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In vitro ruminal fermentation of glycerol, propylene glycol and molasses combined with forages and their effect on glucose and insulin blood plasma concentrations after an oral drench in sheep

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ABSTRACT

The *in vitro* gas production and the products of ruminal fermentation resulting from glycerol, propylene glycol and molasses energy additives combined with corn silage or alfalfa were evaluated. In addition, the effects of drenching with glycerol, propylene glycol and molasses on blood plasma concentrations of glucose and insulin in ewes were measured. Glycerol and propylene glycol showed a later onset of gas production than molasses (3.26 h, 1.46 h and 0.17 h, respectively; $P<0.01$). Regardless of the forage used, propylene glycol had the least volume of gas production (281 ml g^{-1}) compared to glycerol (397 ml g^{-1}) or molasses (402 ml g^{-1}; $P<0.01$). Fermentation with glycerol *in vitro* reduced the proportion of acetate and increased butyrate ($P<0.01$). Propylene glycol increased the proportion of propionate and reduced butyrate ($P<0.01$). Plasma glucose levels *in vivo* increased at 30 ($P<0.01$) and 60 min ($P<0.001$), and remained elevated for 120 and 180 min after of glycerol or propylene glycol oral administration. Similarly, insulin plasma concentrations increased significantly ($P<0.05$) at 30 and 90 min, and remained elevated until 720 min after glycerol and propylene glycol treatment ($P<0.001$). It was concluded that propylene glycol and glycerol have important glycogenic effects that may be facilitated by the long lag time that allows their absorption and direct conversion to glucose in the liver. Furthermore, the forage present during fermentation may influence the dynamics of such fermentation and suggests an association between forage and energy sources.

1. Introduction

The use of glycerol and propylene glycol as glycogenic precursors is based on *in vitro* and *in vivo* studies showing that the concentration of ruminal propionic acid increases when forage is supplemented with these organic compounds (Linke

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et al., 2004; Nielsen and Ingvartsen, 2004; DeFrain et al., 2005; Kristensen and Raun, 2007; Ferraro et al., 2009). Glycogenic precursors such as calcium propionate (Lee et al., 2012; Patton et al., 2004), propylene glycol, glycerol and their combinations have been used in veterinary practice to increase blood glucose and to reduce nutritional problems in dairy cows during the peripartum (Chung et al., 2007; Kristensen and Raun, 2007; Osman et al., 2008; Osborne et al., 2009). The use of glycogenic precursors diminishes the effects of the negative energy balance caused by the high-energy demands and metabolic disorders brought by lactation (Rukkwamsuk et al., 2005; Hippen et al., 2008).

Propylene glycol is frequently used as an energy additive in dairy cows during early lactation to decrease blood concentrations of β-hydroxybutyrate and free fatty acids, to increase propionic acid, glucose and insulin (Nielsen and Ingvartsen, 2004; Chiofalo et al., 2005), and to diminish the risk of fatty liver during the transition period (Rukkwamsuk et al., 2005). However, long periods of oral administration of propylene glycol and doses greater than 500 g per day must be avoided because propylene glycol is metabolised into toxic compounds (Trabue et al., 2007).

Molasses is a by-product of the sugar cane industry widely used as feed additive in ruminants as a rapidly fermented source of soluble carbohydrates that improves food palatability and reduces dustiness (Wiedmeier et al., 1992). The effects of molasses on volatile fatty acid (VFA) production are related to its concentration in the diet; when it exceeds 15% of the diet, propionate production is reduced and an abnormal increase in butyric acid is observed (Olbrich and Wayman, 1972).

The objectives of this study were to determine the *in vitro* gas production and fermentation products of glycerol, propylene glycol and molasses in combination with corn silage or alfalfa, and their glycogenic capacity when given as a single drench to ewes. It was hypothesised that glycerol and propylene glycol will ferment preferentially into propionic acid and that a single oral drench with these substances will produce a glycaemic and insulinemic response greater than molasses.

2. Materials and methods

The Ethics and Animal Welfare Committee of the Facultad de Medicina Veterinaria y Zootecnia, UNAM approved the animal procedures.

2.1. Experiment 1: In vitro gas production and fermentation products

2.1.1. Inoculate and inoculation

Ruminal fluid was extracted from three Suffolk rams with rumen cannula using a vacuum pump. The fluid was filtered through two layers of cheesecloth and maintained at 39 °C until its inoculation into glass flasks. The ruminal fluid was added to culture medium (see Section 2.1.2) to achieve a 10% final dilution (Menke and Steingass, 1988). Samples of dry forages (500 mg) and liquid additives were accurately weighed into 120 ml flasks, which were fitted with stoppers and sealed. Buffered ruminal fluid (90 ml) was pipetted into each flask containing the samples, and immediately placed into a water bath at 39 °C. Blank samples contained buffered ruminal fluid and no forage or liquid additives.

2.1.2. Culture medium

The culture medium was composed of mineral solution 1 (6.0 g dipotassium phosphate per litre of distilled water), mineral solution 2 (6.0 g monopotassium phosphate; 6.0 g ammonium sulphate; 12.0 g sodium chloride; 2.45 g magnesium sulphate and 1.6 g calcium chloride per litre of distilled water), a buffer solution of sodium carbonate 8%, and a reduced solution of cysteine sulphur (2.5 g/l cysteine in 15 ml 2 N sodium hydroxide; 2.5 g of sodium sulphide and 0.1 ml rezarsurin 1%) as described by Ferraro et al. (2009).

2.1.3. Fermentation substrates

Samples of alfalfa and corn silage were oven dried at 60 °C for 24 h and ground (1 mm). Samples in flasks (500 mg) were combined with 320 μl of glycerol, propylene glycol (90% wt/v) or molasses in a final volume of 90 ml, equivalent to 0.36 g glycerol, 0.30 g propylene glycol, and 0.48 g molasses. Forages were obtained from the Experimental Dairy from the University of Chapingo located in the State of Mexico, Mexico (19.48°N 98.89°W) with expected values of chemical composition for alfalfa hay (DM 892; CP 181; NDF 412 g/kg) and corn silage (DM 382; CP 77; NDF 517 g/kg).

2.1.4. Gas production

Gas pressure in the fermentation flask was recorded with a manometer attached to a needle (with a scale of 0–1 kg/cm^2) at 1, 2, 3, 4, 6, 8, 10, 12, 16, 20, 26, 32, 44, 56, 68, 80 and 92 h of incubation. The pressure measurements (kg/cm^2) were transformed to volume; pressure values and volume were adjusted by linear regression. The cumulative gas production was adjusted according to the model proposed by Menke and Steingass (1988) $Y = v/(1 + \exp(2 - 4s(t - L)))$, where Y = total volume of gas produced, v = volume, s = rate of gas production, t = time and L = lag time.

2.1.5. Volatile fatty acid analysis

1 ml samples were extracted from the flasks at 12 h of incubation and conserved with 4 ml of 25% metaphosphoric acid. Samples were centrifuged at 2500 × g for 20 min. The supernatant was obtained and aliquots taken for VFA analysis by gas chromatography (Erwin et al., 1961).