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GLYCEROL IN THE DIET OF RUMINANT ANIMALS: ADVANTAGES OF ITS USE

EL GLICEROL EN LA ALIMENTACIÓN DE ANIMALES RUMIANTES: VENTAJAS DE SU UTILIZACIÓN

A. DELGADO*, JUANA L. GALINDO

Instituto de Ciencia Animal, C. Central, km 47 ½, San José de las Lajas, Mayabeque, Cuba

** Email: alvaro85.del@gmail.com*

This study delves into some topics related to the use of glycerol in ruminant feeding. The main ways to obtain glycerol are discussed and its properties are characterized. Its energetic value is highlighted due to the importance of its inclusion in diets for animals. Some studies are analyzed in which the use of glycerol in dairy and beef cattle was evaluated, as well as its effect on intake and productive indicators. In addition, general aspects related to metabolism are emphasized and the limitations related to its use are exposed. It is concluded that glycerol, which is obtained as a by-product in the manufacture of biofuels, can be included as an energy concentrate in diets intended for ruminants, as long as its methanol level is considered.

Keywords: *glycerol, methanol, ruminants, energy value*

Se profundiza en algunos temas relacionados con el uso de glicerol en la alimentación de rumiantes. Se tratan las vías principales para la obtención del glicerol y se caracterizan sus propiedades. Se resalta su valor energético por la importancia que tiene su inclusión en las dietas destinadas a animales. Se refieren algunos trabajos en los que se evaluó la utilización del glicerol en bovinos de leche y de carne, así como su efecto en el consumo y los indicadores productivos. Además, se enfatiza en aspectos generales vinculados al metabolismo y se exponen las limitaciones relacionadas con su uso. Se concluye que el glicerol, que se obtiene como subproducto en la fabricación de biocombustibles, se puede incluir como concentrado energético en las dietas destinadas a rumiantes, siempre que se considere su nivel de metanol. Se recomienda el desarrollo de investigaciones futuras para demostrar sus características funcionales que permitirá la diversificación de su utilización y comercialización.

Palabras clave: *glicerol, metanol, rumiantes, valor energético*

Introduction

The rising cost of fossil fuels (Benoit and Mottet 2023) and their capacity to generate polluting gases have motivated the interest for searching alternative energy sources, especially renewable ones. In this regard, biodiesel has an important place as a biofuel produced from vegetable oils or animal fats (Kumar Singh *et al.* 2024) through transesterification (Tang *et al.* 2024). Glycerin is the main byproduct resulting from biodiesel production (Bansod *et al.* 2024), so the development of biofuel producing industries has generated considerable volumes of glycerol, which can be used as an ingredient in diets for ruminants (Madrid *et al.* 2019) and non-ruminants, including pigs, laying hens and broilers (Tavernari *et al.* 2022). This contributes to improving economic sustainability of biodiesel industry and reducing the environmental impact caused by the generated waste (Garlapati *et al.* 2016 and Abdul *et al.* 2019).

Initially, glycerol was used in the treatment of bovine ketosis or pregnancy toxemia in sheep. However, its availability and the high price of cereals led to studies in which its effect as an energy component of the diet was evaluated. Generally, glycerol is used to replace corn grain, since both provide similar amounts of energy, so it can be an economically viable alternative in the formulation of rations for ruminants, especially when the price of corn increases.

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Glycerol can be used pure or with a medium level of purity. With the latter, more discreet results are achieved, but without incurring the expense that the refining process entails. However, when using raw glycerol, the existence of some impurities must be considered, which can reduce the beneficial effects of the product and even compromise animal health. The objective of this review is to delve into some topics related to the use of glycerol in ruminant feeding.

Obtaining, properties and uses of glycerol

Glycerol can be obtained from complex lipids, by organic synthesis, through the fermentation of carbohydrates or from synthetic derivatives resulting from petroleum refining. Initially, the main way to obtain glycerol was saponification of fats in the soap manufacturing process, until the development of biofuel production companies began. According to Badia-Fabregat *et al.* (2019), for every 10 kg of biodiesel, approximately 1 kg of crude glycerol are produced through the transesterification of fats from vegetable or animal origin with methanol.

Small amounts of glycerol can also be obtained from marine microalgae, such as *Dunaliella salina* (Celente *et al.* 2022). Animals have an endogenous source from lipolysis of adipose tissue or hydrolysis of triglycerides from blood lipoproteins. Glycerol resulting from lipolysis follows the hepatic gluconeogenesis pathway and can provide up to 15-20 % of total glucose demands (Jeon *et al.* 2023).

Glycerol is a colorless, viscous, almost odorless liquid. It is soluble in water and alcohol and insoluble in ether and chloroform. Currently, it is used in the chemical industry for the synthesis of resins and esters (18 %), pharmaceutical industry (7 %), production of cosmetics (40 %), as a humectant and food preservative, in the preparation of dressings for salads, sweet toppings and frozen desserts (24 %) and others (11 %) (Cardoso *et al.* 2015). It has also been used in the manufacture of explosives (dynamite and nitroglycerin) (Wu *et al.* 2023). Its contribution to the pharmaceutical industry corresponds to its use as a component of capsules, anesthetics, syrups and antiseptics (Wan Azelee *et al.* 2019), while in the production of cosmetics, it improves softness, provides lubrication and has moisturizing properties.

Its gluconeogenic and antiketotic effect explains its use in the treatment of bovine ketosis (Mammi *et al.* 2021) and to prevent fatty liver syndrome (Zhang *et al.* 2023). It can be used in the treatment of pregnancy toxemia in sheep (Cal-Pereyra *et al.* 2015). It can be used as raw material for biopolymers, polyunsaturated fatty acids, production of ethanol, hydrogen and n-butanol (Garlapati *et al.* 2016), as well as in the production of biosurfactants (Trentini Volpato *et al.* 2022) and solketal (Kowalska-Kuś *et al.* 2020).

Most studies with glycerol are based on small proportions added to the diet, due to its gluconeogenic characteristics (Neiva *et al.* 2012 and Soares *et al.* 2012). However, higher quantities have been used as another component of the diet, because production volumes have exceeded utilization capacity (Donkin 2008). This can constitute a way to increase the biological and financial efficiency of biodiesel production and, at the same time, prevent it from being discharged into the environment, becoming another pollutant.

The increase in production volumes suggests a decrease in its price, which strengthens the idea of using it as a substitute for energy concentrates in the diet of ruminants (Khalid and Al-Anbari 2024). There are other properties described by Schröder and Südekum (1999), who refer to its easy absorption in the rumen and intestinal mucosa, as well as its antiseptic power, capable of sanitizing the ration, and its high palatability, which responds to its sweet flavor and its agglomerating effect because it is hygroscopic. It is a normal compound in the metabolism of ruminants, which is found in the blood as well as in the cells.

Metabolism of glycerol

The glycerol that reaches rumen can follow three destinations. It is estimated that 44 % of glycerol that reaches the organ is fermented, 43 % is absorbed through the rumen wall and 13 % passes to the digestive compartments after the rumen, although these proportions can vary (Krehbiel 2008). Excess glycerol can be absorbed by the ruminal and intestinal mucosa, which constitutes a direct gluconeogenic source for the ruminant (Ortega-Cerrilla *et al.* 2018). According to Hejna *et al.* (2016), as a result of the microbial fermentation of glycerol, several chemical compounds could be obtained, such as propionic acid, succinic acid, butanol, propanediol, dihydroxyacetone, among many others.

According to Cabrera-Cruz (2019), replacing glycerol with corn in the diet does not generate a negative effect on the ecology of the rumen, even when there is a modification in the synthesis of volatile fatty acids. Glycerol is capable of increasing total production of volatile fatty acids, *in vivo* (Rémond *et al.* 1993 and Wang *et al.* 2009a) and *in vitro* (Trabue *et al.* 2007). In addition to increasing, fundamentally, the production of propionic acid (Wang *et al.* 2009a and Chanjula *et al.* 2016). It can enter the glycolytic pathway and it transform into pyruvate, which generates propionate via two different routes: succinate or acrylate. This justifies the increase in propionate, by adding glycerol to the ruminant diet (Cardoso *et al.* 2015).

The inclusion of glycerol to the diet can also increase the production of butyric acid (Kupczyński *et al.* 2020), and propionic and butyric acid (Van Cleef *et al.* 2016 and Madrid *et al.* 2019) with decrease in acetic acid (Chanjula *et al.* 2016), which contributes to the decrease in the acetic:propionic ratio (Wang *et al.* 2009a). The majority of glycerol is fermented to volatile fatty acids through the glycolytic pathway, with little production of lactic acid (Trabue *et al.* 2007).

Considering that propionic acid and glycerol itself are potent neoglycogenic agents (McWilliams 2023), it is reasonable to use glycerol as an energy supplement for milk production in the transition period. It could even be more recommended than other energy sources because it has a metabolic advantage over its traditional counterparts, especially propionate and propylene glycol, because it enters gluconeogenesis at the level of phosphate-isomerase, metabolically closer to glucose (Wang *et al.* 2021).

Propionic acid and glycerol are absorbed and reach the liver via the portal vein for subsequent conversion to glucose (Arias-Islas *et al.* 2020). According to Lei and Simões (2021), propionic acid produced by ruminal fermentation is the main substrate for gluconeogenesis in high-producing dairy cows. Between 50 and 60 % of the total glucose required is obtained in this way. Propionic acid production in the rumen is greater in animals that consume concentrate than in those that consume forage. Therefore, in grazing animals, glycerol supplementation could increase energy efficiency (Huerta-Jiménez *et al.* 2018).

According to studies carried out by Rémond *et al.* (1993), the maximum disappearance rates of glycerol in the rumen, determined by *in vitro* fermenters, is 0.52 to 0.62 g.h^{-1}. Other data suggest that, with a dose of 240 g of glycerol, disappearance rates in the rumen are between 1.20 and 2.40 g.h^{-1}. In studies where levels between 15 and 25 % of glycerol were supplemented, most of it disappeared within six hours (Bergner *et al.* 1995). According to Donkin (2008), between 50 and 70 % of the glycerol disappears from the rumen within four hours.

On the other hand, the studies carried out by Chanjula *et al.* (2016) reduced ammoniacal nitrogen levels in the rumen by including 6 % of glycerol in the diet. However, Correa and Moreno (2019) did not modify blood urea nitrogen content.

Effect of the use of glycerol on dry matter intake

Studies in which glycerol was used showed variable results in relation to dry matter intake (DMI). In some other, this indicator was not modified with the inclusion of glycerol in the diet (Moriel *et al.* 2011 and Van Cleef *et al.* 2014). However, Bodarski *et al.* (2005) reported increases in intake by approximately 2 kg of DM at 70 d, while Ogborn (2006) observed a depressive effect in the postpartum stage, Ladeira *et al.* (2016) in young bulls when they used 18 %, and Chanjula *et al.* (2016) used 6 % in goats.

DM intake was also modified in the work carried out by Shin *et al.* (2012) when using glycerol. In addition, they obtained an effect of the inclusion levels of the product, with the highest values of 5 % (28.40 kg. d^{-1}). Neiva *et al.* (2012) did not modify this indicator within the same category (cows and steers), but when comparing cows with steers they observed a reduction in intake for the latter, when glycerol was used at a rate of 6 and 12 % of the DM.

Use of glycerol in animal diet as energy additive

According to Donkin *et al.* (2009) the energy provided by glycerol is similar to that of corn starch, when used in dairy cows. Nevertheless, the energy value of glycerol depends on its purity degree, on the percentage it represents regarding total dry matter (DM) and on the starch content of the used concentrate. Schröder and Südekum (1999) determined net energy of lactation of glycerol and obtained values of 2.30 Mcal.kg^{-1}, when it is offered in diets with low starch content, and between 1.91 and 2.03 Mcal.kg^{-1} in diets with high starch content.

The production of biodiesel generates byproducts with potential use in animal feed (de Souza *et al.* 2014). In this group, glycerol stands out, which can be used as an energy source (Soares *et al.* 2012). It has been used in diets for pigs (Martínez-Miró *et al.* 2021, Dahmer *et al.* 2022 and Li *et al.* 2022) and for broilers (Liu *et al.* 2020).

However, most of the studies carried out are aimed at feeding ruminant animals. Sotgiu *et al.* (2021) used it in sheep and Chanjula *et al.* (2016) in goats. Prado *et al.* (2015) and Ladeira *et al.* (2016) used it in bulls. Likewise, Moriel *et al.* (2011) applied it to the feeding of replacement heifers in breeds intended for meat production. Correa and Moreno (2019) studied its effect in Holstein cows.

There are several studies that include glycerol in the diet of high-producing dairy cows in the transition period (Bodarski *et al.* 2005, Chung *et al.* 2007 and Wang *et al.* 2009b). Some studies refer to its use with a high level of purity (Donkin *et al.* 2009 and Carvalho *et al.* 2011) or raw (Neiva *et al.* 2012). In other research carried out with Holando bulls, glycerol was used as a substitute for corn grain in the ration for dairy cows (Donkin *et al.* 2009 and Carvalho *et al.* 2011) and in fattening (Mach *et al.* 2009). It was also used in the production of rumen activators for the production of beef in feeding systems with fibrous materials (Iriñiz *et al.* 2011).

De Frain *et al.* (2004) studied different levels of glycerol inclusion in the diet, at a rate of 430 and 860 g.cow^{-1}d^{-1}. Bodarski *et al.* (2005) did it in doses of 300 and 500 mL, while Ogborn (2006) included 504 g.cow^{-1}d^{-1}. Chung *et al.* (2007) evaluated lower amounts, by supplying 250 g.cow^{-1}d^{-1}. Wang *et al.* (2009b) introduced 100 and 300 g.cow^{-1}d^{-1}, and Lounglawan *et al.* (2011) worked with 150 and 300 g.cow^{-1}d^{-1}.

Donkin *et al.* (2009) used glycerol levels between 5 and 15 % of DM. Carvalho *et al.* (2011) included it at 11.50 and

10.80 % for prepartum and postpartum, respectively. Similar levels, 0 to 12 %, were used by Mach *et al.* (2009) in the diet. However, other studies surpassed them. D'Aurea *et al.* (2017) applied up to 20 % in combination with urea to evaluate some rumen parameters and the performance of the microbial mass.

Neiva *et al.* (2012) added up to 24 % in diets for dairy breed steers and cows. Van Cleef *et al.* (2014) included up to 30 % in the feeding of confined Nelore bulls.

According to Ortega-Cerrilla *et al.* (2018), the results obtained with the use of glycerol depend on base diet quality, purity degree and inclusion level. Precisely, the latter is one of the main questions generated by the use of said product. Generally, it is used in quantities close to 10 %. According to Donkin (2008), glycerol should be used at least 10 % of DM in diets for dairy cows. Shin *et al.* (2012) stated that they were successful in using diets for dairy cows, whose glycerol content was between 5 and 15 % of the total DM. However, the use of 15 % glycerol in dairy cows in mid-lactation may be accompanied by a temporary decrease in food intake (Donkin *et al.* 2009).

Effect of glycerol on diet digestibility

Studies of Schröder and Südekum (1999) demonstrated that the effect of glycerol on diet digestibility is determined by the amount of starch contained in it. Generally, when high starch diets are used, cell wall digestibility is reduced.

Other studies report that there was no modification in fiber digestibility (Hess *et al.* 2008) with the use of glycerol. Wang *et al.* (2009a) managed to increase the digestibility of organic matter and crude protein with the increase in glycerol supplementation up to an average level of 200 g.animal^{-1}d^{-1}, but with the increase of glycerol levels, digestibility decreased slightly. In studies conducted by Van-Cleef *et al.* (2014) also demonstrated an increase in the digestibility of crude protein with a reduction in the digestibility of neutral detergent fiber, as a result of a decrease in the digestibility of hemicellulose, when using 30 % of glycerol in the diet. Chanjula *et al*. (2016) reduced diet digestibility by using 6 % of glycerol.

Effect of glycerol on live weight and body condition

Several authors found no differences in live weight and body condition (Carvalho *et al.* 2011, Moriel *et al.* 2011 and Shin *et al*. 2012). However, Bodarski *et al.* (2005) observed a positive effect of glycerol on body condition at the end of the evaluation period. Wang *et al.* (2009b) reported its effect on live weight gain, as do Donkin *et al*. (2009) by providing 10 and 15 % of glycerol in the diet. Neiva *et al*. (2012), Van Cleef *et al.* (2014), Chanjula *et al.* (2016) and Ladeira *et al.* (2016) did not modify weight gain with the use of glycerol.

Effect of glycerol on meat quality

In the studies carried out by Lammer *et al.* (2015), carcass quality was not affected when using glycerol in pigs. However, the level of monounsaturated fatty acids in adipose tissue increased by increasing the content of this product in the diet.

There were no major changes when including glycerol in cattle. Elam *et al.* (2008) verified that the addition of up to 15 % in crossbred heifers does not affect intramuscular fat deposition and meat yield. The area of Longissimus dorsi (LD) muscle and its fat content were not modified in the studies carried out by Mach *et al.* (2009), who used 12.1 %. Similarly, Prado *et al.* (2015) reported no damage on the area and composition of the LD, the same with the thickness of the back fat and some indicators of meat quality (marbling, texture and color).

Nevertheless, Van Cleef *et al.* (2014) increased fat levels in carcass and Ladeira *et al.* (2016) favored the marbling of meat with the use of glycerol.

Effect of glycerol on milk production

According to Ogborn (2006), the effects of glycerol on milk production are observed when levels higher than 6 % are used. This may explain why this indicator is not modified in the studies carried out by Chung *et al.* (2007) and Lounglawan *et al.* (2011). However, Bodarski *et al.* (2005) increased milk production with small doses of glycerol, which could be related to the increase of DM intake, although Shin *et al.* (2012) increased dry matter intake, without modifying milk production.

Milk production was also increased in experiments carried out by Khalid and Al-Anbari (2024) and Correa and Moreno (2019). Meanwhile, Donkin *et al*. (2009), Wang *et al.* (2009b) and Carvalho *et al.* (2011) did not report modifications.

Bonis *et al.* (2022) obtained an increment in milk production of 155.91 % in multiparous Siboney cows from Cuba, which were grazing pitilla grass (*Sporobolus indicus* (L.) R. Br) when they used glycerol, obtained from the *Jatropha curcas* biodiesel manufacturing process.

Effect of glycerol on milk composition

Milk components are generally not affected by the use of glycerol (Carvalho *et al.* 2011 and Kupczyński *et al*. 2020). However, there may be some modifications in fat and protein. Bodarski *et al.* (2005) obtained increases in protein with the increase of glycerol in the diet, while Wang *et al.* (2009b) recorded a decrease.

Another indicator that can vary is fat content. Shin *et al.* (2012) confirmed the higher values of fat quantity and concentration, when using 5 % of glycerol. The same occurred in the studies carried out by Correa and Moreno

(2019). However, the incorporation of glycerol in the diet reduced the proportion of fat in milk, as reported by Lounglawan *et al.* (2011). In studies by Donkin *et al.* (2009), a decrease in urea content was recorded.

Main limitations of the use of glycerol

The main limitation of glycerol derived from biodiesel industry is its methanol content. Other impurities, such as soaps, sodium and diethylene glycol, can also have a negative influence. Methanol and diethylene glycol are potent tissue toxicants. However, a dairy cow of 600 kg live weight is capable of consuming 7.44 mg of methanol, that is, 1.24 % for each kg of live weight and converting it into H_2O and CO_2. This is because, under normal conditions, methanogenic bacteria in the rumen transform it into methane (Soares *et al*. 2012). The toxic and limiting effect of methanol intake is more frequently verified in monogastric or pre-ruminant animals (calves).

There are limits for the proportion of methanol in glycerol, which will be used in animal feed. Studies carried out by the US Food and Drug Administration (FDA - USA) indicate that methanol levels greater than 150 p.p.m. can be considered unsuitable for animal feeding. Higher levels have been established in Germany, where a maximum limit of 5,000 p.p.m was defined (Sellers 2008).

Methanol is metabolized in the liver, transforms into formaldehyde, formic acid and finally CO_2 and water. The metabolism of formic acid is slow, so it accumulates in the body and produces metabolic acidosis (Soares *et al.* 2012). The effects related to methanol poisoning manifest themselves with damage to the optic nerve, neurological and renal disturbances, as well as degeneration of liver fat.

According to Soares *et al.* (2012), heavy metals and sodium level could also limit its use in the diet. Excess sodium reduces intake and animal yield. In addition, the incidence and severity of udder edema increases, mainly in prepartum heifers.

To avoid the harmful effects of impurities, some authors recommend purification of the product. However, this process has a high cost (Chol *et al*. 2018), so it is necessary to assess whether it is profitable and to what extent it is more feasible to purify. Evaluations of this glycerin, tested by Schröder and Südekum (1999) and Thompson and He (2006) indicated contents in the order of 63 to 76 % of glycerol in crude glycerin of low purification. The glycerol content increases to 85 % in medium purifications, with a significant reduction in the methanol content, which ends up being less than 0.50 % and can reach 99 % of glycerol, when the purification process continues (Schröder and Südekum 1999).

General considerations

Currently, biofuel production companies are the main source of glycerol. Its low cost, high palatability, gluconeogenic effect and energy content are some of the properties that support its use as animal feed. Several researches demonstrate promising results with the use of this by-product as an alternative energy source for feeding ruminants.

The inclusion of glycerol in ruminant diet could improve the value of meat and milk fat by increasing the anti-cancer, anti-diabetogenic and anti-dipogenic properties due to the presence of conjugated linoleic acid. These reasons place it as a product with functional characteristics. The development of future research that demonstrates this will allow the diversification of its use and commercialization.

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