



## Effect of increasing levels of glycerin on growth rate, carcass traits and liver gluconeogenesis in young bulls<sup>☆</sup>



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### ABSTRACT

This study aimed to evaluate performance, carcass traits, glycerol kinase 1 (*GK1*) and cytoplasmic phosphoenolpyruvate carboxykinase (*PCK1*) gene expression, and glycerol kinase activity in liver of young bulls receiving different levels of crude glycerin. Forty-four cross-bred young bulls (initial body weight of  $368 \pm 4.2$  kg) were used in a completely randomized design, with four treatments and 11 replicates. The experiment period lasted 84 days, preceded by an adaptation period of 28 days. The basal diet was composed of corn silage (300 g/kg) and concentrate (700 g/kg) containing corn and soybean. The experimental treatments were as follows: without glycerin or including 60, 120 or 180 g/kg of crude glycerin in the diet. Blood samples were collected on the last day of the experiment period to evaluate biochemical parameters. After slaughter, carcass traits were measured and liver samples were collected to analyze gene expression and glycerol kinase activity. There was no effect ( $P=0.21$ ) of glycerin on glucose blood concentrations. However, liver glycerol kinase activity was greater ( $P<0.01$ ) and *GK1* and *PCK1* genes expressions were down regulate ( $P<0.01$ ) as glycerin levels in the diet increased. Glycerin did not affect ( $P>0.17$ ) performance and most of the carcass traits. However, there was a greater ( $P=0.02$ ) marbling score in the carcass of animals fed 120 and 180 g/kg of crude glycerin. In conclusion, the use of glycerin at a level of up to 180 g/kg is recommended in diets of feedlot beef cattle, and it increases liver glycerol kinase activity, feed efficiency and beef marbling.

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**Abbreviations:** ADG, average daily gain; AQP 9, aquaglyceroporin; AST, aspartate aminotransferase; CCW, cold carcass weight; CK, creatine kinase; CY, carcass yield; DM, dry matter; DMI, dry matter intake; FBW, final body weight; *GAPDH*, glyceraldehyde 3-phosphate dehydrogenase gene; GGT, gamma-glutamyl aminotransferase; *GK1*, glycerol kinase 1 gene; HCW, hot carcass weight; LMA, longissimus muscle area; ME, metabolizable energy; NFC, non-fibrous carbohydrates; *PCK1*, cytoplasmic phosphoenolpyruvate carboxykinase gene; PEPCK-C, cytosolic phosphoenolpyruvate carboxykinase enzyme; PGC-1 $\alpha$ , peroxisome proliferator-activated receptor- $\gamma$  coactivator 1 $\alpha$ ; PPAR- $\gamma$ , peroxisome proliferator-activated receptor- $\gamma$ ; SFT, subcutaneous fat thickness.

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## 1. Introduction

The use of coproducts to replace commonly used ingredients such as corn and soybean could reduce feeding cost and increase profit in feedlots. Among available coproducts for use in feedlots, glycerin is emerging as an option due to the growth of biodiesel industry around the world. Some studies have shown the potential of this biodiesel industry coproduct for ruminants' feeding, being the first studies performed in dairy cows (Donkin, 2008). In beef cattle, several studies evaluated the effect of crude glycerin on performance and beef quality, but results were ambiguous and inconclusive. In other words, in some cases crude glycerin increased growth performance of the animals (Parsons et al., 2009), but in other cases, there was no positive effect (Egea et al., 2014; Van Cleef et al., 2014), while in some studies, glycerin depressed animal performance and carcass traits (Gunn et al., 2010). In addition, none analyzed performance and liver metabolism at the same time.

Chemical composition of crude glycerin still is quite variable, being important to assess whether the use of this ingredient may have toxic effects in ruminants, especially regarding methanol concentration. According to Elam et al. (2008), it has been hypothesized that glycerin supplementation may cause problems associated with hepatic lipid mobilization by increasing the liver ability to sequester and re-esterify fatty acids from tissues. Besides, glycerol may be a substrate for gluconeogenesis in the liver, increasing glycerol kinase activity and glucose turnover, which could improve beef marbling. It may occur because, according to Gilbert et al. (2003), intramuscular adipose tissue uses a high proportion of glucose for fatty acid synthesis and it is more sensitive to insulin.

Therefore, the hypothesis of this study is that glycerin will not affect negatively feedlot performance, while increases liver gene expression and activity of glycerol kinase. The objective was to evaluate performance, carcass traits, expression of glycerol kinase 1 (*GK1*) and cytoplasmatic phosphoenolpyruvate carboxykinase (*PCK1*) genes, and glycerol kinase activity in the liver of young bulls fed different levels of crude glycerin.

## 2. Materials and methods

Animal care and handling were approved by the Federal University of Lavras Animal Care and Use Committee. The experiment was carried out at the Animal Science Department of the Federal University of Lavras.

### 2.1. Animals, diet and slaughter

Forty-four crossbred young bulls ( $\frac{1}{4}$  Angus,  $\frac{1}{4}$  Nellore,  $\frac{1}{4}$  Senepol,  $\frac{1}{4}$  Caracu), with initial body weight of  $368 \pm 4.2$  kg were used in a completely randomized design, with four treatments (0, 60, 120 and 180 g/kg of crude glycerin in the diet) and 11 replicates. Crude glycerin composition used in the experiment had the following composition: glycerol: 831 g/kg, moisture: 111 g/kg, ash: 60.6 g/kg, chlorides 36.7 g/kg, and methanol: 0.2 g/kg. Animals were housed in open-air pens with an area of 30 m<sup>2</sup> per animal.

This 84-day experiment was preceded by an adaptation period of 28 days, during which animals received the same experimental diet. At the beginning of the adaptation period, animals were treated for ectoparasites and endoparasites (Ivomec, Paulinia, Brazil). Aiming to measure their average daily gain (ADG) using regression analysis, animals were weighed at the beginning, every 28 days and at the end of experimental period, after 16 h of fasting.

Diets were formulated with corn silage, and four different levels of crude glycerin (Table 1) according to the National Research Council – NRC (2000) to make it isonitrogenous. Animals were fed *ad libitum* at 07:30 am and 03:30 pm. Corn gluten meal-21 was included in the diets with crude glycerin to provide similar levels of crude protein and a similar amino acid profile.

Samples of silage and concentrate were collected every 14 days. These samples supplied a composite sample that, after pre-drying in a forced-ventilation oven at 65 °C for 72 h, was ground in a mill with a 1-mm mesh sieve. Chemical analyses of the diets were performed according to AOAC (2000). The dry matter (DM) was obtained by oven drying at 100 °C for 18 h (Method 934.01). The ash content was estimated after a 2-h incineration process in a 600 °C muffle furnace (Method 942.05). The crude protein (CP) content was determined based on the N content multiplied by the 6.25 factor. The N content was obtained by the Kjeldahl procedure (Method 920.87). Evaluation of the ether extract (EE) content was performed with solvent using a Soxhlet extractor apparatus (Method 920.39). Non-fibrous carbohydrates (NFC) were determined according to Sniffen et al. (1992), and metabolizable energy (ME) was calculated according to the NRC (2001) and Mach et al. (2009).

Blood samples were collected from the coccygeal vein using Vacutainer<sup>®</sup> tubes (BD, Juiz de Fora, Brazil) at the end of the experiment after 16 h of fasting. These samples were placed in a cooler with ice and transported directly to the laboratory, where analyses of creatinine and aspartate aminotransferase (AST), gamma-glutamyl aminotransferase (GGT), and creatine kinase (CK) enzymes were performed using commercial kits (Labtest Diagnóstica, Lagoa Santa, Brazil). Blood samples for glucose analysis were collected in another Vacutainer<sup>®</sup> tube (BD, Juiz de Fora, Brazil) containing a fluoride anticoagulant.

Animals were slaughtered at a commercial abattoir by captive bolt and exsanguination, followed by hide removal and evisceration, without electrical stimulus. The carcasses were washed, divided into two equal halves and weighed to obtain the hot carcass weight (HCW) and carcass yield (CY). The subcutaneous fat thickness (SFT) and longissimus muscle area (LMA) were measured between the 12th and 13th ribs of the left half-carcass. Subsequently, they were maintained under

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