with glycerine inclusion in the diet. However, frequency of other activities showed a quadratic effect with glycerine addition. Glycerine inclusion in the diet reduced duration in feed frequency, but the frequency duration for other activities increased linearly. The carcass traits, marbling, texture, color, thawing and cooking loss, sensory analysis and chemical compounds of *Longissimus* muscle were not modified by different glycerine levels in the diets. The saturated fatty acids (SFA) and *n-6:n-3* ratio decrease with glycerine levels. On the other hand, unsaturated fatty acids (PUFA), *n-6* and *n-3* fatty acids, PUFA:SFA ratio increase in muscles of bulls fed on diets with glycerine. The medium purity glycerine is an energetic alternative to total diet replacement (18% of DM), principally the corn replacement (61.3% of DM), on crossbreed bulls finished in a feedlot.

Keywords: glycerol, ruminant, young bull.

INTRODUÇÃO

A utilização do sistema de confinamento para a terminação de bovinos permite controlar e suprimir o déficit de nutrientes encontrado em períodos de baixa disponibilidade de forragem (Restle & Vaz, 1999). O sistema intensivo de produção aumenta a participação de bovinos precoces e super precoces no mercado da carne, elevando o retorno do capital investido devido à redução da idade de abate dos animais (Ito et al., 2010) e a distribuição de carcaças bem acabadas ao longo do ano (Arrigoni, 2003).

No entanto, o sistema de confinamento apresenta maior custo de produção em relação aos demais sistemas produtivos devido à necessidade de aumentar a densidade energética da ração, podendo atingir até 70% do custo total de produção (Restle & Vaz, 1999).

As dietas utilizadas na alimentação de ruminantes em confinamento, normalmente são compostas por grãos de cereais que proporcionam excelentes fontes de amido para o crescimento e manutenção dos microrganismos ruminais (NRC, 2000). O milho (*Zea mays* L.) é utilizado em larga escala na formulação de dietas para animais de produção devido a sua elevada concentração de amido (69 %), que pode atender aproximadamente 85% das necessidades energéticas dos bovinos (Van Soest, 1994).

Segundo ANUALPEC (2012), neste ano, aproximadamente 68,5% (38,7 milhões de toneladas) da demanda interna nacional de milho grão foi destinada a alimentação animal. O maior consumo de milho dentre os setores da cadeia produtiva da carne destinaram-se a avicultura, suinocultura e bovinocultura, sendo produzidos aproximadamente 62 milhões de toneladas de ração (SINDIRAÇÕES, 2012). Segundo a ABIMILHO (2012) o baixo uso de substitutos ao milho na alimentação animal favorece o aumento no consumo de milho por parte da indústria de carnes, justificando o aumento observado no preço da saca de milho (87%) dos últimos cinco anos (ANUALPEC, 2012).

Assim, a terminação de bovinos em confinamento exige estudos sobre o uso de possíveis fontes de energia que permitam substituir a dieta, principalmente os ingredientes de alto valor comercial como o milho. Entre os principais coprodutos agroindustriais com potencial para substituição à fonte energética da dieta (milho) na alimentação de ruminantes, atualmente, destacam-se aqueles oriundos da produção de biodiesel (Lage et al., 2010). Acredita-se que a glicerina possa ser utilizada como fonte energética alternativa em dietas destinadas a terminação de bovinos (Krehbiel, 2008), com isso, torna-se uma estratégia para a redução dos custos alimentares sem afetar a produtividade animal (Mach et al., 2009).

Objetivou-se avaliar o efeito da substituição do milho pela glicerina de média pureza sobre o desempenho, digestibilidade, comportamento ingestivo, características de carcaça e qualidade de carne de bovinos Purunã (¼ Aberdeen Angus + ¼ Caracu + ¼ Charolês + ¼ Canchim) terminados em confinamento.

REVISÃO DE LITERATURA

Glicerina: Coproduto do Biodiesel

A glicerina caracteriza-se por ser líquida, viscosa, higroscópica, incolor e inodora (Abdala, et al., 2008), e pode ser considerada uma fonte energética alternativa para substituir o milho na alimentação animal (Mach et al., 2009). Naturalmente, a glicerina se encontra na forma de triglicerídeos em óleos vegetais e gorduras animais, podendo ser extraída após processo de transesterificação com a utilização de ésteres metílicos durante o processo de fabricação do biodiesel (Hoydonckx et al., 2004).

A utilização de fontes renováveis de energia que possam ser substitutas aos combustíveis fósseis cresceu nos últimos anos (Visser et al., 2011; ANP, 2012b). De acordo com FAPRI (2012), estima-se que a produção mundial de biodiesel em 2012 foi de 21,2 bilhões de litros. Deste mercado, 56,6% serão oriundos da União Europeia, 17% dos Estados Unidos e 12,7% do Brasil.

No último ano, o Brasil comercializou aproximadamente 52,2 bilhões de litros de óleo diesel (ANP, 2012b), sendo necessários cerca de 2,6 bilhões de litros de biodiesel para o cumprimento da Lei Nº 11.097, De 13.1.2005 – DOU 14.1.2005 Art. 2º (ANP, 2012a), que estabelece a adição mínima de 5% (cinco por cento), em volume, de biodiesel ao óleo diesel comercializado em qualquer parte do território nacional. Portanto, a utilização de fontes renováveis de energia e a rápida expansão das indústrias de biodiesel disponibilizaram ao mercado cerca de 260 milhões de litros de glicerina sem destino definido.

A glicerina tem origem durante o processo de transformação química do óleo vegetal ou gordura animal em biodiesel. A adição de álcool (metanol ou etanol) na presença de um catalizador (NaOH ou KOH) aos compostos primários produz o biodiesel e como coproduto a glicerina (Rivaldi et al., 2007). Segundo Ooi et al. (2004) o grau de pureza da glicerina deve-se ao processamento da matéria-prima, determinando o valor deste coproduto no mercado. De acordo com Südekum (2008), a glicerina pode apresentar teores variáveis de glicerol, água, metanol e

ácidos graxos, sendo classificada de acordo com os níveis de glicerol na sua composição (baixa - 50 a 70% de glicerol; média - 80 a 90% de glicerol e alta pureza - acima de 99% de glicerol). Corroborando com Hippen et al. (2008), quando afirmam que nos coprodutos de baixa e média pureza, o teor de glicerol corresponde a 63,3% e 85,3%, respectivamente, além de outros compostos como água, lipídeos, fósforo, sódio e metanol.

De acordo com Knothe et al. (2006) e Beht et al. (2008), a glicerina de alta pureza atende a demanda da indústria de alimentos, sendo utilizada no setor farmacêutico (constituição de xaropes, cápsulas, etc), indústria de cosméticos (fabricação de loções pós-barba, creme dental, etc) e na indústria química (na síntese de propileno glicol, formaldeído, etc). No entanto, a maioria das refinarias nacionais de produção de biodiesel evita purificar o coproduto (glicerina) em função do alto custo do processo, disponibilizando produtos de baixa e média pureza ao mercado (Diniz, 2008). O excedente de glicerina de média pureza torna o coproduto atrativo para utilização em outros seguimentos da cadeia produtiva (ANP, 2012b).

A glicerina é utilizada como aditivo nos alimentos desde 1959 nos Estados Unidos, reconhecida como substância atóxica geralmente reconhecida como segura–"GRAS" pelo Food and Drug Administration (FDA, 2010). No Brasil, a utilização da glicerina como aditivo na alimentação humana e animal é assegurada pela resolução nº 386 de 5 de Agosto de 1999 (ANVISA, 1999). De acordo com o Departamento de Fiscalização de Insumos Pecuários (MAPA, 2010), a glicerina disponibilizada para alimentação animal deve conter um padrão mínimo de qualidade, sendo disponibilizados, no mínimo, 0,8 kg de glicerol para cada kg de glicerina, com valores máximos 130g de umidade e 150 mg de metanol.

A glicerina pode ser utilizada como uma fonte energética alternativa na alimentação animal, particularmente para ruminantes (Mach et al., 2009; Ferraro et al., 2009; Wang et al., 2009; Parsons et al., 2009; Abo El-Nor et al. 2010; Farias et al., 2012; Françozo et al., *in press*). O composto é disponibilizado diretamente para produção de ácidos graxos de cadeia curta que serão absorvidos no rúmen para obtenção de energia. Ou ainda, devido a sua forma líquida, a glicerina pode ser absorvida e metabolizada no fígado, sendo utilizada para a manutenção dos níveis plasmáticos de glicose (Krehbiel, 2008).

De acordo com Bergner et al.(1995), a elevada taxa de fermentação da glicerina no rúmen permite a sua transformação em ácidos graxos voláteis principalmente em propionato e, segundo Abughazaleh et al., (2011), em menor quantidade de butirato e valerato. Os ácidos graxos resultantes da fermentação ruminal, podem ser utilizados pelo trato gastrointestinal como fonte de energia, ou ainda, absorvidos pela veia porta e encaminhados ao fígado (Antunes & Rodriguez, 2006). No fígado uma parte do propionato é convertido a piruvato e o remanescente segue a rota neoglicogênica, sendo convertido em glicose após o ciclo de Krebs (Kozloski, 2009). A glicerina também pode ser diretamente absorvida pelo epitélio ruminal e metabolizada no fígado, da mesma maneira que o propionato oriundo do metabolismo ruminal (Brisson et al., 2011). Portanto, a intensa metabolização da glicerina pelo rúmen / fígado de ruminantes aumenta a concentração de glicose sérica, reduzindo o tempo para a saciedade dos bovinos (Rémond et al., 1993) e, consequentemente, altera a ingestão de MS (Lage et al., 2010).

De acordo com Krehbiel (2008), as taxas de desaparecimento de glicerina no rúmen podem aumentar com a adaptação dos animais, devido à intensa fermentação em ácidos graxos voláteis pelas bactérias ruminais. A suplementação com glicerina na dieta de ruminantes altera o padrão de fermentação ruminal, reduzindo de maneira linear a proporção de acetato / propionato com doses crescentes de glicerina, pois favorecem a produção de propionato no rúmen por bactérias gram-negativas (Wang et al., 2009). Corroborando com Kijora et al (1998), quando afirmaram que após sete dias de adaptação da flora microbiana, a metabolização da glicerina ocorre mais rapidamente. De acordo com Bergner et al. (1995) cerca de 85% da glicerina desaparece em menos de duas horas podendo atingir o total desaparecimento em até seis horas.

A glicerina pode ser adicionada às dietas de bovinos em substituição ao milho ou outra fonte de energia, com o objetivo de reduzir o custo de produção. No entanto, os níveis de glicerina na dieta de ruminantes dependem de diversos fatores, entre eles: composição química do coproduto, tolerância, adaptação e aceitabilidade dos animais à glicerina devido a alta densidade e viscosidade do coproduto. Wang et al. (2009) observaram alterações nas concentrações de ácidos graxos voláteis, com aumento linear na concentração de propionato e

butirato sem afetar os níveis de acetato com o aumento dos níveis de glicerina (1 a 3% da MS). Os autores ressaltam que a glicerina demonstra uma maior degradabilidade ruminal em relação ao amido, corroborando com Schröder & Südekum (1999) quando observaram uma maior degradabilidade em dietas à base de amido de trigo contendo glicerina.

Ilse et al. (2009) observaram que o peso corporal final, o peso de carcaça quente, a ingestão e a conversão alimentar da matéria seca não diferiram entre os níveis de glicerina (0, 6, 12 e 18%) na dieta de 132 novilhas terminadas em confinamento. Os resultados indicam que a glicerina pode ser utilizada como um macro ingrediente de excelente fonte de energia com a inclusão de até 18% com base na MS da dieta total sem afetar negativamente o desempenho animal.

Parsons et al. (2009) avaliaram o desempenho e as características quantitativas da carcaça com a inclusão de glicerina na dieta de novilhas mestiças confinadas por um período experimental de 85 dias. Os tratamentos constituíram de níveis de glicerina bruta, sendo: 0, 2, 4, 8, 12 e 16% com base na matéria seca da dieta. A adição dos níveis de glicerina na dieta reduziu a ingestão de matéria seca (8,84, 8,88, 8,66; 8,61; 8,40 e 7,80 kg), ganho médio diário (12,6; 8,4; 5,0; 1,7 e 13,4 %) e peso corporal final (12,7; 8,1; 5,3; 1,9 e 14,3 kg) dos animais

Mach et al. (2009) avaliaram o desempenho e características físicas da carcaça de touros holandeses alimentados com níveis de glicerina (4, 8 e 12%) na dieta. A inclusão de até 12% de glicerina na dieta não influenciou negativos ao desempenho e características de carcaça e qualidade da carne. Segundo Drouillard (2009), a glicerina bruta reduz a ingestão de matéria seca quando incluída em até 10% das dietas, entretanto apresenta melhoras no ganho médio diário e na eficiência alimentar (16 a 23%) em relação a dietas sem glicerina. O autor demonstra que as melhorias na eficiência alimentar foram maiores quando as dietas continham altos teores de amido e baixos níveis de glicerina, sendo que a inclusão de glicerina (2, 4, 8, 12, 16%) reduziu a eficiência alimentar (11, 10, 8, 3 e -3%, respectivamente) dos animais. O autor conclui que a glicerina é um coproduto promissor para a terminação de bovinos, embora necessite de mais estudos comprobatórios. De acordo com Gunn et al. (2010), a adição de glicerina às dietas de cordeiros machos reduz o ganho médio diário. Todavia, o peso de carcaça quente e área de olho de lombo não diferiram em função dos níveis de

glicerina. Os autores sugerem que a inclusão de até 15% de glicerina bruta nas dietas na terminação de cordeiros não tem influência no desempenho animal.

Lage et al. (2010) avaliaram a inclusão de 0, 3, 6, 9 e 12% de glicerina bruta na dieta de cordeiros machos não castrados da raça Santa Inês. Os autores relatam que a inclusão de até 6% de glicerina bruta melhora a conversão alimentar dos animais e reduz o custo do ganho de carcaça quando o preço do coproduto representa até 70% do preço do milho. Entretanto, a inclusão de glicerina influenciou o desempenho, consumo, digestibilidade e as características quantitativas da carcaça. O provável comprometimento no desempenho pode estar relacionado aos elevados teores de extrato etéreo das dietas com os níveis de inclusão de glicerina e consequentemente pela inibição de bactérias celulolíticas.

Hess et al. (2009) ressaltam a eficiência da glicerina frente a fermentação ruminal, sendo proeminente a sua metabolização em ácidos graxos voláteis, principalmente em propionato e butirato. No entanto, demonstram que o crescimento e as atividades das bactérias celulolíticas foram reduzidas com a concentração de 5% de glicerol puro. Relatam também que a inclusão de 15% de glicerina bruta na dieta não afetou a digestibilidade *in vitro*, podendo ser adicionado a alimentos diversos, sem afetar a digestibilidade da matéria seca ou da fibra. Krueger et al. (2010) avaliaram a cinética ruminal *in vitro* com a adição de glicerina ao longo do tempo e observaram o aumento da população bacteriana (*Megasphaera elsdenii e Selenomona ruminatium*) responsáveis pela fermentação do composto sem afetar a digestibilidade da FDN, quando utilizado níveis de até 20% de glicerina. Abughazaleh et al. (2011) observaram que a ingestão de até 15% de glicerina na MS da dieta não afeta a população e a fermentação ruminal. Entretanto, níveis maiores de glicerina (30 e 45%) diminuíram as populações de bactérias responsáveis pela fermentação de fibra (*Ruminococcus ibus* e *Succinivibrio dextrinosolvens*). Abo El-Nor et al. (2010) encontraram alterações na digestibilidade de fibra e na população bacteriana com a substituição do milho pela glicerina (15, 30 e 45% da MS).

Gomes et al. (2011) observaram ser possível a adição de até 30% de glicerol na dieta total (100% de substituição do milho) de cordeiros em confinamento durante 60 dias sem alterar o desempenho animal, ingestão de alimentos e rendimento dos cortes nobres de cordeiros. Ramos et al. (2012) encontraram uma

redução na ingestão de MS sem influenciar o peso corporal final de novilhos alimentados com níveis de glicerina (5, 10, 15 e 20%) na dieta. Os autores ressaltam que a substituição do milho por até 20% de glicerina não afeta negativamente o desempenho animal. Farias et al. (2012) não observaram efeito dos níveis de glicerina (0,0; 3,8; 6,1 e 9,0%) sobre os coeficientes de digestibilidade da MS, PB, MO, FDN, CNF e CHT de novilhas suplementadas à pasto. Entretanto, observaram redução no ganho médio diário e peso corporal final em função dos níveis de adição. Os autores não recomendam a utilização de glicerina de baixa pureza, pois os elevados teores de álcool e sais podem determinar a redução no consumo e, consequentemente no desempenho animal.

Por outro lado, Françozo et al. (*in press*) não observaram alterações no desempenho animal e características de carcaça de novilhos Nelore alimentados com diferentes níveis de glicerina (0, 5 e 12%) terminados em confinamento, indicando a viabilidade de utilização do coproduto na terminação de bovinos.

Portanto, a indústria de alimentação animal pode ser um consumidor em potencial da glicerina, tendo em vista a disponibilidade e o aumento na produção brasileira de biodiesel. Acredita-se que este coproduto possa ser utilizado como fonte energética alternativa em dietas destinadas a terminação de bovinos confinados, com isso, a glicerina torna-se uma estratégia para a redução dos custos alimentares sem afetar o desempenho animal, além de obter uma destinação segura com diminuição da poluição ocasionada pelos excedentes dos coprodutos da agroindústria.

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CAPÍTULO 1

GLYCERINE LEVELS IN THE DIETS OF CROSSBRED BULLS FINISHED IN FEEDLOT: ANIMAL PERFORMANCE, CARCASS DRESSING, FEED INTAKE AND APPARENT DIGESTIBILITY [1]

1   GLYCERINE LEVELS IN THE DIETS OF CROSSBRED BULLS FINISHED IN

2   FEEDLOT: ANIMAL PERFORMANCE, CARCASS DRESSING, FEED INTAKE

3   AND APPARENT DIGESTIBILITY

4

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19

20

21   **Abstract**

22

23   This work was carried out to study different levels of glycerineas partial

24   replacement of corn on animal performance, feed efficiency and apparent

25   digestibility in young Purunã bulls finished in a feedlot. The bulls were kept in a

26   feedlot for 240 days. This study used 40 Purunã bulls that were 209 ± 33.3 kg in

27   weight and 8 ± 0.9 months old. The diet treatments were as follows: 0% glycerine

28   (G00), 6% glycerine (G06), 12% glycerine (G12) and 18% glycerine (G18). Animal

29   performance, carcass characteristics, dry matter, organic matter and total intake of

30   digestible nutrients were similar among the four diets. However, ether extract,

31   neutral detergent fibre and carbohydrates decreased linearly (P<0.05) as glycerine

32   levels in the diet increased. Feed conversion rates were improved with higher

33   glycerine levels in the diets. The apparent digestibility of nutrients increased

34   linearly (P<0.05) with increasing glycerine in the diet, with the exception of ether

35   extract and neutral detergent fibre.

36

37   *Keys word:* Cattle, animal performance, feed efficiency, energy.

38

39   **1. Introduction**

40

41   High global demand for energy has led to the increasing production and trade
42   of biofuels, especially liquid fuels for transportation, to replace fossil energy
43   sources, improve energy security and counter greenhouse gas emissions (Walter
44   et al., 2008). Rapid growth in biofuel production has led to increased competition
45   for raw materials for food, feed and fuel usage, as well as an increasing quantity of
46   co-product (Robinson et al., 2008). For example, starch and vegetable oils are
47   converted into bioethanol and biodiesel during the production process. Glycerine is
48   the main co-product generated in the production of biodiesel, at a rate of
49   approximately 10 to 13% of the total volume of biodiesel produced (Dasari et al.,
50   2005). The glycerine is produced as a result of transesterification of triglycerides
51   with alcohol and contains impurities, such as water, salts, esters, alcohol, and
52   residual oil, which lower its value (Ooi et al., 2004).

53   One potential application for glycerine is as a gluconeogenic substrate for
54   ruminants (Chung et al., 2007). Glycerine can be converted to glucose in the liver
55   of cattle and can provide energy for cellular metabolism (Goff and Horst, 2001).
56   Glycerine enters the gluconeogenic pathway at the level of dihydroxyacetone
57   phosphate and 3-phosphoglyceraldehyde (Krehbiel, 2008). Glycerine could be
58   included in ruminant rations as an energetic feed ingredient and substituted for
59   other feed ingredients, such as cereals, helping to reduce feed costs.

60   It has previously been reported that feeding crude glycerine had no effect
61   (Mach et al., 2009; Françozo et al., 2013, in press) or decreased (Pyatt et al.,
62   2007; Parsons et al., 2009) dry matter intake (DMI) when fed to beef cattle.
63   Parsons et al. (2009) observed no difference in dry matter conversion with the
64   addition of up to 2% glycerine in the diet and also found that increasing glycerine
65   to 4, 8, 12 and 16% of the diet resulted in a linear decrease in intake by crossbred
66   heifers.

67   In addition, the effects of crude glycerine on average daily gain (ADG) and
68   cattle performance have been variable. As dietary crude glycerine increased, ADG
69   either did not change (Mach et al., 2009; Françozo et al., 2013, in press) or
70   showed a quadratic response (Parsons et al., 2009). The reductions in
71   performance were directly related to a linear decrease in dry matter intake.
72   Parsons et al. (2009) observed an increase in final body weight of 12.7, 8.1 and

73   5.3 kg when glycerine comprised 2, 4 or 8% of the diet, respectively, of crossbred

74   heifers. Mach et al. (2009) used 48 Holstein bulls to evaluate three levels of

75   glycerine (0, 4 or 12% of DM) and observed that DMI, ADG and starch intake were

76   not affected by glycerine level in the diet.

77   Mach et al. (2009) assessed levels of dietary glycerine on carcass

78   characteristics and meat quality in bulls and observed that the dietary treatments

79   (0, 4 or 12%) did not affect hot carcass weight, dressing percentage, fat thickness

80   or conformation. They also found that the *Longissimus* muscle and intramuscular

81   fat were not affected by the treatments. They concluded that the inclusion of up to

82   8% glycerine in the diet can effectively increase the final body weight and hot

83   carcass weight of finished bulls.

84   Digestibility of crude glycerine was reported to be 0.80 after 24 h of

85   incubation *in vitro* (Trabue et al., 2007), 0.90 after 2 h of fermentation (Bergner et

86   al., 1995), and 100% within 4 h *in vivo* (Rémond et al., 1993). Therefore, the

87   addition of crude glycerine to cattle diets would be expected to increase volatile

88   fatty acids (VFA) in the rumen. However, the reported responses to these studies

89   have shown contradictory results. Total VFA production has been reported to have

90   increased (Bergner et al., 1995) or remained unchanged (Mach et al., 2009) due to

91   the addition of crude glycerine to the diet. Researchers reported that 35 to 69% of

92   the crude glycerine administered was used to produce propionate (Rémond et al.,

93   1993). If crude glycerine increased propionate concentration, an increased feed

94   efficiency would be expected (Raun et al., 1976). However, the results of feed

95   efficiency studies have been contradictory. Feed efficiency either did not change

96   (Mach et al., 2009) or was improved (Pyatt et al., 2007) when crude glycerine was

97   fed to cattle. We hypothesise that glycerine is less fermented than corn starch, and

98   this should translate into differences in animal performance. Therefore, the

99   objective of this research was to determine the effects of crude glycerine on

100   ruminal fermentation characteristics and beef cattle performance.

101   Previous research suggests that glycerine is an effective feed source for

102   multiple species, but there are limited data demonstrating the effects of glycerine

103   on carcass characteristics and meat quality of bulls. The aim of this study was to

104   determine the effects of different levels of dietary glycerine (as an energy source)

105   on performance, carcass characteristics and meat quality of Purunã bulls finished

106   in a feedlot.

107 **2. Materials and methods**

108

109 *2.1. Animals, housing and diets*

110

111   This experiment was approved by the Department of Animal Production at
112 the State University of Maringá (CIOMS/OMS, 1985). It was conducted at the
113 Experimental Station at Farm Modelo at the Agronomic Institute of Paraná
114 (IAPAR) in the city of Ponta Grossa, Paraná State, southern Brazil.

115   Forty Purunã bulls (¼ Aberdeen Angus + ¼ Caracu + ¼ Charolais + ¼
116 Canchim) were used in a completely randomised design. The bulls were weighed
117 and distributed into four diet groups with ten replications per group. They were
118 allocated into individual pens (8 m^2 for each animal) in a feedlot system. After an
119 11-d diet adaptation period, the bulls were weighed and started the study with an
120 average initial body weight (BW) of 209 ± 33.3 kg and an average age of 8 ± 0.9
121 months. The bulls' BW and intake of concentrate and corn silage were recorded
122 monthly until day 229 of the experiment when the bulls reached a final BW of 472
123 ± 57.3 kg. After reaching the final BW the bulls were transported to the
124 slaughterhouse. The truck stocking density was 1 animal/m^2 and the transport
125 distance was 10 km. At the slaughterhouse, the bulls were housed in collective
126 pens for approximately 12 hours (overnight) before slaughter. The bulls were
127 slaughtered at a commercial slaughterhouse according to industrial practices in
128 Brazil. Following slaughter, the carcasses were identified, weighed and chilled for
129 24 hours at 4 ℃. After chilling, the right half of the carcass was used to determine
130 the quantitative characteristics.

131   The glycerine was produced in a soy-diesel facility (BIOPAR, Rolândia,
132 Paraná, South Brazil) and the chemical composition (Table 1) was determined at
133 the Institute of Technology of Paraná (TECPAR). In this study, glycerine was used
134 as an energetic ingredient in the diet; therefore, to obtain four isoenergetic diets,
135 the increase in glycerine level was counter balanced, mainly by a decrease in corn
136 grain content (Table 2). All the diets were formulated to be isoprotein (Table 3).

137   The bulls were randomly assigned to 1 of 4 diets containing 0, 6, 12 or 18%
138 glycerine on a DM basis; which represented 18.3; 38.5; and 61.3% of corn
139 replacing. The bulls were fed concentrate and corn silage in separate troughs,
140 both for *ad libitum* intake. The bulls were fed twice a day at 08:00 and 15:00 h.

141 The diets were weighed daily so that the refusals represented 5% of the total. The
142 concentrate intake was fixed at 1.2% of BW and adjusted at 28 days. The diet
143 formulation and quantity supplied were designed to provide a weight gain of 1.2
144 kg/day, according to the NRC (2000) recommendations.

145

146 *2.2. Performance and feed intake*

147

148 To determine animal performance, the animals were weighed once at the
149 beginning of the experiment and then once every 28 days (after a fasting from
150 solid food for a period of 16 hours) for the duration of the experiment (229 days).
151 Daily feed intake was estimated as the difference between the supplied feed and
152 the refusals in the trough. During the collection period, samples of the supplied
153 feed and refusals were collected and a representative composite sample was
154 drafted per animal in each treatment.

155

156 *2.3. Apparent total-tract digestibility*

157

158 Two faecal collections were performed for a period of five days starting on
159 the 57th and another on the 86th days of a feedlot to obtain the apparent
160 digestibility coefficient of dry matter and other nutrients. Faecal samples
161 (approximately 200 g wet weight) were collected for each bull from the rectum two
162 times daily (minimum 3 h intervals between samples) during five consecutive days
163 and pooled by bulls for each 5 day sampling period. After being dried at 55 ^{o}C for
164 48 h, the samples were ground in a feed mill and passed through a 1 mm sieve in
165 preparation for chemical analyses.

166 To estimate the flux of faecal dry matter, indigestible neutral detergent fibre
167 (iNDF) was used as an internal marker (Zeoula et al., 2002). Samples were milled
168 through a 2 mm sieve, packed (20 mg of DM/cm²) in 4 x 5 cm Ankom (filter bags
169 F57) that had been previously weighed, and incubated for 240 h in the rumen of a
170 Holstein bull (Casali et al., 2008) fed a mixed diet of equal parts of forage (corn
171 silage) and concentrate (the same concentrate used in the treatments).

172 After incubation, the bags were removed, washed with water until clean and
173 dried in a ventilated oven at 55 ℃ for 72 h, then removed and oven-dried again at
174 105 ºC. The iNDF was estimated using the difference in sample weight before and

175 after ruminal incubation. Faecal excretion was calculated using the following
176 equations: FE=iNDFI/iNDFCF, where: FE=faecal excretion (kg/day); iNDFI=iNDF
177 intake (kg/day); and iNDFCF=iNDF concentration in faeces (kg/day). The apparent
178 digestibility coefficients (ADC) for DM and nutrients were estimated according to
179 the formula: ADC=[(Intake – Excreted) / Intake] x 100.

180

181 *2.4. Chemical analyses*

182

183 Dry matter content of the ingredients (silage and concentrate mix) was
184 determined by oven-drying at 65 ℃ for 24 h. Analytical DM content of the oven-
185 dried samples was determined by drying at 135 ℃ for 3 h by the methods
186 according to AOAC (1998) (method 930.15). The content of OM was calculated as
187 the difference between the DM and ash contents, with ash determined by
188 combustion at 550 ℃ for 5 h. The heat stable alpha-amylase was utilized to
189 determined NDF and ADF (Mertens, 2002). Nitrogen (N) content was determined
190 by the Kjeldahl method (AOAC, 1998) (Method 976.05). Total carbohydrates (TC)
191 were obtained using the following equation: TC = 100 - (%CP + %EE + %Ash)
192 (Sniffen et al., 1992). Non-fibre carbohydrates (NFC) were determined as the
193 difference between TC and NDF. Total digestible nutrient (TDN) content of the
194 diets was obtained by the methodology described by Kearl (1982). The samples
195 were analysed in the Laboratory of Feed Analyses and Animal Nutrition at the
196 State University of Maringá.

197

198 *2.5. Statistical analysis*

199

200 The experimental design was completely randomised with four treatments
201 and ten replications. The results were statistically interpreted using regression
202 equations performed in SAS (2004) (PROC REG): $Y_{ijk} = \beta_0 + \beta_1 X_i + \beta_2 X_i^2 + \alpha_{ijk}$
203 $+ \varepsilon_{ijk}$,
204 where: Y_{ijk} = dependent variables (glycerine levels); β = regression
205 coefficients; X_{ijk} = independent variables; α_{ijk} = regression deviations; and ε_{ijk} =
206 residual error.

207

208 **3. Results**

209

210   The inclusion of up to 18% glycerine to replace corn as an energy source on
211 the diet of bulls finished in a feedlot did not affect (P>0.05) final body weight,
212 average daily gain (ADG), hot carcass weight or carcass dressing (Table 4). No
213 changes (P>0.05) in dry matter (kg), organic matter and crude protein feed intake
214 occurred when glycerine was included at levels of 0, 6, 12 or 18% on the diet
215 (Table 5). The dry matter (% BW), ether extract, neutral detergent fibre (kg and %
216 BW), non-fibrous carbohydrate and total carbohydrate intake of bulls decreased
217 linearly (P<0.05) as glycerine levels increased in the diet (Table 5). Feed efficiency
218 was affected (P<0.05) by glycerine levels (Table 5).

219   Nutrient digestibility in the total tract is shown in Table 6. Apparent total tract
220 digestibility of DM, OM, CP, NDF, NFC and TC were not affect (P>0.05) to
221 glycerine supplementation. The apparent digestibility of DM, OM, CP, NFC and TC
222 increased linearly (P<0.05) with increasing glycerine in the diet. The digestibility of
223 EE and NDF were similar (P>0.05) among the four diets. Apparent digestibility of
224 EE was high for the bulls that were fed the four diets (0.87).

225

226 **4. Discussion**

227

228   The initial live weight of the bulls was 209 ± 33.3 kg. Generally, the average
229 live weight of bulls finished in feedlot varies from 350 to 380 kg (Maggioni et al.,
230 2009; Rotta et al., 2009b; Maggioni et al., 2010; Fugita et al., 2012). The low initial
231 weight of the bulls in this study can be explained by the bulls' age (8 ± 0.9
232 months). The bulls were finished in a feedlot system from weaning to slaughter the
233 animals in the category of young bulls (16 months). According to Maher et al.
234 (2004) young bulls exhibit better feed efficiency because the transformation of
235 nutrients into the body decreases with the animal's age. Thus, feedlots can be
236 used as an alternative system for animal finishing that increases the participation
237 of young bovine (slaughtered at 14 to 16 months of age), enabling the production
238 of better quality meat (Rotta et al., 2009a; Rotta et al., 2009b; Ito et al., 2012).
239 Consistent with the results of this study, Mach et al. (2009) observed that the
240 inclusion of 12% glycerine on the diets of steers did not affect animal performance.
241 Pyatt et al. (2007) observed an 11.4% increase in the ADG of cattle fed diets

242 supplemented with glycerine and Parsons et al. (2009) observed a quadratic
243 response in the ADG of crossbred heifers to increasing glycerine levels (2, 4, 8, 12
244 and 16%) in the diet. According to the results of these studies, the level of
245 glycerine in the diet of feedlot cattle that are fed high-protein and high-energy diets
246 can vary from 10 to 16%. However, in this study it was possible to include
247 glycerine at levels up to 18% on DM in the diet without altering animal
248 performance. Even at high levels of glycerine (18% on DM), no impairment of body
249 weight, ADG, weight or carcass dressing was observed, perhaps due to the quality
250 of the glycerine product (purity above 80%) and the low concentration fatty acids
251 and methanol. According to Sudekum (2007), glycerine may contain varying
252 amounts of water, methanol and fatty acids and is classified as low purity (50 to
253 70% glycerine), medium purity (80 to 90% glycerine) or high purity (above 90%
254 glycerine). The quality varies according to the nutritional value of the feedstock
255 and the process for producing biodiesel (Vieira et al., 2005). According to Mach et
256 al. (2009) using glycerine with metabolized energy close to 3470 kcal/kg of DM
257 can produce positive results in cattle performance. However, the authors stressed
258 that the presence of methanol in the glycerine at above 0.09% can have
259 detrimental effects on dry matter intake and animal performance. This finding was
260 corroborated by Chung et al. (2007), who found that the composition of glycerine
261 can determine reductions in diet intake. In other studies that used crude glycerine
262 in cattle diets, a reduction in animal performance was observed (Parsons et al.,
263 2009; Lage et al., 2010).

264 Bulls were slaughtered with 16 months old and average final weight body
265 was 472 ± 57.3 kg. These animals were classified as young bulls, which typically
266 have superior carcass conformation and meat quality (Ito et al., 2010). ADG mean
267 was 1.15 ± 0.17 kg, which was low for this category of animal (crossbred, bulls,
268 and slaughtered at 16 months of age). Generally, this type of animal has an ADG
269 from 1.4 to 2.0 kg. According to the NRC (2000), during puberty, cattle need a diet
270 with DM that is 12% CP to reach their total potential body development. In this
271 study, the protein content of DM in the diet (10.8%) was lower than the
272 requirements recommended by the NRC (2000). The low level of CP in the DM of
273 the diet in this study could explain the low ADG of the bulls.

274 Average hot carcass weight carcass (258.3 kg) was consistent with the
275 requirements of the Brazil market (minimum 225 kg) (Costa et al., 2002; Rotta et

276 al., 2009a; Rotta et al., 2009b). Similarly, the average hot carcass dressing
277 (54.7%) was similar to that observed in carcasses from crossbred cattle (*Bos*
278 *Taurus* vs. *Bos indicus*) that were not castrated, finished in a feedlot and
279 slaughtered before reaching two years of age (Ito et al., 2010; Machado Neto et
280 al., 2012).

281 Mach et al. (2009) reported no changes in DMI when glycerine was added at
282 levels of 0, 4, 8 or 12% to the diet of Holstein bulls that were fed high-concentrate
283 diets (8.3 kg/day). In contrast, Parsons et al. (2009) reported a 13% reduction in
284 DMI when glycerine was added at 16% to a steam-flaked corn feed and fed to
285 heifers for the final 85 days before slaughter. Schröder and Südekum (1999)
286 reported a 0.7 kg/day reduction in starch intake of ruminally cannulated steers that
287 were fed a diet with 15% glycerine. Studies conducted on lactating cows that were
288 fed high-forage diets (DeFrain et al., 2004; Chung et al., 2007) have reported no
289 negative effects on feed intake from supplementing the diets with glycerine at
290 rates similar to this study.

291 The reduction in the intake of nutrients (EE, NDF and NFC) can be explained
292 by the decrease in EE, NDF and NFC in the diet due to the inclusion of glycerine
293 (Table 2), which is free of these compounds. Replacing corn by glycerine provided
294 a reduction of 19.2% in EE intake without affecting the total digestible nutrient
295 intake. AbuGhazaleh et al. (2011) observed a decrease in NDF digestibility as
296 glycerine replaced corn in the diet *in vitro* study. The reduction was due to the
297 increased glycerine content in the diet (15, 30 and 45%) replacing the fibrous
298 content. A linear decrease in fibrous compounds was also observed by Farias et
299 al. (2012), who found a reduction in the intake of TC (17.1%) and NFC (21%) with
300 the inclusion of glycerine in the diet.

301 Feed efficiency improved by 10.8, 10.0, 7.2 and 33.1% when glycerine was
302 included at levels of 2, 4, 8, and 12% of the diet, respectively; however, adding
303 glycerine at 16% reduced the efficiency by 2.8% (Parsons et al., 2009). Pyatt et al.
304 (2007) reported a 21.9% improvement in feed efficiency when glycerine was used
305 to replace 10% of the dry-rolled corn content in cattle diets.

306 The apparent digestibility results indicate that glycerine supplementation in
307 the diet potentially improves rumen fermentation through increased feed
308 digestibility in the total digestive tract of beef cattle. Similarly, Wang et al. (2009)
309 found that the total tract digestibility of DM, OM, NDF and CP were increased

linearly with increasing glycerine in the diet. The increased digestibility of nutrients in diets containing glycerine may be explained by the metabolization of glycerine into volatile fatty acids by gram-negative bacteria in the rumen and the absorption of fatty acids by the gastrointestinal mucosa. Biohydrogenation and absorption occur rapidly in the rumen, reducing the amount of material to be transported and metabolised in the gut of the animals. In this study, the rapid transformation of glycerine into propionate is demonstrated by the reduction in feed intake and the conversion of DM without affecting animal performance at different levels of glycerine in the diet. According to (Krehbiel, 2008), the disappearance rates of glycerine in the rumen may increase with the adaptation of animals to intense fermentation of volatile fatty acids by rumen bacteria. Adding glycerine to the diet of ruminants alters the pattern of rumen fermentation, linearly reducing the proportion of acetate/propionate as the dose of glycerine is increased, favouring production of propionate in the rumen by gram-negative bacteria (Wang et al., 2009). Corroborating these findings, Bergner et al. (1995) that after seven days of adaptation by microbial organisms the metabolization of glycerine occurs more rapidly and reaching a total disappearance within six hours. Wang et al. (2009) observed an increase in the digestibility of DM, EE, OM, NDF and ADF (quadratic response) compared with control treatment. The digestibility of CP showed a linear increase as a function of the level of glycerine.

Conclusion

The co-product of biodiesel production glycerine fed to young bulls during 229 days in feedlot system did not affect the animal performance, feed intake and carcass characteristics. However, the increasing amounts glycerine in the diet increased the nutrients apparent digestibility of the diets. Results from current study demonstrated that limiting threshold was up to 18% glycerine on a DM. Likewise, when there is the glycerine production excess with prices below of corn; this product might be utilized in the diets for young bulls finished in feedlot.

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491
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TABLES

531 **Table 1**

532 Chemical composition of the glycerine used in the study

Parameters	Results
Water*	23.2 g/kg
Ash	47.6 g/kg
Glycerol	812 g/kg
Methanol	3.32 mg/kg
Sodium	11.6 g/kg
Potassium	79.1 mg/kg
Chloride	35.8 mg/kg
Magnesium	16.3 mg/kg
Phosphorus	239 mg/kg
Gross energy	14.2 MJ

533 Realised by Institute of Technology of Paraná – TECPAR, Biofuels division, in
534 Curitiba, Paraná.*Karl Fischer.

535 **Table 2**

536 Ingredients and percent composition (g/kg) of the diet treatments

Ingredients	Glycerine levels			
	G00[1]	G06[2]	G12[3]	G18[4]
Corn silage	530	530	530	530
Soybean meal	118	134	150	169
Corn grain	344	268	191	114
Glycerine	0.00	60.0	120	178
Mineral salt[5]	8.30	8.30	8.30	7.60

537 [1]Without glycerine; [2]6% glycerine; [3]12% glycerine; [4]18% glycerine; [5]Guarantee
538 levels (per kg): calcium - 175 g; phosphorus – 100 g; sodium – 114 g; selenium –
539 15 g; magnesium – 15 g; zinc – 6.004 mg; manganese – 1.250 mg; copper –
540 1.875; iodine – 180 mg; cobalt – 125 mg; selenium – 30 mg; fluorine (maximum) –
541 1.000 mg.
542

543 **Table 3**

544 Chemical composition of the base diets (g/kg)

Ingredients	DM[1]	g/kg on DM								
		OM[2]	Ash	CP[3]	EE[4]	TC[5]	NFC[6]	NDF[7]	ADF[8]	TDN[9]
Corn silage	291	973	27.3	60.6	33.6	878	514	364	192	622
Soybean meal	815	929	71.4	489	25.0	415	234	181	116	780
Corn grain	818	977	23.2	103	59.3	814	641	173	47.7	816
Glycerine	943	952	47.6	0.70	1.20	-	-	-	-	806
Mineral salt	980									
Diets										
G00[10]	540	879	27.9	108	36.9	734	475	259	126	702
G06[11]	547	884	30.0	108	33.6	689	438	251	125	701
G12[12]	554	880	32.2	108	30.3	644	401	242	123	670
G18[13]	562	896	34.5	109	27.0	598	364	234	122	699

545 [1]Dry matter; [2]Organic matter; [3]Crude Protein; [4]Ether extract; [5]Total carbohydrates;
546 [6]Non-fibre carbohydrates; [7]Neutral detergent fibre; [8]Acid detergent fibre; [9]Total
547 digestive nutrients; [10]Without glycerine; [11]6% glycerine; [12]12% glycerine; [13]18%
548 glycerine.

549 **Table 4**

550 The effects of glycerine levels on animal performance of Purunã bulls finished in a feedlot.

Item	Glycerine levels				Means	SEM[5]	P-*value*		R²
	G00[1]	G06[2]	G12[3]	G18[4]			L	Q	
Initial body weight, kg	217	204	205	209	$\hat{Y} = 209$	5.33	0.68	0.67	-
Final body weight, kg	472	466	476	474	$\hat{Y} = 472$	9.32	0.84	0.97	-
Average daily gain, kg	1.11	1.14	1.18	1.16	$\hat{Y} = 1.15$	0.02	0.62	0.76	-
Hot carcass weight, kg	255	253	268	257	$\hat{Y} = 258$	5.56	0.70	0.84	-
Dressing carcass, %	54.0	54.3	56.3	54.1	$\hat{Y} = 54.7$	0.29	0.36	0.06	-

551 [1]Without glycerine; [2]6% glycerine; [3]12% glycerine; [4]18% glycerine; [5]Standard error of mean.

560 **Table 6**

561 Glycerine levels on apparent digestibility for Purunã bulls finished in a feedlot

Nutrients, g/kg	Glycerine levels				SEM[5]	P-*value*		R²
	G00[1]	G06[2]	G12[3]	G18[4]		L	Q	
Dry matter[6]	0.66	0.69	0.71	0.75	<0.01	<0.001	<0.001	0.42
Organic matter[7]	0.66	0.70	0.73	0.78	<0.01	<0.001	<0.001	0.54
Crude protein[8]	0.71	0.70	0.74	0.76	<0.01	0.002	0.005	0.21
Ether extract	0.87	0.87	0.87	0.90	<0.01	0.07	0.16	-
Neutral detergent fibre	0.59	0.58	0.59	0.59	<0.01	0.93	0.98	-
Non fibrous carbohydrates[9]	0.82	0.85	0.89	0.93	<0.01	<0.001	<0.001	0.65
Total carbohydrates[10]	0.68	0.70	0.72	0.75	<0.01	<0.001	0.007	0.32

562 [1]Without glycerine; [2]6% glycerine; [3]12% glycerine; [4]18% glycerine, [5]Standard error of mean; Regression equations: [6]$\hat{Y} = 0.660 +$
563 $0.004x$; [7]$\hat{Y} = 0.659 + 0.005x$; [8]$\hat{Y} = 0.696 + 0.002x$; [9]$\hat{Y} = 0.818 + 0.005x$; [10] $\hat{Y} = 0.681 + 0.003x$.

564

CAPÍTULO 2

GLYCERIN LEVELS IN THE DIETS FOR CROSSBRED BULLS FINISHED IN FEED-LOT: INGESTIVE BEHAVIOR, FEEDING AND RUMINATION EFFICIENCY[1]

[1]Artigo submetido ao comitê editorial do periódico científico Acta Scientiarum. Animal Science.

1 **GLYCERIN LEVELS IN THE DIETS FOR CROSSBRED BULLS FINISHED IN**
2 **FEED-LOT: INGESTIVE BEHAVIOR, FEEDING AND RUMINATION EFFICIENCY**
3
4 INGESTIVE BEHAVIOR OF BULLS SUPPLEMENTED WITH GLYCERIN IN THE
5 DIETS
6

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22
23

24 NÍVEIS DE GLICERINA NA DIETA DE BOVINOS MESTIÇOS TERMINADOS EM
25 CONFINAMENTO: COMPORTAMENTO INGESTIVO, EFICIÊNCIA DE
26 ALIMENTAÇÃO E RUMINAÇÃO.
27

28 **RESUMO**
29

30 Objetivou-se avaliar a substituição do milho por níveis de glicerina sobre o
31 comportamento ingestivo e eficiências de alimentação e ruminação de novilhos
32 Purunã terminados em confinamento. Foram utilizados 40 novilhos Purunã com
33 208,8 ± 33,3 kg e oito meses de idade. As dietas utilizadas foram sem glicerina-
34 G00, 6% de glicerina – G06, 12% de glicerina – G12 e 18% de glicerina – G18. A
35 ingestão de MS foi semelhante em todas as dietas. De outra forma, a ingestão de
36 FDN diminuiu linearmente com a suplementação das dietas com níveis de glicerina.
37 As eficiências de alimentação e ruminação de MS e FDN foram semelhantes em
38 todos os tratamentos. As dietas com glicerina alteraram a duração das atividades

39 comportamentais. A suplementação da dieta com glicerina não afetou a frequência

40 de alimentação. De outro modo, a frequência de ruminação foi reduzida linearmente

41 com a inclusão de glicerina na dieta. As frequências de outras atividades apresentou

42 efeito quadrático com a adição de glicerina. A inclusão de glicerina na dieta reduziu

43 o tempo de duração das frequências de alimentação, mas não afetou o tempo

44 despendido nas frequências de ruminação. Entretanto, a duração das frequências de

45 outras atividades aumentou linearmente com a inclusão de glicerina na dieta.

46

47 **Palavras - chave**: bovino, comportamento, confinamento, consumo, glicerol

48

49 **ABSTRACT**

50

51 This work was carried out to study corn substituting by glycerin levels on animal

52 behavior, feeding and rumination efficiency of Purunã young bulls finished in feed-lot.

53 It was utilized 40 bulls Purunã breed with 208.8 ± 33.3 kg and eight months old. The

54 diets were: without glycerin - G00, 6% of glycerin – G06, 12% of glycerin – G12 and

55 18% of glycerin – G18. Dry matter intake was similar among diets. On the other

56 hand, NDF intake decreased linearly with glycerin levels supplementation in the

57 diets. Feeding and rumination efficiency DM and NDF were similar among diets.

58 Glycerin changed activities durations of bulls. Glycerin did not affect feed frequency.

59 At contrary, rumination frequency was reduced linearly with glycerin inclusion. Others

60 activities frequencies showed a quadratic effect with the glycerin addition. Glycerin

61 inclusion in the diet reduced the time duration for feed frequency, but had no effect

62 on the time spent for rumination frequency. However, the frequency duration for other

63 activities increased linearly with glycerin inclusion.

64

65 **Keywords**: behavior, cattle, feed intake, feed-lot, glycerol.

66

67 **INTRODUCTION**

68

69   Factors that regulate dry matter intake by ruminants are complex and not

70 understood fully. Nevertheless, accurate estimates of feed intake are vital to

71 predicting rate of gain of animals. Previous research has established relationships

72 between dietary energy concentration and dry matter intake by beef cattle based on

73   the concept that consumption of less digestible, low-energy (often high-fiber) diets is
74   controlled by physical factors such as rumen fill and digest passage, whereas
75   consumption of highly digestible, high-energy (often low-fiber, high-concentrate) diets
76   is controlled by the animal's energy demands and by metabolic factors (NRC, 2000).

77   The feeding behavior is related of intake, obtaining data to improve animal
78   performance by feed intake (ALBRIGHT, 1993). Thus, the problems related to
79   declining intake in critical times, management practices, quality and quantity of diet
80   offered can be improved by changing the feeding behavior (MARQUES et al., 2008).
81   Animal performance is mainly influenced by dry matter intake that can be affected by
82   the amount of fiber (MISSIO et al., 2010) and energy content in the diet (FREITAS et
83   al., 2010). Feed intake of diet with high concentration of NDF, increases the number
84   and chewing duration and rumination duration due to fill the rumen-reticulum (DADO;
85   ALLEN, 1995). According to Van Soest (1994) rumination duration is influenced by
86   the nature of the diet and seems to be proportional to the cell wall content of forages.
87   Thus, intake of fiber is highly correlated with rumination time, and in general, the
88   nutritional quality of the diet may determine changes in food intake, modifying the
89   ingestive behavior and animal performance (SIGNORETTI et al., 1999). According to
90   Forbes (1988), ruminants can modify in part the ingestive behavior minimizing the
91   effects of unfavorable dietary conditions, reaching their nutritional requirements for
92   maintenance and growth. Bürger et al. (2000) found that period feeding duration of
93   animal finished in feed-lot may vary from one to six hours, depending directly of the
94   energy levels in the diet. The decrease of NDF caused by increased levels of
95   concentrate (energy) in the diet reduces feeding and rumination duration, providing
96   more time to animal performance and other activities that require lower energy
97   expenditures, improving the animal performance (SOUZA et al., 2007).

98   The use of glycerin replacing corn as an energy source in diets for feedlot bulls
99   can change the feeding behavior. Elam et al. (2008) and Farias et al. (2012)
100   observed that animals supplemented with glycerin in the diet needed more time to
101   consume food than control group. The use of glycerin as a corn substitute, an energy
102   source, determines fast rumen fermentation (TRABUE et al., 2007) which modifies
103   intake behavior to the point that animals require more time to consume feed when
104   compared to glycerin-less diets.

105   This study was conducted to evaluate the effects of different glycerin levels as
106 corn substitution in the diets on ingestive behavior, feed intake and rumination
107 efficiency of young bulls breed Purunã finished in feed-lot.
108

109 **MATERIAL AND METHODS**

110

111 *Animals, housing and diets*

112   This experiment was approved by Department of Animal Production at the State
113 University of Maringá (CIOMS/OMS, 1985). It was conducted at the Experimental
114 Station of Farm Modelo at Institute Agronomic of Paraná – IAPAR in Ponta Grossa,
115 city, Paraná State, Brazil South.

116   Forty Purunã bulls breed (¼ Aberdeen Angus + ¼ Caracu + ¼ Charolais + ¼
117 Canchim) were used in a complete randomised design. Bulls were weighed and
118 distributed in four diets with ten replications per group. After an 11-d diet adaptation
119 period, the bulls were weighed and started the study with an average initial BW of
120 208.8 ± 33.3 kg and average age of 8 months. The bulls' BW and concentrate and
121 corn silage intakes were recorded monthly until day 229 of the experiment when the
122 bulls reached a final BW of 471.7 ± 57.3 kg.

123   The glycerin was produced in a soy-diesel facility (BIOPAR, Rolândia, Paraná,
124 Brazil South). Glycerin fed in the current study was used as an energetic ingredient;
125 therefore, to obtain four isoenergetic diets, the increase in glycerin level was
126 counterbalanced, mainly by a decrease in corn grain content (Table 1). All diets were
127 formulated to be isonitrogenous (Table 2).

128

129 Table 1. Ingredients and percent composition (% DM) of the diet treatments

Ingredients, %	Glycerin levels, % DM			
	G00[1]	G06[2]	G12[3]	G18[4]
Corn silage	53.00	53.00	53.00	53.00
Soybean meal	11.78	13.39	14.99	16.87
Corn grain	34.40	26.77	19.14	11.38
Glycerin	0.00	6.00	11.99	17.99
Mineral salt[5]	0.83	0.83	0.83	0.76

130 [1]Diet without glycerin; [2]6% glycerin; [3]12% glycerin; [4]18% glycerin; [5]Guarantee levels
131 (per kg): calcium - 175 g; phosphorus – 100 g; sodium – 114 g; selenium – 15 g;
132 magnesium – 15 g; zinc – 6.004 mg; manganese – 1.250 mg; copper – 1.875; iodine
133 – 180 mg; cobalt – 125 mg; selenium – 30 mg; fluorine (maximum) – 1.000 mg.

134   The bulls were randomly assigned to 1 of 4 diets containing 0, 6, 12 or 18%

135   glycerine on a DM basis; which represented 18.3; 38.5; and 61.3% of corn replacing.

136   The bulls were fed concentrate and corn silage in separate troughs, both for *ad*

137   *libitum*. Bulls were fed twice a day (08:00 and 15:00 h). The diets were weighed daily,

138   so that the refusals represented 5% of the total. The concentrate intake was fixed in

139   1.2% of BW and adjusted every 28-d. The diets formulation and quantity supplied

140   were designed to provide a weight gain of 1.2 kg/day, according to NRC (2000)

141   recommendations.

142

143   Table 2. Chemical composition of the base diets (% DM)

Ingredients, %	DM[1]	%/DM								
		OM[2]	Ash	CP[3]	EE[4]	TC[5]	NFC[6]	NDF[7]	ADF[8]	TDN[9]
Corn silage	29.11	97.27	2.73	6.06	3.36	87.85	51.41	36.44	19.16	62.20
Soybean meal	81.50	92.86	7.14	48.89	2.50	41.47	23.40	18.07	11.65	78.03
Corn grain	81.76	97.68	2.32	10.32	5.93	81.43	64.15	17.28	4.77	81.64
Glycerin	94.27	95.24	4.76	0.07	0.12	-	-	-	-	80.61
Mineral salt	98.00		96.00							
Diets, %										
G00[10]	53.96	87.94	2.79	10.81	3.69	73.44	47.53	25.91	12.61	70.24
G06[11]	54.69	88.45	3.00	10.81	3.36	68.91	43.83	25.07	12.47	70.10
G12[12]	55.42	88.96	3.22	10.81	3.03	64.37	40.14	24.23	12.32	69.96
G18[13]	56.18	89.56	3.45	10.91	2.70	59.84	36.43	23.40	12.20	69.92

144   [1]Dry matter; [2]Organic matter; [3]Crude Protein; [4]Ether extract; [5]Total carbohydrates;
145   [6]Non-fibre carbohydrates; [7]Neutral detergent fibre; [8]Acid detergent fibre; [9]Total
146   digestive nutrients; [10]Diet without glycerin; [11]6% glycerin; [12]12% glycerin; [13]18%
147   glycerin.

148

149   *Samples collection*

150

151   There were two visual assessments of behavioral activities interval of 56 days

152   between observations ones. The data collections were realized during 48

153   consecutive hours, with a record of activities in specific ethogram every five minutes

154   (SILVA et al., 2006). The behavioral activities were collected by eight observers,

155   divided into four teams who alternated every two hours (SILVA et al., 2006).

156   Data were collected to estimate the duration and numbers of the periods spent

157   feeding, ruminating and others activities. The total time of each activity was

158   determined by the sum of repetitions, while the number of periods was accounted for

159   in accordance with the number of consecutive repetitions of each activity. The times

160 of each activity were determined by the ratio between the length and the number of
161 periods for each activity.

162   The efficiencies of feeding and rumination of dry matter and neutral detergent
163 fiber were determined and adapted the methodology proposed by Bürger et al.
164 (2000), according to the formulas described below:

165   FEDM = DMI/FD

166   FENDF = NDFI/FD

167   REDM = DMI/RUD

168   RENDF = NDFI/RUD

169 Where:

170   FEDM– Feeding efficiency of dry matter (kg DM/h);

171   DMI – Dry matter intake (kg DM/day);

172   FD– Feeding duration (h/day);

173   FENDF– Feeding efficiency neutral detergent fiber (NDF kg/h);

174   NDFI–Neutral detergent fiber intake (NDF kg/day);

175   REDM– Rumination efficiency of dry matter (kg DM/h);

176   RUD– Rumination duration (h/day);

177   RENDF– Rumination efficiency of neutral detergent fiber (NDF kg/h).

178

179 *Chemical analyses*

180

181   Dry matter content of the ingredients (silage, concentrate mix) was determined
182 by oven-drying at 105°C for 24h (AOAC, 1990) (method 930.15). The OM content
183 was calculated as the difference between DM and ash contents, with ash determined
184 by combustion at 550°C for 5h. The NDF and ADF contents were determined using
185 the methods described by Van Soest et al. (1991) with heat stable alpha-amylase for
186 solubilization the amylaceous compound (MERTENS, 2002) and sodium sulfite used
187 in the NDF procedure, and expressed inclusive of residual ash. Content of N in the
188 samples was determined by the Kjeldahl method (AOAC, 1990) (method 976.05).
189 The total carbohydrates (TC) were obtained by using the following equation: TC =
190 100 – (% CP + % EE + % Ash) (SNIFFEN et al., 1992). Non-fiber carbohydrates
191 (NFC) were determined by the difference between TC and NDF. Total digestible
192 nutrients (TDN) content of diets was obtained by the methodology descript by Kearl
193 (1982): silage = -17.2649 + 1.2120 (% CP) + 0.8352 (% ENN) + 2.4637 (% EE) +

194 0.4475 (% CF); energetic foods = 40.2625 + 0.1969 (% CP) + 0.4228 (% ENN) +

195 1.1903 (% EE) + 0.1379 (% CF) and protein foods = 40.3227 + 0.5398 (% CP) +

196 0.4448 (% ENN) + 1.4218 (% EE) − 0.7007 (% CF). The samples were analyzed in

197 triplicate at the Laboratory of Feed Analyses and Animal Nutrition at the State

198 University of Maringá.

199

200 *Statistical analysis*

201

202 The experimental design was completely randomized with four treatments and

203 ten replications. Results were statistically interpreted by regression equations using

204 (SAS, 2004) procedure (PROC REG): $Y_{ijk} = \beta_0 + \beta_1 X_i + \beta_2 X_i^2 + \alpha_{ijk} + \varepsilon_{ijk}$.

205 where:

206 Y_{ijk} = dependents variables;

207 β_0 = regression coefficient;

208 X_{ijk} = independents variables;

209 α_{ijk} = regression deviations;

210 ε_{ijk} = residual error.

211

212 **RESULTS AND DISCUSSION**

213

214 Total dry matter intake (7.9 kg/day) was similar ($P > 0.05$) among diets (Table

215 3).Similarly, Mach et al. (2009) reported no changes in DMI when glycerin was

216 included at 0, 4, 8 or 12% in the diet (8.3 kg/day) of Holsteins bulls fed high-

217 concentrate diets. Likewise, some others studies conducted with lactating cows that

218 were fed high-forage diets (CHUNG et al., 2007; DEFRAIN et al., 2004) have

219 reported no negative effects on feed intake when supplementing the diets with

220 glycerin at inclusion rates similar to the present study. On the other hand, Ogborn

221 (2006) reported that 5% glycerin increased DMI in prepartum dairy cows. In contrast,

222 Parsons et al. (2009) reported a 13% reduction in DMI when glycerin was added at

223 16% to a steam-flaked corn fed to heifers for the final 85 days before slaughter.

224 Schröder e Südekum (1999) reported 0.7 kg/day reduction in starch intake in

225 ruminally cannulated steers that were fed 15% glycerin. Thus dry matter intake can

226 be dependent glycerin quality (DONKIN, 2008).

Table 3. Glycerin levels on feed intake, feed efficiency and rumination efficiency of Purunã bulls finished in feed-lot

Item	Glycerin levels, % of DM				Regression equation[5]	SEM[6]	r^2
	G00[1]	G06[2]	G12[3]	G18[4]			
DMI[7], kg/d	8.27	8.47	7.29	7.46	$\hat{Y}=7.87$	0.27	-
NDFI[8], kg/d	2.67	2.64	2.35	2.31	$\hat{Y}=2.699-0.019x$	0.07	0.11
FE_{DM}[9], kg/h	2.36	2.42	2.15	2.59	$\hat{Y}=2.38$	0.09	-
FE_{NDF}[10], kg/h	0.76	0.75	0.69	0.81	$\hat{Y}=0.75$	0.02	-
RE_{DM}[11], kg/h	1.12	1.10	1.08	1.14	$\hat{Y}=1.11$	0.04	-
RE_{NDF}[12], kg/h	0.36	0.34	0.34	0.35	$\hat{Y}=0.35$	0.01	-

[1]Diet without glycerin; [2]6% glycerin; [3]12% glycerin; [4]18% glycerin; [5]Effect of glycerin level; [6]Standard error of mean; [7]Dry matter intake; [8]Neutral detergent fiber intake;[9]Dry matter feeding efficiency; [10]Neutral detergent fiber feeding efficiency; [11]Dry matter rumination efficiency; [12]Neutral detergent fiber rumination efficiency.

On the other hand, NDF intake ($P<0.05$) decreased linearly with glycerin levels supplementation in the diets, which can be explained by the lower content of NDF in the glycerin of the diet offered for bulls (Table 2). However, the reduced NDF intake did not reduce DM intake depending glycerin levels in the diets.

FEDM and FENDF were similar ($P>0.05$) among diets (Table 3). Farias et al. (2012) observed the negative quadratic effect on FEDM and FENDF with replacing corn by glycerin in the diets of heifers supplemented in pasture system.

REDM and RENDF were not changed due to replacement levels, demonstrating that glycerin was effective to replace corn without affecting the performance characteristics. Moreover, Farias et al. (2012) observed a negative quadratic effect on REDM and RENDF due to the inclusion of glycerin in the diet.

Replacing corn by glycerin as energy source in the diets changed($P<0.01$) activities durations of bulls finished in feed-lot (Table 4).The time spent for feeding and ruminating decreases linearly as a function of replacing corn by glycerin in the diets. The behavioral characteristics are mutually excluding, so there was a linear increase ($P<0.01$) in the time spent for other activities.

Table 4. Glycerin levels on duration (minutes) behavior intake of Purunã bulls finished in feed-lot

Item	Glycerin levels, % of DM				Regression equation[5]	SEM[6]	r^2
	G00[1]	G06[2]	G12[3]	G18[4]			
Feeding	217.37	212.75	203.00	177.62	$\hat{Y}=222.03-1.842x$	5.06	0.21
Rumination	445.87	463.50	414.37	400.00	$\hat{Y}=458.95-2.667x$	8.57	0.15
Other activities	776.75	763.50	822.62	862.37	$\hat{Y}=758.91+4.514x$	11.42	0.25

[1]Diet without glycerin; [2]6% glycerin; [3]12% glycerin; [4]18% glycerin; [5]Effect of glycerin level; [6]Standard error of mean.

255　　　　Glycerin supplementation in the diet for bulls finished in feed-lot reduced
256　feeding duration by18.2%. According to Bergner et al. (1995) the increased of rumen
257　fermentation of glycerin allows its transformation into volatile fatty acids, especially
258　propionate (RÉMOND et al., 1993; TRABUE et al., 2007). The rapid metabolism of
259　this compound into energy for maintenance and growth of the animal promotes
260　satiety. Besides that, glycerin can be absorbed by the ruminal epithelium and
261　metabolized into glucose by the liver (DONKIN, 2008). According to Trabue et al.
262　(2007) inclusion of high levels of glycerin levels can inhibit the feed intake for a
263　certain time due to the amount of energy delivered to the animal by the possible
264　presence of salts, impurities, and high methanol levels resulting from the
265　transesterification process (CHUNG et al., 2007; PARSONS et al., 2009). The data in
266　this study corroborate to those of Missio et al. (2010) when evaluating the influence
267　of concentrate levels(22, 40, 59 and 79%) on the ingestive behavior of young bull sin
268　feed-lot. The authors obtained a linear decrease on feed intake due a higher intake
269　(energy) in less time, reaching the nutritional requirements of animals. Likewise, Silva
270　et al (2005) evaluated the feeding behavior of cattle fed different levels of
271　concentrate and observed a linear reduction on feeding duration due to the lower
272　NDF and higher energy intake in the diet. Corroborating to above authors, Farias et
273　al. (2012) observed a reduction in feeding duration depending on glycerin levels (2.8,
274　6.1 and 9.1%) in the diet for heifers supplemented in pasture system.
275　　　　Replacing corn by different glycerin levels in the diets reduced the NDF content
276　in the diets (Table 2). The reduction of NDF in the diet decreased the rumination
277　duration (Table 4). Mendes Neto et al. (2007) observed differences between the
278　rumination duration for roughage and concentrate depending the type and fiber
279　content in the food. According to Kijora et al. (1998), 85% of ingested glycerin may
280　disappear within the first two hours after feeding, agreeing with Bergner et al. (1995)
281　stated that when levels of 15 to 25% of glycerin in the diet of ruminant is modified into
282　six hours. Therefore, the glycerin is rapidly metabolized by bacteria in the rumen or
283　volatile fatty acids, can be absorbed by the ruminal epithelium and promote a
284　negative feedback on the necessary rumination duration (DONKIN, 2008). Farias et
285　al. (2012) observed no difference for rumination duration (382.86 min/day) for heifers
286　fed different glycerin levels in the diets (2.8, 6.1 and 9.1%). According to Missio et al.
287　(2010) decreasing rumination duration, the increased the rest time of animals imply a
288　decrease in physical activity, contributing thus for increases on animal performance.

289   The time spent for other activities were 11% higher for bulls fed with inclusion of

290   glycerin in the diets. The time utilized for other activities (862 min./day) was higher

291   than those found by Bürger et al (2000) when evaluated concentrate levels (30, 45,

292   60 and 75%) in the diet of steers (655, 701, 795 and 841 min./day.) and Farias et al.

293   (2012) evaluating the levels of crude glycerin (2.8, 6.1 and 9.1%) in the diets for

294   heifers (575, 547 and 623 min.). Glycerin of high purity can be better utilized by the

295   body when compared to ruminant diets supplemented with concentrated crude

296   glycerin or not. Fatty acid resulting from ruminal fermentation of glycerin can be

297   metabolized by the gastrointestinal tract into energy, or absorbed through the portal

298   vein and sent to the liver (DONKIN, 2008). Subsequently, propionate derived from

299   the biohydrogenation or glycerin absorbed by the ruminal epithelium is converted into

300   glucose (KIJORA et al. 1998).

301   Glycerin supplementation in the diet did not affect ($P>0.05$) feed frequency (18

302   visits/day) for bulls finished in feed-lot (Table 5). At contrary, rumination frequency

303   was reduced linearly ($P<0.01$) with the substitution of corn by glycerin. Others

304   activities frequencies showed a quadratic effect ($P <0.01$) with the glycerin addition,

305   being 9.5% level glycerin showed higher frequency number (29 visits/day).

306   Glycerin inclusion in the diet of bulls reduced ($P<0.01$) the time duration for feed

307   frequency, but had no effect ($P>0.05$) on the time spent for rumination frequency.

308   However, the frequency duration for other activities increased linearly ($P<0.01$)

309   with glycerin inclusion.

310

311   Table 5. Glycerin levels on frequency and duration frequency per activity of Purunã
312   bulls finished in feed-lot

Item	Glycerin levels, % of DM				Regression equation[5]	SEM[6]	r²
	G00[1]	G06[2]	G12[3]	G18[4]			
FF[7] (visits/d)	17.55	18.62	18.42	18.07	$\hat{Y}=18.16$	0.35	-
RF[8] (visits/d)	17.47	16.85	15.70	14.55	$\hat{Y}=17.63 - 0.141x$	0.33	0.28
OAF[9] (visits/d)	27.47	30.02	28.17	27.15	$\hat{Y}=27.73+0.342x-0.01x^2$	0.36	0.17
FDF[10] (min)	12.48	11.52	11.07	9.85	$\hat{Y}=12.48 - 0.118x$	0.29	0.26
RDF[11] (min)	25.77	27.84	26.53	27.81	$\hat{Y}=26.99$	0.64	-
ODF[12] (min)	28.29	25.70	29.32	31.89	$\hat{Y}=26.63 + 0.206x$	0.58	0.19

313   [1]Diet without glycerin; [2]6% glycerin; [3]12% glycerin; [4]18% glycerin; [5]Effect of glycerin
314   level; [6]Standard error of mean; [7]Feeding frequency, [8]Rumination frequency; [9]Other
315   activities frequency; [10]Feeding duration frequency; [11]Rumination duration frequency;
316   [12]Others activities duration frequency.

317

Glycerin inclusion in the diets reduced the fibrous portion due to the substitution of corn by glycerin. Allowances energy requirements, either by ruminal fermentation or by hepatic metabolism of glycerin, allowed greater availability of cattle to perform other activities during the evaluation period. Thus, the frequency of reduced rumination is related to the rapid disappearance of glycerin in the gastro intestinal tract of ruminants and lower NDF content in bolus regurgitated food by animals (MISSIO et al., 2010).

CONCLUSIONS

Corn partial replacement by glycerin in the diets for bulls finished in feed-lot and fed with 53% corn silage and 47% concentrate can be an alternative due your availability on market and feed utilization for animals.

ACKNOWLEDGEMENTS

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CAPÍTULO 3

GLYCERINE LEVELS IN THE DIETS OF CROSSBRED BULLS FINISHED IN FEEDLOT: CARCASS CHARACTERISTICS AND MEAT QUALITY[1]

1 GLYCERINE LEVELS IN THE DIETS OF CROSSBRED BULLS FINISHED IN
2 FEEDLOT: CARCASS CHARACTERISTICS AND MEAT QUALITY
3

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20 **ABSTRACT**
21   The effect of corn replacement by different glycerine levels on carcass
22 characteristics and meat qualityof40 young Purunã bulls, weighing 209 ± 33.3 kg
23 and 8 + 0.9 months old, finished in feedlot, is analyzed. The diets were G00:
24 without glycerine; G06: 6% glycerine; G12: 12% glycerine; G18: 18% glycerine in
25 the diets. Hot weight, dressing, conformation and length carcass, leg length and
26 cushion thickness were not modified by different glycerine levels in the diets.
27 Glycerine in the diets did not affect fat thickness, Longissimus muscle area,
28 marbling and texture. Muscle (69.4%), fat (18.4%) and bone (12.1%) were not
29 influenced by glycerine levels in the diets. No changes in lightness (L), redness
30 (a*) and yellowness (b*) on LM occurred when glycerine was included at 0, 6, 12
31 or 18% in the diet. There was no difference in moisture, ashes, crude protein and
32 total lipids for different glycerine levels on LM and in most fatty acids in the LM.
33

34 **Keywords:** Carcass characteristics, cattle, glycerine, meat quality

35 **1. Introduction**

36   The rapid expansion of the biodiesel industry over the past decade have

37 increased glycerine availability (FAPRI, 2013). In 2011, the glycerine refining

38 market produced about 2 billion kg of refined glycerine worldwide, and the

39 Brazilian market produced approximately 2.6 million kg (FAPRI, 2013). Increasing

40 availability of glycerine drove prices downward and provided glycerine excess

41 which may be used for other purposes such as animal feed (Donkin, Koser, White,

42 Doane & Cecava, 2009; Farias et al., 2012; Françozo et al., 2013).

43   Glycerol is thought to have gluconeogenic properties (Donkin et al., 2009),

44 metabolized by ruminal microorganism's (Abo El-Nor, AbuGhazaleh, Potu,

45 Hastings & Khattab, 2010) and could potentially improve carcass and meat quality

46 grades (Elam, Eng, Bechtel, Harris & Crocker, 2008). In this study, as hypothesis

47 was forwarded that glycerine supplementation increase lipogenesis and improves

48 marbling and subcutaneous fat. Previous studies reported reduction of acetate to

49 propionate ratio in the rumen, mainly resulting from an increase in rumen molar

50 proportions of propionate, which is a glucose precursor (Kijora et al., 1998;

51 Rémond, Souday & Jouany, 1993). Further, glycerine might be converted to

52 glucose in the liver of cattle. Thus, it was expected that glucose supply would

53 increase in bulls supplemented with glycerine, fostering a rise lipogenesis.

54 However, others studies noted linear decreases in marbling scores when glycerine

55 was included in the diets of cattle (Parsons, Shelor & Drouillard, 2009), which

56 could influence in marbling scores and result in a linear tendency to decrease the

57 percentage in the grading scale of carcasses. Likewise some studies (Purchas,

58 Burnham & Morris, 2002) have associated tenderness with intramuscular fat

59 content that could influence in the meat quality.

60   Current study determines the effects of different glycerine levels as an

61 energy source on the carcass characteristics and meat quality of Purunã bulls

62 finished in feedlot.

63

64 **2. Materials and methods**

65

66 *2.1. Animals, housing and diets*

67   The experiment, approved by the Department of Animal Production of the

68 State University of Maringá (CIOMS/OMS, 1985) was conducted at the

69　Experimental Station of the Paraná Agronomic Institute (IAPAR) in Ponta Grossa
70　city, Paraná State, Brazil South.

71　　　　Forty Purunã bulls (¼ Aberdeen Angus + ¼ Caracu + ¼ Charolais + ¼
72　Canchim) were used in a complete randomised design. Bulls were weighted and
73　distributed into groups, with four diets and ten replications per group. They were
74　allocated into individual pens (8 m2 for each animal) in a feedlot system. After an
75　11-day diet adaptation period, the bulls were weighed, featuring an average initial
76　BW of 209 ± 33.3 kg and average age of 8 + 0.9 months. The bulls' BW and intake
77　of concentrate and corn silage were recorded monthly until day 229 of the
78　experiment when the bulls reached a final BW of 472 ± 57.3 kg.

79　　　　The glycerine, produced in a soya-diesel facility (BIOPAR, Rolândia,
80　Paraná, Brazil) was used as an energetic ingredient (Table1). For four iso-
81　energetic diets, increase in glycerine level was mainly counterbalanced by a
82　decrease in corn grain contents (Table 2). All diets were formulated as iso-
83　nitrogenous.

84　　　　The bulls were randomly assigned to one of the four diets containing 0, 6,
85　12 and 18% glycerine in DM basis of diets, which represented 18.3; 38.5; and
86　61.3% of corn replacing. The bulls were fed ad libitum on concentrate and corn
87　silage in separate troughs, twice a day (08:00 and 15:00 h). The diets were
88　weighed daily, so that the refusals represented 5% of the total. The concentrate
89　intake was fixed in 1.2% of BW and adjusted every 28days. The diet formulation
90　and quantity supplied were designed to provide a weight gain of 1.2 kg/day,
91　according to NRC (2000) recommendations.

92

93　*2.2. Nutrients and diets analyses*

94　　　　Dry matter contents of the ingredients (silage, concentrate mix) were
95　determined by oven-drying at 65℃ for 24 h (Table 3). Analytical DM content of
96　oven-dried samples was determined by drying at 135 ℃ for 3 h by method 930.15
97　(AOAC, 1990). The OM content was calculated as the difference between DM and
98　ash contents, with ash determined by combustion at 550℃ for 5 h (AOAC, 1990).
99　The NDF and ADF contents were determined by methods described by (Mertens,
100　2002). Nitrogen content in the samples was determined by method 976.05 (AOAC,
101　1990). Total carbohydrates (TC) were obtained by the equation of Sniffen,
102　O'Connor, Van Soest, Fox and Russell (1992): TC = 100 - (%CP + %EE +%Ash).

103   Non-fiber carbohydrates (NFC) were determined by the difference between TC

104   and NDF. Total digestible nutrient (TDN) content of diets was obtained by the

105   methodology described by Kearl (1982). Samples were analyzed at the Laboratory

106   of Feed Analyses and Animal Nutrition of the State University of Maringá.

107

108   *2.3. Carcass characteristics measurements*

109   Bulls were slaughtered according to industrial practices in Brazil at a

110   commercial slaughterhouse from 10 km distance from the Ponta Grossa Research

111   Farm. The carcasses were then identified and chilled for 24 h at 4ºC. After chilling,

112   the right part of the carcass was used to determine quantitative characteristics.

113   Hot carcass weight (HCW) was determined soon after slaughter and prior to

114   carcass chilling.

115   Hot carcass dressing (HCD): the percentage of individual animal dressing

116   was defined by the ratio HCW:live weight.

117   Carcass conformation (CONF): Muscle development was determined after

118   excluding fat thickness where the highest value indicated the best conformation

119   (Müller, 1980). CONF may be superior, very good, good, regular, poor, or inferior;

120   ratings may also be reported as plus, average and minus.

121   Carcass length (CAL) was measured from the skull board to the pubic bone

122   on the anterior side of the first rib.

123   Leg length (LEL) was evaluated with a wooden compass with metallic

124   edges that measured the distance from the anterior border of the pubis bone to a

125   middle point on the tarsus bone.

126   Cushion thickness (CUT): Cushion thickness was determined with a

127   wooden compass with metallic edges that measured the distance between the

128   lateral face and the median at the superior part of the cushion. The cushion is the

129   flat muscle (*Biceps femoris*).

130   Fat thickness (FAT) was measured by a calliper averaging three points

131   between the 12th and 13th ribs over the LM.

132   Longissimus muscle area (LMA): The right part of the carcass was

133   measured after a cross-section cut was made between the 12th and 13th ribs

134   using a compensating planimeter that measured the areas of irregular shaped

135   objects.

136   Longissimus muscle area/100kg carcass (LMC): LMC percentage was

137   defined by the ratio LMA:HCW, multiplied by 100.

138   Marbling (MAR): was measured in the LM between the 12th and 13th ribs,

139   following scores described by Müller (1980).

140   Texture(TEX): was determined by fascicle size (muscular "grain" size) and

141   evaluated subjectively on a point scale (Müller, 1980).

142   Colour (COL): the muscle colour was analysed after a 24-hour carcass

143   chilling. Coloration was evaluated according to a point scale 30 minutes after a

144   cross-sectional cut was made on the Longissimus between the 12th and 13th ribs

145   (Müller, 1980).

146   Hydrogen potential (pH): Meat pH was measured before and after chilling

147   using a pH Meter Text Model (Tradelab, Contagem MG Brazil), following LANARA

148   (1981).

149   Percentage of carcass muscle (MP), fat (FP) and bone (BP): Muscle, fat

150   and bone were physically separated from the Longissimus section, which

151   corresponds to the 10th, 11th and 12th ribs, and individually weighed according to

152   Hankins and Howe (1946) as follows:

153   % M = 6.292 + 0.910 X1

154   % F = 1.526 + 0.913 X2

155   % B = 2.117 + 0.860 X3

156   in which X1, X2 and X3 represent muscle, fat and bone percentages,

157   respectively.

158   Colour (Col)was measured on LM samples, removed 48 hours post

159   mortem, with a Minolta Chroma Meter CR-310 (Osaka, Japan), calibrated against

160   a white tile (L* = 92:30, a* = 0.32 and b* = 0.33). Samples were allowed to bloom

161   for 1 hour at 4℃ prior to measurements. The parameters L*, a* and b*,

162   representing lightness, redness and yellowness, were measured at five sites of

163   each LM, and the average was presented. Samples of muscle Longissimus were

164   then frozen at -20ºC for further analyses.

165

166   *2.4. Meat characteristics and sensory analyses*

167   Analyses on the Longissimus were carried out two months after sampling.

168   Thawing loss was determined by water loss. Samples were weighed after thawing

169 at 2 ± 2ºC; 24 hours later thawing loss was determined. Thawing loss is the ratio of

170 sample weight before and after being frozen, multiplied by 100.

171 Muscle samples were weighed (initial weight), separated in individual

172 standardized slices 50 mm thick, placed in an electric oven and cooked at a

173 defined internal temperature (72℃). When the endpo int temperature was reached,

174 the samples were removed from the electric oven and kept at room conditions until

175 equilibrated. The meat was then removed from the plates and weighed. Sensory

176 analysis of meat was performed by a trained panel of 10 judges selected from

177 student and staff members of the Agronomic Institute of Paraná (IAPAR), taking

178 into account their habits, acquaintance with the material to be analysed, sensitivity

179 and ability to judge. Panellists were trained to detect tenderness, juiciness and

180 flavour by triangular discrimination tests. Meat samples were evaluated by a

181 descriptive test, according to the methods by ABNT-NBR 14141for tenderness (7

182 = extremely soft; 1 = extremely firm), juiciness (7 = extremely juicy; 1 = extremely

183 dry) and flavour (7 = palatable; 1 = unpalatable) using a seven-point hedonic scale

184 (ABNT, 1998). Each attribute was discussed and tests were initiated after

185 panellists were familiarized with the scales. Meat samples of the four treatments

186 (G00, G06; G12 and G18%) were grilled and kept at 65℃ until analysis. Samples

187 were served randomly at approximately 60℃. Each pa nellist evaluated two

188 samples per treatment. Unsalted crackers and water at room temperature were

189 provided to clean the palate between sample intakes. The tests were carried out

190 between 9 – 11h am.

191

192 2.5. Chemical composition

193 The samples were ground, homogenised and analysed in triplicate. Beef

194 moisture and ash contents were determined according to AOAC (1990).Crude

195 protein content was obtained through the Kjeldahl method(AOAC, 1990). Total

196 lipids were extracted using the Bligh and Dyer (1959) method with a

197 chloroform/methanol mixture. Fatty acid methyl esters (FAMEs) were prepared by

198 triacylglycerine methylation according to ISO (1978) method. FAMEs were

199 analysed in a gas chromatograph (Varian, USA), equipped with a flame ionisation

200 detector and a fused silica capillary column CP-7420 (100 m, 0.25 mm and 0.39

201 μmo.d., Varian, USA) Select Fame. The column temperature was programmed at

202 165℃ for 18 minutes, 180℃ (30℃ min $^{-1}$) for 22 minutes, and 240℃ (15℃ min $^{-1}$)

203 for 30 minutes with 45-psi pressure. The injector and detector were kept at 220℃
204 and 245℃, respectively. Gas fluxes (White Martins) comprised 1.4 mL min^{-1} for
205 carrier gas (H_2); 30 ml min^{-1} for make-up gas (N_2); and 30 mL min^{-1} and 300 mL
206 min^{-1} for H_2 and synthetic flame gas, respectively. The sample injection split mode
207 was 1/80. Fatty acids were identified by comparing the relative retention time of
208 FAME peaks of the samples with fatty acids methyl ester standards from Sigma
209 (USA) by spiking samples with the standard. The peak areas were determined by
210 Star software (Varian). Data were expressed as percentages of the normalised
211 area of fatty acids.

212

213 *2.6. Statistical analysis*

214 The experimental design was completely randomized with four treatments
215 and ten replications. All characteristics under study were tested for normality.
216 Those that showed normal distribution were analyzed by the regression equations
217 using PROC REG procedure:

218 $Y_{ijk}= \beta 0 + \beta 1 X_i + \beta 2\ X_i{}^2 + \alpha_{ijk} + \varepsilon_{ijk}$,

219 where:

220 Y_{ijk} = dependent variables (glycerine levels);

221 β's = regression coefficient;

222 X_{ijk} = independent variables;

223 α_{ijk} = regressiondeviations;

224 ε_{ijk} = residual error.

225 The characteristics that did not show normal distribution were analyzed by
226 the generalized linear model method (Nelder & Wedderburn, 1972), according to
227 GENMOD procedure. All the statically analysis were performed by SAS (2004).

228

229 **3. Results**

230 Hot weight and dressing carcass were similar ($P>0.05$) in all diets (Table 4).
231 Carcass conformation did not change ($P>0.05$) when glycerine was added to the
232 diets. Glycerine which replaced corn as energy source in the diets for bulls in
233 feedlot did not affect ($P>0.05$) the carcass length, leg length, cushion and fat
234 thickness. Average for Longissimus muscle (LM) area and LM area/100 kg
235 carcass was similar ($P>0.05$) when glycerine was fed to Purunã bulls. No
236 difference ($P>0.05$) was reported in marbling and texture when corn was replaced

237 by glycerine in the diets of bulls finished in feedlot. LM color was not affected
238 (P>0.05) by glycerine level. Muscle, fat and bone were not influenced (P>0.05) by
239 glycerine levels in the diets.

240   No changes (P>0.05) in lightness (L), redness (a*), yellowness (b*) on LM
241 occurred when glycerine was included at 0, 6, 12 or 18% in the diet (Table 5). The
242 LM sensory characteristics did not show any difference (P>0.05) at different
243 glycerine levels in the diets (Table 6).There was no difference (P>0.05) with regard
244 to moisture, ashes, crude protein and total lipids in different glycerine levels on LM
245 (Table 7).

246   There was no difference (P>0.05) in most fatty acids in the LM (Table 8).
247 Fatty acids, such as myristic acid (14:0) and palmitic acid (16:0), were lower
248 (P<0.05) on LM of bulls fed on a diet supplemented with glycerine. Fatty acid
249 levels of cis-vaccenic acid (18:1 n-7), linolenic acid (18:2 n-6), α-linolenic acid
250 (18:3 n-3), arachidonic acid (20:4 n-6), eicosapentanoic acid (20:5 n-3) and
251 docosahexanoic acid (22:6 n-3) increase (P<0.05) LM of bulls fed on diets with
252 glycerine. However, the trans-vaccenic acid presented a quadratic effect (P<0.05)
253 with glycerine levels.

254   Unsaturated fatty acids were higher (P<0.05) in bulls fed on diet with
255 glycerine. The mono-unsaturated (MUFA) and poly-unsaturated (PUFA) fatty acids
256 increase (P<0.05) in muscles of bulls fed on diets with glycerine. On the other
257 hand, saturated fatty acids (SFA), fatty acids n-6 and n-3, PUFA:SFA and n-6:n-3
258 decrease (P<0.05) with glycerine levels (Table 9).The sum of n-6 and n-3 were
259 higher (P<0.05) in bulls fed on diets with glycerine. Difference was reported
260 (P<0.05) with PUFA:SFA ratio among diets, with a 55.5% increase in bulls fed on
261 several glycerine levels. On the other hand, n6:n3 ratio decreases (P<0.05) in
262 diets with glycerine.

263

264 **4. Discussion**

265   Weight and carcass dressing were258 kg and 54.7%, respectively, and
266 values may be considered normal to meet the standards of Brazilian markets
267 (Rotta et al., 2009). In general, Purunã bulls finished in feedlot and slaughtered at
268 a similar age had weight and carcass dressing rates close to results in current
269 experiment (Ito et al., 2010; Prado et al., 2009; Rotta et al., 2009).

270   Carcass conformation was considered very good (13.4 points) for all
271 treatments. According to Müller (1980), score may be considered adequate to
272 meet the standards of Brazilian markets (Rotta et al., 2009). This fact has been
273 corroborated by Mach, Bach and Devant (2009) who reported that bulls fed on a
274 diet with glycerine (4, 8 and 12%) had a 63% satisfactory conformation carcass.

275   The carcass length, leg length and cushion thickness rates were close to
276 those found by other researchers (Maggioni et al., 2010; Prado et al., 2008; Rotta
277 et al., 2009) when they evaluated carcass characteristics of bull breed (*Bos taurus*
278 *taurus vs. Bos Taurus indicus*).

279   Fat thickness (4.49 mm) in the bulls complies with the guidelines of the
280 Brazilian market, which requires the carcass to have between 3 and 6 mm of fat
281 thickness. In similar experimental conditions (Prado et al., 2012; Prado et al.,
282 2009; Rotta et al., 2009)reported similar fat thickness data when bulls were fed on
283 high-concentrate diets and finished in feedlots.

284   Since the LM area showed carcass muscle development, the hot carcass
285 weight and the carcass's comestible portion were directly correlated, with higher
286 weights in commercial cuts. On the other hand, Parsons et al. (2009) observed a
287 linear reduction in the LM area when increasing amounts of glycerine were
288 provided.

289   Marbling was classified as "light" or "small" (6.45 points). Although medium
290 marbling is well accepted within the home market, beef should feature more
291 accentuated marbling to be acceptable in foreign markets. Parsons et al. (2009)
292 observed that the inclusion of glycerine (16%) in the diets for heifers decreased
293 marbling scores. Texture was classified by granulation on the LM surface, with
294 4.49 points, which might be defined as "thin" or "very thin".

295   According to Mancini and Hunt (2005), meat color is an important
296 commercial characteristic that influences consumer behavior. Color was
297 considered good (3.68 points), according to classification by Müller (1980), ranging
298 between "red" and "slightly dark red". Adequate nutrition and low age may have
299 affected meat color (Renerre & Labas, 1987).

300   The glycerine which replaced corn allowed adequate muscle growth, with
301 no differences in the treatments. In this study, muscle (69.4%), fat (18.5%) and
302 bone (12.1%) percentages on LM corroborated rates in other studies performed by
303 the same researchers in conditions close to current study (Rotta et al., 2009).

304　　　　Lightness was influenced by the amount of water on the meat surface and
305　was a consequence of water retention capacity (Pearce, Rosenvold, Andersen &
306　Hopkins, 2011). Therefore, LM water loss was not affected by diet when glycerine
307　levels supplemented the diets. Françozo et al. (2013) corroborated the above and
308　failed to report any difference on LM water loss in bulls fed on crude glycerine-
309　supplemented diets (0; 5 and 12%).Lightness, redness, yellowness on LM were
310　normal for bulls finished in feedlot (Page, Wulf & Schwotzer, 2001).

311　　　　The results for tenderness (6.52 points), juiciness (6.16 points) and flavor
312　(6.49 points) were considered very good (Lepetit, 2008). Tenderness has been
313　associated with intramuscular fat content (Purchas et al., 2002), even though,
314　according to Mach et al. (2009),crude glycerine did not affect tenderness.
315　Tenderness, juiciness and flavor observed in the experiment should result in high
316　consumer acceptance (Hocquette et al., 2012). Consumers did not detect
317　differences in meat acceptability among diets. Indeed, meat acceptability may be
318　altered by fat levels and fatty acids composition (Wood et al., 2008). However, in
319　this study, fat level on the 12th rib and intramuscular fatty acid composition were
320　similar in the treatments. The overall acceptability rates of meat were high (above
321　6.3), with mid-scale at 3.5. As a rule, good quality meat has a rate above 5.0
322　(Campo, Sañudo, Panea, Alberti & Santolaria, 1999). Tenderness is one of the
323　most important criteria for beef quality and consumers are ready to pay a higher
324　price once they are assured that the beef is tender (Boleman et al., 1997). The
325　differences in meat tenderness might be due to the quantity, solubility and space
326　organization of collagen, fatness and calpain and calpastatin activity. In fact,
327　studies by Shackelford, Wheeler and Koohmaraie (1997) and Wulf, O'Connor,
328　Tatum and Smith (1997) suggested that difference in beef tenderness was
329　associated with the variation in the rate and extent of muscle proteolysis that
330　occurs during postmortem storage of fresh beef.

331　　　　Mean moisture, ashes, crude protein and total lipids rates were 74.1%,
332　1.04%, 21.3% and 2.04%, respectively. These results were similar to those
333　obtained by other researchers (Aricetti et al., 2008; Maggioni et al., 2010; Rotta et
334　al., 2009) who studied the chemical composition of different genetic groups of bulls
335　finished in feedlot. However, Françozo et al. (2013) reported total lipids decrease
336　of LM in bulls receiving glycerine levels (0. 5 and 12%). It was expected that, due
337　a rise in blood insulin concentrations and lipogenesis, glucose supply

338 supplemented with glycerine would increase total lipids in bulls (Parsons et al.,
339 2009). In fact, glycerine would increase the tenderness, juiciness and flavor of
340 meat of bulls fed on diet supplemented with glycerine (Parsons et al., 2009).

341 These saturated hypercholesterolemic fatty acids (14:0 and 16:0) are
342 responsible for heart disease owing to an increase in the quantity of low density
343 lipoproteins – LDL (Scollan et al., 2006). Furthermore, glycerine reduction in
344 myristic acid (23.9%) and palmitic acid (9.6%) could be beneficial to human health
345 (Webb & O'Neill, 2008).

346 Cis- and trans-vaccenic fatty acids were modified by glycerine in the diets.
347 Cis-vaccenic fatty acid had a positive linear tendency with level glycerine when
348 replacing corn, with an increase of 24.8% in the muscles of bulls fed on a diet with
349 glycerine. However, trans-vaccenic acid fatty presented quadratic effect with
350 glycerine levels at a maximum of 10.5% glycerine in the diet. This fatty acid is an
351 important intermediate factor produced by microorganisms in the rumen.
352 According to Scollan et al. (2006), these acids may be transformed into conjugate
353 linoleic acid (18:2 c-9, t-11) and later in tissues of ruminants.

354 Fatty linoleic acid increased (25.6%) with glycerine levels. The high
355 presence of this fatty acid in the Longissimus muscle of cattle might be related to
356 the imbalance of n-6/n-3 ratio (Rotta et al., 2009). However, increase to α-linolenic
357 acid (23.5%) would reduce the n-6/n-3 ratio, above its capacity to form other
358 important fatty acids (Wood et al., 2004). The above-mentioned fatty acids are
359 considered essential since most adipose deposits in animal tissues are
360 synthesized by lipogenesis. This is due to the fact that ruminant diets are poor in
361 fat components and elongated fatty acids are important due to their capacity to
362 form other important fatty acids (Wood et al., 2004), partly explained by the bio-
363 hidrogenation that occurs in the rumen (Tamminga & Doreau, 1991).

364 Likewise, fatty acids, namely, arachidonic (20:4 n-6), eicosapentanoic (20:5
365 n-3) and docosahexanoic acid (22:6 n-3) decrease cardiovascular diseases risk in
366 humans due to high production of eicosanoids compounds (Wood et al., 2008).
367 Thus, elongated fatty acids reductions (78.5, 50.0 and 47.4%, respectively) are an
368 asset to human health.

369 Saturated fatty acids (SFA) represented approximately 46.5% of total fatty
370 acid composition of Longissimus muscle of bulls fed with glycerine and finished in
371 feedlot. SFA concentration was lower (9.65%) with glycerine levels in the diets. In

372 fact, SFA of the Longissimus muscle of bulls fed with glycerine was lower than that
373 evaluated in bulls from different crossbreeding systems finished in feedlot.

374   Mono-unsaturated and the poly-unsaturated fatty acids increased 7.74%
375 and 31.9%, respectively. The valiues were higher than those reported by Françozo
376 et al. (2013) when the authors evaluated beef cattle Nellore fed on diets containing
377 glycerine (0, 5 and 12%).

378   The concentration of the sum n-6 fatty acid increased 27.6% because of
379 glycerine levels, which corroborated results by Rotta et al. (2009) who affirmed
380 that percentage ranged between 3.5 and 9.3%. Likewise, n-3 fatty acids presented
381 an increase of 44.9% in treatments with glycerine. The Longissimus muscle of
382 bulls fed on glycerine presented higher values when compared with results by
383 other authors.

384   Glycerine has higher PUFA:SFA ratio than rates found in current study
385 which are below the recommend rate (PUFA:SFA: 0.45, n6:n3: 4;0) by the HMSO
386 (1994), considered to be beneficial to human health. Low rations may be explained
387 by the biohydrogenation process undergone by dietary unsaturated fatty acids in
388 the rumen by microorganisms. In fact, glycerine quality (medium purity) could be
389 more metabolized when compared to crude glycerine. Françozo et al. (2013)
390 observed high rates of n6:n3 ratio (2.63, 2.70 and 2.61%) which evaluated beef
391 cattle Nellore fed on diets with glycerine (0, 5 and 12%, respectively) in feedlot.
392

393 **5. Conclusion**

394   Results from current study demonstrated that diet with up to 18% glycerine
395 might be fed to finishing bulls with no effect on carcass characteristics and meat
396 quality, albeit with only slight effects in fatty acid. The effects of glycerine on fatty
397 acid profiles of the LM might require further research. Glycerine is a viable source
398 of dietary energy, well utilized by bulls, and which could replace corn successfully.
399

purpose of providing specific information and do not imply recommendations or endorsement by the Department of Animal Science, State University of Maringá, Maringá, Paraná, Brazil.

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558

TABLES

Table 1

Chemical composition of the glycerine used in the study

Parameters	Results
Water*	23.2 g/kg
Ash	47.6 g/kg
Glycerol	812 g/kg
Methanol	3.32 mg/kg
Sodium	11.6 g/kg
Potassium	79.1 mg/kg
Chloride	35.8 mg/kg
Magnesium	16.3 mg/kg
Phosphorus	239 mg/kg
Gross energy	14.2 MJ

Realised by Institute of Technology of Paraná – TECPAR, Biofuels division, in Curitiba, Paraná.*Karl Fischer.

Table 2

Ingredients and percent composition (g/kg) of the diet treatments

Ingredients	Glycerine levels			
	G00[1]	G06[2]	G12[3]	G18[4]
Corn silage	530	530	530	530
Soybean meal	118	134	150	169
Corn grain	344	268	191	114
Glycerine	0.00	60.0	120	178
Mineral salt[5]	8.30	8.30	8.30	7.60

[1]Without glycerine; [2]6% glycerine; [3]12% glycerine; [4]18% glycerine; [5]Guarantee levels (per kg): calcium - 175 g; phosphorus – 100 g; sodium – 114 g; selenium – 15 g; magnesium – 15 g; zinc – 6.004 mg; manganese – 1.250 mg; copper – 1.875; iodine – 180 mg; cobalt – 125 mg; selenium – 30 mg; fluorine (maximum) – 1.000 mg.

Table 3

Chemical composition of the base diets (g/kg)

Ingredients	DM[1]	g/kg on DM								
		OM[2]	Ash	CP[3]	EE[4]	TC[5]	NFC[6]	NDF[7]	ADF[8]	TDN[9]
Corn silage	291	973	27.3	60.6	33.6	878	514	364	192	622
Soybean meal	815	929	71.4	489	25.0	415	234	181	116	780
Corn grain	818	977	23.2	103	59.3	814	641	173	47.7	816
Glycerine	943	952	47.6	0.70	1.20	-	-	-	-	806
Mineral salt	980									
Diets										
G00[10]	540	879	27.9	108	36.9	734	475	259	126	702
G06[11]	547	884	30.0	108	33.6	689	438	251	125	701
G12[12]	554	880	32.2	108	30.3	644	401	242	123	670
G18[13]	562	896	34.5	109	27.0	598	364	234	122	699

[1]Dry matter; [2]Organic matter; [3]Crude Protein; [4]Ether extract; [5]Total carbohydrates; [6]Non-fibre carbohydrates; [7]Neutral detergent fibre; [8]Acid detergent fibre; [9]Total digestive nutrients; [10]Without glycerine; [11]6% glycerine; [12]12% glycerine; [13]18% glycerine

Table 4

Fatty acid profile on diets containing different glycerine levels

Fatty acid, %	Glycerine levels				
	SIL[1]	G00[2]	G06[3]	G12[4]	G18[5]
14:0	1.24	0.58	0.07	0.15	0.14
15:0	0.36	0.02	0.03	0.06	0.07
16:0	20.8	13.8	16.5	21.3	22.9
16:1 *n-7*	0.07	0.12	0.12	0.11	0.05
17:0	0.27	0.08	0.10	0.13	0.16
17:1 *n-9*	0.78	0.03	0.01	0.04	0.02
18:0	2.20	2.24	2.67	3.63	4.06
18:1 *n-9*	16.5	29.8	26.7	17.7	14.2
18:1 *t-11*	2.35	0.65	0.92	0.77	1.22
18:2 *n-6*	32.4	51.2	50.6	51.9	52.3
18:3 *n-3*	22.9	1.48	2.25	4.21	4.85
SFA[6]	24.9	16.8	19.4	25.3	27.4
MUFA[7]	19.7	30.6	27.8	18.6	15.5
PUFA[8]	55.4	52.7	52.8	56.1	57.1
n-6[9]	32.4	51.2	50.6	51.9	52.3
n-3[10]	22.9	1.5	2.3	4.2	4.8
PUFA:SFA[11]	2.22	3.14	2.72	2.22	2.09
n-6:*n-3*[12]	1.41	34.5	22.4	12.3	10.8

[1]Corn Silage; [2]Diet without glycerine; [3]6% glycerine; [4]12% glycerine; [5]18% glycerine; [6]Saturated fatty acids; [7]Mono-unsaturated fatty acids; [8]Poly-unsaturated fatty acids; [9]Fatty acids *n-6*, [10]Fatty acids *n-3*, [11]PUFA:SFA; [12]*n-6:n-3* ratio.

.

Table 5

Carcass characteristics of Purunã bulls finished in feedlots and fed on diets containing different glycerine levels

Carcass characteristics	Glycerine levels				Regression equation	SEM[5]
	G00[1]	G06[2]	G12[3]	G18[4]		
Hot carcass weight, kg	255.2	253.1	268.4	256.6	$\hat{Y}=258.3$	5.56
Dressing carcass, %	54.0	54.3	56.3	54.1	$\hat{Y}=54.7$	0.29
[*]Conformation, points	12.85	13.83	13.00	13.80	$\hat{Y}=13.37$	0.21
Carcass length, cm	123.9	120.6	123.2	124.5	$\hat{Y}=123.10$	0.75
Leg length, cm	67.0	62.7	64.7	66.4	$\hat{Y}=65.22$	0.47
Cushion thickness, cm	25.6	30.4	25.7	25.3	$\hat{Y}=26.80$	0.72
Fat thickness, mm	3.78	5.08	3.57	4.60	$\hat{Y}=4.26$	0.22
Longissimus muscle, cm²	62.2	66.5	70.0	67.3	$\hat{Y}=66.52$	1.22
Longissimus muscle, cm²/BW	24.7	28.6	27.1	27.2	$\hat{Y}=29.96$	0.51
[*]Marbling, points	7.10	5.70	5.80	7.20	$\hat{Y}=6.45$	0.30
[*]Texture, points	4.30	4.80	4.20	4.66	$\hat{Y}=4.49$	0.07
[*]Color, points	3.90	3.20	3.90	3.75	$\hat{Y}=3.68$	0.12
pH_{0h}	6.48	6.70	6.41	6.60	$\hat{Y}=6.55$	0.03
pH_{24h}	5.98	6.42	6.07	6.43	$\hat{Y}=6.22$	0.05
Muscle, %	69.5	69.3	69.9	68.9	$\hat{Y}=69.44$	1.67
Fat, %	18.6	18.5	18.2	18.2	$\hat{Y}=18.43$	1.46
Bone, %	11.8	12.1	11.7	12.7	$\hat{Y}=12.12$	0.75

[1]Diet without glycerin; [2]6% glycerin; [3]12% glycerin; [4]18% glycerin; [5]Standard error of mean; [*]Analyzed by the generalized linear models method

Table 6

Lightness (L), red intensity (a*), yellow intensity (b*) on *Longissimus* muscle of Purunã bulls finished in feedlot and fed on diets containing different glycerine levels

Items	Glycerine levels				Regression equation	SEM[5]
	G00[1]	G06[2]	G12[3]	G18[4]		
L, points	35.32	32.88	36.33	33.17	$\hat{Y}$=34.42	0.55
a*,points	17.00	14.29	17.54	14.61	$\hat{Y}$=15.86	0.52
b*, points	6.46	4.32	6.74	4.75	$\hat{Y}$=5.57	0.33

[1]Diet without glycerine; [2]6% glycerine; [3]12% glycerine; [4]18% glycerine; [5]Standard error of mean.

Table 7

Sensory characteristics and loss of *Longissimus* muscle of Purunã bulls finished in feedlot and fed on diets containing different glycerine levels

Items	Glycerine levels				Regression equation	SEM[5]
	G00[1]	G06[2]	G12[3]	G18[4]		
Thawing loss, %	11.67	9.62	12.49	9.55	Ŷ=10.83	0.58
Cooking loss, %	20.69	21.79	23.93	18.64	Ŷ=21.26	0.78
[*]Tenderness, points	6.45	6.90	6.28	6.48	Ŷ=6.53	0.10
[*]Juiciness, points	6.00	6.46	5.80	6.38	Ŷ=6.16	0.11
[*]Flavor, points	6.41	6.86	6.16	6.53	Ŷ=6.49	0.09

[1]Diet without glycerine; [2]6% glycerine; [3]12% glycerine; [4]18% glycerine; [5]Standard error of mean; *Analyzed by the generalized linear models method

Table 8

Chemical composition on *Longissimus* muscle of Purunã bulls finished in feedlot and fed on diets containing different glycerine levels

Items	Glycerine levels				Regression equation	SEM[5]
	G00[1]	G06[2]	G12[3]	G18[4]		
Moisture, %	73.58	74.59	73.90	74.66	Ŷ=74.18	0.20
Ashes, %	1.06	1.03	1.02	1.04	Ŷ=1.03	0.01
Crude protein,%	22.06	21.16	21.14	20.93	Ŷ=21.32	0.21
Total lipids, %	2.11	2.11	1.87	2.08	Ŷ=2.04	0.09

[1]Diet without glycerine; [2]6% glycerine; [3]12% glycerine; [4]18% glycerine, [5]Standard error of mean.

Table 9

Fatty acid profile on muscle *Longissimus* of Purunã bulls finished in feedlot and fed on diets containing different glycerine levels

Fatty acid*	Glycerine levels				Regression equation	SEM[5]	R²
	G00[1]	G06[2]	G12[3]	G18[4]			
14:0	2.59	2.26	2.01	1.97	$\hat{Y}=2.528-0.030x$	0.07	0.24
14:1 *n-7*	0.40	0.47	0.36	0.33	$\hat{Y}=0.39$	0.02	-
15:0	0.38	0.35	0.45	0.41	$\hat{Y}=0.40$	0.01	-
15:1 *n-9*	0.15	0.14	0.16	0.12	$\hat{Y}=0.14$	<0.01	-
16:0	27.60	25.58	25.09	24.95	$\hat{Y}=27.073-0.120x$	0.36	0.16
16:1 *n-7*	0.43	0.40	0.46	0.43	$\hat{Y}=0.43$	<0.01	-
16:1 *n-9*	2.98	3.35	2.90	3.11	$\hat{Y}=3.08$	0.07	-
17:0	1.29	1.09	1.44	1.51	$\hat{Y}=1.33$	0.09	-
17:1 *n-9*	0.93	0.97	1.08	1.22	$\hat{Y}=1.05$	0.07	-
18:0	17.50	14.94	15.93	15.58	$\hat{Y}=15.99$	0.31	-
18:1 *n-7*	1.20	1.51	1.49	1.49	$\hat{Y}=1.302+0.011x$	0.03	0.18
18:1 *n-9*	37.72	39.74	38.53	40.48	$\hat{Y}=39.12$	0.39	-
18:1 *t-11*	0.80	1.06	1.10	0.76	$\hat{Y}=0.793+0.063x-0.003x^2$	0.04	0.29
18:2 *n-6*	3.17	3.29	4.79	3.97	$\hat{Y}=3.222+0.055x$	0.20	0.11
18:2 *c-9, t-11*	0.08	0.09	0.09	0.08	$\hat{Y}=0.09$	<0.01	-
18:3 *n-6*	0.10	0.97	0.12	0.12	$\hat{Y}=0.33$	0.15	-
18:3 *n-3*	0.17	0.22	0.25	0.21	$\hat{Y}=0.191+0.002x$	<0.01	0.14
20:4 *n-6*	0.14	0.20	0.25	0.25	$\hat{Y}=0.153+0.005x$	0.01	0.25
20:5 *n-3*	0.04	0.06	0.07	0.06	$\hat{Y}=0.054+0.0007x$	<0.01	0.12
22:0	0.20	0.24	0.19	0.23	$\hat{Y}=0.22$	0.01	-
22:6 *n-3*	1.16	1.58	2.01	1.71	$\hat{Y}=1.311+0.029x$	0.09	0.15

[1]Diet without glycerine; [2]6% glycerine; [3]12% glycerine; [4]18% glycerine; [5]Standard error of mean

Table 10

Fatty acid on *Longissimus* muscle of Purunã bulls finished in feedlot and fed on diets containing different glycerine levels

Items	Glycerine levels				Regression equation	SEM[5]	R²
	G00[1]	G06[2]	G12[3]	G18[4]			
SFA[6]	49.59	44.48	45.12	44.68	$\hat{Y}=48.085-0.201x$	0.52	0.23
MUFA[7]	44.65	47.69	46.89	47.96	$\hat{Y}=45.428+0.130x$	2.87	0.12
PUFA[8]	4.89	6.42	7.61	6.43	$\hat{Y}=5.473+0.082x$	0.29	0.12
n-6[9]	3.41	4.46	5.17	4.35	$\hat{Y}=3.826+0.050x$	0.19	0.10
n-3[10]	1.38	1.87	2.34	1.99	$\hat{Y}=1.557+0.032x$	0.10	0.15
PUFA:SFA[1]	0.09	0.14	0.16	0.14	$\hat{Y}=0.115+0.002x$	<0.01	0.16
n-6:n-3[12]	2.56	2.38	2.26	2.24	$\hat{Y}=2.527-0.015x$	0.05	0.11

[1]Diet without glycerine; [2]6% glycerine; [3]12% glycerine; [4]18% glycerine; [5]Standard error of mean; [6]Saturated fatty acids; [7]Mono-unsaturated fatty acids; [8]Poly-unsaturated fatty acids; [9]Fatty acids *n-6*, [10]Fatty acids *n-3*, [11]PUFA:SFA; [12]*n-6:n-3* ratio.

CONSIDERAÇÕES FINAIS

A glicerina de média pureza é uma fonte energética alternativa para a substituição parcial do milho grão na terminação de bovinos em confinamento. A suplementação com glicerina aumentou a digestibilidade da dieta e reduziu a ingestão (% PV) de MS pelos animais sem influenciar o desempenho animal. A rápida saciedade alimentar devido à intensa metabolização da glicerina aumentou o tempo destinado a realização de outras atividades que não a alimentação e a ruminação pelos bovinos. A substituição de até 9,5% da dieta total (29,8% do milho) pela glicerina favoreceu a realização de atividades que necessitam menores gastos de energia permitindo a maior efetivação do desempenho animal. Além disso, a glicerina melhorou a composição de ácidos graxos desejáveis no músculo *Longissimus* e reduziu a concentração dos compostos ligados a riscos de doenças cardiovasculares em humanos. Portanto, a glicerina de média pureza foi eficaz ao substituir o milho grão na dieta de bovinos confinados, melhorando a composição de ácidos graxos da carne sem prejudicar as características produtivas e de carcaça dos animais.