

UNIVERSIDADE ESTADUAL PAULISTA - UNESP
CÂMPUS DE JABOTICABAL

**CRUDE GLYCERIN ASSOCIATED WITH STARCH OR FIBER-
BASED ENERGY INGREDIENTS AT TWO LEVELS OF
CONCENTRATE FOR BEEF CATTLE**

Josiane Fonseca Lage

Zootecnista

2014

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Orientador: Profa. Dra. Telma Teresinha Berchielli

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

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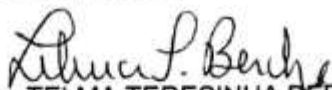
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TÍTULO: CRUDE GLYCERIN ASSOCIATED WITH STARCH OR FIBER-BASED ENERGY INGREDIENTS AT TWO LEVELS OF CONCENTRATE FOR BEEF CATTLE

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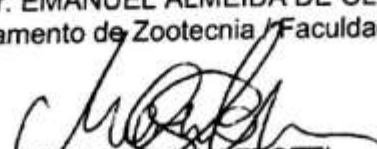
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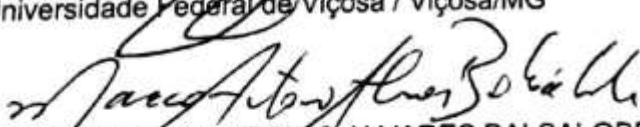
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DADOS CURRICULARES DO AUTOR

JOSIANE FONSECA LAGE - nascida em João Monlevade, Minas Gerais, no dia 29 de abril de 1983. Concluiu o curso de graduação em Zootecnia na Universidade Federal de Viçosa (UFV) em 2007. Na mesma universidade obteve o título de Mestre em Zootecnia em Nutrição e Produção de Ruminantes, em 2009. Ingressou no curso de Doutorado em Zootecnia em março de 2010 na Faculdade de Ciências Agrárias e Veterinárias da Universidade Estadual Paulista “Júlio de Mesquita Filho”, campus de Jaboticabal, sob orientação da Prof^a. Dr^a. Telma Teresinha Berchielli. De outubro de 2012 a julho de 2013 realizou Doutorado - Sanduíche no United States Meat Animal Research Center (USMARC) / USDA, em Clay Center, NE, Estados Unidos, onde desenvolveu estudos na área de qualidade da carne bovina sob orientação de Steven D. Shackelford.

“Para ter sucesso...

é necessário amar de verdade o que se faz...

Caso contrário, levando em conta apenas o lado racional...

...você simplesmente desiste...

É o que acontece com a maioria das pessoas”

Steve Jobs

“Algumas quedas servem para que nos levantemos mais felizes”

William Shakespeare

Dedico

Ào meu avô Inhô (*in memoriam*)...
e a minha avó Zaira (*in memoriam*)...
por despertarem em meu coração...
o amor pelo mundo sertanejo!

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“A realidade é dura, mas é o único lugar onde se pode comer um bom bife...”

Wood Allen

SUMMARY

ABSTRACT	i
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RESUMO	ii
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CHAPTER 1. GENERAL CONSIDERATIONS	1
---	---

1. Literature cited	5
---------------------------	---

CHAPTER 2. DIGESTIBILITY AND RUMINAL FERMENTATION OF NELLORE STEERS FED CRUDE GLYCERIN ASSOCIATED WITH STARCH OR FIBER-BASED ENERGY INGREDIENTS AT TWO LEVELS OF CONCENTRATE

Abstract	10
----------------	----

1. Introduction	11
-----------------------	----

2. Material and Methods	12
-------------------------------	----

3. Results and Discussion	17
---------------------------------	----

4. Conclusions	28
----------------------	----

5. Literature cited	29
---------------------------	----

CHAPTER 3. METHANE EMISSIONS AND GROWTH PERFORMANCE OF NELLORE YOUNG BULLS FED CRUDE GLYCERIN REPLACING STARCH- VS. FIBER- BASED ENERGY INGREDIENTS AT LOW OR HIGH CONCENTRATE DIETS

Abstract	36
----------------	----

1. Introduction	37
-----------------------	----

2. Material and Methods	38
-------------------------------	----

3. Results and Discussion	43
---------------------------------	----

4. Conclusions	56
----------------------	----

5. Literature cited	57
---------------------------	----

CHAPTER 4. FATTY ACID PROFILE, CARCASS AND MEAT QUALITY TRAITS OF YOUNG NELLORE BULLS FED CRUDE GLYCERIN REPLACING ENERGY SOURCES IN THE CONCENTRATE

Abstract	64
1. Introduction	64
2. Material and Methods	66
3. Results and Discussion	72
4. Conclusions	84
5. Literature cited	84

APÊNDICE

Apêndice A. Implicações da inclusão de glicerina bruta na dieta de ruminantes	92
Apêndice B. Composição bromatológica dos ingredientes das dietas	96



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"JÚLIO DE MESQUITA FILHO"
Câmpus de Jaboticabal



CEUA – COMISSÃO DE ÉTICA NO USO DE ANIMAIS

CERTIFICADO

Certificamos que o Protocolo nº 021119/11 do trabalho de pesquisa intitulado "**Balanço de gases de efeito estufa e estratégias de mitigação em pastos de Brachiaria submetidos a diferentes manejos**", sob a responsabilidade da Profª. Drª. Telma Teresinha Berchielli está de acordo com os Princípios Éticos na Experimentação Animal, adotado pelo Colégio Brasileiro de Experimentação (COBEA) e foi aprovado pela COMISSÃO DE ÉTICA NO USO DE ANIMAIS (CEUA), em reunião ordinária de 07 de Outubro de 2011.

Jaboticabal, 11 de Outubro de 2011.


Prof. Dr. Jeffrey Frederico Lui
Presidente - CEUA


Med. Vet. Maria Alice de Campos
Secretária - CEUA

CRUDE GLYCERIN ASSOCIATED WITH STARCH OR FIBER-BASED ENERGY INGREDIENTS AT TWO LEVELS OF CONCENTRATE FOR BEEF CATTLE

ABSTRACT - This trial aimed to evaluate the effects of feeding crude glycerin (CG) - 80% glycerol - included on 10% of DM diet, associated with corn or soybean hulls (SH) in different concentrate level (CL; 40 or 60%) on digestibility, ruminal fermentation, performance, methane emission, carcass and meat quality traits of Nellore young bulls fed in feedlot. Twelve ruminally cannulated Nellore steers (401.0 ± 41.5 kg) were used in a replicated truncated Latin Square arrangement of treatments with six animals in six treatments and four periods to evaluate the ruminal fermentation. Experimental periods were 19 d (14 d for adaptation and 5 d to sampling). Diets were: CO - without CG and corn as ingredient of concentrate; CGC - inclusion of CG (10% of DM) associated with corn; and CGSH - inclusion of CG (10% of DM) associated with SH. Differences in DMI ($P = 0.47$), DM digestibility ($P = 0.29$) and NDF digestibility ($P = 0.77$) were not observed among the diets. The propionate concentrations ($P < 0.01$) and A:P ratio ($P < 0.01$) were affected by inclusion of CG in diets. The bacteria or protozoa species were not affected by inclusion of CG in the diets ($P > 0.05$). Seventy Nellore bulls with 18 months of age were used to evaluate the performance and meat quality traits. The DMI ($P = 0.89$) and ADG ($P = 0.98$) were similar among the diets. The CL and the diets had a tendency an interaction for methane emissions (g) per kg of DMI ($P = 0.07$). Animals fed CGC had a greater G:F (g carcass gain/kg DMI; $P < 0.01$). Animals fed diets with CGC or CGSH showed meat with greater deposition of monounsaturated fatty acids (MUFA; $P < 0.01$) and CLA (18:2 *cis*-9, *trans*-11) contents ($P < 0.01$). CG can be used to replace corn or SH in 10% of diet DM, without affect the DMI, digestibility and growth of microorganisms in the rumen. The inclusion of CG in diets associated with SH in LC diets tends to decrease the methane emission than animals fed with CGSH in HC diets. When CG is associated with SH in the diets tends to decrease the CrG and decrease the G:F ratio in relation to CrG. The MUFA and CLA content increases in beef from animals fed CG.

Keywords: feedlot, glycerol, meat quality, methane, microbiology, performance

GLICERINA BRUTA ASSOCIADA A INGREDIENTES ENERGÉTICOS Á BASE DE AMIDO OU FIBRA EM DOIS TEORES DE CONCENTRADO PARA BOVINOS DE CORTE

RESUMO - Objetivou-se neste trabalho avaliar os efeitos da alimentação com glicerina bruta (GB; 80% de glicerol) - incluída em 10% da MS da dieta, associada ao milho ou casca de soja (CS) em diferentes teores de concentrado (40 - BC ou 60% - AC) sobre a digestibilidade, fermentação ruminal, desempenho, emissão de metano, características da carcaça e qualidade da carne de bovinos Nelore alimentados em confinamento. Doze novilhos Nelore ($401,0 \pm 41,5$ kg) canulados no rúmen foram usados em um delineamento de quadrado latino truncado replicado, com seis animais em seis tratamentos e quatro períodos para avaliar a fermentação ruminal. Períodos experimentais possuíam 19 dias (14 para adaptação e 5 dias para amostragem). Dietas utilizadas foram: CO - sem GB e milho como ingrediente do concentrado; GBM - inclusão de GB (10% na MS) associada ao milho; GBCS - inclusão de GB (10% na MS) associada á CS. Diferenças em consumo de MS (CMS; $P = 0,47$), digestibilidade da MS ($P = 0,29$) e digestibilidade da FDN ($P = 0,77$) não foram observadas entre dietas. As concentrações de propionato ($P < 0,01$) e relação acetato:propionato ($P < 0,01$) foram afetadas pela inclusão de GB nas dietas. As espécies de bactérias e protozoários não foram afetadas pela inclusão de GB nas dietas ($P > 0.05$). Setenta tourinhos Nelore ($373,70 \pm 24,70$ kg) com 18 meses de idade foram utilizados para avaliar o desempenho e a qualidade da carne. O CMS ($P = 0,89$) e o GMD ($P = 0,98$) foram similar entre as dietas. Houve tendência a interação entre teor de concentrado e dietas para emissão de metano (g/kg de MS ingerida; $P = 0,07$). Animais alimentados com GBM apresentaram melhor eficiência alimentar (g ganho de carcaça (GCr) /kg MS ingerida; $P < 0,01$). Animais alimentados com GBM ou GBCS apresentaram maior porcentagem de ácidos graxos monoinsaturados (AGM; $P < 0,01$) e ácido linoléico conjugado (CLA, 18:2 *cis*-9, *trans*-11; $P < 0,01$). GB pode ser utilizada associada ao milho ou CS em 10% da MS da dieta, sem afetar o CMS, digestibilidade e crescimento de microrganismos no rúmen. A inclusão de GB em dietas associada á CS em BC diminui a emissão de metano em

relação a animais alimentados com CGCS em AC. Quando a GB substitui a CS em dietas, tende a diminuir o GCr. O conteúdo de AGM e CLA é maior em animais alimentados com GB.

Keywords: confinamento, desempenho, glicerol, metano, microbiologia, qualidade da carne

CHAPTER 1 - GENERAL CONSIDERATIONS

Glycerin, also known as glycerol, is a colorless, odorless, hygroscopic and sweet-tasting viscous liquid, formed by a three carbon compound that can be found in animals and plants. It is a carbohydrate molecule ($C_3H_8O_3$) with a net energy concentration of 1.98 - 2.29 Mcal/kg which is approximately equal to the energy contained in corn starch (Schroder and Sudekum, 1999) and has a wide range of applications in the food, pharmaceutical and cosmetic industries (Donkin, 2008). Glycerin can be obtained through biodiesel production from plant oils or animal fats and this case, is called crude glycerin. In Brazil, great variation is observed in crude glycerin composition with levels of glycerol ranging from 40 to 80%, containing impurities as a methanol, heavy metal (Lage et al., 2014), crude fat (Lage et al., 2010) and water. This product, which is not pure, does not satisfy the legal requirements for pharmaceutical use and could thus be used as a feed additive for ruminants.

According National Biodiesel Board (2008), on each 100 kg of oil or fat yields approximately 10 kg of glycerin, thus the increase of biodiesel production has led to increased stocks of glycerol with a subsequent price reduction. Despite crude glycerin have an energetic value similar to corn (DeFrain et al., 2004), this by product have been researched in ruminant nutrition replacing corn or ingredients that contain rapidly fermentable starch. Corn prices are high because of increased demand from ethanol industry and agroindustrial byproducts may be an economical alternative to replace corn grain in ruminant diets.

Glycerol improves glucose status in ruminants as it is readily absorbed through the rumen wall and converted to glucose in the liver (Remond et al., 1993), or fermented to propionate in the rumen, a gluconeogenic precursor that increases blood glucose levels after absorption in cattle (Chung et al., 2007) and sheep (Johns, 1953). Therefore, the inclusion of glycerol in ruminant diets modifies the ruminal acetate:propionate ratio due to the increase of propionate levels (Drouillard, 2008; Avila et al., 2011). According to Ramos and Kerley (2012) propionate increase and acetate decrease when crude

glycerin is fermented compared with starch. Thus, the crude glycerin is an ingredient potential that can be used as a substrate gluconeogenic to ruminants.

The rumen microflora is also able to metabolize glycerol (Wright, 1969), which enters into the composition of plant cell wall phospholipids and into that of the reserve lipids of plant seeds. Ingested in this form, however, glycerol represents only a small proportion of total feed intake, being 2 to 4 g/kg of dry matter ingested (Roger et al., 1992). Glycerol in a concentration of 5%, inhibit the cellulolytic activity in the rumen due to the inhibitory effect of glycerol on the growth of the microorganisms, which inhibited and retarded severely the growth of *Ruminococcus flavefaciens* and *Fibrobacter succinogenes* (Roger et al., 1992). This negative effect on cellulolytic activity can be explained despite the lower ruminal pH when animals are fed with crude glycerin replacing corn (Mach et al., 2009; Ramos and Kerley, 2012) and can be attributed due to shift in acetate to propionate in rumen.

Pathways to propionate production are known to act as a hydrogen sink and would therefore reduce methane emissions (Boadi et al., 2004). The quantity of hydrogen available in the rumen can be used to produce in methane by methanogenic bacteria and its depends of volatile fatty acids proportions produced in the rumen, as a acetate, butyrate and propionate. During the formation of acetic and butyric acid, the hydrogen is produced while the formation of propionic acid uptake the hydrogen. Thus, animals fed with diets that will provide an increase of propionate in the rumen, could have lower methane emissions. Lee et al. (2011) reported reduced methane emissions by including glycerol in *in vitro* incubations of corn grain and alfalfa hay; but Meale et al. (2013) did not observe an effect when they evaluated glycerol as a replacement for whole wheat grain in up to 12% of the diet DM in *in vitro* study. Avila-Stagno et al. (2013) reported that the inclusion of glycerol up to 21% of the diet DM did not affect lamb methane emissions in *in vivo* study. The use of crude glycerin replacing starch or fiber based energetic ingredients would be evaluated because carbohydrate type in the diet and their manipulation can reduce cattle methane emissions (Johnson and Johnson, 1995).

Due to impact of crude glycerin on ruminal microorganisms, changes in *in vivo* digestibility would be expected in ruminants. These effects of crude glycerin on cellulolytic activity decreases the fiber digestibility and reduces the dry matter intake by ruminants (Abo El-Nor et al., 2010; Lage et al., 2010; Ramos and Kerley, 2012). These observations could have important implications for diets that contain higher proportion of forage than concentrate and diets with high or lower amount of starch. The apparent differential effects of glycerin on fiber digestion in diets with or without starch are supported by observations of Schroder and Sudekum (2009), who reported improvements in fiber digestion in low-starch diets, while digestibility of fiber in high-starch diets was decreased with glycerin addition. However, the quantity of crude glycerin that can be added in the diets is limited because the crude glycerin affects the fiber digestion and compromise the feed efficiency.

Recent studies (Drouillard, 2012; Parsons et al., 2009; Barton et al., 2013) reported that excessive levels of glycerin are deleterious to growth of beef cattle, as a result of to decrease feed intake, while levels in diets up to 10% or less of diet DM generally yields positive effects. The inclusion of 10% of crude glycerin in diets replacing corn improved the feed efficiency by 19.2% compared to diets without crude glycerin (Pyatt et al., 2007). Other researchers have reported a linear increase in average daily gain when steers were fed finishing diets with crude glycerin until 9% of diet DM (Moore et al., 2011). Versemann et al. (2008) and Parsons et al. (2009) also demonstrated increased average daily gain in finishing cattle fed crude glycerin at up to 10% of dietary DM.

Regards to carcass and meat quality, it is plausible that glycerin may have effects on intramuscular fat depots than that associated with 12th-rib subcutaneous fat depth, because glycerin is rapidly converted to propionate and because the acetate:propionate ratio is likely decreased in beef cattle fed crude glycerin. Smith and Crouse (1984) demonstrated that glucose provides 50 to 75% of the acetyl units for *in vitro* lipogenesis in the intramuscular fat depot. Therefore, glycerin fermentation, resulting in elevated blood glucose and insulin, may be a key component in triggering intramuscular adipocyte development. Mach et al. (2009), reported that bulls fed with high concentrate

level and crude glycerin included in 0, 4, 8 and 12% of diet DM did not had differences in carcass and meat quality. The intramuscular fat increase when animals are fed with 10% of crude glycerin in diet DM of beef cattle (Verseemann et al., 2008). The inclusion of crude glycerin (36.2% of glycerol) in 12% of diet DM with higher concentration of crude fat and methanol, replacing corn in the diets of lambs does not affect negatively the meat safety, tenderness and intramuscular fat content on longissimus muscle (Lage et al., 2014).

Strategies to enrich ruminant-derived foods with unsaturated fatty acids are desired as they are considered beneficial for good human health. Krueger et al. (2010) reported that glycerol likely inhibit lipolysis decreasing the accumulation of free fatty acids in the ruminal environment of animals, leading to a greater amount for unsaturated fatty acids available to be incorporated in meat products. According to Abo El-Nor et al. (2010) the DNA concentration for *Clostridium proteoclasticum* and *Butyrivibrio fibrisolvens* decrease when glycerol replace corn in concentrate. Recent studies have demonstrated that these two bacteria play a fundamental and central role in the rumen biohydrogenation process (Wallace et al., 2006; Maia et al., 2007). Thus, the use of crude glycerin in diets to ruminants can be an alternative to improve the fatty acid profile in beef. The fatty acid profile has been reported in subcutaneous fat from animals fed with crude glycerin (Avila-Stagno et al., 2013) but in meat needs to be studied.

Almost all studies that included crude glycerin in ruminant diets have reported the use of high concentrate diets with crude glycerin replacing rapidly fermentable starch. Soybean hulls is widely used in feedlot in Brazil as a substitute for corn in concentrate diets (Mendes, et al., 2005; Silva, et al., 2002) mainly due its lower cost as feedstuff. Schroder and Sudekum, (2009) suggested that glycerol can replace up to 10% of readily fermentable starches but that its energetic value when fed in conjunction with a high-starch concentrate diet (60% concentrate) was approximately 85% of its value when fed with a low-starch concentrate diet. According to Drouillard (2008), propionate production is typically greater for concentrate-fed animals, and thus there may be less opportunity to improve energetic efficiency when glycerol is combined with concentrates compared to feeding with forages.

The objective of this study was to evaluate the effects of 10% of crude glycerin (diet DM) associated with starch or fiber-based energy ingredients at two levels of concentrate on the digestibility, ruminal fermentation, methane emissions, blood parameters, growth performance, carcass and meat quality of beef cattle finished in feedlot.

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CHAPTER 2

O artigo a seguir está redigido conforme normas de publicação do *Journal of Animal Science*, exceto o posicionamento das tabelas e figuras.

Digestibility and ruminal fermentation of Nellore steers fed crude glycerin associated with starch or fiber-based energy ingredients at two levels of concentrate

ABSTRACT: Twelve ruminally cannulated Nellore steers (401.0 ± 41.5 kg) and 24 months of age were used in a replicated truncated Latin Square arrangement of treatments with six animals in six treatments and four periods to evaluate the effect of crude glycerin (CG; 80.34% of glycerol) associated with starch or fiber-based energy ingredients in the concentrate on DMI, DM (DMD) and NDF digestibility (NDFD), ruminal pH, ammonia-N concentration, VFA's and ruminal microbiology of the steers fed in feedlot. Experimental periods were 19 d (14 d for adaptation and 5 d to sampling). Diets were: CO - without CG and corn as ingredient of concentrate; CGC - inclusion of CG (10% of DM) associated with corn in the concentrate; and CGSH - inclusion of CG (10% of DM) associated with soybean hulls (SH) in the concentrate. All three diets were offered at a low (LC) or high concentrate (HC; 40 or 60%). Animals fed LC or HC diets had similar DMI ($P = 0.64$), DMD ($P = 0.85$) and NDFD ($P = 0.61$). Differences in DMI ($P = 0.47$), DMD ($P = 0.29$) and NDFD ($P = 0.77$) were not observed among the diets. The ruminal pH was lower to animals fed HC diets than ruminal pH from animals fed LC diets ($P < 0.01$). No differences were detected in our experiment in pH when CG was associated with corn or SH in 10% of diet DM. Animals fed diets with CG associated with SH had lower ammonia-N concentration than animals fed diets without CG or with this by product associated with corn ($P < 0.01$). The propionate concentrations ($P < 0.01$) and A:P ratio ($P < 0.01$) were affected by inclusion of CG in diets associated with corn or SH in the concentrate. Animals fed diets with CG associated with corn or SH had higher propionate concentrations and lower A:P ratio in ruminal fluid ($P < 0.05$). There was an interaction between concentrate level and the diets for acetate ($P = 0.03$) and butyrate concentrations ($P = 0.05$). The species or total protozoa were not affected by inclusion of CG in the diets ($P > 0.05$). The species of bacteria did not differ from animals fed LC or HC diets ($P = 0.56$) and among diets ($P > 0.05$). Diets with HC reduce the pH, increase the propionate but do not affect the NDF digestibility of diets and DMI by animals. CG

can be used associated with corn or SH in 10% of diet DM, without affect the DMI, digestibility and growth of microorganisms in the rumen. Moreover, the inclusion of CG in diets reduces the A:P ratio as a result of increases of propionate concentrations.

Keywords: glycerol, microbiology, propionate, protozoa, soybean hulls

1. INTRODUCTION

Glycerol is a byproduct from biodiesel agroindustry and has been used in diets to ruminants as an energy source (Donkin, 2009; Hales et al., 2013; Meale et al., 2013). The reductions in NDF digestibility has been reported by inclusion of glycerin in diets to sheep (Lage et al., 2014), dairy cattle (Donkin et al., 2009) and beef cattle (Parsons and Drouillard, 2010). The reduction in NDF digestibility in diets with glycerol has been associated with a growth inhibition of cellulolytic bacteria. AbuGhazaleh et al. (2011) reported that *Butyrivibrio fibrisolvens* and *Selenomonas ruminantium* in ruminal fluid decreased when increased concentrations of glycerol, and *Ruminococcus flavefaciens* and *Fibrobacter succinogenes* were inhibited when glycerol was included in cultures at 5% of DM basis (Roger et al., 1992) both in *in vitro* study. The apparent differential effects of glycerin on fiber digestion in diets with or without starch are supported by observations of Schroder and Sudekum (2009), who reported improvements in fiber digestion in low-starch diets, while digestibility of fiber in high-starch diets was decreased with glycerin addition.

The propiogenesis has been shown to substantially increase when glycerol is added to high-fiber diets than when added to high-starch diets incubated *in vitro* (Rémond et al., 1993). Thus, the addition of glycerol could improve energetic efficiency of ruminants fed forage more than in those fed concentrates. Although the effects of feeding glycerol to ruminant animals either as an energy supplement (Fisher et al., 1973; DeFrain et al., 2004; Bartoň et al., 2013) on performance of animals and feed intake are well documented, little is known about the effects of glycerol on growth microorganisms

in the rumen. Moreover, it is unknown how glycerin feeding influences protozoa populations.

Concerns about decreases in fiber digestibility with glycerin feeding are limited for feedlot cattle fed high-concentrate finishing diets because fiber concentrations are normally low (Hales et al., 2013). Thus, the objective of this study was to evaluate the effects of crude glycerin associated with starch or fiber-based energy ingredients in two levels of concentrate on the ruminal fermentation, digestibility and growth of protozoa and bacteria in the rumen of Nellore steers.

2. MATERIAL AND METHODS

The trial was performed at the São Paulo State University (UNESP, Jaboticabal, SP, Brazil) following the humane animal care and handling procedures according to the guidelines of the São Paulo State University (UNESP, Brazil). Pre-harvest handling was in accordance with good animal welfare practices, and slaughtering procedures followed the Sanitary and Industrial Inspection Regulation for Animal Origin Products (Brasil, 1997).

Twelve ruminally cannulated Nellore (401.0 ± 41.5 kg) and 24 months of age were used in a replicated truncated Latin Square arrangement of treatments with six animals in six treatments and four periods to evaluate dietary intake, apparent total tract digestibility, ruminal pH, ammonia-N concentration, volatile fatty acids and ruminal microbiology of the steers fed in feedlot. The steers were treated for internal and external parasites at the beginning of the experiment and kept in individual pens of approximately 21 m² for adaptation and sampling period, with protected feeders and water. Experimental periods were 19 d: 14 d for adaptation to the diet and five days to sampling. The DMI was estimated in 5 d, sampling to collect feces in 3 d and sampling to collect liquid and ruminal contents in 1 d.

Animals were fed with one of the following experimental diets: CO - without crude glycerin and corn as ingredient of concentrate; CGC - inclusion of crude glycerin (10% of DM) associated with corn in the concentrate; and CGSH - inclusion of crude glycerin

(10% of DM) associated with soybean hulls in the concentrate. All three diets were offered at two levels of concentrate (40 or 60%). Crude glycerin was acquired from a soybean oil-based biodiesel production company, i.e., ADM, Rondonópolis, Brazil (80.34% of glycerol; 1.59% of ether extract; 5.03% of ash and 12.02% of water). Corn silage was used as the only source of roughage and the concentrates comprised grounded corn or soybean hulls, soybean meal, urea/ammonium sulfate and a mineral mixture. Soybean meal was used as an alternative protein source, and a mixture of urea and ammonium sulfate was used to adjust the diet CP content. All diets were balanced to provide 14.4 ± 0.2 of CP (Valadares Filho et al., 2006). The ingredient proportions and chemical compositions of the experimental diets are presented in Table 1.

Table 1. Diet composition (DM basis)¹

Item	40% of concentrate			60% of concentrate		
	CO	CGC	CGSH	CO	CGC	CGSH
Ingredient, % of DM						
Corn silage	60.00	60.00	60.00	40.00	40.00	40.00
Corn	26.30	16.00	-	46.40	36.10	-
Soybean meal	10.00	10.00	10.00	10.00	10.00	10.00
Soybean hulls	-	-	16.20	-	-	36.60
Crude glycerin	-	10.00	10.00	-	10.00	10.00
Urea/ammonium sulfate	0.70	1.00	0.80	0.60	0.90	0.40
Mineral premix ²	3.00	3.00	3.00	3.00	3.00	3.00
Nutrient composition ³ , %						
DM	57.60	57.90	58.60	67.08	67.38	68.90
CP	14.30	14.70	14.60	14.45	14.20	14.10
NDF	30.14	28.53	37.60	25.19	23.59	42.95
EE ⁴	3.13	3.01	2.92	3.18	3.05	2.83
NFC ⁵	47.00	49.46	40.14	53.93	55.48	35.46
ME, Mcal/kg	2.56	2.54	2.50	2.68	2.67	2.60

¹CO = corn, without crude glycerin; CGC = crude glycerin associated with corn; CGSH = crude glycerin associated with soybean hulls.

²Mineral mixed contained Ca, 210 g; P, 20 g; S, 37 g; Na, 80 g; Cu, 490 mg; Mn, 1.424 mg; Zn, 1.830 mg; I, 36 mg; Co, 29 mg; Se, 9 mg; F máx., 333 mg;

³All nutrients were analyzed. ME were calculated.

⁴Ether extract.

⁵Non fiber carbohydrates = $100 - (\text{CP} + \text{EE} + \text{Ash} + \text{NDF})$.

Diets were fed as a total mixed ration, in which corn silage and concentrate (previously mixed) were weighed and mixed before feeding. Cattle were fed one time daily at 0700 h and feed refusals were recorded daily for each pen. Amounts of feed offered to animals were calculated according to previous DMI and adjustments were made daily at ad libitum intake. Steers had free access to water. Feed refusal weights were performed for the diet quantities provided from each animal. To estimate intake, samples of diets (forage and concentrate) and orts from each animal were collected in 5 d of sampling period (d 15 to 19), then composited every period. DMI and NDF nutrient intake were calculated as the difference between amounts offered and refused based on chemical analysis of the composited sample within steers on each period. Feed samples and orts were frozen at - 18°C for later analysis of DM (method 934.01; AOAC, 1990), ether extract (AOAC, 1990), N was determined by combustion (Leco Instruments Inc., method 976.06, AOAC, 1990) and multiplied by 6.25 to obtain CP, ash (method 924.05; AOAC, 1990) and NDF was determined by method of Van Soest et al. (1991) using Ankon bags (Ankon Technology Corp., Fairport, NY, USA).

Total feces collect were realized in three days between d 15 and 17. Feces were collected immediately after each spontaneous defecation, stored in 20 L buckets, and at the end of each 24-h collection period the buckets were changed and the feces were weighed, manually blended, and aliquots of the daily feces excretion (approximately 300 g) were collected. Each fecal sample obtained per day, per animal, and per period was predried in a forced-air oven at 60°C for 72 to 96 h and ground in a Wiley mill (1 mm screen; model MA680, Marconi, Piracicaba, SP). Then, 10 g of each of the predried samples from each day were used to compose the final sample. All composite samples were stored in plastic flasks for subsequent analysis of DM and FDN as described above.

Rumen pH, ammonia-N, volatile fatty acids were measured for one day during the d 18 of each the period. Ruminal contents were obtained at 0, 2, 4, 6, 8, 10 and 12 h after 0700 h feeding. Rumen fluid was obtained from several sites within the rumen and were subsequently strained through 2 layers of cheesecloth. Ruminal pH was immediately measured using an electric pH meter (Nova Técnica, PHM, Piracicaba, SP).

After pH measurement, the samples were poured into 50 mL plastic flasks with 1 mL of 9.3 M H_2SO_4 and frozen at -20°C for subsequent analysis of $\text{NH}_3\text{-N}$ concentration. Ruminal fluid NH_3 was analyzed by distilling with 2 MKOH in a micro-Kjeldahl system, according to the original procedures of Fenner (1965). The samples collected for analysis of volatile fatty acids were centrifuged at $13.000 \times g$ (4°C) for 30 min and quantified by gas chromatography (GC Shimatzu model 20-10, automatic injection) using capilar column (SP-2560, $100 \text{ m} \times 0.25 \text{ mm}$ of diameter and 0.02 mm of thickness, Supelco, Bellefonte, PA) according (Palmquist; Conrad, 1971) methods.

Ruminal microbiology (bacteria and protozoa) samples were collected on the d 19 after 3 h of feeding. Cell counts were obtained from rumen content aliquots that were preserved in formalin (a solution of equal parts water and 370 ml/L formaldehyde) according to D'Agosto and Carneiro, (1999). Ciliate protozoa species were identified and quantified the in chamber Sedgewick-Rafter, according to Dehority (1984). Each sample was homogenized and 1 mL of ruminal content was pipetted and transferred to vials with lugol, according modified methodology from D' Agosto and Carneiro (1999). After 15 min, 9 mL of glycerin at 30% was added in vials. To quantify the protozoa, from each vials was pipetted 1 mL of content to fill the chamber of Sedgewick-Rafter. The ciliates were measured according Dehority (1984).

Fifty grams of the ruminal contents were weighed to identify bacterial population and immediately added to 50 ml of phosphate saline buffer (pH 7.4), stirred vigorously for 3 minutes, and then filtered with a mesh fabric (100 microns). The filtrate was subjected to centrifugation at $16.000 \times g$ for 10 minutes at 4°C . The supernatant was discarded and the remaining precipitate was resuspended in 4 ml of Tris-EDTA buffer (10X, pH 8.0). The resuspended content was centrifuged at $16.000 \times g$ for 10 min at 4°C , the supernatant was discarded, and the precipitate was immediately stored in refrigeration (-20°C) for a period of three months. DNA extraction was conducted in 250 mg of sample using the extraction kit FastDNA® SPIN Kit for Soil (MP Biomedical, LLC). The integrity and quantity of the DNA was checked by electrophoresis on agarose gel (0.8%), and complementary DNA was assessed by spectrophotometry (Thermo Scientific NanoDrop™ 1000) for evaluation of its quality and quantity. For quantification

of total bacteria and relative quantification of cellulolytic bacteria (*Ruminococcus albus*, *Fibrobacter succinogenes*, and *Ruminococcus flavefaciens*), the technique used was qPCR. The primers used in this study are shown in Table 2.

The amplifications were performed in triplicate and negative controls were run in the assay, omitting the total DNA. The reactions were conducted in the 7500 Real Time PCR System. The qPCR reaction was carried out using 20 ng of total DNA in a reaction containing: 7.5 µl of SYBR® Green PCR Master Mix (Bio-Rad, Hercules, California, USA), 10 pmol of primer pair, and H₂O to a final volume of 25 µl. Cycling conditions were 50 °C for 2 min, 95 °C for 10 minutes; and 40 cycles of 95 °C for 15 seconds, 60 °C for 1 minute, and 78 °C for 30 seconds. After cycles of amplification, a step was added in increasing temperature from 60 to 95 °C to obtain dissociation curve of the reaction products, used for analyzing the specificity of amplification. The relative quantification was determined using the amplification of total bacteria as reference gene.

Table 2. PCR primers used in this study for the quantification of specific rumen microbes by qPCR

Primer	Sequency (5' to 3')
Total bacteria ¹	F: CGG CAA CGA CAA CCC R: CCA TTG TAG CAC CTG TGT AGC C
<i>Fibrobacter succinogenes</i> ¹	F: GTT CGG AAT TAC TGG GCG TAA A R: CGC CTG CCC CTG AAC TAT C
<i>Ruminococcus flavefaciens</i> ¹	F: CGA ACG GAG ATA ATT TGA GTT TAC TTA GG R: CGG TCT CTG TAT GTT ATG AGG TAT TAC C
<i>Ruminococcus albus</i> ¹	F: CCCTAAAAGCAGTCTTAGTTCG R: CCTCCTTGCGGTTAGAACA
<i>Total Archaeas</i> ²	F: TTC GGT GGA TCD CAR AGR GC R: GBA RGT CGW AWC CGT AGA ATC C

F = "forward"; R = "reverse"; ¹ Denman e McSweeney. (2006); ² Denman et al. (2007)

The experimental design was a truncated double Latin Square and the data were analyzed using the MIXED procedure (Sas Inst. Inc., Cary, NC). The model included the fixed effects of concentrate level, diets and the interaction of concentrate level and diets. For all data, the animal and period was included in the model as a random effect. The pH, ammonia-N and VFA, were analyzed as a repeated measurements in time using

MIXED procedure. Several covariance structures were tested, and the structure resulting in the smallest Akaike and Schwarz Bayesian criteria was considered the most appropriate for analysis. The fixed effects of concentrate level, diet and the treatment by time interaction were included in the model. The animal and period was included in the model as a random effect. The least-squares means were generated for main-effects and significant interactions were compared ($P \leq 0.05$) using Tukey's test.

3. RESULTS AND DISCUSSION

Animals fed low (LC; 40%) or high concentrate (HC; 60%) diets had similar DMI ($P = 0.64$), DM digestibility ($P = 0.85$) and NDF digestibility ($P = 0.61$; Table 3). The negative effect on DMI in high concentrate diets can occur due to low fiber digestibility and it is important because it could promote the limitation of voluntary feed intake. Sarwar et al. (1992) reported that the extent of digestion of NDF decreased as the proportion of concentrate in the diet increased. However, in the present study, DM and NDF digestibility did not differ between two levels of concentrate used and negative effects did not observed in DMI, probably because the reduced feed intake generally occurs at a 70 to 75% concentrate (Tremere et al., 1968) and the maximum concentrate level used in this study was 60% of diet DM.

Differences in DMI ($P = 0.47$), DM digestibility ($P = 0.29$) and NDF digestibility ($P = 0.77$) were not observed among the diets (Table 3). Although the crude glycerin has been reported to affect negatively the fiber digestibility in diets to ruminants (Abo El-Nor et al., 2010; Shin et al., 2012), studies has reported that crude glycerin can be included up to 10% of the DM, replacing rapidly fermentable starch for beef cattle finished in feedlots without affect the intake or nutrient digestibility (Ramos and Kerley, 2012; Barton et al., 2013). Our results of digestibility in the diets with inclusion of crude glycerin replacing starch-based energy ingredients in the concentrate are consistent with those reports.

Table 3. Effect of crude glycerin associated with corn or soybean hulls in two levels of concentrate on DMI and nutrient digestibility

Item	Concentrate level		SEM	Diets ¹			SEM	<i>P</i> -value ²		
	40%	60%		CO	CGC	CGSH		CL	D	CLxD
DMI, kg/d ³	6.87	7.04	0.35	7.17	7.02	6.70	0.40	0.64	0.47	0.22
DMD, % ⁴	55.29	55.86	2.13	55.06	58.57	53.10	2.35	0.85	0.29	0.17
NDFD, % ⁵	39.08	41.34	3.60	38.64	42.08	39.91	4.01	0.61	0.77	0.53

¹CO = corn, without crude glycerin; CGC = crude glycerin associated with corn; CGSH = crude glycerin associated with soybean hulls.

²CL = concentrate level effect; D = diets effect; CLxD = concentrate level and diets interaction effect.

³Dry matter intake;

⁴Dry matter digestibility;

⁵Neutral detergent fiber digestibility;

^{a,b,c}Means within a row that do not have a common superscript differ ($P \leq 0.05$).

The DMI has been variable in studies where crude glycerin replaced rapidly fermentable starch (Parsons et al., 2009; Avila-Stagno et al., 2013; Meale et al., 2013), but DMI influence occurs between 10 and 20% of glycerol concentration in the diet, which would tend to agree with the literature (Ramos and Kerley, 2012).

In the present study, we hypothesized that the inclusion of crude glycerin replacing fiber-based energy ingredients in the concentrate could decrease the NDF digestibility and consequently the DMI, due to soybean hulls have 66.3% of NDF concentration in DM basis (NRC, 2000). However, the soybean hulls has a small feed particle size (Mertens, 1997) and could result in a more rapid ruminal escape and in a reduction of the ruminal fill (Iraira et al., 2013).

Although the soybean hulls have high NDF content and the experimental diets which crude glycerin replaced soybean hulls increases NDF concentration (Table 1), the digestibility was not affected in these diets because the soybean hulls had lower time to retention in the rumen. Moreover, Schroder and Sudekum (2009) reported that fiber digestion was increased in low-starch diets when glycerol was included at a concentration of 150 g/kg DM.

The ruminal pH was lower to animals fed high concentrate diets than ruminal pH from animals fed low concentrate diets ($P < 0.01$; Table 4). The DMI is a major determinant of rumen pH and individual animal difference in rumen pH depends on the capacity of the animal to buffer and to absorb organic acids produced in the rumen which determines the drop of rumen pH after feeding large amounts of fermentable carbohydrates (Krause and Oetzel, 2006). The DMI from animals fed with different concentrate levels (40 or 60%) was similar among these diets, but generally, increasing the grain content of the diet usually results in a decline in rumen pH as a result of an increase in the supply of rapidly fermentable carbohydrates (Van Kessel and Russell, 1996; Lana et al., 1998; Walsh et al., 2009).

Some researchers have reported that ruminal pH decreases when crude glycerin replaces starch in diets to ruminants (Mach et al., 2009; Ramos and Kerley, 2012), because the ruminal fermentation of glycerol is faster than starch. Contrary to these experiments, no differences were detected in pH, in the present study, when crude glycerin replaced corn or soybean hulls in 10% of diet DM.

Table 4. Effect of crude glycerin associated with corn or soybean hulls in two levels of concentrate on pH, ammonia-N and volatile fatty acids concentrations

Item	Concentrate level			Diets ¹				<i>P</i> -value ²			
	40%	60%	SEM	CO	CGC	CGSH	SEM	T	CL	D	CLxD
pH	6.22 ^a	5.94 ^b	0.10	6.10	6.11	6.04	0.11	<0.01	<0.01	0.50	0.65
NH ₃ -N (mg/dl)	16.57	15.95	1.61	17.30 ^a	16.98 ^a	14.50 ^b	1.65	<0.01	0.43	<0.01	0.95
Acetate	64.86	62.54	0.71	67.16	60.97	62.98	0.78	<0.01	0.01	<0.01	0.03
Propionate	20.20 ^b	22.65 ^a	1.05	20.24 ^b	22.25 ^a	21.80 ^a	1.06	<0.01	<0.01	<0.01	0.13
Butyrate	16.89	16.40	0.82	14.45	18.69	16.80	0.85	<0.01	0.27	<0.01	0.05
A:P ratio ³	3.20 ^a	2.83 ^b	0.13	3.33 ^a	2.74 ^b	2.97 ^b	0.14	<0.01	0.01	<0.01	0.34

¹CO = corn, without crude glycerin; CGC = crude glycerin associated with corn; CGSH = crude glycerin associated with soybean hulls.

²T = time effect; CL = concentrate level effect; D = diets effect; CLxD = concentrate level and diets interaction effect.

³Acetate:propionate ratio

Likewise, DeFrain et al. (2004) reported no differences in pH when crude glycerin replaced corn starch in diets to ruminants.

Differences in ammonia-N concentration was not observed in animals fed low or high concentrate diets ($P = 0.43$; Table 4). However, animals fed diets with crude glycerin replacing soybean hulls had lower ammonia-N concentration than animals fed diets without crude glycerin or with this by product replacing corn ($P < 0.01$). The suppressed microbial fermentation activity can be induced by ruminal transit (Cole and Hutcheson, 1985) and the soybean hulls exhibit a greater rate of ruminal passage than corn (Ipharraguerre and Clark, 2003), remaining less time at the rumen, reducing the microbial fermentation activity and favoring the lower production of ammonia-N in the rumen.

Animals fed high concentrate diets had greater propionate concentrations than animals fed low concentrate diets ($P < 0.01$; Table 4). Despite this fact, the acetate:propionate ratio was lower in ruminal fluid from animals fed high concentrate diets ($P = 0.01$). When propionate concentrations increase in ruminal fluid, the pH decreases (Orskov, 1986). Linear decrease in the molar proportion of acetic acid and simultaneous increase in propionic acid concentration occurs in response to increasing the grain content (Mc Geough et al., 2010). Likewise, in the present study, the propionate concentrations increase and the pH were reduced in ruminal fluid from animals fed high concentrate diets. It has also been well established (Moe and Tyrrell, 1979; Johnson and Johnson, 1995) that altering the dietary forage-to-concentrate ratio, specifically the fiber to-starch ratio, affects the proportion of the individual VFA in the rumen.

The propionate concentrations ($P < 0.01$) and A:P ratio ($P < 0.01$) were affected by inclusion of crude glycerin in diets replacing starch- or fiber-based energy ingredients in the concentrate. Animals fed diets with crude glycerin replacing corn or soybean hulls had higher propionate concentrations and lower A:P ratio in ruminal fluid than animals fed diets without crude glycerin. The increase in molar proportion of propionate resulted in reduction of acetate:propionate ratio, according with Lee et al. (2011) that reported the propionate as an mainly end product from fermentation of glycerol. The increase of propionate concentrations by feeding with glycerol has been reported when crude

glycerin replaced roughage (Hales et al., 2013) or rapidly fermentable starch in the concentrates (Avila et al., 2011; Ramos and Kerley, 2012), confirming the glycogenic properties of glycerol.

There was an interaction between concentrate level and the diets for acetate ($P = 0.03$; Fig. 1) and butyrate concentrations ($P = 0.05$; Fig. 2). Acetate concentrations were higher in ruminal fluid from animals fed diets with low concentrate without crude glycerin than diets with high concentrate without crude glycerin. In diets with high proportion of roughage, the acetate concentrations are greater because increase the quantity of fiber fermented. We hypothesized that animals fed diets with crude glycerin replacing corn or soybean hulls in low concentrate diets may produce lower acetate concentrations than animals fed diets with crude glycerin replacing corn or soybean hulls in high concentrate diets because crude glycerin may produce others VFA's more efficiently at low-concentrate diets.

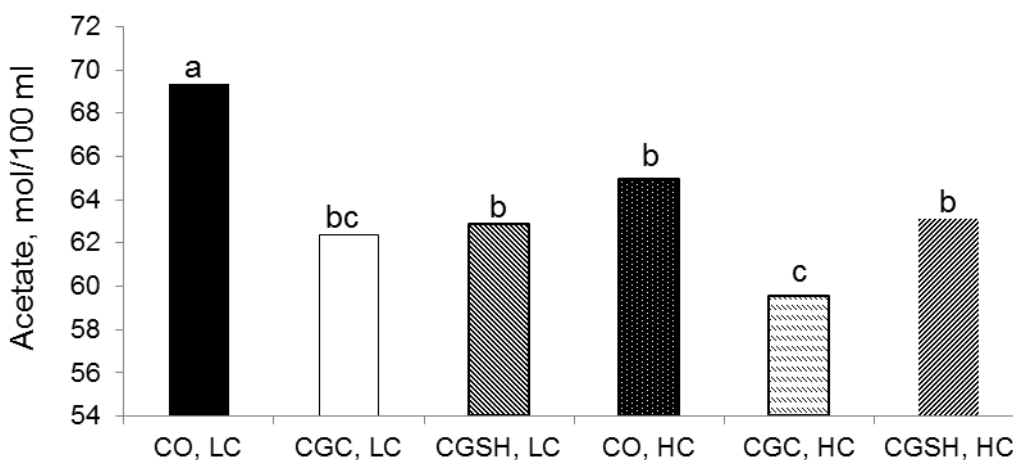


Fig. 1. Acetate concentration in the ruminal fluid of steers fed crude glycerin associated with corn or soybean hulls in two levels of concentrate. Significant effects of time ($P < 0.01$), concentrate level ($P = 0.01$), diet ($P < 0.01$) and concentrate level x diet interaction ($P = 0.03$) were detected.

The decrease in acetate concentrations were observed in animals fed crude glycerin replacing starch- or fiber-based ingredients in low concentrate than animals fed low concentrate diets without crude glycerin. Although the acetate concentrations from

animals fed crude glycerin replacing starch- or fiber-based ingredients in low concentrate were not lower than acetate concentrations from animals fed high concentrate diets, these concentrations were similar between these diets, reporting the glycogenic property of glycerol in low concentrate diets.

Effects of glycerol on ruminal fermentation are a shift in VFA production in favor of propionate, with an even greater increase in butyrate at the expense of acetate both *in vitro* and *in vivo* (Rémond et al., 1993). Starch fermentation in the rumen yields more propionate, less acetate production (Church, 1988), unlike the soybean hulls that are low in lignin and composed of a large proportion of potentially digestible fiber (Quicke et al., 1959; Hsu et al., 1957). Therefore, when soybean hulls are fermented in the rumen, more acetate than propionate is produced.

Evaluating diets with crude glycerin replacing corn or soybean hulls (low or high concentrate), was observed that acetate concentrations were similar in animals fed crude glycerin replacing corn or soybean hulls both in low concentrate diets, but it is not observed in high concentrate diets, which acetate concentrations were higher in diets with crude glycerin replaced soybean hulls than diets with crude glycerin replacing corn. Despite these results, can be observed that crude glycerin had a greater efficiency to reduce the acetate concentrations replacing fiber-based ingredients in low concentrate, because similar results did not achieved in high concentrate diets.

Butyrate concentrations were greater in diets with crude glycerin replacing corn or soybean hulls, mainly in low concentrate diets ($P = 0.05$). Glycerol is metabolized by *Megasphaera elsdenii*, *Streptococcus bovis*, and *Selenomonas ruminantium* (Stewart et al., 1997), and *Megasphaera elsdenii* has been associated with increases in butyric acid in ruminal fluid (Hales et al., 2013).

The increase in the molar proportion of butyrate with glycerol substitution level is consistent with the findings of others (Rémond et al., 1993; Wang et al., 2009; Abo El-Nor et al., 2010) who reported that the molar proportion of butyrate in the VFA mixture increased when glycerol was added in the diets. However, when CG replaced roughage sources, butyrate concentration was similar in diets without or with 10% of glycerol in DM basis (Hales et al., 2013).

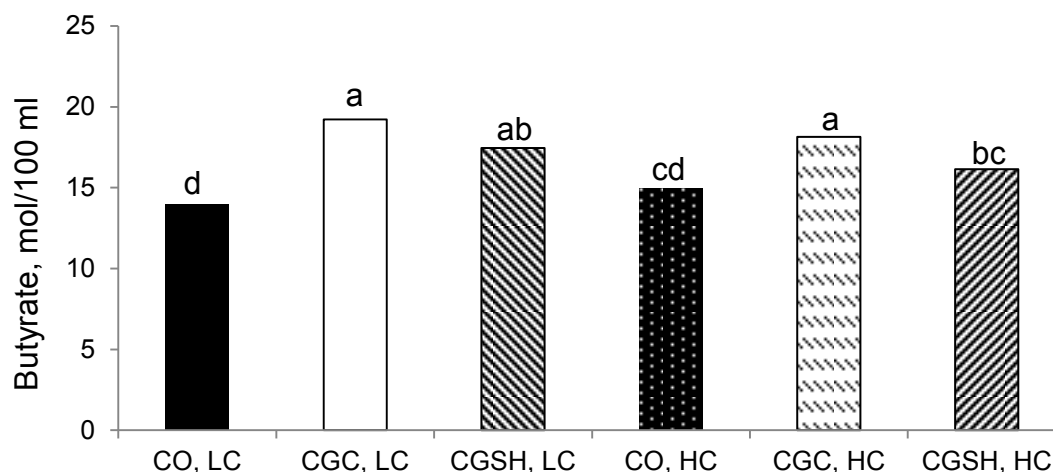


Fig. 2. Butyrate concentration in the ruminal fluid of steers fed crude glycerin associated with corn or soybean hulls in two levels of concentrate. Significant effects of time ($P < 0.01$), diet ($P < 0.01$), concentrate level x diet interaction ($P = 0.05$) but not significant differences were detected for concentrate level ($P = 0.27$).

The species or total protozoa were not affected in animals fed low or high concentrate diets ($P > 0.05$; Table 5). The amount of feed intake had more influence than the type of diet on the ciliate protozoa (Franzolin and Dehority, 2010) and greater numbers of ciliated protozoa are also in greater concentrations under butyrate concentrations (Whitelaw et al., 1972).

The DMI's and butyrate concentrations was similar by animals fed low or high concentrate diets, thus, the protozoa populations in the rumen were not affected after 3 h of feeding. On high concentrate diets, the prevalence of protozoa in the rumen typically declines, probably due to the lack of a fibrous floating mat in the rumen where the ciliate remain attached in order to multiply (Owens et al., 1998). However, in the present study, diets considered as a high concentrate had 40% of roughage in DM basis, allowing the presence of fibrous particles floating in the rumen, without affect negatively the protozoa growth.

Rumen pH plays an important role on the survival of rumen ciliated protozoa as researchers have observed differences in rumen protozoa under different pH levels in several feeding systems (Williams & Coleman, 1992; Dehority, 2003).

Table 5. Effect of crude glycerin associated with corn or soybean hulls in two levels of concentrate on rumen fluid protozoa numbers ($1 \times 10^5 \text{ ml}^{-1}$)

Item	Concentrate level		SEM	Diets ¹			SEM	<i>P</i> -value ²		
	40%	60%		CO	CGC	CGSH		CL	D	CLxD
Entodinium	20.37	23.24	3.96	21.15	23.74	20.52	4.25	0.39	0.71	0.23
Dasytricha	0.11	0.22	0.05	0.19	0.16	0.14	0.06	0.13	0.84	0.34
Isotricha	0.22	0.33	0.08	0.26	0.32	0.24	0.09	0.28	0.84	0.21
Eudiplodinium	0.20	0.18	0.09	0.06	0.25	0.25	0.11	0.85	0.25	0.65
Protozoa Total	20.93	24.10	4.07	21.78	24.51	21.28	4.39	0.35	0.72	0.26

¹CO = corn, without crude glycerin; CGC = crude glycerin associated with corn; CGSH = crude glycerin associated with soybean hulls.

²T = time effect; CL = concentrate level effect; D = diets effect; CLxD = concentrate level and diets interaction effect.

The ruminal pH in animals fed high concentrate diets were lower than ruminal pH from animals fed low concentrate diets (Table 4). However, some researchers have concluded that *Entodinium* species (greater concentrations in the rumen) are more tolerant to low pH than other genera of rumen protozoa (Mackie et al, 1978; Lyle et al., 1981). Likewise, Dehority (2005) reported death of in vitro protozoa at pH values below 5.4, being lower than value of ruminal pH found in this present study for high concentrate diets (5.94).

The species or protozoa total were not affected by inclusion of crude glycerin in the diets ($P > 0.05$; Table 5). Data reporting influences of crude glycerin on protozoa populations from ruminants fed in feedlot are scarce. Therefore, considering the similar pH and DMI's among diets with or without crude glycerin, the results of protozoa are consistent.

The species of bacteria did not differ from animals fed low or high concentrate diets ($P = 0.56$; Table 6). Ruminal pH < 6.0 is the main limitation of fiber digestibility and current work with molecular techniques has shown that even cows with very low pH can maintain normal populations of cellulolytic bacteria (Palmonari et al., 2010). Low ruminal pH could be reducing binding by cellulolytic to particulate matter in the rumen, allowing acid-tolerant bacteria to initially adhere and therefore have a more favorable competition for new feed particles (Mouriño et al., 2001). The present study are consistent with those report, because the pH was lower than 6.0 in high concentrate diets and populations of cellulolytic bacteria evaluated as a *Ruminococcus albus*, *Ruminococcus flavefaciens* and *Fibrobacter succinogenes*, remaining similar in low or high concentrate diets.

Bacteria populations were similar among diets ($P > 0.05$; Table 6). Researchers has reported that glycerin has the potential to produce negative effects on cellulolytic activity in the rumen, ultimately decreasing fiber digestion and large reductions in cellulose degradation, due to adhesion and cellulolytic activity of *R. flavefaciens* and *F. succinogenes* were inhibited when glycerol was included in cultures at 5% of DM basis, in *in vitro* study (Roger et al., 1992). Although in the present study were used 10% of crude glycerin in the DM diet, the NDF digestibility and ruminal pH were not different among diets ($P > 0.05$).

Table 6. Effect of crude glycerin associated with corn or soybean hulls in two levels of concentrate on relative proportion (%) of cellulolytic bacteria and methanogenic arqueas

Item	Concentrate level			Diets ¹				<i>P</i> -value ²		
	40%	60%	SEM	CO	CGC	CGSH	SEM	CL	D	CLxD
<i>Ruminococcus albus</i>	0.152	0.362	0.31	0.003	0.54	0.22	0.35	0.56	0.47	0.26
<i>Ruminococcus flavefasciens</i>	0.012	0.001	0.01	0.001	0.017	0.001	0.01	0.32	0.35	0.46
<i>Fibrobacter succinogenes</i>	0.028	0.041	0.02	0.051	0.021	0.031	0.02	0.41	0.29	0.39
<i>Methanogenic</i>	0.061	0.044	0.05	0.051	0.062	0.043	0.05	0.41	0.72	0.18

¹CO = corn, without crude glycerin; CGC = crude glycerin associated with corn; CGSH = crude glycerin associated with soybean hulls.

²T = time effect; CL = concentrate level effect; D = diets effect; CLxD = concentrate level and diets interaction effect.

The lack of effects in bacteria populations of *Ruminococcus albus*, *Ruminococcus flavefaciens* and *Fibrobacter succinogenes* evaluated are consistent with these results. Likewise, Abo El-Nor et al. (2010) reported that the glycerol supplemented did not impact the DNA concentrations of *R. albus*.

Considering the inverse relationship between CH₄ and propionate production and due to glycolytic properties of glycerol achieved in the present study by lower propionate concentrations ($P < 0.01$) in diets which crude glycerin replaced corn or soybean hulls, it was expected that the methanogenic populations were reduced in these diets. However, the methanogenic populations were not different among diets ($P = 0.72$). A certain level of CH₄ production is necessary to remove reducing equivalents, thereby allowing the overall ruminal fermentation to proceed. As a result, rumen fermentations will likely always continue to produce at least some CH₄ (Krause et al., 2013). Therefore, the quantity of crude glycerin included in these diets (10% of DM) was not enough to change the methanogenic populations and the presence of these microorganisms in these diets are consistent with the function of metabolism in ruminant that produces methane as energy loss.

4. CONCLUSIONS

Increasing concentrate content (40 to 60%) reduces the pH, increases the propionate but does not affect the NDF digestibility of diets and DMI by animals. Neither bacteria and protozoa species were affected by increases of concentrate in diets. Crude glycerin can be used to replace corn or soybean hulls in 10% of diet DM, without affecting the DMI, NDF digestibility and growth of microorganisms in the rumen. Moreover, the inclusion of crude glycerin in diets replacing starch- or fiber-based energy ingredients reduces the acetate:propionate ratio as a result of increases of propionate concentrations.

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CHAPTER 3

O artigo a seguir está redigido conforme normas de publicação do *Journal of Animal Science*, exceto o posicionamento das tabelas e figuras.

Methane emissions and growth performance of Nellore young bulls fed crude glycerin replacing starch- vs. fiber-based energy ingredients at low or high concentrate diets

ABSTRACT: Seventy Nellore bulls at 18 months of age were used to determine the effects of crude glycerin (CG) replacing starch- vs. fiber-based energy ingredients in low (LC) or high concentrate (HC) diets on the animals' DMI, methane emissions, glucose and insulin profiles, G:F ratio and growth performance. Ten animals were slaughtered (reference group) to obtain the carcass gain (CrG). The 60 remaining animals, housed in individual pens, were weighed after a 16-h solid fast to obtain the shrunk BW values (373.70 ± 24.70 kg). The animals were allotted by BW in a 2 x 3 factorial arrangement (two concentrate levels - CLs, and three diets) and exposed to one of six treatments: CO - without CG and corn as an ingredient of concentrate; CGC - inclusion of CG (10% of DM) replacing corn in the concentrate; and CGSH - inclusion of CG (10% of DM) replacing soybean hulls (SH) in the concentrate. All three diets were offered as LC or HC diets (40 or 60%). The animals fed LC or HC had similar DMI (kg/d, $P = 0.88$), ADG ($P = 0.96$), final BW ($P = 0.60$), methane emissions (g/kg DMI; $P = 0.76$), glucose ($P = 0.11$) and insulin ($P = 0.82$) profiles, longissimus muscle area (LMA; $P = 0.96$) and rib fat thickness (RFT; $P = 0.18$). The DMI ($P = 0.89$) and ADG ($P = 0.98$) were similar among the diets. The CL and the diets had a tendency an interaction for methane emissions (g) per kg of DMI ($P = 0.07$). The animals fed CG as a SH replacement in the LC diet had a tendency to produce lower methane emissions (g/kg DMI) than animals fed CG as a SH replacement in the HC diet and similar methane emissions to animals fed the HC diet without CG. Animals fed CGC had a greater G:F (g CrG/kg DMI; $P < 0.01$) than animals fed CO or CGSH diets. Animals fed CGSH had greater serum glucose concentrations ($P < 0.01$) than those fed CO diets and had lower CrG ($P = 0.09$) and LMAD ($P = 0.02$) than animals fed CGC. Increasing the concentrate content did not affect the intake, methane emissions or growth performance. The inclusion of CG in diets to replace SH in LC diets tends to decrease the methane emissions more than from animals fed with CG as a replacement for SH in HC diets. Moreover, this diet promotes similar methane emissions

from animals fed diets with or without CG as a replacement for corn in the HC diet. However, when CG replaces SH in the diets, decreases in CrG, G:F in relation to the CrG and LMAD compared to animals fed CGC.

Keywords: beef cattle, feedlot, glucose, glycerol, soybean hulls, ultrasound

1. INTRODUCTION

Numerous factors influence cattle methane emissions, including the level of feed intake and carbohydrate type in the diet, and their manipulation can reduce cattle methane emissions (Johnson and Johnson, 1995). The increase in the diet starch content, has been reported to reduce methane emissions (Moss et al., 2000) and can be an effective method without compromising the animal's performance (Mc Geough et al., 2010). However, corn prices are high because of an increased demand from the ethanol industry, and agroindustrial byproducts may thus be an economical alternative to replace corn grain in ruminant diets.

Crude glycerin is a byproduct from biodiesel agroindustry and can be used in ruminant diets up to 10% of diet DM without compromise intake and performance (Drouillard, 2012; Parsons et al., 2009; Schroder & Sudekum, 2009). This byproduct has been reported as a potential energy source because glycerol decreases the acetate:propionate ratio in the rumen (Abo-El Nor et al., 2010; Avila et al., 2011; Ramos and Kerley, 2012). Considering the inverse relationship between CH₄ and propionate production (Van Soest and Demeyer, 1995; Mitsumori and Sun, 2008), feeding glycerol can reduce methane emissions for ruminants. Studies that examine the effects of crude glycerin on methane emissions in vivo are rare. Avila-Stagno et al. (2013a) reported that the inclusion of glycerol up to 21% of the diet DM did not affect lamb methane emissions. Lee et al. (2011) reported a reduction of methane per unit of digestible energy, but Meale et al. (2013) did not observe an effect when they evaluated glycerol as a replacement for whole wheat grain in up to 12% of the diet DM; both of these were in vitro studies.

Almost all studies that included crude glycerin in ruminant diets have reported the use of high concentrate diets with crude glycerin replacing rapidly fermentable starch. Schroder and Sudekum, (2009) suggested that glycerol can replace up to 10% of readily fermentable starches but that its energetic value when fed in conjunction with a high-starch concentrate diet (60% concentrate) was approximately 85% of its value when fed with a low-starch concentrate diet. According to Drouillard (2008), propionate production is typically greater for concentrate-fed animals, and thus there may be less opportunity to improve energetic efficiency when glycerol is combined with concentrates compared to feeding with forages.

Therefore, we hypothesized that providing crude glycerin in conjunction with soybean hulls in low-concentrate diets to beef cattle should reduce methane emissions in comparison with animals fed starch-based energy ingredients diets without compromising their growth performance. The objective of this study was to evaluate the effects of crude glycerin in combination with starch- vs. fiber-based energy ingredients in low or high-concentrate diets on the methane emissions, blood parameters and growth performance of beef cattle finished in feedlot.

2. MATERIAL AND METHODS

The trial was performed at the Sao Paulo State University (UNESP, Jaboticabal, SP, Brazil) following the humane animal care and handling procedures according to the guidelines of the Sao Paulo State University (UNESP, Brazil). Pre-harvest handling was in accordance with good animal welfare practices, and slaughtering procedures followed the Sanitary and Industrial Inspection Regulation for Animal Origin Products (Brasil, 1997).

Seventy Nellore bulls at 18 months of age were used. Ten animals were slaughtered (383.6 ± 41.0 kg) and served as the reference group at the beginning of the experiment as the initial dressing percentage (51.9%), which estimated the initial carcass weight to obtain the carcass gain (CrG) and CrG in relation to the ADG (CrG/ADG) at the end of experiment. The 60 remaining animals, housed in individual

pens with automatic drinkers, were weighed after a 16-h solid fast to obtain the shrunk BW values (373.70 ± 24.70 kg). The animals were allotted by BW into a 2 x 3 factorial arrangement (two concentrate levels - CLs, and three diets) with ten animals per treatment. Animals were submitted to 21 d of adaptation to experimental installations and diets and 94 d experimental period to evaluate their intake, methane emissions, blood parameters and growth performance.

Young bulls were fed with one of the following experimental diets: CO - without crude glycerin and corn as ingredient of concentrate; CGC - inclusion of crude glycerin (10% of DM) replacing corn in the concentrate; and CGSH - inclusion of crude glycerin (10% of DM) replacing soybean hulls in the concentrate. All three diets were offered at a low or high concentrate (40 or 60%). Crude glycerin was acquired from a soybean oil-based biodiesel production company, i.e., ADM, Rondonópolis, Brazil (80.34% of glycerol; 1.59% of ether extract; 5.03% of ash and 12.02% of water). Corn silage was used as the only source of roughage, and the concentrates comprised grounded corn or soybean hulls, soybean meal, urea/ammonium sulfate and a mineral mixture. Soybean meal was used as an alternative protein source, and a mixture of urea and ammonium sulfate was used to adjust the diet CP content. All diets were balanced to provide 14.4 ± 0.2 of CP (Valadares Filho et al., 2006). The ingredient proportions and chemical compositions of the experimental diets are presented in Table 1.

Cattle were fed two times daily at 0800 h and 1600 h and feed refusals were recorded daily for each pen. Amounts of feed offered to animals were calculated according to previous DMI and adjustments were made when needed so that refused feed did not exceed 10% of daily intake. Orts daily weights and samplings were performed for the diet quantities provided from each animal. To estimate intake, samples of diets (forage and concentrate) and Orts from each animal were first composited weekly and then composited every month. Feed samples were frozen at -18°C for later analysis of DM, ether extract (AOAC, 1990), N was determined by combustion (Leco Instruments Inc., method 976.06, AOAC, 1990) and multiplied by 6.25 to obtain CP and NDF was determined by method of Van Soest et al. (1991) using Ankon bags (Ankon Technology Corp., Fairport, NY, USA).

Table 1. Diet composition (DM basis)¹

Item	Low concentrate (40%)			High concentrate (60%)		
	CO	CGC	CGSH	CO	CGC	CGSH
Ingredient, % of DM						
Corn silage	60.00	60.00	60.00	40.00	40.00	40.00
Corn	26.30	16.00	-	46.40	36.10	-
Soybean meal	10.00	10.00	10.00	10.00	10.00	10.00
Soybean hulls	-	-	16.20	-	-	36.60
Crude glycerin	-	10.00	10.00	-	10.00	10.00
Urea/ammonium sulfate	0.70	1.00	0.80	0.60	0.90	0.40
Mineral premix ²	3.00	3.00	3.00	3.00	3.00	3.00
Nutrient composition ³ , %						
DM	57.60	57.90	58.60	67.08	67.38	68.90
CP	14.30	14.70	14.60	14.45	14.20	14.10
NDF	30.14	28.53	37.60	25.19	23.59	42.95
EE ⁴	3.13	3.01	2.92	3.18	3.05	2.83
NFC ⁵	47.00	49.46	40.14	53.93	55.48	35.46
ME, Mcal/kg	2.56	2.54	2.50	2.68	2.67	2.60

¹CO = corn, without crude glycerin; CGC = crude glycerin replacing corn; CGSH = crude glycerin replacing soybean hulls.

²Trace mineral mixed contained Ca, 210 g; P, 20 g; S, 37 g; Na, 80 g; Cu, 490 mg; Mn, 1.424 mg; Zn, 1.830 mg; I, 36 mg; Co, 29 mg; Se, 9 mg; F máx., 333 mg;

³All nutrients were analyzed. ME were calculated.

⁴Ether extract.

⁵Non fiber carbohydrates = 100 – (CP + EE + Ash + NDF).

Coccygeal blood samples from all animals were taken after a 16-h solid fast before the first feeding in the morning from the coccygeal vein on (0 d), 59 and 94 d after feeding period. Blood samples were collected into commercial blood collection tubes (10 mL; Vacutainer) and EDTA-coated glass tubes (10 mL; Vacutainer). Blood samples were placed on ice and centrifuged at 2.500 x g for 20 min at 4°C and the resulting serum or plasma was decanted and stored at - 20°C until laboratory analysis. Preprandial serum

samples were analyzed for glucose (Glicose Liquiform Vet Kit, Labtest Diagnostica S.A., Lagoa Santa, Brazil) and insulin (ADVIA Centaur CP Insulina - IRI, manufactured in Japan by Kyowa Medex Co., Ltd. For Siemens Healthcare Diagnostics Inc., Tarrytown, NY, USA) through commercial kits.

The animals were evaluated through ultrasound on (0 d), 27, 59 and 94 d after feeding. Ultrasound measurements of 12th-13th rib fat thickness and longissimus muscle area (LMA) were taken on the right side of the animals, using an Aloka 500V real time ultrasound machine equipped with a 17.2-cm, 3.5-MHz linear transducer. Images were taken by a technician who is certified by the Ultrasound Guideline Council and images were interpreted using the software Biosoft Toolbox® II for Beef (Biotronics, Inc., Ames, Iowa, USA).

The methane emissions were assessed using the sulfur hexafluoride (SF_6) tracer technique (Johnson et al., 1994), where each animal was sampled daily for 5 consecutive days, beginning at 87 days of feeding. Thirty-six animals (six treatment replicates) were fitted with gas collection halters at 10 days before methane sampling to allow animals to adapt and facilitate sampling. Brass permeation tubes (Fig. 1 a) filled with SF_6 and known release rates were administered orally to each of the 36 animals 72 h before methane sampling to allow the tracer gas to equilibrate in the rumen. The release rate of the gas from a permeation tube is known before its insertion into the rumen. The permeation tubes were maintained in water bath at 39°C, being weighed for 8 weeks in laboratory.

The animals were fitted with gas collection halters connected to pre evacuated polyvinyl chloride canisters designed to fill halfway over 24 h. Sampling started at 0600 h daily, when each animal individually were removed from the pens, and was conducted at the management center (stockyard) to facilitate sampling. The collection canisters were located above each animal to reduce the risk of equipment damage and were connected to the halter by tubing inside airline flexible-coil tubing. The pressure of the canister removed from the animal was measured after 24 hours of collection and if the final pressure was out of the expected range, the halter was preventively replaced. If the final pressure was above the range, the halter was probably blocked or disconnected; if the

final pressure was below the expected possibly has a leaking in the system. In both situations the new halter was placed in the animal with an average absorption rate within the stipulated range (fill halfway over 24 h). After sampling (approximately 30 minutes), each animal were returned at the original pen for feeding. The pressure readings were recorded, and the canisters were pressurized using pure N₂. The ambient air samples in the feedlot were collected two samples for day to determine the background concentrations of methane and SF₆. These values were subtracted from the animals' values to calculate the net output in the expired breath. Methane emission rate is calculated as follows: $QCH_4 = QSF_6 \times [CH_4] / [SF_6]$; where QCH₄ is the emission rate of methane in liters/hour, QSF₆ is the known release rate of SF₆ from the permeation tube, [CH₄] and [SF₆] are the measured concentrations in the canister. Methane emissions (g/head per d) and g of methane per kg of DMI (g/kg DMI) were calculated by dividing the daily methane output of each animal by daily intake (during methane sampling) and methane emissions (g/head per d) in relation to kg of ADG and kg of CrG (throughout the entire experimental period). The variable CH₄, g/kg CrG = g CH₄ produced per day / kg of daily carcass gain.

At the beginning of the trial all the animals were weighed after a 16-h solid fast before the first feeding in the morning. The ADG was obtained by weighing the animal at the beginning and the end of the experiment, always after a 16-h solid fast. After 94 d of feeding, all the animals were slaughtered at a commercial beef plant. For obtain the CrG, ten animals were selected as a reference group and slaughtered to obtain the dressing percentage (DP) in the day 0 of the experiment. The DP of reference group was used in conjunction with the BW initial (day 0) of 60 animals to estimate the carcass weight initial (CWi) these 60 animals as describe: $CWi = (BW_i \times DP \text{ of reference group}) / 100$. At the end of experiment these 60 animals were slaughtered and the carcass weight final (CWf) was recorded from each animal, thus we did $CrG = (CWf - CWi / d \text{ of feeding})$.

All data were analyzed using the SAS PROC MIXED procedure (SAS Inst. Inc., Cary, NC). The model tested the fixed effects of the concentrate level, diets (i.e., without crude glycerin, crude glycerin plus corn and crude glycerin plus soybean hull) and the

interaction of concentrate level and diets. For all data, the animal was the experimental unit and was included in the model as a random effect. When the interaction term was not significant, it was removed from the model. The ultrasound measures and serum concentrations of glucose and insulin were analyzed as repeated measurements in time using the MIXED procedure. This model included the effects of the concentrate level, diets, time and concentrate level x diets x time interactions as fixed effects and the animals as random effects. The best (co)variance matrix was chosen based on Akaike's Information Criteria. The least-squares means were generated for main-effects and significant interactions and compared ($P \leq 0.05$) using Tukey's test. For all variables, P -values less than or equal to 0.10 were considered tendencies.

3. RESULTS AND DISCUSSION

Animals fed low- or high-concentrate diets had similar DMI ($P = 0.88$; Table 2). Increasing the level of supplementation with concentrates often causes a decline in the rate of forage digestion in the rumen (Forbes, 2007). However, reduced feed intake generally occurs at a 70 to 75% concentrate (Tremere et al., 1968) because the lactate-utilizing bacteria increase dramatically (Mackie et al., 1978), suggesting that more acid conditions prevail in the rumen (Yokoyama and Johnson, 1988) thereby, decreasing the fiber digestibility and consequently the feed intake. Differences were not observed in DMI, probably because the reduced feed intake generally occurs at a 70 to 75% concentrate and the maximum concentrate level used in this study was 60% of diet DM. Due to the similarity in the DMI, animals had a similar ADG ($P = 0.96$), final BW ($P = 0.60$) and G:F, g ADG/kg DMI ($P = 0.78$; Table 3).

DMI differences were not observed among the diets ($P = 0.89$; Table 2). These results are consistent with those obtained in other studies, suggesting that crude glycerin can be included up to 10% of the DM, replacing rapidly fermentable starch for beef cattle finished in feedlots without promoting effect deleterious on intake or performance (Pyatt et al., 2007; Parsons et al., 2009; Drouillard, 2012; Barton et al., 2013). The NDF concentration in the experimental diets increased when soybean hulls

were included in concentrate (Table 1). In the present study, we hypothesized that the inclusion of crude glycerin as a replacement for fiber-based energy ingredients would decrease DMI, possibly because glycerin in the rumen can reduce cellulolytic activity of ruminal bacteria thereby potentially decreasing fiber digestibility (Roger et al., 1992; Abo El-Nor et al., 2010). However, the inclusion of crude glycerin in fiber-based diets did not affect the animal DMI, possibly because the soybean hulls rapidly escaped the rumen due to their small feed particle size (Mertens, 1997). Food intake directly influences the ADG and due to a similar DMI in animals fed with these diets, animals had a similar ADG ($P = 0.98$), final BW ($P = 0.97$) and G:F, g ADG/kg DMI ($P = 0.85$; Table 3). The partial dietary replacement of rapidly fermentable starch with crude glycerin (10% of DM) did not affect the feed conversion ratio in relation to ADG of bulls fed in the feedlot (Barton et al., 2013).

The daily methane emissions (g/head per d; $P = 0.98$), and g/head per d in relation to kg of DMI ($P = 0.76$), g/head per d in relation to kg of ADG ($P = 0.23$) or g/head per d in relation to kg of CrG ($P = 0.79$), did not differ from cattle offered low or high-concentrate diets (Table 2). According Johnson and Johnson (1995) and Yan et al. (2000), the DMI increase may be attributable to an increased digesta outflow rate from the rumen, thus reducing the time available for microbial fermentation of the ingested feed and thereby limiting methane production. Animals fed low-concentrate diets had similar DMIs to animals fed high-concentrate diets and, therefore, had similar methane emissions ($P > 0.10$).

Neither daily methane emission (g/head per d) differences were observed ($P = 0.85$) in relation to kg of ADG ($P = 0.78$) or kg of CrG ($P = 0.63$) among the diets. However, there was a tendency in an interaction between the concentrate level and the diets for methane emissions (g/head per d) per kg of DMI ($P = 0.07$; Fig. 1). In an in vivo study, Avila-Stagno et al. (2013a) did not observe an effect of crude glycerin replacing barley grain in 21% of diet DM on methane emissions from lambs. Lee et al. (2011) observed in an in vitro study that the inclusion of glycerol with corn or alfalfa reduces the methane production after 24 h. In the present in vivo study, we expected that the inclusion of crude glycerin in the diets would reduce the methane emissions because

glycerol fermentation favors propionate over acetate production (Wang et al., 2009; Avila et al., 2011). However, animals fed glycerol in the high-concentrate diets had a tendency to produce greater methane emissions (g/kg DMI) than animals fed high-concentrate diets without crude glycerin. Glycerol failed to decrease methane emissions as hypothesized, but tended to increase it in high concentrate diets ($P = 0.07$).

Glycerin fermentation does not always produce a hydrogen sink because it promotes a shift in carbohydrate fermentation that may affect the overall electron balance in the rumen and not promote reduction in the availability of hydrogen for methane formation. Glycerol is a more reduced substrate than sugars and releases two electron pairs for each mole of glycerol converted to pyruvate (Zhang and Yang, 2009), one in the oxidation of glycerol to dihydroxyacetone, which is then phosphorylated and enters glycolysis, and the other in glycolysis itself, in the oxidation of 3-phosphoglyceraldehyde to 3-phosphoglycerate (Biebl et al., 1999). Thus, according to Avila-Stagno et al., (2013b) this compensates for electron incorporation in the conversion of pyruvate or phosphoenolpyruvate to propionate, and there is no net electron incorporation in the conversion of glycerol to propionate. Moreover, this author reported that there is a linear increase in butyrate production when glycerol is included up to 15% of DM, thus, butyrate production from both carbohydrates and glycerol would result in a release of reducing equivalents and contribute to increasing methane emissions.

Animals fed crude glycerin as a replacement for corn in low-concentrate diets had a tendency to produce greater methane emissions (g/kg DMI) than animals fed high-concentrate diets without crude glycerin. Increasing the starch content of the diet shifts the rumen fermentation towards propionic acid production at the expense of acetic acid (McGeough et al., 2010). The production of propionic acid is a hydrogen-utilizing process, which deprives the methanogenic bacteria of the hydrogen necessary to produce methane (Moss et al., 2000).

Table 2. Effect of crude glycerine replacing corn or soybean hulls in low- or high-concentrate diets on methane emissions from cattle

Item	Concentrate level		SEM	Diets ¹			SEM	<i>P</i> -value ²		
	Low (40%)	High (60%)		CO	CGC	CGSH		CL	D	CLxD
DMI, kg/d	9.2	9.2	0.33	9.1	9.1	9.4	0.53	0.88	0.89	0.92
CH ₄ , g/head per d	173.6	173.3	19.94	160.7	188.7	170.8	32.77	0.98	0.85	0.49
CH ₄ , g/head per d/kg DMI	20.8	21.2	0.80	19.3	22.3	21.4	0.98	0.76	0.09	0.07
CH ₄ , g/head per d/kg ADG	138.7	127.4	13.23	123.5	145.7	130.0	21.06	0.23	0.78	0.60
CH ₄ , g/head per d/kg CrG ³	188.6	185.1	9.31	180.5	184.7	195.3	11.40	0.79	0.63	0.51

¹CO = corn, without crude glycerin; CGC = crude glycerin replacing corn; CGSH = crude glycerin replacing soybean hulls.

²CL = concentrate level effect; D = diets effect; CLxD = concentrate level and diets interaction effect.

³CrG = carcass gain.

^{a,b,c}Means within a row that do not have a common superscript differ ($P \leq 0.10$).

This suggests that a dietary starch threshold must be achieved to sufficiently stimulate glucogenic VFA production to a concentration that could provide a viable hydrogen sink alternative to methanogenesis (Lovett et al., 2005), thus reducing methane emissions.

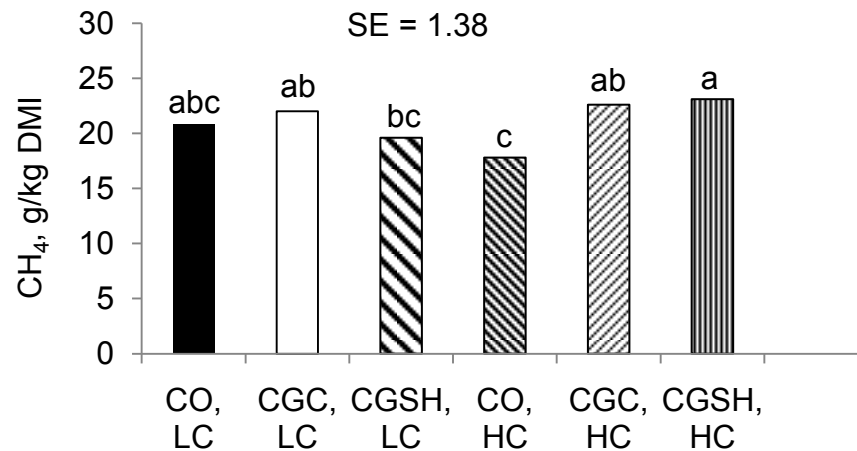


Figure 1. Daily methane emissions g/kg of DMI of young bulls fed diets CO = corn, without crude glycerin; CGC = crude glycerin replacing corn; CGSH = crude glycerin replacing soybean hulls at low or high concentrate diets during 94 days of the experiment. There was an interactions between concentrate level x diet ($P = 0.07$).

We hypothesized that animals fed crude glycerin as a replacement for fiber-based energy ingredients in low-concentrate diets may emit less methane than animals fed starch-based ingredients at high concentrate (with or without crude glycerin) because crude glycerin may produce propionate more efficiently at low-concentrate diets with lower starch concentrations. Animals fed crude glycerin as a replacement for soybean hulls in low-concentrate diets had similar methane emissions (g/head per d/kg DMI) than animals fed starch-based ingredients at high concentrate with or without crude glycerin. In addition, these animals had a tendency to emit less methane (g/head per d/kg DMI) than animals fed fiber-based energy ingredients in high-concentrate diets with crude glycerin ($P = 0.07$; Fig. 2). The addition of glycerol plus forage (alfalfa)

reduces the acetate:propionate ratio more than glycerol plus corn (Lee et al., 2011) in an in vitro study. Therefore, we hypothesized that animals fed crude glycerin as a replacement for soybean hulls in low-concentrate diets may be using glycerol more efficiently for propionate production in the rumen, because these animals had similar methane emissions (g/head per d/kg DMI) than animals fed high-concentrate diets without crude glycerin and had a tendency to reduce the methane emissions (g/head per d/kg DMI) than animals fed crude glycerin as a replacement for soybean hulls in high-concentrate diets.

The carcass gain, CrG/ADG and G:F, g CrG/kg DMI, were similar in animals fed low or high-concentrate diets ($P > 0.10$; Table 3), and these animals had similar blood glucose concentrations ($P = 0.11$; Fig. 2), insulin ($P = 0.82$; Fig. 3) and an insulin:glucose ratio ($P = 0.99$; Table 4). Differences were not detected for concentrate level x diets x time ($P = 0.25$; $P = 0.93$; $P = 0.44$; data not show) in the blood. Moreover, these animals did not differ in the LMA ($P = 0.96$) and rib fat thickness (RFT; $P = 0.18$; Table 5). Animal growth directly correlates with tissue growth, as it is dependent upon the rate and extent of cellular hypertrophy and hyperplasia comprising the respective tissues. Cellular enlargement occurs due to nutrient uptake by the cell and the balance between the anabolic and catabolic process that regulate accretion of component and structural cellular materials (Etherton, 1982). These findings demonstrate that these animals had similar glucose uptake by the peripheral tissue and did not exhibit significant growth differences. Likewise, Lage et al. (2012) did not observe differences in the LMA or RFT from the carcasses of animals fed with low or high-concentrate diets.

Although we did not identify differences in the ADG, animals fed diets with crude glycerin as a replacement for starch-based energy ingredients had a tendency to obtain greater CrG than animals fed diets with crude glycerin as a replacement for fiber-based energy ingredients ($P = 0.09$; Table 3).

The change in live weight is an imprecise growth indicator, considering accretion of protein and fat tissue (Owens et al., 1995). McLeod and Baldwin (2000) reported that the digestive tract mass increases significantly when ruminants are fed fiber-based diets.

Table 3. Effects of the addition of crude glycerine replacing corn or soybean hulls in low- or high-concentrate diets on cattle growth performance and feed efficiency

Item	Concentrate level		SEM	Diets ¹			SEM	P-value ²		
	Low (40%)	High (60%)		CO	CGC	CGSH		CL	D	CLxD
Final BW, kg	501.2	496.5	11.67	495.7	498.6	502.3	18.69	0.60	0.97	0.70
ADG, kg/d	1.37	1.37	0.07	1.38	1.36	1.38	0.11	0.96	0.98	0.28
CrG, g/d ³	940	932	0.02	928 ^{ab}	988 ^a	892 ^b	0.03	0.84	0.09	0.65
CrG/ADG, % ⁴	69.61	68.83	2.12	66.2 ^b	75.4 ^a	66.0 ^b	3.37	0.65	0.10	0.48
G:F, g ADG/kg DMI ⁵	147.3	148.7	0.01	150.2	151.1	142.6	0.01	0.78	0.85	0.15
G:F, g CrG/kg DMI ⁶	103.6	103.7	0.01	103.9 ^b	111.2 ^a	96.0 ^c	0.01	0.98	<0.01	0.39

¹CO = corn, without crude glycerin; CGC = crude glycerin replacing corn; CGSH = crude glycerin replacing soybean hulls.

²CL = concentrate level effect; D = diets effect; CLxD = concentrate level and diets interaction effect.

³CrG = carcass gain.

⁴Carcass gain in relation to average daily gain.

⁵Gain to feed ratio, g average daily gain / kg dry matter intake.

⁶Gain to feed ratio, g carcass gain / kg dry matter intake.

^{a,b,c}Means within a row that do not have a common superscript differ ($P \leq 0.10$).

Table 4. Effect of crude glycerin replacing corn or soybean hulls in low or high concentrate diets on serum glucose and insulin concentrations from cattle

Item	Concentrate level		SEM	Diets ¹			SEM	P-value ²			
	Low (40%)	High (60%)		CO	CGC	CGSH		T	CL	D	CLxD
Glucose, mg/dl	74.8	78.1	1.42	72.3 ^b	76.5 ^{ab}	80.6 ^a	1.74	0.03	0.11	<0.01	0.58
Insulin, uIU/ml	4.2	3.9	0.85	2.5	5.4	4.3	1.04	0.04	0.82	0.13	0.63
Insulin:glucose	5.2	5.2	1.09	3.2	6.7	5.6	1.34	0.03	0.99	0.15	0.47

¹CO = corn, without crude glycerin; CGC = crude glycerin replacing corn; CGSH = crude glycerin replacing soybean hulls.

²T = time effect; CL = concentrate level effect; D = diets effect; CLxD = concentrate level and diets interaction effect.

^{a,b,c}Means within a row that do not have a common superscript differ ($P \leq 0.10$).

When higher fiber levels are fed throughout the finish period, the percentage of weight as gastrointestinal fill may not decrease (Buckley et al., 1990), thus explaining the similar ADG compared to animals fed other diets but tendency to reduce the carcass deposition ($P = 0.09$).

Animals fed diets with CG as a replacement for starch-based energy ingredients had a tendency in greater CrG/ADG than animals fed diets with CO or CG replacing fiber-based energy ingredients ($P = 0.10$). Due to similar DMIs but greater CrGs, animals fed diets with CGC had better G:F, g CrG/kg DMI than animals fed diets CO, as these animals fed diets without crude glycerin had greater G:F, g CrG/kg DMI than animals fed diets with CGSH ($P < 0.01$).

These findings can be attributed to the effects of starch on serum concentrations of glucose and insulin in cattle. Glucose is derived from ruminal (Schoonmaker et al., 2003) and postruminal (Huntington et al., 2006) starch digestion. In contrast to corn, soybean hulls are low-starch energy supplements that are high in digestible fiber (Sharman et al., 2013). Starch fermentation in the rumen yields more propionate, less acetate production, and more glucose supply to the animal compared with fiber digestion (Church, 1988). An increased supply of glucose from intestinal digestion of starch promotes lipogenesis via the citrate cleavage pathway, so lipogenesis in the total body could thus be enhanced (Owens et al., 1995). Therefore, intestinal fat synthesis could explain why an increased supply of intestinal starch often enhances the efficiency of finishing cattle (Owens et al., 1986). This effect may have been enhanced by the inclusion of crude glycerin into the diet due to the gluconeogenic property of this byproduct.

Glycerin levels of up to 10% of the DM as a replacement for rapidly fermentable starch in feed for bulls finished in feedlots did not affect glucose serum concentrations (Barton et al., 2013). Likewise, in this study, animals fed crude glycerin as a replacement for corn had similar serum glucose concentrations to animals fed diets without crude glycerin and corn. However, when crude glycerin replaced fiber-based ingredients, serum glucose concentrations increased more than when animals were fed diets without crude glycerin plus corn ($P < 0.01$; Table 4).

The increase in serum glucose in animals fed crude glycerin as a replacement for soybean hulls is likely due to the suppressed microbial fermentation activity induced by fasting and ruminal transit (Cole and Hutcheson, 1981; 1985).

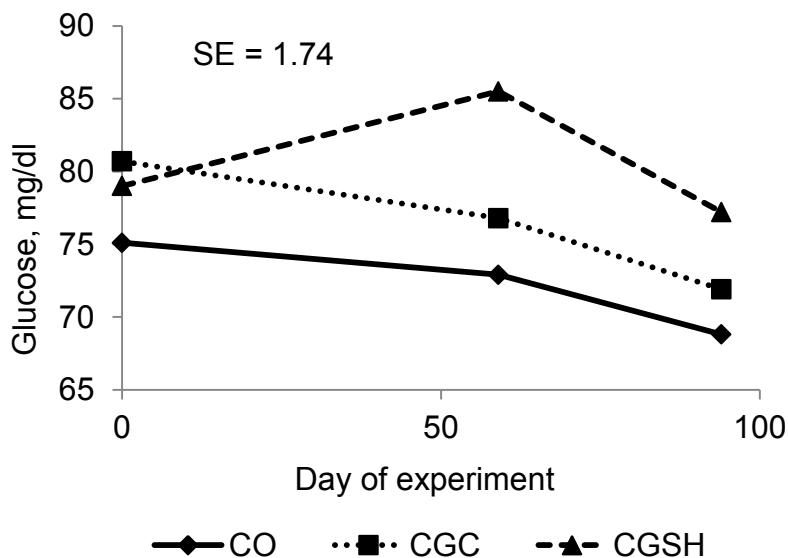


Figure 2. Glucose serum concentrations of young bulls fed diets CO = corn, without crude glycerin; CGC = crude glycerin replacing corn; CGSH = crude glycerin replacing soybean hulls at low or high concentrate diets during 94 days of the experiment. Significant effects of time ($P = 0.03$) and diet ($P < 0.01$) but not significant differences for concentrate level ($P = 0.11$) and concentrate level x diet interaction ($P = 0.58$) were detected.

The soybean hulls exhibit a greater ruminal rate and degradation extent than corn (Ipharraguerre and Clark, 2003), so the reduction in microbial fermentation activity could have increased the undergradable intake protein to the small intestine and thus increased the amount of gluconeogenic precursors available and thereby intestinal glucose absorption (Mueller et al., 2011).

Differences in the insulin ($P = 0.13$) and insulin:glucose ratio ($P = 0.15$) among the diets were not observed (Table 4). However, a significant effect of time (sampling period) was detected for glucose ($P = 0.03$; Fig. 2), insulin ($P = 0.04$; Fig. 3) and the insulin:glucose ratio ($P = 0.03$).

Serum glucose concentrations on d 94 were less than those on d 0 (72.7 ± 1.56 vs. 78.3 ± 1.51 mg/dL, respectively; $P = 0.06$) and d 59 (78.4 ± 2.1 mg/dL; $P = 0.05$) but did not differ ($P = 0.99$) between d 0 and 59 (Fig. 2). The serum insulin concentrations on d 94 were greater than those on d 0 (5.64 ± 1.03 vs. 2.0 ± 1.03 uIU/ml; $P = 0.04$) but did not differ ($P = 0.74$) between d 94 and 59 (4.6 ± 1.03 uIU/ml; Fig. 3).

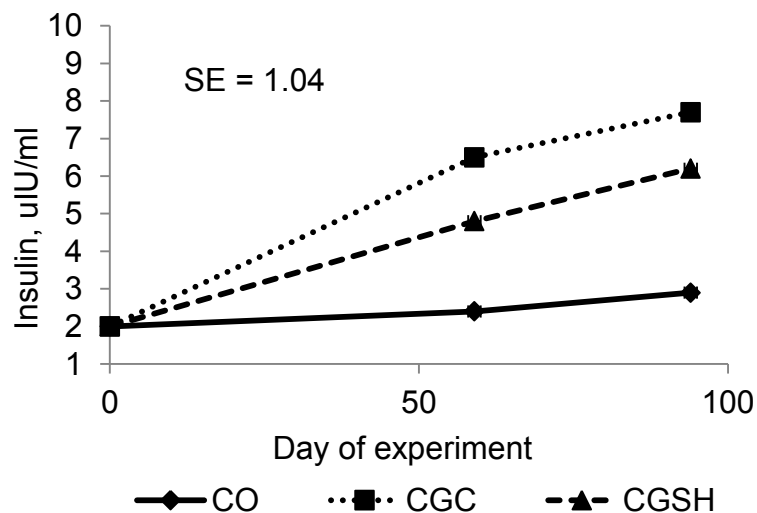


Figure 3. Insulin serum concentrations of young bulls fed diets CO = corn, without crude glycerin; CGC = crude glycerin replacing corn; CGSH = crude glycerin replacing soybean hulls at low or high concentrate diets during 94 days of the experiment. Significant effects of time ($P = 0.04$) but not significant differences for diet ($P = 0.13$), concentrate level ($P = 0.82$) and concentrate level x diet interaction ($P = 0.63$) were detected.

The animals fed diets with crude glycerin as a replacement for fiber-based energy ingredients had numerically lower serum insulin concentrations (Fig. 3) than animals fed crude glycerin as a replacement for starch-based energy ingredients. Increased serum insulin likely leads to increased glucose uptake by peripheral tissues (Schoonmaker et al., 2003). Insulin exerts many diverse actions that range from acute effects, such as glucose transport stimulation, to long-term effects, such as increasing rates of protein and lipid synthesis (Etherthon, 1982).

We hypothesized that animals fed CGSH diets would improve their energy efficiency and obtain greater tissue deposition because Schroder and Sudekum (2009) suggested that glycerol can readily replace up to 10% of readily fermentable starches, but its energetic value when fed in conjunction with a high-starch concentrate diet was approximately 85% of its value when fed with a low-starch concentrate diet. However, animals fed fiber-based energy ingredients had smaller LMA ($P = 0.02$) than did animals fed diets without or with crude glycerin as a replacement for corn (Table 5).

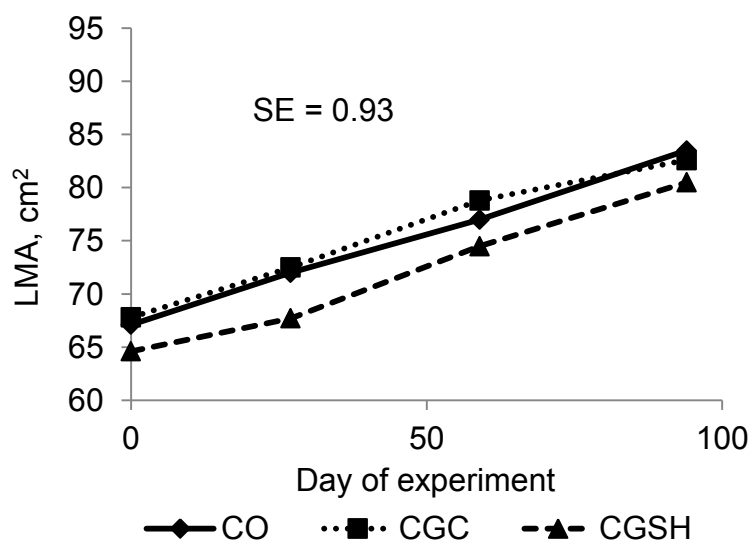


Figure 4. Longissimus muscle area of young bulls fed diets CO = corn, without crude glycerin; CGC = crude glycerin replacing corn; CGSH = crude glycerin replacing soybean hulls at low or high concentrate diets during 94 days of the experiment. Significant effects of time ($P < 0.01$) and diet ($P = 0.02$), but not significant differences for concentrate level ($P = 0.96$) and concentrate level x diet interaction ($P = 0.41$) were detected.

These results can be attributed to the greater serum glucose concentrations of animals fed diets with CGSH ($P < 0.01$) and thus had lower glucose uptake by tissues (Fig. 2) and less lean tissue development (Fig. 4).

Animals fed diets with corn received a more than adequate supply of glucose from dietary sources, suggesting that ruminal starch fermentation to provide other

nutrient needs (energy substrates, amino acids) would improve the efficiency of starch use for growth (Huntington, 1997).

Unlike the LMA, no differences were observed for rib fat thickness ($P = 0.11$) among the diets (Table 5). Soybean hulls are low in lignin and composed of a large proportion of potentially digestible fiber (Quicke et al., 1959; Hsu et al., 1957). Therefore, when soybean hulls are fermented in the rumen, more acetate than propionate is produced. The contribution of acetate to lipogenesis is greatest in subcutaneous adipose tissue (Smith and Crouse, 1984; Smith et al., 2009).

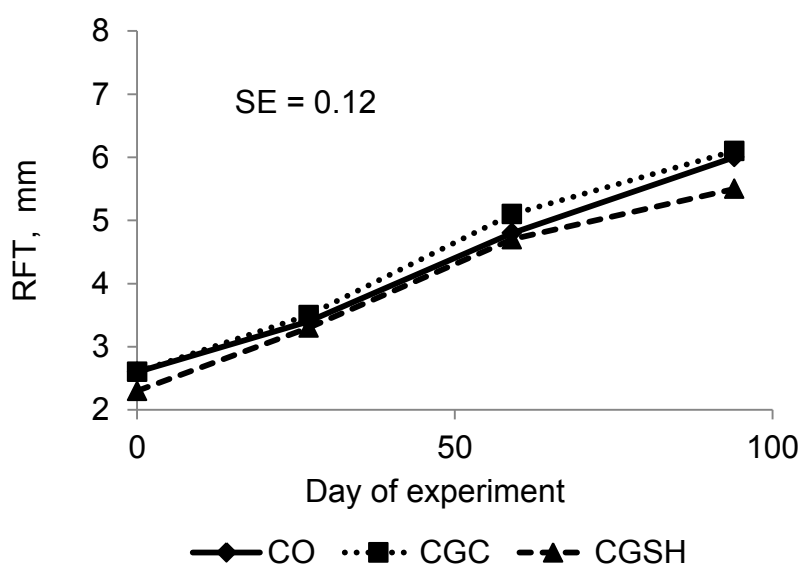


Figure 5. Rib fat thickness of young bulls fed diets CO = corn, without crude glycerin; CGC = crude glycerin replacing corn; CGSH = crude glycerin replacing soybean hulls at low or high concentrate diets during 94 days of the experiment. Significant effects of time ($P < 0.01$), but not significant differences for diet ($P = 0.11$), concentrate level ($P = 0.18$) and concentrate level $\times$ diet interaction ($P = 0.39$) were detected.

Table 5. Effect of crude glycerin replacing corn or soybean hulls in low or high concentrate diets on cattle tissue deposition evaluated through ultrasound

Item	Concentrate level		SEM	Diets ¹			SEM	P-value ²			
	Low (40%)	High (60%)		CO	CGC	CGSH		T	CL	D	CLxD
LMA, cm ³	74.0	74.1	0.76	74.9 ^a	75.4 ^a	71.9 ^b	0.93	<0.01	0.96	0.02	0.41
RFT, mm ⁴	4.2	4.1	0.10	4.2	4.3	3.9	0.12	<0.01	0.18	0.11	0.39

¹CO = corn, without crude glycerin; CGC = crude glycerin replacing corn; CGSH = crude glycerin replacing soybean hulls.

²T = time effect; CL = concentrate level effect; D = diets effect; CLxD = concentrate level and diets interaction effect.

³Longissimus muscle area.

⁴Rib fat thickness.

^{a,b,c}Means within a row that do not have a common superscript differ ($P \leq 0.10$).

Likewise, Sharman et al., (2013) reported no differences in subcutaneous fat from carcasses of animals fed fiber-based supplements (soybean hulls) compared to carcass from animals fed starch-based supplements (corn), but differences in mRNA expressions of genes involved in lipogenesis as a fatty acid synthase (FASN), adipocyte fatty acid binding protein 4 (FABP4) and stearoyl-CoA desaturase 1 ($\Delta 9$ -desaturase) were detected in perirenal adipose tissue of growing Angus steers fed fiber-based supplements (soybean hulls) compared to expression of genes in the perirenal adipose tissue from animals fed starch-based supplements (corn). Therefore, these observations explain why animals fed CGSH, despite a lower carcass gain and lean tissue (LMA), had similar thickness of subcutaneous fat (Fig. 6).

4. CONCLUSIONS

Increasing concentrate content (40 to 60%) did not affect the intake, methane emissions or growth performance. Animals fed crude glycerin as a replacement for soybean hulls in low-concentrate diets had similar methane emissions (g/kg DMI) compared to animals fed starch-based ingredients in high-concentrate diets with or without crude glycerin. Moreover, the inclusion of crude glycerin as a replacement for soybean hulls in low-concentrate diets tends to decrease the animal methane emissions more than animals fed with crude glycerin as a replacement for soybean hulls in high-concentrate diets.

Although when crude glycerin replaces soybean hulls in the diets, it does not compromise the DMI and ADG, the animals' growth is compromised, decreasing the carcass gain, feed efficiency in relation to carcass gain and lean tissue more than in animals fed crude glycerin as a replacement for corn. Thus, crude glycerin replacing corn in 10% of DM can be used in diets to Nellore young bulls in feedlot, promoting greater growth and carcass deposition.

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CHAPTER 4

O artigo a seguir está publicado e redigido conforme normas do *Meat Science*, exceto o posicionamento das tabelas e figuras.

Fatty acid profile, carcass and meat quality traits of young Nellore bulls fed crude glycerin replacing energy sources in the concentrate

ABSTRACT - Carcass and meat quality traits of 60 Nellore young bulls were evaluated fed diets without crude glycerin (CG); with CG replacing corn (CGc; 10% of dry matter - DM) in the concentrate; with CG replacing soybean hull (CGsh; 10% of DM) in the concentrate. Diets were evaluated at two concentrate levels (CL). The CL did not affect cold carcass weight (CCW; $P = 0.6074$), cold carcass dressing (CCD; $P = 0.9636$), rib fat thickness (RFT; $P = 0.8696$) and longissimus muscle area (LMA; $P = 0.7524$). Animals fed diets with CGc or CGsh showed meat with greater deposition of monounsaturated fatty acids (MUFA; $P = 0.0022$) and CLA (18:2 *cis*-9, *trans*-11) contents ($P = 0.0001$) than animals fed diets without CG. The inclusion of 10% of CG in diets CGc or CGsh does not affect the carcass and meat quality traits, however, increases the MUFA and CLA content in beef, although these changes are very small in nutritional terms.

Keywords: corn, glycerol, feedlot, soybean hulls, tenderness

1. INTRODUCTION

Crude glycerin is a biodiesel byproduct that has been reported as a potential energy source for ruminant animals, particularly when included at levels up to 10% of total dry matter of the diet (Drouillard, 2012; Schroder & Sudekum, 2009; Versemann, Wiegand, & Kerley, 2008).

Changes in carcass and meat quality traits of animals fed crude glycerin may occur as a result of the increase in the availability of gluconeogenic compounds that would be used as precursors for fatty acid synthesis leading to an increase in marbling of meat (Evans, Wiegand, & Kerley, 2008; Versemann, et al., 2008). This phenomenon occurs because glycerol can be converted to propionate in the rumen or absorbed by the ruminal epithelium and thus converted into glucose (Krehbiel, 2008) which in turn is

the main source of carbon used for the fatty acid synthesis (Schoonmaker, Fluharty, & Loerch, 2004).

The inclusion of crude glycerin in diets of ruminant animals may also increase the level of unsaturated fatty acid in meat. Krueger et al. (2010) reported that glycerol likely inhibit lipolysis - a prerequisite for rumen biohydrogenation, which is responsible for the saturation of dietary fatty acids consumed by ruminant animals - decreasing the accumulation of free fatty acids in the ruminal environment of animals by 48 and 77% supplementing 2 or 20% of glycerol in vitro to mixed populations of ruminal microbes. Thus, feeding with crude glycerin has a potential to increase the amount for unsaturated fatty acids available to be incorporated in meat products.

According to Edwards et al. (2012) supplementing glycerol at or near 8 to 15% of diet DM would be expected to achieve effective reductions in lipolysis without adversely affecting rumen DM digestion, however, results obtained during culturing of mixed populations of ruminal microbes and from assays of pure cultures of rumen bacteria have not adequately established the mechanism by which glycerol inhibits ruminal lipolysis. The glycerol (up to 21% of diet DM) has the potential to beneficially affect fatty acid profiles from subcutaneous adipose tissue in lambs, increasing the 18:0 and 9c-18:1 and reducing 10t-18:1 and the n-6/n-3 ratio (Avila-Stagno et al., 2013), but the effects on beef has not been assessed and needs to be studied.

Several studies that have shown the effect of crude glycerin on carcass and beef quality traits using diets with high levels of starch (Mach, Bach, & Devant, 2009; Parsons, Shelor, & Drouillard, 2009; Ramos & Kerley, 2012). However, the production of propionate is typically greater in animals fed high concentrate diets which reduce the chances to improve the energy efficiency by the inclusion of glycerol (Drouillard, 2008), when compared to roughage based diets or diets with low levels of starch. Soybean hulls is widely used in feedlot in Brazil as a substitute for corn in concentrate diets (Mendes, Ezequiel, Galati, & Feitosa, 2005; Silva, et al., 2002) mainly due its lower cost as feedstuff. As such, this study aimed to evaluate the inclusion of crude glycerin at 10 % of total dry matter as a replacement for different energy sources (corn or soybean

hulls) in diets with two different concentrate:roughage proportions on carcass and meat quality traits.

2. MATERIAL AND METHODS

2.1. Animals and management

The trial was carried out at the Sao Paulo State University (UNESP, Jaboticabal, SP, Brazil), following the humane animal care and handling procedures, according to the guidelines of the Sao Paulo State University (UNESP, Brazil). Sixty Nellore bulls with average initial body weight of 373.70 ± 24.70 kg and age of 18 months were used. Initially, cattle were weighed, identified and housed in individual pen with feeders and automatic drinkers. Cattle were submitted to 21 days of adaptation to experimental installations and diets.

After adaptation, cattle were randomly assigned to one of the following experimental diets: Control - without crude glycerin and corn as ingredient of concentrate; inclusion of crude glycerin (10% of DM) that replaced corn in the concentrate; and inclusion of crude glycerin (10% of DM) that replaced soybean hulls in the concentrate. All the three diets were offered at two roughage:concentrate proportion (60:40 and 40:60). Crude glycerin was acquired from a soybean oilbased biodiesel production company ADM, Rondonópolis, Brazil (80.34% of glycerol; 1.59% of ether extract; 5.03% of ash and 12.02% of water). Corn silage was used as the only source of roughage and concentrates were composed of ground corn or soybean hulls, soybean meal, urea/ammonium sulphate and mineral mixture. The ingredients proportion and chemical composition of the experimental diets are presented in Table 1. The fatty acid profile of the ingredients of the experimental diets is presented in Table 2.

At the beginning of the experiment animals were weighed after 16-h solid fast to obtain the values of shrunk body weight (SBW) and assigned in a completely randomized design in 2 x 3 factorial schemes (two roughage:concentrate proportions and three feeding regime) with 10 animals per treatment.

Table 1. Diet composition (DM basis)¹

Item	Low concentrate (40%)			High concentrate (60%)		
	CO	CGC	CGSH	CO	CGC	CGSH
Ingredient, % of DM						
Corn silage	60.00	60.00	60.00	40.00	40.00	40.00
Corn	26.30	16.00	-	46.40	36.10	-
Soybean meal	10.00	10.00	10.00	10.00	10.00	10.00
Soybean hulls	-	-	16.20	-	-	36.60
Crude glycerin	-	10.00	10.00	-	10.00	10.00
Urea/ammonium sulfate	0.70	1.00	0.80	0.60	0.90	0.40
Mineral premix ²	3.00	3.00	3.00	3.00	3.00	3.00
Nutrient composition ³ , %						
DM	57.60	57.90	58.60	67.08	67.38	68.90
CP	14.30	14.70	14.60	14.45	14.20	14.10
NDF	30.14	28.53	37.60	25.19	23.59	42.95
EE ⁴	3.13	3.01	2.92	3.18	3.05	2.83
NFC ⁵	47.00	49.46	40.14	53.93	55.48	35.46
ME, Mcal/kg	2.56	2.54	2.50	2.68	2.67	2.60

¹CO = corn, without crude glycerin; CGC = crude glycerin replacing corn; CGSH = crude glycerin replacing soybean hulls.

²Trace mineral mixed contained Ca, 210 g; P, 20 g; S, 37 g; Na, 80 g; Cu, 490 mg; Mn, 1.424 mg; Zn, 1.830 mg; I, 36 mg; Co, 29 mg; Se, 9 mg; F máx., 333 mg;

³All nutrients were analyzed. ME were calculated.

⁴Ether extract.

⁵Non fiber carbohydrates = 100 – (CP + EE + Ash + NDF).

Table 2. Percentages of the principal fatty acids in the corn silage, corn, soybean meal, soybean hulls and crude glycerin

Item ¹	Corn silage	Corn	Soybean meal	Soybean hulls	Crude glycerin
Capric C10:0	0.10	-	-	-	3.12
Lauric C12:0	0.19	-	-	-	4.87
Myristic C14:0	0.21	0.04	0.11	0.17	0.46
Palmitic C16:0	15.30	13.15	16.12	14.16	10.71
Margaric C17:0	0.15	0.08	0.11	0.32	1.45
Stearic C18:0	2.96	2.40	4.20	4.10	6.53
Palmitoleic C16:1	0.16	0.14	0.14	0.42	-
Oleic C18:1 <i>cis</i> -9	30.58	34.87	18.74	22.85	35.79
Linoleic C18:2	45.32	46.25	51.50	45.37	32.27
Linolenic C18:3	2.37	0.89	6.08	7.45	2.48
SFA	20.34	16.84	21.70	20.19	27.14
UFA	79.66	83.16	78.30	79.81	72.86
MUFA	31.97	36.02	20.72	26.99	38.11
PUFA	47.69	47.14	57.58	52.82	34.75

¹SFA = saturated fatty acids; UFA = unsaturated fatty acids; MUFA = monounsaturated acids fatty; PUFA = polyunsaturated fatty acids.

2.2. Slaughter, carcass data and sample collection

After 94 days of feeding, all the animals were slaughtered at a commercial beef plant with 498.35 ± 33.55 kg of shrunk body weight. Pre-harvest handling was in accordance with good animal welfare practices, and slaughtering procedures followed the Sanitary and Industrial Inspection Regulation for Animal Origin Products (Brasil, 1997).

After the slaughter, carcasses were weighed and then refrigerated at 0°C for approximately 24 h. After the postmortem chill period, the cold carcass weight (CCW), ultimate carcass pH (pHu), 12th rib fat thickness (RFT) and 12th rib longissimus muscle area (LMA) were measured on the left side of each carcass. The LMA were traced on transparencies and measured later with a planimeter and RFT measurements were taken $\frac{3}{4}$ the length ventrally over the longissimus muscle (Greiner, Rouse, Wilson, Cundiff, & Wheeler, 2003). Cold carcass dressing percent (CCD) was calculated using CCW divided by final SBW and then multiplying the result by 100.

A boneless longissimus section 10 cm thick was removed from the posterior end of the wholesale rib. Longissimus muscle samples were individually vacuum-packaged and held at -20 °C for 2 days. After that, each frozen Longissimus sample was standardized from the posterior end into one 2.54 cm thick steak sample (AMSA, 1995) for Warner-Bratzler shear force measurement and other analyses as described later. All steaks were vacuum package and held at -20 °C for 10 days until the analysis were performed.

2.3. Meat and subcutaneous fat color

The determination of meat and fat color was performed as described by Houben, Van Dijk, Eikelenboom, and Hoving-Bolink (2000), using a Minolta colorimeter (Model CR 300, Minolta Camera Co. Ltd., Osaka, Japan) evaluating the lightness (L^*), redness (a^*), and yellowness (b^*). The color aspects were assessed by the CIE $L^*a^*b^*$ color system using 0°/45°. Thirty minutes prior to the assessment, samples were removed from vacuum package and surface samples were exposed to air for oxygenation of myoglobin. Same procedure was made for the fat color measurement. After this step the color was measured at three different points and average values were calculated.

The colorimeter was calibrated before analyzing the samples against white and black standards.

2.4. Warner–Bratzler shear-force measurement and cook loss

Warner–Bratzler shear force (WBSF) steaks were thawed at 4 °C for 24 h and oven-broiled in an electric oven (Layr, Luxo Inox) preheated to 150 °C. Internal steak temperatures were monitored by 20-gauge copper-constantan thermocouples (Omega Engineering, Stamford, CT) placed in the approximate geometric center of each steak and attached to a digital monitor. When internal steak temperature reached 35 °C, the steak was turned over and allowed to reach an internal temperature of 70 °C before removal from the oven. Cooked WBSF steaks were cooled for 24 h at 4 °C (AMSA, 1995). Eight round cores (1.27 cm diameter) were removed from each steak parallel to the long axis of the muscle fibers (AMSA, 1995). Each core was sheared once through the center, perpendicular to the fiber direction by a Warner-Bratzler shear machine (G-R Manufacturing Company, Manhattan, KS - USA). Cook loss were evaluated on the steaks that were also used for WBSF measurement. Total cooking loss was calculated as the difference between the weight of the steaks before and after oven-broiling.

2.5. Proximate analysis

For proximate analysis, the epimysium was removed from the samples prior the lyophilization for 36 h. Samples were then ground and analyzed for ether extract (EE; Method 920.85) (AOAC, 1990) in order to determine the chemical composition of each longissimus sample.

2.6. Lipid oxidation

For determination of thiobarbituric acid (TBA) reactive substances, meat samples (50 g) were collected, identified, and vacuum packaged in polyethylene bags. A 10 g sample was ground in a multiprocessor and 0.2 mL of antioxidant BHT (0.03%) was added along with 50 mL of distilled water and 1 mL of an antifoaming solution (Sigma A5758, São Paulo, Brazil). The samples were then ground again and homogenized for 1 min. After homogenization, the samples were transferred to a 250 mL volumetric flask

containing pieces of porcelain, to which 50 mL of a 4 M HCl solution was added. Subsequently, samples were distilled in a blanket heater at 100°C until 50 mL of the distillate was collected. From the distillate, 5 mL was transferred to a test tube and 5 mL of a 0.02 M TBA solution was then added. The test tubes remained in a water bath at the boiling point for 35 min. The test tubes were then cooled with tap water, and the absorbance was measured at 538 nm with a spectrophotometer. The TBA reactive substance values, expressed in milligrams of malonaldehyde per kilogram of meat, were obtained by multiplying the absorbance by 7.8.

2.7. Fatty acid profile

To determine the fatty acid composition of the fresh meat, samples of the transversal section were collected from the longissimus muscle, lyophilized, and frozen for lipid extraction and methylation. The fatty material was extracted using a mixture of chloroform–methanol, as reported by Bligh and Dyer (1959) and the fatty acids methyl esters (FAME) were obtained by ISO 5509 method (1978). Qualitative and quantitative measurements of fatty acid content were performed by gas chromatography using a chromatograph (Shimadzu, Kyoto, Japan — Model GC-14B with a Communication Bus Module - CBM 102) with a flame ionization detector (FID) and fused silica capillary column (Omegawax 250), which was 30 m in length and 0.25 mm in diameter and had a film thickness of 0.25 μm (Supelco SP-24136). Helium was used as a carrier gas at a flow of 1 mL/min. A 1 μL aliquot of the sample was injected into a “split” at a division ratio of 1/100 and a temperature of 250 °C. The temperature of the oven was programmed to remain at 100 °C for 2 min and then increase to 220 °C at 4 °C/min for 25 min, while the detector was at 280 °C. Identification and quantification of the methyl esters of the fatty acids was achieved by comparison with the retention times and concentrations of methyl esters of standard fatty acids.

2.8. Statistical analysis

Statistical analyses were performed using the PROC GLM procedure of SAS (SAS Inst. Inc., Cary, NC), using a 2×3 factorial arrangement - two concentrate levels (40 or 60% of concentrate) and three feeding regimes (without crude glycerin, crude

glycerin plus corn and crude glycerin plus soybean hulls) - as the following statistical model:

$$Y_{ijk} = \mu + \alpha + \beta + \alpha\beta + \varepsilon.;$$

where μ is the mean, α is the effect of the concentrate level, β is the effect of the feeding regimes, $\alpha\beta$ is the interaction of the concentrate level and the feeding regimes, and ε is the random error. Tukey's test was used to compare the least square means, with significance considered at $P < 0.05$.

3. RESULTS AND DISCUSSION

There was no interaction ($P > 0.05$) between concentrate levels (CL) and feeding regimes (FR) for any of the variables evaluated. Thus, effects of CL and FR were discussed independently.

No differences were found for CCW ($P = 0.6074$), CCD ($P = 0.9636$), RFT ($P = 0.896$), LMA ($P = 0.7524$) and LMA/100 kg ($P = 0.5405$) among cattle fed different levels of concentrate. The feeding regimes did not affect CCW ($P = 0.6937$), CCD ($P = 0.0744$), RFT ($P = 0.1234$), LMA ($P = 0.3040$) and LMA/100 kg ($P = 0.2727$; Table 3).

According to Ferreira et al. (1998), cattle fed high concentrate diets may increase the size of organs and weight of internal fat, which may decrease values of the carcass dressing when animals are fed diets with greater proportion of concentrate (Lage, et al., 2012). However, no differences were observed in this present study. Moreover, the similar carcass weight among animals fed different concentrate levels ($P = 0.6074$) may also have contributed for the lack of differences for CCD. Similarly, Oliveira et al. (2009) reported similar values of carcass dressing percentage among animals fed diets with 40 and 60% of concentrate. The lack of differences for LMA, LMA/100 kg, and RFT for animals fed different CL may be attributed to the similarity of the genetic composition of the animals.

The inclusion of CG in the diets does not promote greater carcass traits ($P > 0.05$) than animals fed with diets without crude glycerin.

Table 3. Means and SEM for cold carcass weight (CCW), cold carcass dressing (CCD), rib fat thickness (RFT), longissimus muscle area (LMA) and longissimus muscle area in relation to cold carcass weight (LMA/100kg) from young Nellore bulls fed crude glycerin

Item	Concentrate Level (CL)		<i>P</i> -value	Feeding regimes (FR) ¹			<i>P</i> -value	CL x FR (<i>P</i> -value)
	60:40	40:60		Cn	CGc	CGsh		
CCW, kg	282.17±7.98	279.40±9.17	0.6074	280.43±5.80	283.77±3.87	278.15±3.69	0.6937	0.8890
CCD, %	56.36±1.52	56.34±1.80	0.9636	56.15±0.38	57.00±0.24	55.88±0.40	0.0744	0.6165
RFT, mm	5.10±0.25	5.16±0.26	0.8696	4.61±0.38	5.16±0.30	5.62±0.34	0.1243	0.1840
LMA, cm ²	75.72±2.40	76.50±2.65	0.7524	78.65±2.66	75.66±1.86	74.01±1.57	0.3040	0.8313
LMA/100kg	26.89±0.83	27.38±0.93	0.5405	28.04±0.74	26.71±0.66	26.66±0.58	0.2727	0.8442

^{a,b,c} Means within a row that do not have a common superscript differ ($P < 0.05$).

¹/Cn = without crude glycerin; CGc = crude glycerin plus corn; CGsh = crude glycerin plus soybean hulls.

Similar results were reported by Mach et al. (2009) in a study that the inclusion of CG in beef cattle diets (up to 12 % of DM) did not improve carcass weight, carcass dressing percentage, RFT and LMA.

Schröder and Südekum (2009) reported that the energy value of glycerol is greater for animals fed roughage based diets with low starch ingredients. As such, it would be expected a greater tissue deposition in animals fed with diets (crude glycerin plus soybean hulls). However, the inclusion of crude glycerin in diets containing soybean hulls did not showed greater carcass traits ($P > 0.05$) than animals fed with diets (crude glycerin plus corn).

Carcass ultimate pH evaluated was not affected by the CL ($P = 0.4158$) or FR ($P = 0.4062$; Table 4). Usually cattle fed grain-based diets present greater availability of glycogen at the slaughter and lower carcass ultimate pH values (Neath, et al., 2007). Nonetheless, the increase in the levels of concentrate or the inclusion of CG in the diets did not change ultimate pH values of the carcass. Similarly, Duarte et al. (2011) have found no differences in carcass ultimate pH among animals fed diets with 40 and 80 % of concentrate. In unstressed animals with large reserves of muscle glycogen, pH normally decreases from an initial 7.0-7.2 postmortem to final values of 5.4-5.8, 48 hours postmortem (Young, West, Hart, & van Otterdijk, 2004). However, the average values for carcass ultimate pH observed in this trial (6.2) are greater than recommended values which may be attributed to stressor factors during pre-slaughter handling such as transportation, long time starvation and sexual behavior of non-castrated males (Felício, 1997). The carcass ultimate pH is an important characteristic to be evaluated as it is responsible for changes in meat quality traits such as color (Jeremiah, Tong, & Gibson, 1991; Wulf, O'Connor, Tatum, & Smith, 1997).

Similar values of lightness (L^* ; $P = 0.4090$), redness (a^* ; $P = 0.8282$) and yellowness (b^* ; $P = 0.7200$) were observed for beef from animals fed different CL. With regard to fat color, animals fed lower CL had greater values of b^* than those fed higher CL whereas no differences were observed for L^* and a^* among animals fed different CL (Table 4).

Table 4. Means and SEM for meat color, subcutaneous fat color (L*, a*, b*)¹ and carcass ultimate pH from young Nellore bulls fed crude glycerin

Item ¹	Concentrate Level (CL)		P-value	Feeding regimes (FR) ²			P-value	CL x FR (P-value)
	60:40	40:60		Cn	CGc	CGsh		
pH	6.24±0.06	6.16±0.07	0.4158	6.11±0.08	6.26±0.08	6.23±0.09	0.4062	0.1496
<i>Meat colour</i>								
L*	33.91±0.46	34.68±0.79	0.4090	34.89±0.77	34.17±0.92	33.83±0.68	0.6284	0.2561
a*	14.12±0.41	13.99±0.40	0.8282	14.36±0.38	13.80±0.44	14.00±0.64	0.7292	0.4477
b*	6.88±0.37	7.07±0.41	0.7200	7.43±0.38	6.79±0.46	6.71±0.55	0.4932	0.1703
<i>Fat colour</i>								
L*	78.56±0.52	79.34±0.61	0.3416	78.89±0.65	78.43±0.75	79.54±0.69	0.5467	0.7808
a*	1.52±0.23	1.72±0.24	0.5075	1.85±0.31 ^a	2.07±0.32 ^a	0.95±0.10 ^b	0.0116	0.5555
b*	12.53±0.23 ^a	11.66±0.23 ^b	0.0135	12.14±0.28	12.13±0.29	12.03±0.34	0.9586	0.8409

^{a,b,c} Means within a row that do not have a common superscript differ ($P < 0.05$).

¹L* = luminosity; (0 = black and 100 = white); a* = index from green (-) to red (+); b* = index from blue (-) to yellow (+);

²Cn = without crude glycerin; CGc = crude glycerin plus corn; CGsh = crude glycerin plus soybean hulls.

Moloney et al. (2008) have shown that the animal feed intake as well as the length of the feeding period affects the fat color. Fresh and conserved pasture swards and other green forages contain chemical compounds called carotenoids that cause yellow color to develop in the fat of bovines when such forages are eaten regularly (Dunne, Monahan, O'Mara, & Moloney, 2009). Therefore, it was expected greater values of b^* in the fat of animals fed diets with greater proportion of roughage due to the greater deposition of carotenoids. French et al. (2000) also reported that carcass fat yellowness was inversely and linearly related to the proportion of concentrate in the diet.

Feeding regime did not affect the variables evaluated with regard to meat color L^* ($P = 0.6284$), a^* ($P = 0.7292$), and b^* ($P = 0.4932$). However, FR affected the color of fat ($P = 0.0116$) where animals fed diets without CG and those fed diets containing corn and CG had greater values of a^* than those fed diets with CG and soybean hulls. No differences were observed for L^* ($P = 0.5467$) and b^* ($P = 0.9586$) among animals fed at different FR (Table 4). Ladeira et al. (2012b) reported inclusion of crude glycerin in beef cattle diets up to 18 % of DM positively affected the beef color. On the other hand, in the present study the level of inclusion of CG in the diets (10 % DM) was not enough to promote changes in beef color. Differences found in this study for redness of fat may have occurred due to unexpected blood contamination on the carcass since values of a^* usually is not affected by the diet of the animals (Dunne, et al., 2009).

Due to the high values of pH observed in this study (6.2), it was expected greater challenges in the variables such as L^* and a^* in comparison to meat normal pH values (5.4 and 5.8). A higher muscle pH, proteins are able to bind more strongly with water, allowing less free water. When the proteins bind more water, the muscle fibers are swollen, leaving less space between muscle fibers (Page, Wulf, & Schwotzer, 2001). Therefore, meat that has a higher pH will be darker in color because there is less free water to reflect light (Ledward, Johnston, & Knight, 1992). Furthermore, at a higher muscle pH, enzymes that use oxygen are more active, resulting in less oxygenation of the surface myoglobin and a darker color (Ledward, et al., 1992; Price & Schwiebert, 1987). However, the high pH value did not promote negative effects on meat color. The average values of L^* , a^* , and b^* corroborates those reported by Muchenje et al. (2009),

who observed values of 33 to 41 for L^* , 11.1 to 23.6 for a^* , and 6.1 to 11.3 for b^* in beef.

Differences no were observed ($P = 0.3524$) for cooking loss and Warner-Bratzler shear force (WBSF; $P = 0.6149$) for beef from animals fed different CL. Several studies have shown that the use of high concentrate diets do not cause changes in beef tenderness (Duarte, et al., 2011; Lage, et al., 2012; Missio, et al., 2010). Glycerin level used (10% of DM) and the use of low starch levels in the current study had no effect ($P = 0.3129$) on WBSF measurements of LM (2.99 kgf) and CKL ($P = 0.6695$; Table 5). Nevertheless, the obtained WBSF results (< 4.6 kg) ensure a tenderness that should result in high consumer acceptance (Shackelford, Morgan, Cross, & Savell, 1991).

There was no effect ($P = 0.3764$) of FR on the EE content of Longissimus muscle. We hypothesized that the production of propionate would be typically greater in animals fed high concentrate diets with high levels of starch leading to a fewer chances to improve the energy efficiency when glycerol is added to the diet (Drouillard, 2008) compared roughage based diets or concentrate with low levels of starch, such as soybean hulls. Thus, it was expected an increase in intramuscular fat deposition (since propionate is glucose precursor) in beef from animals fed diets with greater roughage proportion or low level of starch. However, the level of inclusion of glycerin (10% DM) in the diets was not enough to increase marbling independently of the level of starch of the diet.

With regard to fatty acid profile, differences were observed on deposition of saturated fatty acids (SFA; $P < 0.05$) among animals fed different concentrate levels as well as animals from different feeding regimes (Table 6). Meat from animals fed lower CL had greater ($P = 0.0184$) level of palmitic acid (C16:0) compared to meat from animals fed greater CL. The corn silage used had high level of palmitic acid and thus, it was expected a greater level of palmitic acid in beef from animals fed diets with greater proportion of roughage.

Table 5. Means and SEM for Warner-Bratzler shear force (WBSF), cooking loss (CKL), malonaldehyde (MDA; mg/kg of meat) and ether extract of the longissimus muscle from young Nellore bulls fed crude glycerin

Item	Concentrate level (CL)		<i>P</i> -value	Feeding regimes (FR) ¹			<i>P</i> -value	CL x FR (<i>P</i> -value)
	60:40	40:60		Cn	CGc	CGsh		
WBSF, kgf	2.93±0.18	3.05±0.17	0.6149	3.00±0.19	3.22±0.27	2.76±0.15	0.3129	0.1024
CKL, %	14.64±0.96	15.95±1.04	0.3524	15.42±0.98	16.01±1.38	14.46±1.33	0.6695	0.1157
MDA mg/kg	0.40±0.03	0.44±0.04	0.4464	0.39±0.04	0.39±0.04	0.49±0.05	0.1828	0.5473
Ether Extract,%	3.06±0.20	2.49±0.21	0.0527	2.49±0.25	2.97±0.27	2.86±0.25	0.3764	0.2287

^{a,b,c} Means within a row that do not have a common superscript differ ($P < 0.05$).

¹/Cn = without crude glycerin; CGc = crude glycerin plus corn; CGsh = crude glycerin plus soybean hulls.

Animals fed with diet without CG had lower ($P = 0.0001$) levels of margaric acid (C17:0) in meat compared to animals fed diets with CG (Table 6), which can be explained by the greater content of margaric acid in the CG and soybean hulls compared to other feedstuff that composed the experimental diets (Table 2). Differences were observed ($P = 0.0274$) for levels of stearic acid in meat among cattle fed at different FR. Greater levels of stearic acid were detected in meat from animals fed diets without CG compared to meat from animals fed diets with CG and soybean hulls. This might have occurred due to the challenges in ruminal fermentation as a result of CG inclusion in the diets. Stearic acid is originated from C18:1 *trans*-11 and there are evidences that the inclusion of CG in diets reduces the ruminal biohydrogenation (Kruger et al., 2010) leading to a lower formation of stearic acid for deposition in meat from animals fed diets with CG.

Differences were observed ($P < 0.05$) for levels of myristoleic acid (C14:1 *cis*-9) and palmitoleic acid (C16:1 *cis*-9) in meat from animals fed different CL (Table 6). Meat from animals fed lower CL had greater levels of myristoleic acid ($P = 0.0218$) and palmitoleic acid ($P = 0.0026$) than meat from animals fed greater CL. A high level of palmitoleic acid was observed in corn silage (Table 2) and thus, it was expected that meat from animals fed diets with greater proportion of roughage would have higher levels of palmitoleic acid mainly due to the escape of unsaturated fatty acids from ruminal biohydrogenation.

Feeding regime affected the level of oleic acid (18:1 *cis*-9) in meat ($P = 0.0119$; Table 6). Meat from animals fed diets without CG had lower levels of oleic acid (38.21%) than meat from animals fed CG. The utilization of glycerol in cattle diets could potentially inhibit bacterial fat degradation and may promote ruminal passage of the total lipid, thereby providing greater proportions of beneficial unsaturated fat for incorporation into beef products (Krueger et al., 2010). The increase of this fatty acid has been associated with greater beneficial HDL cholesterol (HDL-C) plasma concentrations in humans (Gilmore, et al., 2011). Similar results were reported by Avila-Stagno et al., (2012) and Ladeira et al. (2012a) who observed an increase in levels of oleic acid in meat of lambs and beef cattle, respectively, fed diets with glycerol.

Table 6. Means and SEM for principal fatty acids saturated and unsaturated (%) found in the muscle Longissimus of young Nellore bulls fed crude glycerin

Fatty acid ¹	Lipid number	Concentrate level (CL)		<i>P</i> -value	Feeding regimes (FR) ²			<i>P</i> -value	CL x FR (<i>P</i> -value)
		60:40	40:60		Cn	CGc	CGsh		
SFA									
Capric	10:0	0.06±0.01	0.05±0.01	0.8531	0.06±0.01	0.05±0.01	0.05±0.01	0.2791	0.3957
Lauric	12:0	0.06±0.01	0.05±0.01	0.7374	0.07±0.01	0.06±0.01	0.06±0.01	0.5209	0.1829
Myristic	14:0	3.42±0.11	3.14±0.01	0.0630	3.33±0.11	3.37±0.15	3.14±0.13	0.4113	0.7867
Palmitic	16:0	27.66±0.31 ^a	26.68±0.26 ^b	0.0184	27.62±0.33	26.71±0.44	27.16±0.29	0.1911	0.7099
Margaric	17:0	1.01±0.04	0.98±0.03	0.4986	0.82±0.02 ^b	1.07±0.03 ^a	1.10±0.04 ^a	<0.0001	0.2797
Stearic	18:0	15.14±0.33	15.70±0.39	0.2498	16.35±0.49 ^a	15.17±0.45 ^{ab}	14.74±0.31 ^b	0.0274	0.4489
MUFA									
Myristoleic	14:1 <i>cis</i> -9	0.82±0.04 ^a	0.70±0.02 ^b	0.0218	0.70±0.04	0.82±0.06	0.75±0.03	0.1966	0.9933
Palmitoleic	16:1 <i>cis</i> -9	3.48±0.09 ^a	3.07±0.09 ^b	0.0026	3.08±0.14	3.42±0.14	3.32±0.06	0.0972	0.8434
Heptadecenoic	17:1	0.94±0.04	0.90±0.04	0.3351	0.71±0.02 ^b	1.00±0.04 ^a	1.06±0.04 ^a	<0.0001	0.9232
Oleic	18:1 <i>cis</i> -9	39.78±0.40	39.75±0.63	0.9586	38.21±0.60 ^b	40.57±0.69 ^a	40.52±0.52 ^a	0.0119	0.4660
Eicosenoic	20:1 <i>cis</i> -9	0.18±0.01	0.19±0.01	0.3972	0.16±0.01 ^b	0.19±0.01 ^{ab}	0.21±0.01 ^a	0.0111	0.1964

^{a,b,c} Means within a row that do not have a common superscript differ ($P < 0.05$).

^{1/}SFA = saturated fatty acids; MUFA = monounsaturated fatty acids.

^{2/}Cn = without crude glycerin; CGc = crude glycerin plus corn; CGsh = crude glycerin plus soybean hulls.

Differences were observed in meat from animals fed different CL ($P = 0.0442$) and FR ($P = 0.0246$) for the level of linoleic acid (18:2 *cis*-9, *cis*-12; Table 7). Lower level of linoleic acid was observed in meat from animals fed lower CL than meat from those fed greater CL. High concentrate diets are associated with lower ruminal pH, which decreases hydrogenase activity, producing less conversion of linoleic to stearic acid (Tove & Matrone, 1962). Glycerol addition to the diet linearly reduce the proportions of linoleic acid (Avila-Stagno et al., 2012), which justify the results of the current study where meat from animals fed diets without CG had the highest level of linoleic acid compared to meat from animals fed CG. These results are in accordance with Diaz et al. (2005) who found a negative correlation between linoleic and oleic acid. This is attributable to the inhibiting effect of linoleic acid on of $\Delta 9$ -desaturase, the enzyme responsible for oleic acid synthesis (JeffCoat & James, 1984).

Level of CLA (18:2 *cis*-9, *trans*-11) of meat was affected by the FR evaluated ($P = 0.0001$; Table 7). Meat from animals fed diets without CG had lower CLA content compared to meat from animals fed diets with CG and corn and soybean hulls. Supplemental glycerol inhibits rumen lipolysis, a prerequisite for rumen biohydrogenation, which is responsible for the saturation of dietary fatty acids consumed by ruminant animals (Edwards et al., 2012). According to Bauman et al. (Bauman, Baumgard, Corl, & Griinari, 1999), the CLA content depends on 2 processes: ruminal biohydrogenation and endogenous desaturation by the $\Delta 9$ -desaturase enzyme (intramuscular fat tissue). After ruminal production originating from linoleic acid (18:2 *cis*-9, *cis*-12) the CLA is biohydrogenated to vaccenic acid very quickly and the vaccenic acid (C 18:1 *trans*-11) produces stearic acid (C 18:0) in rumen in normal conditions by biohydrogenation process.

As presented in Table 7, the level of linoleic acid (18:2 *cis*-9, *cis*-12) in meat was lower in animals fed diets with CG which demonstrates a possible increase in ruminal production of CLA (18:2 *cis*-9, *trans*-11) originating from linoleic acid, being converted to vaccenic acid, absorbed in the small intestine and re-converted in CLA (18:2 *cis*-9, *trans*-11) by $\Delta 9$ -desaturase enzyme in the intramuscular fat tissue. The effect of CG in the biohydrogenation process can be observed by lower concentration of stearic ($P = 0.0274$) in the longissimus.

Table 7. Means and SEM for principal fatty acids polyunsaturated (%) found in the muscle Longissimus of young Nellore bulls fed crude glycerin

Fatty acid ¹	Lipid number	Concentrate level (CL)		P-value	Feeding regimes (FR) ²			P-value	CL x FR (P-value)
		60:40	40:60		Cn	CGc	CGsh		
PUFA									
Linoleic	18:2 <i>cis</i> -9, <i>cis</i> -12	3.05±0.23 ^b	3.84±0.32 ^a	0.0442	4.19±0.44 ^a	2.93±0.27 ^b	3.21±0.27 ^{ab}	0.0246	0.7352
α-Linolenic	18:3 n-3	0.28±0.02	0.33±0.03	0.1505	0.34±0.04	0.26±0.02	0.31±0.03	0.2450	0.6717
CLA	18:2 <i>cis</i> -9, <i>trans</i> -11	0.36±0.02	0.38±0.02	0.2593	0.30±0.01 ^b	0.43±0.02 ^a	0.38±0.02 ^a	0.0001	0.4373
Eicosatrienoic	20:3 n-6	0.15±0.01	0.19±0.02	0.1188	0.18±0.02	0.17±0.02	0.16±0.02	0.8151	0.7661
Arachidonic	20:4 n-6	0.53±0.07	0.73±0.11	0.1446	0.72±0.14	0.61±0.11	0.56±0.10	0.6237	0.9980
EPA	20:5 n-3	0.09±0.01	0.14±0.02	0.1454	0.13±0.03	0.12±0.02	0.10±0.02	0.6202	0.9634
DTA	22:4 n-6	0.06±0.01	0.07±0.01	0.2674	0.08±0.01	0.06±0.01	0.06±0.01	0.5733	0.7862
DHA	22:6 n-3	0.02±0.01	0.03±0.01	0.3004	0.02±0.01	0.03±0.01	0.02±0.01	0.8727	0.9603
Total SFA		47.85±0.53	47.09±0.56	0.3238	48.67±0.59	46.97±0.70	46.77±0.65	0.0887	0.5910
Total MUFA		47.51±0.46	47.06±0.71	0.5671	45.24±0.72 ^b	48.30±0.73 ^a	48.31±0.54 ^a	0.0022	0.5581
Total PUFA		4.65±0.35	5.85±0.51	0.0588	6.09±0.69	4.73±0.47	4.93±0.43	0.1663	0.9201
Total UFA		52.15±0.53	52.91±0.56	0.3238	51.33±0.59	53.03±0.70	53.23±0.65	0.0887	0.5910
UFA/SFA		1.10±0.02	1.13±0.02	0.3214	1.06±0.03	1.14±0.03	1.14±0.03	0.0972	0.6073
n-6/n-3		10.79±0.57	11.11±0.55	0.6806	11.99±0.64	10.33±0.72	10.52±0.65	0.1697	0.2540

^{a,b,c} Means within a row that do not have a common superscript differ ($P < 0.05$).

¹/SFA = saturated fatty acids; MUFA = monounsaturated fatty acids; PUFA = polyunsaturated fatty acids; UFA = unsaturated fatty acids.

²/Cn = without crude glycerin; CGc = crude glycerin plus corn; CGsh = crude glycerin plus soybean hulls.

It can be concluded that has lower conversion of vaccenic acid to stearic acid in the rumen from animals fed CG. The mechanism by which glycerol inhibits ruminal lipolysis have not been adequately established (Edwards et al., 2012). According to Abo El-Nor et al. (2010) the DNA concentration for *Clostridium proteoclasticum* and *Butyrivibrio fibrisolvens* decrease when glycerol replace corn in concentrate. Recent studies have demonstrated that these two bacteria play a fundamental and central role in the rumen biohydrogenation process (Wallace et al., 2006; Maia et al., 2007).

Although the beef from animals fed CG has an increase in the CLA content (18:2 *cis*-9, *cis*-12), these changes are very small in nutritional terms. Ip et al. (1994) estimated that a 70-kg human should consume 3.0 g of CLA/d to achieve maximum health benefits. Therefore, although there was an increase ($P = 0.0001$) in the CLA content of beef from animals fed CG, this difference (3 g/kg in beef from animals fed control diet to about 4 g/kg in beef from animals fed CG diets) will not be enough to promote a greater differences in benefits to human health.

Meat from animals fed diets without CG had lower values of monounsaturated fatty acids (MUFA; $P = 0.0022$) than meat from animals fed diets with CG and corn and soybean hulls, which corroborates those reported by (Krueger et al., 2010). No differences were detected for SFA ($P = 0.0887$), unsaturated fatty acids (UFA; $P = 0.0887$), UFA/SFA rate ($P = 0.0972$) and n6/n3 ratio ($P = 0.1697$) among feeding regimes.

No effects of CL ($P = 0.4464$) and FR ($P = 0.1868$) were observed for levels of mg malonaldehyde for kg of meat (MDA; Table 5). The UFA's are directly related to the development of unfavorable odors resulting from their oxidation, which contributes to a drastic reduction in their acceptance and, consequently, the product shelf life (Oliveira, et al., 2011). One of the products resulting from this process is malonaldehyde, which is measured by the TBA reaction and is used to estimate the development of rancidity in meat products (Pearson, 1994). The inclusion of CG in the diets does not increase the UFA content in meat ($P = 0.0887$), therefore it was not enough to promote changes in values of MDA ($P = 0.1828$), which would promote the rancidification of meat. Nonetheless, Ladeira et al. (2012b) reported a tendency to increase TBARS value in meat when cattle are fed diets with levels up to 18% of CG on diet DM.

4. CONCLUSIONS

The levels of concentrate evaluated in the present study did not affect carcass and meat quality traits, but animals fed high concentrate diets had subcutaneous fat color with less yellow intensity. Feeding with high concentrate levels promote better fatty acid profile, decreasing the palmitic acid and increasing linoleic acid content in beef. The inclusion of crude glycerin as a substitute of corn or soybean hulls with levels up to 10% of DM does not affect the carcass or meat quality traits, however, increases the monounsaturated acids content and conjugated linoleic acid in beef, although these changes are very small and insignificant in nutritional terms.

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APÉNDICE

APÊNDICE A – IMPLICAÇÕES DO USO DA GLICERINA BRUTA NA DIETA DE RUMINANTES

“Este texto foi preparado para relatar a minha experiência com a condução de pesquisas que utilizam glicerina bruta na dieta de ruminantes”

A glicerina bruta tem sido um ingrediente energético avaliado constantemente nas pesquisas brasileiras, sendo que na maioria dos trabalhos realizados, substitui o milho presente no concentrado das dietas de ruminantes. Há trabalhos sendo conduzidos avaliando seu uso em dietas de bovinos de corte, ovinos, caprinos e bovinos de leite.

No Brasil tem ocorrido grande variação na composição deste produto, encontrando-se glicerinas brutas com 40 ou 80% de glicerol. Isto ocorre devido ao processo de extração do óleo e sua transformação em biodiesel ainda não ser padronizado entre as empresas produtoras. Portanto, a glicerina bruta que contém 40% de glicerol possui algumas impurezas como metanol ou etanol (utilizado no processo de produção do biodiesel) e os ácidos graxos não convertidos em biodiesel. Esta glicerina com alto teor de ácidos graxos (acima de 40%), apesar de possuir baixo preço no mercado, tem limitações de inclusão na dieta dos ruminantes devido ao fato de aumentar o teor de extrato etéreo da dieta. Tem-se concluído que o nível de extrato etéreo na dieta de ruminantes não deve exceder 7% na matéria seca para não reduzir o consumo e consequentemente o crescimento dos animais e, portanto, foi constatado em dietas de ovinos de corte terminados em confinamento, que este co produto deve ser incluído em até 3% na MS da dieta (Lage et al., 2014).

Com relação a glicerina bruta que contém aproximadamente 80% de glicerol, a recomendação bovinos terminados em confinamento está em aproximadamente 10% da MS da dieta. Em condição de pasto, estudos conduzidos na Unesp / Jaboticabal tem nos mostrado que esta pode ser incluída em 28% na MS (concentração máxima avaliada) do suplemento (recria e terminação de novilhos Nelore não castrados), em ambos os casos substituindo o milho do concentrado. Nestas concentrações a glicerina bruta era adicionada ao concentrado através do uso de um misturador adquirido para este fim.

O equipamento possui um recipiente externo no qual se colocava a glicerina bruta que aos poucos era misturada automaticamente aos demais ingredientes do concentrado. O equipamento possui capacidade máxima para misturar 500 kg de concentrado, entretanto, devido ao fato da mistura com glicerina bruta ficar mais densa dentro do equipamento, fizemos alguns testes e decidimos por misturar 300 kg de concentrado por vez. Após finalizar a mistura, os concentrados eram armazenados em sacos reaproveitados (próprios para concentrado) e dessa forma, ficavam estocados por aproximadamente 3 dias. Portanto a cada semana, fazia-se duas ou três misturas de ração (experimento com 60 animais confinados).

Neste experimento a glicerina bruta foi associada ao milho ou casca de soja em 10% na MS da dieta. Os concentrados nos quais a glicerina bruta foi associada com o milho não apresentaram problemas durante a mistura. Entretanto, os concentrados nos quais a glicerina bruta substituiu a casca de soja apresentou problemas nos primeiros testes realizados. O concentrado utilizado neste experimento foi formulado na universidade e portanto, os ingredientes foram adicionados separadamente a mistura, ou seja, não era uma mistura completa na qual se adicionou apenas a glicerina bruta.

A casca de soja normalmente é produzida e transportada na forma de “pellets” o que dificultou a mistura dos ingredientes do concentrado na qual a glicerina bruta foi associada a casca de soja, que substituiu todo o milho do concentrado. Portanto, a alternativa foi moer primeiramente a casca de soja (processamento realizado na fábrica de ração) e após a moagem, a casca foi adicionada aos demais ingredientes do concentrado e realizado o procedimento de mistura com a glicerina bruta.

Um fato importante a se destacar é que quando a mistura estava pronta, os concentrados se encontravam farelados e adequados para serem colocados nos sacos e armazenados. Após os dois ou três dias de armazenagem (tempo que demorava para ser consumido pelos animais), os concentrados que continham glicerina bruta formavam blocos no formato do saco, o que dificultava a sua remoção e pesagem individual de acordo com o consumo dos animais. Portanto, antes de manusear cada saco para pesar a quantidade necessária, era preciso desintegrar o bloco de concentrado formado dentro de cada saco, que voltava novamente ao seu estado totalmente farelado. Ainda não foram constatadas pesquisas que avaliassem o tempo

de armazenagem de um concentrado com glicerina bruta, uma vez que dependendo do tipo de glicerina utilizada, pode haver grande concentração de extrato etéreo e ocorrer rancidez do produto, ou mesmo proliferação de fungos devido ao fato do concentrado ficar mais úmido no momento da retirada do misturador e armazenado.

Na pesquisa que foi conduzida na Universidade Federal de Viçosa (dissertação de mestrado) com a glicerina bruta que continha alto teor de extrato etéreo, esta era misturada manualmente e diariamente de acordo com o consumo de cada animal, portanto, a ração não foi armazenada com a glicerina bruta misturada aos demais ingredientes, entretanto, este trabalho foi conduzido com ovinos de corte. Quando se trabalha com bovinos de corte, torna-se mais trabalhoso em uma pesquisa na universidade, pesar e misturar diariamente a glicerina bruta no cocho, uma vez que a maioria dos serviços são manuais e devido ao fato da glicerina bruta ser um produto líquido. Portanto, quando se trabalha com bovinos fistulados (experimento com baixo número de animais) a técnica de mistura da glicerina bruta manualmente aos demais ingredientes é possível de ser realizada com menor dificuldade, mas quando se trata de animais submetidos a avaliação do crescimento, como no caso deste experimento com 60 animais, torna-se inviável e demorado o manejo das dietas no confinamento.

No caso de experimentos em pasto, onde o suplemento é fornecido para um conjunto de animais em um cocho único, esta prática de mistura manual pode ser feita com mais facilidade em relação ao confinamento, mas tem-se a dificuldade de transporte do ingrediente até o cocho. Além de ser uma prática mais trabalhosa, uma outra desvantagem de pesquisas realizadas com a glicerina bruta misturada manualmente a dieta é o fato de que a adição deste ingrediente pode não promover uma mistura homogênea e ocorrer seleção dos ingredientes pelos animais, principalmente em dietas para bovinos de corte, onde se usa alto teor de concentrado, tornando mais difícil a mistura manual.

Um outro ponto a se destacar é a mistura de glicerina bruta com outros ingredientes que também são líquidos como por exemplo, o óleo de soja. Foi conduzido na Unesp / Jaboticabal, um experimento em confinamento com machos não castrados Nelore, avaliando a inclusão de 10% de glicerina bruta na MS da dieta associada com fontes de óleo, sendo uma das fontes líquidas, o óleo de soja. O concentrado foi

utilizado em 70% na MS da dieta. A mistura dos ingredientes do concentrado foi realizado no mesmo misturador (que contém um recipiente) e a glicerina bruta foi adicionada a este recipiente juntamente com o óleo de soja para ser misturada aos demais ingredientes farelados do concentrado (milho moído, farelo de soja, uréia e suplemento mineral). Com o uso do misturador garantiu-se uma mistura homogênea e a ração foi armazenada por dois a três dias, apresentando os mesmos problemas de formar “blocos” dentro dos sacos e procedia-se da mesma forma como nos demais experimentos para desintegrar os “blocos” e pesar os concentrados individualmente para cada animal.

Com relação ao transporte, a glicerina bruta é transportada em bombonas plásticas (250 L) ou pequenos tanques (1.000 L) contendo uma torneira própria. O transporte em bombonas e manuseio deste recipiente se torna trabalhoso na universidade, uma vez que para descarregar foi necessário utilizar máquina, devido ao seu elevado peso. A retirada da glicerina bruta de dentro desta embalagem era muito trabalhosa, uma vez esta possui apenas um orifício em cima para saída do líquido. Portanto, era necessário três pessoas e uso de cordas para amarrar, tombar a embalagem e retirar o líquido. No caso dos pequenos tanques que foram utilizados em pesquisas posteriores, facilitou muito o manuseio do material uma vez que já possui uma torneira própria para saída líquido.

O uso de glicerina em fazendas pode ser realizado através de vagões misturadores da dieta completa. A realização de mistura manual em fazendas é inviável devido ao fato de ser um ingrediente líquido, dificultando o manuseio e uma mistura eficaz da dieta.

APÊNDICE B – TABELA DE COMPOSIÇÃO DOS INGREDIENTES DA DIETA

Tabela 1. Composição dos ingredientes utilizados nas dietas

Item	Silagem de milho	Milho	Farelo de soja	Casca de soja
Composição química, % MS				
Matéria seca	38,00	85,40	86,60	89,70
Proteína bruta	9,02	7,00	52,05	12,20
Fibra em detergente neutro	40,32	15,55	18,60	71,20
Extrato etéreo	2,89	2,28	2,41	1,11
Matéria Mineral	4,45	1,31	8,51	4,01