# Effect of Close-up Dry Period Protein Level on Preparturiental Nitrogen Balance and Lactating Performance of Primigravid and Multiparous Holstein Cows

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**ABSTRACT :** This study compared the effects of two levels of crude protein (CP) supplementation during a close-up dry period on the productive performance, nitrogen balance, and blood profiles of Holstein cows. Eighteen cows (8 primigravid and 10 multiparous), 21 days prior to calving, were divided into four groups and fed a diet containing a low (11.5 to 12.7% CP) or high (14.1 to 15.3% CP) level of protein. Following parturition, all cows were offered the same diet. Nitrogen balance was measured 12 d to 10 d prior to the expected calving date. Whole feces and urine were collected. The higher close-up CP did not improve milk production during the early lactation period (14 w) in multiparous cows but tended to increase production by 2 kg of milk per day in primigravid cows. There were no differences in the weight of calves, the weight of the placenta, or the incidence of calving difficulty. Nitrogen intake, urine nitrogen and nitrogen retention were increased by the higher CP diet in primigravid and multiparous cows. All groups of cows showed a positive nitrogen balance. In particular, higher nitrogen retention was observed in primigravid cows fed the higher CP diet. With the exception of plasma urea nitrogen (PUN), non of the blood profiles were influenced by the CP level. The higher close-up CP increased the PUN during the period studied in primigravid and multiparous cows. These results indicate that a CP level of 14% during a close-up dry period is optimal for primigravid cows, and 12% CP level is sufficient for multiparous cows. ***(Asian-Aust. J. Anim. Sci. 2006. Vol 19, No. 6 : 831-836)***

**Key Words :** Holstein Cow, Transition Period, Protein Level, Nitrogen Balance, Milk Production, Primigravid Cow

## INTRODUCTION

The dry period, in particular the close-up period as well as the early lactation period, imposes physiological and nutritional stress on dairy cows, and dry matter intake, milk production, and the herd health of cows may be affected (Bertics, 1992; Grant and Albright, 1995; Grummer, 1995). An appropriate diet for close-up cows could reduce the risk of metabolic disorders during early lactation and improve lactation performance. Several studies have been conducted to examine the effects of dietary crude protein (CP) during the period on the health and production of postpartum dairy cows. Most studies have shown that milk yield is not influenced by the CP content of prepartum diets (Putnam, 1998; Greenfield, 2000; Moorby, 2000; Robinson, 2001). Several studies have shown that milk protein concentration (VanSaun, 1993) and milk yield have increased in primigravid cows (Santos, 2001) fed high CP diets with protein sources rich in rumen undegradable protein (RUP; 40% of CP), though this result has not been observed in the majority of studies.

In the publication Nutrient Requirements of Dairy Cattle (National Research Council, 2001), separate nutrient density guidelines were given for far-off dry cows (between 4 to 9 weeks prepartum) and close-up cows (from 3 weeks prepartum to parturition). The recommendations for CP density in diets fed to multiparous cows and primigravid cows in the period are 12.0 to 12.8% and 13.1 to 15.0%, respectively. However, the amino acid requirements for pregnancy have not been defined. Also, CP requirements for mammary growth were not included in the model. Little is known about the effect of prepartum protein nutrition on nitrogen balance (Putnam, 1998; Moorby, 2000) and performance in primigravid cows (Santos, 2001). Some data suggest that excessive feeding of CP to dairy cows results in increased fecal and urinary nitrogen excretion (Putnam, 1998), which is an environmental concern. Kaku et al. (2004) reported that livestock industry should avoid nitrogen overload to environment, especially in Japan.

Therefore, further research is required to more clearly define the protein requirements during the close-up period. The objective of this study is to compare the effects of two levels of CP diet during the close-up dry period on the milk production, nitrogen balance, and health related to parturition of Holstein cows.

## MATERIALS AND METHODS

### Animals and diets

Data from 8 primigravid Holstein cows and 10 multiparous Holstein cows kept at Ibaraki Prefecture Livestock Research Center (Ishioka, Japan) were used for the experiment. The term "primigravid" refers to cows that were pregnant for the first time and have started their first lactation, and the term "multiparous" refers to cows that

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**Table 1.** Ingredient of diets fed to Holstein cows in close-up period (% of DM)

| Composition | Far-off[1] | Close-up[2] | | Postpartum[3] |
|---|---|---|---|---|
| | | Low | High | |
| Ingredient | | | | |
| Timothy hay | 60.0 | 40.0 | 40.0 | 26.0 |
| Alfalfa hay | 30.0 | 25.0 | 25.0 | 14.0 |
| Corn | 7.5 | 26.1 | 19.3 | 30.8 |
| Barley | - | - | - | 3.9 |
| Flour | 0.2 | 0.7 | 0.7 | - |
| Wheat bran | 0.4 | 1.4 | 0.8 | 1.3 |
| Soybean hull | 0.3 | 0.9 | 0.9 | - |
| Soybean meal | 0.4 | 1.4 | 5.3 | 7.5 |
| Soy plus | 0.3 | 1.1 | 5.3 | 2.9 |
| Corn gluten meal | - | - | - | 0.9 |
| Fish meal (CP65%) | - | - | - | 1.2 |
| Molasses | 0.2 | 0.6 | 0.6 | - |
| Beet pulp | 0.5 | 1.8 | 1.8 | 3.6 |
| Cottonseed whole | - | - | - | 6.0 |
| Alfalfa meal | 0.2 | 0.7 | - | 0.9 |
| Calcium carbonate | 0.1 | 0.2 | 0.2 | 1.0 |
| Magnesium oxide | 0.1 | 0.2 | 0.2 | - |
| Mineral, vitamin premix[4] | 0.05 | 0.05 | 0.05 | 0.1 |

[1] Far-off: between 4 to 9 weeks prepartum.
[2] Close-up: from 3 weeks prepartum to parturition.
[3] Modofication of diet on the second year for primigravid cows; corn 19.8, barley 7.2, wheat bran 3.5, brewers grain (dry) 2.7, soy-plus 2.7, corn gluten meal 1.8, fish meal 0, beet pulp 7.2, calcium soaps of fatty acid 0.9, alfalfa meal 0, calcium carbonate 0.8%, respectively.
[4] Mineral and vitamin premix: vitamin A, 500,000 IU; vitamin $D_3$, 60,000 IU; vitamin E, 10 g; B-carotene 2 g; vitamin $B_1$, 12.3 g; niacinamide, 90 g; sodium choline, 230 g; Zn, 9.4 g; Cu, 1.4 g; Mn, 4 g; and filler (added to produce a total of 1 kg).

**Table 2.** Chemical composition of diets (% of DM)

| Composition | Primigravid | | | | | Multiparous | | | |
|---|---|---|---|---|---|---|---|---|---|
| | Far-off | Close-up | | Post-partum | | Far-off | Close-up | | Post-partum |
| | | Low | High | 1st Y | 2nd Y | | Low | High | |
| Chemical | | | | | | | | | |
| CP (%) | 10.3 | 11.5 | 14.1 | 15.7 | 16.0 | 12.0 | 12.7 | 15.3 | 17.0 |
| RUP (%) | 29.1 | 34.4 | 34.4 | 37.8 | 36.9 | 29.1 | 34.4 | 34.4 | 37.8 |
| EE (%) | 1.9 | 2.5 | 2.5 | 4.4 | 3.5 | 2.3 | 2.5 | 2.5 | 4.3 |
| NFC (%) | 23.6 | 34.9 | 32.1 | 39.1 | 37.6 | 22.8 | 33.5 | 29.9 | 33.5 |
| NDF (%) | 55.2 | 43.2 | 43.1 | 34.1 | 35.4 | 53.6 | 42.1 | 41.7 | 33.7 |
| TDN (%) | 62.6 | 69.2 | 69.1 | 75.5 | 75.2 | 63.4 | 69.6 | 69.5 | 75.8 |
| Ca (%) | 0.7 | 0.6 | 0.6 | 0.7 | 0.8 | 0.6 | 0.6 | 0.6 | 0.7 |
| P (%) | 0.2 | 0.3 | 0.3 | 0.4 | 0.3 | 0.2 | 0.3 | 0.3 | 0.3 |

[1] CP: crude protein; RUP: rumen degradable protein (% of CP); EE: ether extracts; NFC: nonfibrous carbohydrates; NDF: neutral detergent fiber; TDN: total digestible nutrient.
[2] NFC = OM-(CP+EE+NDF).
[3] TDN is calculated from Japanese Feeding Standard for Dairy Cattle (1999), and Standard Tables of Feed Composition in Japan (1999).

have started their second or later lactation. The treatments were characterized as low CP (11.5% for 4 primigravid cows, 12.7% for 5 multiparous cows) and high CP (14.1% for 4 primigravid cows, 15.3% for 5 multiparous cows) according to the guideline of NRC. The cows were managed in individual tie stalls and in a paddock throughout the experimental period from 9 weeks prepartum to 14 weeks postpartum.

The ingredients and chemical composition of the diets are shown in Table 1 and 2. The chemical compositions of the diets were analyzed by the Dairy Related Technical Research Institute (Nishishirakawa, Japan). In what was the second year of this study for the primigravid cows, Japan's first case of Bovine Spongiform Encephalopathy was confirmed at a dairy farm. Because of this case, the use of animal protein sources including fish meal was banned as feed for cows in Japan. Consequently, the composition of postpartum diets for primigravid cows was modified. We replaced the fish meal with plant protein so as to maintain a similar composition in the postpartum diets for primigravid cows.

The dry matter (DM), CP, neutral detergent fiber (NDF), nonfibrous carbohydrate (NFC), total digestible nutrient (TDN) and ether extract (EE) contents were determined according to the methods of AFFRCS (1999). The diets were formulated to contain either low protein or high protein content in the close-up period. Low CP diets were offered that met 100% of the CP requirements and high CP diets were offered that met 120% of the CP requirements for primigravid cows. For multiparous cows, low CP diets were offered that met 110% of the CP requirements and high CP diets were offered that met 140% of the CP requirements (AFFRCS 1999).

The diets in the far-off part of the dry period and in the postpartum period were formulated to contain the same amounts of CP, NDF, NFC, TDN, and ether extract. From 9 weeks prepartum to parturition, isocaloric feed was offered that met 100% of the TDN requirements (AFFRCS, 1999) of each cow. Daily dry matter intake (DMI) was calculated for each cow using a weekly average. Commercial mineral and vitamin premix was given to each cow in the amount of 50 g per day in the prepartum and 100 g per day in the postpartum period.

The gestation length was assumed to be 280 d. After parturition, cows were fed *ad libitum* in the total mixed rations. The amounts of feed offered and the residuals were measured daily. The body weights (BW) and body condition scores (BCS) (Ferguson, 1994) of cows were measured once weekly from 9 weeks prepartum to 14 weeks postpartum. The body weight ratio (BWR) was calculated using the percentage of basal body weight on the starting line in each of the prepartum and postpartum periods. The cows were milked twice daily, and the milk weights were recorded. Milk samples were collected each week from 1 to 14 weeks postpartum, and the milk was analyzed as a composite sample of the milk obtained at the morning and evening milkings. The milk fat and protein contents were analyzed by a Milko-Scan automated spectrophotometer (Foss Electric, Denmark).

Blood samples were collected via jugular vein at 4 h after feeding on -9 w, -1 w, 0 and 7 w relative to the day of calving. Blood was collected into evacuated tubes containing heparin. The samples were immediately cooled and centrifuged at 3,000 rpm for 20 min. Blood plasma was evaluated for glucose, triglyceride (TG) and plasma uria nitrogen (PUN) using an automated dry chemistry analyzer (Spotchem SP4430, Arkray, Japan). Nonesterified fatty acid (NEFA) was measured using diagnostic kits (NEFA C-Test Wako, Wako Pure Chemical industries, Ltd.). A calving difficulty score was recorded on a five-point scale (Van Amburgh 1998). After calving, the placentas were immediately weighed and the body weights of the calves were recorded with the amnion fluid removed. Health observations were recorded daily.

### Nitrogen balance trial

The nitrogen balance was measured on d 12 to d 10 prior to the expected calving date. After 4 days of an adaptation period, the 3 days of the collection period followed. The total amount of feces was recorded daily during the trial. The feces collected from each cow were pooled, sealed in a plastic container, and weighed. The samples were immediately subjected to nitrogen analyses, and sub-samples that were dried in a forced air drying oven at 60°C for 24 h were ground in a mill into granules up to 1mm in size to be used in chemical analyses.

The total amount of urine was collected each time a cow urinated using plastic hoses leading into plastic vessels containing 300 ml of 20% $H_2SO_4$ to prevent the volatilization of ammonia in the urinary nitrogen. The total amount of urine was weighed and filtered through paper, and samples were immediately analyzed for nitrogen. Feed samples were collected and ground through a 1 mm screen for chemical analysis. The dry matter content of the feed and feces was determined using a forced air drying oven at 135°C for 2 h. The nitrogen concentrations were analyzed by the steam distillation method using an automated nitrogen analyzer (KJEL-AUTO DPE-4, Nakayama-Rika, Japan).

### Statistical analysis

BW, feed intake, milk yield and milk composition, parturition score, and the nitrogen balance of primigravid and multiparous cows were analyzed by the least squares ANOVA using the general linear models procedure of SAS (1988). The model was as follows:

**Table 3.** Effect of close-up dry period protein level on body weight, DMI during 9 weeks prepartum

| Item | Primigravid | | | | Multiparous | | | |
|---|---|---|---|---|---|---|---|---|
| | Low | High | SE | Effect | Low | High | SE | Effect |
| | X | X | | P | X | X | | P |
| Number | 4 | 4 | | | 5 | 5 | | |
| BW (kg)[1] | 627.2 | 657.4 | 28.5 | 0.249 | 669.1 | 654.5 | 26.1 | 0.798 |
| BWR (%) | 105.6 | 106.9 | 1.4 | 0.665 | 104.2 | 103.8 | 1.2 | 0.629 |
| BCS (1-5)[2] | 3.4 | 3.6 | 0.1 | 0.154 | 3.3 | 3.3 | 0.1 | 0.942 |
| DMI (kg/d) | 10.3 | 11.0 | 0.3 | 0.029 | 9.2 | 9.1 | 0.2 | 0.843 |
| CPI (kg/d) | 1.10 | 1.27 | 0.05 | 0.001 | 1.07 | 1.15 | 0.05 | 0.035 |

[1] BW: body weight; BWR: body weight ratio; BCS: body condition score; DMI: dry matter intake; CPI: crude protein intake.

[2] The BCS were evaluated using a five-point scale where 1 = thin to 5 = fat.

$Y_{ijk} = \mu + T_i + B_k + E_{ijk}$

Where
$\mu$ = overall mean,
$T_i$ = treatment effects,
$B_k$ = block effects,
$E_{ijk}$ = residual error.
Significance was declared at p<0.05.

## RESULTS AND DISCUSSION

### Body weight, feed intake and lactation performance

No differences among treatments were observed for prepartum BW, BCS, or DMI, but differences in CP intake were observed (Table 3). Higher CP intakes were recorded in the high CP group of primigravid cows than in the low CP group, 1.27 kg vs. 1.10 kg (p<0.01) respectively, and higher intakes were also recorded in the high CP group of multiparous cows than in the low CP group, 1.15 kg vs. 1.07 kg (p<0.05) respectively. The high CP treatments resulted in higher CP intakes in primigravid and multiparous cows. The restricted offering of prepartum diets was reflected in this result.

The effect of dietary CP on the postpartum BW, feed intake and lactation performance of cows is presented in Table 4. Prepartum dietary protein levels had no significant effects on BW, BWR or BCS. The high CP treatment did not increase the postpartum DMI of multiparous cows. Putman (1998) also found that postpartum intake was unaffected by the prepartum protein intakes in multiparous cows. However, for primigravid cows in this study, the postpartum DMI was higher (p<0.01) in cows fed high CP compared to those fed low CP (19.9 vs. 17.6 kg/day).

Milk production tended to affect BWR, as evidenced by the reduction of the BW after calving. The BWR decreased 1.5% in the higher milk-yielding group. This difference was not significant. These results suggest that the mobilization of body tissue supports milk synthesis. Prepartum dietary protein levels had no significant effects on milk production in multiparous cows. Contrary to what was observed for multiparous cows, primigravid cows that were fed the high CP diet tended to produce approximately 2 kg more milk (p = 0.07) than those fed the low CP diet during the early lactation period. The increase in milk yield was probably due to the higher postpartum DMI (19.9 vs. 17.6 kg/day) in primigravid cows. Milk fat and milk protein were not affected by the prepartum treatment for multiparous and primigravid cows. Santos (2001) also found that a higher protein diet (14.7% CP) in the close-up period improved the lactation performance of primigravid cows, but this effect was not observed in multiparous cows. These results indicate that 14% CP during the close-up dry period is optimal for primigravid cows, and that 12% CP is sufficient for multiparous cows in early lactation.

**Table 4.** Effect of close-up dry period protein level on body weight, DMI, milk production during 14 weeks postpartum

| Item | Primigravid | | | | Multiparous | | | |
|---|---|---|---|---|---|---|---|---|
| | Low | High | SE | Effect | Low | High | SE | Effect |
| | X | X | | P | X | X | | P |
| Number | 4 | 4 | | | 5 | 4 | | |
| BW (kg)[1] | 565.7 | 592.8 | 14.9 | 0.252 | 628.4 | 599.0 | 9.9 | 0.094 |
| BWR (%) | 97.5 | 96.0 | 1.5 | 0.498 | 99.2 | 100.6 | 2.0 | 0.666 |
| BCS (1-5)[2] | 3.0 | 3.1 | 0.1 | 0.773 | 3.0 | 2.9 | 0.1 | 0.505 |
| DMI (kg/d) | 17.6 | 19.9 | 0.4 | 0.007 | 23.1 | 22.4 | 0.5 | 0.408 |
| Milk (kg/d) | 32.0 | 34.3 | 0.7 | 0.074 | 38.4 | 35.8 | 2.6 | 0.495 |
| Fat (%) | 3.95 | 3.89 | 0.1 | 0.627 | 3.88 | 3.99 | 0.2 | 0.623 |
| Protein (%) | 3.15 | 3.13 | 0.1 | 0.773 | 3.16 | 3.29 | 0.1 | 0.339 |
| MU (mg/dl) | 31.5 | 31.8 | 1.2 | 0.882 | 33.9 | 29.7 | 2.2 | 0.192 |

[1] BW: body weight; BWR: body weight ratio; BCS: body condition score; DMI: dry matter intake; MU: milk urea.
[2] The BCS were evaluated using a five-point scale where 1 = thin to 5 = fat.

**Table 5.** Effect of close-up dry period protein level on nitrogen balance on prepartum

| Item | Primigravid | | | | Multiparous | | | |
|---|---|---|---|---|---|---|---|---|
| | Low | High | SE | Effect | Low | High | SE | Effect |
| | X | X | | P | X | X | | P |
| Number | 3 | 4 | | | 5 | 5 | | |
| N intake (g/d)[1] | 184.5 | 261.7 | 14.9 | 0.007 | 178.7 | 220.3 | 3.8 | 0.0001 |
| Urine N (g/d) | 76.8 | 100.5 | 0.1 | 0.248 | 88.3 | 120.2 | 2.2 | 0.0001 |
| Fecal N (g/d) | 66.9 | 64.5 | 0.4 | 0.730 | 60.1 | 62.1 | 2.4 | 0.585 |
| N digestibility (%) | 64.2 | 75.2 | 0.7 | 0.004 | 66.5 | 71.9 | 1.0 | 0.007 |
| TNO (g/d) | 143.7 | 164.9 | 0.1 | 0.279 | 148.4 | 182.3 | 3.3 | 0.0002 |
| N retention (g/d) | 40.7 | 96.8 | 0.1 | 0.017 | 30.3 | 38.0 | 3.4 | 0.150 |

[1] N: nitrogen; TNO: total nitrogen output.

**Table 6.** Effect of close-up dry period protein level on parturition difficulty, calf body weight and placenta weight

| Item | Primigravid | | | | Multiparous | | | |
|---|---|---|---|---|---|---|---|---|
| | Low | High | SE | Effect | Low | High | SE | Effect |
| | X | X | | P | X | X | | P |
| Number | 4 | 4 | | | 5 | 5 | | |
| Parturition score[1] | 1.25 | 1.75 | 0.22 | 0.175 | 1.18 | 0.98 | 0.15 | 0.361 |
| Placenta weight (kg) | 5.79 | 5.46 | 1.5 | 0.882 | 3.57 | 5.34 | 0.5 | 0.155 |
| Calf BW (kg) | 42.0 | 43.0 | 1.9 | 0.719 | 42.6 | 41.5 | 2.4 | 0.755 |

[1] Parturition scores, 1 = no assistance, 2 = minor assistance, 3 = rigorous calf manipulation, 4 = extreme difficulty, 5 = cesarean or dead calf.

### Nitrogen balance

The effect of dietary CP on the nitrogen balance of cows is presented in Table 5. The nitrogen intake increased as the protein concentration in the diet increased (p<0.01). Fecal nitrogen excretion was not affected by the treatment. The fecal nitrogen excretion was attenuated by the improvement in nitrogen digestibility as the nitrogen intake increased.

The urine nitrogen excretion was higher in the high CP diet in multiparous cows (p<0.001). However, no differences between treatments were observed in urine nitrogen excretion in primigravid cows. All cows were in a positive nitrogen balance. Nitrogen retention was 30.3 g for multiparous cows fed low CP diet compared to 38.0 g for those fed the high CP diets. This difference was not significant. In contrast, nitrogen retention was 40.7 g for primigravid cows fed low CP diet compared to 96.8 g for those fed the high CP diet (p<0.05). Putnam (1998) found that the positive nitrogen balance on the dry period did not affect milk production in multiparous cows. Kojima (2005) reported that the dietary nutrient levels that decrease nutrient excretion without adverse effects on milk production should be investigated. Thus, the high (15.3%) CP was excessive for multiparous cows, and the excessive nitrogen was excreted into the urine. In contrast, the high (14.1%) CP was not excessive for primigravid cows. This differences between the two groups of cows showed that primigravid cows may require an additional amount of protein for their growth, and for the accretion of their gravid uterine and mammary gland.

Tamminga (1996) showed that excessive CP produces environmental waste including ammonia lost into the air and nitrate contamination of the surface and ground water. Thus, the dairy cow diets during the dry period as well as the lactation period should be formulated to optimize the CP content for requirements and for efficiency of nitrogen utilization.

### Health and blood profiles around parturition

The effect of dietary CP on the parturition score, calf body weight and placenta weight is presented in Table 6. The average calf weight at birth and placenta weight were unaffected by the treatment. Bell (1995) reported that the nutrient requirements of the growing fetus are a high priority for the mother’s body, and that maternal tissues are repartitioned to serve the fetus and mammogenesis if the dietary supply of nutrients is limited. In this study, the prepartum BW, BWR and BCS were unaffected by the treatments, and prepartum BCS loss was not observed. Thus the low CP diet might not be insufficient for the cows. No incidence of metabolic disorders was observed. One multiparous cow that had been fed low CP diets experienced retained placenta.

No differences among treatments were observed in prepartum and postpartum blood glucose, TG, or NEFA, though a difference was observed in PUN. The PUN concentration in the close-up period increased as the protein concentration in the diets increased (p<0.01) in multiparous cows. The observation that there were higher prepartum PUN concentrations in cows fed high CP diets than in cows fed low CP diets was similar to the findings of Greenfield (2000) and Doepel (2002). This finding showed that the effects of CP level on the blood profiles were expressed in part through changes in the concentration of the PUN. The PUN concentrations for primigravid cows tended to increase as a result of the high CP diet in the period studied. However, this difference was not significant. These findings showed that the concentration of PUN closely matched urinary nitrogen excretion and nitrogen retention in the nitrogen trial.

Primigravid cows have lower DMI, additional CP and energy requirements for growth; therefore, we can conclude that 14% CP during the close-up dry period is optimal for primigravid cows, and that 12% CP is sufficient for multiparous cows. Further research is required to more clearly define the protein and amino acid requirements during the prepartum transition period.

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