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Inclusion of Crude Glycerin in Diets for Sheep

Marco Túlio Costa Almeida and Josimari Regina Paschoaloto

Abstract

Crude glycerin is the main by-product of biodiesel industry. It has a great potential for reducing the feed costs in ruminant feedlot systems without affecting animal health and performance, mainly as a replacement for conventional food energy sources, such as corn grain. In the past years, great advancements have been achieved with crude glycerin utilization. This by-product is mainly composed of glycerol, an energetic compound of great assimilation by rumen microorganisms, being extensively metabolized in the liver. Recent studies with ovine species have demonstrated that high concentrations of glycerol (more than 76% of crude glycerin) can be used without detrimental effect for animals. In the rumen, glycerol is rapidly metabolized by microorganisms to form volatile fatty acids (VFA), mainly propionate and butyrate. In this way, glycerol constitutes an excellent substrate for gluconeogenesis and animal energy generation. At present, the inclusion of up to 20% of dry matter (DM) in a total diet seems to be the most interesting strategy, as it promotes greatest animal performance. However, other studies suggest that high inclusions of crude glycerin (30% of dry matter) could be possible depending on market price and the structure of farm operation, with favorable economic results.

Keywords: by-product, energy source, glycerol, metabolism, performance, small ruminant

1. Introduction

An increasing worldwide interest in alternative fuel sources and in a more diversified energy matrix has led researchers to search for alternatives to fossil fuels [1, 2]. In this scenario, biodiesel appears as the main substitute for this energetic matrix. In addition to being a renewable energy source, biodiesel has also lower power pollution [3]. However, because of government incentives, there was a huge growth in the production of this biofuel. Consequently, the availability of by-products (cakes, meals, and glycerin) also increased, being now considered a residual with disposal costs [4]. With this concern, the use of crude glycerin in animal diets has been explored as an economically and environmentally acceptable way to utilize this by-product.

Crude glycerin represents the main by-product of biodiesel production. Approximately 10% of the total volume of biodiesel produced becomes crude glycerin [5, 6]. The energy content of crude glycerin turns it into a promising ingredient for animal feed. The energy value of crude glycerin is similar to corn grain, being estimated as 3.47 Mcal/kg of dry matter (DM) for ruminants [7]. In this way, this by-product has been studied as a macro-ingredient since the 1950s [8, 9].

Crude glycerin is considered safe for use as animal feed since 2016 [10]. Due to the increased availability and relatively low cost, it became again the focus of studies as an alternative energy source in ruminants' diets [1, 11, 12], with promising results.

Crude glycerin is mainly composed of glycerol (more than 76%), an energetic compound of great assimilation by rumen microorganisms, being extensively metabolizable in the liver [13]. Once ingested and in the animal rumen, glycerol mostly disappears in the first 24 h [14]. It may be directly absorbed by the epithelium of the digestive system via passive diffusion (probably without facilitated diffusion carriers) and act as a gluconeogenic substrate in the liver [15, 16]. Glycerol may be transformed into propionate and butyrate [17, 18] by ruminal microorganisms, or it may outflow from the rumen through the omasal orifice without transformation [15, 16].

The fermentation of crude glycerin may promote better stability to rumen environment preventing metabolic disorders. It could also avoid severe reduction of ruminal pH and the development of rumen acidosis. These preventive actions are possible mainly by the reduction in lactic acidosis due to an increase in the population of lactate-consuming bacteria and undue fermentations [19].

On the other hand, crude glycerin may have a detrimental effect on the growth of structural carbohydrate-fermenting bacteria [17, 20]. Previous studies have shown a negative impact of crude glycerin on cellulolytic bacteria growth of [20], resulting in reductions in fiber digestibility [12, 21, 22], consequently decreasing DM intake and methane production [23]. However, in other studies these effects on rumen fermentation were not observed, as reported by [24, 25] with inclusions up to 12% of crude glycerin.

There are many controversies and inconsistent outcomes about crude glycerin inclusion in ruminant diets. Most of them are related to the end products of glycerol fermentation and its effects on ruminal microorganisms. However, promising results regarding growth performance and feed efficiency have been reported for inclusion up to 20% of crude glycerin in diets for feedlot lambs [26–28]. Therefore, this chapter discusses important aspects of different inclusions and associations of crude glycerin, including glycerol digestibility and metabolism and practical applications in sheep nutrition and production.

2. Crude glycerin

In general, glycerin is derived from renewable biological sources (such as vegetable oils or animal fats), occurring as triglycerides. Hydrolysis of these triglycerides results in crude glycerin and fatty acid (**Figure 1**). However, crude glycerin from animal source is prohibited for ruminants. A ban feeding ruminants with animal residues to ruminants is due to mad cow disease (bovine spongiform

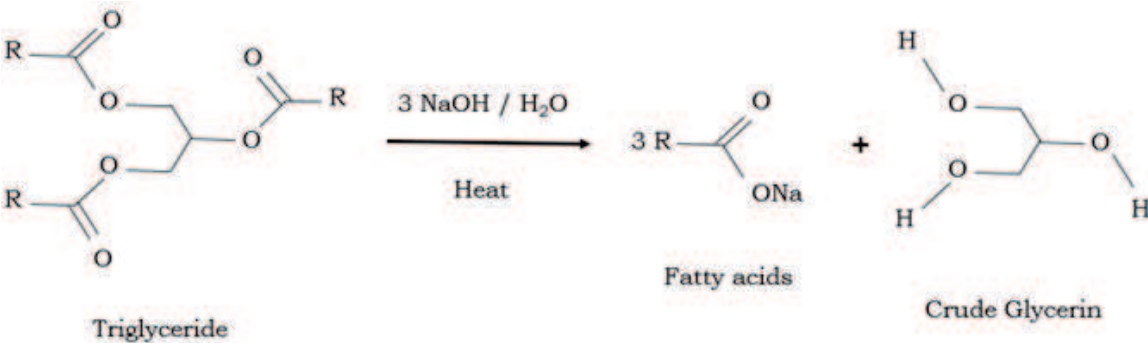


Figure 1.
Hydrolysis of triglyceride to fatty acids and crude glycerin.

Reference	Glycerol	Protein	Fat	Ash	Salts	Na	Methanol
Mach et al. [7]	857.0	—	—	—	55.0	—	0.09
Egea et al. [60]	875.0	—	—	59.0	—	20.0	0,05
Eiras et al. [64]	812.0	—	—	47.5	—	—	0.03
Paschoaloto et al. [74]	860.0	12.0	2.00	—	60.0	—	<0.01
Almeida et al. [26]	830.0	11.0	—	—	60.0	—	<0.01

Table 1.
Composition and nutritional value of crude glycerin used in ruminant nutrition (g/kg DM).

encephalopathy). This neurodegenerative disease has been linked to the practice of feeding the cattle with meat-and-bone meal putatively contaminated with scrapie agent [29]. For this reason, only crude glycerin derived from vegetable oils can be used for ruminant feeding.

Approximately 10% of the total volume of biodiesel produced becomes crude glycerin [5, 6]. Crude glycerin is a colorless, hygroscopic, and sweet-tasting viscous liquid. It is composed of glycerol and trace amounts of water and methanol. However, the chemical composition is dependent on glycerin purity and on the oil source utilized [30]. On average, crude glycerin contains 83% glycerol, 0.4% fat, 0.9% crude protein, 5.3% ash, 7.3% salts, and <0.05% of methanol (**Table 1**).

The energy value of crude glycerin is similar to corn grain, being estimated as 3.47 Mcal/kg DM for ruminants [7]. For this reason, crude glycerin becomes a promising ingredient for ruminant feed.

Methanol content in crude glycerin is very low, and in a total mixed ration, it is even lower. Thus, there are no problems associated with ingestion of methanol from crude glycerin by ruminants. According to [31], the high health risk associated with methanol consumption due to the inclusion of crude glycerin in diets is not expected in ruminant because methanol can be naturally produced in the rumen as a result of pectin fermentation. Normally, due to the type of crude glycerin used in ruminant diets (with more than 76% glycerol), it is not a problem. In Ref. [32], authors observed problems in the performance of feedlot lambs related to high impurity contents in crude glycerin (i.e., methanol, NaCl), even at low inclusions (12% DM). However, besides the high impurity, the crude glycerin used had low content of glycerol (<40%), which is not common to be seen on the market. Anyway, the methanol content should be monitored to avoid future problems.

3. Ruminal digestion and fermentation of crude glycerin

Glycerol constitutes an excellent substrate for gluconeogenesis and animal energy generation. It can be converted to glucose in the liver providing energy for cellular metabolism [33]. Once ingested, glycerol is totally metabolizable by the animal in the first 24 h [14]. Other authors reported even faster metabolization by microorganisms between 4 and 6 h [34]. It demonstrates the potential of crude glycerin as a rapid energetic source for ruminants.

Into the rumen, glycerol may be fermented and transformed in volatile fatty acids (VFA) by ruminal microorganisms (25–45%) or be directly absorbed through the rumen papillae (~43%) or escape the rumen by outflow through the omasal orifice without transformation due to its high solubility [15].

Glycerin fermentation changes the VFA profile of the rumen (total concentration and molar proportion) because it is preferentially converted to propionate and butyrate [35, 36]. Thus, the acetate/propionate rate is altered [37–39]. The

proportion of propionate and butyrate generally increases at the expense of acetate when diets are supplemented with crude glycerin. Recent studies with ruminal fermentation continuous culture [25] and feedlot goats [40] showed linear increases in propionic acid with linear decreases in acetic acid concentrations when crude glycerin replaced dietary corn up to 20%. No changes were observed for total VFA concentration in this work. However, several *in vivo* and *in situ* studies have reported increases in total VFA production [34, 41–43].

Differently, other authors evaluating the increasing inclusions of crude glycerin in diets for crossbred lambs (up to 30% DM, in [28, 44]) and Nellore bulls (up to 30% DM, [36]) observed a linear decrease in total VFA with no changes in propionic acid concentration. However, a pronounced reduction in acetic acid concentration was observed. These results can be explained mainly due to the deleterious effect of crude glycerin on rumen fibrolytic microorganisms, as described by [13, 20]. They reported reductions on *Butyrivibrio fibrisolvens* and *Ruminococcus flavefaciens* bacterial groups. These authors have also found a decrease in neutral detergent fiber (NDF) digestibility and in *Selenomonas ruminantium* and *Clostridium protoclasticum* population by increasing concentrations of glycerin. However, no effects were observed in rumen pH, in $\text{NH}_3\text{-N}$ concentrations, and in DM digestibility.

Reduction in methane (CH_4) production has been reported as a detrimental effect of crude glycerin inclusion on the growth of structural carbohydrate-fermenting bacteria. It also happened due to low production of total VFA and acetic acid in the rumen [28, 36, 45]. This reduction probably occurs because the amount of H_2 in the rumen decreases. Hence, availability for methanogenic microorganisms is used to reduce CO_2 to CH_4 [46, 47]. For this reason, the inclusion of crude glycerin in ruminants' diets becomes an interesting alternative for mitigating CH_4 emission by livestock.

The deleterious effect of crude glycerin on fiber fermentation was confirmed by [28, 36] when high inclusions of crude glycerin (up to 30% DM) in diets for feedlot crossbred lambs and Nellore bulls were studied, respectively. The authors observed a linear decrease in fiber fraction *in vitro* total tract digestibility when crude glycerin was included. Although the absolute yield of propionate was not affected by the increasing inclusion of crude glycerin, a linear increase in propionate proportion was observed.

The change in propionate relative proportion happened, probably, due to crude glycerin fermentation characteristics. Glycerin is fermented, mainly by bacteria of the genus *Selenomonas*, being mostly used by animals in the first hours after ingestion [7, 48]. Propionate produced in the rumen is readily absorbed through the ruminal wall, getting into the portal vein. Then, when reaching the liver, it is transformed into glucose and is called glucogenic VFA. The glucogenic pathway in ruminants is shown in **Figure 2**.

The mechanisms that determine the VFA production in quantity and quality can influence the rumen pH and their buffering [49]. When energetic ingredients (starch or soluble sugars) are added to ruminant diets, a reduction in ruminal pH is frequently observed. When ruminal pH is lower than 5.6, strong organic acids (like lactate, $\text{pK}_a = 3.86$) are produced on a major scale, which can cause a nutritional disorder, named ruminal acidosis. The consequences of ruminal acidification process can range from decrease of DM intake up to the animal's death.

Crude glycerin can prevent metabolic disorders, avoiding severe reduction on ruminal pH and the development of rumen acidosis due to glycerin fermentation profile. Inclusions in ruminal pH were observed when lambs were fed with up to 20 or 30% of crude glycerin [28, 44], respectively. During glycerol ruminal fermentation, there is no generation of lactic acid. This happens because propionic acid is produced by an alternative fermentative pathway via succinate [50]. For this reason,

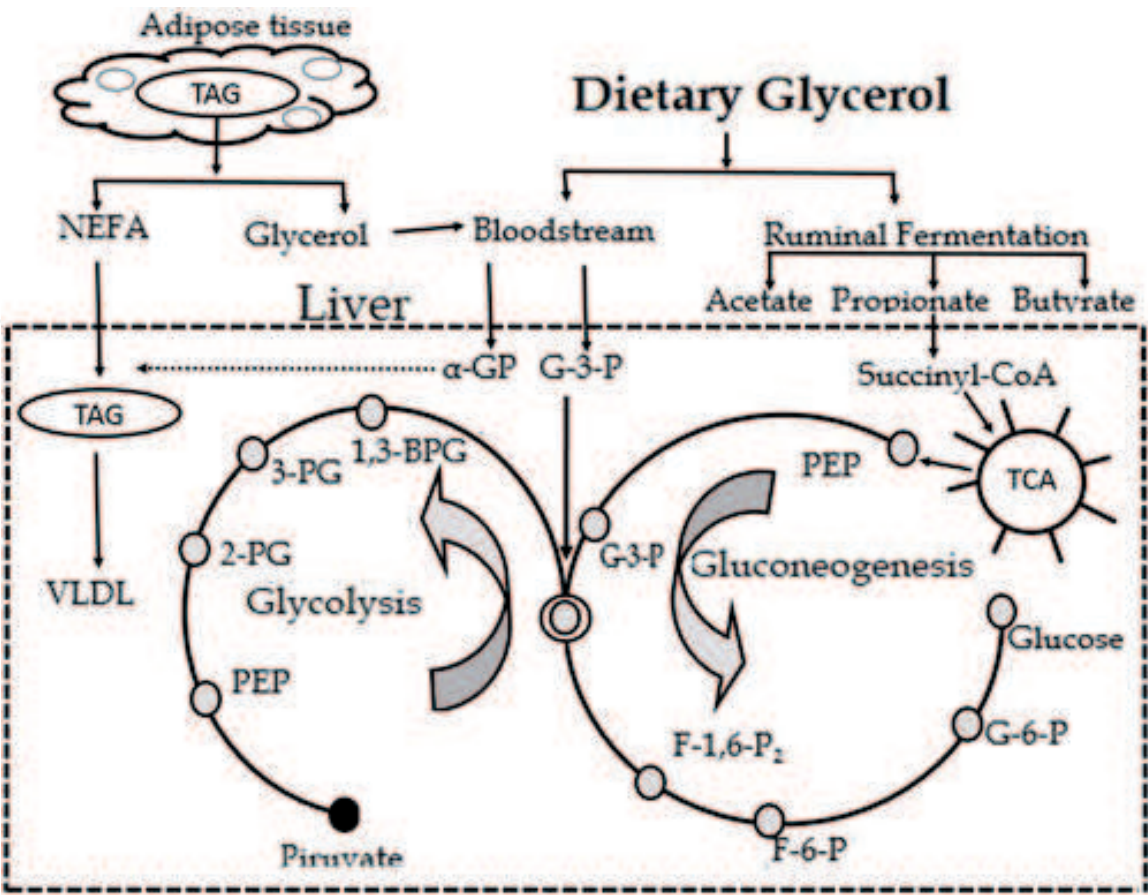


Figure 2.
Proposed metabolism of glycerol in ruminant animals (adapted of Osman et al. [41]).

crude glycerin may promote better stability to rumen environment compared with starch-rich ingredients. Lactic acidosis is reduced due to an increase in the population of lactate-consuming bacteria and undue fermentation [19]. As a consequence, some benefits are gained, such as rumen development and feed efficiency improvement [15, 16].

Authors in Ref. [50] evaluated the effects of increasing inclusions of crude glycerin (up to 30% DM) in diets for crossbred lambs in two different periods of feedlot (adaptation and finishing). The authors observed that crude glycerin did not compromise the stomach compartments and rumen papillae measurements in both periods of feedlot. No clinical manifestations resulted from ruminal acidosis (such as liver abscess, ruminitis, and lesions in ruminal mucosa) in any period and treatment studied. The authors concluded that the replacement of corn grain by crude glycerin (up to 30% DM) was effective in the animals' adaptation to concentrate-based diets. The good results were possible due to the better ruminal conditions provided by crude glycerin fermentation.

4. Effects on dry matter intake and growth performance

The use of crude glycerin has been investigated in diets for feedlot lambs [1, 27, 51–53]. The results are varied, regarding intake, digestibility, and performance. Probably, this may be related to the degree of purity of the crude glycerin used.

Authors in Ref. [1] evaluated the effects of high concentrations of crude glycerin (up to 30% DM basis) in diets for feedlot lambs. The authors reported positive results in animal performance when crude glycerin was included in 10% DM. Linear reductions

in dry matter intake (DMI) were also observed by the authors. However, when all crude glycerin treatments were compared with the control diet (10, 20, and 30% vs. control diet), no significant difference was observed. Authors [42, 54, 55], working with feedlot beef cattle, also observed a reduction in DMI when levels of glycerin were higher than 10%. In others works, even in low concentrations (<10% DM), the inclusion of crude glycerin promoted a drop in DMI for lambs and goats [32, 56, 57].

The lower value of DMI observed when crude glycerin was included in the diets may be explained by the deleterious selection of fibrolytic microorganisms, known to be sensitive to glycerin. Previous studies have shown that glycerol provides a selection of rumen microorganisms, mainly fibrolytic ones, affecting NDF digestibility. The reduction in NDF digestibility is attributed to the decrease in DNA concentration from bacteria *Selenomonas ruminantium* and *Butyrivibrio fibrisolvens* [13]. Furthermore, according to [20], glycerin decreases microorganism growth, cell membrane permeability, and adhesion of bacteria in feed. Thus, these facts can reduce the feed passage rate through the rumen. This results in ruminal filling, known as repletion state [58], consequently reducing DMI. Another plausible explanation for DMI reduction is the fact that ingestion of glycerol can improve animal metabolic status. This improvement occurs due to the increased energy intake from glucose with increased propionate and reduced acetate/propionate ratio in the rumen, satiating the animal in terms of energy [1, 15].

Authors in Ref. [40] fed goats with up to 20% DM and in Ref. [51] fed lambs with up to 30% DM of crude glycerin. The authors, contradicting the above mentioned, observed no difference in DMI in any inclusions offered to the animals. On the other hand, authors in Ref. [27] reported that inclusion of crude glycerin up to 15% DM in diets of finishing lambs increased DMI and feedlot performance in the first 14 days. According to these authors, the controversies observed in DMI are probably due to the purity level of the glycerin used. Other important factors were the forage/concentrate ratio, the amount of dietary starch and fiber used, and the tolerance and acceptability of the ingredient by each animal species. Thus, the best crude glycerin association should be used to avoid deleterious effects.

Nevertheless, despite the reductions in DMI, authors in Ref. [59] reported average daily gain (ADG) of 250 g/day with the inclusion of 12.5% DM of crude glycerin. Similarly, authors in Ref. [1] observed ADG of 332 g/day with the inclusion of 10% DM. In both studies, the crude glycerin used contained ~83% of glycerol, and the forage/concentrate ratio used was 40:60, the corn silage being the forage source. Furthermore, other researchers reported improvements in feed efficiency when crude glycerin was included up to 12% DM in diets for sheep and beef cattle [54, 55, 57].

5. Effects on carcass characteristics and meat quality

Recent studies did not present negative effects of crude glycerin inclusion on carcass and meat traits [7, 27, 60]. The inclusion up to 30% crude glycerin in diets for feedlot lambs promoted lower carcass weights [1, 61]. However, other studies have demonstrated that the inclusion of this by-product has effects on carcass grade [1, 62]; increases the intramuscular fat and oleic acid content [63]; decreases myristic, palmitic, and stearic acids in *Longissimus muscle* [64]; increases the monounsaturated fatty acid (MUFA) content and conjugated linoleic acid content [65]; decreases saturated fatty acid; and increases unsaturated and odd-chain fatty acid contents [62].

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 precursor absorption [66]. The change in these features can also be achieved by increasing unsaturated fatty acid content on meat [63, 64, 67] possibly due to ruminal lipolysis inhibition [19, 68].

It is known that inclusion of crude glycerin in diets decreased the DNA concentration for *Selenomonas ruminantium* and *Butyrivibrio proteoclasticus* [13, 17], important bacteria in biohydrogenation process [69–72]. This fact suggests that when glycerol is incorporated in diets, it may reduce lipolysis and biohydrogenation in the rumen, promoting a greater flow of unsaturated fatty acids to be incorporated in meat [19, 68]. For these reasons, the crude glycerin inclusion in diets for ruminants promotes a healthier meat for humans.

6. Crude glycerin as a strategy ingredient

Crude glycerin has been recently studied as a great ingredient to prevent metabolic disorders when high inclusions of concentrate were offered to animals in feedlot systems [26, 50, 73, 74]. Because of its own fermentation profile, crude glycerin can avoid severe reduction of ruminal pH and the development of rumen acidosis. The larger portion of crude glycerin (~43%) is rapidly absorbed by rumen papillae, whereas 25–45% are fermented to butyrate and propionate by alternative fermentative pathway (via succinate) and do not generate lactic acid, benefiting the rumen development and thus improving the feed efficiency [15, 16, 50].

When animals are submitted to a feedlot management, abrupt changes occur in their diets. Generally, high inclusions of starch-rich cereal grains are used. Then, a correct adaptation to diets should be done, mainly in the first 2 weeks [75]. When animals are not correctly adapted to diets, changes in feeding behavior with reduced DMI [76]; clinical manifestations such as laminitis, liver abscesses, and ruminitis; and inappropriate ruminal fermentation [77, 78] are prevalent. Recent studies have shown that crude glycerin is effective in the animals' adaptation to concentrate-based diets [27, 50]. Authors in Ref. [50] evaluated the effects of increasing inclusions of crude glycerin (up to 30% DM) in diets for crossbred feedlot lambs in two different periods (adaptation and finishing). The authors observed that crude glycerin did not compromise the stomach compartments and rumen papillae measurements in both periods of feedlot. No clinical manifestations resulted from ruminal acidosis in any period and treatment studied. Authors in Ref. [27] reported that the feed efficiency during the first 14 days of the feedlot was greater for all glycerin-fed lambs when compared with lambs not fed with glycerin.

Apparently, crude glycerin may have stimulated rumen growth due to its fermentative characteristic, mainly by increasing the total rumen VFA [14]. The presence of VFA in ruminal lumen promotes the growth and proliferation of papillae. With increased absorption area, the capacity of removing these acids is improved as well as the provision of greater energy absorption for the animal [79–81], thus improving the animal performance. In this way, crude glycerin presents good aspects to the adaptation physiological process and can be indicated to adapt the animal to concentrate-based diets, mainly in the first 2 weeks of the feedlot.

7. Crude glycerin practical recommendations

Because of its high viscosity (~8.5 cSt), crude glycerin can be used to change the physical form of diets. It helps in particle aggregation, thus facilitating the feed intake. Inclusions up to 30% can be used for this purpose. However, depending on the diet ingredients composition, an accumulation of the ingredient in the feed bunk bottom may occur.

8. Conclusions

Based on what has been discussed in this chapter, crude glycerin can be considered as a good energy ingredient for sheep nutrition. At present, inclusions up to 20% DM in a total diet seem to be the most interesting strategy. They promote greater animal performance and a healthier meat for humans. However, other studies suggest that higher inclusions of crude glycerin (30% of dry matter) could be possible depending on market price and the farm operation structure with favorable economic results.

Conflict of interest

The authors declare no known conflict of interest.

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