Asian-Aust. J. Anim. Sci.
Vol. 19, No. 10 : 1415 - 1421
October 2006
www.ajas.info

The Effect of Prepartum Diet on Nitrogen and Major Mineral Balance of Dairy Cows during Parturition in Summer

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ABSTRACT : Proper nutritional management during the dry period is required to prevent metabolic disorders during the time of parturition and for potential increase milk yield during early lactation, especially under the heat of summer. The effect of prepartum diets on partitioning of nitrogen (N), calcium (Ca), phosphorus (P), magnesium (Mg) and potassium (K) during dry period and early lactation in summer was investigated. Nine cows were assigned to two groups and fed either control (group C: four cows) or high concentrate (group H: five cows) diets to meet 110% of their requirements before parturition. The proportion of concentrate in control diet was 35%, and that in the high concentrate diet was 45%. After parturition, all cows were fed the same diets *ad libitum* during lactation. Balance trials were conducted at 9, 8 and 7 days before parturition and at 12, 13 and 14 days after parturition. Before parturition, dry matter intake (DMI), DM and NFE digestibility in group C tended to be lower than those in group H. The retention of N ($p<0.01$) and P ($p<0.05$) in group C during the dry period was significantly lower than those in group H. The retention of Mg in group C during the dry period tended to be lower than in group H. The concentration of plasma NEFA in group C tended to be higher than in group H during dry period. The prepartum diet did not have an apparent effect on DMI and milk yield at 2 weeks after parturition and N, Ca, P, Mg and K balance after parturition. (**Key Words :** Balance Trial, Dairy Cow, Nitrogen, Mineral, Heat Stress, Parturition)

INTRODUCTION

The negative effect of heat stress on dry matter intake (DMI), milk yield and composition of dairy cows has been widely demonstrated (Rodriguez et al., 1985; Kume et al., 1990). Kamiya et al. (2005) reported that the high ambient temperature reduced DMI intake and N utilization for milk production. Kume et al. (1987) suggested that the major mineral absorption of lactating cows was affected by heat stress.

The period of transition from late pregnancy to early lactation stage imposes an enormous metabolic challenge to the dairy cows (Bell, 1995a; Goff and Horst, 1997). Many reports showed that DMI of dairy cows started to decline at approximately 3 weeks before parturition and dropped dramatically in the last few days of gestation (Bertics et al., 1992; Grummer, 1995). The decline of DMI has caused many problems in the post-parturient cow, including metabolic diseases, infertility, and decline of milk production (Vandehaar et al., 1999; Holtenius et al., 2003; Khan et al., 2004). The periparturient cows might be affected by heat stress more seriously, therefore the proper and more stringent nutritional management is required to prevent the decrease of DMI during a hot summer (Grant and Albright, 1995). However, the effects of prepartum diet on nutrient metabolism of heat stressed dairy cows in periparturient period have not been well clarified.

The supplementation of concentrate in prepartum diet is one of the strategies to increase the energy density in diet and prevent the decline of DMI. In the previous report (Kamiya et al., 2006), we investigated the effects of prepartum diet on DMI during parturition and milk production and showed that supplementation of concentrate to prepartum diet prevented the decline of DMI before parturition. In this report, we investigated the effects of forage to concentrate ratio in prepartum diet on nutrient balance and plasma metabolites in dairy cows around parturition during a hot summer.

MATERIALS AND METHODS

Experimental design and animals

Nine pregnant Holstein cows due to calve under the hot ambient temperatures (July and August) were used in this experiment. They were assigned to one of two groups and

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Received October 11, 2005; Accepted March 3, 2006

Table 1. Ingredients of experimental diet

Ingredient	Dry period		Lactation
	Control	High	
Italian ryegrass hay	47.0	32.0	10.0
Alfalfa hay	3.0	3.0	8.0
Corn silage	15.0	20.0	30.0
Corn	11.0	13.0	13.0
Barley	11.0	13.0	13.0
Wheat bran	4.0	8.0	8.0
Soybean meal	4.5	5.0	9.0
Soybean hulls	3.8	5.2	3.1
Cotton seed	-	-	3.0
Fatty acid calcium salt	-	-	1.0
Vitamin & Mineral mix	0.7	0.8	1.9

Table 2. Chemical composition of experimental diets

Component	Dry period		Lactation
	Control	High	
TDN[1]	64.2	68.4	71.3
CP	13.0	13.3	14.9
NFE	51.0	54.8	57.4
CF	25.4	21.9	16.6
EE	2.4	2.6	4.0
NDF	48.4	43.4	33.7
ADF	29.1	25.3	19.9
Ca	0.42	0.43	0.78
P	0.31	0.35	0.52
Mg	0.20	0.20	0.20
K	2.89	2.54	1.94

[1] TDN is calculated by balance trial

fed either control diet (group C: four cows) or high concentrate diet (group H: five cows) from 18 days before expected calving date to parturition. The cows were offered an experimental diet three times a day at 08:30 h, 16:00 h and 18:00 h to meet the 110% of their energy requirement recommended by Japanese Feeding Standard for Dairy Cattle (1999) before parturition. After parturition all cows were offered the same diet for lactation. The cows were fed preliminary diet for 5 days after parturition. After 5 days postpartum all cows were offered the same experimental diet for lactation four times a day at 08:30 h, 13:00 h, 16:00 h, and 18:00 h *ad libitum*. They received water *ad libitum*. Ingredients and chemical composition of experimental diets are given in Table 1 and 2.

All cows were managed in individual tie stalls and rested in a paddock from 10:00 h to 16:00 h daily before parturition and around the calving day and from 10:00 h to 13:00 h daily after parturition. All cows had cooling from fans at tie stalls in the daytime. This study was carried out in accordance with the guide for care and use of laboratory animals for National Agricultural Research Center for Kyushu Okinawa Region.

Nutrient balances

Body weight was measured at the start of collection period. The DMI was measured daily during experimental period. Feces and urine were collected totally at 9, 8, and 7 days before parturition and 12, 13, and 14 days after parturition. Urine was collected by a plastic urine cup which drained into a container during dry period and by a sterile Foley catheter placed into the urethra during early lactation. Feces and urine were weighed daily and stored frozen for later analyses. Rectal temperature was measured at 09:00 h and 16:00 h during the collection period. Blood samples were obtained by jugular puncture into heparinized tubes at 10:00 h on the last day of collection period. Blood was centrifuged (1,500×g for 15 min, 4°C) to separate the plasma. Each cow was milked at calving, 08:30 h and 18:00 h after parturition. Milk weight was recorded daily and milk samples were obtained at 12, 13, and 14 days postpartum. Plasma and milk samples were stored frozen until the analyses were performed.

Sample analysis

Milk fat, protein and lactose were analyzed by Milko-Scan (133b, Foss Japan, Tokyo, Japan). The nitrogen (N) in diets, feces and urine were measured using the macro-Kjeldahl methods. For analysis of calcium (Ca), phosphorus (P), magnesium (Mg) and potassium (K), diets, milk, feces and urine were digested in nitric-perchloric acid and plasma was deproteinized with 10% trichloroacetic acid. The P content in each sample was determined by colorimetric methods of Gomori (Gomori, 1942). The concentrations of Ca, Mg and K in each prepared sample were determined by atomic absorption spectrophotometry (SOLAAR M6, Nippon Jarrell-Ash Co., Ltd. Kyoto, Japan). The concentrations of plasma NEFA, glucose, and TP were determined by an automatic analyzer (7070, HITACHI, Tokyo, Japan). The concentration of plasma insulin was measured using a radioimmunoassay kit (Eiken Chemical Co., Ltd. Tokyo, Japan). The concentration of plasma osteocalcin (OC) was measured using a commercially available radioimmunoassay kit (Yuka Medias Co., Ltd, Ibaraki, Japan). The urinary deoxypyridinoline (DPD) content was determined with a competitive enzyme immunoassay kit (Metra Biosystems, Inc., Mountain View, CA, USA).

The general linear models procedure of SAS (1999) was used to analyze the effects of prepartum diet on N and major mineral balances and plasma metabolites. The model was as follows:

$$Y_{ijk} = \mu+D_i+C_{(i)j}+T_k+DT_{ik}+E_{ijk}$$

where Y_{ijk} is the observed dependent variable, μ the overall mean, D_i the effect of diet, $C_{(i)j}$ the random variable caused by cow nested in diet, Tk the effect of sampling day, DT_{ik} the interactions and E_{ijk} residuals.

Table 3. Rectal temperature of dairy cows at collection period

	Dry period		Lactation	
	Control	High	Control	High
Rectal temperature				
09:00 h	38.8±0.1	38.7±0.0	39.5±0.2	39.5±0.2
16:00 h	39.1±0.1	39.2±0.1	39.7±0.3	40.0±0.2

Means±Standard error.

RESULTS AND DISCUSSION

The upper critical temperature for dairy cows is defined as 24°C when relative humidity is over 70% (Japanese Feeding Standard for Dairy Cattle, 1999). The mean temperature and relative humidity during the experimental period were 27.7°C, 79.7%, respectively. The rectal temperature (RT) at 09:00 h and 16:00 h during collection period is shown in Table 3. The prepartum diet did not affect the RT at 09:00 h and 16:00 h during both dry and lactation period. Toharmat et al. (1996) has reported that RT at 15:00 h was significantly higher in cows fed at maintenance level than when fed at maintenance plus pregnancy level during late dry period. However, the prepartum diet seemed not to affect the thermoregulation mechanism of cows in both groups.

The BW, DMI, milk yield, feces and urine output are shown in Table 4. The DMI in group C tended to be lower (p<0.08) than in group H during the dry period. Some researchers (Vandehaar et al., 1999; Rabelo et al., 2003) showed that DMI decreased as dietary NDF increased, similarity in the study, higher NDF content in the prepartum diet seemed to result in the decline of DMI in group C. The DM (p<0.08), NFE (p<0.07) and Mg (p<0.05) digestibility in group C tended to be lower than those of group H during the dry period. Doepel et al. (2002) reported that DM digestibility was higher for high energy diet than low energy diet, which was agreed with the findings of our data. The N, CF, EE, Ca, P and K digestibility were not affected by prepartum diet during the dry period. The TDN intake of cows in groups C and H during dry period were calculated as 6.2 kg/day and 7.8 kg/day, respectively (Tables 2 and 4).

Table 4. Body weight (BW), DMI, milk yield, feces and urine output and nutrient digestibility of dry, pregnant and lactating cow fed two level of concentrate in diets before parturition

	Dry period		Lactation	
	Control	High	Control	High
BW (kg)	801.1±30.8	777.3±27.6	699.4±36.6	675.8±32.6
DMI (kg)	9.6±0.7	11.5±0.6	14.3±0.7	14.3±0.6
Milk (kg)	-	-	34.0±1.6	34.9±1.5
Feces (kg)	3.3±0.2	3.5±0.2	4.1±0.2	4.2±0.2
Urine (kg)	12.7±1.1	11.7±0.9	16.8±3.1	12.3±2.7
Digestibility (%)				
DM	65.6±1.3	69.3±1.2	70.9±1.0	70.6±0.9
N	64.3±1.1	66.4±0.9	73.1±2.1	71.3±1.9
NFE	72.7±1.5	77.0±1.3	77.6±0.9	77.7±0.8
CF	57.0±2.1	57.6±1.9	49.5±2.2	49.3±2.0
EE	79.4±0.8	81.3±0.7	87.6±1.1	85.2±1.1
Ca	33.5±5.5	42.1±5.0	37.0±4.8	40.8±4.3
P	15.3±5.2	28.6±4.7	46.0±3.4	46.8±3.0
Mg	14.2±3.4[a]	26.1±3.1[b]	29.2±4.1	31.2±3.7
K	84.9±1.2	84.3±1.1	89.3±1.5	85.9±1.4

Means±standard error.

[a, b] Means in the same raw with different superscript are significantly different from each other (p<0.05).

The TDN was calculated to supply 86% of their energy requirement in group C and 110% in group H. The cows in group C seemed to be energy deficienct even before parturition, indicating the mobilization of fat deposit from maternal tissues to support the fetal growth. The DMI and nutrient digestibility after parturition were not affected by prepartum diet, which was agreed with the data of Doepel et al. (2002). Milk production at collection period was not affected by prepartum diet.

The N balance is shown in Table 5. The N intake (p = 0.05), apparent absorption (p<0.05) and retention (p<0.05) in group C was lower than those in group H. The N retention in groups C and H was 21.2 g/day and 74.5 g/day, respectively. Bell et al. (1995b) calculated that the gravid uterus accretes approximately 19 g/day of nitrogen at 270 days of gestation. This was approximately equal to those accumulated by the cows in group C. The cows in group H

Table 5. The N balance of dry, pregnant and lactating cow fed two level of concentrate in diets before parturition

Prepartum diet	Dry period		Lactation	
	Control	High	Control	High
N balance (g/day)				
Intake	199.0±14.1	243.5±12.6	339.9±13.2	332.7±11.8
Excretion				
Feces	71.0±5.3	81.7±4.7	91.0±7.9	95.8±7.0
Urine	106.8±11.9	87.3±10.6	110.4±5.3	100.0±4.8
Milk	-	-	146.2±5.5	156.3±5.0
Apparent absorption	128.1±9.7[c]	161.8±8.7[d]	248.9±12.3	236.9±11.0
Retention	21.2±9.0[a]	74.5±8.1[b]	-7.8±16.1	-19.2±14.4

Mean±standard error.

[a, b] Means in the same raw with different superscript are significantly different from each other (p<0.01).

[c, d] Means in the same raw with different superscript are significantly different from each other (p<0.05).

Table 6. The Ca balance of dry, pregnant and lactating cow fed two level of concentrate in diets before parturition

Prepartum diet	Dry period		Lactation	
	Control	High	Control	High
Ca balance (g/day)				
Intake	40.5±3.1	50.1±2.7	112.1±5.7	112.7±5.1
Excretion				
Feces	26.6±2.7	29.1±2.4	69.3±3.5	66.7±3.1
Urine	0.4±0.1	0.6±0.1	1.7±0.5	0.6±0.4
Milk	-	-	33.3±2.1	35.4±1.9
Apparent absorption	14.0±2.9	21.0±2.6	42.8±7.2	46.0±6.4
Retention	13.6±2.8	20.4±2.5	7.9±7.3	10.0±6.5

Mean±standard error.

Table 7. The P balance of dry, pregnant and lactating cow fed two level of concentrate in diets before parturition

Prepartum diet	Dry period		Lactation	
	Control	High	Control	High
P balance (g/day)				
Intake	29.1±2.3[a]	40.1±2.0[b]	74.4±3.3	74.0±3.0
Excretion				
Feces	24.3±2.0	28.8±1.8	39.8±2.8	39.5±2.5
Urine	0.3±0.0	0.2±0.0	0.1±0.1	0.2±0.1
Milk	-	-	31.9±1.7	32.7±1.5
Apparent absorption	4.9±1.5[c]	11.4±1.3[d]	34.6±3.3	34.5±3.0
Retention	4.6±1.5[c]	11.2±1.4[d]	2.5±3.6	1.6±3.2

Mean±standard error.

[a, b] Means in the same raw with different superscript are significantly different from each other (p<0.01).

[c, d] Means in the same raw with different superscript are significantly different from each other (p<0.05).

Table 8. The Mg balance of dry, pregnant and lactating cow fed two level of concentrate in diets before parturition

Prepartum diet	Dry period		Lactation	
	Control	High	Control	High
Mg balance (g/day)				
Intake	18.6±1.4	22.8±1.2	30.2±1.4	30.0±1.2
Excretion				
Feces	15.9±1.3	16.8±1.2	21.2±1.2	20.7±1.1
Urine	1.1±0.3[a]	2.3±0.2[b]	2.9±0.3[a]	2.0±0.3[b]
Milk	-	-	2.8±0.2	3.2±0.2
Apparent absorption	2.6±0.8[a]	6.0±0.7[b]	9.0±1.5	9.4±1.3
Retention	1.5±0.7	3.7±0.6	3.3±1.6	4.2±1.4

Means±standard error.

[a, b] Means in the same raw with different superscript are significantly different from each other (p<0.05).

seemed to gain maternal body N during the dry period. During lactation, N balance was not significantly different between treatments. The N retention was negative in both groups, indicating the mobilization of protein from maternal tissues to support milk production. This result was in agreement with the finding of Belyea et al. (1978), who showed that body protein of dairy cows reach a minimum at approximately 6 weeks of lactation.

The Ca balance is shown in Table 6. The Ca intake during the dry period was tended to be lower in group C (p<0.06). The Ca absorption and retention were not affected by prepartum diet. Braithwaite (1983a) reported in the ewes that Ca balance was hardly affected by dietary Ca level before parturition. House and Bell (1993) showed that Ca accretion in the fetus at 280 days of gestation is 10.3 g/day. The Ca retention of cows in groups C and H was 13.6 g/day and 20.4 g/day, respectively, therefore, maternal Ca balance was possibly positive in both groups. The Ca balance during lactation period was not affected by prepartum diet. The apparent absorption of Ca was 37.0% in group C and 40.8% in group H (Table 3) which agrees with the data of Van't Kloster (1976). While most cows in early lactation are in negative Ca balance (Braithwaite et al., 1976), the cows in this study were in positive Ca balance at 2 weeks postpartum. The positive Ca balance during early lactation might suggest that dietary Ca intake is sufficient to meet the Ca requirement of lactating cows (Knowlton and Herbein, 2002).

The P balance is shown in Table 7. The P intake (p<0.01), P absorption (p<0.05) and P retention (p<0.05) in group C were significantly lower than those in group H during the dry period. Braithwaite (1983b) reported in ewes that absorption of P was directly related to P intake during late pregnancy, which might support our results. House and Bell (1993) showed that P accretion in the fetus at 280 days of gestation is 5.4 g/day. The P retention was 4.6 g/day in group C and 11.2 g/day in group H. This result indicates that cows in C group might mobilize P from maternal tissues to support fetal P requirement. The P retention was not affected by prepartum diet during lactation. The P retention was 2.5 g/day in group C and 1.6 g/day in group H. Knowlton (2002) reported that P retention was negative at 3 weeks of lactation; this was not agree with our data. Milk P was relatively low (31.9 g/day in group C and 32.7 g/day in group H), therefore, P retention seems to be positive even during the early lactation in this experiment.

The Mg balance is shown in Table 8. The Mg intake (p<0.07) and retention (p<0.06) in group C tended to be lower than in group H during dry period. The Mg output into urine and apparent absorption in group H were

Table 9. The K balance of dry, pregnant and lactating cow fed two level of concentrate in diets before parturition

Prepartum diet	Dry period		Lactation	
	Control	High	Control	High
K balance (g/day)				
Intake	277.8±25.3	304.3±22.6	279.1±18.5	286.2±16.6
Excretion				
Feces	41.2±3.6	47.5±3.2	30.1±5.0	40.4±4.5
Urine	102.9±20.8	81.4±18.6	96.0±20.6	80.2±18.4
Milk	-	-	58.7±2.8	59.9±2.5
Apparent absorption	236.6±23.2	256.8±20.7	249.0±16.6	245.8±14.9
Retention	133.7±33.4	175.4±29.9	94.3±30.1	105.7±26.9

Means±standard error.

Table 10. The concentrations of plasma NEFA, glucose, TP, insulin, Ca, P, Mg and Osteocalcin (OC) and urinary deoxypyridinoline (DPD) excretion

Prepartum diet	Dry period		Lactation	
	Control	High	Control	High
NEFA (mEq/L)	0.43±0.08	0.19±0.07	0.31±0.08	0.41±0.07
Glucose (mg/dL)	62.0±3.1	54.2±2.8	55.8±1.9	54.4±1.7
TP (g/dL)	6.2±0.3	5.3±0.3	6.6±0.3	7.1±0.3
Insulin (μU/mL)	8.1±1.6	6.4±1.4	8.2±1.7	6.8±1.5
Ca (mg/dL)	9.0±0.2	9.4±0.1	8.8±0.2[a]	10.2±0.2[b]
P (mg/dL)	5.2±0.4	4.9±0.4	4.3±0.6	4.4±0.5
Mg (mg/dL)	1.8±0.1	2.0±0.1	2.3±0.1	2.2±0.1
OC (ng/mL)	5.3±1.7	6.6±1.5	5.0±0.9	4.4±0.8
DPD (nmol/day)	41.5±10.4	31.7±9.3	64.4±23.3	107.6±20.8

Means±atandard error.

[a, b] Means in the same raw with different superscript are significantly different from each other (p<0.05).

significantly higher (p<0.05) than in group C during the dry period. Jittakhot et al. (2004) reported that absorption and urinary excretion of Mg significantly increased after the intake of supplemental Mg when K intake was the same level. The retention of Mg was 2.6 g/day in group C and 6.0 g/day in group H. The Mg accretion in the fetus was estimated at 0.18 g/day (House and Bell, 1993), which suggest that the maternal Mg retention in both groups seems to be positive during dry periods. The prepartum diet did not affect the Mg balance during early lactation except the Mg excretion into urine.

The K balance is shown in Table 9. The prepartum diet did not affect the K balance during the dry and lactation periods. House and Bell (1993) reported that K retention in the conceptus was 1.03 g/day at 280 day of lactation. The K retention was 133.7 g/day in group C and 175.4 g/day in group H. Sanchez et al. (1994) reported that heat stressed cows lost more K in sweat than those that were not heat stressed. Thus, K retention of cows in the present experiment might be overestimated by including the sweat K loss.

The plasma metabolites and urinary DPD excretion are summarized in Table 10. The concentrations of plasma NEFA in group C tended to be higher (p = 0.10) than group H during dry period. These data would support the hypotheses that the cows in group C did not have enough feed and that fat deposit were lost during the dry period. The concentrations of plasma glucose, TP and insulin were not affected by prepartum diet. The concentrations of plasma P and Mg were not affected by prepartum diet. The concentrations of plasma Ca in group C during early lactation was lower than group H, however, plasma Ca of both groups was within the normal range. The concentration of plasma OC was measured as bone formation marker (Farrugia et al., 1989; Naito et al., 1990) and urinary DPD excretion was analyzed to determine bone resorption activity (Liesegang et al., 1998). These data indicate that the prepartum diet did not have apparent effects on the marker of bone formation and resorption activity.

Nardone et al. (1997) reported that the DMI of pregnant heifers was 9.8 kg/day under thermal comfort and 8.3 kg/day under high air temperatures during the late dry period. Heat stress adversely affects DMI and this decline of DMI may cause metabolic disorders and health problem during early lactation in summer. The cows fed the control diet (group C) showed decline of DMI and seemed to be in nutrient deficiency even before parturition. On the other hand, cows fed the high concentrate diet (group H) seemed to have enough DMI and satisfy the energy, nitrogen and mineral requirement of the pregnant cows. The present study indicates that adequate feeding would prevent the decline of DMI and improve the N and major mineral balance in cows during a dry period. However, the prepartum diet did not have apparent effect on milk production at 2 weeks of lactation. Further study is needed to clarify nutritional level and feeding methods for dry cows under the heat of summer.

ACKNOWLEDGMENTS

The authors thank to the stuff of dairy farm in the National Agricultural Research Center for Kyushu Okinawa Region for technical help and assistance in sample collection. The authors greatly appreciate the assistance of Y. Sugita and K. Oyama for sample analyses.

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