Short Note [Nota corta]

EFFECT OF PROPYLENE GLYCOL ON BLOOD METABOLITES, RUMINAL AND PRODUCTIVE PARAMETERS OF GROWING-FINISHING LAMBS

[EFECTO DEL PROPILENGLICOL SOBRE METABOLITOS SANGUÍNEOS Y PARÁMETROS RUMINALES Y PRODUCTIVOS DE CORDEROS EN CRECIMIENTO-FINALIZACIÓN]

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SUMMARY

Background. Propylene glycol has been used successfully since the 1950’s for him acute and prophylactic treatment of ketosis in dairy cows however; its use has been poorly evaluated in beef cattle and meat sheep. Objective. Evaluate the effects of different doses of propylene glycol on the productive parameters, blood metabolites and ruminal parameters in fattening lambs. Methodology. Twenty lambs 14.70 ±0.57 kg of weight, 2 months old, males and Katahdin-Black Belly breed, were randomly assigned to one of four treatments, a control mixed ration and an experimental diet with three different levels of propylene glycol: 1.0, 1.5 and 2.0 g/20 kg live weight/day. The effects of propylene glycol on blood metabolites, ruminal and productive parameters were assessed. Results. Propylene glycol supplementation did not affect (P>0.05) glucose, total lipids, triglycerides and cholesterol concentration as well as increased (P<0.05) the concentration of beta hydroxybutyrate. In addition, its inclusion resulted in an increase in pH and protozoa population and in a decrease of reductive activity (P<0.05). No effect was observed (P>0.05) on feed intake, weight gain and feed conversion ratio of lambs but carcass yield was improved without increasing the feeding cost of production. Implications. Propylene glycol is a viable option as an energy source in fattening lambs. Conclusion. Inclusion of PPG increased the pH and the population of ruminal protozoa, reduced the formation of ketone bodies and improved the carcass yield of lambs without increasing the meat production cost.

Key words: Blood metabolites; feed additives; meat lambs; productive performance; ruminal parameters.

RESUMEN

Antecedentes. El propilenglicol se ha utilizado con éxito desde la década de 1950 para el tratamiento agudo y profiláctico de la cetosis en vacas lecheras, sin embargo, su uso ha sido poco evaluado en ganado bovino y ovino productor de carne. Objetivo. Evaluar el efecto de diferentes dosis de propilenglicol sobre los parámetros productivos, metabolitos sanguíneos y parámetros ruminales en corderos de engorda. Metodología. Veinte corderos de 14.70 ± 0.57 kg de peso, 2 meses de edad, machos y raza Katahdin-Black Belly, fueron asignados aleatoriamente a uno de cuatro tratamientos, una ración mixta de control y una dieta experimental con tres niveles diferentes de propilenglicol: 1.0, 1.5 y 2.0 g/20 kg peso vivo/día. Se evaluó el efecto del propilenglicol sobre la concentración de metabolitos sanguíneos, parámetros ruminales y productivos. Resultados. La suplementación con propilenglicol no afectó (P>0.05) la concentración de glucosa, lípidos totales, triglicéridos y colesterol, mientras que aumentó (P<0.05) la concentración de beta hidroxibutirato. Además, su inclusión dio como resultado un incremento del pH ruminal y la población de protozoos, así como una disminución de la actividad reductora.
Glucose is the main source of energy in animals, it is necessary for both maintenance and to comply with the production requirements. In the case of ruminants, about 90% of the total glucose required is contributed by the gluconeogenesis process from the volatile fatty acids absorbed after ruminal fermentation (Aschenbach et al. 2010). In dairy cows in particular, during the periparturient period, due to diminished dry matter intake, the demand for propionate and glucogenic amino acids from the rumen increases significantly (Gualdrón-Duarte and Allen, 2017).

Propylene glycol (PPG) is an organic compound with a brute energy content of 5.8 kcal/g that is characterized by its glycogenic activity (Ferraro et al. 2016) and has been shown to increase the concentration of propionate and decrease the ratio of acetate to propionate, thereby resulting in a ruminal volatile fatty acids pattern that is more glucogenic (Chung et al. 2009).

Propylene glycol has been used since the 1950s as an acute and prophylactic treatment of ketosis in dairy cows (Nielsen and Ingvarsen, 2004; Bjerre-Harpøth et al. 2015), as well as in different reproductive protocols in small ruminants and dairy cattle (Hackbart et al. 2017; Mikula et al. 2020), however has been poorly evaluated in beef cattle and meat sheep. In this last, the effect of the inclusion of propylene glycol on intake, digestibility, and forage selection has been studied (Costa et al. 2019), however, there are no studies that evaluate the effect of its supplementation on productive parameters.

Therefore, the objective of this study was to evaluate the effects of different doses of propylene glycol on blood metabolites and ruminal and productive parameters in growing-fattening lambs.

**INTRODUCTION**

**MATERIALS AND METHODS**

The study was carried out in the Behavioral Testing Unit, Department of Zootechnics of the Autonomous University of Aguascalientes, located in Jesús María, Aguascalientes, México. Twenty Katahdin x Black Belly male lambs with an average weight of 14.70 ±0.57 kg of weigh, 2 months old, males and Katahdin-Black Belly breed, were randomly distributed into four experimental groups, with five replications per treatment (n=5). Treatments consisted in a control mixed ration without PPG (PPG 0) and an experimental diet in which 7 to 8% of grain (whole sorghum and rolled corn) was substituted with hay and three levels of propylene glycol (Nombre comercial del producto®, Laboratorio): 1.0, 1.5 and 2.0 g PPG/20 kg live body weight (PPG 1, PPG 1.5, and PPG 2, respectively), formulated according to the NRC (2007) specifications for fattening sheep (Table 1). Lambs were housed in individual pens, fed ad libitum, and had free access to water throughout the experiment which lasted 90 days.

**Table 1. Physical and chemical composition of control and experimental diets administered to growing lambs.**

| Ingredient (g/100 g) | Control diet | Experimental diet |
|---------------------|--------------|-------------------|
| Whole sorghum       | 40.00        | 35.00             |
| Soy meal            | 13.00        | 13.00             |
| Alfalfa meal        | 7.00         | 7.00              |
| Mineral nucleus     | 0.50         | 0.50              |
| Calcium carbonate   | 1.00         | 1.00              |
| Sodium bicarbonate  | 1.50         | 1.50              |
| Corn, rolled        | 34.00        | 30.00             |
| Hay                 | 3.00         | 12.00             |
| Analyzed composition|              |                   |
| Dry matter (%)      | 95.00        | 97.00             |
| Crude protein (%)   | 15.00        | 15.00             |
| Crude fiber (%)     | 4.00         | 10.00             |
| Crude fat (%)       | 3.50         | 4.50              |
| Ash (%)             | 3.50         | 9.00              |
| Nitrogen Free       | 69.00        | 56.50             |
| Extract (%)         |              |                   |
| Acid Detergent Fiber (%) | 17.00 | 28.00            |
| Neutral Detergent Fiber (%) | 42.00 | 44.00            |
| Metabolic energy (Mcal/kg) | 3.00 | 2.80              |

Sampling and evaluation of production parameters was carried out on days 0, 30, 60 and 90 of the study. Blood samples were obtained by jugular venipuncture 2 h after feeding using tubes (i.e., without anticoagulant) and serum was separated by centrifugation at 2500 g for 10 min. Ruminal fluid
was taken 3 h after feeding by means of an oropharyngeal probe.

Blood glucose and β-hydroxybutyrate (BHBA) concentrations were determined using a glucometer with their respective test strips (Free Style Optium Neo, Abbott, Mexico City, Mex). The measurement of serum triglycerides and cholesterol were performed with the Triglycerides Reagent kit and the Cholesterol Reagent kit (Pointe Scientific Inc., Michigan, USA) respectively, using a semiautomatic analyzer BTS-350 (BioSystems S.A., Barcelona, Spain). Total lipids concentration was also measured the Total Lipids kit (Cod. 8001602, MexLab Group, Guadalajara, Mex) in a spectrophotometer RA-50 (Bayer A.G., Leverkusen, Germany).

Immediately after ruminal fluid collection, the pH was determined using an electric potentiometer (Hanna Instruments, Mexico City, Mex). Bacterial reductive activity was evaluated by methylene blue reduction test and subsequently, 20 µL of rumen fluid was placed in a Neubauer chamber and observed in an optical microscope at 40x magnification to determine protozoa population (Dirksen, 1969).

To determine the productive parameters feed offered and their respective refusals were recorded daily, and the lambs were weighed monthly in the morning before feeding. Feed intake was estimated as daily feed intake × the days of confinement. Total weight gain (TWG) was determined for difference between the final and initial body weight, and the daily weight gain (DWG) was calculated as the quotient of the total weight gain and the confinement time (90 days). Feed conversion ratio (FCR) was calculated as the amount of feed consumed (kg) per body weight gain (kg), and the feed cost per kilogram of meat produced as the feed conversion ratio × the cost of the diet. Lambs were slaughtered at a commercial slaughterhouse at the end of the trial and carcass yield was calculated as the quotient of the hot carcass weight and the live weight at slaughter × 100.

Data were analyzed using the statistical software SPSS (2017). The effect of PPG inclusion on blood metabolites and ruminal parameters was statistically analyzed using repeated-measurements ANOVA. Data from productive performance were subjected to one-way ANOVA including the initial weight of the lambs as a covariate. Post hoc comparisons were made using Tukey test and results were considered significant when P < 0.05.

RESULTS

Blood metabolites

Average serum glucose concentration was 67.85, 69.05, 66.70 and 71.25 mg/dL for PPG0, PPG1, PPG1.5 and PPG2 lambs respectively. Although during the first 30 days the serum glucose concentration increased in the supplemented lambs to later show a gradual decrease, while that of the unsupplemented lambs decreased from the beginning of the test, this was similar (P>0.05) between the treatments and throughout the study period. Average serum BHBA concentration in PPG0, PPG1, PPG1.5 and PPG2 lambs was 0.51, 0.37, 0.35, 0.32 mg/dL respectively. BHBA concentration of the unsupplemented lambs increased significantly (P<0.05) at 60 days, also showing from that moment, a higher concentration (P<0.05) of BHBA than those lambs that received 1.5 and 2.0 g/20 kg BW of PPG. Average serum total lipids concentration was 271.00, 218.31, 201.34 and 232.87 mg/dL for PPG0, PPG1, PPG1.5 and PPG2 lambs respectively. Despite the fact that serum concentration of this metabolite in unsupplemented lambs showed a significant increase at 60 days, it did not present significant changes (P>0.05) throughout the study or between treatments. Average serum triglycerides concentration in PPG0, PPG1, PPG1.5 and PPG2 lambs was 37.85, 33.10, 31.05, 32.35 mg/dL respectively. In all treatments, the concentration of serum triglycerides increased during the first 30 days to later show a slight decrease, being statistically similar (P> 0.05) throughout the study period. Average serum cholesterol concentration was 144.40, 127.05, 123.30 and 129.03 mg/dL for PPG0, PPG1, PPG1.5 and PPG2 lambs respectively. Serum cholesterol concentration was similar between all treatments (P> 0.05), however it was significantly higher (P <0.05) at 60 days with respect to that observed at the beginning and at the end of the test (Figure. 1).

Ruminal activity

Average ruminal pH in PPG0, PPG1, PPG1.5 and PPG2 lambs was 6.07, 6.63, 6.62 and 6.69 respectively. Ruminal pH of PPG0 lambs were lower (P<0.05) at days 30 and 60 than that of the rest of lambs. At day 90, pH of PPG1 and PPG2 lambs was higher (P<0.05) than that of the PPG0 lambs while pH of PPG 1.5 lambs showed intermediate values. Average time of reductive activity in PPG0, PPG1, PPG1.5 and PPG2 lambs was 3.83, 3.71, 3.55 and 3.46 minutes respectively. Time of reductive activity increased significantly (P<0.05) in all treatments on day 30 to later show values slightly lower to those found at the beginning of the trial. At day 30, reductive activity of PPG0 lambs were higher (P<0.05) than those observed in the rest of the lambs. Average protozoa population per mL of ruminal fluid was 341.50, 373.50, 380.00 and 393.00 x 10³ for PPG0, PPG1, PPG1.5 and PPG2 lambs respectively. Protozoa population of PPG1.5 and PPG2 lambs increased rapidly during the first 30 days of the trial to later remain stable. Protozoa population of PPG1 lambs showed an initial decrease to later increase rapidly and show values similar to those of the PPG1.5 and PPG2 lambs. Potozoa population of the PPG0 lambs showed
alternating increases and decreases to eventually, being significantly lower (P<0.05) than that of the rest of the lambs (Figure 2).

**Productive parameters**

The inclusion of PPG in the diet did not affect (P>0.05) feed intake, weight gain and feed conversion ratio of the lambs as it improved (P<0.05) the carcass yield without increasing the feeding cost of production (Table 2).

**DISCUSSION**

In the present study PPG supplementation with PPG did not increase the concentration of glucose which coincides with the studies carried out by Pickett et al. (2003) and Lomander et al. (2012) and, is contrary to that described by Nielsen and Ingvartsen (2004), Kristensen and Raund (2007) and Ferraro et al. (2016) who mention that increased plasma glucose concentration is indicative of the efficacious glucogenic effect of PPG. Ferraro et al. (2009) mention that PPG is rapidly metabolized to propionate in the rumen from where it escapes through the rumen wall and is converted to glucose by the liver increasing plasma concentrations of glucose and insulin.

**Figure 1.** Effect of propylene glycol supplementation on serum metabolites of growing-finishing lambs.
Figure 2. Effect of propylene glycol supplementation on ruminal pH, reductive activity, and protozoa population of growing-finishing lambs.

Table 2. Effect of time and propylene glycol supplementation on productive performance of growing lambs.

| Treatments | Total Feed intake (kg) | Daily weight gain (g/d) | Total weight gain (kg) | Feed conversion ratio (kg) | Hot carcass yield (%) | Feeding cost/kg meat produced² |
|------------|------------------------|-------------------------|------------------------|---------------------------|----------------------|-------------------------------|
| PPG 0      | 14.63±1.00             | 244.92±24.98            | 3.87±0.25              | 4.08±0.38                 | 49.85±1.18           | $1.50±0.13                   |
| PPG 1      | 15.81±1.10             | 267.01±19.67            | 4.12±0.46              | 3.87±0.39                 | 57.80±0.86¹          | $1.57±0.09                   |
| PPG 1.5    | 14.54±1.09             | 228.78±24.67            | 3.58±0.37              | 4.29±0.36                 | 58.32±1.17²          | $1.37±0.14                   |
| PPG 2      | 16.54±1.08             | 286.99±33.75            | 4.29±0.46              | 4.01±0.35                 | 56.40±0.89³          | $1.65±0.14                   |

P value

Means in the same column with different superscript are different (P<0.05); ¹ PPG 0 control diet without propylene glycol, ² PPG 1 experimental diet + 1.0 g PPG/20 kg BW, ³ PPG 1.5 experimental diet + 1.5 g PPG/20 kg BW, ⁴ PPG 2 experimental diet + 2.0 g PPG/20 kg BW; ² US Dollars.

Total lipids, triglycerides and cholesterol concentration was similar between treatments which agrees with the findings of Toghdory et al. (2009) and Kabu and Civelek (2012) who found that the addition of PPG did not affect the concentration of total lipids, triglycerides and cholesterol.

At the end of the test period which coincides with a higher energy demand of the lambs, a decrease in BHBA concentration together with a decrease in triglycerides concentration was observed in lambs supplemented with the highest doses of PPG. Nielsen and Ingvartsen (2004), Kristensen and Raund (2007), Bjerre-Harpøth et al. (2015) and Chalmeh et al. (2020) stated that PPG caused an increase in the glucogenic status and a reduction of adipose tissue mobilization, which leads to decrease NEFA in the liver and reduction in the formation of ketone bodies. In this way, Kalyesubula et al. (2019) concluded that treatment with PPG effectively reduced hyperketonemia and lipolysis. Contrary, non-supplemented lambs showed a higher concentration of serum BHBA which suggests that to support the energy requirement, the body fat and protein are mobilized for hepatic gluconeogenesis, which leads to the increase of NEFA, BHBA and ammonia in plasma (Moore and DeVries (2020). Addition of PPG increased the ruminal pH, a situation contrary to that described by Nielsen and Ingvartsen (2004) since, according to their study, it could be expected that the inclusion of PPG in the diet would lead to a decrease in ruminal pH because of an increase in the concentration of ruminal
propionate. Chung et al. (2009) found a decrease in ruminal pH in cows receiving PPG as an oral drench, but not in cows that received it as a part of a total mixed ration or as a rumen drench. For their part, Kristensen and Raund (2007) found that ruminal pH was not affected by PPG supplementation.

The inclusion of PPG increased rumen protozoa which, as several studies pointed out, is closely related to ruminal pH. According to Dehority (2005) and Francisco et al. (2019), the abundance of total ciliates and entodiniomorphid protozoa was highly influenced and positively correlated with rumen pH, and values below 5.5 above 15 h/day generally causes protozoa death (Dehory, 2005; Franzolin and Dehority, 2010).

Time of reductive activity decreased with the inclusion of PPG observing values less than 4 minutes which was indicative of an adequate bacterial activity. Concurring to Karapinar et al. (2008) a methylene blue reduction time greater than 6 minutes is considered as evidence of acute ruminal lactic acidosis, a rumen fermentative disorder that occurs in sheep feed with large amounts of seeds rich in ruminal fermentable carbohydrates such as grain whose inclusion level in the diets used in this study ranged between 65 and 74%.

Recapitulating, the addition of PPG increases the number of ruminal protozoa which would appear limit the accumulation of lactate, which leads to an increase in the pH and in the number and activity of bacteria with the consequent increasing of reductive activity (Brossard et al. 2004; Karapinar et al. 2008).

Notwithstanding that PPG has a high energy content and consequently the potential to increase food intake, this was not observed in the present study. Studies carried out in cows, bull calves and sheep coincide that food intake is generally not affected by the administration of PPG (Pickett et al. 2003; Kim et al. 2005; Chung et al. 2009; Yazdi et al., 2015; Costa et al. 2019). The foregoing is attributed to the fact that the doses used are not sufficient to improve the energy density of the food and induce an increase in food intake. Also, PPG is considered unpalatable and generally does not stimulate food intake (Nielsen and Ingvartsen, 2004).

Addition of PPG did not increase TWG and DWG of the lambs, which is consistent with the study carried out by Kim et al. (2005), who did not observe differences in the initial and final weight in steers supplemented with propylene glycol. However, it should be noted that the lambs achieved the DWG for which the control diet was formulated (250 – 300 g/d), and the decrease in the energy content of the experimental diets did not affect the growth of the lambs or the feeding cost of production, which could be considered a positive effect of the PPG addition. This is very important if it is considered that in Mexico the energy of the diets used for the fattening of lambs comes mainly from cereal and soybean meal, which are mostly imported and their price is subject to frequent fluctuations that could negatively affect the profitability of the farms (Reynoso et al. 2017).

FCR did not have a significant effect between treatments while the study of other gluconeogenic substrates showed that feed conversion efficiency of finishing cattle was improved by 21.9 and 33% when glycerine was used to replace 100 g/kg DM of the dry-rolled corn content and when it was included at 120 g/kg DM of the diet respectively (Pyatt et al. 2007; Eiras et al. 2014). However, FCR obtained in this study is within the reported range (4.11 – 4.99) for Katahdin lambs crossed with various meat breeds (Vázquez et al. 2011).

Carcass yield was significantly improved with the inclusion of PPG, effect not found by Kim et al. (2015) who reported that PPG did not affect carcass weight of Korean native steers.

**CONCLUSION**

Inclusion of PPG increased the pH and the population of ruminal protozoa, reduced the formation of ketone bodies and improved the carcass yield of lambs without increasing the meat production cost.

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**Conflict of interests.** The authors hereby declare that they have no conflict of interest.

**Compliance with ethical standards.** This study was approved by the Ethics Committee for the Use of Animals in Teaching and Research (CEADI-UAA as stand in Spanish). The study was carried out in compliance with the provisions established in the Ethics regulations for the use of animals in teaching and research at the Autonomous University of Aguascalientes Code: DI-PL-NO-37.

**Data availability.** Data are available with the corresponding author (teodulo.quezada@edu. ua a.mx) upon reasonable request.

**Author contribution statement (CRediT).** Araceli López-Vargas – Conceptualization, Methodology, Formal Analysis, Writing – original draft. Teódulo Quezada Tristán – Conceptualization, Methodology, Formal Analysis, Writing – original draft. Carlos Haubi-Segura – Formal Analysis, Writing – review & editing. Rafael Macedo-Barragán – Formal Analysis, Writing – review & editing. Raúl Ortiz-Martínez – Formal Analysis, Writing – review & editing. Arturo Valdivia-Flores - Formal Analysis, Writing – review & editing.
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