



## Effects of Propylene Glycol on Milk Production, Serum Metabolites and Reproductive Performance during the Transition Period of Dairy Cows

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**ABSTRACT :** The objective of this study was to investigate the effects of an oral drench of propylene glycol (PG) on milk production, serum metabolites and reproductive performance during the transition period of animals. Twenty-four 2-3 multiparous Holstein cows (average body weight 565 kg, body condition score about 3.6, at the 9<sup>th</sup> month of gestation) were selected, blocked, and then randomly assigned into a PG and a control group. The control and the PG group cows were orally drenched with water or 50 ml sugarcane molasses mixed with 500 ml PG from 7 days pre-partum to 30 days post-partum, respectively. Experimental results indicated that the oral drench PG had no effect on dry matter intake (DMI). The milk yield of the PG group was significantly higher than that of the control group ( $p < 0.05$ ), whereas milk fat content, milk protein and somatic cell counts (SCC) were not significantly different between groups. Concentration of plasma glucose in the PG group was significantly higher than that of the control group ( $p < 0.05$ ). Conversely, the concentrations of non-esterified fatty acids (NEFA), and blood urea nitrogen (BUN) in the PG group were lower than those of the control group ( $p < 0.05$ ). Concentrations of insulin and ketone bodies were not significantly difference between groups. Body condition score (BCS) in the PG group was significantly higher than that of the control group ( $p < 0.05$ ). In reproductive performance there was no difference between groups. The experimental results indicate that supplementation of PG during the transition period of dairy cows can supply energy rapidly, resulting in reduced catabolism of body tissue and increased milk yield. (**Key Words :** Dairy Cows, Propylene Glycol, Milk Production, Serum Metabolites)

### INTRODUCTION

The period of 3 weeks prepartum to 3 weeks postpartum in dairy cows is the transition period. During this period, feeding and management are critical factors for cows. Good feeding and management during this period benefits future milk production. However, if feeding and management are inadequate, metabolic syndromes such as ketosis and milk fever can develop in the early period of postpartum (Curtis et al., 1985; Goff and Horst, 1997).

The DMI of dairy cows starts declining at 3 weeks prepartum, as during this period a fetus grows rapidly and various stresses can adversely impact hormone secretion (Grummer, 1995). This reduction in DMI is typically apparent at 7 days prepartum. Grummer (1995) and Robinson and Garrett (1999) indicate that the DMI declines during the prepartum period can be up to 30-35%, especially in subtropical area like Taiwan, where the summer temperatures usually reach 35°C during the day

time.

The milk production peak is at 5-8 weeks postpartum while the diet consumption peak is at 10-14 weeks postpartum. Thus, dairy cows will typically suffer a 6-8 week period of negative energy balance during the postpartum period (Butler and Smith, 1989). This negative energy balance consequently induces catabolism of body tissue to meet energy requirements, resulting in reduced BCS, rumen fermentation, and milk production; and even worse, the possibility of metabolic syndrome (Grant and Albright, 1995; Hutjens, 1996; Robinson, 1997; Kim and Suh, 2003). Thus, we propose that supplying extra energy during this crucial transition period will benefit the energy balance of dairy cows.

Propylene glycol, which is rich in energy (4.7 Mcal NE/L) (Miyoshi et al., 2001), can rapidly supply transition dairy cows with energy. Propylene glycol is easily and rapidly absorbed and metabolized in the rumen. Roughly 50% can be metabolized 1-2 h after feeding, with approximately 80-90% usually metabolized 3h after feeding. Propylene glycol can also be converted to propionic acid in the rumen and transported to liver, where it is converted to

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Received October 26, 2006; Accepted April 30, 2008

**Table 1.** The composition of experimental concentrate

Ingredient (DM basis)	%
Corn	66.5
Soybean meal	20
Wheat bran	6
Molasses	5
Dicalcium phosphate	1
Salt	0.5
Sodium bicarbonate	0.6
Premix	0.4
Total	100
Analyzed value	
Dry matter (%)	89.8
Crude protein (%)	16
Crude fat (%)	7
Crude fiber (%)	3.2
Crude ash (%)	7.4
NDF (%)	35.5
ADF (%)	42.3
Gross energy (MJ/kg)	17.2

\* Per kg of premix contain: Vitamin A, 10,000,000 IU; Vitamin E, 70,000 IU; Vitamin D<sub>3</sub>, 1,600,000 IU; Fe, 50 g; Mn, 40 g; Cu, 10 g; Zn, 40 g; Se, 0.1 g; Co, 0.1 g.

glucose through the glyconeogenesis pathway (Emery et al., 1967; Clapperton and Czerkawski, 1972; Kristensen et al., 2002; Nielsen and Ingvarsen, 2004). Some investigators have indicated that PG supplementation can reduce serum NEFA and ketone body concentrations (Christensen et al., 1997; Miyoshi et al., 2001; Pickett et al., 2003), and has been reported to increase milk production (Formigoni et al., 1996; Lucci et al., 1998). Additionally, reproduction performance was improved (Miyoshi et al., 2001). Thus, we hypothesize that PG may improve available energy during the transitional period of cows, offering beneficial effects, especially environments of high temperature and humidity. This study, therefore, investigates the effects of adding PG during the transitional period on milk production, serum traits, and reproductive performance of dairy cows in high temperature, high humidity climates.

## MATERIALS AND METHODS

### Animals and treatments

This study conducted in the summer season of the subtropical area of Taiwan (averaged day time temperature was 33.25±2.56°C). Twenty-four 2-3 multiparous Holstein dairy cows were selected for this study (average body weight 565 kg, BCS about 3.6; 9 months pregnant). Selected cows were blocked by parity, month of calving, 305 days mature equivalent milk production and BCS, then randomly divided into 2 groups: a control group (water 550 ml) and a PG (500 ml PG mixed with 50 ml molasses) group. The water and the PG were orally drenched from 7 days prepartum to 30 days postpartum once a day. All cows were housed in a tie stall facility for the duration of the

study and separated for individual feeding and measurement. During the dry period (30 days of prepartum) cows were fed with 4 kg concentrate and 4kg alfalfa hay daily, and bermuda hay was accessed freely. After calving, the amount of concentrate fed was based on the milking level using a 1:3 concentrate to milk ratio (upper limit was 10 kg of concentrate). The concentrate composition is shown in Table 1. Alfalfa hay, supplied for roughage, had an upper limit of 8 kg, while bermuda hay was accessed freely. The nutrient supply followed NRC (1989). Mineral salt and water were supply *ad libitum*. Cows were milked twice daily and milk production was recorded for 90 days. The feed intake, calving, placenta retention, and first heat data were recorded. Cows were bred by artificial insemination at second heat; conception and pregnancy were then checked by a veterinarian each month.

### Experimental procedure, sampling and analysis

About 15 ml of blood samples were taken from the tail vein at 7 and 1-3 days prepartum, and 0-7, 14, 21 and 28 days postpartum at a fixed time (16:00). Milking samples (100 ml) from am. and pm. milking were taken on days 14, 21 and 28 postpartum. Milk fat, protein and somatic cells counts (SCC) were determined by an infra-red machine (Foss electric Co. Milko Scan 255 A/B type, USA). BCS was discerned by three professionals and classed on a 1-5 scale (1 = thin, 5 = obese) following Wildman et al (1982).

Plasma glucose concentration was measured by a glucose-oxidase strip with a glucose analyzer (Bayer, Germany). Insulin concentration was examined using an enzyme immunoassay kit by ELISA method (Boehringer Mannheim, Germany). Blood urea nitrogen (BUN) concentration was analyzed by a kit with a serum autoanalyzer (Roche COBAS MISA, Switzerland). NEFA was determined by a modified procedure described by Chromy et al. (1977). Serum samples 100µL were added 3mL of extraction reagent (chloroform:heptane:methanol; 49:49:2), and 1 ml copper reagent (1.0 M Cu(NO<sub>3</sub>)<sub>3</sub>H<sub>2</sub>O and 5 ml triethanolamine, diluted to 100 ml with saturated NaCl; pH 8.3). Next, the mixture was shaken for 5 min and centrifuged at 1,500×g for 5 min. We next took 1 ml of supernatant added color reagent (0.25 ml of 0.1% 1-2-thiazolylazo-2-naphol, TAN) and measured at 570nm with a spectrophotometer (Hitachi, U-2000, Japan). Palmitic acid (C16:0) was used as a standard.

Ketone body concentration was measured using the method described by Reid (1960). 5% ZnSO<sub>4</sub>·7H<sub>2</sub>O and 0.3 N barium hydroxide were added to serum samples, which were filtered to remove protein. Put 8 ml of 7 N H<sub>2</sub>SO<sub>4</sub> into distillation bottle then start to distillation and collected 5 ml for acetoacetate and acetone determination. Next, 5 ml of 0.2% K<sub>2</sub>Cr<sub>2</sub>O<sub>7</sub> was added and distillation was continued. 5 ml was also collected for β-hydroxybutyric acid

**Table 2.** Effect of propylene glycol supplementation on production traits of cows in the transition period

Items	Control	Propylene glycol
Dry matter intake (kg/day)	18.25±0.28	19.72±0.37
Body condition score <sup>1</sup>	2.73±0.01	3.08±0.02*
Milk yield <sup>2</sup> (kg/day)	26.63±1.6	27.24±0.13*
Milk fat (%)	3.53±0.09	3.62±0.11
Milk protein (%)	3.22±0.13	3.28±0.19
Somatic cell count (×1,000)	332.1±58.2	315.2±30.2

Means±SE (n = 12).

\* Means in the same row differ significantly (p&lt;0.05).

<sup>1</sup> 1 to 5 scale where 1 = thin, 5 = fat (Wildman et al., 1982).<sup>2</sup> Milk was collected from calving to 90 days milking period.

determination. Then, 4 ml of 10 N NaOH and 2 ml color reagent solution (100 ml ethanol and 20 ml salicylaldehyde) was added to the 5 ml collected ketone body samples. After shaking, the mixture was placed in a 55°C water bath for 20 min and then rested at room temperature for 1 h. It was then measured by a spectrophotometer at 530 nm. Sodium acetoacetate and β-hydroxybutyrate were used as standard, and both ketone body values were pooled.

### Statistical analysis

Experimental data were then analyzed using the mixed model of SAS (1998) with repeated measures, according to the following model.

$$Y_{ij} = \mu + \alpha_i + \beta_j + w_k + \beta w_{jk} + e_{ijk}$$

Where  $\mu$  is the mean,  $\alpha_i$  the effect of the *i*th treatment,  $\beta_j$  the effect of the *j*th block,  $w_k$  the effect of the *k*th week,  $\beta w_{jk}$  the interaction between week and block, and  $e_{ijk}$  is the residual error.

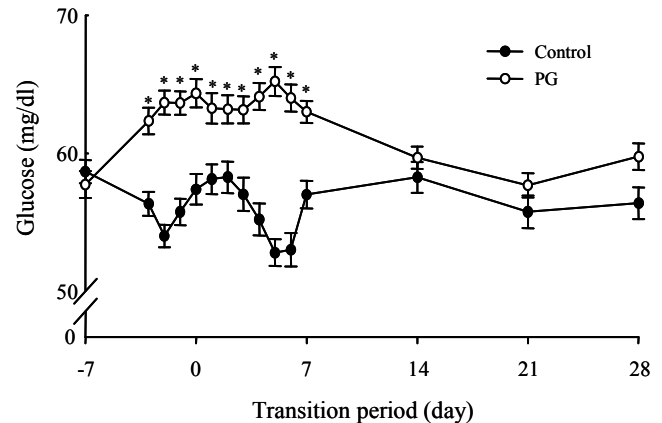
## RESULTS

### Effect of administration of PG on the production traits of transitional dairy cows

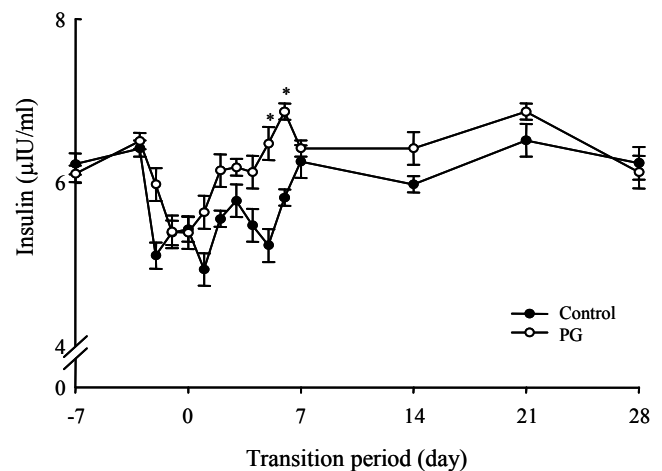
Table 2 lists the effect of PG supplementation on the production traits of cows during the transition period. BCS and milk yield of the PG group were significantly greater (p<0.05) than that of the control group. Dry matter intake, milk fat, milk protein and SCC were not significantly different (p>0.05) between the two groups.

### Effect of administration of PG on serum metabolites

Figure 1 shows the effect of PG supplementation on blood glucose concentration of cows in the transitional period. Analytical results reveal that the PG group had a higher (p<0.05) blood glucose concentration than that of the control group during the period of 3 days prepartum to 7 days postpartum.



**Figure 1.** Effect of propylene glycol supplementation on plasma glucose concentration of cows in the transition period. Means±SE (n = 12). \* Means differ significantly between groups (p<0.05).

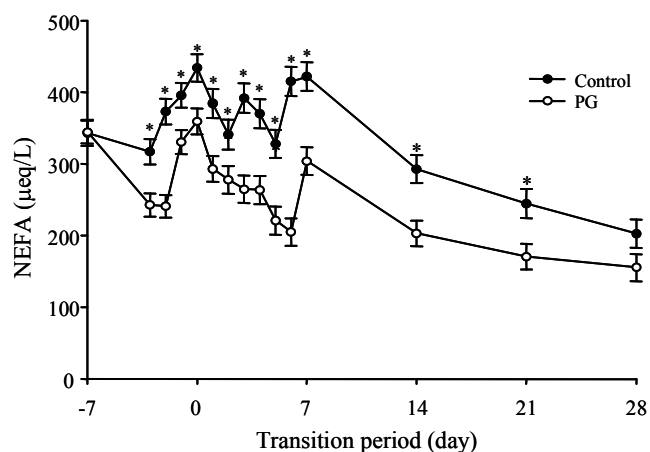


**Figure 2.** Effect of propylene glycol supplementation on serum insulin concentration of cows in the transition period. Means±SE (n = 12). \* Means differ significantly between groups (p<0.05).

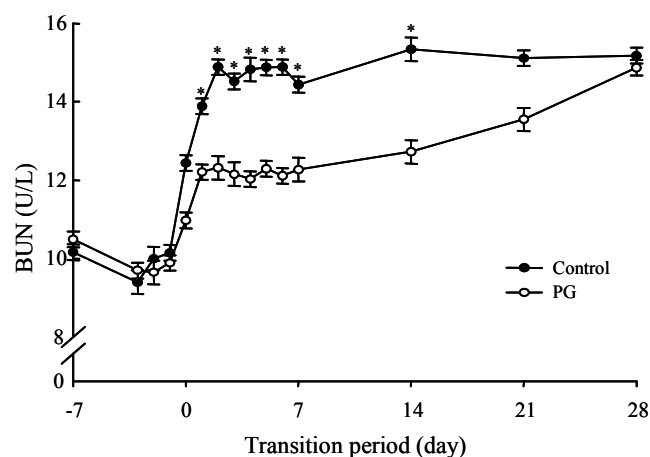
Figure 2 displays the effect of PG supplementation on serum insulin concentration in the two groups of cows in the transition period. Over most of the sample period no significant difference (p>0.05) existed between two groups. However, serum insulin concentration on day 5 and 6 postpartum in the PG group was higher than in the control group.

Figure 3 exhibits the effect of PG supplementation on serum NEFA concentration of the cows in the transition period. Serum NEFA concentration in the PG group was markedly different (p<0.05) than in the control group across all sampling points, except for the last sampling point (day 28 postpartum).

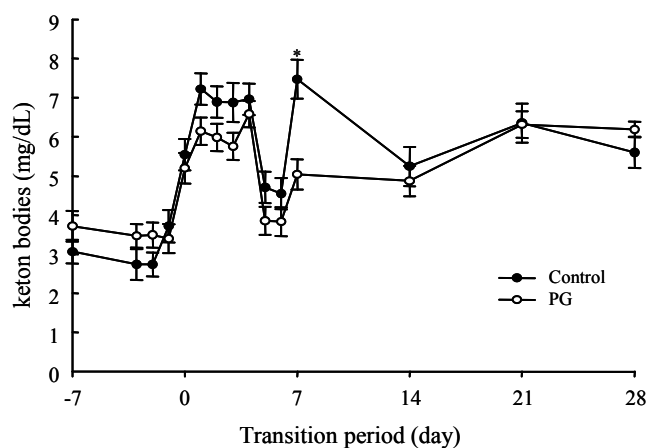
Figure 4 shows the effect of PG supplementation on the serum ketone body concentration in the cows over the transition period. The two groups did not exhibit significant differences in serum ketone body concentration at most



**Figure 3.** Effect of propylene glycol supplementation on serum nonesterified fatty acid concentration of cows in the transition period. Means $\pm$ SE (n = 12). \* Means differ significantly between groups (p<0.05).



**Figure 5.** Effect of propylene glycol supplementation on blood urea nitrogen concentration of cows in the transition period. Means $\pm$ SE (n = 12). \* Means differ significantly between groups (p<0.05).



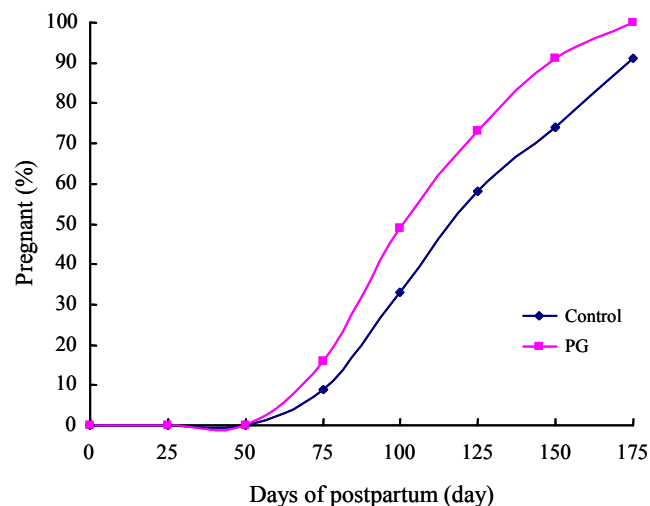
**Figure 4.** Effect of propylene glycol supplementation on serum ketone bodies (acetoacetate+ $\beta$ -hydroxybutyrate) concentration of cows in the transition period. Means $\pm$ SE (n=12). \* Means differ significantly between groups (p<0.05).

sampling points (p>0.05). However, on day 7 postpartum the ketone body levels in the PG group were lower (p<0.05) than those of the control group.

Figure 5 displays the effect of administration of PG on BUN concentration of cow in the transition period. The BUN concentration in the PG group was lower (p<0.05) than that in the control group during days 1-14 postpartum.

#### Effect of administration of PG on the reproductive traits

Figure 6 shows the effect of PG supplementation on the percentage of pregnancy in the two groups of cows. Although no significantly different (p>0.05) existed between the two groups, the PG group cows had a higher percentage of pregnant cows (about 20%) than the control group.



**Figure 6.** Effect of propylene glycol supplementation on pregnant percentage of cows in the transition period.

Table 3 displays the effect of PG supplementation on the reproductive traits of the cows. No significant different (p>0.05) between the two groups was noted.

## DISCUSSION

#### Effect of supplementation of PG on production traits

The palatability of PG is poor and its supplementation to the diet may negatively affect feed consumption (Dhiman et al., 1993; Girschewski et al., 1977). Thus, we employed oral drenching PG mixed with molasses. DMI was not significantly different between the two groups in our study, consistent with the results of Miyosh et al. (2001) and Pickett et al. (2003), who found that feed intake was not affected by PG supplementation.

**Table 3.** Effect of propylene glycol supplementation on reproductive traits of cows in the transition period

Item	Control	Propylene glycol
Days of first oestrus (day)	77.44±5.83	63.88±7.92
Days open (day)	115.33±9.01	105.56±10.71
Retain placenta (%)	25 (3/12)	8 (1/12)

Means±SE (n = 12).

Throughout the study period, the BCS of cows in the PG group was approximately 3.0, higher than that of the control group. Thus, PG supplementation appeared to have a positive effect on cow BCS. BCS is a methodology used to estimate the quantity of body reserves of fat, as it is the largest source of energy, but also the major reserve of body protein. Thus, BCS may be used as an indicator of the nutritional status of a cow or ewe. According to Caldeira et al. (2007a), ewe BCS at 3.0 indicates a balanced metabolic status. PG supplies energy rapidly, thereby improving the negative energy balance, reducing the catabolism of body tissue. The experimental results were similar to those obtained by Barllard et al. (2001).

Cows in the transition period have a negative energy balance, and the supplementation of PG can rapidly supply the energy required for milk production. Thus, the milk yield of the PG group was higher than that of the control group. Miyoshi et al. (2001) also indicated that PG supplementation increases milk production. Salem et al. (2005) reported that supplementing PG to goats increased the apparent digestibility of crude protein. Nitrogen retention and daily gain also appeared to increase.

Notably, analytical results showed that PG supplementation did not affect milk composition. Pickett et al. (2003) also had similar experiment results. Cozzi et al. (1996) and Barllard et al. (2001) reported that PG supplementation during weeks 7 and 8 of the milking period reduces SCC. However, no reduction in SCC of the PG group was observed in this study.

#### Effect of supplementation of PG on serum metabolites

Cows fed a diet supplemented with oral PG had significantly elevated plasma glucose concentration. Grummer et al. (1994), Miyoshi et al. (2001) and Juchem et al. (2004) found that cows fed with PG in the transition period exhibited significantly increased plasma glucose concentration. Propylene glycol is rapidly absorbed and metabolized by cows. In the rumen, PG is converted to propionic acid (Grummer et al., 1994), then transported to the liver and metabolized into glucose through glycconeogenesis.

Shingfield et al. (2002) and Pickett et al. (2003) reported that feeding transitional cows 518 g or 200 g PG daily did not influence serum insulin concentration; their findings are consistent with those obtained in this study. However, Grummer et al. (1994) fed transitional cows with 0, 307,

613 and 918 g PG, and found that as PG intake increased, serum insulin concentration increased.

Transitional cows fed PG had significantly reduced serum NEFA concentration, consistent with the results of Grummer et al. (1994), Formigoni et al. (1996), Miyoshi et al. (2001) and Hoedemaker et al. (2004). The experimental results indicated that transitional period cows had a negative energy balance, with degraded body fat, and released NEFA to use as energy. Since PG rapidly supplies energy, this improved the degree of negative energy balance, resulting in decreased body fat catabolism and serum NEFA levels (Miyoshi et al., 2001). When degraded body fat releases large amounts of NEFA, as the quantity of NEFA exceeds the liver burden or sufficient glucose is unavailable, NEFA is converted to ketone bodies. Grummer et al. (1994) indicated that when cows received 613-919 g PG daily during the milking peak period, markedly reduced serum ketone body concentrations resulted. In this study, the serum ketone body concentration in the two groups were not significantly different at most measured times. However, on day 7, ketone bodies in the PG-treated group were lower than in the control group.

If the negative energy balance increases, then the catabolism of body tissue also increases, resulting in degradation not only the body fat but also the body protein. BUN levels are then increased. In the PG group the BUN level was lower than that of the control group. Since both groups of cows received the same diet, elevated BUN levels may be the result of degraded body protein, which implies that PG can improve negative energy balance, a finding in agreement with that of Barllard et al. (2001).

Caldeira et al. (2007a) indicated that ewes in poor nutritional condition will have lower plasma glucose, serum insulin, and higher serum NEFA and BUN. When in a balanced status, plasma glucose and insulin are at intermediate levels, and BUN will be low. Thus, when nutrition is balanced, it will be reflected in the metabolite profile. Chimonyo et al. (2002) also reported that plasma glucose declined and NEFA and BUN levels increased, when BCS of cows was reduced. In a state of inferior nutrition, glucose is insufficient for energy expenditure. As a result, body fat and protein will degrade, yielding high plasma NEFA and BUN. Thus, plasma glucose, NEFA and BUN concentrations offer valuable diagnostic information for the interpretation of the nutritional status of animals and may be used to improve the nutritional management and prevent metabolic disorders (Caldeira et al., 2007b).

#### Effect of supplementation of PG on the reproductive traits

Butler and Smith (1989) and Britt (1992) indicated that negative energy balance can reduce progesterone secretion, prolong postpartum anestrus, and interfere with cow

pregnancy. Negative energy balance in the early postpartum period affected follicular development (Britt, 1992). Markusfeld et al. (1997) demonstrated that reduction in the body condition score during the transition period increased the incidence of postpartum reproductive disease and inactive ovaries. Gillund et al. (2001) and Domecq et al. (1997) reported that loss of BCS during early lactation had negative effects on reproductive performance. Kim and Suh (2003) also found that the number of days to first breeding after calving was longer in cows with marked BCS loss than in those with moderate BCS loss. In addition, Miyoshi et al. (2001) found that transition cows fed PG have an earlier estrus onset, because insulin is important in normal ovarian function.

In this study, days to first oestrus (77.44 vs. 63.88 days), and placental retention (25 vs. 8 heads) were reduced, and the pregnancy ratio was higher in the PG group than in the control group. This may explain why the insulin level in the PG group at day 5 and 6 of early postpartum was increased. However, the difference between the PG and control groups was not significant. Perhaps the sample size was not large enough.

## CONCLUSION

Feeding PG to transition period cows resulted in reduced serum NEFA and BUN levels, implying reduced catabolism of body tissue, increased plasma glucose concentration, BCS and milk yield, and did not affect DMI. This study offers evidence that oral drenching with PG of cows in the transition period could reduce negative energy balance and benefits dairy cows. However, long-term study is need for future research.

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