

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

**DIFFERENT SOURCES OF FORAGE WITH CRUDE
GLYCERIN IN DIETS WITH HIGHER PERCENTAGE OF
CONCENTRATE TO BEEF CATTLE**

**Andressa Ferreira Ribeiro
Médica Veterinária**

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TÍTULO: DIFFERENT SOURCES OF FORAGE WITH CRUDE GLYCERIN IN DIETS WITH HIGHER PERCENTAGE OF CONCENTRATE TO BEEF CATTLE

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“Todo o conhecimento humano começou com intuições, passou daí aos conceitos e terminou com ideias.”

Immanuel Kant

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Aos meus pais e a minha irmã pelo amor incondicional.

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(Churton Collins)

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DIFFERENT SOURCES OF FORAGE WITH CRUDE GLYCERIN IN DIETS WITH HIGHER PERCENTAGE OF CONCENTRATE TO BEEF CATTLE

ABSTRACT - This trial aimed to evaluate the effects of feeding different sources of forage in diets with crude glycerin included in 10% of DM diet, on intake, digestibility, ruminal fermentation and microbiology, performance, methane emission, carcass and meat quality traits of Nellore cattle finished in feedlot. The treatments consisting in different sources of forage: Corn silage (CS), Sugar cane (SC) and Sugar cane bagasse (SB), in diets with crude glycerin (10% DM) with higher concentrate proportion. For such study two experiments were conducted: Experiment 1: Nine ruminally cannulated Nellore steers ($300.0 \pm 30\text{kg}$ and 20 ± 2 months of age) were used in a 3×3 Latin Square experimental design with three treatments and three animals in three simultaneous triplicates. There was no effect of different sources of forage with crude glycerin on dry matter (DM), organic matter (OM) and crude protein (CP) intake. The intake of neutral detergent fiber (NDF) increased ($P < 0.05$) in animals fed CS that did not differ from animal fed with SB. Furthermore, animals fed with SB decreased non-fibrous carbohydrate (NFC) intake ($P < 0.05$). Animals fed with CS increased digestibility of DM and NDF compared with the other diets ($P < 0.05$). The NFC digestibility increased in animals fed with SC ($P < 0.05$). Animals fed with SB increased pH values which not differed from animals fed with SC ($P < 0.05$). Animals fed with SC decreased $\text{NH}_3\text{-N}$ values ($P < 0.05$). The protozoa population was not influenced by the different sources of forage in diets with crude glycerin, except *Dasytricha* and *Isotricha*. The population of fibrolytic bacteria (*Ruminococcus flavefaciens*, *Ruminococcus albus* and *Fibrobacter succinogenes*) were similar ($P > 0.05$) among diets. On the other hand, the population of *Selenomonas ruminantium*, was higher ($P < 0.01$) in animals fed with CS. Experiment 2: There were used 40 Nellore young bulls, however, 10 animals ($396.6 \pm 34.7\text{ kg}$ 24 ± 2 months of age) were slaughtered as reference animals in the beginning of the experiment. The remaining 30 animals were randomly assigned to three treatments with 10 replicates during 84 day to evaluate intake, performance, blood parameters, enteric methane emissions, carcass and meat quality traits. The intake of DM, OM, CP, NDF, gross energy and metabolizable energy, average daily gain (ADG), longissimus muscle area (LMA), rib fat thickness (RTF) and blood parameters were similar ($P > 0.05$) among the diets. Enteric methane emission, were not affected by different sources of forage in diets with crude glycerin ($P > 0.05$). No effects of different sources of forage in diets with crude glycerin were observed ($P > 0.05$) on carcass traits and *longissimus* fatty acid profile. The yellow index was greater ($P = 0.03$) in fat of animals fed with CS. The heptadecenoic fatty acid was lower in the meat of animals fed with SB ($P = 0.04$). These results suggests that alternatives forages to CS such as SC and SB included in a similar fNDF level in diets with crude glycerin changed the NDF intake and ruminal parameters, however, there were no impact on animal performance, enteric methane emission and meat quality.

Key words: bacteria, cattle, digestibility, greenhouse gases, silage, sugar cane

DIFERENTES FONTES DE FORRAGEM COM GLICERINA BRUTA EM DIETAS COM ALTA PORCENTAGEM DE CONCENTRATO PARA BOVINOS DE CORTE

RESUMO - Este trabalho teve como objetivo avaliar os efeitos da alimentação com diferentes fontes de forragem em dietas com 10% glicerina bruta incluída na MS sobre o consumo, digestibilidade, fermentação ruminal, desempenho, características de carcaça, qualidade da carne e emissão de metano entérico de bovinos Nelore terminados em confinamento. Os tratamentos consistiam em diferentes fontes de forragem (FDN da forragem fixado em 15%): Silagem de milho (SM), Cana de açúcar (CA) e Bagaço de cana (BC) em dietas com glicerina bruta (10% MS). Para tal estudo foram conduzidos dois experimentos: Experimento 1: Nove novilhos Nelore canulados no rúmen (300 ± 30 kg e 18 ± 2 meses de idade) foram utilizados em delineamento de quadrado latino (3×3), três tratamentos e três períodos em três repetições simultâneas para avaliar o consumo, digestibilidade, fermentação e microbiota ruminal. Não houve efeitos das diferentes fontes de forragem em dietas com glicerina bruta no consumo de matéria seca (MS), matéria orgânica (MO) e proteína bruta (PB) ($P > 0.05$). O consumo de fibra em detergente neutro (FDN) foi maior em animais alimentados com SM que não diferiu de animais alimentados com BC ($P < 0.05$). Além disso, animais alimentados com BC apresentaram menor consumo de carboidratos não fibrosos (CNF; $P < 0.01$). Animais alimentados com SM apresentaram maior digestibilidade da matéria seca MS e FDN comparada com as outras dietas. Animais alimentados com CA apresentaram maior digestibilidade de CNF ($P < 0.05$). Adicionalmente, os animais alimentados com BC apresentaram maior digestibilidade da PB e não diferiram dos animais alimentados com silagem de milho. Animais alimentados com BC apresentaram os maiores valores de pH que não diferiu de animais alimentados com CA. Além disso, os animais alimentados com CA apresentaram os menores valores de nitrogênio amoniacal ($\text{NH}_3\text{-N}$). A população de protozoários foi similar entre os tratamentos exceto as populações de *Dasytricha* e *Isotricha*. A população de bactérias fibrolíticas (*Ruminococcus flavefaciens*, *Ruminococcus albus* e *Fibrobacter succinogenes*) foi similar ($P > 0.05$) entre as dietas. Entretanto, a população de *Selenomonas ruminantium* foi maior ($P < 0.01$) em animais alimentados com SM. Experimento 2: Foram utilizados 40 novilhos Nelore, entretanto, 10 animais (396.6 ± 34.7 kg 20 ± 2 meses de idade) foram abatidos como animais referencia no começo do experimento. Os 30 animais restantes (416.70 ± 24.74 kg) foram aleatoriamente distribuídos em um dos três tratamentos com 10 repetições cada durante 84 dias para avaliar o consumo, desempenho, parâmetros sanguíneos, emissão de metano, características de carcaça e qualidade de carne. O consumo de MS, MO, PB, FDN, energia bruta, energia metabolizável e ganho de peso diário (GD) foi similar entre as dietas ($P > 0.05$). Não houve diferença na área de olho de lombo (AOL), espessura de gordura subcutânea (EGS) e parâmetros sanguíneos ($P > 0.05$). A emissão de metano entérico não foi afetada pelas diferentes fontes de forragem em dietas com glicerina bruta. As características de carcaça foram similares entre os animais alimentados com diferentes fontes de forragem em dietas com glicerina bruta. Não houve efeito das diferentes fontes de forragem em diets com glicerina bruta sobre a qualidade da carne e o perfil de ácido graxo do

músculo *longissimus* ($P>0.05$). O índice de amarelo foi maior ($P=0,03$) na gordura de animais alimentados com SM. O ácido graxo heptadecenóico foi menor em animais alimentados com BC ($P=0,04$). Estes resultados sugerem que forragens alternativas a SM como a CA e o BC inclusos no mesmo nível de fNDF com glicerina bruta alteram o consumo de FDN e parâmetros ruminais, porém, não causam impacto no desempenho, emissão de metano e qualidade da carne dos animais.

Palavras chave: bactérias, bovinos, cana de açúcar, digestibilidade, gases de efeito estufa, silagem de milho

CHAPTER 1 - GENERAL CONSIDERATIONS

The current world population of 7.2 billion is projected to increase by 1 billion over the next 12 years and reach 9.6 billion by 2050 (UN, 2013) consequently world meat production is projected to double by 2050, most of which is expected in developing countries, such as Brazil (FAO, 2011). Beef is considered as an essential ingredient for human diet, due to highly nutritious and valued food (SCOLLAN et al., 2006). However, the fatty acid profile of beef has been criticized, mainly due to the high ratio of saturated to polyunsaturated fatty acids, which is a risk factor for the development of vascular and coronary diseases (BARTOŇ et al., 2007). In addition total greenhouse gases (GHG) emissions from livestock production systems and agriculture will increase as world population and food demands increase (O'MARA, 2011). Therefore, the current challenge for livestock sector in Brazil is develop nutritional strategies to increase production and economic efficiency, improve meat quality with lower environmental impact.

In this sense, feedlot has become the alternative for Brazilian farmers to increase efficiency gain, decrease age at slaughter and decrease environmental impact of livestock system, however, feed costs are high due to the grain prices. According to Oliveira and Millen et al. (2014), approximately 11.72% or 3.377 millions of slaughtered cattle were finished in feedlot in Brazil. Due to the use of this production strategy diets with higher percentages of concentrate have been used, which may affect ruminal fermentation and microbial population.

According to Zebeli, Metzler-Zebeli and Ametaj (2012), feeding cattle more than 44.1% of concentrate could lead to high concentration of volatile fatty acids

(VFAs) and decreases ruminal pH due to the rapid degradation of non-fibrous carbohydrates (NFC). The low ruminal pH (<6.0) may impact on the activity of fibrolytic bacteria such as *Ruminococcus albus* and *Ruminococcus flavefaciens* and *Fibrobacter succinogenes*, which could decrease diet digestibility and feed intake (OWENS et al., 1998; STOCK et al., 1995). Although, there is no agreement about the thresholds of ruminal pH which indicate ruminal upset, one indicative generally accepted of ruminal disorders is pH depressed below 5.6 to 5.8 or <6.0 during 96.5 min/day (LECHARTIER; PEYRAUD, 2010; ZEBELI; METZLER-ZEBELI; AMETAJ, 2012). Additionally, animals fed with higher percentages of concentrate have lower ability to buffer the rumen by inadequate salivary secretion which contributes to lower pH. However, this situation may be minimized by the inclusion of forage in these diets.

According to Galyean and Deffoor (2003) the inclusion of NDF supplied by forage in diets with higher percentage of concentrate might be expected more stable ruminal pH compared with low-fiber diet. The positive relationship between pH and dietary fiber content can be explained by the positive effect of fiber on stimulating chewing activity (TAF AJ et al., 2005), which in turn would result in an increased saliva output, whose bicarbonate neutralizes the VFAs and consequently increases the pH (ERDMAN, 1988). According to Tafaj et al. (2007), forage fiber content expressed as forage NDF (fNDF) seems to be the main governing factor of chewing and ruminating when corn silage was fed. Furthermore, when forage sources are substituted into the diet on an equal NDF basis, differences among forages in feeding value became small, or non-significant, not affecting intake. Galyean and Deffoor (2003) further suggested that to equalize DMI, forages in finishing diets could be

exchanged on an equivalent NDF level. Ware and Zinn (2004) in a study with different sources of forage and similar forage NDF level did not observed different dry matter intake, performance and gain efficiency in animals finished in feedlot. According to the recommendations of Goulart and Nussio (2011), 15% of fNDF ensures the minimum requirements of rumen health and maximizes feed efficiency in *Bos Indicus* which contrast with the North American typical formulations ranging from 6 to 9% of fNDF for *Bos Taurus* (Zinn and Ware, 2007).

There is wide variety of forages used in feedlots in Brazil but the main sources of forage used in feedlot in Brazil are corn silage, sorghum silage, sugar cane bagasse and sugar cane (OLIVEIRA and MILLEN, 2014). These sources of forage vary according to many factors, including nutritional value, cost and availability. Corn silage has high nutritional value and ability to be stored for long periods of time while maintaining a good nutritional value and palatability (SEGERS et al., 2013). Moreover, corn silage has high digestibility and cell content (starch, non-fibrous carbohydrate) which provide high ruminal fermentation and metabolic products but the high cost of production seen as a negative factor. The increase in global demand for corn in animal feed and ethanol production has increased the price of corn silage (ROTTA et al., 2014). Thus, other forage sources such as sugar cane and sugar cane bagasse from ethanol biofuel plants are used in feedlots in order to increase the efficiency of this production system.

Sugar cane present characteristics such as high saccharose (non-fibrous carbohydrate), moderate level of insoluble neutral detergent fiber (NDFi), high dry matter production per unit area and increased digestibility with increasing maturity, maintaining its nutritional value long period of time after maturity (CREEK; SQUIRE,

1976; PATE; FAIRHURST; MUNTHALI, 1985; LASCANO et al., 2012). However, sugar cane as a main feed for ruminants, has nutritional limitations related to low levels of protein, minerals and high content of fiber with low ruminal degradation (LENG, 1993; QUEIROZ et al., 2012).

Sugar cane bagasse a by-product of the milling of sugar cane has been used as a source of fiber in cattle feed. According to Henrique et al. (2007), this by-product is a feedstuff rich in cell wall constituents contains low cell content and has low digestibility and low density. In diets with high concentrate levels, is safer to use a minimum content of fiber, able to stimulate chewing and allow adequate ruminal environment not harming animal performance. Some studies indicate that the use of small amount of sugar cane bagasse as a source of fiber in diets for beef cattle can prevent metabolic problems without prejudice performance when high concentrate diets are used (DE MEDEIROS BULLE et al., 2002). However, sugar cane bagasse have been used to generate electric energy in biofuel plants, which may difficult the supply of this fiber source to farmers and increase the its price.

Another alternative which has been common in feedlot is the utilization of agro industrial byproducts which is an economic and ecological alternative to replace ingredients in ruminant diets. Crude glycerin, a byproduct from biodiesel agroindustry, which has energetic value similar than corn (DEFRAIN et al., 2004), have been used to replace corn in ruminant diets up to 10% of diet DM without compromise intake and performance (EIRAS et al., 2014). According to Pyatt, Doane and Cecava (2007), the inclusion of 10% of crude glycerin in diets replacing corn improved the feed efficiency by 19.2% compared to diets without crude glycerin. Moreover, Shin et al. (2012) suggests that crude glycerin have a faster fermentation

rates compared to starch. Furthermore, the inclusion of this byproduct has been reported to decrease the acetate:propionate ratio in the rumen which considering the inverse relationship between CH_4 and propionate production can reduce methane emissions by ruminants. Although, there is a lack of information about the use of crude glycerin in high concentrate diets with low quality forages this byproduct seems to be a good alternative as a feedstuff.

Feedlot besides increasing efficiency of livestock system decreases enteric methane emissions (BERNDT; TOMKINS, 2013). High concentrate diets used in feedlot replace fibrous carbohydrates from forages (cellulose, hemicellulose) by non-fibrous carbohydrates (starch and sugars) contained in concentrates most energy-rich. Diets with high percentage of concentrate is associated with increases in feed intake, higher rates of ruminal fermentation and accelerated feed turnover, which results in large modifications of rumen physico-chemical conditions and microbial populations (PETRI et al., 2012). A shift of VFA production from acetate towards propionate occurs with the development of starch-fermenting microbes, such as *Selenomonas ruminantium*. This results in a lower CH_4 production because the relative proportion of ruminal hydrogen sources declines whereas that of hydrogen sinks increases.

According to Knapp et al. (2014) CH_4 emissions can be affected by the level of feed intake, type of carbohydrate, forage quality and species. In this context, it has been widely accepted that of all nutrients, fibrous carbohydrates have the highest methane-producing potential (MARTIN; MORGAVI; DOREAU, 2010). Additionally, when nonstructural carbohydrates are evaluated, saccharose (present in sugar cane) has higher methanogenic potential than starch (present in corn silage;

HINDRICHSEN; KREUZER, 2009) due to the butyrate formation which is the major product from sugar cane fermentation that provides hydrogen for methanogens (HINDRICHSEN et al., 2004).

Additionally, forages have been the major and also cheapest source of fatty acids in ruminant diets (KALAČ, 2011). Among available forage options in Brazil, sugar cane has high amount of n-3 PUFAs compared with corn silage (FERNANDES et al., 2009). The beneficial effects of the longer chain n-3 PUFAs, in reducing the risk of cardiovascular disease, cancer and type-2 diabetes, and their critical roles for proper brain function, visual development in the foetus and for maintenance of neural and visual tissues throughout life are well recognized (BARCELÓ-COBLIJN; MURPHY, 2009; LOPEZ-HUERTAS, 2010). Moreover, the polyunsaturated (PUFA) and monounsaturated fatty acids (MUFA) found in beef are generally regarded as beneficial for human health (Schollan et al., 2006). Among the PUFAs, linoleic (C18:2 c9c12) and α -linolenic (C18:3 n3) fatty acids are considered the most important because they are not synthesized by the organism and they are the principal precursors of conjugated linoleic acid (CLA) (Oliveira et al., 2011). The dominant CLA in beef is the cis-9, trans- 11 isomer, which has being identified as possessing a range of health promoting biological properties including antitumoral and anticarcinogenic activities (De la Torre & Debiton et al., 2006). In this sense strategies to enrich nutritional values of meat with functional components that play important roles in health maintenance and disease prevention are highly desired.

Thus, the objectives of this study were to evaluate corn silage, sugar cane and sugar cane bagasse in diets with crude glycerin on the intake, performance, ruminal fermentation and meat quality from Nellore young bulls finished in feedlot.

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CHAPTER 2

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

DIFFERENT SOURCES OF FORAGE IN DIETS WITH CRUDE GLYCERIN INFLUENCE FERMENTATION PARAMETERS AND RUMINAL MICROBIOTA OF NELLORE FEEDLOT STEERS

ABSTRACT

This study investigated the effects of different sources of forage in diets with crude glycerin on intake, digestibility, rumen fermentation and rumen microbiota of Nellore feedlot steers. Nine ruminally cannulated Nellore steers ($300.0 \pm 30\text{kg}$ and 18 ± 2 months of age) were used in a 3x3 Latin Square experimental design with three treatments and three animals in three simultaneous triplicates. The treatments were different sources of forage (fixed 15% of NDF from forage; fNDF): corn silage (CS), sugar cane (SC) and sugar cane bagasse (SB), in diets with 10% (DM) of crude glycerin. The intake of NDF was higher ($P<0.05$) in animals fed corn silage that did not differ from animal fed with sugar cane bagasse. Furthermore, animals fed with sugar cane bagasse presented lower NFC intake ($P<0.05$). Animals fed with corn silage showed higher digestibility of DM and NDF compared with the other diets ($P<0.05$). Additionally, the higher CP digestibility was observed in animals feed with sugar cane bagasse, which not differed from animals fed with corn silage ($P<0.05$). NFC digestibility was higher in animals fed with sugar cane ($P<0.05$). Animals fed with sugar cane bagasse showed higher values of pH which not differed from animals fed with sugar cane ($P<0.05$). Animals fed with sugar cane presented lower values of $\text{NH}_3\text{-N}$ ($P<0.05$). The protozoa population was not influenced by the different sources of forage in diets with crude glycerin, except *Dasytricha* and *Isotricha*. The population of fibrolytic bacteria (*Ruminococcus flavefaciens*, *Ruminococcus albus* and *Fibrobacter succinogenes*) were similar ($P>0.05$) among diets. On the other hand,

the population of *Selenomonas ruminantium*, was higher ($P < 0.01$) in animals fed with corn silage. Sugar cane and sugar cane bagasse included in 15% of fNDF in diets with crude glycerin (10% DM) altered ruminal parameters, however, maintained adequate conditions for animal performance.

Keywords: bacteria, byproducts, NDF, protozoa, silage, sugar cane

1. INTRODUCTION

On condition that afford high-producing to beef cattle the use of diets containing higher percentages of concentrate can ensure appropriate energy levels and result in greater efficiency (Zebeli et al., 2012). Conversely, the rapidly degradation of non-fibrous carbohydrates (NFC; concentrate) lead to high concentration of volatile fatty acids (VFAs) causing low pH. In spite of fact, there is no consensus about thresholds of pH that may cause ruminal upset, one indicative generally accepted of ruminal disorders is pH depressed bellow 5.6 to 5.8 (Lechartier & Peyraud, 2010) which could shift microbiome (Hook et al., 2011; Saleem et al., 2012) .

The healthy rumen function has been recognized by adequate structure of fiber content in high concentrate diets which stimulate formation of particle mat and chewing activity, hence increasing salivary output (Tafaj et al., 2006). The effectiveness of forage in stimulating chewing activity, rumen fermentation and ruminal retention time and, therefore, ruminal microbiota vary with different botanical origin, processing, particle size, and NDF content (Owens et al., 1998). According to Tafaj et al. (2007), forage fiber content expressed as forage NDF (fNDF) seems to be the main governing factor of chewing and ruminating when corn silage was fed.

However, there was a lack of information about ruminal fermentation and microbiota of low quality forages such as, sugar cane and sugar cane bagasse, which would be used as an alternative to corn silage (high nutritional value forage) in feedlots in Brazil (Oliveira & Millen, 2014). Thus, evaluate forages with different quality in similar fNDF inclusion could increase feedlot forage options.

However, due to the unstable prices of grains researches have been searching for alternatives feedstuff for feedlot diets. In this sense, crude glycerin (or glycerol) a byproduct from biodiesel have been used as energy source to replace corn in ruminant diets up to 10% of diet dry matter (Lage et al., 2013, Silva et al. 2013) without compromise intake and performance (Eiras et al., 2014). 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). In addition crude glycerin may have a faster fermentation rates compared to starch (Shin et al., 2012). Furthermore, 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 NDF digestibility (Ramos and Kerley, 2012). However, there is a lack of information about the use of low quality forages in diets with 10% of crude glycerin.

So, we hypothesized that the inclusion of low quality forages associated with crude glycerin may be used in diets with high percentage of concentrate to modulate ruminal fermentation of Nellore feedlot steers. Thus, the present study aimed to evaluate corn silage, sugar cane and sugar cane bagasse in diets with crude glycerin

on intake, digestibility, rumen fermentation, and rumen microbiota of Nellore feedlot steers.

2. MATERIAL AND METHODS

Animals and management

The trial was carried out at the Faculdade de Ciências Agrárias e Veterinárias, UNESP – Univ Estadual Paulista, Jaboticabal, following the humane animal care and handling procedures, according to the UNESP guidelines. All experimental procedures were approved by the Commission on Ethics and Animal Welfare (CEBEA) of the College of Agrarian and Veterinary Sciences (process nº 021118/11).

Six months prior to beginning the experiment, nine Nellore steers were housed in individual pens and prepared for fistulation surgery (12 ± 2 months of age and 170kg of live weight). These animals were feed with corn silage and concentrate (F:C ratio 60:40; CP: 14.30%, NDF 30.14%; concentrate composition: ground corn, soybean meal, urea, mineral supplement) and kept in this management until the beginning of the experiment.

Nine ruminally cannulated Nellore steers (300.0 ± 30 kg of body weight and 18 ± 2 months of age) fitted with ruminal 4" of diameter silicone- type cannulas were used in a triple latin square design 3x3 to evaluate dietary intake, apparent total tract digestibility, ruminal pH, ammonia-N (N-NH_3) concentration, and ruminal microbiology of Nellore steers finished in feedlot. The steers were treated for internal and external parasites at the beginning of the experiment and kept in individual pens of approximately 21m^2 for adaptation and sampling period, with protected feeders and water. Initially, animals were weighed, identified, and housed in individual pen with

feeders and drinkers. The animals spent 21 days adapting to the facilities and management. After animals adaptation three consecutive 19-day periods were used. Each study period consisted of 14 days for adaptation, 4 days for DMI recording and feces collection and 1 day for ruminal fluid sampling. The latter was collected for measurement of ruminal pH, and ammonia N ($\text{NH}_3\text{-N}$), as well as bacteria and protozoa quantification.

Animals were fed with one of the following experimental diets: three sources of forage were used: corn silage (CS), sugar cane (SC) and sugar cane bagasse (SB, Table 1).

Table 1. Chemical composition of corn silage (CS), sugar cane (SC) and sugar cane bagasse (SB)

	Forage		
	CS	SC	SB
Chemical Composition, % DM			
Dry Matter	31.08	29.32	63.03
Organic Matter	83.16	90.46	83.16
Crude Protein	8.68	3.03	3.32
Neutral Detergent Fiber	54.40	55.50	86.50
Indigestible Neutral Detergent Fiber	16.99	25.73	54.19
Ether Extract	2.38	0.42	0.40
Gross Energy, MJ	17.99	17.53	17.20

The neutral detergent fiber from forage (fNDF) was fixed at 15% of DM to ensure ruminal fiber requirements avoiding ruminal disturbs. Crude glycerin was included on 10% of DM, replacing corn in the concentrate (Table 2).

Corn silage used in the present study was obtained from UNESP farm. A corn hybrid (whole plant) (hybrid 2B688Hx - Dow AgroSciences) was harvested at about 31% DM and chopped at 5cm (50mm). The chopped corn material was placed in a

trench- type silo, covered with black plastic and ensiled for at least 2 months. The sugar cane was obtained from a local farm. Sugar cane was chopped in particles 2–3cm (20mm). Sugar cane bagasse in particles 3–4cm (30mm) was obtained from a private biofuel plant. The forage particle sizes were different due to their precedence. Crude glycerin was acquired from soybean oil-based biodiesel production company ADM, Rondonópolis, Brazil (80.34% glycerol; 1.59% ether extract; 5.03% ash, and 12.02% water).

Table 2. Ingredients proportion and chemical composition of the experimental diets (DM basis)

	Diet		
	CS	SC	SB
Ingredient proportion,% DM			
Corn Silage	28.8		
Sugar Cane		27.5	
Sugar Cane Bagasse			17.3
Corn	35.7	33.7	45.1
Soybean Meal	22.5	25.8	24.6
Crude Glycerin	10.0	10.0	10.0
Mineral Supplement [†]	3.00	3.00	3.00
Chemical Composition, % DM			
Dry Matter	70.97	73.66	84.29
Organic Matter	90.95	91.80	92.00
Crude Protein	18.13	17.86	17.89
NDF [‡]	31.69	30.70	33.27
fNDF [§]	15.0	15.0	15.0
Ether Extract	2.64	2.12	2.60
Non-Fibrous Carbohydrates [‡]	40.89	43.83	41.05
Ash	6.65	5.49	5.19
Gross Energy, MJ	17.53	17.34	17.41
Metabolizable Energy, MJ	10.15	10.39	10.91

*CS: Corn silage; SC: Sugar cane; SB: Sugar cane bagasse.

[†]Composition = calcium: 210g; phosphorus: 20g; sulfur: 37g; sodium: 80g; copper: 490mg; manganese: 1.424mg; zinc: 1.830mg; iodine: 36mg; cobalt 29mg; selenium: 9mg; fluorine (max):333mg.

[‡]NDF: Neutral detergent fiber.

[§]fNDF: NDF from forage.

[‡]Non-fiber carbohydrates = 100 – (CP + EE + ash + NDF) NRC, 2001.

The concentrates were composed by ground corn, soybean meal, crude glycerin and mineral supplement (Table 2). The ingredients of concentrate were ground in a hammer mill fitted with strainers and 2mm sieves. Continuous homogenization of the diets was performed in a horizontal mixer for 15 min. The ingredient proportions, chemical composition of the experimental diets are showed in Table 2. The diets were calculated using AFRC (1993) for 1.5kg of average daily gain.

Animals 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 adjusted to allow a surplus of approximately 10% in relation to the total amount consumed on the previous day. Feed refusals were collected before feeding and weighed. Subsamples were obtained and frozen at -20°C .

Total feces collect were realized in four days between d 15 and 18 (Barbosa et al., 2014). Feces were collected immediately after each spontaneous defecation, stored in 20L 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 300g) were collected. After dry and milled feces were mixed proportionate to daily excretion for laboratory analyses.

Laboratory analyses

Feed offerings, feed refusals, feces, ingredients were dried at 55°C for 72h and ground in a Wiley mill (Thomas Scientific, Swedesboro, NJ, USA) to pass through a 1mm screen. Samples of corn silage, sugar cane, sugar cane bagasse, concentrates and feed refusals were analyzed to determine dry matter (DM; method

934.01) and mineral matter (MM; method 942.05) and to obtain ether extract (EE; method 920.85) according to AOAC (1990). Nitrogen was determined using an LECO FP-528 nitrogen analyzer (LECO Corp., St. Joseph, MI, USA).

Neutral detergent fiber (NDF) was determined using α -amylase without the addition of sodium sulfite following Van Soest et al. (1991) and adapted for the Ankom200 Fiber Analyzer (Ankom Technology, Fairport, NY). The gross energy (GE) content of feeds and refusals was determined using an adiabatic bomb calorimeter (PARR Instrument Company 6300, Moline, IL, USA).

Ruminal parameters

Rumen pH, and ammonia N ($\text{NH}_3\text{-N}$) were measured for one day during the d 18 of each period. To assess rumen fermentation parameters, rumen fluid samples (around 80mL) were collected manually, both before supplying the diet (time zero) and 1, 2, 4, 6, 8, 10, and 12h after feeding. Immediately after collection, the pH of rumen fluid was determined using a digital potentiometer (ORION 710A, Boston, MA). 1 aliquot of collected fluid (40mL each) was placed into a plastic bottle and frozen at -20°C $\text{NH}_3\text{-N}$ analysis following the methodology adapted by Fenner (1965). Ruminal fluid NH_3 was analyzed by distilling with 2M KOH in a micro-Kjeldahl system, according to the original procedures of Fenner (1965).

Microbiology

Ruminal microbiology (bacteria and protozoa) samples were collected on the 19 d after 3 h of feeding. For protozoa population cell counts were obtained from rumen content aliquots that were preserved in formalin (a solution of equal parts

water and 370mL/L formaldehyde) according to D'Agosto & Carneiro, (1999). Ciliate protozoa species were identified and quantified the in chamber Sedgewick-Rafter, according to Dehority (1984). Each sample was homogenized and 1mL of ruminal content was pipetted and transferred to vials with lugol, according modified methodology from D' Agosto & Carneiro (1999). After 15 min, 9mL of glycerin at 30% was added in vials. To quantify the protozoa, from each vails was pipetted 1mL of content to fill the chamber of Sedgewick-Rafter.

Bacterial population was quantified using fifty grams of the rumen contents which were weighed and immediately added to 50mL 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 x g for 10 minutes at 4°C. The supernatant was discarded and the remaining precipitate was resuspended in 4mL of Tris-EDTA buffer (10X, pH 8.0). The resuspended content was centrifuged at 16,000 x 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 two months.

DNA extraction was conducted in 250mg of sample using the extraction kit AxyPrep™ Bacterial Genomic DNA Miniprep (Axygen-Biosciences). 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*), *Selenomonas ruminantium* and Total Archeas the technique used was qPCR. The primers used in this study are shown in Table 3. Four concentrations

(200, 400, 600 and 800nM) of forward and reverse primers were tested to determine minimum primer concentration giving the lowest threshold cycle (Ct) and to reduce nonspecific amplification before start the reaction.

The amplifications were performed in triplicate and negative controls were run in the assay, omitting the total DNA. Real-time PCR was performed with Applied Biosystems 7500 Real-time PCR System (Applied Biosystems). Rox was used as a passive reference dye. The qPCR reaction was carried out using 100ng of total DNA in a reaction containing: 6,25µL of SYBR Green PCR Master Mix (Bio-Rad, Hercules, California, USA), 400 or 600nM of primer pair, and H₂O to a final volume of 12,5µL. 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.

Relative quantification was used to determine species proportion. The results were expressed as a 16S rDNA ratio of general bacteria, following the equation:

$$\text{Relative quantification} = 2^{-(\text{Ct target} - \text{Ct total bacteria})};$$

Where Ct is defined as the number of cycles required for the fluorescent signal to cross the threshold.

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

Primer	Sequência (5` to 3`)
<i>Ruminococcus albus</i> *	F: CCCTAAAAGCAGTCTTAGTTCG R: CCTCCTTGCGGTTAGAACA
<i>Ruminococcus flavefaciens</i> *	F:GGACGATAATGACGGTACTT R:GCAATC(CT)GAACTGGGACAAT
<i>Fibrobacter succinogenes</i> *	F: GGTATGGGATGAGCTTGC R:GCCTGCCCCTGAACTATC
<i>Selenomonas ruminantium</i> †	F: GGCGGGAAGGCAAGTCAGTC R: CCTCTCCTGCACTCAAGAAAGACAG
Total Archaeas‡	F: TTC GGT GGA TCD CAR AGR GC R: GBA RGT CGW AWC CGT AGA ATCC

F = "forward"; R = "reverse"; *Denman & McSweeney. (2006); †Khafipour et al. (2009); ‡ Denman et al. (2007).

Statistical analyses

The data of intake, digestibility, and protozoa populations were analyzed as triple 3x3 Latin square design using the PROC MIXED procedure of SAS (Statistical Analysis System, version 9.2). The general mathematical model was represented as follows:

$$Y_{ijk} = \mu + \alpha_i + \beta_j + s_k + \alpha\beta_{ij} + e_{ijk}$$

in which Y_{ijk} represents the observation on steers k given diet i at period j ; α_i represents the fixed effect of the i -th diet, $i = 1, 2, \dots, nt$; β_j represents the fixed effect of the j -th period, $j = 1, 2, \dots, np$; and s_k represents the random effect of the k -th steers, $k = 1, 2, \dots, ns$, with variance component σ^2_c (Tempelman, 2004).

Ruminal pH and NH₃-N data were analyzed as triple 3x3 Latin square design with repeated measures over time using the PROC MIXED procedure of SAS (Statistical Analysis System, version 9.2). The model included fixed effects of diet, time, diet x time interaction and random effects of steers and periods. The structure of errors that best fitted the data according to the Bayesian information criterion (BIC) was used. Differences between treatment means were determined by Tukey's test. Differences among means with $P < 0.05$ were accepted as representing statistically significant differences.

Relative quantities of 16S rRNA as determined from real-time PCR were analyzed using R software (version 3.1.1) as a triple Latin square 3 x 3, with 3 treatments and 3 periods. The fixed effects were treatments and Latin Square, and random effects were time, animal and error. The statistical test used was Tukey, and the significance was $P < 0.05$.

3. RESULTS

There was no effect of different sources of forage in diets with crude glycerin on DM, OM and CP intake ($P > 0.01$). However, NDF intake was increased ($P < 0.05$) in animals fed corn silage which did not differ from animal fed with sugar cane bagasse (Table 4). Animals fed with sugar cane bagasse decreased NFC intake ($P < 0.05$) compared with animals fed with corn silage and sugar cane.

Animals fed with corn silage diet increased ($P < 0.05$) digestibility of DM and NDF compared with the other diets (Table 4). Additionally, CP digestibility was increased in animals feed with sugar cane bagasse, which not differed from animals

fed with corn silage ($P<0.05$). NFC digestibility increased in animals fed with sugar cane ($P<0.05$).

Table 4. Effect of different sources of forage in diets with crude glycerin on intake and digestibility of dry matter (DM), organic matter (OM), crude protein (CP), neutral detergent fiber (NDF) and non-fiber carbohydrates (NFC)

Parameters	Diet [*]			SEM	P-value
	CS	SC	SB		
Intake, kg/day					
DM	7.13	6.25	6.44	0.557	0.501
OM	7.02	5.91	6.10	0.473	0.231
CP	1.33	1.10	1.37	0.107	0.187
NDF	2.36 ^a	1.71 ^b	1.72 ^{ab}	0.188	0.035
NFC [†]	3.05 ^a	3.15 ^a	2.16 ^b	0.283	<.001
Digestibility, % DM					
DM	80.78 ^a	75.94 ^b	75.00 ^b	1.232	0.006
OM	82.57	77.26	78.74	1.965	0.134
CP	77.17 ^{ab}	73.73 ^b	79.60 ^a	1.704	0.020
NDF	70.77 ^a	45.40 ^b	46.83 ^b	2.771	<.001
NFC [†]	91.40 ^b	94.68 ^a	90.61 ^b	1.119	<.001

^{a, b, c} Within a row, means with different letters differ by Tukey test ($P<0.05$).

^{*} CS: Corn silage; SC: Sugar cane; SB: Sugar cane bagasse.

[†] Non-fiber carbohydrates = $100 - (\text{CP} + \text{EE} + \text{ash} + \text{NDF})$, NRC 2001.

There was no interaction between diet and time for pH and $\text{NH}_3\text{-N}$ ($P>0.01$; Table 5). Animals fed with sugar cane bagasse increased pH values ($P<0.05$) which not differed from animals fed with sugar cane. Animals fed with sugar cane decreased $\text{NH}_3\text{-N}$ values ($P<0.05$).

Table 5. Effect of different sources of forage in diets with crude glycerin on pH, and NH₃-N

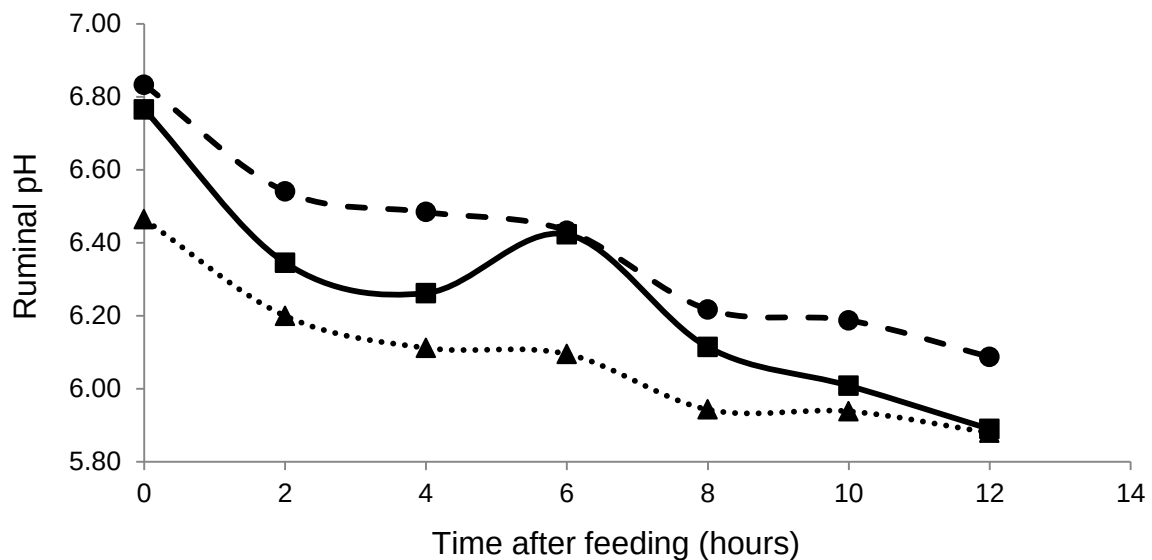
Parameters	Diet [*]			SEM	P-value [†]		
	CS	SC	SB		D	T	DxT
pH	6.08 ^b	6.27 ^{ab}	6.40 ^a	0.085	<.001	<.001	0.617
NH ₃ - N(mg/dl)	15.42 ^a	11.54 ^b	15.40 ^a	1.227	<.001	<.001	0.586

^{a, b, c} Within a row, means with different letters differ by Tukey test (P<0.05).

^{*}CS: Corn silage; SC: Sugar cane; SB: Sugar cane bagasse.

[†]D = diet; T = time effect; DxT = diet and time interaction effect.

Ruminal pH fell below 6 for animals fed with corn silage and sugar cane after 8 and 10 hours after feeding respectively (Figure 1). Moreover, an increase concentration of NH₃-N was observed before feeding. In addition, animals fed with sugar cane decreased NH₃-N level during the period (0 to 12 hours) after feeding (Figure 2).

**Figure 1.** Ruminal pH of Nellore steers fed different sources of forage in diets with crude glycerin. Corn silage (▲); Sugar cane (■); Sugar cane bagasse (●); Standard error of means (SEM= 0.085); diets x time interaction (P=0.617).

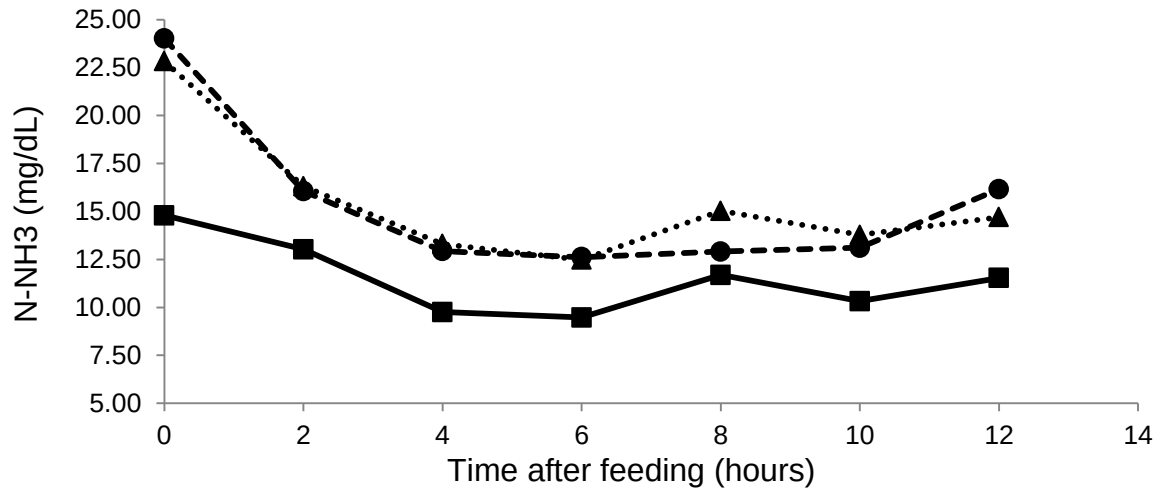


Figure 2. Ruminal $\text{NH}_3\text{-N}$ of Nellore steers fed different sources of forage in diets with crude glycerin. Corn silage (▲); Sugar cane (■); Sugar cane bagasse (●); Standard error of means (SEM= 1.227); diets x time interaction ($P=0.586$).

The protozoa population was not influenced by the different sources of forage in diets with crude glycerin, with exception of *Dasytricha* and *Isotricha* (Table 6). The concentration of *Dasytricha* population higher in animals fed with sugar cane which not differed from animals fed with corn silage diet ($P>0.05$). Additionally, *Isotricha* population concentration were higher ($P<0.05$) in animals fed with sugar cane.

Table 6. Effect of different sources of forage in diets with crude glycerin on rumen fluid protozoa numbers

Protozoa (n x 10 ⁵ /mL)	Diet [*]			SEM	P-value
	CS	SC	SB		
<i>Entodinium</i>	6.18	6.04	6.05	0.09	0.140
<i>Dasytricha</i>	4.08 ^{ab}	4.48 ^a	3.63 ^b	0.23	0.026
<i>Isotricha</i>	3.88 ^b	4.56 ^a	3.66 ^b	0.14	<.005
<i>Diploplastron</i>	3.88	4.40	3.88	0.23	0.437
<i>Polyplastron</i>	4.15	4.08	3.87	0.24	0.224
Protozoa total	17.76	19.08	17.54	1.53	0.637

^{a, b, c} Within a row, means with different letters differ by Tukey test (P<0.05).

^{*}CS: Corn silage; SC: Sugar cane; SB: Sugar cane bagasse.

The population of fibrolytic bacteria (*Ruminococcus flavefaciens*, *Ruminococcus albus* and *Fibrobacter succinogenes*) were similar (P>0.05) among diets (Table 7). On the other hand, the population of *Selenomonas ruminantium*, a lactic acid consumer bacteria, was higher (P<0.01) in animals fed with corn silage. Moreover, *methanogenic arqueas* population was similar (P>0.05) among diets.

Table 7. Effect of different sources of forage in diets with crude glycerin on relative proportion (%) of cellulolytic bacteria and methanogenic arqueas of Nellore steers in feedlot

Parameters	Diet [*]			SEM	P-value
	CS	SC	SB		
<i>Ruminococcus albus</i>	0.007	0.002	0.001	0.001	0.205
<i>Ruminococcus flavefaciens</i>	0.003	0.007	0.001	0.001	0.524
<i>Fibrobacter succinogenes</i>	0.570	0.440	0.450	0.042	0.363
<i>Selenomonas ruminantium</i>	0.005 ^a	0.002 ^b	0.001 ^b	0.001	<.001
Archeas total	0.054	0.067	0.052	0.003	0.076

^{a, b, c} Within a row, means with different letters differ by Tukey test (P<0.05).

^{*}CS: Corn silage; SC: Sugar cane; SB: Sugar cane bagasse.

4. DISCUSSION

According to Piatkowski et al. (1990) and Hoffmann (1990) to an adequate ruminal fermentation is necessary at least 400g of structural crude fiber per 100kg of animal body weight, to proper adequate structure fiber content in the concentrate-rich diet. Thus, to avoid ruminal disturbs in the present study the NDF from forage (fNDF) was fixed in 15% in the diet. This fNDF inclusion is recommended by Goulart & Nussio (2011) who suggested that at this inclusion level ruminal functions may be not affected in Nellore cattle fed diets with high percentage of concentrate. Thus, in the present study forage:concentrate ratio was different among the diets, CS=28.8%, SC=27.5% and SB=17.3%, due to the different content of NDF from the sources of forage studied (CS 54.4%, SC 55.5% and SB 86.5%).

Decreased NDF intake by animals fed with low quality forages could be due lower fiber digestion rate of this forage (Correa et al., 2003; Rotta et al., 2014), which limited voluntary intake (Ørskov & Hovell, 1978). However, in diets with high percentages of concentrate the intake is rarely limited by physical fill (Galyean & Defoor, 2003). Thus, our results of NDF intake were possibly due to the feeding sorting by animals, which are able to sort mixed rations more in diets with low forage content (DeVries et al., 2007, 2008). In addition, the NDF content of refusals were 0.53, 0.83 and 1.25kg DM/day for corn silage, sugar cane and sugar cane bagasse diets respectively, which suggest that animals fed with corn silage sorted for particles with high NDF content trying to increase intake of physically effective fiber and reduce intake of starch indicating that cattle may adapt their feed selection to minimize ruminal disturbs (DeVries et al., 2014).

Different sources of forage in diets with higher percentages of concentrate changed DM and NDF digestibility which could be relationated with forage fiber

quality. Sugar cane and sugar cane bagasse have low digestibility and high concentration of indigestible NDF (iNDF) which associated with high percentages of concentrate may increase passage rate limiting fiber degradation resulting in lower NDF digestibility (Galyean & Defoor, 2003).

Ruminal pH was affected by forage source due to the positive effect of fiber on stimulating chewing activity leading to an increase in saliva production (bicarbonate neutralizes the VFAs) increasing pH (Tafaj et al., 2005). Thus, forages with greater fermentability such as corn silage, are physically fragile due to the its NDF digestibility (more than 70% in this study; Taylor & Allen, 2005), which could lead to a lower chewing stimulating and consequently lower pH compared to forages with low quality and digestibility such as sugar cane bagasse (digestibility 46%). Additionally, due to the high fermentability of corn silage NDF animals fed with this diet sorted for fibrous particles, which were high in NDF, to reduce discomfort associated with low ruminal pH conditions, suggesting that cattle will attempt to select a diet to help stabilize rumen conditions (DeVries et al., 2008). Our study demonstrated this effect because animals fed with SB were more efficient in keep pH in adequate values, on the other hand ruminal pH of animals fed with CS dropped to 6 after 8 hours post feeding. In addition, the minimum ruminal pH value of this study was 5.88 which not characterize a sub-acute ruminal acidosis we could attribute this result to the inclusion of crude glycerin in the diets and forages particle size. According to Shi et al. (2012) glycerin is more rapidly fermented than starch in the rumen which may avoid lower ruminal pH. Furthermore, according to DeVries et al. (2014), diets with higher percentage of concentrate and forage particle size <1.9 cm tend to attenuate the effects of acidosis in cattle.

Carbohydrate availability determines the rate of microbial growth in the rumen and efficiency of ruminal $\text{NH}_3\text{-N}$ utilization. It have been shown that increasing amounts of readily fermentable carbohydrates, such as sugar, decreased $\text{NH}_3\text{-N}$ concentrations because of improved N uptake by ruminal microbes (Bach et al., 2005). In addition, Chamberlain et al. (1985) reported that sucrose was more effective than starch in reducing ruminal ammonia concentration which is in accordance with our results.

Diet is one of the major factors influencing the rumen microbial composition due to substrate preferences of microbes and indirectly by modifying the rumen environment due to fermentation of the ingested substrates. The genus *Entodinium* is the most resistant ruminal low pH protozoa, as it represents 90 to 99% of the total protozoa population, in cattle fed high grain diets (Hristov et al., 2001). Additionally, *Holotrichid* protozoa (*Isotricha* and *Dasytricha*) use soluble sugars for metabolism and cattle fed with sugar cane tend to have a greater population these protozoa (Ryle & Orskov, 1987).

Fibrobacter succinogenes and other cellulolytic species such as *Ruminococcus albus* and *Ruminococcus flavefaciens* did not completely disappear from diets with high proportion of concentrate (Petri et al., 2012), which could be noted in this study.

Increased NFC intake has been associated with acidic rumen conditions which reduce the activity of fibrolytic bacteria and increase the activity of amylolytic and lactic acid utilizing bacteria in the rumen such as *Selenomonas ruminantium* (Nagaraja & Titgemeyer, 2007). According to Nagaraja and Titgemeyer, (2007) *S. ruminantium* can tolerate low pH, thus, the increased concentration of *S. ruminantium*

in CS diets could be due to the lower ruminal pH of animals fed with this diet. Furthermore, the different sources of forage in diets with crude glycerin not influenced *Archeas* total population which is agreement with methane emissions measurements in chapter 3.

In summary, this study suggests that, the inclusion of low quality forages, included in similar fNDF in diets with crude glycerin do not affect intake, digestibility of organic matter, fibrolytic bacterias and total *Archeas*. The good news is that these sources of forage allowed average ruminal pH to be above 5.8 for up to 12 hours after feeding, possibility these sources of forage are taken into account when formulating diets to prevent ruminal disorders.

5. CONCLUSION

Sugar cane and sugar cane bagasse included in a similar fNDF level in diets with crude glycerin altered ruminal parameters, however, maintained adequate conditions for animal performance.

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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.

**ENTERIC METHANE EMISSION, INTAKE AND PERFORMANCE OF NELLORE
YOUNG BULLS FED DIFFERENT SOURCES OF FORAGE IN DIETS WITH
CRUDE GLYCERIN**

ABSTRACT: Forty Nellore young bulls were used to determine the effects of different sources of forage in diets with crude glycerin on intake, performance, blood metabolite and enteric methane emission in diets with high percentage of concentrate. Ten animals (396.6 ± 34.7 kg) were slaughtered (reference group) to obtain the carcass gain (CG). The 30 remaining animals (416.70 ± 24.74) were randomly assigned to three treatments with 10 replicates. The treatments were different sources of forage (fixed 15% of NDF from forage; fNDF): corn silage (CS), sugar cane (SC) and sugar cane bagasse (SB), in diets with 10% (DM) of crude glycerin. There were no differences on intake of dry matter (DM), organic matter (OM), crude protein (CP), neutral detergent fiber (NDF), gross energy (GE) and metabolizable energy (ME; $P > 0.05$). No effects of different sources of forage in diets with crude glycerin were observed on performance, blood metabolites and enteric methane emission ($P > 0.05$). These results suggest that the alternative forages for corn silage such as sugar cane and sugar cane bagasse which have high fiber content and low protein provide similar intake, performance and enteric methane emission.

Key words: cattle, feedlot, greenhouse gases, silage, sugar cane, sugar cane bagasse

1. INTRODUCTION

Methane (CH₄) produced by ruminants from enteric fermentation, which has global warming potential, represents 11.6% of methane generated globally (Ripple et al., 2014). This process represents 2–15% of the gross energy intake being one of most important inefficiencies in livestock systems (Johnson et al., 1991; Holter and Young, 1992; Bodas et al., 2012). According to Martin et al. (2010), decreasing enteric methane emission from ruminants without compromise animal performance is desirable both as a strategy to reduce global greenhouse gas emissions and as a means of improving feed efficiency.

Finishing animals in feedlot have been used as a strategy to reduce enteric methane emissions and increase energy utilization (e.g., performance) by the animal (Berndt and Tomkins 2013). However, higher percentages of concentrate in the diets could lead to ruminal acidosis and systemic inflammation decreasing animal performance (Zebeli et al., 2012). To ensure the minimal requirements of rumen health and maximize feed efficiency of animals finished in feedlot a ranging between 10 to 18% of NDF from forage is recommended by Goulart and Nussio (2011). Additionally, sources of forage used in feedlots vary in chemical-physical characteristics of its fiber which could affect ruminal fermentation, performance and CH₄ emission (Shibata and Terada, 2010). Forages with high cell wall constituents and low digestibility such as, sugar cane and sugar cane bagasse (Ezequiel et al., 2006; Nussio et al., 2006) would be used as an alternative to corn silage (high nutritional value forage) in feedlots in Brazil (Oliveira and Millen., 2014). However, little is known about their potential on enteric methane emission by beef cattle (Nelore). Thus, evaluate forage sources which could be used in feedlot is important

to increase forage options in this diets and increase livestock efficiency (kg meat/kg CH₄ emitted).

Another alternative feedstuff for feedlot diets is crude glycerin (glycerol), a byproduct from biodiesel agroindustry, which could replace corn in ruminant diets up to 10% of diet DM without compromise intake and performance (Eiras et al., 2014). In addition, glycerol modifies the ruminal acetate:propionate ratio due to the increase of propionate levels (Drouillard, 2008; Avila et al., 2011) which could reduce CH₄ emissions by ruminants.

So, we hypothesized that low quality forages with crude glycerin may be used in diets with high percentage of concentrate decreasing enteric CH₄ emission without affect performance of Nellore finished in feedlot. Therefore, the present study aimed to evaluate the corn silage, sugar cane and sugar cane bagasse in diets with crude glycerin on intake, performance, enteric CH₄ emissions and blood parameters from Nellore young bulls finished in feedlot.

2. MATERIAL AND METHODS

Animals and management

The trial was carried out at the Faculdade de Ciências Agrárias e Veterinárias, UNESP – Univ Estadual Paulista, Jaboticabal, following the humane animal care and handling procedures, according to the UNESP guidelines. All experimental procedures were approved by the Commission on Ethics and Animal Welfare (CEBEA) of the College of Agrarian and Veterinary Sciences (process nº 021118/11).

Two mo prior to beginning the experiment, forty Nellore young bulls at 18 ± 2 mo of age were grazed on *Brachiaria brizantha* cv. Marandu pasture and were supplemented with concentrate (CP 38.8% and GE 15.1 MJ/kg; composition: ground

corn, cotton cake, urea, mineral supplement) at 0.5 kg/head/d during the rainy season (December to March). Initially, animals were weighed, identified, and housed in individual pen with feeders and drinkers. The animals spent 21 d adapting to the diets, facilities and management. After adaptation, ten animals were slaughtered (396.6 ± 34.7 kg and 20 ± 2 months of age) to estimate the initial carcass weights and dressing percentage of all bulls to obtain the carcass gain (CG) and CG in relation to the ADG (CG/ADG) at the end of experiment. The 30 remaining animals with average initial body weight of 416.70 ± 24.74 kg were randomly assigned to one of the following experimental diets. Three sources of forage were used: corn silage (CS), sugar cane (SC) and sugar cane bagasse (SB). Chemical compositions of forages are shown in table 1. The neutral detergent fiber from forage (fNDF) was fixed at 15% of DM to ensure ruminal fiber requirements avoiding ruminal disturbs. Crude glycerin was included on 10% of DM, replacing corn in the concentrate.

Table 1. Chemical composition of corn silage (CS), sugar cane (SC) and sugar cane bagasse (SB)

	Forage		
	CS	SC	SB
<i>Chemical Composition, % DM</i>			
Dry Matter	31.08	29.32	63.03
Organic Matter	83.16	90.46	83.16
Crude Protein	8.68	3.03	3.32
Neutral Detergent Fiber	54.40	55.50	86.50
Ether Extract	2.38	0.42	0.40
Gross Energy, MJ/kg	17.99	17.53	17.20

Corn silage used in the present study was obtained from UNESP farm. A corn hybrid (whole plant) (hybrid 2B688Hx - Dow AgroSciences) was harvested at about 31% DM and chopped at 5cm (50mm). The chopped corn material was placed in a trench- type silo, covered with black plastic and ensiled for at least 2 months. The

sugar cane was obtained from a local farm. Sugar cane was chopped in particles 2–3cm (20mm). Sugar cane bagasse in particles 3–4cm (30mm) was obtained from a private biofuel plant. The forage particle sizes were different due to their precedence. Crude glycerin was acquired from soybean oil-based biodiesel production company ADM, Rondonópolis, Brazil (80.34% glycerol; 1.59% ether extract; 5.03% ash, and 12.02% water).

The concentrates were composed by ground corn, soybean meal, crude glycerin and mineral supplement (Table 2). The ingredients of concentrate were ground in a hammer mill fitted with strainers and 2 mm sieves and mixed by continuous homogenization in a horizontal mixer for 15 min. The ingredient proportions, chemical composition of the experimental diets are showed in Table 2. The diets were calculated using AFRC (1993) for 1.5 kg of average daily gain.

Table 2. Ingredients proportion and chemical composition of the experimental diets (DM basis)

	Diet ¹		
	CS	SC	SB
Ingredient proportion,% DM			
Corn Silage	28.8		
Sugar Cane		27.5	
Sugar Cane Bagasse			17.3
Corn	35.7	33.7	45.1
Soybean Meal	22.5	25.8	24.6
Crude Glycerin	10.0	10.0	10.0
Mineral Supplement ²	3.00	3.00	3.00
Chemical Composition, % DM			
Dry Matter	70.97	73.66	84.29
Organic Matter	90.95	91.80	92.00
Crude Protein	18.13	17.86	17.89
NDF ³	31.69	30.70	33.27
fNDF ⁴	15.0	15.0	15.0
Ether Extract	2.64	2.12	2.60
Gross Energy, MJ	17.53	17.34	17.41
Metabolizable Energy, MJ	10.15	10.39	10.91

¹CS: corn silage; SC: sugar cane; SB: sugar cane bagasse.

²Composition = calcium: 210 g; phosphorus: 20 g; sulfur: 37 g; sodium: 80 g; copper: 490 mg; manganese: 1.424 mg; zinc: 1.830 mg; iodine: 36 mg; cobalt 29 mg; selenium: 9 mg; fluorine (max): 333 mg.

³NDF: neutral detergent fiber.

⁴fNDF: NDF from forage.

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 three treatments with 10 animals per treatment. Animals 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 adjusted to allow a surplus of approximately 10% in relation to the total amount consumed on the previous day. Intermediate weights were taken after a 16-h solid fast before the first feeding in the morning on 28(d), 56 and 84 d of experiment to follow animals weight gain.

Feed refusals were collected before feeding and weighed. Subsamples were obtained and frozen at -20°C . Feed offerings and feed refusals were dried at 55°C for 72 h and ground in a Wiley mill (Thomas Scientific, Swedesboro, NJ, USA) to pass through a 1-mm screen. Samples of corn silage, sugar cane, sugar cane bagasse, concentrates and feed refusals were analyzed to determine dry matter (DM; method 934.01) and mineral matter (MM; method 942.05) and to obtain ether extract (EE; method 920.85) according to AOAC (1990). Nitrogen was determined using an LECO FP-528 nitrogen analyzer (LECO Corp., St. Joseph, MI, USA).

Neutral detergent fiber (NDF) was determined using α -amylase without the addition of sodium sulfite following Van Soest et al. (1991) and adapted for the Ankom200 Fiber Analyzer (Ankom Technology, Fairport, NY). The gross energy (GE) content of feeds and refusals was determined using an adiabatic bomb calorimeter (PARR Instrument Company 6300, Moline, IL, USA).

Blood Metabolites

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), 28 and 84 d after feeding period. 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 $2.500 \times g$ 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.

Ultrasound Measurements

The animals were evaluated through ultrasound on 0 (d), 28, 56 and 84 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).

Methane Measurements

The methane emissions were assessed using the sulfur hexafluoride (SF₆) tracer technique (Johnson et al., 1994), where each animal was sampled daily for 6-d consecutive 24-h, beginning at 78th d of feeding. Thirty animals (10 treatment replicates) were fitted with gas collection halters at 14-d before methane sampling to allow animals to adapt and facilitate sampling.

The release rate (RR) 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 7 wk in laboratory. The average RR was similar among the treatments (RR 1.90 ± 0.2 mg SF₆/d, mean $\pm$ SD). Brass permeation tubes filled with SF₆ and known release rates were administered orally to each of the 30 animals 72-h before methane sampling to allow the tracer gas to equilibrate in the rumen. The

animals were fitted with gas collection halters connected to preevacuated polyvinyl chloride (PVC) canisters designed to fill halfway over 24-h. Eructated gas sampling started at 0600 h daily, when the animals were removed from the pens, and were 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. Collection canisters, constructed of PVC pipe, were attached to a vacuum pump in the laboratory to create a negative pressure. As the vacuum in the sampling canister was slowly dissipated, the negative pressure steadily drew the sample of air from around the mouth and nose of the animal. The pressure of the canister removed from the animal was measured after 24-h 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 min), each animal were conducted at the original pen for feeding. The pressure readings were recorded, and the canisters were pressurized using pure N₂. Additional canisters were placed near de experimental pasture to monitor background levels of methane and SF₆ daily during each sampling period. These values were subtracted from the animals' values to calculate the net output in the expired breath. The concentrations of CH₄ and SF₆ in the collection tubes were measured at the Laboratory of Animal Nutrition – UNESP (Jaboticabal, SP, Brazil) using a Shimadzu CG-2014 ATF model gas chromatograph

(Agilent, San Jose, CA, USA), capillary column Porapak Q, FID detector for methane and ECD detector to hexafluoride, as described by Johnson et al. (1994).

CH₄ flux produced by animals was calculated in relation to the SF₆ tracer gas flux from a permeation capsule lodged in the rumen minus the basal CH₄ concentration in the air (Westberg et al., 1998). Following eq. was used:

$$Q_{CH_4} = Q_{SF_6} \times ([CH_4]_y - [CH_4]_b) / [SF_6]$$

Where QCH₄ is the animal methane emission rate in liters/h, QSF₆ is the known release rate of SF₆ from the permeation tube. [CH₄]_y measured concentration of CH₄ in the canister, [CH₄]_b measured concentration of CH₄ in ambient air samples, [SF₆] measured concentration of sulphur hexafluoride in the canister. Methane emissions (g/d) were considered the average emission during 6 d sampling. The ambient air samples in the feedlot were collected twice daily to determine the background concentrations of CH₄ and SF₆. These values were subtracted from the animals' values to calculate the net output in the expired breath.

Methane emissions (g/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 carcass gain (throughout the entire experimental period). In addition methane emission calculated in MJ/d was calculated multiplying methane per kg/d by methane energy content (55.65 MJ/kg; Crutzen et al., 1986). The variable CH₄, g/kg CrG = g CH₄ produced per d/kg of daily carcass gain.

Slaughter Procedure

At the end of the experimental period, the animals were transported to a slaughterhouse. The next day, after fasting (from solids) for 24 h, slaughter took place using a compressed air pistol to cause a cerebral concussion, and the animals were then bled by cutting the jugular and carotid veins. After slaughter, the carcasses were identified and weighed to obtain the weight and hot carcass yield. Carcass yield was calculated based on the hot carcass weight and body weight ratio after fasting.

Statistical Analysis

The experimental design was completely randomized design with 3 treatments and 10 replicates. The data were analyzed using the PROC MIXED procedure of SAS (Statistical Analysis System, version 9.1). The ultrasound measures and serum concentrations of glucose and insulin were analyzed as repeated measurements in time using the MIXED procedure. The best (co)variance matrix was chosen based on Akaike's Information Criteria. Tukey's test was used to compare the least square means considering 5% as the level of significance.

3. RESULTS

There was no effect ($P > 0.05$) of different sources of forage in diets with crude glycerin on DM, OM, CP, NDF, GE and ME intake (Table3). Additionally, there was no difference on DM intake expressed in g/kg of BW and % of BW among diets.

Table 3. Effects of feeding diets different sources of forage in diets with crude glycerin on nutrient intake of young Nellore bulls finished in feedlot

Parameters	Diet ¹			SEM	P-value
	CS	SC	SB		
<i>Intake, kg/d</i>					
DM	9.10	10.31	9.77	0.40	0.130
OM	8.61	9.76	9.42	0.50	0.870
CP	1.51	1.69	1.67	0.06	0.255
NDF	4.03	3.92	4.35	0.17	0.180
GE, MJ/d	163.02	181.54	172.27	7.09	0.203
ME ² , MJ/d	96.31	108.76	107.94	4.47	0.103
<i>Intake, g/kg of BW</i>					
DM	16.8	18.24	17.51	0.64	0.329
<i>Intake, % of BW</i>					
DM	1.70	1.77	1.75	0.65	0.715

¹CS= corn silage; SC= sugar cane; SB= sugar cane bagasse.

²ME calculation= DE (based on GE digestibility) x 0.82 (ARC, 1980).

The sources of forage in diets with crude glycerin did not influence ($P > 0.05$) the variables related to animal performance, such as final body weight (BW), average daily gain (ADG), feed efficiency (FE), hot carcass weight (HCW) and carcass gain (CG; Table 4).

Table 4. Effects of feeding diets different sources of forage in diets with crude glycerin on final body weight (BW), average daily gain (ADG), feed efficiency (FE), hot carcass weight (HCW), hot carcass dressing (HCD) and carcass gain (CG) of young Nellore bulls finished in feedlot

Parameters	Diet ¹			SEM	P-value
	CS	SC	SB		
Nº of Bulls	10	10	10		
Final BW, kg	541.70	564.44	558.40	12.22	0.420
ADG, kg/d	1.49	1.59	1.63	0.12	0.386
FE ²	0.16	0.18	0.17	0.06	0.129
HCW, kg	297.50	306.74	303.56	8.38	0.733
HCD	54.86	54.98	54.38	0.69	0.810
CG, g/d	0.83	0.96	0.87	0.08	0.547

¹CS= corn silage; S=: sugar cane; SB= sugar cane bagasse; ²Feed efficiency = kg ADG/kg DM intake;

There were no effect ($P > 0.05$) of different sources of forage in diets with crude glycerin on blood concentrations of glucose and insulin (Table 5).

Table 5. Effects of feeding diets different sources of forage in diets with crude glycerin on serum glucose and insulin concentrations of young Nellore bulls finished in feedlot

Parameters	Diet ¹			SEM	P-value ²		
	CS	SC	SB		D	P	DxP
Nº of Bulls	10	10	10				
Glucose, mg/dl	75.72	75.60	72.73	2.13	0.530	0.788	0.389
Insulin, uIU/ml	0.10	0.09	0.09	0.12	0.736	0.159	0.018

¹CS= corn silage; SC= sugar cane; SB= sugar cane bagasse; ² D= diet; P= period; DxP= diet and period interaction effect.

There were no effect ($P > 0.05$) of different sources of forage in diets with crude glycerin on LMA and RFT (Table 6). However, these parameters were influenced ($P < 0.05$) by feeding period (Figure 1 and 2).

Table 6. Effects of feeding diets different sources of forage in diets with crude glycerin on longissimus muscle area (LMA), Rib fat thickness (RFT)

Parameters ³	Diet ¹			SEM	P-value ²		
	CS	SC	SB		D	P	DxP
Nº of Bulls	10	10	10				
LMA, cm ²	70.06	71.63	71.14	1.45	0.692	<.001	0.252
RFT, mm	4.69	4.58	4.38	0.28	0.734	<.001	0.248

¹CS= corn silage; SC= sugar cane; SB= sugar cane bagasse; ² D= diet; P= period; DxP= diet and period interaction effect.

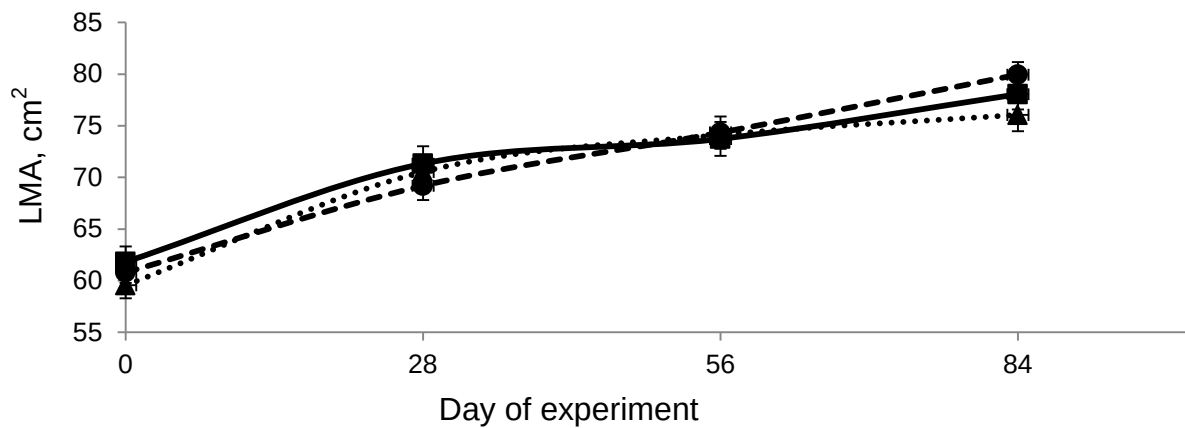


Figure 1. Longissimus muscle area of Nellore young bulls fed different sources of forage in diets with crude glycerin. Corn silage (▲); Sugar cane (■); Sugar cane bagasse (●); Standard error of means (SEM= 1.45). Significant effects of time ($P < 0.01$) but not significant differences for treatment ($P = 0.692$) and time x treatment ($P = 0.252$) were detected.

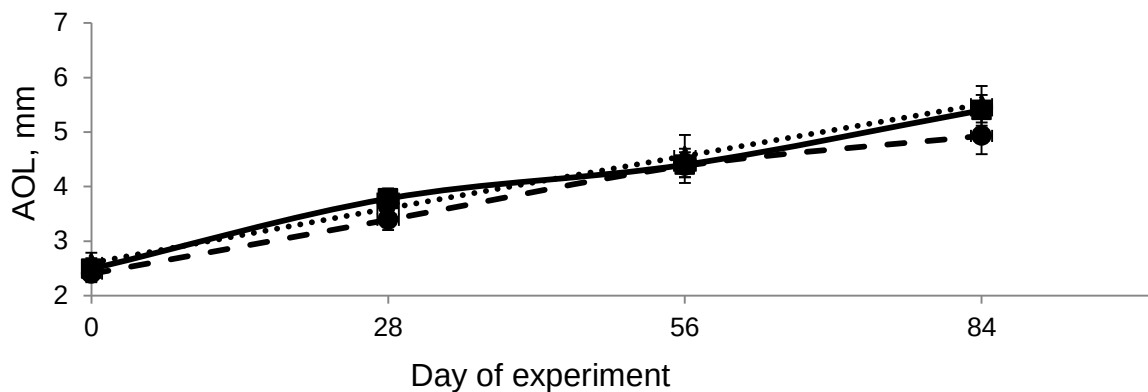


Figure 2. Rib Fat Thickness of Nellore young bulls fed different sources of forage in diets with crude glycerin. Corn silage (▲); Sugar cane (■); Sugar cane bagasse (●); Standard error of means (SEM= 0.28). Significant effects of time ($P < 0.01$) but not significant differences for treatment ($P = 0.734$) and time x treatment ($P = 0.248$) were detected.

Enteric methane emission, expressed in g/d, g/kg DM intake, g/kg NDF intake, % GE intake and g/kg CG were not affected by different sources of forage in diets with crude glycerin ($P > 0.05$; Table 7).

Table 7. Effects of feeding diets different sources of forage in diets with crude glycerin on CH₄ emission of young Nellore bulls finished in feedlot

Parameters	Diet ¹			SEM	P-value
	CS	SC	SB		
N ^o of Bulls	10	10	10		
CH ₄ , g/d	138.87	153.32	150.37	9.34	0.516
CH ₄ , g/kg DMI	14.44	14.43	14.66	0.78	0.972
CH ₄ , g/kg NDF intake	32.80	38.17	33.86	2.12	0.178
CH ₄ , % GE intake	3.37	3.34	3.48	0.19	0.857
CH ₄ , g/kg CG ²	167.40	160.21	171.02	10.06	0.628

¹ CS= corn silage; SC= sugar cane; SB= sugar cane bagasse; ² CG= carcass gain.

4. DISCUSSION

In the present study forage:concentrate ratio was different among the diets, CS=28.8%, SC=27.5% and SB=17.3%, due to the different content of NDF from the sources of forage studied (CS 54.4%, SC 55.5% and SB 86.5%). The chose to fix NDF from forage (fNDF) in 15% due to the recommendations of Goulart and Nussio (2011) whose stated that this content of fNDF ensures the minimum requirements of rumen health and maximizes the efficiency of Nellore cattle finished in feedlot. Animals finished in feedlot has ruminal acidosis as the most common digestive disorder which is due to the rapidly degradation of non-fibrous carbohydrates (NFC; concentrate) However, this process could be minimized increasing NDF from forage in high concentrate diets. According to Tafaj et al. (2007) chewing and rumination activities were positively correlated to forage NDF content. This is desirable because stimulating chewing activity, which in turn would result in an increased saliva output,

whose bicarbonate neutralizes the VFAs and consequently increases the pH (Tafaj et al., 2005).

According to Magalhães et al. (2006), regardless of the feeding strategy used, forages of different qualities affect DMI and consequently the ingestion of dietary components. However, when forages sources are substituted into the diet on an equal NDF basis, such as in the present study, differences among forages in feeding value become small, or non-significative, not affecting intake (Ware and Zinn 2004). Thus, the lack of difference on intake of animals fed with different sources of forage in diets with crude glycerin could be explained by equivalent NDF forage level among the diets (Galyean and Defoor, 2003). These results corroborate with Ware and Zinn (2004) who did not observed different DMI in animals fed different forage sources at the same fNDF level.

Moreover, the replacement of corn by crude glycerin could decrease fiber degradation because glycerol do not have cellular wall and is fermented faster than corn in rumen which reduce energy substrate to fibrolytic bacterias. Thus, this effect could lead to lower digestibility and intake of diets which had forages, such as sugar cane and sugar cane bagasse with low degradation rate, fiber availability and fiber digestibility (Daniel et al., 2013). However, no difference for nutrient intake was observed in the present study. According to Ramos and Kerley (2012), and Lage et al., (2014) inclusion crude glycerin included up to 10% on DM not affect intake and digestibility.

The lack of effect of forage sources in diets with crude glycerin on intake was reflected in similarity in the results of animal final body weight, average daily gain and feed efficiency, which might be explained by the similar levels of fNDF in the diets.

These results could be explained by the absence of apparent ruminal disturbs and similar nutrient intake, which could be due to the fNDF level fixed at 15%. When diets containing low forage inclusions are formulated based on fNDF there are no limitations on rumen function due to secretion of buffers by the chewing activity and consequently performance is not affected by forage source (Ware and Zinn, 2004; Benton et al., 2007). In addition, the diets were formulated to 1.5 of ADG but animals fed with sugar cane and sugar cane bagasse gained 11% and 9% more than the predicted ADG. According to Schroder and Sudekum, (2009) crude glycerin can replace up to 10% of readily fermentable starches but its energetic value when fed in conjunction with a higher amount of high starch (CS diet) was approximately 85% of its value when fed diet with lower amount of starch (SC and SB) which could explain the higher percentage of ADG when animals were fed with low quality forages.

The main factor responsible for the differences in carcass weight of cattle is the content and development of the digestive tract that could be influenced by feeding cattle with more fibrous forage (Di Marco, 1998). However, differences among forage sources are minimized when diets are formulated to equivalent fNDF levels (Ware and Zinn, 2004) which can explain the lack of difference in carcass weight and carcass gain (Table 4).

It is known that as grain in the diet increases the production of propionate is increased (Sutton, 1980), which is a significant source of the glucose (Reynolds, 1995). Despite the diets in the present study had different forage:concentrate ratio this lack of difference in glucose concentration could be due to the suppressed microbial fermentation activity induced by fasting and ruminal transit which may be induced by similar fNDF amount. The reduction in microbial fermentation activity

could have increased diet flow to intestine and thus increased the amount of gluconeogenic precursors available and thereby intestinal glucose absorption (Mueller et al., 2011). Besides, these findings suggest that these animals had similar glucose uptake by the peripheral tissue and did not exhibit significant growth differences (Table 5). In growing animals, glucose requirement is determined by growth rate (Reynolds, 2005), which is dependent of uptake and nutrient utilization by the cells in skeletal muscle. In addition, insulin facilitates glucose transports activity in skeletal muscles and in adipose tissues (Simpson and Cushman 1986) and increases glucose utilization by muscles and adipose tissues (Brockman, 1993). Thus, the lack of difference in glucose and insulin concentration in the present study could be due to the animals' growth rate which needs to use these blood metabolites for tissue growth.

The Longissimus muscle area is indicative of muscle development in animals and is correlated with carcass commercial cut yields (Luchiari Filho, 2000). The lack of difference for this characteristic in the present study may be due to the similarity on animal's performance. The rib fat thickness (RFT) is an important characteristic because it influences carcass visual aspect and serve as a protection against dehydration during carcass cooling (Moletta and Restle, 1996). There were in the present study similar deposition of RTF among diets (Average 4.55 mm), which were in the range for Brazilian market (from 3 to 6 mm; Costa et al., 2002).

Animals fed with different sources of forage in diets with crude glycerin had no differences on enteric CH₄ emissions. According to Knapp et al. (2014) CH₄ emissions can be affected by the level of feed intake, type of carbohydrate, forage quality and species. In this sense, it has been widely accepted that of all nutrients,

fibrous carbohydrates have the highest methane-producing potential. Additionally, when nonstructural carbohydrates are evaluated, saccharose (present in sugar cane) has higher methanogenic potential than starch at high pH (present in corn silage; Hindrichsen and Kreuzer, 2009). This was underlined by the fact that butyrate, a major product from sugar fermentation, provides hydrogen for methanogens other than propionate formation (Hindrichsen et al., 2004). However, physical chemical differences, such carbohydrate type (starch or saccharose) among forage sources are minimized in diets with higher percentage of concentrate when formulated to equivalent fNDF levels (Ware and Zinn, 2004). Thus we can infer that forages, with different carbohydrates, could be used in a similar NDF level in feedlot without increases in enteric methane emissions.

In addition, enteric CH₄ emission and DMI are often highly correlated (Cottle et al., 2011). The increased DMI could limit methane production by the increase on digesta outflow rate from the rumen, reducing the time available for microbial fermentation (Johnson and Johnson, 1995). Thus, in the present study similar DM intake was observed possibly due to the fNDF which provided similar rate of passage leading to lower ruminal fermentation (Galyean and Defoor, 2003) resulting in similar enteric methane emission.

Energy lost in the form of CH₄ (expressed as % of GEI) in the present study was slight higher than values suggested by Intergovernmental Panel on Climate Change (IPCC, 2006), which is 3% for feedlot animals (> 90% concentrate). However, this value may be not consistent with livestock conditions observed in Brazil, where is hardly to observe inclusion levels over than 90% of concentrate in ruminant diets. Therefore the range of 10 to 90% of concentrate to estimate methane

emission by cattle is low specific for most of the ruminant herd management in Brazil (Machado et al., 2010). On the other hand, the average emission of all animals in the present study was 53.87 kg of CH₄/yr. Lima et al. (2007) cited by Cedeberg et al. (2009), reported that the average emission of Nellore bulls above 500 kg of BW in southeastern Brazil (similar description to the animals in the present study) is 69.7 kg CH₄/yr thus, the animals used in this study showed 22.71% lower emissions compared to the emissions of those authors studies. Additionally, the average CH₄ emission obtained in the present study values was slight lower to the 56 kg/CH₄/yr estimated by the IPCC (2006) for Latin America for cattle.

Furthermore, 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). Pathways to propionate production are known to act as a hydrogen sink and would therefore reduce CH₄ emissions (Boadi et al., 2004). The quantity of hydrogen available in the rumen can be used to produce in CH₄ 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 could provide an increase of propionate in the rumen, may have lower CH₄ emissions. Lee et al. (2011) reported reduced methane emissions by including glycerol in in vitro incubations of corn grain and alfafa hay. Moreover, Lage et al. (2013) and Silva et al. (2013) reported that the inclusion of 10% of crude glycerin not affected CH₄ emission of Nellore young bulls finished in feedlot. Thus, the lack of difference on CH₄ emission may be due to the similar inclusion of fNDF and crude glycerin among diets.

In our study CH₄ emissions per kg of product was not affected. The good news is that mean calculated value of CH₄ emission for the treatments employed here falls below the estimated IPCC (2006) values. So we could infer that low quality forages may be used at 15% of fNDF in diets with crude glycerin without compromise production system efficiency and not increasing enteric methane emission per kg of meat.

5. CONCLUSION

Sugar cane and sugar cane bagasse included in 15% of fNDF in diets with crude glycerin can be used as alternative forages for corn silage in feedlot without modify intake, performance, blood metabolites and enteric methane emissions.

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CHAPTER 4

O artigo a seguir está redigido conforme normas de publicação do *Meat Science*, exceto o posicionamento das tabelas.

FATTY ACID PROFILE, MEAT QUALITY AND CARCASS TRAITS OF NELLORE YOUNG BULLS FED DIFFERENT SOURCES OF FORAGE IN DIETS WITH CRUDE GLYCERIN

ABSTRACT: Thirty Nellore young bulls with 416.70 ± 24.74 kg initial average body weight were randomly assigned to three treatments consisting different sources of forage: Corn silage (CS), Sugar cane (SC) and Sugar cane bagasse (SB), in diets with crude glycerin (10% DM) to evaluate the effects on carcass traits, meat quality and fatty acid profile. No effects of different sources of forage in diets with crude glycerin were observed ($P>0.05$) on performance and carcass traits. The heptadecenoic fatty acid was lower in the meat of animals fed with sugar cane bagasse ($P=0.04$). The yellow index was greater ($P=0.03$) in the fat of animals fed with corn silage. Our results indicate that sugar cane and sugar cane bagasse could be used in feedlot as a viable forage alternative for corn silage.

Key words: beef cattle, feedlot, forage NDF

1. INTRODUCTION

Beef is considered to be a highly nutritious and valued food (Scollan et al., 2006). However, saturated fatty acids present in meat have been criticized due to the risk factor of vascular and coronary diseases (Bartoň et al., 2007). On the other hand, the polyunsaturated fatty acids (PUFA) such as linoleic (C18:2 c9c12) and α -linoleic (C18:3 n3) present in beef are considered essentials for humans because they are not synthesized by the organism and they are the principal precursors of conjugated linoleic acid (CLA; Oliveira et al., 2011). The dominant CLA in beef is the cis-9 trans-

11 isomer (80%), which has antitumoral and anticarcinogenic properties (De la Torre et al., 2006). Thus strategies to enrich nutritional values of meat with functional properties are highly desired.

Several studies have shown that meat fatty acid profile can be significantly influenced by feedstuff used in animal diets (Pereira & Vicente, 2013). So, feeding animals with high percentages of concentrate such as feedlot diets is a possibility to modify meat fatty acid profile by decreasing biohydrogenation, which is responsible for the saturation of dietary fatty acids consumed by ruminant (Harfoot & Hazlewood, 1988). The inclusion of concentrate in the diets has high potential to manipulate the process of ruminal biohydrogenation, by inhibition of lipolysis (Doreau & Ferlay, 1994; Latham et al., 1972). Nevertheless, the unstable prices of grains have been leading researches to search for alternatives feedstuff to feed animals. Among them, crude glycerin, a byproduct from biodiesel agroindustry, has been used to replace corn in ruminant diets up to 10% of diet DM (Mach et al., 2009). Moreover, glycerin can be used as a gluconeogenic substrate to ruminants, due to the rumen wall absorption and conversion to glucose in the liver (Remond et al., 1993), or fermentation to propionate in the rumen, a gluconeogenic precursor that increases blood glucose levels after absorption in cattle (Chung et al., 2007). Thus, it was expected that a glucose supply would increase in animals fed with glycerin, which could increase lipogenesis in intramuscular fat (Smith & Crouse, 1984).

On the other hand, feedlot diets with high percentages of concentrate may cause ruminal upsets which have been avoided using a minimal amount of NDF from forage (fNDF; Galyean & Defoor, 2003). Among available forage options in Brazil, for ruminant feeding, sugar cane and sugar cane bagasse has lower nutritional value

and higher cell wall constituents with low digestibility compared with corn silage (Ezequiel et al., 2006; Nussio et al., 2006; Pate et al., 2001) which could influence fatty acid flow to duodenum and interfere in their deposition in muscle (Scollan et al., 2014). Furthermore, sugar cane has high amount of n-3 PUFAs compared with corn silage (Fernandes et al., 2009), but due to the lower digestibility and ruminal rate passage the PUFAs could be modified by biohydrogenation process. Therefore, these differences in the nutritional value of forages may limit their utilization and consequently modify the quality of the meat having an impact on human health.

We hypothesized that low quality forages (sugar cane and sugar cane bagasse) in diets with crude glycerin may be used as an alternative for corn silage without compromise carcass traits and meat quality of Nellore young bulls. Thus, the objectives of this study were to evaluate the effect of forages with different nutritional values, such as corn silage, sugar cane and sugar cane bagasse in diets with crude glycerin on carcass traits, meat quality and fatty acid profile from young Nellore bulls finished in feedlot.

2. MATERIAL AND METHODS

2.1 Animals and management

The trial was carried out at the Faculdade de Ciências Agrárias e Veterinárias, UNESP – Univ Estadual Paulista, Jaboticabal, following the humane animal care and handling procedures, according to the UNESP guidelines. All experimental procedures were approved by the Commission on Ethics and Animal Welfare (CEBEA) of the College of Agrarian and Veterinary Sciences (process nº 021118/11).

Two months prior to beginning the experiment, forty Nellore young bulls at 18 ± 2 months of age were grazed on *Brachiaria brizantha* cv. Marandu pasture and were supplemented with concentrate (CP 38.8 % and GE 3.64 MJ/kg; composition: ground corn, cotton cake, urea, mineral supplement) at 0.5kg/head/d during the rainy period (December to March).

Initially, animals were weighed, identified, and housed in individual pen with feeders and drinkers. The animals spent 21 days adapting to the diets, facilities and management. After adaptation, ten animals were slaughtered (396.6 ± 34.7 kg and 20 ± 2 months of age) to estimate the initial carcass weights and dressing percentage of all bulls to obtain the carcass gain (CG) and CG in relation to the ADG (CG/ADG) at the end of experiment. The 30 remaining animals with average initial body weight of 416.70 ± 24.74 kg were randomly assigned to one of the following experimental diets. Three sources of forage were used: corn silage (CS), sugar cane (SC) and sugar cane bagasse (SB) 9. The neutral detergent fiber from forage (fNDF) was fixed at 15% of DM to ensure ruminal fiber requirements avoiding ruminal disturbs. The chemical composition of forages and fatty acid profile of ingredients are shown in table 1 and 2 respectively. Crude glycerin was included on 10% of DM, replacing corn in the concentrate.

Table 1

Chemical composition of corn silage (CS), sugar cane (SC) and sugar cane bagasse (SB)

	Forage		
	CS	SC	SB
Chemical Composition, % DM			
Dry Matter	31.08	29.32	63.03
Organic Matter	83.16	90.46	83.16
Crude Protein	8.68	3.03	3.32
Neutral Detergent Fiber	54.40	55.50	86.50
Ether Extract	2.38	0.42	0.40
Gross Energy, MJ	17.99	17.53	17.20

Corn silage used in the present study was obtained from UNESP farm. A corn hybrid (whole plant) (hybrid 2B688Hx - Dow AgroSciences) was harvested at about 31% DM and chopped at 5cm (50mm). The chopped corn material was placed in a trench- type silo, covered with black plastic and ensiled for at least 2 months. The sugar cane was obtained from a local farm. Sugar cane was chopped in particles 2–3cm (20mm). Sugar cane bagasse in particles 3–4cm (30mm) was obtained from a private biofuel plant. The forage particle sizes were different due to their precedence. Crude glycerin was acquired from soybean oil-based biodiesel production company ADM, Rondonópolis, Brazil (80.34% glycerol; 1.59% ether extract; 5.03% ash, and 12.02% water).

Table 2

Percentages of the principal fatty acids in the corn silage, sugar cane, sugar cane bagasse, corn, soybean meal and crude glycerin

Fatty Acid	Lipid number	Corn silage	Sugar Cane	Sugar Cane Bagasse	Corn	Soybean meal	Crude glycerin
Capric	10:0	0.10	0.76	1.74	-	-	3.12
Lauric	12:0	0.19	1.48	1.53	-	-	4.87
Myristic	14:0	0.21	1.89	1.43	0.04	0.11	0.46
Palmitic	16:0	15.30	24.47	30.47	13.15	16.12	10.71
Margaric	17:0	0.15	0.33	1.87	0.08	0.11	1.45
Stearic	18:0	2.96	3.28	5.64	2.40	4.20	6.53
Palmitoleic	16:1	0.16	1.30	1.88	0.14	0.14	-
Oleic	18:1 cis-9	30.58	16.32	23.49	34.87	18.74	35.79
Linoleic	18:2	45.32	30.56	15.66	46.25	51.50	32.27
Linolenic	18:3	2.37	0.07	0.10	0.89	6.08	2.48
SFA ¹		20.34	33.29	44.85	16.84	21.70	27.14
UFA ²		79.66	64.80	51.69	83.16	78.30	72.86
MUFA ³		31.97	19.95	31.7	36.02	20.72	38.11
PUFA ⁴		47.69	44.85	19.99	47.14	57.58	34.75

¹SFA - saturated fatty acids = C10:0; C12:0; C14:0; C15:0; C16:0; C17:0; C18:0; C20:4 n6;

²UFA - unsaturated fatty acids = MUFA+PUFA;

³MUFA - monounsaturated fatty acids = C14:1c9; C16:1c9; C17:1; C18:1c9, C18:1n7; C20:1c9;C24:1n9;

⁴PUFA - polyunsaturated fatty acids = C18:2c9c12; C18:3n6; C18:3n3 C18:2c9t11; C20:2; C20:3n6; C20:4n6; C20:5n3; C22:4n6; C22:6n3;

The concentrates were composed by ground corn, soybean meal, crude glycerin and mineral supplement (Table 3). The ingredients of concentrate were ground in a hammer mill fitted with strainers and 2mm sieves. Continuous homogenization of the diets was performed in a horizontal mixer for 15 min. The ingredient proportions, chemical composition of the experimental diets are showed in Table 3. The diets were calculated using AFRC (1993) for 1.5 kg of average daily gain.

Table 3

Ingredients proportion and chemical composition of the experimental diets (DM basis)

	Diet ¹		
	CS	SC	SB
Ingredient proportion,% DM			
Corn Silage	28.8		
Sugar Cane		27.5	
Sugar Cane Bagasse			17.3
Corn	35.7	33.7	45.1
Soybean Meal	22.5	25.8	24.6
Crude Glycerin	10.0	10.0	10.0
Mineral Supplement ²	3.00	3.00	3.00
Chemical Composition, % DM			
Dry Matter	70.97	73.66	84.29
Organic Matter	90.95	91.80	92.00
Crude Protein	18.13	17.86	17.89
NDF ³	31.69	30.70	33.27
fNDF ⁴	15.0	15.0	15.0
Ether Extract	2.64	2.12	2.60
Gross Energy, MJ	17.53	17.34	17.41
Metabolizable Energy, MJ	10.15	10.39	10.91

¹CS: Corn silage; SC: Sugar cane; SB: Sugar cane bagasse.

²Composition = calcium: 210 g; phosphorus: 20 g; sulfur: 37 g; sodium: 80 g; copper: 490 mg; manganese: 1.424 mg; zinc: 1.830 mg; iodine: 36 mg; cobalt 29 mg; selenium: 9 mg; fluorine (max): 333 mg.

³NDF: Neutral detergent fiber.

⁴fNDF: NDF from forage.

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 three treatments with 10 animals per treatment. Animals 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 adjusted to allow a surplus of approximately 10% in relation to the total amount consumed on the previous day.

2.2 Growth performance and sample collection

After 85 days of feeding, all animals were slaughtered at a commercial beef plant with 554.51 ± 38.51 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 (Brazil, 1997).

After slaughter, the carcasses were identified, washed and divided into halves which were individually weighed. All carcasses were refrigerated at 0°C for approximately 24 h and then taken from the cooling chambers and weighed again in order to determine the cold carcass weight (CCW). The difference between the chilled carcass weight and hot carcass was used to calculate carcass shrink loss (CSL). Dressing percent (DP) was calculated using hot carcass weight divided by final SBW and then multiplying the result by 100. After 24 h postmortem chill, final pH (pH_f), 12th rib fat thickness (RFT) and 12th rib longissimus muscle area (LMA) were measured on the left side of each carcass. The final pH was measured at approximately 4 cm deep on longissimus muscle of the left side of each carcass (12th rib) using a pH-meter (Testo 230, Testo GmbH & Co, Germany). Longissimus muscle area was traced on transparencies and measured later with a planimeter (Greiner, Rouse, Wilson, Cundiff, & Wheeler, 2003) and rib fat thickness (RFT)

measurements were taken 3/4 the length ventrally over the longissimus muscle by using a digital paquimeter.

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. Each frozen longissimus sample was standardized from the posterior end into one 2.54 cm thick steak sample (AMSA (American Meat Science Association), 1995) for Warner–Bratzler shear force measurement and two 1 cm thick steaks, for the determination of the other analysis. All steaks were vacuum-packaged and held at -20°C for 10 days until the analysis was performed.

2.3 Proximate analysis

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

2.4 Color measurements

The determination of meat and fat color was performed as described by Houben, Van Dijk, Eikelenboom, & Hoving-Bolink (2000), using a Hunterlab colorimeter (Hunterlab MiniScan EZ, Hunterlab, Reston, Virginia USA) evaluating the lightness (L^*), redness (a^*), and yellowness (b^*). The color aspects were assessed by the CIE $L^*a^*b^*$ color system using $0^{\circ}/45^{\circ}$. Thirty minutes prior to the assessment, cross sections were made at the samples' surface to expose the myoglobin to

oxygen, the same steps were 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 analysing the samples against white and black standards.

2.5 Warner–Bratzler shear-force measurement and cook loss

Warner–Bratzler shear force (WBSF) steaks were thawed at 4°C for 24h and oven-broiled in an electric oven (LTedesco, Caxias do Sul, RS, Brazil) preheated to 175°C. Internal steak temperatures were monitored by 20-gauge copper–constantan thermocouples (Omega Engineering, Stamford, CT, USA) 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 (American Meat Science Association), 1995). Five round cores (1.27 cm diameter) were removed from each steak parallel to the long axis of the muscle fibers (AMSA (American Meat Science Association), 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 was 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, thawing loss was calculated as the difference between the weight of the steaks before and after thawing, and total loss was calculated by adding up the cooking loss and thawing loss.

2.6 Lipid oxidation

For the 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 4M HCl solution was added. Subsequently, the 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 530 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 composition

To determine the fatty acid composition of the fresh meat, samples of the transversal section were collected from the longissimus muscle, freeze-dried, and frozen for lipid extraction and methylation. The fatty material was extracted using a mixture of chloroform–methanol, as reported by Bligh & Dyer (1959) and the fatty acid 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 (Omega wax 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 were achieved by comparison with the retention times and concentrations of methyl esters of standard fatty acids.

2.8 Statistical analysis

The experimental design was completely randomized design with 3 treatments and 10 replicates. The data were analyzed using the PROC MIXED procedure of SAS (SAS Inst. Inc., Cary, NC, USA). The model tested the fixed effects of diets. Tukey's test was used to compare the least square means, with significance considered at $P < 0.05$.

3. RESULTS

The carcass traits of the Nellore young bulls were not influenced ($P>0.05$) by the different sources of forage in diets with crude glycerin (Table 4).

Table 4

Means for cold carcass weight (CCW), cold carcass dressing (CCD), 24-h carcass shrink loss (CSL), rib fat thickness (RFT) and longissimus muscle area (LMA) Nellore young bulls fed different sources of forage in diets with crude glycerin

Parameters	Diet ¹			SEM	P-value
	CS	SC	SB		
N ^o of Bulls	10	10	10		
CCW, kg	288	298	295	8.33	0.71
CCD, %	53.2	53.4	53.0	0.74	0.90
CSL, %	2.94	2.82	2.55	1.04	0.12
RFT, mm	5.90	4.24	4.87	0.36	0.18
LMA, cm ²	75.9	80.2	79.2	1.59	0.53

¹CS: Corn silage; SC: Sugar cane; SB: Sugar cane bagasse.

Similar results were found for values of pH ($P=0.64$), meat color (L^* $P=0.20$; a^* $P=0.43$; b^* $P=0.39$) and fat color (L^* $P=0.59$; a^* $P=0.12$) but corn silage diet increased b^* of fat ($P=0.03$), which not differed from animals fed with sugar cane (Table 3). The sources of forage in diets with crude glycerin did not influence ($P>0.05$) any of the variables related to meat quality of the longissimus muscle (Table 5).

Table 5

Means for pH, Warner–Bratzler shear force (WBSF), cooking loss (CKL), ether extract (EE), and malonaldehyde (MDA mg/kg of meat) and color characteristics (L^* , a^* , b^*) of meat and subcutaneous fat of longissimus of Nellore young bulls fed different sources of forage in diets with crude glycerin

Parameters ¹	Diet ²			SEM	P-value
	CS	SC	SB		
Nº of Bulls	10	10	10		
pH	5.34	5.92	5.81	0.79	0.64
WBSF, <i>kgf</i>	3.50	3.52	3.74	0.13	0.73
CKL, %	12.94	12.82	12.55	0.08	0.12
EE, %	3.90	3.52	4.53	0.21	0.17
MDA, <i>mg/kg</i>	0.49	0.44	0.49	0.02	0.39
<i>Meat</i>					
L^*	41.7	38.1	39.4	0.83	0.20
a^*	17.3	16.4	17.0	0.28	0.43
b^*	14.7	13.3	13.8	0.41	0.39
<i>Fat</i>					
L^*	67.7	67.7	69.1	0.63	0.59
a^*	10.0	9.70	8.20	0.39	0.12
b^*	17.8 ^a	16.7 ^{ab}	15.5 ^b	0.35	0.03

^{a,b} Within a row, means with different letters differ by Tukey test ($P < 0.05$).

¹ L^* : luminosity (0=black and 100=White); a^* : index from green (-) to red (+); b^* = Index from blue (-) to yellow (+).

² CS: Corn silage; SC: Sugar cane; SB: Sugar cane bagasse.

The sources of forage in diets with crude glycerin did not change ($P > 0.05$) the profile of saturated and monounsaturated fatty acids in longissimus muscle (Table 6), with the exception of heptadecenoic acid ($P = 0.04$), which was lower in animals fed with sugar cane bagasse.

Table 6

Means for principal saturated and unsaturated fatty acids (%) found in the longissimus of Nellore young bulls fed different sources of forage in diets with glycerin

Fatty acid	Lipid number	Diet ¹			SEM	P-value
		CS	SC	SB		
Nº. of Bulls		10	10	10		
<i>Saturated fatty acids (SFA)</i>						
Capric	10:0	0.05	0.04	0.04	0.03	0.77
Lauric	12:0	0.05	0.05	0.05	0.03	0.77
Myristic	14:0	2.57	2.3	2.31	0.16	0.93
Palmitic	16:0	24.3	24.1	26	0.47	0.2
Margaric	17:0	1.01	0.89	0.48	0.09	0.15
Stearic	18:0	16.4	16	16.2	0.52	0.96
<i>Monounsaturated fatty acids (MUFA)</i>						
Myristoleic	14:1 <i>cis</i> -9	0.48	0.58	0.6	0.05	0.92
Palmitoleic	16:1 <i>cis</i> -9	2.5	2.66	2.7	0.12	0.79
Heptadecenoic	17:1	0.83 ^a	0.88 ^a	0.70 ^b	0.03	0.04
Oleic	18:1 <i>cis</i> -9	38.6	39.4	36.8	0.93	0.51
Eicosenoic	20:1 <i>cis</i> -9	0.18	0.2	0.14	0.01	0.12

^{a, b, c} Within a row, means with different letters differ by Tukey test ($P < 0.05$).

¹ CS: Corn silage; SC: Sugar cane; SB: Sugar cane bagasse.

None of the treatments affected ($P > 0.05$) the polyunsaturated fatty acid profile of longissimus muscle (Table 7).

Table 7

Means for principal polyunsaturated fatty acids (%) found in longissimus of Nellore young bulls fed different sources of forage in diets with crude glycerin

Fatty acid ¹	Lipid number	Diet ¹			SEM	P-value
		CS	SC	SB		
Nº of Bulls		10	10	10		
<i>Polyunsaturated fatty acids (PUFA)</i>						
Linoleic	18: 2 <i>cis</i> -9, <i>cis</i> -12	6.33	6.44	7.22	0.42	0.93
α-Linolenic	18:3 n-3	0.41	0.37	0.44	0.03	0.72
CLA	18:2 <i>cis</i> -9, <i>trans</i> -11	0.33	0.29	0.29	0.01	0.20
Eicosatrienoic	20:3 n-6	0.54	0.64	0.82	0.03	0.63
Arachidonic	20:4 n-6	0.10	0.10	0.10	0.16	0.99
EPA	20:5 n-3	0.30	0.29	0.39	0.02	0.65
DTA	22:4 n-6	0.34	0.44	0.58	0.02	0.41
DHA	22:6 n-3	0.07	0.09	0.12	0.01	0.85
Total SFA ²		43.7	40.8	40.6	0.84	0.75
Total MUFA ³		44.8	46.0	43.2	1.01	0.52
Total PUFA ⁴		11.0	12.0	14.4	0.66	0.81
Total UFA ⁵		56.3	59.1	59.4	0.84	0.75
UFA/SFA		1.30	1.45	1.43	0.04	0.71
n-6/n-3 ⁶		12.4	13.3	12.3	0.40	0.49

¹CS: Corn silage; SC: Sugar cane; SB: Sugar cane bagasse;

²SFA - saturated fatty acids = C10:0; C12:0; C14:0; C15:0; C16:0; C17:0; C18:0; C20:4 n6;

³MUFA - monounsaturated fatty acids = C14:1c9; C16:1c9; C17:1; C18:1c9, C18:1n7; C20:1c9; C24:1n9;

⁴PUFA - polyunsaturated fatty acids = C18:2c9c12; C18:3n6; C18:3n3 C18:2c9t11; C20:2; C20:3n6; C20:4n6; C20:5n3; C22:4n6; C22:6n3;

⁵UFA - unsaturated fatty acids = MUFA+PUFA;

⁶n-6 = C18:2c9c12; C18:3 n6; C20:3 n6; C20:4n6; C22:4n6; n-3 = C18:3 n3 + C20:6 n3 + C20:5n3;

Altogether, our results indicate that none of the treatments here investigated changed the carcass and meat quality traits of Nellore young bulls finished in feedlot.

4. DISCUSSION

The NDF content of forages used in this study were different, 86.5%, 55.5% and 54.4% for sugar cane bagasse (SB), corn silage (CS) and sugar cane (SC) respectively, and it is known that this fraction affects the energetic utilization of

feedstuff by the animals. Then, we chose to fix NDF from forage (fNDF) at 15%. According to the recommendations of Goulart & Nussio (2011), this content of fNDF ensures the minimum requirements of rumen health and maximizes the efficiency of the animal feed. Thus, forage:concentrate ratio was different among the diets, CS=28%, SC=27% and SB=17%, to attend the amount of fNDF.

In the present study different sources of forage in diets with crude glycerin (CG) unchanged carcass traits of Nellore young bulls. This may be attributed to similarity of growth rate due to the similar genetic and nutritional historic among the animals used in this study. Thus, the lack of differences for CCD may be attributed to similarity of CCW. Macitelli et al. (2007) also did not report differences in CCD among crossbreed bulls fed with CS or SC. Furthermore, the inclusion of CG replacing corn (10% DM) in beef cattle diets did not change carcass traits, such as CCW, CCD, RFT and LMA of animals finished in feedlot (Lage et al., 2014), which could be due to glycerin has similar energy content of corn, approximately 1.04 MJ^{-1} (San Vito et al., 2015).

LMA is an indicative of the muscle development of animals and increases as the edible portion of carcass increases. In addition, this parameter is correlationated with CCW and high commercial value cuts. Thus, we can infer that there was no difference in muscle development in animals fed with different sources of forage with CG (Table 3). The RFT of carcass acts such as a thermal insulator that directly affects the cooling rate and rigor mortis, which is the conversion of muscle into meat (Savell et al., 2005). In the present study a similar deposition of RFT was observed among diets (Table 3) and it was greater than 3mm that is minimum value recommended in Brazil (Diniz et al., 2010). Agreeing with our results, Brondani et al.

(2006) did not report differences for LMA and RFT of animals finished in feedlot fed with CS or SC. Moreover, Lage et al. (2014) in a study using CS with or without CG also did not found differences in LMA and RFT.

The RFT can play a significant role in the reduction of CSL, such as reducing carcass dehydration, during the chilling process of beef (Dolezal, Smith, Savell, & Carpenter, 1982). So, according to similar deposition of RFT in the present study no differences were observed for CSL values.

The lack of effects of diets in the final pH (Table 4) found in the carcass reflects accumulated lactic acid due to the ATP resulting from glycogen reserves. It has been shown that cattle finished in feedlots have a greater availability of glycogen at the time of slaughter and a lower final pH in the meat (Neath et al., 2007). In addition, CG as gluconeogenic precursor could increase the levels of animals' energy reserves, as glycogen. The carcass pH is directly correlated to meat quality traits such as meat color (Jeremiah, Tong, & Gibson, 1991). Nevertheless, pH values in the present study were out of the optimum range (5.4–5.8) suggested for beef cattle, as previously reported (Abularach et al., 1998; Mach et al., 2008), which according to Felício et al. (1997) could be due to the stressor factors during pre-slaughter handling such as long time starvation, transportation and sexual behavior of non-castrated males.

The values of WBSF obtained in the present study were within the range proposed by Shackelford et al. (1991), who classified as tender longissimus muscle having less than 4.5 kg of shear force. The average WBSF values were lower (3.58 kgf) than those obtained by Zorzi et al. (2013), who reported 4.25 kgf for WBSF from Nellore bulls finished in feedlot. According to Crouse et al. (1993) meat from *Bos*

Indicus cattle also has been shown to be more variable in tenderness than meat from *Bos Taurus* cattle.

Lipid content which is relationated to juiciness, aroma and tenderness showed no difference in the present study. Additionally, marbling fat is the fat depot of most interest in relation to fatty acid due to its average composition of of saturated 0.45–0.48, monounsaturated 0.35–0.45 and up to polyunsaturated 0.05 of total fatty acids (Scollan et al., 2006).

Color is the attribute of meat that has most influence in consumers' decision to buy and could vary according to age and sexual condition of animal (Costa et al., 2002; Ramos & Gomide, 2007). However, in the present study animals had similar age and sexual condition which led no differences in meat color.

The color of bovine subcutaneous adipose tissue (carcass fat) is an important component of beef carcass quality (Wood & Fisher, 1997). The b* values (Table 4) are higher on the fat of animals fed with CS and SC than those fed with SB, which is explained by the fact that these forages contains higher levels of carotene than SB (Krishna, 1985; Pickworth et al., 2012). Deposition of carotenoids in the fat comes strictly from the diet in ruminants, which do not achieve de novo synthesis of carotenoids (Goodwin, 1992). Dunne et al. (2009) stated that the yellow coloration of fat is related to the amount of carotenoids in the diet. Carotenoids are present in most photosynthetic organisms, including higher plants such as the whole plant of corn used to make the silage and sugar cane used in this study, which supports the results of the present study (Table 4).

In the present study there was no difference in the fatty acid profile of meat from animals fed different sources of forage with CG. Changes in passage of dietary

components from the rumen could be related to changes in DMI resulting from differences in forage source and level. If fNDF increases passage of the grain portion of the diet, less fermentation would occur in the rumen (Galyean & Defoor, 2003). Thus, we could infer that different sources of forages used on an equal forage level basis in the diets with high percentages of concentrate prevent digestive upsets and promote low ruminal biohydrogenation which leading to a greater passage of unsaturated fatty acids (UFA, MUFA and PUFA) to intestine. Moreover, according to Zinn & Ware (2007) ruminal fiber digestion may be more limited by ruminal fibrolytic capacity (condition of ruminal pH), which is relationated with biohydrogenation, than by the native degradability of the fiber (digestive quality of the fiber). Thus, low quality forage, such as SB and SC could be used in feedlot promoting similar meat quality than animals fed CS, which is considered good quality forage. Additionally, glycerol, present in CG, may improve glucose level in ruminants due to the rapidly absorption through rumen wall and conversion to glucose in liver (Remond et al., 1993), which provides 50 to 75% of the acetyl units for in vitro lipogenesis in the intramuscular fat (Smith & Crouse, 1984) increasing the amount of unsaturated fatty acids in beef.

Meat of animals fed with different sources of forage with CG showed similar amount of saturated fatty acids. This result is important because these fatty acids can raise low density lipoprotein (LDL) in human blood (Wood et al., 2004) and they could interfere in the normal operation of the LDL receptors in the liver, increasing LDL concentration in the plasma (Woollet et al., 1992). Despite of stearic acid (C18:0) be a saturated fatty acid, it has a neutral role on cardiovascular disease risk. Furthermore, considering that humans can convert stearic acid to oleic acid, whose consumption has been associated with several health benefits (Covas, 2007), is

desirable meat with higher amount of this fatty acid. In this context, higher mean value of stearic acid were observed in the present study (16.2%) compared with Fernandes et al. (2009; 14.32%) who evaluated the fatty acid profile of meat from animals fed with CS or SC, without CG, in different forage:concentrate ratio 40:60 and 60:40.

The PUFA and MUFA are generally regarded as beneficial for human health (Schollan et al., 2006). Among the PUFA, linoleic (C18:2 c9c12) and α -linolenic (C18:3 n3) fatty acids are considered the most important because they are not synthesized by the organism and they are the principal precursors of CLA (Oliveira et al., 2011). The meat of animals fed with different sources of forage showed similar amount of oleic and linoleic fatty acids, which are important results due to these fatty acids relationship with health, such as hypocholesterolemic properties (Mir et al., 2003).

In the present study, similar deposition of MUFA was observed among diets except for heptadecenoic fatty acid (C17:1) which had similar amount in meat of animals fed CS and SC. This could be due to the inhibition of biohydrogenation, by which increased ruminal proportions of propionate, leading to a saturation of C17:0 to C17:1 by the enzyme Δ^9 desaturase (Fievez et al., 2003) in muscles of animals fed with CS and SC. The amount of oleic fatty acid, which is the predominant MUFA, obtained in the present study were in higher (38.2%) than that reported by Fernandes et al. (2009) who found 33.7% in meat of animals fed with CS or SC. According to Mir et al. (2003) this fatty acid is highly desirable because it has hypocholesterolemic properties.

Ruminant products such as beef and milk are dietary sources of CLA (Ritzenthaler et al., 2001). The dominant CLA in beef is the *cis*-9, *trans*-11 isomer, which has been identified as possessing a range of health promoting biological properties including antitumoral and anticarcinogenic activities (De la Torre et al., 2006). The CLA (*cis*-9, *trans*-11) content in ruminants' products is a consequence of two processes: a partial biohydrogenation of the dietary fatty acids (linoleic and linolenic) or an endogenous desaturation by Δ^9 -desaturase enzyme of *trans* vaccenic fatty acid (subcutaneous or intramuscular fat tissue). Contrasting of our results Lage et al. (2014) found higher levels of CLA and lower levels of linoleic and linolenic fatty acids in meat from animals fed with CS with CG in different forage:concentrate ratio 40:60 and 60:40. Thus, we could infer that the lack of difference on conjugated linoleic acid (CLA) amount in meat of animals fed with different source of forage with CG may be due to the similar amount of fNDF among diets which increased the passage rate (Galyean & Defoor, 2003) leading to a lower ruminal fermentation of dietary fatty acids and consequently higher deposition of them in muscle. Additionally, CLA content in beef was not related to final body score, but was influenced more specifically by the rate of fattening of animals which is related to intrinsic factors linked to animals such as age, sex, breed and type of muscles (De La Torre et al., 2006). The animals used in this study had similar genetics, age and sexual conditions which could explain the lack of difference in CLA content.

The n-6/n-3 ratio in the present study was above the recommended ratio of 5:1 or less (World Health Organization, 2003). This result could be due to the meat in the present study had higher amount of linoleic fatty acid (C18:2) than linolenic fatty acid (C18:3), precursors of the n-6 and n-3 respectively.

Meat from animals fed different sources of forage with CG presented similar values for MDA and the average value were 0.47 for all treatments inferior a 2.28 considered critical by Campo et al. (2006), because they indicate a level of lipid oxidation products which produce a rancid odor and taste that can be detected by consumers.

5. CONCLUSION

Sugar cane and sugar cane bagasse included in 15% of fNDF in diets with crude glycerin can be used as alternative forages for corn silage in feedlot without alter carcass traits, meat quality and longissimus fatty acid profile.

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