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Utilization of Crude Glycerin in Ruminant Diets

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Abstract: The intensive use of grains as feed in ruminant production systems raises concerns about future food security. The replacement of food raw materials used in animal nutrition with industrial by-products and other waste biomass that are not suitable for human consumption is viewed as the most important potential strategy that can decrease food-feed competition and increase the profitability of animal production. Crude glycerin (CG) is a byproduct of the biodiesel industry and has been used as an alternative to grains in ruminant diets in recent years. Glycerol, which is the main component of CG, rapidly participates in energy metabolism by undergoing fermentation to propionate and butyrate in the rumen. Depending on the biodiesel production method, the effect of CG, which is obtained at varying levels of purity, on ruminants may vary according to the amount of glycerol, and other impurities in it and the amount of CG added to the diet. Due to this variability, inconsistent results are obtained regarding the efficiency of CG usage in ruminants. Based on the literature, it was concluded that CG could be used in ruminant diets at levels of 10-15% of dry matter after taking into account the levels of impurities without adversely affecting the performance.
Keywords: Biodiesel, by-product, crude glycerin, glycerol, ruminant

Ruminant Rasyonlarında Ham Gliserin Kullanımı

Öz: Ruminant beslemede tahılların yoğun olarak kullanılması geleceğin gıda güvenliği konusunda endişelere yol açmaktadır. Hayvan beslemede kullanılan gıda hammaddelerinin insan tüketimine uygun olmayan sanayi yan ürünleri ve bazı diğer atık biyokütleler ile değiştirilmesi gıda-yem rekabetini ve hayvansal üretimin karlılığını artırabilecek en önemli potansiyel strateji olarak görülmektedir. Ham gliserin (HG), biyodizel endüstrisinin yan ürünüdür ve son yıllarda ruminant rasyonlarında tahıllara alternatif bir enerji kaynağı olarak kullanılmaktadır. HG'nin ana bileşeni olan gliserol, rumende hızlı bir şekilde propiyonat ve bütirata fermente olarak enerji metabolizmasına katılmaktadır. Biyodizel üretim metoduna bağlı olarak değişken saflıklarda elde edilen ham gliserinin ruminantlar üzerindeki etkisi içerisindeki gliserol ve diğer kirliliklerin miktarına ve rasyona eklenme düzeyine göre değişkenlik gösterebilmektedir. Bu değişkenlik sebebiyle ham gliserinin ruminantlarda kullanım etkinliği konusunda çok çeşitli sonuçlar elde edilmektedir. Literatür bulguları değerlendirildiğinde HG'nin içeriğindeki kirlilikler gözetilerek ruminant rasyonlarında kuru maddenin %10 ila 15 düzeylerinde performansı olumsuz etkilemeden kullanılabileceği sonucuna varılmıştır.
Anahtar kelimeler: Biodizel, gliserol, ham gliserin, ruminant, yan ürün

Introduction

Biodiesel is a liquid fuel, a mono alcohol ester obtained in the reaction of vegetable, animal, or waste oils (e.g., frying oil, grease trap) with alcohol (methanol or ethanol) in the presence of catalysts (acid, alkali, or enzyme). As a result of this process called transesterification, methyl or ethyl esters (biodiesel) are obtained as the final product, and approximately 0.1 m^3 of crude glycerin (CG) is obtained as a byproduct for every 1 m^3 of biodiesel produced (Kholif, 2019). Glycerol obtained during biodiesel production is called CG because it mixes with contaminants such as alcohol, catalysts, salts, and fatty acids. Whereas the term glycerol (1,2,3-propanetriol) is used to describe the pure substance whose physical and chemical structure is known, the term glycerin is generally used for commercial products containing more than 95% glycerol in aqueous solution (Knothe et al., 2005). The percentage of glycerol in CG can vary from 45–90% depending on the raw material used for production, reaction conditions, and to what extent CG is refined by the biodiesel plant.

Decreasing fossil fuel resources in the world, increasing greenhouse gas emissions, and unstable oil prices direct the policies of different countries to seek alternative energy resources that are sustainable, renewable, cost-effective, and environmentally friendly. According to the European Union's "2030 Framework for Climate and Energy Policies" statement (Parliament Regulation, 2018), it is aimed to increase the share of renewable energy sources in the global

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energy supply to 32% and reduce greenhouse gas emissions by 40% by the year 2030. Biodiesel production has increased rapidly in recent years due to the lower cost of biodiesel compared to that of many other renewable energy sources such as wind and solar energy. Global biodiesel production, which was 39 billion liters by 2020, is expected to increase to 46 billion liters by 2029 (OECD/FAO, 2020). Biodiesel production in Turkey increased by 112% in the last five years, reaching 134 million liters in 2019 (BSD, 2019).

With the rapidly increasing biodiesel production globally, there exist some difficulties in terms of sustainable utilization of crude glycerol, which constitutes nearly 10% of the final product. Generally, glycerol has more than 1500 applications in various categories and industries, such as personal care, cosmetics, medicine, and food industries. Although these widespread applications are limited by the purity of the product, attaining 99% purity of CG requires additional costs. Therefore, as is the case with many biofuel by-products such as distillers dried grains and soluble obtained from ethanol production (Şahin et al., 2013), the feed industry is viewed as one of the potential markets in which CG may be utilized without additional purification.

Feed costs constitute the largest share of expense in livestock production systems (Elmalı et al., 2010). Besides, the intensive use of grains in traditional animal nutrition raises concerns about future food security. Therefore, it is advantageous to include economic products such as CG in ruminant diets as an alternative to grains in terms of profitability and reducing food–feed competition. In ruminant feeds, glycerol was first used as a drench in the ketosis treatment of dairy cows with negative energy balance as a glucogenic precursor (Goff and Horst, 2001; Linke et al., 2005). As CG became a surplus product in the global market and its price decreased considerably, it was started to be used as an alternative energy source in animal diets instead of starch-based ingredients such as corn, which are generally widely used in ruminant diets (Musselman et al., 2008; Gunn et al., 2010). Moreover, based on the hypothesis that glycerol reduces rumen biohydrogenation and lipolysis (Edwards et al., 2012), its application in increasing beneficial fatty acids in meat and milk has been the subject of research in recent years (Carvalho et al., 2015; Fiorentini et al., 2018). In this review, the effects of CG added to ruminant diets on performance, digestibility, and levels of the certain rumen and blood parameters, as well as that of meat and milk fatty acid composition are presented to evaluate its effectiveness in ruminant nutrition.

Nutritional content of crude glycerin

The energy content of glycerol is very close to that of corn with an estimated 1.98–2.27 Mcal/kg dry matter (DM) net energy lactation (NEL) and 4.32 Mcal/kg DM gross energy values (Schröder and Südekum, 1999) for ruminants. The energy value of CG can vary according to the amount of glycerol present. As long as the composition of methanol or other pollutants in CG is at acceptable levels, their use in ruminant feeding is considered safe (FDA, 2006). The most important factor limiting the use of CG in animal nutrition is the level of methanol present in it. Methanol in CG can be found up to 37.5%, depending on the method used during biodiesel production. While EFSA (2010) determined the upper limit of methanol in raw glycerin obtained from vegetable origin raw materials that can be used in feed as 0.2%, FDA (2006) determined the maximum upper limit of methanol in animal feed as 150 ppm. Methanol at the specified levels is considered extremely safe for ruminants as it is rapidly metabolized by the rumen flora to methane, carbon dioxide, and water. However, when more than the amount of methanol that can be metabolized in the rumen is consumed, severe effects such as a decrease in milk yield, anorexia, dullness, and sudden death could occur (Nazato et al., 2019). However, in studies where CG was used in animal diets, no toxic effects were reported due to high methanol levels in CG. In a study investigating the effect of crude glycerin containing 26.7% methanol in beef cattle, it was reported that there was no change in the nutrient digestibility and DM consumption of animals fed with 1.1 L/day CG compared to the control group (Schröder and Südekum, 1999). In another study, 12% CG (containing 8.7% methanol) was added to lamb diets, and no adverse effects were reported (Lage et al., 2014).

The second important pollutant in CG after methanol is sodium. Salt (NaCl), which is used as a catalyst in biodiesel production, can be found in CG at levels of up to 6.6% (Knothe et al., 2005). High sodium levels in the diet can be tolerated as long as the animal consumes sufficient amount of water; however, in some cases, it can negatively affect rumen fermentation and reduce appetite and energy use efficiency (Knothe et al., 2005). High dietary salt and sodium levels may cause electrolyte imbalances in animals and reduce the acceptability of the diet with CG. Ezequiel et al. (2015) indicated that diets containing 300 g/kg DM of CG increased the dietary content of NaCl by 500% and reduced feed intake of dairy cows.

CG can be obtained from any renewable biological oil source (such as vegetable oils or animal fats) consisting of triglycerides. However, as it is known, feeds of animal origin are prohibited in ruminant feeding due to their association with bovine spongiform encephalopathy. Hence, only CG obtained from vegetable oils can be used in the diet of ruminant animals.

Glycerol metabolism

In ruminants, most of the glycerol is fermented by rumen bacteria, mainly propionic acid and butyric acid. Whereas glycerol converted to butyrate is metabolized to β-hydroxybutyrate (BHBA) by the rumen epithelium (ketogenic), propionate can be directly absorbed from the rumen wall and participate in glucose synthesis in the liver (glycogenic). The low amount of glycerol that passes through the rumen and reaches the small intestine can be absorbed from there and used in glucose synthesis in the liver as a glycogenic substrate (Kholif, 2019). In the liver, glycerol is converted into triose phosphate and then to glucose via gluconeogenesis (Figure 1.) or catabolized by glycolysis (Carvalho et al., 2015). In a study examining the metabolic pathways of glycerol in rumen-cannulated cows, it has been reported that 25% of the glycerol entering the rumen is fermented in the rumen, 45% is absorbed by passive diffusion through the rumen wall, and 30% leaves the rumen through the omasal groove (Werner Omazic et al., 2015). In another study by Rémond et al. (1993) investigating glycerol metabolism in the rumen with cannulated cows, it has been reported that 32 g out of 250 g of glycerol ingested were passed to the omasum, 105 g were fermented, and 103 g were absorbed from the rumen wall. However, the mode of glycerol administration to the animal is another factor affecting the rumen metabolic pathway. It has been reported that a large quantity of glycerol is fermented in the rumen if administered as mixed diet, whereas most of it is absorbed from the rumen wall when administered as a drink (DeFrain et al., 2004).

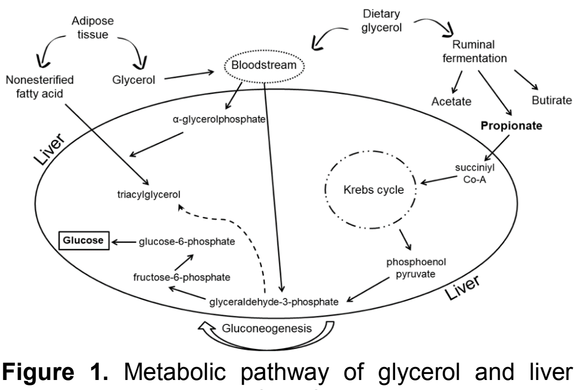

Figure 1. Metabolic pathway of glycerol and liver gluconeogenesis, modified from Kupczyński et al. (2020).

Effect of crude glycerin on dry matter intake and performance

The effects of CG on DM intake (DMI) and fattening performance vary depending on the degree of purity and the level at which it is added to the diet. In many studies, it has been reported that the use of CG at a level over 10% of the DM diet negatively affects DMI (Musselman et al., 2008; Gunn et al., 2010; Chanjula et al., 2016). In related studies, it has been suggested that high levels of CG supplementation cause a decrease in DMI by negatively affecting rumen fermentation. Furthermore, it is suggested that glycerol fermentation increases propionate concentration in the rumen, and propionate at increased levels acts as a DMI inhibitor in ruminants (Allen, 2000). According to another hypothesis, CG increases the energy density in the diet by inducing a chemical feeling of satiety, and eventually, DMI is suppressed (Saleem and Singer, 2018). Fiorentini et al. (2018) stated that the DMI decrease might be related to the decrease in the taste of the diet due to the presence of impurities such as methanol in CG.

The effects of CG on daily body weight gain (DBWG) and feed conversion rate (FCR) are mostly compatible with that on DMI. Versemann et al. (2008) and Parsons et al. (2009) reported that the addition of CG up to a level of 10% in beef cattle diets increases DBWG and the addition of CG at levels over 10% caused a decrease in DBWG by suppressing DMI. In some studies using CG, an increase in fattening performance was observed despite the decrease in DMI. This situation generally occurs due to balancing of the decreasing DMI with a high metabolisable energy value and high nutrient digestibility in the glycerol groups (Saleem and Singer, 2018). Moore et al. (2011) observed a positive increase in DBWG and FCR in fattening cattle fed with diets containing CG at 0%, 3%, 6%, and 9% levels, although DMI decreased. In their study, Gunn et al. (2010) added 0%, 5%, 10%, 15%, and 20% CG to lamb diets, and they associated the increase in DBWG, up to 15% CG addition to the increases in DMI, and the decrease in DBWG observed in the group with 20% CG to the decrease in DMI due to the decreased rumen fermentation. Consistent with this study, in a study where 0%, 15%, 30%, and 45% levels of CG were added to lamb diets, the addition of more than 15% CG to the diets was reported to negatively affect DMI, DBWG, and FCR (Musselman et al., 2008). In a study by Avila-Stagno et al. (2013), they added 0%, 7%, 14%, and 21% CG to lamb diets and reported that DMI and DBWG were decreased, although no significant effect was observed in FCR at a level of over 7% CG addition.

In general, current studies report that the addition of CG up to levels of 10%–15% does not harm fattening performance; however, its use above these levels damages rumen fermentation.

Effect of crude glycerin on nutrient digestibility

CG can adversely affect cellulose digestion by suppressing cellulolytic bacterial activity in the rumen. Paggi et al. (1999) reported that as the *in vitro* glycerol concentration in rumen culture increased from 50 to 300 mM, the cellulolytic activity decreased by 8–15%. El-Nor et al. (2010) investigated the effect of different levels of glycerol (0, 36, 72, and 108 g glycerol/kg DM) on bacteria by measuring the DNA concentrations of some bacteria in *in vitro* culture of rumen samples. They observed that the number of cellulolytic bacteria (*Butyrivibrio fibrisolvens* and *Selenomonas ruminantium*) decreased in diets containing 72 g and 108 g glycerol. However, it has been reported that increased glycerol amount has no effect on acid detergent fiber (ADF) digestibility, and it negatively affects neutral detergent fiber (NDF) digestibility. In studies by Chanjula et al. (2014) and Lage et al. (2017), respectively, on beef cattle diets containing CG up to 20 and 10%, CG level did not have a significant effect on DM, crude protein (CP), crude fat, NDF, and ADF digestibility. On the other hand, in a study by Ribeiro et al. (2018) in which 0, 7, 14, and 21% CG was added to lamb diets, they reported that the digestibility of DM, CP, and NDF decreased in proportion to the increasing amount of CG.

Literature survey reveals that high CG level in ruminant diets suppresses the activity of cellulolytic bacteria. In this context, it should be considered that the use of glycerol, especially in concentrate diets, may create an unsuitable environment for cellulolytic bacterial species and may adversely affect NDF and ADF digestion.

Effect of crude glycerin on ruminal fermentation

Ruminal pH

The diet composition of ruminants is the most important factor that shapes the microbial flora and fermentation parameters in the rumen (Kaya, 1997). High starch diets decrease ruminal pH by increasing the amount of lactate produced by homolactic species such as *Streptococcus bovis* and *Selenomonas spp.,* thereby promoting their growth. Glycerol is metabolized in the rumen mainly by lactate-metabolizing bacteria. Thus, lactate can be removed from the rumen environment, and the decrease in ruminal pH can be prevented. However, there are conflicting reports about ruminal pH findings in studies using CG. Van Cleef et al. (2018) reported that the ruminal pH increased with increasing levels of CG in lamb diets; Chanjula et al. (2014) reported that CG addition does not affect ruminal pH; whereas Lage et al. (2017) reported that ruminal pH decreases with the addition of CG. The differences between the ruminal pH values obtained in the related studies are believed to be caused by diet composition rather than CG levels.

Ruminal ammonia nitrogen

Paggi et al. (1999) determined that glycerol reduced the proteolytic activity in rumen fluid by 20% and suggested that it may also decrease the ruminal NH_3–N level. Consistent with this study, Wang et al. (2009) reported that the ruminal NH_3–N concentration decreased in parallel with the increase in the CG ratio in beef cattle diets. Syahniar et al. (2016) reported that ruminal NH3–N level decreased with the increase in the glycerol level in the diet, and this situation may be caused by lower levels of proteolytic and/or deaminating activity during ruminal fermentation. Kijora et al. (1998) found that the addition of glycerol at the level of 10% of the DM in the diet to rumen and duodenal cannulated bulls reduced the total NH_3–N concentration and the proportion of bacterial nitrogen in the rumen. Chanjula et al. (2014) reported that CG added to goat diets at a rate of 20% decreased the ruminal NH_3–N concentration. However, El-Nor et al. (2010) did not find a significant change in the NH_3–N concentration between the groups in their study in which they added different levels of glycerol (0, 36, 72, and 108 g glycerol/kg DM) in *in vitro* fermenters.

Rumen microbial population and rumen volatile fatty acids

Glycerol is metabolized in the rumen mainly by *Megasphaera elsdenii*, *Streptococcus bovis,* and *Selenomonas ruminantium* (Hobson et al., 1997). *Selenomonas ruminantium* and *Streptococcus bovis* are the main fermenters of glycerol, and the main products of fermentation are propionate, lactate, succinate, and acetate. *Megasphaera elsdenii*, on the other hand, metabolizes the lactate formed by species such as *Streptococcus bovis* and *Selenomonas ruminantium* to propionate and butyrate with acrylate as an intermediate and indirectly takes part in glycerol metabolism (Shin et al., 2012). Therefore, as a result of the rumen metabolism of glycerol, the amount of propionate increases mainly in the rumen, whereas that of butyrate increases to a lesser extent. However, fermentation of glycerol in the rumen is largely dependent on the ratio of roughage to concentrate in the diet. For example, when high levels of carbohydrate sources such as easily fermentable starch are present in the diet, the contribution of the acrylate pathway is high, and when the level of cellulose in the diet is high (roughage-based diet), the contribution of the acrylate pathway is negligible (El-Nor et al., 2010). Hence, the increase caused by glycerol in the ratios of propionate and butyrate in concentrated feed diets in the rumen may be higher.

In many *in vivo* and *in vitro* studies investigating the effect of glycerol in ruminant feeding, an increase in the total volatile fatty acid (TVFA) levels, mainly those of propionic and butyric acid, and a decrease in acetate/propionate ratios were observed in glycerol fermentation (El-Nor et al., 2010). In addition, with the increasing amount of CG in the diet, there was an increase in butyric acid and valeric acid concentrations, although no difference was observed in iso-butyric and iso-valeric acid levels (Van Cleef et al., 2015). In a study in which CG was added to goat diets at 0, 5, 10, and 20% levels, there was no difference between the ruminal TVFA concentration and the molar ratios of butyrate and other volatile fatty acids (iso-butyrate, iso-valerate, valerate, and caproate). However, with increasing CG levels, a decrease in the molar ratio of acetate and an increase in that of propionate have been reported (Chanjula et al., 2014). In another study in which CG was added to lamb diets, a linear decrease was observed in total molar concentrations of TVFA, acetic, butyric, isobutyric, and iso-valeric acids, whereas no change was observed in propionic and valeric acid levels (Van Cleef et al., 2018). In an *in vitro* study with experimental groups fed with diets containing 0, 10, and 30% CG on the DM basis, propionate and TVFA concentrations increased linearly in parallel with the increase in the amount of CG, whereas acetate, butyrate, iso-valerate, and acetate:propionate ratios were decreased (Benedeti et al., 2015).

Effect of crude glycerin on blood parameters

Glycerol is used as a glucose precursor in hepatic gluconeogenesis. Therefore, an increase in serum glucose concentration is expected as a result of the addition of CG to ruminant diets (Gunn et al., 2010). Chanjula et al. (2014) and Gomes et al. (2011) added CG to goat and lamb diets, respectively, and reported that there was no significant change in glucose levels between groups, although there was a numerical increase in serum glucose levels in parallel with the increasing CG level in the groups. Contrary to these studies, Gunn, Schultz, et al. (2010) reported a significant decrease in serum glucose concentrations with increasing glycerol levels in lamb diets. Researchers have reported that the unexpected decrease in blood glucose level due to glycerol supplementation may be due to the decrease in DMI. In contrast, in studies on lambs by Saleem and Singer (2018) and Terré et al. (2011), it was reported that CG does not affect serum total protein, albumin, globulin, total lipid, cholesterol, and glucose concentrations.

The effects of CG on serum glucose concentration are directly related to the mode of CG administration to animals. Linke et al. (2005) and Goff and Horst (2001) compared two different administration methods, namely glycerol as a drink and as an additive to feed, and reported that ingestion of glycerol by drinking was more effective in increasing plasma glucose and insulin concentrations, whereas the administration of glucose in the form of feed supplements increased blood BHBA levels. When glycerol is administered as a feed supplement, it gets fermented to butyrate, which has a ketogenic nature in the rumen, and therefore glycerol may show a ketogenic effect rather than a glycogenic effect. However, the rumen fermentation of glycerol administered via drench is more limited, and glycerol, which reaches the intestines bypassing the rumen without being converted to butyrate, is absorbed from the intestine and used as an effective glycogenic substrate in the liver (Linke et al., 2005).

Previous reports support that administration of CG to animals via the drench route is the most effective in increasing serum glucose levels. Therefore, drench glycerol application may be advantageous for dairy cows that experience ketosis as a result of negative energy balance during the transition period.

Effect of crude glycerin on meat fatty acid composition

After understanding the beneficial effects of polyunsaturated fatty acids (PUFA) on human health in recent years, it is aimed to increase the polyunsaturated/saturated fatty acid ratio (PUFA/SFA) in ruminant meat and dairy products with various dietary modifications. Ruminants transform the dietary unsaturated fatty acids into SFAs by hydrogenating them using the biohydrogenation (BH) mechanism in their rumen. Glycerol has the potential to inhibit the lipolysis stage, which is the preliminary stage of BH (Edwards et al., 2012). With this effect of glycerol that disrupts the BH mechanism, and more PUFA flow occurs from the rumen to the abomasum. With the increase in the amount of PUFA that passes from the rumen without hydrogenation, the availability of PUFAs in meat and dairy products derived from the animal could increase.

Based on the suppressive effect of glycerol on BH, it has been hypothesized that CG supplementation may increase the amount of PUFA in ruminant products. Terré et al. (2011) investigated the effect of 0, 5, and 10% CG addition to lamb diets on the fatty acid profile of lamb meat. They reported that although CG did not affect the levels of PUFA, SFA, and n-6/n-3 ratio in meat, the amounts of C12:0 and C17:0 fatty acids were higher, and that of C18:1 fatty acid was lower in the groups that were administered glycerol. Avila-Stagno et al. (2013) added CG to lamb diets at levels of 0, 7, 14, and 21%, reported that linoleic acid (C18:2 n-6) percentage in total fatty acids (TFAs) in meat decreased linearly with increasing glycerol concentration, whereas that of α-linolenic acid (C18:3 n-3) did not change. Additionally, among SFAs, the level of palmitic acid (C16:0), which leads to cholesterol accumulation in humans (Carvalho et al., 2015) , was reported to decrease and those of stearic acid (18:0), which has no adverse effects on cholesterol accumulation, and oleic acid (C18:1), which is associated with the concentrations of cholesterol (high-density lipoprotein) known as beneficial cholesterol in humans, were reported to increase. Carvalho et al. (2015) supplemented lamb diets with 0, 7.5, 15, 22.5, and 30% CG and reported that CG decreased stearic, palmitic, transvaccenic, and total SFA percentage in TFAs in meat, while increased odd-chain fatty acids, oleic, palmitoleic, total unsaturated, and monounsaturated fatty acids percentage. Borghi et al. (2016) added 0, 10, and 20% CG to lamb diets, and they did not observe any significant changes in other fatty acids, except for an increase in margaric acid percentage in TFAs in meat. Fiorentini et al. (2018) supplemented beef cattle diets with soybean oil and CG. They found that animals given soybean oil incorporated 32% more linoleic acids and 40% more conjugated linoleic acids into their muscles than those given CG.

Effect of crude glycerin on lactation performance and milk composition

Because CG increases the density of energy in a lactating animal's diet, it may improve lactation performance (Kholif, 2019). Lomender et al. (2012) observed an enhanced milk yield in cows supplemented with 450 g glycerol/day. Also, Thoh et al. (2017) reported that increasing CG supplementation from 0 to 5% of DM in goats' diet increased milk yield from 2.38 to 2.64 kg/goat/day. Inconsistent with these findings, in a study by Ezequiel et al. (2015), in which 0, 15, and 30% CG was added to dairy cow diets, they reported that DMI and milk yield decreased numerically in proportion to the increasing amount of CG. They also indicated that feeding diets that replace corn with crude glycerin at 30% of dietary DM significantly reduced animal performance. Similarly, Pavia et al. (2016) added 0, 70, 140, and 210 g of CG/kg DM to lactating cows' diets and observed a linear decrease in DMI (15%) and milk yield (3.1 kg/day) as the glycerol content in the diet increased. The authors described CG's negative effects on animal performance in four ways: 1) the impurities in CG, such as methanol and salts (Ezequiel et al., 2015), 2) the rate at which glycerol is fermented in the rumen (Rémond et al., 1993), 3) the rumen epithelium's ability to absorb glycerol to be metabolized in the liver (Krehbiel, 2008), 4) increasing the production of Krebs cycle intermediates that stimulating satiety (Allen, 2000).

Several studies indicated that the addition of glycerol to ruminant diets depressed milk fat yield and milk fat content (Ezequiel et al., 2015; Thoh et al., 2017; Freitas et al., 2020). The main lipogenic precursor in the mammary gland is acetate, and it is absorbed and incorporated into milk fat. As previously stated, glycerol increases ruminal propionate concentration and decreases the acetate/propionate ratio, which may be the primary cause of milk fat depression (Kholif, 2019). On the other hand, glycerol feeding can improve milk protein synthesis by increasing dietary energy and blood insulin levels (Willbert et al., 2013). Insulin has been shown to play an important role in the synthesis of milk protein in dairy cows (Hurley et al., 2012). Another factor that might be related to the increase in milk protein content is the greater production of propionate in the rumen. The increase of propionate in the rumen decreases the use of gluconeogenic amino acids for glucose synthesis or oxidation for energy production, allowing it to be used for protein synthesis in the mammary gland.

Ruminant milk fat is a major source of odd-chain fatty acids (OCFAs), which are primarily produced by rumen microorganisms. Glycerol can stimulate the production of OCFAs by increasing the available primers (e.g., propionate) for rumen microorganisms. Ezequiel et al. (2015) reported that OCFAs and conjugated linoleic acid in milk fat increased linearly with the addition of CG to cow diets. On the other hand, adding CG to ruminant diets can increase beneficial fatty acids like unsaturated fatty acids (UFA) and biohydrogenation intermediates (trans-11 C18:1 and cis–9 C18:1) in milk because glycerol inhibits rumen biohydrogenation. Freitas et al. (2020) observed that supplementing lactating goats' diets with CG at 0, 7, 14, and 21% of dietary DM linearly increased UFA, UFA/SFA, and cis-9, C18:1 while linearly decreasing SFA concentration in milk.

Conclusion

With the rapid surge in global biodiesel production, the surplus amounts of CG obtained as a byproduct threatens the sustainability of the biodiesel sector by causing environmental concerns. Meanwhile, the animal feed sector is under pressure due to food-feed competition and food security discussions associated with the intensive use of grain. Therefore, the effective utilization of CG as an alternative to grains in ruminant feed is strategically important in alleviating environmental problems and improving the profitability of both sectors. In conclusion, CG can be used in ruminant diets at levels of 10-15% of DM without adversely affecting the performance. However, elucidating the level of impurities and glycerol in CG is critical in determining the optimal level of CG to be added to the diet without adversely affecting performance, nutrient digestion, and rumen fermentation. Moreover, although there is a limited number of studies investigating the effects of CG on meat fatty acid profile, current literature data support that CG supplementation partially increases the levels of beneficial fatty acids in meat.

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