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*Original Research Article*

# The Effects of Different Glucose Precursors on Rumen and Blood Parameters in Dairy Cows

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## ABSTRACT

**The purpose of this research is to determine the drenching different glucose precursors, which are propylene glycol and glycerol, on rumen volatile fatty acids (VFA), blood glucose and insulin levels and dry matter intake (DMI) of dairy cows. Three rumen cannulated lactating Holstein cows were divided into propylene glycol, glycerol and control groups, according to the 3x3 Latin square method. Blood and rumen samples were taken before and at certain minutes after drinking the liquids. The amount of DMI by the cows was recorded daily. The measured DMI value was found to be the highest (22,9 kg/day) in the glycerol group and the lowest (17,3 kg/day) in the propylene glycol group (P<0,02). The blood insulin level (22,2 ng/dL) in the propylene glycol group was higher than the glycerol (18,5 ng/dL) and control (16,8 ng/dL) group (P<0,05). Rumen propionic acid values of the propylene and glycerol groups were higher than the control group (P<0,05). From this research, it is concluded that these effects should be considered in the use of propylene glycol due to the decrease in DMI along with the increase in blood glucose and insulin levels.**

**Keywords: Glycerol, propylene glycol, dry matter intake, blood parameters**

## INTRODUCTION

Dairy cattle have been subjected to genetic selection for the last 50-60 years to obtain more milk yield, and significant increases in the milk yield have been achieved by providing better care and feeding conditions. With the increase in milk yields, it has been observed that the animals' dry matter intake (DMI) is insufficient to meet the increasing energy needs. This is especially true in the so-called transitional period, which includes the three weeks before birth (prepartum) and the three weeks after birth (postpartum). During this period, the significant live weight and body condition (which are caused by the rapid growth of the calf, decreased DMI, the onset of colostrogenesis and the rapid increase in the need for nutrients in cows in parallel with the increased milk yield with birth). The condition that manifests itself with the loss of body condition is called negative energy balance (NEB)(Hayırlı *et al.* 2012).

Transition management aims to eliminate NEB. The increase in the incidence and frequency of diseases in the transition period is an important factor affecting the lactation performance in the following period (Mulligan *et al*. 2008). In this period, adequate glucose support is important to meet the production requirements of the tissues. For this purpose, glucogenic substances have been increasing in recent years to reduce energy and glucose deficit in high-yielding dairy cows. The most commonly used preparations are propylene glycol and glycerol, which offer additional propionate for gluconeogenesis in the liver (Drackley *et al*. 2005).

Lemley *et al*.(2010) and Hidalgo *et al.* (2004) found in their studies that drinking propylene glycol once a day increased serum insulin, progesterone and pregnancy rates and decreased NEB. In their study, Hayirli *et al.* (2001) reported that PG administration caused changes in some metabolic parameters, increased glucose and insulin levels, and decreased non-esterified fatty acids (NEFA) and Beta-hydroxy butyric acid (BHBA) levels in dairy cattle.

Fisher *et al*. (1971) reported that giving glycerol once a day to dairy cows has potential benefits and may be a preventative agent for ketosis. Studies show that the part of glycerol that enters the rumen is directly absorbed and contributes to glucose synthesis, helping to regulate energy metabolism. (Remond *et al.*1993)

In our study, certain parameters in rumen fluid and blood were evaluated by giving propylene glycol and glycerol to dairy cattle. We aim to determine which one is more effective by observing the changes in the body

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and rumen of dairy cattle, which are frequently used glucose precursors to meet the increasing energy need in dairy cattle, and to contribute to the solution of transition period problems and NEB.

## MATERIALS AND METHODS

This study was carried out in 2016 in the cattle breeding unit of Uludağ University Faculty of Veterinary Medicine, Animal Health and Animal Production Research Application Center. Three dairy cows were used as animal material. These were determined as Holstein cows with rumen cannula inserted and multiple births. The body condition scores of these animals used in the study are in the range of 3.25-3.75, and the number of days to milk is 150 days and above. Corn silage with 31.86% dry matter and alfalfa hay stored in the second form, 20-25 kg bales cut in mid-flowering were used as roughage material.

The daily nutrient needs of animals are arranged according to the recommendations of NRC (2001) for a dairy cattle with a body weight of 600 kg, a milking day of 200, daily milk production of 30 kg with 3.70% fat, and consuming 22 kg of dry matter. The ration was prepared with a rough-concentrated feed ratio of 48:52, and its composition is given in Table 1.

**Table 1.** Total mixed ration and nutrient content.

| | **% DM basis** |
|---|---|
| Corn Silage | 31,86 |
| Alfalfa Hay | 16,00 |
| Concentrate mixture | 52,14 |
| **Nutrient Content (Based on DM)** | |
| Dry Matter, % | 52,61 |
| Crude Protein, % | 16,89 |
| Crude Ash, % | 7,55 |
| Ether extract, % | 3,41 |
| NDF (Neutral Detergent Fiber),% | 34,35 |
| ADF (Acid Detergent Fiber),% | 21,32 |
| Forage NDF,% | 24,17 |
| eNDF, % | 25,26 |
| Starch,% | 25,73 |
| NEL (Net Energy Lactation), Mcal/kg | 1,54 |

Cows used in the study; 1 animal in each group was randomly selected and divided into three groups according to the 3x3 Latin square method. These groups are; in 13-day periods, with each animal staying for 13 days in each group; propylene glycol, glycerol and control (water). The animals were given mono propylene glycol in the form of a 25 kg drum belonging to the "Kartal Kimya" company as propylene glycol. Pharma quality glycerin was given to the animals as glycerol in the form of a 30-kilogram drum belonging to the "Kartal Kimya" company. In the group in which the cows took place for 13 days, 10 days of acclimatization was applied, and samples were taken for the next 3 days. Blood and rumen contents were sampled from these animals, which were given propylene or glycerol for 10 days before the feed was added in front of them at 07:30 in the morning on the 11th, 12th and 13th days, then their feed was added and 300 cc was added into the propylene group as in the field conditions. Propylene glycol was given to the glycerol group with 450 cc of glycerol and to the control group, 400 cc of water with the help of a mouth pump. Blood samples were taken from the vena subcutanea abdominis into serum tubes at 0, 10, 20, 30, 60, 90, 120, 180, 240 and 360 minutes during the day after the orally ingestion was finished.

The blood samples were put into ice without losing time for glucose measurement and stored at -20°C until analyzed by removing their serum under the cold chain. Afterwards, it was dissolved and serum glucose level was determined with the help of glucose kit (Biolabo Reagents, Glucose GOD-PAP, Reference No: 87109) in Bursa Uludağ University Faculty of Veterinary Medicine Department of Biochemistry. Likewise, the

determination of serum insulin concentration from blood samples was done with Elisa kits (SunRed Bovine Insulin (Ins) ELISA Kit, Catalog No: 201-04-0019).

Rumen fluid samples were taken at 10, 20, 30, 60, 90, 120, 150, 180, 210, 240 and 360 minutes from these animals whose rumen cannulas were placed after the oral drinking process was completed. pH changes in the rumen were measured from fresh rumen fluid samples with the help of a digital pH meter (Mettler Toledo AG 8603 SevenGo pH meter SG2, Schwerzenbach, Switzerland).

In order to determine the amount of volatile fatty acid, 1,5 ml of rumen fluid samples were filtered 4 times, transferred to 2 ml eppendorf tubes containing 30 µl of 50 % sulfuric acid (H2SO4) and stored at -20°C. Then it was dissolved and centrifuged for 3 minutes, then 0,6 ml was taken into another violet, 0,12 ml of 25% metaphosphoric acid was added, centrifuged for 3 minutes and then read in Gas Chromatography (Gc).
For nylon bag analysis, feed samples taken from TMR, incubated and ground beforehand, were taken at 0, 2, 4, 8, 16, 30 and 48 hours separately for each cow, and 5 g feed samples were placed in 3 separate bags for each hour and rumen. It is placed in the rumen of our cannulated animals (Orskov et al.1979). It was taken at 0, 2, 4, 8, 16, 30 and 48 hours and put into cold water, stopping the microorganism activities, and then put in the oven again. The rumen degradability of the total mixed rations used in the study in terms of DM was determined by taking the difference with the first values on the basis of dry matter (DM).

In order to determine the DMI, the amount of feed left over from the amount of feed given to the animals was subtracted on the basis of DM and daily consumption was determined. The animals included in the study were fed individually and their daily feed consumption was recorded. This process was continued for 39 days as 10 days of acclimatization and 3 days of sampling.

For the nutrient analysis of the feeds, a sample was taken from the forage and concentrate mixture once in each data period. These samples were mixed and turned into a single sample, and nutrient analyzes were made. Methods specified in AOAC (1990) for the determination of raw nutrients (DM, crude protein, crude oil, crude ash); polarimetric method in starch analysis; Ankom Fiber Analyzer was used in NDF, ADF and ADL analyzes, operating according to the principles stated by Van Soest *et al* 1991. While the GLM procedure and Full Factorial model were preferred for the comparison of DMI, rumen pH, blood insulin and glucose, VFA, data, Boferroni was chosen for the confidence interval correction for the comparison of the main effects. Kruskal-Wallis Analysis of Variance was used for comparison of experimental groups according to time, while Mann-Whitney U test was used for pairwise comparisons between groups.

The significance level was $P < 0.05$, and the SPSS (Version 20.0, SPSS Inc, USA) program was used to evaluate the data.

## RESULTS AND DISCUSSION

In Table 2 which includes the blood glucose and insulin values after the application, the 120th minute control group was found to be the lowest, the propylene group the highest, and no significant difference was found between the glycerol group and the other 2 groups. The propylene group was found to be the highest at 180 minutes, and there was no significant difference between the glycerol and control groups. The control group was found to be the highest at 360 minutes, and there was no significant difference between propylene and glycerol. There was no significant difference between the mean values. The propylene group was found to be the highest, and the control group was the lowest at 60th minute in the insulin data. No significant difference was found between the glycerol group and the other two groups. While the glycerol group was the lowest at 90 minutes, there was no significant difference between the control and propylene groups. At 180. min, the control group was found to be the lowest, and there was no significant difference between the glycerol group and the propylene group. At 240 minutes, the glycerol group was the highest, the control group was the lowest, and there was no significant difference between the propylene group and the other two groups. At 360 min, the propylene group was found to be the highest, and there was no significant difference between the control and glycerol groups. When the mean values were examined, the propylene group was found to be the highest, and there was no significant difference between the other two groups.

**Table 2.** Post-application blood glucose (mg/dL) and insulin (µU/mL) values.

| | | 0 | 10 | 20 | 30 | 60 | 90 | 120 | 180 | 240 | 360 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **Control** | **Glucose** | 68,2 | 67,5 | 63,3 | 74,6 | 64,5 | 61,2 | 57,2$^{b}$ | 63,7$^{b}$ | 66,0 | 73,8$^{a}$ |
| | **Insulin** | 13,5$^{b}$ | 19,6$^{a}$ | 17,1$^{a}$ | 16,9 | 15,4$^{b}$ | 18,6$^{a}$ | 14,3 | 14,5$^{b}$ | 13,7$^{b}$ | 15,4$^{b}$ |
| **Propilene** | **Glucose** | 67,2 | 74,4 | 67,0 | 71,2 | 64,9 | 61,6 | 67,6$^{a}$ | 73,9$^{a}$ | 69,0 | 62,9$^{b}$ |
| | **Insulin** | 17,3$^{a}$ | 23,5$^{a}$ | 23,8$^{a}$ | 18,3 | 22,0$^{a}$ | 26,2$^{a}$ | 17,9 | 22,3$^{a}$ | 20,8$^{ab}$ | 31,5$^{a}$ |
| **Glycerol** | **Glucose** | 68,6 | 69,5 | 65,0 | 78,1 | 80,5 | 67,5 | 61,7$^{ab}$ | 59,2$^{b}$ | 55,1 | 62,9$^{b}$ |
| | **Insulin** | 16,8$^{a}$ | 15,3$^{b}$ | 15,6$^{b}$ | 18,2 | 18,9$^{ab}$ | 17,2$^{b}$ | 17,9 | 21,6$^{a}$ | 23,6$^{a}$ | 19,6$^{b}$ |

$^{a, b}$: Differences between means with different superscripts in the same column are significant (P<0,05)

In Table 3 acetic acid data, propylene is the highest at 60. min, glycerol is the lowest at 120. min, the glycerol group is the lowest at 210. min, the control is the highest at 210. min, the propylene group is the lowest at 360. min. It was observed that the glycerol group had the lowest level. When we look at the mean values, the control group has the highest, and the glycerol group has the lowest.

**Table 3**. Acetic acid (AA), Propionic acid (PA) and Butyric acid (BA) values in rumen fluid after application (mmol/L).

| | 0 | 10 | 20 | 30 | 60 | 90 | 120 | 150 | 180 | 210 | 240 | 360 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **AA-Control** | 47,9 | 60,9 | 67,1 | 58,8 | 54,1$^{ab}$ | 48,1 | 52,5$^{a}$ | 53,5 | 52,0 | 97,2$^{a}$ | 42,7 | 50,8$^{a}$ |
| **AA-Propilene** | 46,5 | 95,0 | 103,1 | 46,9 | 48,9$^{a}$ | 73,0 | 53,9$^{ab}$ | 47,2 | 49,4 | 53,0$^{b}$ | 39,6 | 53,4$^{a}$ |
| **AA-Glycerol** | 43,5 | 50,3 | 75,2 | 43,9 | 39,8$^{b}$ | 41,7 | 43,2$^{b}$ | 44,2 | 50,1 | 52,7$^{ab}$ | 51,0 | 42,8$^{b}$ |
| **PA-Control** | 21,4 | 17,0 | 18,7 | 15,8 | 15,2$^{b}$ | 22,1 | 24,2 | 17,5$^{b}$ | 24,8 | 18,2$^{b}$ | 18,1$^{b}$ | 16,6$^{c}$ |
| **PA-Propilene** | 21,6 | 41,4 | 45,9 | 22,5 | 24,4$^{a}$ | 28,2 | 29,4 | 25,6$^{ab}$ | 25,7 | 30,0$^{a}$ | 19,0$^{b}$ | 27,8$^{b}$ |
| **PA-Glycerol** | 22,9 | 26,1 | 37,7 | 24,4 | 23,2$^{a}$ | 24,5 | 27,3 | 28,1$^{a}$ | 28,5 | 33,5$^{a}$ | 30,0$^{a}$ | 30,0$^{a}$ |
| **BA-Control** | 12,6 | 14,1 | 15,5 | 15,8 | 16,1 | 15,1 | 16,9 | 19,2 | 18,3 | 36,3$^{a}$ | 14,1$^{b}$ | 13,6$^{b}$ |
| **BA-Propilene** | 15,5 | 24,8 | 26,7 | 14,6 | 15,1 | 24,1 | 18,9 | 16,6 | 16,6 | 20,4$^{b}$ | 12,6$^{b}$ | 18,1$^{a}$ |
| **BA-Glycerol** | 13,9 | 15,2 | 21,6 | 15,3 | 15,9 | 17,6 | 20,4 | 21,4 | 24,2 | 22,4$^{ab}$ | 21,8$^{a}$ | 17,9$^{a}$ |

$^{a, b}$: Differences between means with different superscripts in the same column are significant (P<0,05)

In Table 3 propionic acid data, the lowest in the control group at 60. min, the lowest in the control group, the highest in glycerol, the lowest in the control group at 210. min, and the lowest in the glycerol group at 240 and 360. min was found to be high. Considering the mean values, the control group was the lowest, and there was no significant difference between the glycerol and propylene groups.

In Table 3, it was found that butyric acid levels were highest in the control group at 210.min, lowest in the propylene group, highest in the glycerol group at 240.min, and lowest in the control group at 360.min. Considering the mean values, no significant difference was found between the three groups.

In Table 4, the highest value in the DMI data was the group given glycerol, while the lowest value was in the propylene group.

**Table 4.** Dry matter intake (kg).

| | Dry matter intake |
|---|---|
| **Control** | 21,2$^{b}$ |
| **Propilene** | 17,3$^{c}$ |
| **Glycerol** | 22,9$^{a}$ |

$^{a, c}$: Differences between means with different superscripts in the same column are significant (P<0,00)

In our study, when we evaluated the levels of glucose and insulin data in three groups at determined times; Although PG and glycerol have superiority over each other at different minutes, the increases in the PG group are evident. In a study by Holtenius *et al.* (1996) on PG administration, it was shown that it increased blood glucose levels, as in our study. In a study by Miyoshi *et al.* (2001), it was determined that serum insulin concentration increased between the 30th and 90th minutes after administering PG to cattle on the 7th and 42nd days postpartum. Similarly, in our study, a significant increase was noted at the 60th and 360th minutes. In a study conducted by Grummer *et al.* (1995) in pregnant cattle, they applied PG and found that the insulin concentration increased. In

this study, they determined that glucose increased until the 30th minute, remained just above the initial level before the 100th minute, and that these levels were maintained for 200 minutes, and then began to decrease. Insulin, on the other hand, has been reported to show a linear regression after the first half-hour rises above the initial levels in PG patients. On the other hand, in a study by Moallem *et al.* (2009), they applied 500 g/day of pure PG for 21 days during the transition period and determined lower insulin concentrations in the PG group compared to the control group.

When examined in similar studies, an increase in blood glucose levels and a decrease in the amount of NEFA and ketone bodies were reported with the administration of glycerol to dairy cows (Bodarski *et al* 2005, Goff *et al.* 2001, Osman *et al.* 2008). Piantoni *et al.* (2015) reported that plasma glucose concentrations increase linearly as glycerol supplementation increases, and cows supplemented with glycerol have 7% higher blood glucose than control cows. Although there was an increase in blood glucose at the 2nd and 3rd hours of PG supplementation in our study, as seen in many studies, the effect of glycerol supplementation on plasma glucose level was not at the expected level. It can be thought that the animals in our study were not in NEB, but in positive energy balance, which caused these results.

In our study, in the acetic acid data measured in the rumen fluid, glycerol was found to be at the lowest level at 60, 120 and 360 minutes, and the propylene group was the lowest at 210. Minutes. Traube *et al.*(2007) reported that the acetate concentration was higher in the control samples, similar to our study in animals given propylene glycol and glycerol in 2 different forms. This indicates that the addition of propylene glycol and glycerol suppressed acetate formation. When the data of propionic acid were evaluated, the control group was found to be lowest at 60, 150 and 210. minutes, and the glycerol group was highest at 150. minutes, 240 and 360. minutes. Some older publications show that glycerol is largely propionate fermented (Johnson R. 1954). When rumen fluid from cows previously acclimated to a glycerol diet was added to glycerol in vitro, it resulted in higher production of propionate and butyrate compared to acetate (Remond *et al* 1993). In different studies, it was determined that the administration of glycerol into the rumen did not affect the molar percentage of propionate (Kristensen *et al.*2007). Traube *et al.* (2007) found that adding propylene glycol resulted in much greater propionate concentration increases over time than adding of glycerol. When the analysis of the data of butyric acid is evaluated; The highest in the control group, the lowest in the propylene group, the highest in the glycerol group at 240.min, and the lowest in the control group at 360.min. There are also studies with different results on this subject; addition of PG sharply reduced butyrate formation; an increase in butyrate was observed with the addition of glycerol (Remond *et al.*1993). De Frein *et al*.(2004) found a higher proportion of butyrate in ruminal fluid obtained from cows using glycerol by oral ingestion.

When we look at the results of DMI in our study, the BMT value of the group fed with PEG was lagged compared to glycerol. Previous studies have shown no reduction in KM intake for glycerin-fed animals(Carvalho *et al*. 2011). Wang *et al*. (2009) found no difference in KM intake in Nelore bulls fed 300 g/kg glycerin. Likewise, in the study of Vanessa *et al*.(2015) on this subject, the addition of 200 g/day glycerin to cattle diets did not affect KM intake. There are also studies reporting a decrease in KM intake in dairy cattle fed with glycerin (Elam *et al* 2008). In the study of Drackley *et al.* (1999), among the effects of glycerol-supplemented prepartum diet, there was a 17% decrease in DMI, regardless of the amount of glycerol administered. In Ogborn's 2006 study, glycerol feeding tends to reduce DMI by about 1 kg/day. In our research, on the contrary, there was a significant increase in DMI in the glycerol group. We think that the changes in administration and amount of glucose precursors are effective in the differences in our study. The possibility that frequent samples may cause stress in our seas may be one of the factors limiting our results. Our aim in this study is to evaluate the effects of the glucose precursors we use on rumen fluid and blood parameters, to open new horizons in the solution of common metabolic problems, and to provide the expected increase in productivity in dairy cattle.

Based on the data we obtained in our study, it would be beneficial to use propylene glycol especially in dairy cattle feeding, considering the decrease it causes in DMI due to the significant increase in blood glucose and insulin values and rumen parameters in certain minutes. This and similar studies with glucose precursor supplements will bring more guiding results, especially in large groups of animals in the transitional period and NEB.

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