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Plasma 3-Methylhistidine Concentration in Peripartum Dairy Cows Given Diets with Two Protein Levels

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Abstract: In this study, plasma 3-Methylhistidine (3-MH) and other Amino Acids (AA) were measured in high yielding dairy cows between 1 week prepartum and 4 weeks postpartum to evaluate the effect of different protein levels in a postpartum diet on milk performance, metabolites and feed intake. Eleven multiparous cows were used in the study and all received the same prepartum diet. Postpartum, they were divided into two groups; five cows received a diet of 19% CP (CP19) and six cows received a diet of 17% CP (CP17). Plasma 3-MH and also other AA concentrations were significantly lower in the CP19 cows than in the CP17 cows. The peak plasma 3-MH concentration postpartum in CP17 and CP19 were 15.5 and 9.9 μM, respectively while the mean plasma 3-MH concentration throughout the trial in CP17 and CP19 were 11.7 and 7.6 μM, respectively. Milk yield and composition tended to improve in CP19 cows, however, these were not significant. Blood Urea Nitrogen (BUN) was significantly higher in CP19 than that in CP17 (14.3 vs. 9.9 mg dL^{-1}, respectively). The kinetics of plasma 3-MH were the same as a earlier study where it increased after parturition for 1 week and then progressively decreased until 4 weeks postpartum. These results indicate that muscle protein mobilization can be lowered by protein in the diet as evidenced by plasma 3-MH. Lower AA might be an indication of their efficient utilization from the diet, so that an increased consumption of AA for milk production is reflected in lower AA levels in blood. This study suggested that even for cows receiving 19% CP in early lactation, if the AA utilization into milk protein is efficient, BUN can be kept within a normal range. In conclusion, plasma 3-MH is a sensitive and useful index for protein nutrition in early lactation and a 19% CP diet might be effective in preventing body protein degradation as evidenced by plasma 3-MH.

Key words: 3-methylhistidine, transition, myofibrillar protein, protein, diet

INTRODUCTION

It is generally accepted that plasma 3-Methylhistidine (3-MH) concentration increases transiently during the 1st week after parturition (Blum *et al*., 1985; Doepel *et al*., 2002; Akamatsu *et al*., 2007). This increase is an indication of the mobilization of body protein to compensate for the rapid increase of milk protein demand after parturition which is not met due to lower feed intake (Bell, 1995) because 3-MH is released particularly from the breakdown of skeleton muscle and not reutilized in protein synthesis (Nishizawa *et al*., 1979; Harris and Milne, 1981). After peaking during the 1st week postpartum, it decreases gradually until 4 weeks postpartum due to the recovery of appetite and feed intake. It is known that excessive body protein mobilization often causes metabolic disorders and reproductive problems in dairy cows, so researchers hypothesized that lower peak 3-MH levels might better express the condition of dairy cows (Butler and Smith, 1989; Komaragiri and Erdman, 1997; Staples *et al*., 1990).

On the other hand, plasma 3-MH might be more sensitive to accurately reflect protein metabolite status than urinary 3-MH in cattle and other animals (Rathmacher and Nissen, 1998; Yamashita, 2007). Phillips *et al*. (2003) reported that the plasma 3-MH kinetics after parturition differed based on the prepartum protein level; the peak plasma 3-MH concentration was significantly higher in a prepartum diet of 11% CP than 14% CP. Moreover, Doepel *et al*. (2002) reported that a prepartum protein level of 17% CP produced a lower prepartum plasma 3-MH concentration than 12.5% CP. In addition, plasma 3-MH concentration is also affected by its sensitivity to somatotropin, monensin, energy booster, feed intake and heat stress (Ndibualonji *et al*., 1997;

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Vallimont *et al*., 2001; Kamiya *et al*., 2006; Chibisa *et al*., 2008). In the earlier study (Sawada *et al*., 2012), researchers showed that plasma 3-MH concentration is also affected by forage intake. Thus, based on these findings it was considered that plasma 3-MH is a simple and sensitive index for protein metabolites, especially in early lactating dairy cows. To the knowledge, there have been no studies on how postpartum protein level affects plasma 3-MH concentration.

In general, 17% CP in early lactation diets is a widely accepted protein level for dairy cows. This is because numerous studies have shown that excess protein from the diet has negative effects on fertility and that over 19% CP in the diet lowered the conception rate (Jordan and Swanson, 1979; Bruckental *et al*., 1989; Canfield *et al*., 1990; McCormick *et al*., 1999). These studies showed that Urea Nitrogen in the Blood (BUN) increased from excess protein in the diet which negatively affected the reproductive tissues.

The hypothesis was that during the plasma 3-MH increase after parturition and within 4 weeks postpartum, cows require more protein than current standard protein level of 17% CP and it might even be possible to reduce the plasma 3-MH level. The aim of this study was to investigate whether plasma 3-MH used as an index to expresses body protein mobilization after parturition, could be lowered by modifying the feed protein level. Thus, researchers designed the experiments using two protein levels in the postpartum diet (17 vs. 19% CP) in early lactating dairy cows to investigate plasma 3-MH kinetics with other AA and BUN concentrations.

MATERIALS AND METHODS

Animals and treatments: The experimental procedures used here complied with the Guide for the Care and Use of Agricultural Animals of the Kagoshima Prefectural Institute for Agricultural Development. Eleven multiparous early-lactation Holstein dairy cows were housed in individual tie stalls with free access to water at the Dairy Department of the Kagoshima Prefectural Institute for Agricultural Development. These animals calved between August and December. Six cows were assigned to a control group (CP17) receiving 17% CP in the diet and five cows were assigned to a high protein group (CP19) receiving 19% CP in the diet. The mean parity and body weight at initiation of the experiments in CP17 and CP19 cows were 4.3±1.9 vs. 3.2±2.2 and 735±90.8 versus 694.4±44.5 kg, respectively. Milking was performed twice daily (0900 and 1530 h). Cows were fed diets (for dry cows and lactating cows (Table 1)) as a Total Mix Ration (TMR) given twice daily at 0900 and 1600 h before calving and *ad libitum* once at 0900 h after calving and formulated to meet the recommendations of the National Research Council (NRC, 2001). The two experimental diets supplied the same levels of energy, NDF and minerals and vitamins and varied only in protein level.

Table 1: Ingredient and nutrient composition of prepartum and postpartum dietary treatments (DM basis)

		Postpartum	
Composition	Prepartum	CP17	CP19
Ingredient composition (%)			
Corn silage	34.0	22.0	16.5
Grass silage (Italian)	11.0	4.5	8.0
Alfalfa meal (dehy)	11.0	9.0	9.0
Beat pulp	8.0	3.0	3.0
Barley	-	7.0	7.0
Corn	15.0	15.0	8.4
Rice bran	11.3	10.0	13.5
Soy bean meal	4.3	9.0	14.6
Cotton seed meal	-	14.0	7.5
Soy bean hull	-	4.0	8.0
Tofu meal mix (dry)*	(10)	(12)	(18)
Fat calcium salt	-	-	1.95
Vitamin/Mineralmix[1]	5.4	2.5	2.5
Nutrient composition			
ME (Mcal kg^{-1})	2.5	2.6	2.6
CP (%)	13.0	17.1	19.0
RUP (%)[2]	4.4	5.9	6.7
NDF (%)[3]	37.0	35.0	35.0
Forage NDF (%)[4]	22.6	12.9	12.7
F:C[5]	56:44	35:65	35:65

*The three cows in each group received this by-products the amount of the number in parentheses with reducing soy bean meal, corn, rice bran, cotton seed meal and soy bean hull to meat same nutrient composition; [1]Vitamin/mineral mixes contain vitamin ADE, Dicalcium phosphate and Limestone at the level of NRC (2001) recommendation and contain Magneciunsalfate prepartum diet; [2]Ruminaly undegradable protein; [3]Neutral detergent fibers; [4]NDF from forage; [5]Forage concentrate ratio in feed

Sample analysis: Feed samples were taken before the start of treatment from each cow and were oven-dried to determine Dry Matter (DM) according to the Japanese Feeding Standard for Dairy Cattle. CP was measured by the Kjeldahl Method. Acid Detergent Fiber (ADF) and Neutral Detergent Fiber (NDF) were assayed according to the JFS which are the same as the methods described by Van Soest (1963) and Van Soest and Wine (1967). Feed refusals were weighed individually every morning thereby allowing calculation of Dry Matter Intake (DMI) per cow. Blood samples were collected via the jugular vein before feeding and milking at 0900 h at weeks -1, 0, 0.2, 1 and 4, relative to calving. Blood was collected into evacuated tubes containing sodium heparin. BUN was determined immediately after taking blood on an automated clinical chemistry analyzer (FUJI DRI-CHEM 3030; FUJIFILM Medical K.K., Tokyo, Japan). Blood samples were centrifuged at 3,000 g for 20 min at 4°C and stored at -80°C until analysis. Before analyzing 3-MH and Adeproteinized with one volume of a 10% solution of sulfosalicylic acid,

separated by centrifugation at 8,000 g for 15 min at 4°C and the supernatants were then passed through 0.45 μm membrane filters and processed through an automated amino acid analyzer. Analyses of 3-MH and AA in plasma were performed on a Hitachi Industries Ltd. model L-8800, automated AA analyzer (Hitachi Industries Ltd. Tokyo, Japan). Samples from two cows in the LF group were measured on a JOEL Ltd. model JLC-500/V2, automated AA analyzer (JOEL Ltd., Tokyo, Japan). Analysis of Nonesterified Fatty Acids (NEFA) was performed on an automated blood analyzer (Model 7020; Hitachi Medico K.K., Tokyo, Japan).

During the 1st 28 days of lactation, the milk yield was electronically recorded daily with a milk meter (Milcon MC-6-B; Orion Machinery Co., Ltd. Nagano, Japan). Milk samples were taken at milking time, twice a day and their compositions were immediately determined with an analyzer (MilcoScan FT120; Foss Japan K.K., Tokyo, Japan).

Statistical analysis: Data on 3-MH, AA and metabolites between CP17 and CP19-group cows were compared by repeated measures using ANOVA with the MIXED procedure of the JMP Software package (Version 7; SAS Institute, Tokyo, Japan). When there were no interactions, the mean values of both groups were analyzed by the Tukey-Kramer multiple comparison to see the effect of parturition. Milk and milk protein yield during the experimental period were analyzed by the Student's t-test. Results are expressed as the mean±Standard Deviation (SD). Significance was declared at $p<0.05$.

RESULTS AND DISCUSSION

Plasma amino acids and metabolites: The mean plasma concentrations of AA, 3-MH and BUN from CP17 and CP19-group cows are shown in Table 2. There were no interactions between groups or weeks in all plasma AA, BUN and 3-MH ($p<0.05$), showing that the dynamics of both groups were the same. Furthermore, the CP19 diet significantly lowered all plasma AA and 3-MH levels compared to that of the CP17 diet and BUN was inversely higher. When both groups are taken together, the amino acids threonine, valine, alanine and glycine were affected by parturition significantly where lysine, threonine, valine and glycine appear to decrease before parturition and increase after parturition significantly. The amino acids histidine, leucine, methionine, phenylalanine and alanine remained at the same level throughout the trial and only alanine increased significantly from 1-4 weeks after parturition.

The mean plasma 3-MH change was similar to the earlier study (Sawada *et al*., 2012) increasing after parturition and reaching its peak level at 0.2 weeks postpartum (15.5 μM for CP17 and 9.9 μM for CP19) then decreasing until 4 weeks postpartum to nearly the same level at prepartum (5.4 μM for CP17 and 5.7 μM for CP19). These values were calculated by the loss in body weight of CP17 and CP19 cows (61.3±23.3 vs. 26.6±36.5 kg day^{-1}, respectively, $p = 0.088$). The loss of body weight in CP19 cows was much lower than that of CP17 cows, even though it was not significant.

The mean plasma BUN significantly increased after parturition and the total mean values were 14.3 mg dL^{-1} for CP19 and 9.9 mg dL^{-1} for CP17.

Milk performance and DMI: Total milk yield, mean daily milk, protein yield, fat yield and DMI during the experimental period are presented in Table 3. The DMI from days 0-28 after calving for the CP17 and CP19 groups did not differ (14.0±4.0 vs. 16.8±2.3 kg day^{-1}). The milk, milk protein and fat yield from days 1-28 after calving increased in the CP19 group, however, there were no significant differences (28.0±8.8 vs. 31.5±4.9, 0.93±0.25 vs. 1.12±0.20, 1.34±0.4 vs. 1.45±0.43 kg day^{-1}, respectively).

Plasma 3-MH was significantly lower in the CP19 diet indicating that body protein mobilizations were lowered by the added protein in the postpartum diet. In fact, body weight loss after parturition was lower in CP19 than that in CP17. Higher milk and milk protein yield in CP19, although, it is not significant, suggested that there were sufficient AA for milk production and mammary uptake confirmed by the lower AA which indicates AA consumption. Taken together, it was considered that the AA utilization for milk in this stage was very efficient in the CP19 group. Earlier studies investigating body weight changes from dietary protein sources were limited. Principally, body proteins are mobilized to support the synthesis of milk protein in early lactation of high producing dairy cows (NRC, 1989). Komaragiri and Erdman reported in 1997 on the effect of dietary protein on mobilization of body protein using high protein diets (19% CP). And they used 16% CP and 19% CP diets which contained 6 and 9% Ruminally Undegradable Protein (RUP) (DM basis) from 2 weeks before calving to 12 weeks after calving. The results in body protein mobilization showed no change in either diet and they concluded that tissue mobilization in early lactation is not affected by changes in dietary RUP under adequate feeding conditions. This result was reached without measuring 3-MH but by measuring body condition score, empty body protein, empty body energy and empty body fat. Researchers can not compare the results because of

Table 2: Plasma amino acids concentrations, BUN and 3-MH in CP17 and CP19 groups

		Weeks							p-value		
AA (μM)[1]	Groups	-1	0	0.2	1	4	Mean	RMS[2]	Diet (D)	Time (T)	D×T
His	CP17	63.40	58.90	64.90	61.70	59.60	61.8[x]	9.530	<0.0001	0.4660	0.9970
	CP19	25.10	20.00	28.50	22.80	22.80	23.8[y]				
Leu	CP17	149.70	141.40	154.50	161.50	130.70	147.6[x]	30.800	0.0002	0.9170	0.5300
	CP19	57.70	46.80	52.50	47.00	63.80	53.6[y]				
Lys	CP17	63.40	51.40	72.90	79.80	77.60	69.0[x]	14.180	0.001	0.2960	0.2790
	CP19	28.50	18.60	23.50	23.50	32.30	25.3[y]				
Met	CP17	38.70	30.60	29.40	31.30	36.30	33.3[x]	7.600	<0.0001	0.4390	0.6310
	CP19	9.01	7.70	9.50	7.50	9.70	8.7[y]				
Phe	CP17	63.30	60.10	68.10	70.50	57.90	64.0[x]	9.370	<0.0001	0.5820	0.3030
	CP19	21.70	17.20	18.80	18.90	22.80	19.9[y]				
Thr	CP17	52.70	28.00	44.80	59.60	99.30	56.9[x]	17.880	0.004	<0.0001	0.0670
	CP19	25.60	18.20	29.00	35.00	45.70	30.7[y]				
Val	CP17	217.50	196.00	264.00	268.80	251.80	239.6[x]	46.850	0.0004	0.0302	0.5236
	CP19	92.30	60.10	90.20	92.50	126.20	92.2[y]				
Ala	CP17	203.80	182.40	140.60	158.50	245.60	189.6[x]	36.180	<0.0001	0.001	0.1615
	CP19	93.60	85.50	71.40	81.90	103.80	87.2[y]				
Gly	CP17	548.80	377.20	451.70	596.60	470.90	489.1[x]	103.050	0.0001	0.0237	0.2612
	CP19	171.80	174.30	230.20	266.00	202.10	208.9[y]				
Metabolites BUN	CP17	6.38	8.15	11.55	11.73	11.63	9.9[y]	2.487	0.0165	<0.0010	0.1156
(mg dL^{-1})	CP19	8.90	9.94	18.26	17.24	17.20	14.3[x]				
3-MH (μM)	CP17	10.90	12.80	15.50	13.93	5.40	11.7[x]	2.860	0.0377	<0.00010	0.06180
	CP19	7.00	8.60	9.90	7.00	5.70	7.6[y]				

Means in the same row having different superscripts are significantly different ($p<0.05$); [1]AA = Amino Acid, [2]RMS = Roots Mean Square

Table 3: Effect of CP level on total milk yield, average of milk yield and milk protein, DMI and body weight during the experimental period

Items	CP17 (n = 6)	CP19 (n = 5)	p-value
Total milk yield (kg)	813.2±255.1	915.0±142.5	0.449
Average daily milk yeid (kg day^{-1})	28.0±8.800	31.5±4.900	0.449
Average daily protein yield (kg day^{-1})	0.93±0.25	1.12±0.20	0.215
Average daily fat yield (kg day^{-1})	1.34±0.40	1.45±0.43	0.682
Caving body weight	666.2±85.10	651.0±48.30	0.733
Final body weight	604.9±103.8	624.4±23.80	0.693
Body weight loss (kg day^{-1})	61.3±23.30	26.6±36.50	0.088
DMI (kg day^{-1})	14.0±4.000	16.8±2.300	0.202

Values are expressed by averages±standard deviation

the different index of body protein mobilization but the cows fed 19% CP in his study produced 42 kg of milk which was 10 kg higher than the cows fed 19% CP in the experiment. Thus, researchers can imagine if he had determined plasma 3-MH also, it could also have been higher than the values. To the best of the knowledge, the experiment is the first examination of the effect of a 19% CP postpartum diet on lowering plasma 3-MH in early lactation.

This experiment showed that even when cows receive a high protein (19% CP) diet, protein utilization for milk became more efficient and BUN did not increase to the level considered to adversely affect fertility. Unfortunately, in this study, reproductive performance was not measured; therefore, it is not clear whether CP19 cows were estrus cycling better than CP17 cows. However, the normal ranged BUN and less body weight changes all indicated that CP19 cows would have few problems for reproduction. The protein level of 19% CP is higher than the JFS, however, it is not very high compared to the requirement table described in NRC (2001).

Numerous researchers reported that diets >17% CP negatively influenced fertility because BUN values greater than 20 mg dL^{-1} directly influenced the reproductive tissues. However, in this study although BUN was higher in CP19, it was still within the normal range around 14 mg dL^{-1} and this level are consider to have any adverse affect on fertility.

Although, most studies have indicated an adverse effect on reproductive performance from feeding high CP diets, others indicate no effect of diet CP on fertility. Carroll *et al.* (1988) observed no differences in pregnancy rate or first service conception rates of dairy cows fed 20 or 13% CP diets. Howard *et al.* (1987) also reported no difference in fertility between multiparous cows fed 15 or 20% CP diets.

There are two main theories why excess dietary protein decreases reproductive performance. The first theory is related to the energy costs associated with metabolism disposal of excess Nitrogen (N). In early lactation, energy is closely associated with ovulation and progesterone concentration (Beam and Butler, 1997; Butler, 1998). The second theory is that BUN

concentration could have a toxic effect on sperm, ova or embryos, resulting in a decrease in fertility (Canfield *et al*., 1990). High BUN may also reduce the binding of leutinizing hormone in serum progesterone concentration and fertility (Barton *et al*., 1996). In the reviews of Laven and Drew (1999) although a high protein intake has been postulated to have an effect on fertility for over 30 years, the evidence still remains inconclusive and the aetiology and pathogenesis of the effect remains obscure. Taken together, there is no conclusive evidence relating high protein and lower fertility.

In early lactation of high producing dairy cows, mobilized body protein contributes a significant amount of milk protein, however, excessive mobilization can lead to health problems such as ketosis (Akamatsu *et al*., 2007) and poor reproductive performance (Butler and Smith, 1989; Staples *et al*., 1990). Akamatsu measured plasma 3-MH of healthy and ketosis cows and found it was higher in ketosis cows. Bulter and Staples showed only body weight changes after parturition but it was significantly related to poor first ovulation or conception rate and large body weight loss. Thus, reducing body protein mobilization in early lactation is very important for dairy cow management and until DM intake reaches a certain amount, cows may need more protein their diet.

CONCLUSION

Dietary CP level in early lactation may control body protein mobilization and we have shown that feed containing CP 19% can reduce the muscle protein mobilization within 4 weeks postpartum. Plasma 3-MH concentration is a sensitive index for protein metabolites in early lactation and a 19% CP diet might be effective to suppress body protein degradation as evidenced by 3-MH in plasma.

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