Growth, Nutrient Utilization and Amino Acid Digestibility of Dairy Calves Fed Milk Replacers Containing Different Amounts of Protein in the Preruminant Period*

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ABSTRACT : This study was designed to examine the effects of different dietary protein levels on growth performance, nutrient utilization, amino acid (AA) digestibility and serum biochemical parameters of preruminant dairy calves. Fifteen healthy new-born calves were randomly allotted to three experimental groups and fed with different milk replacer that contained 18% (LP), 22% (MP) or 26% (HP) of protein. The results showed that final BW, net gain and ADG were significantly higher in the MP group than in LP and HP groups ($p<0.05$). In addition, the apparent digestibility of CP in the MP group was significantly higher than in the other two groups ($p<0.05$). The values of N intake and fecal N excretion were significantly increased following the increase of dietary protein content ($p<0.05$). However, in all three groups of animals, dietary protein content had no significant effect on urinary N concentration ($p>0.05$). BUN concentration, on the other hand, decreased as calves grew but increased following the increase of dietary CP content. Furthermore, no significant differences in digestibility of amino acids were observed among these three groups of animals ($p>0.05$). We concluded that calves fed with milk replacer containing 22% of protein had better growth performance and nutrient utilization as compared to animals treated with milk replacer containing either 18% or 26% of protein. (**Key Words :** Calves, Dietary Protein Level, Amino Acid, Digestibility, Growth)

INTRODUCTION

One of the major challenges of rearing preruminant calves on the dairy farm is to reduce feed costs (Babu et al., 2003). One effective strategy is using milk replacer (MR) instead of whole milk for young calves. Protein is the most expensive and key component in formulating rations for animals. However, the optimum level of protein in the diet remains to be determined. The level of protein in the diet of calves is critical, because excessively low or high protein content can cause animal production, welfare and environmental problems. Indeed, several researchers have stated the absolute need to accurately define the protein requirement for dairy calves as well as the optimal protein level in milk replacer (Blome et al., 2003; Lohakare et al., 2006).

The Nutrient Requirements of Dairy Cattle, established by NRC (2001), is the widely accepted feeding standard.

However, this system was established based upon limited earlier body composition data of young calves that may not reflect the composition of current Holstein calves (Bartlett et al., 2006). Because of the differences between dairy farms (e.g., environment, management, feedstuff conditions, etc), this system may not be suitable to all countries. Most importantly, those previous studies were based upon a supplement of animal proteins but not vegetable proteins; therefore, it does not reflect the function of current milk replacers supplied with vegetable proteins.

Previous studies on calves fed with different levels of dietary protein mainly emphasized growth performance (Donnelly and Hutton, 1976a; Blome et al., 2003), body composition (Donnelly and Hutton, 1976b; Jagusch et al., 1970; Bartlett et al., 2006) and health (Nyachoti et al., 2006; Quigley et al., 2006). These studies lack unanimity on optimum dietary CP content (Lohakare et al., 2006). In the current study, we performed a complete panel of experiments to determine the influence of different protein contents of milk replacers on growth performance, nutrient utilization, amino acid (AA) digestibility and blood metabolism in preruminant calves.

Table 1. Ingredient and chemical composition of diets

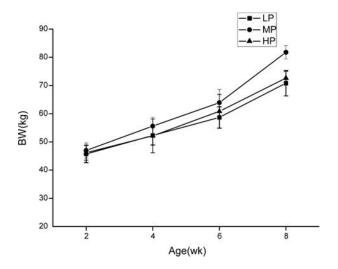| Nutrients	Milk replacer			Concentrate feed	Hay
	LP	MP	HP		
Ingredient composition (% as fed basis)					
Soy protein concentrate	33.0	43.5	55.5	-	-
Whey powder	40.0	30.0	21.8	-	-
Skim milk	6.5	8.3	7.0	-	-
Fat	10.5	8.2	5.7	-	-
Compound-premix	10.0	10.0	10.0	-	-
Calculated composition					
ME (MJ/kg)	17.8	17.8	17.8	-	-
CP (%)	18.1	22.1	26.0	-	-
Analyzed composition (%)					
DM	97.4	97.2	96.8	87.4	91.6
CP	17.2	21.4	25.8	21.8	6.6
EE	15.0	16.1	17.0	1.7	3.6
Ash	8.2	7.7	7.5	6.8	4.6
Ca	1.5	1.5	1.5	0.6	0.4
P	0.7	0.7	0.7	0.7	0.2
AA composition (% as DM basis)					
Asp	1.8	2.2	2.7	2.2	0.6
Thr	0.7	0.8	1.0	0.8	0.3
Ser	0.8	1.0	1.2	1.1	0.3
Glu	3.0	3.7	4.5	4.2	0.7
Gly	0.6	0.7	0.9	0.9	0.3
Ala	0.7	0.8	1.0	1.1	0.4
Val	0.8	1.0	1.2	1.0	0.4
Ile	0.8	0.9	1.1	0.9	0.3
Leu	1.3	1.5	1.9	1.8	0.5
Tyr	0.6	0.7	0.9	0.7	0.2
Phe	0.8	0.9	1.2	1.0	0.3
His	0.6	0.7	0.8	0.7	0.3
Lys	1.5	1.5	2.1	1.2	0.3
Arg	0.3	0.4	0.4	1.5	0.3
Pro	1.2	1.5	1.8	1.2	0.4
Cys	0.3	0.4	0.5	0.4	0.1
Met	0.5	0.7	1.0	0.3	0.1
Trp	0.9	1.0	1.3	0.2	0.2
Total	16.9	20.3	25.3	21.2	5.7

MATERIALS AND METHODS

Animals, diets, and management

Fifteen healthy new born Holstein calves were used to evaluate growth performance, nutrient utilization and AA digestibility of preruminant calves less than 8 weeks age. All the animals received 3 kg of colostrum within 12 h after birth, and were fed whole milk with nipple buckets at a rate of 8% body weight (BW) during the first week. The calves were stratified on bodyweight and then randomly divided into three groups of five animals. All the groups had an adaptation period over days 8-14 when they were fed mixed milk (milk replacer and whole milk), with the ratio of milk replacer to whole milk increased from 1:3 to 3:1 gradually during this period. From day 15 to 56, each group was fed one of the three milk replacers with different protein levels.

The milk replacers contained 18% (low protein, LP),

22% (medium protein, MP), and 26% (high protein, HP) of crude protein (CP), and were reconstituted to 12.5% solid content and fed at 10% of BW daily; feeding was allocated across three times at 8:30, 14:00, and 20:30 and the amount fed to each calf was adjusted with growth. Milk replacers were formulated to be isocaloric and were based on soy protein concentrate, whey powder, skim milk, fat and compound premix, but did not contain antibiotics. Nutrient composition of the milk replacers is shown in Table 1. Milk replacers were sampled weekly and pooled by group of calves. The calves were weighed twice weekly in the morning before feeding in order to assess changes in BW and average daily gain (ADG).

Concentrate feed was offered when calves were 3 weeks of age, and 300 g, 500 g, or 1,000 g concentrate feed was offered tor each calf per day during weeks 3-4, 5-6 or 7-8, respectively, and any residue was recorded daily. Hay was

Figure 1. Effects of different protein content in milk replacers on BW of calves. Three groups were labeled by LP (■), MP (●) and HP (▲) respectively.

offered *ad libitum* and intake was recorded daily. Fresh water was provided to all the calves once each morning and afternoon throughout the study.

Each group of calves was housed individually in a well ventilated, dry-floored shed (5×5 m² large) before the metabolism test. The sheds were cleaned regularly and disinfected fortnightly. Calf health was monitored daily, fecal score was recorded using the following system: 0 = dry, hard; 1 = soft, formed; 2 = pudding-like; 3 = runny; and 4 = liquid, splatters.

Digestibility study

To obtain an accurate measure of the utilization of the diets throughout the study period, a metabolism trial of 10 d duration was conducted on all the experimental calves from day 46 to 56. During this period, all the calves were held individually in special metabolism stalls. Samples were not collected in the first 5-d to ensure the calves adapted to the environment. Collections of feces and urine of each calf were conducted during the last 5-d.

Total feces of each calf was collected and weighed twice daily (9:00 and 16:00), and after thorough and uniform mixing in a clean plastic basin, representative samples were taken and mixed with a few ml of 10% hydrochloric acid solution, then stored at -20°C immediately. The feces samples from each calf were pooled over the 5-d collection period and dried at 65°C for 48 h. The pooled sample was sub-sampled for analysis of dry matter (DM), nitrogen (N), ether extract (EE), ash, calcium (Ca) and phosphorus (P) (AOAC, 1990), and AA.

Total urine was collected in a plastic bucket placed under the metabolism stall and measured twice a day at the same time as feces. 1% of total urine was collected and filtered through two-double pledgets into plastic bottles containing a few ml of 10% hydrochloric acid solution to maintain pH below 3. Samples were pooled for each calf after the collection period and stored at -20°C until analysis for N, Ca and P.

Samples of milk replacer, dry feed and hay were collected daily in the morning during the collection period, pooled over the 5 d of collection, then ground to pass through a 2 mm sieve and stored in plastic bags at -20°C. All the diet samples were assayed for DM, N, EE, ash, Ca, P and AA.

Blood sample collection

Blood was sampled fortnightly before the morning feeding from each calf by jugular venipuncture into separate evacuated tubes and centrifuged within 1 h at 3,000 rpm for 20 min at room temperature to obtain the serum. Serum was frozen at -20°C and used to determine concentrations of urea N (BUN), total protein (TP), albumin (ALB) and globulin (GLOB).

Statistical analyses

The growth (Initial BW, Final BW, Net Gain and ADG), digestion (Nutrients and AA digestibility) and utilization (N, Ca and P retention) data were analyzed using the one-way ANOVA procedure of SPSS 13.0. The fortnightly body weight and the blood variables (BUN, TP, ALB, GLOB concentrations and A/G ratios) were analyzed using the general linear models procedure (GLM) of SPSS 13.0. Differences were identified using the Duncan's multiple range test (Duncan, 1955). Significance was declared at $p < 0.05$ for all comparisons and the results were presented as mean values and standard deviation (SD).

RESULTS AND DISCUSSION

Growth performance

Initial BW did not differ among the three groups. Fortnightly BW gain of all calves increased from 2 to 8 wk (Figure 1). The highest values of final BW, gain and ADG were observed in the MP group ($p < 0.05$) with average values of BW gain and ADG which reached 34.8 kg and 829 g/d, respectively, throughout the study. From the 2nd to the 4th wk, the changes in body weight gain were similar between the HP and the LP groups. However, by the 6th wk of age, calves of the HP group were heavier than the LP group. A growth response in calves by increasing dietary protein has been reported previously. Blome et al. (2003) reported that the final BW of calves increased linearly as dietary CP increased from 16% to 26%; similar results were obtained in calves fed skim milk-based replacers (Donnelly and Hutton (1976a). Furthermore, Brosh et al. (2000) reported higher BW gain in Holstein-Friesian calves fed a high CP diet as compared to those fed medium or low CP

Table 2. Effects of different CP levels on nutrient digestibility of calves

Variable	CP content		
	LP	MP	HP
Dry matter			
Intake (g/d)	1,874.5±199.1	2,114.9±128.8	2,019.1±148.5
Digested (g/d)	1,397.9±132.5	1,542.7±91.3	1,490.8±107.6
Digestibility (%)	74.6±0.9	72.96±0.9	73.87±2.8
Crude protein			
Intake (g/d)	307.0±40.7[b]	374.35±9.0[a]	397.2±23.5[a]
Digested (g/d)	235.9±29.9[b]	291.7±11.6[a]	280.3±23.5[ab]
Digestibility (%)	76.7±1.0[a]	77.9±1.2[a]	70.5±2.9[b]
Ether extract			
Intake (g/d)	134.7±3.7[b]	157.5±4.6[a]	161.2±3.7[a]
Digested (g/d)	121.8±7.0[b]	142.3±5.5[a]	143.3±7.9[a]
Digestibility (%)	90.4±2.8	90.3±1.1	88.8±2.8
Ash			
Intake (g/d)	129.3±13.5	140.6±6.0	133.2±8.9
Digested (g/d)	71.4±4.6[b]	84.7±4.3[a]	77.0±7.0[ab]
Digestibility (%)	55.5±3.9	60.3±2.4	57.8±1.8

diets. In addition, the elevation of BW gain could be induced by increased availability in protein and energy levels in diets containing normal or high level of protein but not in diets with low level of protein (Singh et al., 1994; Verma, 1998). In contrast, it has been noted that there was no significant difference in BW gain of growing calves fed diets containing varied levels of protein (Sengar et al., 1985; Gonzalez et al., 1990; Lohakare et al., 2006). In the current study, we fed the calves according to their body weight, and the diet supply was adjusted as calves grew. We found that the calves in the LP group gained less weight than the other groups during the whole experiment period. This might have been due to the reduced MR intake, and the lower level of dietary protein content that could not meet the requirement of their growth. The calves fed with the MP milk replacer had the best growth performance among the three groups.

Intake and digestibility of nutrients

The digestibility of DM, EE and ash were not affected by dietary CP concentration (Table 2) although the digestibility of CP declined as the CP content of the diet increased. The DM intake tended to be lower in the LP group than the other groups, however no significant differences between the three groups existed (p>0.05). The average value of DM digestibility was 74.6, 73.0 and 73.9% in the LP, the MP and the HP groups, respectively, which was similar to previous observations (Krishna Mohan et al., 1987; Babu et al., 2003). Compared to the MP or the HP groups, the LP group had the lowest level of digested EE (p<0.05); however, digestibility of EE did not differ significantly between the three groups (p>0.05). The apparent digestibility of ash did not differ significantly among the three groups (p>0.05). As expected, CP intake of calves increased with elevated diet protein level. The

highest level of digested CP was found in the MP and HP groups, and the digestibility of CP was highest in the LP and MP groups (p<0.05). This might have been due to calves in the LP group not receiving adequate amounts of protein in their diet; while the calves fed with higher protein were unable to utilize dietary N as efficiently as the calves fed with medium CP content.

Balances of nutrients

Nitrogen balance : Gross efficiency of dietary CP usage (retained N as a proportion of dietary CP intake) for calves in the MP group was higher than in the other groups (Table 3). The average daily N intake varied significantly (p<0.05) in calves under different dietary regimes that matched the CP levels applied in the experiments. Following increased CP level in the diet, N intake increased linearly according to the experimental design. Fecal N increased with the increase of dietary CP (p<0.05), and similarly with protein intake. Pattanaik et al. (2003) reported that there was a positive relationship between N intake and excretion in feces. In contrast, Blome et al. (2003) reported that fecal N for calves showed a significant quadratic relationship from 16.1 to 25.8% CP when animals were fed with whey protein-based milk replacers at 1.5% of BW daily. In the present study, the absorbed N value was used as an index for the percentage of intake N (i.e., N apparent digestibility); surprisingly, this value was significantly lower in the HP group than the MP or LP groups (p<0.05).

Moreover, contrary to the values of fecal N, there were no significant differences in urine N among the three groups of animals (p>0.05). Some authors reported that urine N apparently showed a positive relationship with intake (Dabiri and Thonney, 2004; Lohaare et al., 2006). Retained N expressed either as a percentage of intake N or as a percentage of absorbed N which increased linearly as the

Table 3. Effects of different CP levels on N, Ca and P utilization of calves

Variable	CP content		
	LP	MP	HP
N			
Intake N (g/d)	49.1±6.5[b]	59.9±1.4[a]	63.6±3.8[a]
Fecal N (g/d)	11.4±1.8[b]	13.2±0.4[b]	18.7±1.9[a]
Urine N (g/d)	16.5±1.0	13.9±2.1	18.9±3.9
Absorbed N (g/d)	37.7±4.8[b]	46.7±1.9[a]	44.8±3.8[ab]
Absorbed N (% of intake N)	76.9±1.0[a]	77.9±1.2[a]	70.5±2.9[b]
Retained N (g/d)	21.3±5.2[b]	32.8±3.0[a]	26.0±0.7[ab]
Retained N (% of absorbed N)	55.8±7.3[b]	70.2±4.7[a]	58.2±5.3[b]
Retained N (% of intake N)	42.9±5.6[b]	54.7±4.2[a]	41.0±3.3[b]
Ca			
Intake Ca (g/d)	16.7±1.2	18.4±0.5	17.7±0.8
Fecal Ca (g/d)	8.0±0.5[a]	5.8±0.7[b]	7.7±1.0[a]
Urine Ca (g/d)	2.3±0.4[a]	2.0±0.4[ab]	1.4±0.5[b]
Retained Ca (g/d)	6.4±0.3[c]	10.6±0.3[a]	8.7±1.2[b]
Retained Ca (% of intake Ca)	38.5±1.2[c]	57.5±1.0[a]	48.7±4.6[b]
P			
Intake P (g/d)	10.5±1.3	11.7±0.3	11.5±0.8
Fecal P (g/d)	3.1±0.2[a]	2.8±0.4[b]	3.8±0.5[a]
Urine P (g/d)	4.0±0.5[a]	0.7±0.0[c]	2.4±0.2[b]
Retained P (g/d)	3.4±0.7[c]	8.2±0.2[a]	5.4±0.9[b]
Retained P (% of intake P)	32.3±3.5[c]	69.7±3.0[a]	46.6±6.1[b]

Data are expressed as mean±SD. [a, b, c] Values with different letter in the same row are different ($p<0.05$).

dietary CP increased probably induced the greater growth rates associated with feeding higher amounts of dietary CP (Blome et al., 2003). However, in the current study, retained N as a proportion of absorbed N or of N intake was significantly higher in the MP group than the other groups ($p<0.05$). On the other hand, the significantly lower value of the ratio between the retention of N and the absorbed N in the HP group indicated a higher N excretion through urine; hence, it further suggests that the N intake was in excess of the animals' capacity of energy availability (Lohaare et al., 2006). This data demonstrated that the calves in the MP group had more effective dietary protein utilization and, therefore, more rapid growth. Indeed, the average gross efficiency of N usage in the MP group achieved the highest value (54.66%) of the three groups. This was similar to the average value reported by Bartlett et al. (57.6%) (2006) even through the latter was obtained from calves fed with 22% CP at 1.75% of BW daily, which were much higher than the levels of these parameters used in the current study.

Calcium and phosphorus balance : The mean values of daily intake of dietary calcium and phosphorus remained unaffected by different dietary treatments (Table 3). However, the excretion of Ca and P in the feces or urine in the MP group were significantly lower than the other groups ($p<0.05$). Consequently, the values of retained Ca and P were significantly higher in the MP group than the LP or the HP groups ($p<0.05$). Although several previous reports suggested that the diet protein levels had no effect on Ca or

P utilization (Sengar et al., 1985; Lohaare et al., 2006), we found in the current study that, as a percentage of intake, the retained Ca or P were significantly higher in the MP group than in the LP or the HP groups ($p<0.05$). These results suggested that medium protein content may favor transporting of dietary calcium and phosphorus.

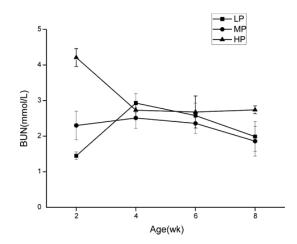**Digestibility of dietary amino acids**

Although research on amino acid digestibility in the young calf is deficient compared to studies with swine and poultry, a few studies have indicated that lysine and methionine plus cysteine are the first and second limiting factor for growth (Williams and Hewitt, 1979; Tzeng and Davis, 1980). Several researchers have estimated the amino acid profile required for the growth of calves (Williams and Hewitt, 1979) and pigs (Chung and Baker, 1992; Hansen et al., 1993). However, we argued that the requirements for young calves fed with restricted amounts of milk replacer would differ from those of rapidly growing young pigs and veal calves that were fed at high intakes (Davis and Drackley, 1998). In the current study, we found that, among the indispensable amino acids, histidine had the lowest apparent digestibility and tryptophan had the highest digestibility within all three groups, whereas, among the dispensable amino acids, the digestibilities of alanine and cystine were the lowest (Table 4). Except for alanine, there were no differences in the fecal digestibility of amino acids among the three groups ($p>0.05$). The average digestibility of the total AA was 82.7%, 83.4% and 79.4% in the LP, MP

Table 4. Effects of different CP levels on AA digestibility of calves

Variable	CP content		
	LP	MP	HP
Indispensable amino acids			
MET	84.2±1.0	86.9±2.0	85.1±1.9
ARG	86.6±0.2	86.5±2.2	81.6±4.1
LYS	84.5±1.3	84.3±1.6	82.7±2.4
HIS	66.3±2.9	63.8±2.1	63.1±4.4
PHE	82.6±0.6	84.3±2.6	78.7±4.3
VAL	78.7±1.1	78.9±2.8	74.8±3.5
ILE	80.1±0.9	81.1±2.8	76.4±3.7
LEU	82.3±1.1	82.9±2.5	78.1±4.1
THR	78.1±1.6	78.1±2.3	73.0±3.3
Trp	94.2±1.2	95.8±0.7	93.1±1.9
Dispensable amino acids			
ASP	82.8±0.9	83.5±2.6	79.7±3.4
SER	83.8±1.1	84.1±2.1	79.3±3.3
GLU	87.7±0.9	88.1±1.9	84.1±3.4
GLY	77.3±0.9	77.4±3.0	73.4±3.1
ALA	72.4±1.4[ab]	73.9±2.9[a]	67.8±3.6[b]
TYR	81.8±0.5	84.3±2.7	78.7±4.0
CYS	72.4±1.9	76.1±6.7	74.1±5.3
PRO	88.6±1.6	89.4±1.9	85.8±2.9
Total	82.7±1.0	83.4±2.2	79.5±3.2

Data are expressed as mean±SD.
[a, b, c] Values with different letter in the same row are different ($p<0.05$).

and HP groups, respectively. However, these values were a little lower than those reported previously by Wang et al. (2006).

Figure 2. Effects of different protein content in milk replacers on BUN concentrations of calves. Three groups were labeled by LP (■), MP (●) and HP (▲) respectively.

Blood metabolisms

One of the important objectives of the current study was to determine the impact of different dietary CP levels on blood metabolites. We found that the value of BUN was affected by both age and dietary protein levels (Figure 2). The BUN concentration decreased as calves grew in the current study, which was similar to a previous report in lambs (Holcombe, 1994). Similarly, we found that BUN increased following the increased dietary CP content, which agrees with several previous reports (Blome et al., 2003; Bartlett et al., 2006).
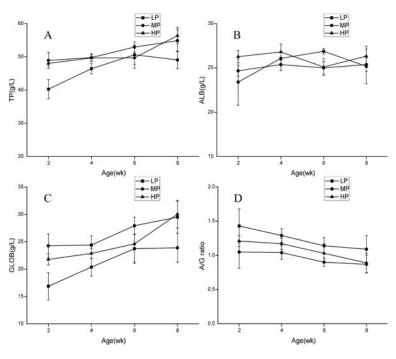
Previous investigations lacked unanimity in the

Figure 3. Effects of different protein content in milk replacers on TP (A), ALB (B), GLOB (C) concentrations and A/G ratios (D) of calves. Three groups were labeled by LP (■), MP (●) and HP (▲) respectively.

influence of dietary CP content on serum protein metabolism. Several researchers have suggested that serum concentrations of total protein, albumin, globulin, and albumin: globulin ratios were unaffected by dietary CP content (Sahoo et al., 2002; Blome et al., 2003). However, Sykes and Field (1973) reported that higher protein intake could increase serum albumin concentration. In the current study, we found that total protein and globulin concentrations in the LP group were lower than other groups (p<0.05) (Figure 3); however, albumin concentration was unaffected statistically (p>0.05). On the other hand, the total protein and globulin concentrations increased with age, but the albumin concentrations remained constant during the whole test. These results suggested serum albumin may have better adaptation tolerance in the face of protein deficiency than total protein or globulin.

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