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# Digestibility and palatability of adding crude glycerin to the diet of ponies

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ABSTRACT

Crude glycerin has a potential for use as a nutritional additive and/or substitute for some feed components. Although proven in some animal species, there are no reports of its use in horses. The aim of this study was to determine the effect of adding different levels of crude glycerin on the digestibility and palatability of nutrients in the equine diet. Eight pony geldings were used in this study, aged 9 years, weighing 149.80 ± 17.20 kg and body condition score 5.4 ± 0.7. A contemporary Latin square 4 × 4 double experimental design was used. Diets differed in the level of inclusion of glycerin (0, 4, 8 and 12%) over the concentrate. To verify the effect of adding crude glycerin to the diet, the total apparent digestibility of nutrients in the diet was assessed. Data were analyzed using the Statistical Analysis System (SAS Institute Inc., 2010). For statistical significance, Tukey test was used with $P < 0.05$. Digestibility analyses showed that glycerin addition, even at different levels in the diet, did not affect the feed intake nor the apparent variables of digestibility and fermentation of the total tract ($P > 0.05$). Additionally, no differences were found between treatments for glucose and insulin values ($P > 0.05$). In this study the addition of glycerin, even at the amount of 12%, did not affect the acceptability and digestibility of nutrients in the diet. These results are encouraging, yet more research is needed to clarify the appropriate levels of inclusion and/or substitution using this product.

## 1. Introduction

The use of renewable inputs that can reduce or replace the use of fossil fuels is becoming increasingly encouraged due to socioeconomic development, environmental appeal and due to the depletion of easily extractable oil reserves (Mota et al., 2009; Peiter et al., 2016). Considered renewable due to the reabsorption of emitted carbon dioxide by plants, biofuels have a less associated impact on global warming (Leung et al., 2010). Brazil is a pioneer in the utilization of this type of fuel, with biodiesel as the main class (Apolinário et al., 2012).

Glycerin is a by-product of biodiesel production processes, whereby each ton of biodiesel produces 100 kg of glycerol, making the search for new ways of utilizing glycerin necessary in order to help its incorporation in the market (Batista, 2007). Due to its characteristics and sweet taste, crude glycerin is considered a promising material for animal feed (Gomes et al., 2011), with the main interest being due to its energetic value, with metabolizable energy values being very close to those found in corn, which in Brazil is the main energy component of an animal feed (Pereira and Rossi Junior, 1995; Silva et al., 2012).

The nutritional requirement of horses is directly linked to live body weight, level and intensity of work, physiological stage, level of performance, environment and metabolic efficiency (Martin-Rosset and Martin, 2015). Energy level adequacy is one of the variables used to determine the quantity needed to be supplied in a diet. In order to meet these requirements using only soluble carbohydrates, it is necessary to intake large quantities which can potentially lead to digestive disorders (Morgado and Galzerano, 2006; Ribeiro et al., 2009).

Research on the use of glycerin by-products for use in animal feed has proven positive in various species, mainly in chickens and pigs. To the best of our knowledge this is the first report of the inclusion of glycerin in the equine diet. Thus, the aim of this research was to determine the effect of adding different levels of crude glycerin on the digestibility and palatability of nutrients in the equine diet.

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## 2. Material and methods

All animal care and handling procedures were approved by the Ethics Committee on the Use of Animals of the Faculty of Veterinary Medicine and Animal Science of the University of São Paulo, protocol number 8,156,080,517.

Eight pony geldings, weighing 149.80 ± 17.20 kg, aged 9 years and 5.4 ± 0.7 BCS (Body Condition Score) (Henneke et al., 1983), were used in this study.

A replicated 4 × 4 Latin square design with four dietary treatments was used. The experimental period included a 14-day period for dietary adaptation to feed type, and five days for sampling of feces for the total diet digestibility and other variables, totaling 19 days for each period.

Diets differed in the level of inclusion of glycerin (0, 4, 8 and 12%) over the concentrate. The experimental diets were 1) control diet, where no glycerin was added (GLY0), 0 mL / d; 2) a diet containing 4% crude glycerin (GLY4), 45.23 ± 5.61 mL / d; 3) a diet containing 8% crude glycerin (GLY8), 87.60 ± 8.84 mL / d; and 4) a diet containing 12% crude glycerin (GLY12), 132.50 ± 11.54 mL / d. Experimental diets were distributed by the level of glycerin included, and were calculated relative to the body condition per animal and per period. Diets were formulated to meet or exceed NRC requirements, and were adjusted by period for changes in body weight (Martin-Rosset and Martin, 2015). The ponies were kept in maintenance conditions throughout the study. Glycerin was added as a top dressing to the concentrate directly into the trough. Composite samples of concentrate and hay were analyzed for nutrient content (Table 1) and ingredients (Table 2). Glycerin was analyzed by a commercial laboratory for composition and found to be composed of: 77.82% glycerol, 22.1% moisture, 0.08% ash and 0.0% methanol.

During the collection period, the amount of feed offered, refusals and feces excreted were weighed and recorded daily to measure the feed intake and total tract digestibility of each feed. Leftover concentrate remaining after the treatments was used as an acceptability index. Total feces were collected from the concrete floor, stored in plastic containers every 12 h, and then weighed and mixed per individual. Aliquot samples (10% of the total) of the feed, feces and refusals were obtained daily and placed in identified plastic bags and stored in a freezer at - 20°C which, after five days of fecal collection, formed the composite samples. Feces contaminated with urine were weighed separately and then discarded.

At the end of the protocol a sample composed of each animal was generated and sent to the multiuser Laboratory of Animal Nutrition and Bromatology of the Animal Production and Nutrition Department of FMVZ / University of São Paulo (USP), where they were processed and analyzed.

Feces were analyzed for DM, Ash, CP ($N \times 6.25$), Ether extract (EE), NDF, ADF and OM. Feed was analyzed for the same factors, with the addition of Ca, P and starch. The determination of DM, Ash, CP ($N \times 6.25$), Ether extract, OM, Ca and P of the diets and feces followed AOAC (1996 Methods 930.15; 942.05; 988.05; 954.02) recommendations. Fiber fractions (NDF and ADF) were determined according to the methods described by Van Soest et al. (1991). Starch was analyzed following the enzymatic method of Pereira and Rossi Junior (1995). The coefficient of apparent total tract digestibility of DM was calculated according to Coelho da Silva and Leão, (1979). The coefficient of apparent total tract digestibility (CATTD) were calculated by the methodology described by Schneider and Flatt (1975).

**Table 1**
Nutrient composition of concentrate and hay (% in DM) fed to horses.

| | Concentrate | Coast cross hay |
|---|---|---|
| DM | 93.1 | 95.0 |
| Ash | 9.8 | 6.9 |
| CP | 12.4 | 7.5 |
| Ether extract | 0.6 | 3.1 |
| NDF | 25.8 | 80.8 |
| ADF | 7.8 | 47.4 |
| Ca | 2.0 | 0.5 |
| P | 0.5 | 0.2 |
| Starch | 47.9 | 1.3 |

**Table 2**
Ingredient composition of concentrate fed to horses.

| Ingredient | % |
|---|---|
| Wheat bran | 43 |
| Corn meal | 46.5 |
| Soybean meal | 4 |
| Limestone | 2.5 |
| Premix composition* | 3 |
| Salt | 1 |

*calcium 190 - 215 g / kg; chlorine 105 g / kg; cobalt 12 mg / kg; copper 250 mg / kg; sulfur 2 g / kg; iron 1000 mg / kg; fluorine 720 mg / kg; phosphor 72 g / kg; iodine 20 mg / kg; magnesium 28 g / kg; manganese 1000 mg / kg; selenium 2 mg / kg; sodium 68 g / kg; zinc 1500 mg / kg.

Fecal pH was measured from the juice of fresh fecal samples of approximately 20 g that were collected at the first moment of spontaneous defecation on the last day of total collection, according to the methodology adapted from Zeyner et al., 2004 and Goachet et al. (2014). This was then filtered on 100 µm nylon cloth, and analysis was carried out at the collection site using an electronic pH meter, according to Goachet et al. (2014).

For the analysis of volatile fatty acids (acetic, propionic, isobutyric, butyric, isovaleric and valeric), 10 g of fresh feces was collected into a lidded plastic container at the first moment of spontaneous defecation on the penultimate day of total collection. Samples were diluted in 20 mL of distilled water and, after homogenization, the material was strained. An aliquot of 4 mL was transferred to 10 mL tubes without anticoagulant containing 1 mL of pre-PA formic acid 98–100% HPLC (High-Performance Liquid Chromatography) grade. Tubes were then centrifuged for 12 min (Model 80–2B-15ML centrifuge, Centribio) at 4000 rpm (1800 x g), and 2 mL of the supernatant was transferred to Microtubo Axygen® with a 2 mL capacity and frozen at - 20°C. Analysis was carried out at the Ruminal Fermentability Laboratory of FZEA-USP.

Fecal lactic acid analysis was performed using a sample of 1 g of feces at the first moment of spontaneous defecation on the last day of total collection and was conditioned in test tubes with a rubber cap, pre-weighed and frozen at - 20º C. Samples were sent to the multiuser Laboratory of Animal Nutrition and Bromatology of the Department of Animal Production and Nutrition of FMVZ/USP where they were processed and analyzed. The Spectrophotometric method for biological fluids was used for acid determination (Pryce, 1969).

To determine levels of plasma glucose and serum insulin, blood was collected by venipuncture in the jugular vein, taken at 30 min (before feeding) 60, 120, 180 and 240 min, using a 2 mL BD Vacutainer sodium fluoride/EDTA. For serum insulin analysis, collection of blood samples in a 2 mL BD Vacutainer without anticoagulants was used and homogenized by inversion of 5 to 8 times in order to avoid hemolysis. The tubes, both glucose and insulin, were kept at room temperature and centrifuged (centrifugal model 80–2B-15ML, Centribio) at 4000 rpm (1800 x g) for the separation of plasma and serum. Following this procedure the plasma and serum were transferred to 1.5 ml plastic tubes, identified and maintained in freezers at - 20º C until processed (Stull and Rodiek, 1988). The samples were sent for analysis in a private laboratory, using kits (Bioclin Diagnostics, Belo Horizonte, Brazil).

Data was analyzed by the Statistical Analysis System, and the normality of the residues was verified by the Shapiro-Wilk test and the homogeneity of the variances compared by the Hartley test.

Consumption data and total apparent digestibility, volatile fatty acids, pH and lactic acid were subjected to analysis of variance, which separated as causes of variation the fixed effect of treatment, in addition to the random effects of square period within square, animal within the square and residue. The glycemic and insulinemic response data were analyzed with the same causes of variation, but for the analysis of these response variables, the factor repeated measurements related to the different moments of collection used, using the SAS MIXED procedure. Means were compared by the Tukey test, with significance set at $P < 0.05$.

## 3. Results and discussion

The objective of the current study was to determine whether glycerin could be included in the equine diet. The inclusion of glycerin in the equine diet was successful in that no leftover concentrate was found in any of the total collection periods and therefore did not decrease overall intake. Since the inclusion of foods with a sweet flavor makes the concentrate more palatable (Frape, 2008; Martin-Rosset and Martin, 2015), we believe this could be due to the sweet taste of glycerin.

Through visual monitoring, none of the animals in this study showed any type of hypersensitivity or food sensitivity to the product, nor did they present changes in feces consistency or gastrointestinal disorders during the experimental period. In addition, there were no changes in the body condition score. These observations suggest that the use of glycerin does not abruptly affect the ponies' gastrointestinal tract.

Even at the highest level of glycerin supplementation (12%), no differences were observed on the digestibility parameters of the treatments in the present study (Table 3). These data differ from most species, where values greater than 10% can cause metabolic disturbances or decrease the nutrient digestibility (Lima et al., 2014; Polizel et al., 2017).

The pH value and short chain fatty acids are used as indicators of gastrointestinal health and play an important role in maintaining digestive performance and development in equines. The fermentative process may suffer instabilities caused by nutritional supplementation, that will lead to changes in these concentrations (Kabe et al., 2016). In the current study, there was no difference observed between the pH values in the treatments ($P = 0.2477$). Normal values of intestinal pH vary from 6.4 to 6.7 in horses (Hoffman et al., 2003). The fact that there were no differences in the amount of lactic acid ($P = 0.5665$) helped in the stabilization of pH, since the accumulation of this acid can suppress the mechanisms of tamponade (Goachet et al., 2014; Hoffman et al., 2003).

Glycerol can be metabolized by the microbiota to produce acetate, propionate and butyrate (Martin-Rosset and Martin, 2015; Wright, 1969). Acetate, propionate and butyrate contribute up to 80% of the horse's energy requirement (Al Jassim and Andrews, 2009). They are responsible for the synthesis of lipids, in the case of acetate and butyrate, and the glyconeogenic substrate, in the case of propianate, contributing to the glycolytic metabolism. However, no differences were observed in the fermentation parameters of the treatments ($P < 0.05$) (Table 3), thought to be due to the amount of glycerin used in the diet not being able to interfere with these parameters. The propionate produced in the cecum accounts for about 7% of blood glucose (Frape, 2008). Ponies tend to have delayed reach of the glycemic and insulinemic peak and a slower return to the basal values (Harris et al., 2006). Studies with other species indicate that glycerol increases blood glucose values, since glycerol can be converted to glucose by the liver, providing energy for cellular metabolism (Nelson and Cox, 2014). In this study, no differences were observed between treatments for glucose and insulin values (Fig. 1).

Authors affirm that the glycemic index contributes to the adoption of prophylactic management practices and the nutritional metabolic modifications, as this is a physiological response directly linked to the ingestion of foods, mainly carbohydrates. However, basing chemical analysis solely on carbohydrate composition, for example, is not able to accurately indicate the glycemic response. Therefore, the measurement of pre- and post-prandial glycemic and insulinemic levels is the best form of evaluation (Stull and Rodiek, 1988; Williams et al., 2001). Fasting glycemic levels remain between 80 and 100 mg / dL, postprandial levels tend to rise to 150 mg / dL within two to three hours, returning to baseline values by up to six hours (Harris et al., 2006). Gobesso et al. (2009) observed that by using diets with different sources of starch, peak values of postprandial glucose occurred around 150 min, accompanied by the gradual increase of insulin.

The changes in glucose and insulin values, as well as the response times are directly related to the type and amount of food consumed, the composition and absorption digestibility capacity and post-absorptive use (Casalecchi et al., 2012). The use of components with high glycemic indexes does not necessarily cause changes in glucose and insulin concentrations. Nunes Gil et al. (2012), using different levels of maltodextrin instead of starch, observed that there was no difference between glycemic and insulinemic concentration values.

**Table 3**
Coefficients of apparent nutrient digestibility (CATTD, %), pH, lactic acid and volatile fatty acids (mmol / L), in feces of ponies supplemented with different levels of crude glycerin.

| Item | Treatment* | | | | | |
|---|---|---|---|---|---|---|
| | GLY0 | GLY4 | GLY8 | GLY12 | SEM | *P*-value |
| CANTTDDM | 60.1 | 56.6 | 59.92 | 60.4 | 3.17 | 0.2381 |
| CANTTDCP | 70.3 | 68.6 | 69.7 | 69.6 | 3.37 | 0.8491 |
| CANTTDEE | 71.3 | 70.1 | 68.0 | 70.6 | 2.09 | 0.4526 |
| CANTTDASH | 49.3 | 46.6 | 50.1 | 50.5 | 3.11 | 0.3623 |
| CANTTDAOM | 60.3 | 57.3 | 60.9 | 61.0 | 3.55 | 0.2421 |
| CANTTDNDF | 53.3 | 49.1 | 53.3 | 53.8 | 4.07 | 0.2980 |
| CANTTDADF | 80.4 | 78.0 | 80.0 | 82.3 | 1.89 | 0.0747 |
| pH | 6.37 | 6.46 | 6.51 | 6.59 | 0.18 | 0.2477 |
| Lactic acid | 6.07 | 5.84 | 5.40 | 6.90 | 1.49 | 0.5665 |
| Acetic acid | 7.02 | 7.13 | 10.33 | 7.61 | 1.83 | 0.1959 |
| Propionic acid | 3.15 | 2.99 | 3.90 | 3.33 | 0.59 | 0.3123 |
| Isobutyric acid | 0.29 | 0.30 | 0.39 | 0.28 | 0.07 | 0.2796 |
| Butyric acid | 0.67 | 0.65 | 1.20 | 0.91 | 0.32 | 0.3931 |
| Isovaleric acid | 0.36 | 0.20 | 0.55 | 0.19 | 0.19 | 0.2474 |
| Valeric acid | 0.24 | 0.13 | 0.49 | 0.18 | 0.09 | 0.1216 |

*GLY0 = control diet, no glycerin; GLY4 = *a* diet containing 4% crude glycerin; GLY8 = *a* diet containing 8% crude glycerin; GLY12 = diet containing 12% crude glycerin.

## 4. Conclusions

From this study, we can observe that the addition of glycerin, even at the amount of 12%, did not affect the digestibility of nutrients in the diet nor did it affect the general acceptability of the concentrate.

This work serves as a pioneer study for the inclusion of glycerin in horse feed. Further research is needed to clarify the appropriate levels of inclusion as well as substitution, as is the case with other species. Additionally, other areas to explore would be verifying the stability of glycerin use in feed manufacturing, as well as research into better understanding its physiological role.

## Declaration of Competing Interest

None

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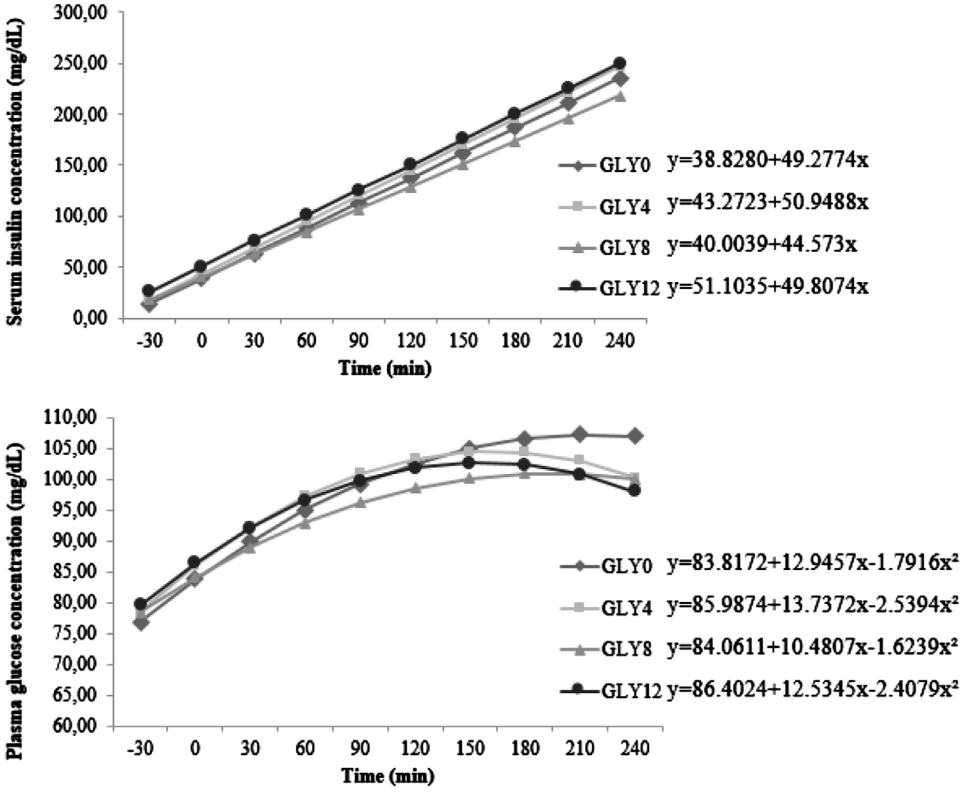


**Fig. 1.** Plasma glucose and serum insulin responses curves (regression) of equines fed experimental diets containing different levels of glycerin for 240 min ($P > 0.05$). GLY0 = control diet, no glycerin; GLY4 = *a* diet containing 4% crude glycerin; GLY8 = *a* diet containing 8% crude glycerin; GLY12 = diet containing 12% crude glycerin.

## Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.livsci.2020.104159.

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