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*Full Length Research Paper*

# Effect of oral administration of propylene glycol during periparturient period on blood biochemical parameters and liver triacylglycerol accumulation in postparturient dairy cows

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**Effect of propylene glycol administered during the periparturient period on blood biochemical parameters and liver triacylglycerol accumulation was studied in 35 dairy cows. Sixteen control cows were drenched with 400 ml of water per day starting from 7 days before expected calving date to 7 days after calving, while 19 treated cows were drenched with 400 ml of propylene glycol. Body condition scores and milk yield were recorded. Blood samples were collected at 2 weeks prepartum and at 2 and 4 weeks postpartum. Liver samples were collected at 2 weeks prepartum and 4 weeks postpartum. Control cows tended to lose more body condition scores than did treated cows during the first 4 weeks of lactation. Milk yield did not differ between control and treated cows. Serum nonesterified fatty acids concentrations in control cows were significantly higher than the concentrations in treated cows at 2 and 4 weeks postpartum. Serum glucose, triacylglycerol and urea nitrogen concentrations did not differ between the two groups at all sampling periods. Liver triacylglycerol concentrations were similar between 2 groups at 2 weeks prepartum. The concentrations in both groups increased at 2 weeks postpartum, and were significantly higher in control cows than in treated cows. Propylene glycol had an advantage to reduce adipose tissue lipolysis postpartum; as a consequence, reducing liver triacylglycerol accumulation.**

**Key words:** Dairy cow, fatty liver, propylene glycol.

## INTRODUCTION

Negative energy balance (NEB) is a common phenolmenon in periparturient dairy cows due to an inability of the cows to match their energy requirement for maintenance and lactation with energy intake from the diet (Harrison et al., 1990). As a consequence, dairy cows need to increase their fat mobilization from the body reserves, resulting in increased nonesterified fatty acids (NEFA) in the blood (Rukkwamsuk et al., 1998). This notion is partly explained by a decline of blood insulin concentration during the NEB period (Holtenius, 1993). Blood NEFAs are further metabolized in the liver, where they could be re-esterified to form triacylglycerol (TAG) or be completely oxidized to generate ATP or be partially oxidized to produce ketone bodies (Bruss, 1993). Increased TAG synthesis in the liver may cause an increase in TAG accumulation in the liver, resulting in fatty liver (Van den Top, 1995). It is documented that dairy cows with a severe NEB secreted greater amount of fatty acids through the milk, which affected the milk composition (Rukkwamsuk et al., 2001). NEB, fatty liver and ketosis are evidence to be associated with health (Wentink et al., 1997), production (Rukkwamsuk et al., 2001) and reproduction (Heinonen et al., 1988; Rukkwamsuk et al., 1999) problems.

In Thailand, most observable problems in small-holder

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**Table 1.** Composition of the diets fed to both control and treated cows.

| Diets fed | During 90 days of lacation | Dry period |
|---|---|---|
| | g/kg as fed | |
| Rice straw | 69.3 | 83.6 |
| Corncob | 449.5 | 542.7 |
| Cassava chip | 120.4 | 159.3 |
| Ricebran | 54.6 | 28.9 |
| Soybean meal | 107.1 | 40.8 |
| Palm meal | 170.8 | 115.5 |
| Sugar cane molasses | 15.4 | 13.9 |
| Urea | 2.9 | 4.1 |
| NaCl | 3.5 | 5.0 |
| Sulfer | 0.6 | 0.5 |
| Premixes | 2.9 | 2.6 |
| Dicalcium phospate | 2.9 | 3.1 |

dairy farms include clinical and subclinical mastitis and postpartum infertility (Poolket et al., 2001; Yawongsa et al., 2003). These problems might affect production performance of the dairy farm and cause economic loss to the farmers. Although these problems are caused by many factors, NEB might play a crucial role because physiological changes during the NEB period directly affect milk production and reproduction. Therefore, prevention of NEB in periparturient period would be essential to help improve postparturient dairy cow performance.

The principles of prevention of NEB are to increase glucose synthesis and reduce lipolysis from adipose tissue by administration of ammonium propionate (Fürll and Leifen, 2002), calcium propionate (Goff et al., 1996), choline (Hartwell et al., 2000), monensin (Duffield et al., 2003) and propylene glycol (Pickett et al., 2003; Rukkwamsuk et al., 2005). Other studies used hormones to stimulate hepatic gluconeogenesis such as glucagons (Nafikov et al., 2002) and glucocorticoids (Fürll et al., 1993).

However, most studies were conducted in Western countries, which have different conditions from tropical countries like Thailand. Application of those results to Thai dairy cows is limited or may not be appropriate. Therefore, our aim was to study the effect of propylene glycol orally administered from 7 days before expected calving to 7 days after calving on blood biochemical parameters and liver triacylglycerol accumulation in postparturient dairy cows.

## MATERIALS AND METHODS

### Animals and diets

Thirty five dry crossbred Holstein Friesian dairy cows were selected from one commercial dairy farm in Kanchanaburi Province, Thailand. They were randomly divided into 2 groups, 16 control cows and 19 treated cows. The diets of the cows are presented in Table 1. During the dry period, all cows were fed with the dry cow diet and, after calving, they were fed with the lactating diet according to their milk yield. From approximately 7 days before expected calving date, treated cows were drenched with 400 ml of propylene glycol while control cows were drenched with 400 ml of water. Body condition score of all cows was recorded at 1 month before expected calving date and 1 month after calving. Daily milk yield was record for 4 weeks of lactation.

### Sampling procedures and sample analyses

Liver samples were collected at 2 weeks before expected calving date and at 2 weeks after calving using percutaneous biopsy technique as described previously (Van den Top, 1995). At collection, liver samples were placed in physiological saline and kept on ice. In laboratory, these liver samples were dried on the filter paper and were freed from any blood clots and connective tissue.

The liver samples were weighed in a separate tube for each triacylglycerol determination, and were then kept at -20°C until analysis. Blood samples were collected from jugular vein at 2 weeks before expected calving date and at 2 and 4 weeks after calving. In brief, 5 ml of blood were put in the serum separated tube (Vacuette® Z serum clot activator, Bangkok inter products Co., Ltd., Bangkok, Thailand), were allowed to clot at room temperature for 30 min, and were centrifuged for 10 min at 1300 ×g. Serum samples were harvested and stored at -80°C until further analyses.

Triacylglycerol concentrations in the liver sample were determined using the method described by Van den Top (1995). In brief, the liver samples were destroyed overnight with 20% KOH and then were saponified with absolute ethanol. In this solution, the triacylglycerol concentrations were determined by enzymatic spectrophotometry using a commercial kit (RA-130-20 triglyceride enzyme set, biotechnical Co., Ltd., Bangkok, Thailand).

Serum NEFA (RDX-FA115 NEFA, medical-diagnostics Co., Ltd., Bangkok, Thailand), triacylglycerol (RA-130-20 triglyceride enzyme set, biotechnical Co., Ltd.), glucose (RA-122-10 glucose enzyme,

biotechnical Co., Ltd.) and urea nitrogen (RA-115-12 BUN enzyme, biotechnical Co., Ltd.) were determined by enzymatic spectrophotometry with commercially available kits as indicated.

#### Statistical analyses

Data were tested for normal distribution using Shapiro-Wilk W test (Patrie and Watson, 1999). Normally distributed data were subjected to ANOVA using propylene glycol administration as a fixed main effect and sampling period as a repeated measure (Patrie and Watson, 1999). Liver triacylglycerol concentrations and serum NEFA, triacyglycerol, glucose and urea nitrogen concentrations between control and treated cows were compared using the student's t test or, when data were not normally distributed, with the Mann-Whitney U test (Patrie and Watson, 1999). When data had a normal distribution, homogeneity of variances was determined using the Levene's test (Patrie and Watson, 1999). The differences were considered to be significant if $P \leq 0.05$.

## RESULTS

### Body condition score and milk production

Average body condition scores measured at 4 weeks before expected calving were 3.46 ± 0.17 (SEM) and 3.23 ± 0.14 for control and treated group, respectively. At 4 weeks after calving, average body condition score for control cows was 3.07 ± 0.14 and for treated cows was 3.16 ± 0.15, which were not different between the two groups. However, changes in the body condition score (value measured at 4 weeks before expected calving – value measured at 4 weeks after calving) were 0.43 ± 0.16 and 0.13 ± 0.06 for control and treated cows, respectively. The control cows tended to lose more body condition score than did the treated cows ($P = 0.06$).

During the first 4 weeks of lactation, average milk yields were 17.02 ± 0.17 kg/day and 17.60 ± 0.18 kg/day for control and treated cows, respectively. The average milk yield did not differ between the two groups (Figure 1).

### Blood biochemical parameters

Average concentrations of serum NEFA, triacylglycerol, glucose and urea nitrogen are presented in Figures 2, 3, 4 and 5. At 2 week before expected calving, serum NEFA did not differ between control and treated cows (Figure 2). At 2 weeks after calving, the concentrations increased in both groups. As compared with the concentration before calving, the concentrations increased 132% for control group and 57% for treated group. The concentrations at 2 and 4 weeks after calving in control cows were higher than treated cows.

Average serum triacylglycerol concentrations did not differ between control and treated cows at 2 week before calving (Figure 3), and the average concentrations of serum triacylglycerol were 17.07 ± 1.21 mg/dL for control cows and 14.97 ± 0.86 mg/dL for treated cows. The concentrations decreased after calving and did not differ between the two groups at either 2 or 4 weeks.

Average serum glucose concentrations at 2 weeks before calving and at 2 and 4 weeks after calving did not differ between control and treated cows (Figure 4). However, the concentrations at 2 week after calving decreased as compared with the concentrations at 2 week before calving. The decreases of serum glucose concentrations were 26% for control cows, whereas only 7% for treated cows.

Average serum urea nitrogen concentrations at 2 weeks before calving were 11.31 ± 1.14 mg/dL and 13.05 ± 0.79 mg/dL for control and treated cows, respectively. These concentrations did not differ between the two groups (Figure 5). After calving, serum concentrations slightly increased in both groups, and did not differ between control and treated cows.

### Liver triacylglycerol

Liver triacylglycerol concentrations are presented in Figure 6. The concentrations at 2 weeks before parturition did not differ between groups and were 13.37 ± 1.26 and 14.86 ± 2.27 mg/g of liver (wet weight) for control and treated cows, respectively. As compared with the concentrations at 2 weeks before calving, the concentrations at 2 week after calving increased 288% for control cows and 61% for treated cows. In addition, liver triacylglycerol concentrations at 2 weeks after calving were higher for control than for treated cows.

## DISCUSSION

Dairy cows in control and in treated groups entered a period of negative energy balance after parturition. Negative energy balance induces mobilization of body fat reserves, which was related to the loss of body condition score. Although dairy cows in both groups lose their body condition score after parturition, cows drenched with propylene glycol during periparturient period lost less body condition score than did control cows which were drenched with water. It therefore suggested that propylene glycol could improve energy status by providing additional gluconeogenic precursor for the hepatic production of glucose (Nielsen and Ingvartsen, 2004). In this study, oral administration of propylene glycol during periparturient period had no effect on milk production during 4 weeks of lactation, which was in agreement with other studies (Butler et al., 2006; Hoedemaker et al., 2004; Miyoshi et al., 2001; Rukkwamsuk et al., 2005)

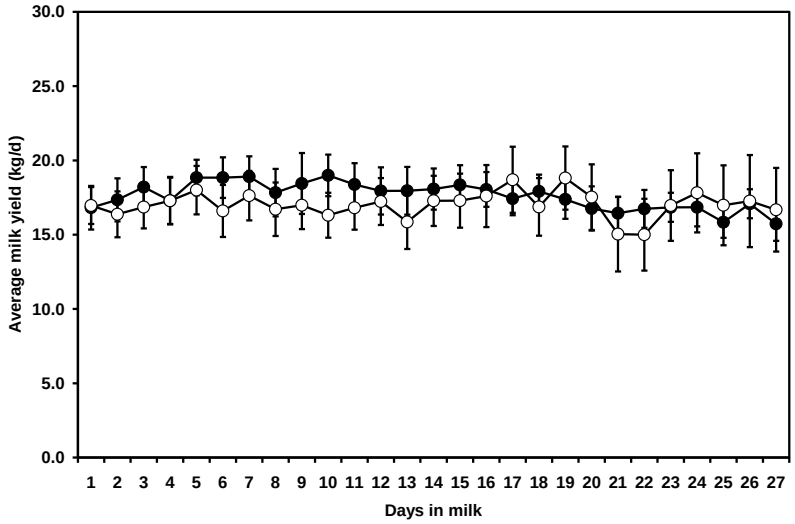


**Figure 1.** Comparison of milk yield (kg/d) during the first 4 weeks of lactation between control cows (○; n = 16) and cows drenched with propylene glycol (●; n = 19). Data represented means and SEM as error bars.

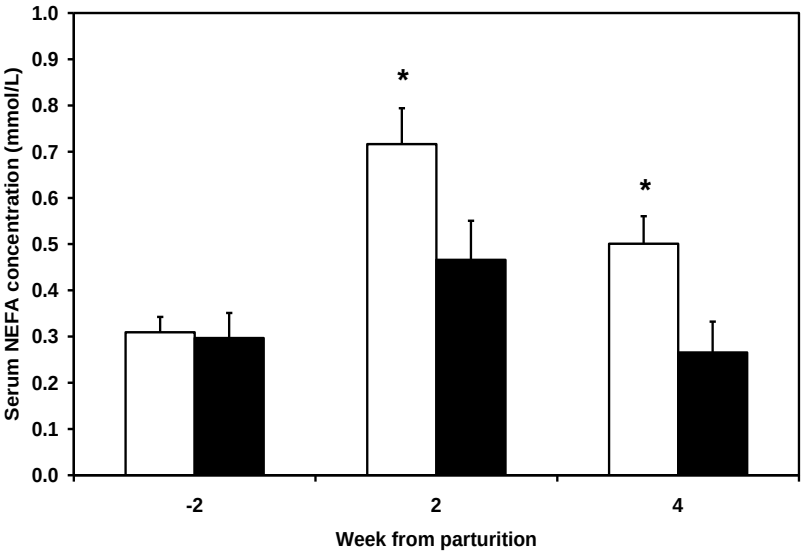


**Figure 2.** Comparison of serum nonesterified fatty acids (NEFA) concentrations (mmol/L) between control cows (□; n = 16) and cows drenched with propylene glycol (■; n = 19). Data represented means and SEM as error bars. Asterisks indicated means between the two groups were significantly different at $P < 0.05$.

In this study, dairy cows drenched with propylene glycol had a lower concentration of serum NEFA as compared with control cows. Dairy cows drenched with propylene glycol reduce adipose tissue lipolysis because propyleneglycol could increase rumen production of propionic acid, which is then used for synthesis of glucose in the liver. In addition, propylene glycol could be absorbed from the gastrointestinal tract and be converted to lactate, which is also used for hepatic gluconeogenesis (Nielsen and Ingvartsen, 2004). Therefore, oral administration of propylene glycol could induce insulin secretion from the pancreas and hence inhibiting lipolysis of adipose tissue.

Drenching propylene glycol to the cow at a dosage of 400 ml/day had no obvious effect on serum glucose

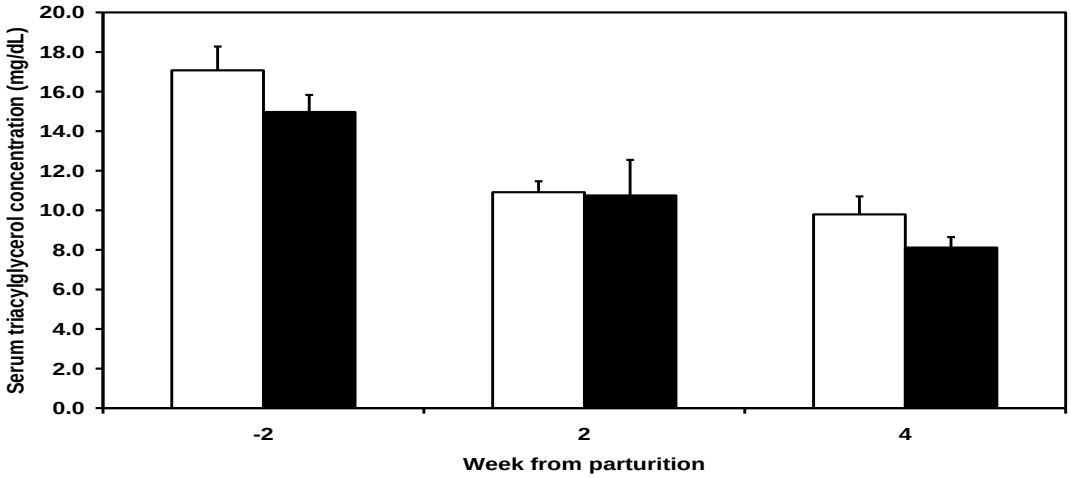


**Figure 3.** Comparison of serum triacylglycerol concentrations (mg/dL) between control cows (□; n = 16) and cows drenched with propylene glycol (■; n = 19). Data represented means and SEM as error bars.

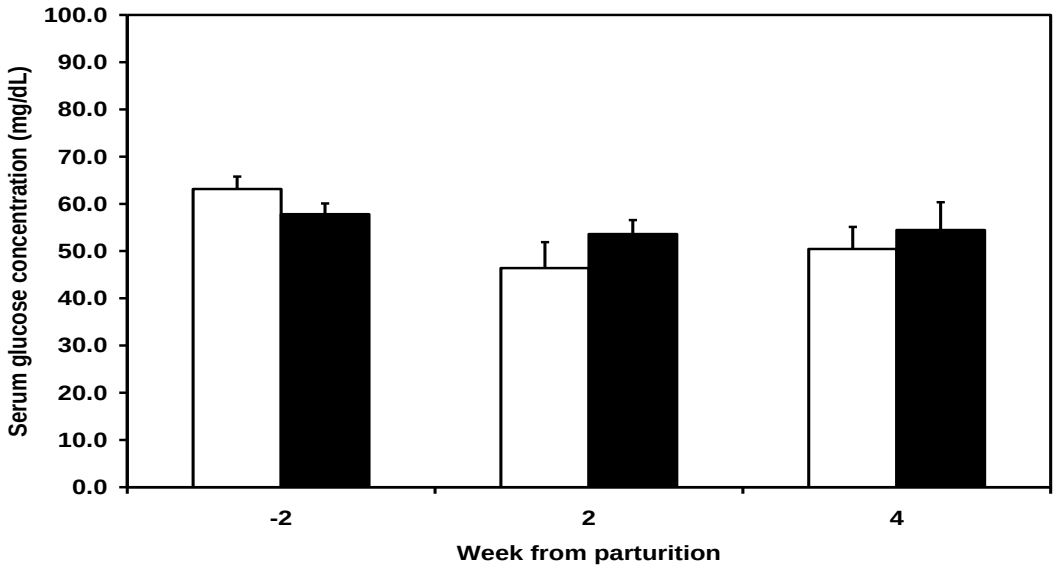


**Figure 4.** Comparison of serum glucose concentrations (mg/dL) between control cows (□; n = 16) and cows drenched with propylene glycol (■; n = 19). Data represented means and SEM as error bars.

concentration, which was similar to the observation by Chibisa et al. (2008). However, cows drenched with propylene glycol had 7% depletion of serum glucose concentrations as compared with 26% depletion in control cows. Administration of propylene glycol had no effect on serum triacylglycerol concentrations. A decrease in serum triacylglycerol concentration after calving was corresponded with lactation as also described by Basoglu et al. (1998).

With respect to the lactation diet, postparturient dairy cows were offered a high protein diet. Increased protein uptake was associated with increased serum urea nitrogen concentration (Chibisa et al., 2008). However, serum urea nitrogen concentrations of control and treated cows were not different and were in an acceptable level of dairy cows during postparturient period (Peterson and Walern, 1981). It suggested that drenching with propylene glycol did not influence blood urea nitrogen concentration.

The concentration of triacylglycerol in the liver

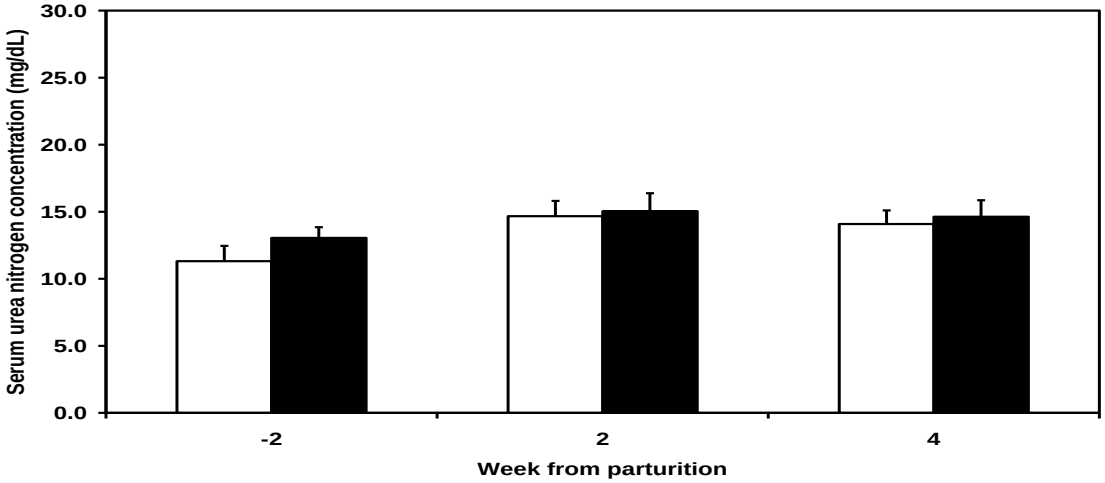


**Figure 5.** Comparison of serum urea nitrogen concentrations (mg/dL) between control cows (□; n = 16) and cows drenched with propylene glycol (■; n = 19). Data represented means and SEM as error bars.

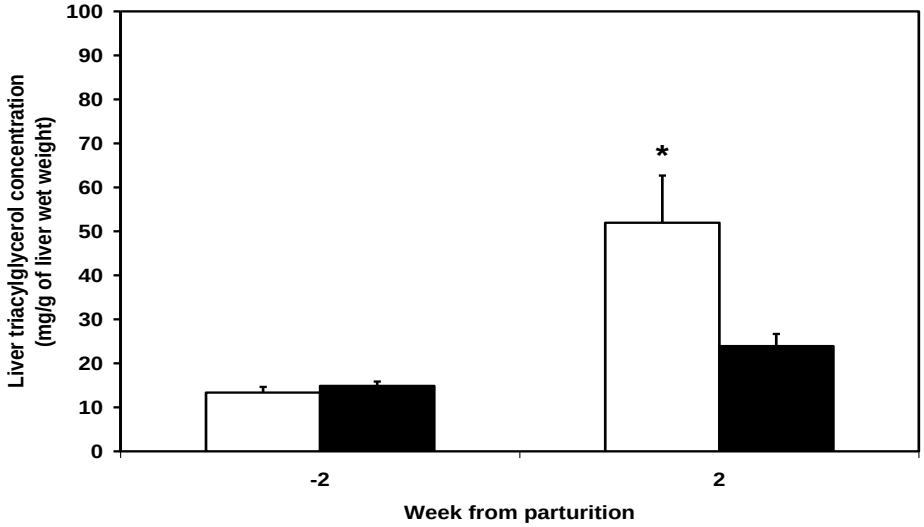


**Figure 6**. Comparison of liver triacylglycerol concentrations (mg/g of liver wet weight) between control cows (□; n = 16) and cows drenched with propylene glycol (■; n = 19). Data represented means and SEM as error bars. Asterisks indicated means between the two groups were significantly different at $P < 0.05$.

measured during the dry period was similar to previous reports in Thailand (Rukkwamsuk et al., 2004; Rukkwamsuk et al., 2005). Increased triacylglycerol concentration in the liver was positively correlated with negative energy balance (Rukkwamsuk et al., 1999). The level of accumulation of triacylglycerol in the liver was related to the rate of lipolysis in adipose tissue. In this study, control cows had a greater lipolysis, which resulted in higher serum NEFA concentration (Figure 3), and thus increasing hepatic triacylglycerol accumulation. In contrast, oral administration of propylene glycol reduced lipolysis, and thus reducing triacylglycerol accumulation in the liver (Pickett et al., 2003; Rukkwamsuk et al., 2005).

## Conclusion

Oral administration of propylene glycol to the dairy cows from 7 days before calving to 7 days after calving could

improve energy status. Propylene glycol provided additional energy source for propionic acid production in the rumen. This propionic acid was used by the liver to produce glucose, which initiated the process of reducing lipolysis from adipose tissue. As a consequence, hepatic uptake of NEFA was lowered and the hepatic synthesis and accumulation of triacylglycerol were alleviated. Therefore, propylene glycol could prevent fatty liver development in periparturient dairy cows, which, in turn, may improve health and production of the dairy cows.

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