

*Full Length Research Paper*

# Effects of Alfalfa Hay and Peanut Hay in Total Mixed Ration on the Performance of Periparturient Dairy Cows: Insights from a Field Trial in Thailand

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Forty Holstein Friesian dairy cows were used to compare alfalfa (ALF) and peanut hay-base (PNT) total mixed rations on performance in periparturient period. Twenty cows were fed ALF diet (16.5% crude protein) and 20 cows were fed PNT diet (19% crude protein). All cows were routinely drenched once daily with propylene glycol as early as 1 week prior to anticipated calving date, until 7 days after calving. Blood samples were collected at -2, 1, 2, 3 and 4 weeks from parturition. Milk yields were recorded daily and milk samples were collected twice a week to determine urea nitrogen concentrations and milk composition. During the 4 weeks postpartum, daily dry matter intake of cows did not differ between the two groups. Serum glucose, non-esterified fatty acid,  $\beta$ -hydroxybutyrate and urea nitrogen concentrations did not differ between the two groups at any sampling times. After calving, decreased glucose and increased nonesterified fatty acid and  $\beta$ -hydroxybutyrate concentrations in the blood indicated that cows in both groups went into negative energy balance status. Serum urea nitrogen concentrations did not change during the sampling period. Cows fed PNT diet seemed to have higher urea nitrogen concentrations in the milk than cows fed ALF diet. Average milk production during the 30 days postpartum was greater for cows fed ALF diet than those fed PNT diet. Milk composition did not differ between groups. Although, average days from calving to first service did not differ between groups, cows fed ALF diet had better conception rate at first service than cows fed PNT diet. In conclusion, cows fed ALF diet could improve milk yield and conception rate. However, in Thailand, replacing PNT with ALF hay in total mixed ration would depend on the economic analysis because ALF hay was exported from a foreign country.

**Key words:** Alfalfa hay, dairy cow, peanut hay, performance.

## INTRODUCTION

In Thailand, dairy farmers widely use traditional by-products from agricultures such as forages, pineapple peels, peanut by-products, corn by-products and rice straw to feed their cows. Peanut by-products including peanut skins, peanut hulls, peanut hay and silages are

economically priced, and they can be incorporated into a variety of diets for dairy cattle (Hill, 2002). It is accepted that quality of roughages influences to a greater extent the milk yield and milk quality and to lesser extent the reproductive performance and health status of the cows.

Dairy cows during periparturient period suffer some degrees of negative energy balance, which is associated with general health problems (Wentink et al., 1997), reproductive disorders (Heinonen et al., 1998; Rukkwamsuk et al., 1999) and low milk production

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(Rukkwamsuk et al., 2001). In Thailand, Rukkwamsuk et al. (2006) reported negative energy balance problem in dairy cows raised in small-holder farms. Propylene glycol is a substance used in prevention and treatment of ketosis in dairy cows. The metabolism of propylene glycol and its effects on physiology parameters, feed intake, milk production and risk of ketosis are reviewed by Nielsen and Ingvertsen (2004). Propylene glycol is therefore used to prevent negative energy balance in periparturient dairy cows (Studer et al., 1993; Grummer et al., 1994; Christensen et al., 1997; Rukkwamsuk et al., 2005).

High quality roughage, alfalfa, is rarely grown in Thailand because of unsuitable climate; therefore a large part of alfalfa hay used in the country must be imported from foreign countries. Due to the high cost, alfalfa hay is restrictedly used by some dairy farmers. Data concerning the use of alfalfa are considerably reported in western countries and are limited in Thailand. Before any assumptions to use alfalfa in the diet of dairy cows in Thailand could be made, comparative study of using alfalfa and other common forages in the total mixed ration was required. The objective of this study was to evaluate the use of alfalfa hay and peanut hay in total mixed ration on performance of periparturient dairy cows.

## MATERIALS AND METHODS

### Animals and diets

The study was conducted in a commercial dairy farm in Nakhonratchasima Province, Northeastern Part of Thailand. The farm consisted of 503 lactating cows, 171 dry cows, and 414 replacement calves and heifers. The average milk yield was 20.5 kg/cow daily. Close-up and lactating cows were kept in a free-stall housing with an evaporative cooling system, which controls the inside temperature between 25 - 28°C. Forty healthy pregnant cows were selected from this farm, and were randomly divided into 2 groups: 20 cows were fed alfalfa-base (ALF) diet during the transition and lactating periods, and the other 20 cows were fed peanut-base (PNT) diet. At the start of experiment, average ages were 3.44 (0.36; SD) and 3.44 (0.41) years; average 305-day milk yields were 7320 (1512) and 7581 (1134) kg; average dry periods were 59.6 (16.8) and 57.9 (13.6) days, for cows in ALF and PNT diet groups, respectively.

During the early dry period, cows in both groups were fed the same dry period total mixed ration. Thereafter, cows in each group were assigned to the total mixed rations in their transition and lactating periods as indicated in Table 1. The chemical compositions of the rations are shown in Table 2. All cows were drenched once daily with 400 ml of propylene glycol starting from 7 days before anticipated calving date to 7 days after calving. Dry matter intake of cows was estimated every day and milk yields were recorded daily. Cows received their first artificial insemination in the second estrus postpartum and conception rate at first service was recorded.

### Sampling procedures

Blood samples were collected from all cows at -2, 1, 2, 3, and 4

weeks from parturition. At sampling, 20 ml of blood were collected from the jugular vein in evacuated tubes. The tubes were kept on ice and were centrifuged at 1200 × g for 15 min within 2 to 3 h after collection, and serum samples were harvested and stored at -20°C until analysis for glucose, non-esterified fatty acids (NEFA), β-hydroxybutyrate, and urea nitrogen concentrations.

Milk samples were collected from all cows twice weekly for 4 weeks after parturition. For determination of urea nitrogen in the milk, 1.5 ml of composite milk of each cow was placed in a microcentrifuge tube. For determination of milk composition, 30 ml of composite milk was kept in a plastic bottle with potassium dichromate at 0.1% (wt/vol) as a preservative. All milk samples were stored at 4°C and were analyzed within 7 days.

### Assay procedures

Concentrations of serum glucose (Glucose GOD-PAP, Class-1 Laboratories Co., Ltd., Bangkok, Thailand), NEFA (NEFA C, Wako Pure Chemical Industries Ltd., Osaka, Japan), β-hydroxybutyrate (RB 1007, Randox Laboratories, San Diego, CA, USA), and urea nitrogen (Urea Nitrogen Reagent, Class-1 Laboratories Co., Ltd.) were measured enzymatically with commercially available kits as indicated. Milk composition (protein, fat, lactose, and solid non fat) were measured automatically using Fourier Transform Infrared Spectrophotometer (MilkoScan FT6000 Spectrophotometer). For milk urea nitrogen, samples in microcentrifuge tubes were centrifuged at 1200 × g for 15 min. The fat layer was discarded and the defatted milk was measured for urea nitrogen using the same kit as used for the serum urea nitrogen concentration.

### Statistical analysis

Data were explored for normal distribution using Kolmogorov-Smirnov test. Normally distributed data were subjected to analysis of variance using dietary treatment as a fixed main effect and sampling day as a repeated measure (Patrie and Watson, 1999). The homogeneity of variances was verified using the Levene's test. Within group, comparison of data between sampling days was performed using the pairwise comparisons of repeated measure analysis of variance. First service conception was compared between groups using Chi-square test. The two-sided level of statistical significance was preset at  $P \leq 0.05$ .

## RESULTS

### Feed intake

Dry matter intake of cows in both groups is demonstrated in Figure 1. During the first 30 days postpartum, average dry matter intake in cows fed ALF diet was  $17.6 \pm 1.2$  (mean ± SD) kg/d and in cows fed PNT diet  $18.2 \pm 0.9$  kg/d.

### Milk yield and compositions

Milk yield during the 30 days of lactation of cows in both groups is presented in Figure 2. Throughout the

**Table 1.** Composition of total mixed rations as fed basis.

Ingredient	Dry period	Transition period		Lactating period	
		Alfalfa-base	Peanut-base	Alfalfa-base	Peanut-base
kg as fed					
Concentrate <sup>1</sup>	1.3	...	...	...	...
Concentrate <sup>2</sup>	...	2.11	...	...	...
Concentrate <sup>3</sup>	...	...	2.0	...	...
Concentrate <sup>4</sup>	...	...	...	4.61	...
Concentrate <sup>5</sup>	...	...	...	...	3.8
Wet brewer grain	8.0	3.0	7.0	6.0	10.0
Corn silage	8.0	12.1	12.5	10.65	12.5
Alfalfa hay	...	3.0	...	4.0	...
Peanut hay <sup>6</sup>	2.0	...	6.0	...	6.0
Rice straw <sup>7</sup>	3.5	...	...	...	...
Cassava chips <sup>8</sup>	...	0.9	1.0	1.8	1.3
Whole cotton seed	...	0.5	0.6	1.5	2.0
Fish meal	...	0.05	...	0.1	...
Blood meal	...	0.05	...	0.1	...
Ground corn	...	...	...	...	1.0
Molasses <sup>9</sup>	1.0	0.75	0.5	1.0	0.5
Premixes <sup>9</sup>	0.5	0.5	0.5	1.0	1.0

<sup>1</sup> Consisting of 33.0% soybean meal, 27% canola meal, 22.5% wheat bran, 11% dried brewer grain, 4.1% limestone, and 2.4% salts.

<sup>2</sup> Consisting of 64% extruded corn, 31.3% soybean meal, and 4.7% energizer-RP10.

<sup>3</sup> Consisting of 54.5% soybean meal, 15.0% canola meal, 13.0% dried brewer grain, 11.0% corn gluten meal, 4.7% salts, and 1.8% biophos.

<sup>4</sup> Consisting of 58.6% extruded corn, 25.1% soybean meal, 10.9% corn gluten meal, and 5.4% energizer-RP10.

<sup>5</sup> Consisting of 50.0% soybean meal, 27.0% corn gluten meal, 14.0% canola meal, 5.5% dried brewer grain, 1.9% salts, and 1.6% limestone.

<sup>6</sup> Consisted of 90.4% of dry matter, 11.1% of crude protein, 1.3% of crude fat, 24.7% of crude fiber, 34.3% of acid detergent fiber, and 37.6% of neutral detergent fiber.

<sup>7</sup> Consisted of 93.9% of dry matter, 4.4% of crude protein, 2.3% of crude fat, 35.6% of crude fiber, 52.2% of acid detergent fiber, and 26.4% of neutral detergent fiber.

<sup>8</sup> Consisted of 89.8% of dry matter, 2.5% of crude protein, 0.4% of crude fat, 3.4% of crude fiber, 5.5% of acid detergent fiber, and 10.7% of neutral detergent fiber, and 73.96% of starch.

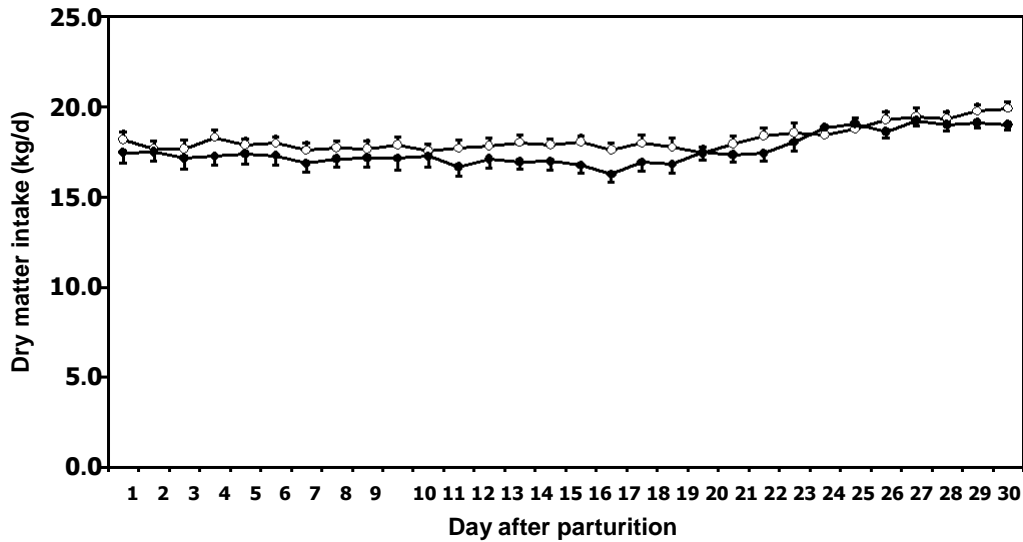
<sup>9</sup> Commercial premixes containing trace minerals and vitamins.

**Table 2.** Chemical compositions of the total mixed ration.

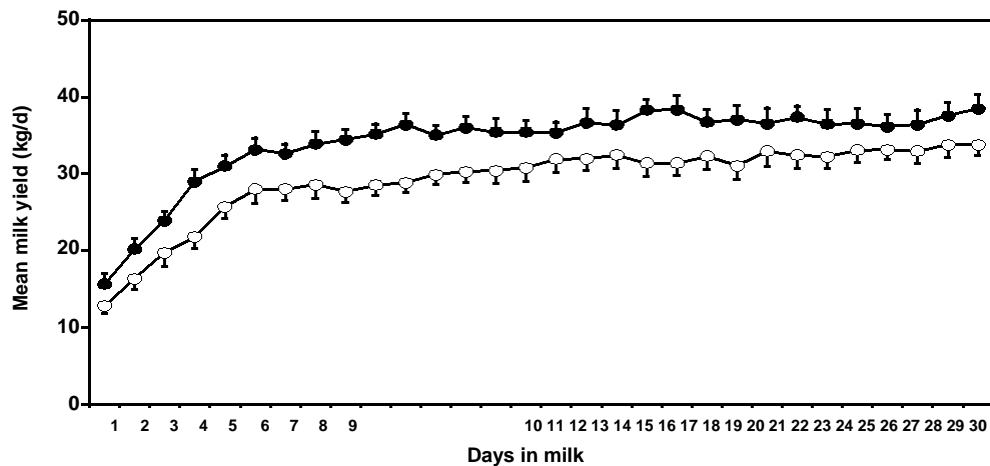
Item (%)	Dry period	Transition period		Lactating period	
		Alfalfa-base	Peanut-base	Alfalfa-base	Peanut-base
Dry matter	49.51	51.15	53.69	55.91	55.99
Ash	7.29	7.41	11.75	9.18	10.35
Crude protein	11.44	12.90	14.15	16.48	19.25
Crude fat	2.56	3.96	4.18	4.83	4.34
Neutral detergent fiber	61.36	39.13	45.54	42.21	41.49
Acid detergent fiber	31.75	27.66	40.35	23.38	27.33

experimental period, cows fed ALF diet produced greater amount of milk when compared with cows fed PNT diet. Average milk yields during 30 days in milk were 34.8 ±

8.7 kg/d and 29.1 ± 8.4 kg/d for cows fed ALF and PNT diets, respectively. Milk composition of cows during the 30 days of lactation is presented in Table 3. At the first



**Figure 1.** Average dry matter intake (kg/d) during the first 30 d after parturition of cows in alfalfa-base diet (●; n = 20) and in peanut-base diet (○; n = 20). Data represent means and the error bars are S.E.M.



**Figure 2.** Comparison of milk yield during the first 30 d in milk between cows in alfalfa-base diet (●; n = 20) and cows in peanut-base diet (○; n = 20) groups. Data are means ( $\pm$  SEM).

week of lactation, percentages of fat and protein in the milk were high and the percentages declined in the second week and became almost at a constant levels at week 4 of lactation.

**Serum glucose,  $\beta$ -hydroxybutyrate, NEFA, and urea nitrogen**

Serum glucose, NEFA,  $\beta$ -hydroxybutyrate, urea nitrogen concentrations were demonstrated in Figure 3, 4, 5, and 6. Two weeks before parturition, serum glucose concen-

trations were  $59.5 \pm 1.1$  and  $58.7 \pm 5.6$  mg/dL for cows fed ALF and cows fed PNT diets, respectively. After parturition, serum glucose concentrations decreased sharply for cows in both groups, indicating that cows suffered some degrees of negative energy balance. Serum glucose concentrations remained at lower levels until 4 weeks after parturition, and did not differed between the two groups (Figure 3).

Serum NEFA concentrations did not differ between cows fed ALF diet and cows fed PNT diet. However, the concentrations in both groups increased after parturition as cows entered a period of high energy requirement.

**Table 3.** Compositions of milk during the 4 wk postpartum of cows in alfalfa-base diet (n = 20) and in peanut-base diet (n = 20) groups. Data represent means (SEM).

Item	Week after parturition							
	0.5	1	1.5	2	2.5	3	3.5	4
<b>Milk fat (%)</b>								
Alfalfa-base	4.73 (0.33)	4.85 (0.35)	4.47 (0.27)	4.18 (0.39)	4.62 (0.61)	3.28 (0.19)	4.31 (0.74)	3.04 (0.27)
Peanut-base	4.61 (0.41)	4.52 (0.17)	4.49 (0.41)	4.08 (0.37)	3.56 (0.21)	3.99 (0.54)	3.27 (0.45)	3.20 (0.29)
<b>Milk protein (%)</b>								
Alfalfa-base	3.72 (0.09)	3.43 (0.06)	3.14 (0.05)	3.02 (0.03)	2.89 (0.04)	2.81 (0.05)	2.57 (0.05)	2.60 (0.05)
Peanut-base	4.00 (0.13)	3.59 (0.09)	3.21 (0.07)	3.00 (0.06)	2.90 (0.06)	2.76 (0.04)	2.71 (0.05)	2.71 (0.07)
<b>Milk lactose (%)</b>								
Alfalfa-base	4.74 (0.05)	4.87 (0.04)	4.92 (0.02)	5.02 (0.03)	4.95 (0.05)	4.99 (0.05)	4.93 (0.05)	4.99 (0.04)
Peanut-base	4.54 (0.12)	4.79 (0.07)	4.92 (0.05)	4.93 (0.06)	4.95 (0.05)	4.93 (0.06)	5.00 (0.04)	4.98 (0.04)
<b>SNF* (%)</b>								
Alfalfa-base	9.14 (0.07)	9.00 (0.07)	8.78 (0.07)	8.75 (0.04)	8.55 (0.07)	8.59 (0.07)	8.45 (0.08)	8.53 (0.06)
Peanut-base	9.20 (0.07)	9.08 (0.07)	8.83 (0.07)	8.65 (0.07)	8.55 (0.07)	8.38 (0.08)	8.41 (0.07)	8.38 (0.07)

\* Solid non fat.

The concentrations increased sharply right after calving and remained at high levels and slightly reduced very close to the prepartum levels at week 4 postpartum (Figure 4). Serum  $\beta$ -hydroxybutyrate concentrations were changed in the same manner as serum NEFA concentrations. The prepartum concentrations were at the lowest concentrations, whereas the concentrations during 2 to 4 weeks postpartum were higher; however, concentrations between groups were not different (Figure 5). Serum urea nitrogen concentrations did not differ between groups during prepartum and postpartum periods (Figure 6). The mean concentrations in both groups remained in a narrow range between 12 and 15 mg/dL.

### Milk urea nitrogen

Concentrations of milk urea nitrogen of cows fed ALF and PNT diets are shown in Figure 7. Milk urea nitrogen con-

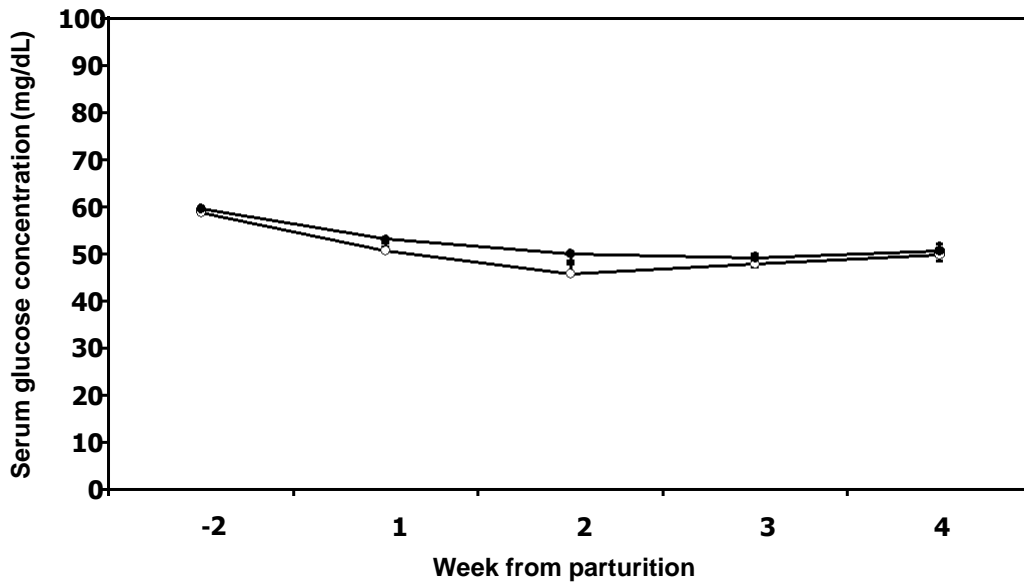
centrations measured during the first 2 weeks of lactation tended to be higher in cows fed PNT diet than in cows fed ALF diet. The means of milk urea concentrations during the first 4 week of lactation were  $14.9 \pm 3.8$  and  $13.7 \pm 3.4$  mg/dL for cows fed PNT and cows fed ALF diets, respectively.

### Conception at first service

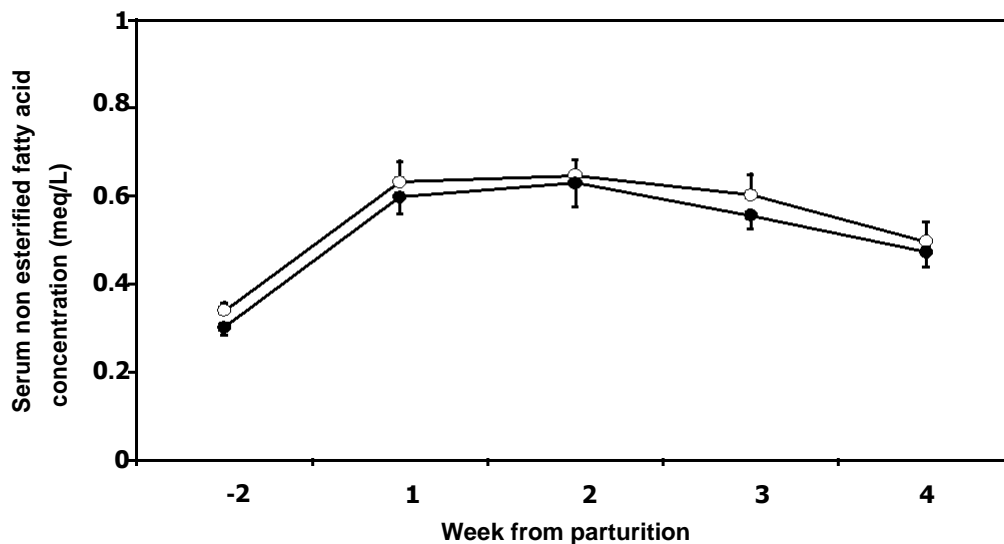
Average days from calving to first service did not differ between cows fed PNT diet ( $88 \pm 25$  days) and cows fed ALF diet ( $86 \pm 24$  days). Eleven cows (55%) fed ALF diet were conceived at first insemination, but only 3 (15%) cows fed PNT diet were conceived. Therefore, cows fed ALF diet had higher conception rate than cows fed PNT diet (Chi-square = 7.03, P = 0.008).

### DISCUSSION

Dry matter intake did not differ between both groups



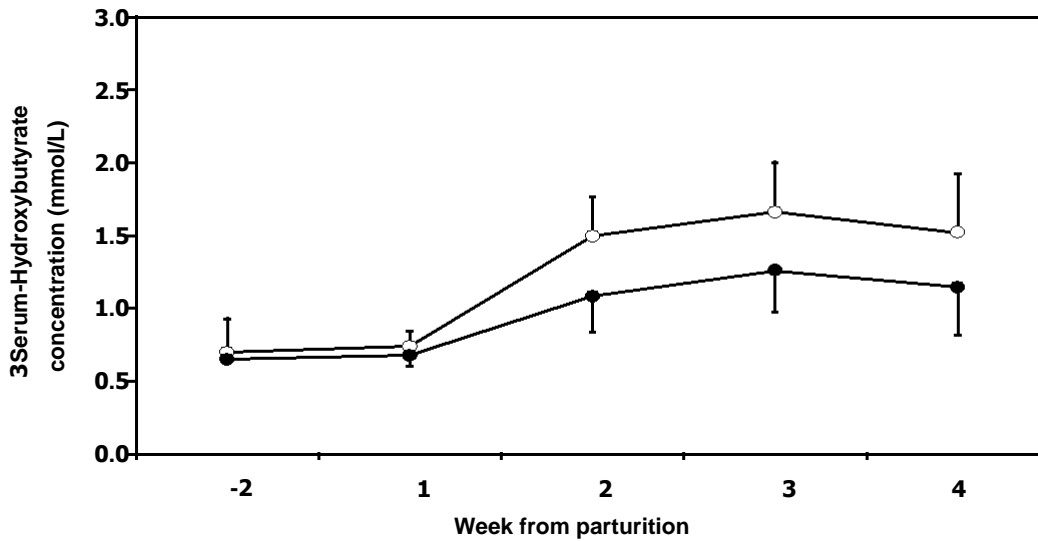
**Figure 3.** Comparison of glucose concentrations in the serum measured before and after parturition between cows in alfalfa-base diet (●; n = 20) and cows in peanut-base diet (○; n = 20) groups. Data are means ( $\pm$  SEM).



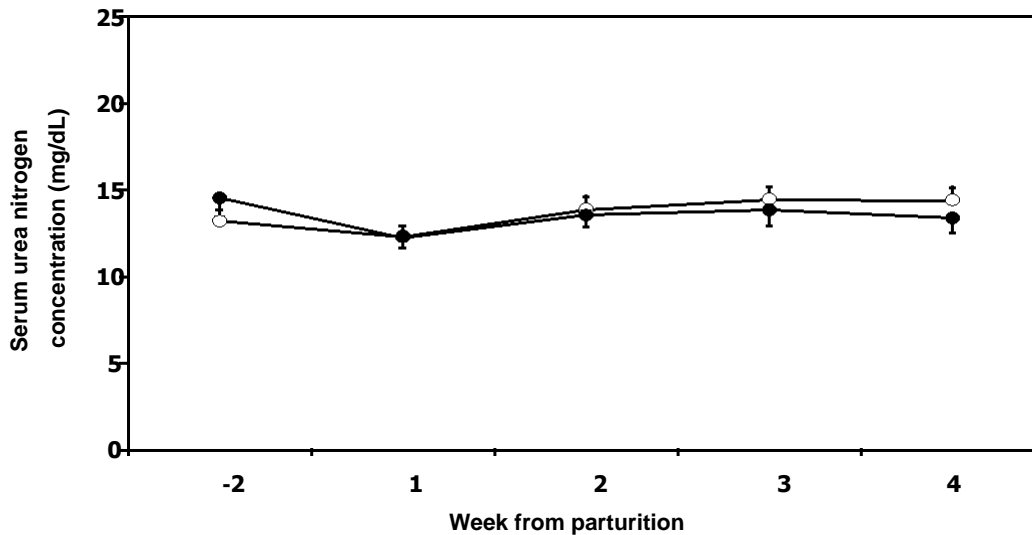
**Figure 4.** Comparison of NEFA concentrations in the serum measured before and after parturition between cows in alfalfa-base diet (●; n = 20) and cows in peanut-base diet (○; n = 20) groups. Data are means ( $\pm$  SEM).

during 4 wk of lactation, indicating that both 16.5% crude protein (ALF diet) and 19% crude protein (PNT diet) had similar palatability to dairy cows. In a previous study, Rukkamsuk et al. (2005) demonstrated that propylene glycol does not affect the dry matter intake of the cows (Miyoshi et al., 2001).

glycol at a dosage of 400 ml/day did not stimulate or reduce dry matter intake as compared with their control cows. In addition, it has been reported that propylene glycol does not affect the dry matter intake of the cows. The increased fat percentages in the milk might be



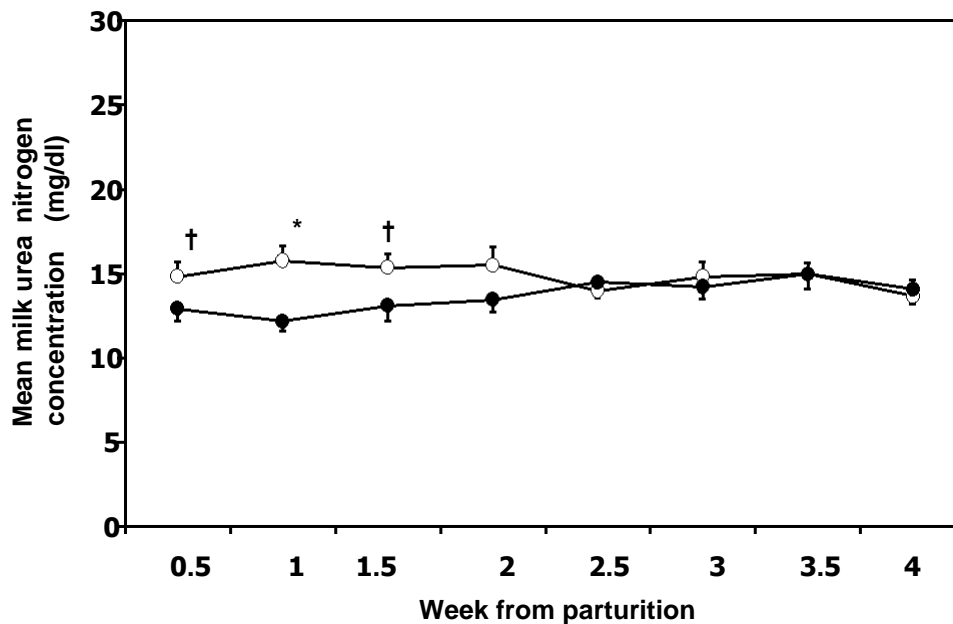
**Figure 5.** Comparison of  $\beta$ -hydroxybutyrate concentrations in the serum measured before and after parturition between cows in alfalfa-base diet (●; n = 20) and cows in peanut-base diet (○; n = 20) groups. Data are means ( $\pm$  SEM).



**Figure 6.** Comparison of urea nitrogen concentrations in the serum measured before and after parturition between cows in alfalfa-base diet (●; n = 20) and cows in peanut-base diet (○; n = 20) groups. Data are means ( $\pm$  SEM).

related to increase lipolysis in adipose tissue as corresponding to negative energy balance (Rukkamsuk et al., 2001). Unlike fat and protein percentages, milk lactose percentages did not change much during the 4 weeks of lactation. Comparing the milk composition between cows fed ALF diet and cows fed PNT diet, no difference between

weeks of lactation. As explained by increased fat and protein percentages in the first week of lactation, percentages of solid non fat were high at the first week and declined as the protein percentages declined. percentages of fat, protein, lactose as well as solid non fat was found. These results suggest that cows in both



**Figure 7.** Comparison of urea nitrogen concentrations in the milk measured after parturition between cows in alfalfa-base diet (●; n = 20) and cows in peanut-base diet (○; n = 20) groups. Data are means ( $\pm$  SEM). Asterisk indicates mean milk urea nitrogen concentrations between the two groups were different ( $P \leq 0.05$ ). Daggers indicate mean milk urea nitrogen concentrations between the two groups tended to be different ( $P < 0.1$ ).

groups could produce satisfactory compositions in the milk at any time of lactation. The levels of crude protein in the diet did not influence the milk composition. Propylene glycol did not effect the milk composition in this study as was also observed in the other study (Cozzi et al., 1996). The finding that serum glucose concentrations decreased after calving and remained at lower levels until 4 weeks after calving corresponded well with other previous studies (Van den Top, 1995; Rukkwamsuk et al., 2005). However, postpartum concentrations of serum glucose in this study were higher than the previous observation by Rukkwamsuk et al. (2005), indicating that propylene glycol did improve energy balance of the post parturient dairy cows.

Drenching propylene glycol to cows in both groups reduced degree of lipolysis as also reported earlier (Studer et al., 1993). During the first week of lactation where the serum NEFA concentrations were at the highest levels, the milk fat percentages in both groups were also at the highest levels (Table 3). Therefore, milk fat percentages could be used as a practical parameter for explaining negative energy balance in post parturient dairy cows, which is also suggested elsewhere (Rukkwamsuk et al., 2001). Increased serum NEFA concentrations were usually followed by increased ketone

bodies levels in the blood. This is in agreement with other previous studies (Van den Top, 1995; Hoedemaker et al., 2004). Apart from being a glucogenic precursor, propylene glycol might improve protein utilization and metabolism in the rumen. Therefore, cows fed high protein diet (19%) had similar concentrations of serum urea nitrogen to cows fed 16.5% crude protein in the diet. In addition, milk urea nitrogen concentrations in cows fed 16.5% crude protein diet were also similar to that in cows fed 19% crude protein diet. The mean milk urea nitrogen concentrations in both groups were in a normal range of postpartum levels, suggesting a normal protein metabolism in these groups of cows. Although the conception rate of cows fed ALF-diet was greater than cows fed PNT-diet (55 versus 15%), the mechanism of ALF in improving postpartum fertility was still unknown and needed further study. However, it might be related to the better quality of ALF-diet as compared to PNT-diet.

### Conclusions

Cows fed ALF diet seemed to produce more milk than cows fed PNT diet. Milk composition was not affected by the diets in this study. Furthermore, cows fed ALF diet showed better conception rate at first insemination after calving that cows fed PNT diet. Due to the routine use of



propylene glycol administered orally to a cow 7 days before expected calving to 7 days after calving, negative energy balance was reduced in both groups of cows during the periparturient period. In Thailand, replacing PNT hay with ALF hay would require further study.

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