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*Original Research Article*

# The Effects of Different Glucose Precursors on Milk Production and Composition in Dairy Cows

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## ABSTRACT

**The aim of this study is to investigate the effects of glucose precursors on blood parameters, milk yield and composition in dairy cows. For this study, 26 multiparous and 34 primiparous Holstein cows were used in randomized block design to evaluate the feeding glycerol (G), propylene glycol (PG), and water (C) from 7 d prepartum to 21 d in milk (DIM). Treatments were 300ml water (C), 450 ml glycerol (G), and 300 ml propylene glycol were drenched after morning feeding. Milk yields were recorded as daily, milk samples were analyzed for fat, protein, lactose from all cattle for two consecutive days every week compositions until the 100 DIM. In other hand, blood samples were taken for glucose at last week of gestation, day calving, week 1, 2 and 3 in postpartum. There was no difference between the groups in glucose levels. However, milk yield of C (35.06 L/day) group was 1 L higher than G (34.18 L/day) group and 1,5 L higher than PG (33.46 L/day) group (P<0,05). Milk fat was found to be higher in PG group cows (3.67%) compared to C (3.44%) and G (3.46%) cows (P<0.05). However, milk proteins of the C (3.32%) group were higher than the PG (3.15%) group. Milk yield and milk protein were found to be higher in the untreated C group than in the G and PG groups, and differences were found between the groups in terms of milk fat. It was concluded that there is a need for further investigation of the effects of glucose procurers such as G and PG, especially on the rumen.**

**Keywords: Glucose Precursors, glycerol, propylene glycol, cows**

## INTRODUCTION

In order to meet the increasing demand for animal products in parallel with the increasing world population, it has become inevitable to raise high-yielding cattle and to use existing breeds more effectively (Meral and Kara, 2013). High milk yielding cows have been bred with genetic breeding studies, but this has brought some disadvantages with it. Especially in the transition period, with the start of lactation, despite the increase in milk yield, the insufficient increase in dry matter consumption cannot meet the increased energy need of the animal in this period. As a result, the animal enters the Negative Energy Balance (NEB) and various diseases occur along with it. Therefore, in recent years, the use of glucogenic substances has been the focus of attention in order to meet the energy and glucose deficiency in the peripartum period in order to prevent the negative consequences of NEB in cattle with high milk yield (Erdoğan 2014, Liu *et al*. 2009). The most well-known glucose precursors are propylene glycol and glycerol. Many studies have been carried out in this area over the years and different results have been obtained. In studies conducted with PG at various doses, there are studies reporting that an increase in milk yield was observed in the first week of lactation in groups treated with PG (Overton and Waldron 2004, Stokes and Goff 2001). Although there was a tendency to increase milk yield with PG application, but no statistically significant increase was observed. (Emery *et al.*1964, Liu *et al*. 2009). When we look at the publications made with glycerol in the literature, it was stated in a study that 500 ml G administration between the prepartum 21st day and the postpartum 70th day increased milk yield in the first 10 weeks of lactation (Bodarski *et al*. 2005). In another study, 20 g/L of G was added to drinking water between prepartum 7 and postpartum 7 days, and as a result of this short application, the desired glycogenic effect was not observed and no change in milk yield was observed (Osborne *et al*., 2009).

In our study, the effects of the use of propylene glycol and glycerol in dairy cattle on milk yield, milk composition and certain blood parameters were evaluated. The aim of this study is to examine the effects of propylene glycol (PG) and glycerol on some blood parameters, milk production and composition in dairy cows.

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## MATERIALS AND METHODS

Experimental studies were carried out between January – December 2016 in Orhan Agriculture and Food and Livestock, operating under Orhan Holding in Bursa Yenişehir, which has 250 milking cows. The animals used in the research were kept in a semi-open, free-stall barn. As animal material, a total of 60 Holstein pregnant cows, 34 of which gave birth for the first time and 26 cows that gave birth more than once, were used. The average lactation number of the animals used in the study was 1,97. The mean BCS of the studied animals was 2.0-4.0 and 3.41. Experimental animals were included in the groups 7 days before the end of the dry period, and the applications were continued until the 21st day after birth and followed up until the 100th day. The dairy cattle used in the study were classified according to their lactation numbers and BCS at the beginning of the transition period, and care was taken to distribute them equally to the groups according to these criteria. Cows are divided into 3 groups. Two trials, a control group, were set up to have 20 cattle in each group. Goup C: cows in the control group did not receive any intervention. Group PG: cows in the PG group were administered 300 ml of PG orally once a day between the 7th day of prepartum and the 21st day of postpartum. Group G: cows in group G were administered 450 ml of glycerol orally once a day during the same time period. In the study, care was taken to apply the above-mentioned practices at the same times every day, when the morning portions are poured and by the same person.
The cows used in the study were fed with the ration specified in Table 1 during the first 40 days of the dry period, and in the last 20 days of the dry period, the ration given to the fresh group (Table 2) and the mixture obtained by taking half a portion of each of the dry period ration, and the mixture obtained was given to the fresh group after calving. When they were taken into the group, they were fed with the ration indicated in Table 2. While preparing the specified rations, they were arranged to meet the daily nutrients specified by the National Research Council (NRC, 2001). The roughage and concentrate feeds were mixed in TMR and given to the animals as 2 meals a day ad libitum. While calculating the portion, 10% of the feed given was adjusted to remain. Dry matter (DM), crude protein (CP), crude oil (CO), crude ash, Ca and P analyzes of the prepared rations were prepared according to the methods specified in the Association of Official Analytical Chemists (AOAC 1990). Neutral Detergent Fiber (NDF), Acid Detergent Fiber (ADF) and Acid Detergent Lignin (ADL) analyzes were performed by Soest *et al.* (1991) was applied as reported in his study.

**Table 1.** The TMR content and nutrient composition of dry period.

| **Items** | **%DM** |
|---|---|
| Straw | 33.50 |
| Clover | 8.8 |
| Corn Silage | 23.4 |
| Sunflower Seed Meal | 17.3 |
| Barley | 8.5 |
| Bran | 8.5 |
| **Nutrient Content** | **%DM** |
| Crude Protein | 13.5 |
| Ether Extract | 2.4 |
| Crude ash | 6.66 |
| NFC | 26.6 |
| Starch | 14.8 |
| NDF | 50.9 |
| ADF | 33.2 |
| NEL Mkal/kg | 1.22 |

**Table 2.** The TMR content and nutrient composition of lactation period.

| **Items** | **%DM** |
|---|---|
| Oat Hay | 7.2 |
| Alfalfa Silage | 8.2 |
| Alfalfa Dry Grass | 13.5 |
| Corn Silage | 21.9 |
| Soybean Meal | 7 |
| Canola Meal | 3.8 |
| Wheat bran | 2.9 |
| Sweetcorn | 6.6 |
| Barley | 7.6 |
| Wheat | 3.3 |
| Sunflower Seed Meal | 1.3 |
| Rice Bran | 1 |
| DDGS | 7.8 |
| Corn Bran | 1 |
| Corn Gluten | 1.3 |
| Molasses | 2.5 |
| Limestone | 0.8 |
| Salt | 0.3 |
| Vit-Min Premix | 0.1 |
| By-Pass Oil | 1.9 |
| **Nutrient Content** | **%DM** |
| Crude Protein | 17.5 |
| Crude oil | 6.2 |
| Ash | 7.29 |
| NFC | 36.7 |
| Starch | 23.2 |
| NDF | 33 |
| ADF | 19.7 |
| NEL Mkal/kg | 1.69 |

The blood sample to be analyzed in the study was taken from Vena Subcutanea Abdominis into 10 ml tubes. Prepartum -7. Day and postpartum 7th, 14th and 21st days were taken at the same time. The blood samples taken were centrifuged at 10000 rpm for 5 minutes with a centrifuge machine of Elevtro-Mag-M4812, Istanbul, Turkey brand and model, and blood serums were obtained. The obtained serums were taken into 2 ml Eppendorf tubes with the help of an automatic pipette. It was stored in a UDD-500 BK brand and model freezer at -20$^0$C until the day the tests were to be analyzed. 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.

In our study, lactating dairy cattle were milked three times a day, in the morning, noon and evening, at the same time and daily milk data were recorded. From the 4th day of lactation to the 100th day of lactation, milk samples were collected individually and homogeneously from each milking two days in a row every week through the sample collection chamber of the milking parlor equipment. Milk samples taken into 50 ml skirted falcon tubes were analyzed on the same day with cold chain in Uludag University, Faculty of Veterinary Medicine, Department of Animal Nutrition and Nutritional Diseases. In the analysis, Foss brand FT1 device (Denmark) device was used for the measurements of total dry matter, non-fat dry matter, fat, protein, lactose and milk urea nitrogen values in milk.

The milk yields of the dairy cattle included in the study for the first 100 days will be followed and the lactation milk yield for 305 days has been determined according to the peak times, peak milk yield, periods of staying at peak, milk yield persistence, milk averages for the first 100 days and lactation peak milk yield among

the groups. The daily milk data of the dairy cattle used in the study were taken daily from the herd management program Alpro used in the enterprise.

The dairy cattle used in the study were followed up and the lactation was 0-100. Clinical and subclinical mastitis rates were determined and recorded. Clinical mastitis in the cows included in the study was diagnosed according to the signs of inflammation in the udder, changes in milk and changes in the general condition. In order to detect subclinical mastitis in cows in the study group, somatic cell count of milk (SCC) was determined by taking consecutive and three-meal milk samples two days a week. SCC was measured with the help of Bentley Instruments-Somacount 20, USA machine in Uludağ University Faculty of Veterinary Medicine Department of Animal Science. Thus, the individual SCC of the cows in the groups was followed.

The Chi Square test was used to evaluate the milk production and composition data. Pearson Chi-square or Fisher's Exact Test was chosen for the interpretation of the results. SPSS (Version 23) statistical program was used to compare the data. The significance level was P<0,05.

## RESULTS AND DISCUSSION

According to the results in Table 3, there was no statistically significant difference blood glucose levels between the groups at 0 and 3 weeks (P>0.05). There was no statistically significant difference between the C groupand the other groups (P>0.05), but there was a significant difference between the PG group and the G group (P<0.05). The group with the highest blood glucose level was determined as group G (70.20 mg/dl), and the group with the lowest blood glucose level was determined as PG group (53.06 mg/dl). At week 1, there was a statistically significant difference between the PG group (55.36 mg/dl) and the G group (39.97 mg/dl) (P<0.03), while the P group (55.36 mg/dl) and C group (63.92 mg/dl) no significant difference was found (P>0.05). The group with the lowest blood glucose level in this week was determined as group G, and the group with the highest blood glucose level was determined as group K. In the 2nd week, although there was no statistically significant result, it was observed that the animals in the PG group had a tendency to decrease in blood glucose levels and it was determined that it had the lowest value (P<0.07).

**Table 3.** Blood glucose analysis results.

| Week | Control(mg/dL) | Propilene Glycol mg/dL) | Glycerol (mg/dL) | SE | P |
|---|---|---|---|---|---|
| **-1** | $58.7^{ab}$ | $53.0^{a}$ | $70.2^{b}$ | 2.98 | <0.05 |
| **0** | 66.9 | 68.6 | 72.0 | 4.06 | NS |
| **1** | $63.9^{a}$ | $55.3^{a}$ | $39.9^{b}$ | 2.64 | <0.03 |
| **2** | 50.0 | 36.6 | 46.1 | 2.46 | NS |
| **3** | 53.6 | 50.4 | 40.8 | 2.56 | NS |

Not Significant: NS (P>0.05)
a,b: Differences between means with different superscripts in the same row were significant

Average daily milk yields of cows throughout the study are presented in Table 4. No statistically significant difference was found between the PG group and the G group (P>0.05), but there was a statistically significant difference between the C group and the other groups (P<0.05).

**Table 4.** Milk production of animals.

| Groups | Milk Yield (L) |
|---|---|
| **Control** | $35.06^{b} \pm 0.21$ |
| **Propylene Glycol** | $33.46^{a} \pm 0.27$ |
| **Glycerol** | $34.18^{a} \pm 0.32$ |

[a, b]: Differences between means with different superscripts in the same column are significant (P<0.05)

According to the results of somatic cell analysis of the milk samples taken, no statistically significant difference was found between the C groupand the other groups (P>0.05), but there was a significant difference between the PG group and the G group (P<0.05). It was determined that the group with the lowest average was the PG group, and the group with the highest SCC average was the G group.

According to the results of the analysis, the general averages of the milk fat ratios of the groups are given in Table 5. According to the results of the analysis, a statistically significant difference was found between the PG group and the other groups (P<0.05). During the first 100-day lactation period, PG group had 0.22% higher milk fat ratio than the other groups.

**Table 5.** Milk fat ratios of the groups.

| Groups | Milk Fat Ratio (%) |
|---|---|
| **Control** | $3.44^{b} \pm 0.03$ |
| **Propylene glycol** | $3.67^{a} \pm 0.03$ |
| **Glycerol** | $3.46^{b} \pm 0.03$ |

a, b: Differences between means with different superscripts in the same column are significant (P<0.05)

According to the analysis results obtained from the milk samples in our study, the general averages of the milk protein ratios of the groups are given in Table 6. According to the results of the analysis, no statistically significant difference was found between the G group and the other groups (P>0.05). However, a significant difference was determined between the PG group and the C group(P<0.05). As a result of the study, it was determined that the PG group had a lower milk protein rate of 0.17% compared to the C group.

**Table 6.** Milk protein ratio of groups.

| Groups | Milk Protein Ratio (L) |
|---|---|
| **Control** | $3.32^{b} \pm 0.05$ |
| **Propylene Glycol** | $3.15^{a} \pm 0.05$ |
| **Glycerol** | $3.20^{ab} \pm 0.05$ |

According to the milk protein analysis results, no statistically significant difference was found between the G group and the other groups (P>0.05). However, a significant difference was determined between the PG group and the C group(P<0.05). As a result of the study, it was determined that the PG group had a lower milk protein rate of 0.17% compared to the C group.

In this study, no difference was found in blood glucose levels of animals treated with PG compared to the C group (P>0.05). Compared to the C group of animals treated with G, a difference was observed in blood glucose levels only in the first week, and the blood glucose levels of animals in the G group were lower in the first week (P<0.03). There was a significant difference between PG and G at the prepartum period (P<0.05) and at the postpartum 1st week (P<0.03). In the prepartum period, G increased the blood glucose level more, while PG increased the blood glucose level better in the first week of lactation.

As in our study, there are studies that conclude that PG administration does not affect blood glucose concentration (Chibisa *et al.* 2008, Chung *et al*. 2009, Laranja *et al*. 2004, Stokes and Goff 2001). In some studies with PG, it was stated that the blood glucose concentration increased (Grummer *et al*. 1994, Liu *et al*. 2009, Pieper *et al*. 2005). 500 ml was administered orally for 10 days before birth and 25 days after birth, and it was determined that it was not effective on blood glucose concentration in the prepartum period, but increased significantly in the postpartum period (Butler *et al.* 2006). The time of collection of blood samples from animals treated with PG affects insulin and blood glucose levels. The results of some scientific studies on G support our study. In one of the studies closest to our study, it was determined that there was a decrease in blood glucose values in the 3rd week of lactation in animals treated with 500 ml G, and considering the closeness of the administered G dose in our study, it supports the results we obtained (Bodarski *et al*. 2005).

It was determined that the average of the first 100 days of milk yield of the animals studied was higher than the average of the C groupthan the PG and G groups (P<0.05). There was no significant difference between

the PG and G groups (P>0.05). In a study, 20 g/L of G was added to drinking water between prepartum 7 and postpartum 7 days, and as a result of this short application, the desired glycogenic effect could not be observed and no change in milk yield was observed (Osborne *et al.* 2009). In another study, it was stated that 500 ml G application between the prepartum 21st day and the postpartum 70th day increased milk yield in the first 10 weeks of lactation (Bodarski *et al*. 2005). In one of the studies close to our study, milk yield was not affected in general, but it differed in the same weeks as our study (Laranja *et al.* 2004). In our study, while a decrease in milk yield was observed in the 5th, 6th and 7th weeks, an increase was observed in the same weeks in this study. The dose of PG administered is approximately the same. The only difference in the applications is that water is given to the C group by oral resistance application. In our study, no application was made to the C group. It can be thought that the difference occurred due to the fact that the C groupwas exposed to less stress. Milk yield and milk components in dairy cattle are mostly affected by nutritional conditions, but they are also affected by other environmental factors such as genetic parity, season and disease status. It can invalidate the effect of PG on milk yield in large enterprises with a good ration application. In addition, while oral administration was applied to the PG and G groups every day in the specified time period, no application was made to the control group. It is thought that the difference occurred due to the fact that the C groupwas exposed to less stress.

In our study, it was determined that PG and G application did not make a significant difference on SCC (P>0.05). However, when the PG and G groups were evaluated among themselves, it was determined that the mean SCC of the PG group was significantly lower than the G group (P<0.05). In terms of SCC, PG can be considered a better option. Many studies with PG and G in the past support our results. (Chung *et al*. 2007, DeFrain *et al*. 2004, Formigoni *et al.* 1996, Hoedemaker *et al*. 2004, Ogborn *et al.* 2004, Studer *et al.* 1993).
It was determined that the application of G had no effect on the milk fat ratio (P>0.05), while the application of PG significantly decreased it in the first 3 weeks and increased it in the mean of a total of 100 days (P<0.05). The reason of this; It is thought that it may first be formed due to the decrease in plasma NEFA concentration. Because the decrease in plasma NEFA concentration means a decrease in the amount of NEFA reaching the mammary gland, and therefore milk fat synthesis decreases. Another reason may be the decrease in the amount of acetate required for fatty acid synthesis in the mammary gland, since PG administration causes a decrease in the acetate ratio in the rumen. After the 3rd week, an increase in the milk fat ratio of the animals in the P group is observed.

It was determined that G application did not affect the milk protein ratio (P>0.05), but there was a significant decrease in milk protein ratio in animals treated with PG (P<0.05). In a similar study by Fisher *et al*.28 in 1971, the lack of difference between these two precursors supports us. Many studies have been conducted that show results of milk protein ratio as a result of G application in parallel with our study (Chung *et al*. 2007, DeFrain *et al*. 2004, Donkin and Doane 2007, Khalili *et al.*1997, Ogborn *et al*. 2004, Osborne *et al.* 2009). There are many publications stating that PG application has no effect on milk protein ratio (Butler *et al.* 2006; Formigoni *et al*. 1996, Moallem *et al*. 2007, Nielsen and Ingvartsen 2004, Studer *et al*. 1993)

There was no difference between the groups in glucose levels from blood samples taken from cows. However, according to the results obtained, milk yield and milk protein were found to be higher in the K group, which did not receive any treatment, compared to the G and PG groups, and differences were found between the groups in terms of milk fat. This shows that there may be some changes in the rumen after administration and that both glycerol and propylene glycol have some effects on rumen microorganisms. For this reason, there is a need to further investigate the behavior of glucose precursors such as glycerol and propylene glycol, especially on rumen fermentation and microorganisms.

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