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UNIVERSIDADE ESTADUAL PAULISTA – UNESP

CÂMPUS DE JABOTICABAL

PERFORMANCE, CARCASS TRAITS AND RUMEN FERMENTATION FROM FEEDLOT NELLORE CATTLE FED CRUDE GLYCERIN AND VIRGINIAMYCIN

Pablo de Souza Castagnino

Zootecnista

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

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DADOS CURRICULARES DO AUTOR

PABLO DE SOUZA CASTAGNINO, filho de Angelo Carlo Bragança Castagnino e Círia de Souza Castagnino, nasceu em 02 de Janeiro de 1988 em Roca Sales - RS. Em fevereiro de 2012, graduou-se no curso de Zootecnia pela Universidade Federal de Santa Maria. Em março de 2012, ingressou no mestrado em Zootecnia pela Faculdade de Ciências Agrárias e Veterinárias, Universidade Estadual Paulista, Câmpus de Jaboticabal (FCAV–UNESP/Jaboticabal) sob orientação da Profa. Dra. Telma Teresinha Berchielli. De Outubro a Dezembro de 2013 realizou estágio no Rowett Institute of Nutrition and Health, Aberdeen, Scotland sob orientação do Dr. Robert John Wallace. Em março de 2014 ingressou no curso de Doutorado em Zootecnia na Faculdade de Ciências Agrárias e Veterinária da Universidade Estadual Paulista "Júlio de Mesquita Filho", campus de Jaboticabal, sob orientação da Profa. Dra. Telma Teresinha Berchielli. De novembro de 2016 a Agosto de 2017 realizou Doutorado – Sanduíche no "Department of Animal Sciences/The Ohio State University, Columbus, USA", sob orientação do Prof. Dr. Jeffrey L. Firkins.

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CEUA – COMISSÃO DE ÉTICA NO USO DE ANIMAIS

CERTIFICADO

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

Jaboticabal, 11 de Outubro de 2011.

Prof. Dr. Jeffrey Frederico Lui
Presidente - CEUA

Med. Vet. Maria Alice de Campos
Secretária - CEUA

DESEMPENHO, CARACTERISTICAS DE CARCAÇA E FERMENTAÇÃO RUMINAL DE BOVINOS NELORE ALIMENTADOS COM GLICERINA BRUTA E VIRGINIAMICINA

RESUMO – O glicerol é um substrato utilizado por bactérias que metabolizam o lactato ruminal e a virginiamicina é um antibiótico não ionóforo que inibe o crescimento de bactérias gram-positivas produtoras de lactato. Foram realizados dois experimentos para avaliar os efeitos da glicerina bruta (GB) e da virginiamicina (VM) na ingestão, digestibilidade, fermentação ruminal, população microbiana, desempenho, características de carcaça e perfil de ácidos graxos da carne de bovinos Nelore. Os tratamentos experimentais foram organizados em um arranjo fatorial 2 × 2: dietas sem virginiamicina (VM-) ou 25 mg de virginiamicina/kg de matéria seca (VM+) combinadas com dietas sem glicerina bruta (GB-) ou 100 g de glicerina bruta/kg de matéria seca (GB+). O bagaço de cana-de-açúcar foi usado como forragem na proporção de 20% na matéria seca (MS) da dieta e a GB substituiu o milho na formulação da dieta. No primeiro experimento, foram utilizados oito bovinos Nelore fistulados no rúmen (Peso corporal = 600 ± 34 kg, 26 ± 3 meses) em um quadrado latino 4×4 replicado (período= 21 dias) para se avaliar a digestibilidade dos nutrientes, fermentação ruminal e população microbiana. A ingestão de MS teve uma tendência a aumentar em dietas com GB ($P = 0,07$). As digestibilidades aparentes totais dos nutrientes foram semelhantes entre as dietas ($P \geq 0,10$). As dietas com GB ou VM apresentaram valores de pH similares (média = 6,15; $P \geq 0,10$). A proporção de propionato aumentou 27,5% nas dietas com GB+, independentemente da inclusão VM ($P = 0,01$). No segundo experimento, foram utilizados quarenta e oito bovinos com peso corporal inicial (408,4 ± 22,2 kg, 21 ± 2 meses) em um delineamento de blocos completos ao acaso para avaliação das características de carcaça e do desempenho animal. A ingestão de MS, o peso da carcaça fria e o rendimento de carcaça fria foi superior nas dietas com GB ($P < 0,05$). VM teve uma tendência para aumentar ganho médio diário e a eficiência alimentar ($P < 0,1$). A concentração total de ácidos graxos insaturados (AGI) no músculo longissimus aumentou em 6,08% nas dietas dietas com GB ($P < 0,05$), porém a relação n-6/n-3 permaneceu constante entre todos os tratamentos ($P > 0,10$). A GB teve efeitos positivos sobre os produtos de fermentação e pode substituir a virginiamicina com aumento na abundância de *Megasphaera elsdenii* e da deposição de AGI na carne. No entanto, a administração simultânea de VM e GB não interfere positivamente nos produtos de fermentação ruminal, desempenho e características de carcaça de bovinos Nelore em confinamento.

Palavras-chave: glicerina bruta, microbiologia, qualidade da carne, virginiamicina

PERFORMANCE, CARCASS TRAITS AND RUMEN FERMENTATION FROM FEEDLOT NELLORE CATTLE FED CRUDE GLYCERIN AND VIRGINIAMYCIN

ABSTRACT – Glycerol is a substrate used for bacteria that metabolize ruminal lactate and virginiamicyn is a non-ionophore antibiotic that inhibits the growth of gram-positive lactate-producing bacteria. Two experiments were conducted to evaluate the effects of crude glycerin (CG) combined with virginiamycin (VM) on intake, digestibility, ruminal fermentation, microbial population, performance, carcass traits and fatty acid profile of meat from feedlot Nellore cattle. Treatments were arranged in 2 × 2 factorial design: diets without virginiamycin (VM-) or virginiamycin at 25 mg/kg DM (VM+) combined with diets without crude glycerin (CG-) or CG (80% glycerol) at 100 g/kg DM (CG+). The sugar cane bagasse was used as the exclusive roughage in the proportion of 20% in the dry matter (DM) of diet and crude glycerin replaced corn in the diet formulation. In the first experiment, eight rumen fistulated bulls (BW= 600 ± 34 kg; 26 ± 3 months) were used in a replicated 4 × 4 Latin square (21-d periods) to evaluate the digestibility, ruminal fermentation and microbial population. The intake of DM had a tendency to be greater in CG+ than CG- diets ($P = 0.07$). Apparent total tract digestibilities of nutrients were similar among diets ($P \geq 0.10$). Diets with CG or VM had similar values of pH (mean=6.15; $P \geq 0.10$). The proportion of propionate increased 27.5% in CG+ diets, regardless of VM inclusion ($P = 0.01$). In the second experiment, forty-eight bulls with initial BW (408.4 ± 22.2 kg; 21 ± 2 months) were used in a randomized complete block design for carcass traits and animal performance evaluation. The intake of DM, cold carcass weight and cold carcass dressing was greater in crude glycerin diets ($P < 0.05$). VM had a slightly tendency to increase ADG and feed efficiency ($P < 0.1$). Total unsaturated fatty acids (UFA) concentration in the longissimus muscle increased 6.08% in diets CG+ diets ($P < 0.05$), however n-6/n-3 ratio remained constant among all treatments ($P > 0.10$). Glycerin had a positive effects on fermentation products and could replace virginiamycin with increment of *Megasphaera elsdenii* abundance and UFA deposition on meat. However, simultaneous administration of VM and CG does not interfere positively on rumen fermentation products, performance and carcass traits of feedlot Nellore cattle.

Keywords: crude glycerin, meat quality, microbiology, virginiamycin

CHAPTER 1 – GENERAL CONSIDERATIONS

Beef production from Brazilian feedlots has increased in recent years with market demands on efficiency and consumer satisfaction. According to Anualpec (2015) between 2006 and 2014 there was an increase of more than 100%, in feedlot finished cattle in Brazil with an approximate contribution of 10% of total cattle slaughtered. In addition to system efficiency, as ingredient costs are currently high, there is concern about the risk of metabolic disorders (e.g. acidosis) caused by a high intake of carbohydrates with faster rate of degradation. In a survey of Brazilian nutritionists, Millen et al. (2009) showed that acidosis represents the second largest health problems in feedlots.

High-concentrate diets increase the accumulation of VFA and potentially lactate in the rumen fluid by altering composition and metabolic activity of rumen microbiota, leading to an increment of lactate accumulation and metabolic disorders (e.g. ruminal acidosis) (NAGARAJA; TITGEMEYER, 2007; FERNANDO et al., 2010; ZEBELI et al., 2015).

The majority of bacteria stimulated by feeding high concentrate diets were *Proteobacteria*, *Megasphaera elsdenii*, *Streptococcus bovis*, *Selenomonas ruminantium*, and *Prevotella bryantii* populations followed by *Rumminococcus* spp reduction during high grain adaptation (FERNANDO et al., 2010; ZEBELI; METZLER-ZEBELI, 2012). In addition to bacteria fermentation ciliate protozoa can influence the rate and site of starch degradation by engulfment of starch granules (NAGARAJA et al., 1992). Strains of lactate utilizer and producers bacteria can metabolize starch in different pathways (Figure 1).

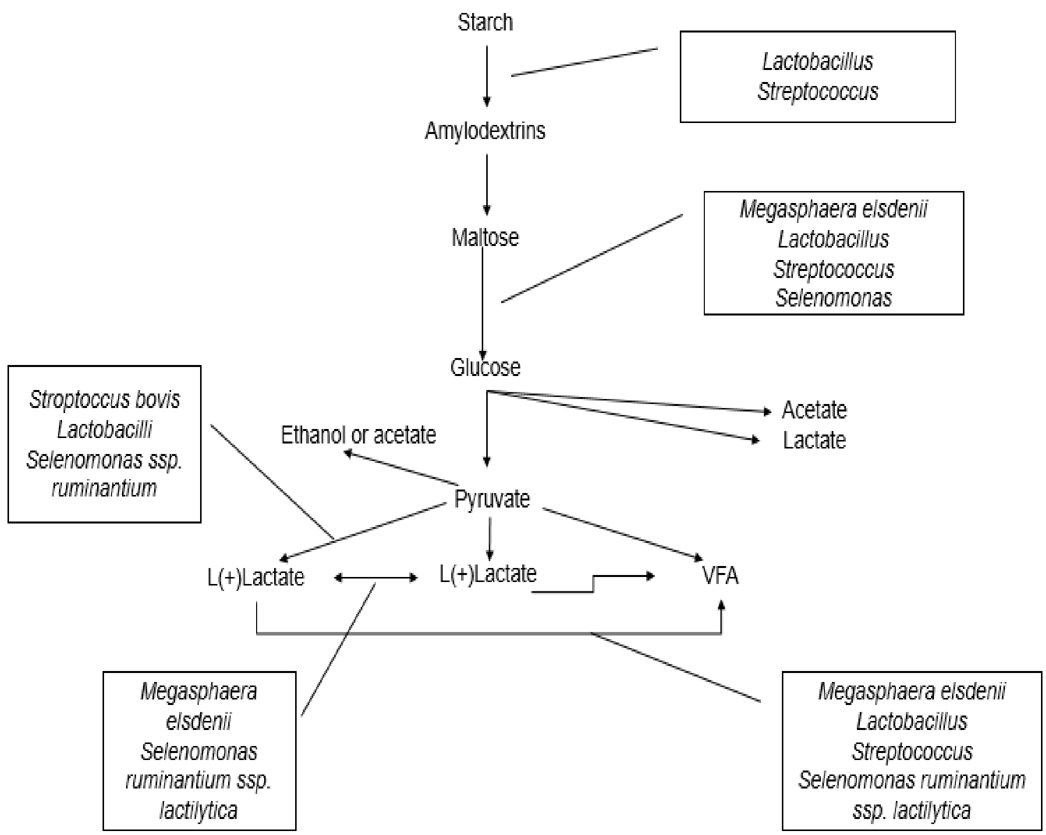

Figure 1- Starch fermentation by different strains of bacteria that produce and utilize lactate. Adapted from Nagaraja and Titgemeyer (2007).

Megasphaera elsdenii belongs to the phylum *Firmicutes* and metabolize lactate to propionate by the acrylate pathway (MAROUNEK; FLIEGROVA; BARTOS, 1989). During glucose fermentation, butyric and caproic acids are produced from an acetic acid intermediate (HINO; KURODA, 1993). Studies with *Megasphaera elsdenii* administration have demonstrated different results on acidosis manifestation both in beef and dairy cattle (KLIEVE et al., 2003; HAGG et al., 2010; MEISSNER et al., 2010; AIKMAN et al., 2011; MUYA et al., 2015). Some general effects of *Megasphaera elsdenii* supplementation include acetate:propionate ratio reduction and lower daily pH fluctuation.

The rumen influx of high fermentable carbohydrate (e.g. starch) may exceed the production of VFA during fermentation without the same removal synchronism by the epithelium or saliva neutralization, and therefore cause a reduction of pH below 6. This in turn, will lead to a development of lactate producing species such as *Streptococcus bovis* and *Lactobacilus* spp (AL JASSIM; GORDON; ROWE, 2003). *Streptococcus bovis* is a gram-positive bacteria homofermentative that grows extremely rapidly and becomes predominant in the rumen with lactate accumulation (HERNANDEZ et al., 2007). Some other microbes' species involved in lactate production in the rumen include *Bifidumbacterium* spp. and *Lactobacilus* spp. (STEWART; FLINT; BRYANT, 1997).

The decrease of pH changes the proportions of ruminal microbial populations. Cellulolytic bacteria can not develop in low pH environments due to the accumulation of VFA anions in the cell compared to bacteria resistant to acidification of the medium, and an increase in the pH gradient can cause anionic toxicity (RUSSEL; WILSON, 1996). In an experiment with high and low concentrate diets Fernando et al. (2010) found an average of 398 taxonomic units (OTU's) in cattle fed high-forage diets and approximately 315 OTU's in high-concentrate diets. However, they only share 24 OTU´s in similarities.

The main accumulation of lactic acid or VFA may cause two forms of acidosis; (1) an acute ruminal lactic disorder presenting ruminal pH less than 5.0 or (2) a subacute ruminal acidosis that is characterized by a range of 5.2-5.5 for at least 3 hours per day (METTE DANSCHER et al., 2015). The subacute ruminal acidosis is associated with the production of immunogenic factors in the rumen environment when the balance between host and rumen bacteria is disrupted. For example, the increase of Gram-negative bacteria and their subsequent lysis is accompanied with dramatic rises in the concentration of lipopolysaccharide, a cell-wall component known as endotoxins that can be translocated through disrupted epithelial junctions of the rumen epithelium and activate a cascade of inflammation events expressed through general signs like fever, lowered feed intake, changes in metabolism, lipolysis and stress (ZEBELI; METZLER-ZEBELI, 2012)

Problems linked with acidosis often follow an abrupt change in the diet such as from a fiber-rich roughage to one of a starch-rich concentrate diet (lacking physically effective fiber). Its severity is a function of the extent of decline in rumen pH and the duration of exposure to low pH (HERNANDEZ et al., 2007).

The contribution of starch present in cereal grains to the acidogenic power depends on size, shape and embedment within the protein matrix (GONZÁLEZ et al. 2017). Furthermore, processing methods such as reduction of particle size, thermal treatment (e.g. steam flaking) and shear forces are known to increase the rate of cleavage of starch to glucose (OWENS et al., 1998; GONZÁLEZ et al., 2017). The increase of starch degradation in the small intestine by feeding processed grains or using diets rich in corn versus barley and wheat or grains can relieve the generation of protons in the rumen environment and increase glucose pools, which may prevent excessive lipolysis and keep concentration of non-esterified fatty acids (NEFA) and beta-hydroxy-butyrate (BHBA) at rather normal levels (ZEBELI et al., 2015).

Several strategies have been used to modulate ruminal fermentation to increase performance and mitigate rumen acidosis risks in high concentrate diets of beef cattle: (1) feeding management (BEVANS et al., 2005); (2) use of buffers (CRAWFORD et al., 2008) and antibiotics such as virginiamycin (VM) or ionopheres (COE et al., 1999); and (3) administration of direct fed microbes such as *Megasphera elsdenii* or *Sacchararomyces cerevisiae* (MEISSNER et al., 2010). Furthermore, to reduce costs associated with cereal grains, the utilization of co-products from bio-diesel plants such as crude glycerin can be an alternative to replace part of corn in the diet formulation.

The use of antibiotics such as VM in sub-therapeutic doses has been used in the feeding of several species of animals as a growth promoter. The VM is a non-ionophore antibiotic of the streptogramins class, produced by *Streptomyces virginiae* that inhibits the growth of gram-positive lactate-producing bacteria. The VM structure has two major factors, M and S (GOTTSCHALL; WANG; KINGSTON, 1988) that function synergistically impairing bacteria protein synthesis by blocking peptide chain elongation (COCITO, 1979).

In the gastrointestinal tract of pigs VM acts decreasing inhibition of gram-positive bacterial growth, degradation of protein and ammonia production and therefore enhance nutrient availability for the animal. The majority of VM effects on ruminal environment are similar to those of monensin, such as increasing propionate content at the expense of acetate and methane (HEDDE; SHOR; QUACH, 1983; NAGARAJA et al., 1997) and protecting ruminal protein degradation (IVES et al., 2002). Virginiamycin has demonstrated to be very efficient in the inhibition of lactic-acid producing microorganisms (e.g., *Lactobacillus and Streptococcus spp.*) without interfering in the growth of lactic-acid consuming micro-organisms like *Megasphaera elsdenii* (ARAÚJO, 2016). The incidence of liver abscess decreased with VM administration by inhibiting *Fusobacterium necrophorum* and *Actinomyces pyogenes* (NAGARAJA; CHENGAPPA, 1998).

Rogers et al. (1995) have shown in a series of dose–response trials (19 mg to 27 mg VM/kg of the dry matter [DM]) with steers and heifers that VM enhanced average daily gain (4.6%) and gain to feed ratio (3.6%) and reduced the incidence of liver abscess (38%). Coe et al. (1999) evaluated different doses of VM (17.5 or 25mg/kg of DM) in diets with 85% of concentrate on the fermentative parameters as VFA, lactate and ammonia. The concentration of 25 mg/kg of DM was efficient in reducing ammonia (-1.40%), increasing propionate (+22.05%) and reducing butyrate (-15.89%), but in this study the lactate concentration was not altered.

Salinas-Chavira et al. (2009) did not show any effects on carcass characteristics (hot carcass weight, dressing percentage, fat thickness, quality grade and retail yield) of Holstein feedlot steers fed with control (no antibiotic) compared with VM (16 or 22.5 mg/kg of the DM). However VM inclusion tended to increase longissimus muscle area which was likely on the final indirect effect of virginiamycin on final body weight. In a second trial with cannulated Holstein steers VM administration increased molar proportion of acetate and tended decreased propionate molar proportion.

According to VAN BOECKEL et al. (2015) the top countries consuming antimicrobials, such as VM, in livestock production are China, the U.S, India, Brazil and Germany. By 2030, the authors project that antimicrobial consumption will rise by 50% in Brazil, Russia, India, China and South Africa due to consumer demand for livestock products and a shift to large-scale farms where antimicrobials are used routinely. The use of medically antimicrobials in food animals for production purposes is an important global issue due to the possible transfer of resistance genes and bacteria among animals and animal products and the environment (MCEWEN; FEDORKA-CRAY, 2002). On January 2006 the EU banned the use of antibiotics, including VM in animal feed. In 2017, the U.S Food and Drug Administration (FDA) published a Veterinary Feed Directive (VFD) regulation for medicated feeds, which restricts the use of antimicrobial drugs in feeds without veterinary prescription.

Another strategy to prevent or attenuate excessive lactate production and increase animal performance would be to increase the number of lactate-utilizing bacteria (e.g., *Megasphaera elsdenii* and *Selenomonas ruminantium*) in the diet. According to Owens et al. (1988) the maintenance of *Megasphaera elsdenii* or *Selenomonas ruminantium* (lactate utilizers) for a long-term phase in the rumen can be limited by substrate availability and competition from other microbial species (i.e. lower turnover time compared with *Streptococcus bovis*). Furthermore, many of the candidate microbes are obligate anaerobes, limiting cell yield and complicating their culture in commercial fermentation facilities (MCALLISTER et al., 2011). One alternative would be the use of a substrate, such as glycerol that makes *Megasphaera elsdenii* or *Selenomonas ruminantium* grows in the rumen environment (STEWART; FLINT; BRYANT, 1997).

Crude glycerin (65-85% pure glycerol), a byproduct from the biodiesel industry, could be used to modulate ruminal fermentation and lactate metabolism. The growth of the biofuels industry has increased availability of crude glycerin and has directed its use to livestock production (DONKIN, 2008; MACH et al., 2009; LAGE et al., 2014). Glycerol can be fermented by ruminal microrganisms, be absorbed in the epithelium or flow to the duodenum (REMOND; SOUDAY; JOUNAY, 1993). Ruminants can ferment glycerol to propionate in the rumen and utilize its molecules at gluconeogenesis via hepatic metabolism.

Glycerol and starch from corn grains present similar attributes as glucose suppliers (BAJRAMAJ et al., 2017). Ruminal degradation of glycerol and corn in the rumen increase the proportions of propionate and butyrate at the expense of acetate. The small intestine digestion of starch in grain corn (30-55%) may release glucose to be partially absorbed into the bloodstream (RÉMOND et al., 2004). Glycerol can be absorbed at the half percentage in the rumen wall and enhance glucose circulation via gluconeogenesis in the liver (REMOND; SOUDAY; JOUNAY, 1993).

Rumen bacteria oxidizes glycerol to dihydroxyacetone by glycerol dehydrogenase and then phosphorylatizes to dihydroxyacetone phosphate by dihydroxyacetone kinase. Triose phosphate isomerase in the glycolysis directs the dihydroxyacetone phosphate to glyceraldehyde-3-phosphate. Each mole of glycerol converted to pyruvate generates two moles of NADH, which must then go through the propionic acid synthesis pathway in order to completely convert the NADH back to NAD^+ and maintain the redox balance (ZHANG; YANG, 2009).

In vitro trial with pure culture of cellulolytic microbes and glycerol addition at 0.1, 0.5, 1, 2 and 5%, inhibited the growth of *Ruminococcus flavefasciens* and *Fibrobacter succinogenes* and impaired growth and cellulolytic activity of fungus *Neocallimastix frontalis* when included at 5% using cellobiose as the sole energy source (ROGER et al., 1992). ABO EL-NOR et al. (2010) evaluated diets consisting of 600 g/kg alfalfa hay and 400 g/kg concentrate (DM basis) with glycerol replacing corn at 0, 36, 72 and 108 g/kg dry matter in a continuous fermenter. They found a decreased of NDF digestibility and population of *Butirivibrio fibrisolvens*, as well as *Selenomonas ruminantium* and *Clostridium protoclasticum* by increasing concentrations of glycerol. However no effects were observed on pH, NH_3-N and dry matter digestibility.

Crude glycerin has demonstrated variable results when administered in livestock diets. Dry matter intake has been shown to decrease in diets containing more than 40 g glycerol /kg of DM of a steam-flaked corn finishing diet of crossbred heifers (PARSONS et al., 2009). Inclusion of crude glycerin up to 150 g/kg of DM in diets of finishing lambs increased DMI and feedlot performance in the first 14 days (GUNN et al., 2010). In feedlot diets of beef cattle, glycerin levels up to 100 g/kg of DM have also been used to replace cereals grains with positive results on performance of beef cattle (MACH et al., 2009; PARSONS et al., 2009; LAGE et al., 2014).

Carcass and meat traits can be altered in the meat of animals fed with crude glycerin by increasing marbling deposition as a function of glycogenic precursors absorption (SCHOONMAKER et al., 2003) or by increasing unsaturated fatty acid content on meat (CARVALHO et al., 2014; EIRAS et al., 2014; FAVARO et al., 2016) possibly due to the ruminal lipolysis inhibition (KRUEGER et al., 2010; EDWARDS et al., 2012).

Improvements of beef cattle performance through manipulation of rumen fermentation may be an alternative to increase feedlots' profitability and to reduce acidosis risks. The VM is a non-ionophore antibiotic that inhibits the growth of gram-positive lactate-producing bacteria (e.g., *Streptococcus bovis* and *Lactobacillus spp*). Crude glycerin (CG) is a substrate that stimulates the growth by bacteria that metabolize lactate, such as *Megasphaera elsdenii* and *Selenomonas ruminantium*.

We hypothesize that CG could replace VM without impairing ruminal fermentation, carcass traits and fatty acid profile of meat from feedlot Nellore cattle or that combining VM and CG would increase the positive effects on ruminal fermentative parameters and animal performance .

The objective of this study was to evaluate the effect of VM and CG on rumen fermentation parameters, performance, carcass traits and fatty acid profile of meat from feedlot Nellore cattle. In order to evaluate these parameters we quantified the following variables: intake and digestibility of nutrients, average daily gain, rumen VFA, pH, NH_3-N, ruminal microbial population, meat and fat color and fatty acid composition of longissimus muscle.

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5. Conclusions

Crude glycerin had positive effects on rumen fermentation products and can replace virginiamycin with increment of *Megasphaera elsdenii* abundance. However, combining virginiamycin and glycerin does not affect positively rumen fermentation and the growth of bacteria that metabolize lactate.

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5. Conclusions

Crude glycerin at 100 g/kg DM could be a suitable replacement for VM, as it led to an increase in UFA deposition in meat, although this increase was probably too small to have significant health benefits to human consumers of the meat. Simultaneous administration of VM and CG did not positively affect performance and carcass traits of feedlot Nellore cattle.

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