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Impacts of dietary metabolizable protein on performance and ruminal parameters of Holstein cows at early lactation

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Abstract

The effects of different levels of metabolizable protein (MP) on production efficiency and ruminal parameters of early post-partum dairy cows were examined. Forty-two multiparous and 16 primiparous Holstein cows were assigned to 3 diet treatments in a completely randomized block design immediately after parturition. . Diets were formulated to provide three concentrations of dietary MP [Low MP, 1893; Medium MP, 2023; and High MP, 2149 g/day of dry matter (DM)] while rumen-degraded protein (RDP) remained constant (11.3% of DM). Dry matter intake was linearly increased by treatment. Whole milk yield, fat corrected milk and protein content increased significantly when increasing MP content in the diet fed to cows. Digestibilities of DM and CP increased significantly with the increase of MP. Ruminal concentrations of acetate, propionate and butyrate and total Volatile fatty acidss were increased significantly ($P<0.05$) by the increase of dietary MP, however the rumen pH did not differ among treatments. Generally it can be concluded that, increasing the level of MP in the diet of cows at early lactation improved the productive performance of the cows as well as their rumen function and digestibility of feeds.

Keywords: metabolizable protein, fresh cow, performance, ruminal parameters

Abbreviation key: MP = metabolizable protein, **HMP** = high MP experimental diet, **MMP** = medium MP experimental diet**, LMP** = low MP experimental diet, **MUN** = milk urea nitrogen, **FM =** fish meal**, CGM =** corn gluten meal, **SBM** = soybean meal, **EAA** = essential amino acids**, GI =** gastro intestinal.

Introduction

Dairy cows in postpartum period, have increasing demands to metabolizable protein (MP) to meet their requirements of milk production (NRC, 2001). Invariably, the early-lactating cow faces glucose and amino acid deficit (Phillips et al., 2003). To ameliorate this nutrient deficit, body adipose and protein reserves are mobilized to support the energy requirements for high milk production in early lactation. Although body fat depots are recognized as the major source of energy reserves, the catabolism of both body fat and protein contribute to nutrient requirements in early lactation (NRC, 2001). During this period, body fat mobilization ranges from 41 to 90 kg (Erdman and Andrew, 1989), and protein mobilization ranges from 21 to 24 kg (Komaragiri and Erdman, 1997; Komaragiri et al., 1998). Therefore, in addition to being in a negative energy balance, dairy cows experience a negative nitrogen (N) balance in early lactation (Plaizier et al., 2000). Body protein mobilization is driven by the overwhelming need to supply amino acids for hepatic gluconeogenesis and for milk protein synthesis during early lactation (Bauman and Currie, 1980). Propionate is the major precursor for gluconeogenesis (Drackley et al., 2001); however, limited feed intake during early lactation limits ruminal propionate supply to the liver, raising the requirement for alternative gluconeogenic precursors. Besides amino acids, there is an increased contribution of lactate, pyruvate, and glycerol to hepatic gluconeogenesis, which augment the limited propionate supply (Lomax and Baird, 1983). Although skeletal muscle is the primary labile source of amino acids, only a few studies have investigated protein metabolism in this tissue during lactation (Meijer et al., 1995; Komaragiri et al., 1998; Phillips et al., 2003; Chibisa et al., 2008). Skeletal muscle protein mass has been shown to decrease in early-lactating dairy cows

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(Komaragiri et al., 1998; Phillips et al., 2003). Although the mobilization of protein reserves is necessary to augment the inadequate dietary supply of energy and protein, excessive mobilization can lead to an increased incidence of metabolic disorders, and poor reproductive and lactational performance (Drackley, 1999). Overton et al. (1998) used alanine as an indicator of gluconeogenesis from amino acids and found that propionate conversion to glucose at 1 and 21 d postpartum was 119 and 129% of that at 21 d prepartum, but that alanine conversion to glucose at 1 and 21 d postpartum was 198 and 150% of that at 21 d prepartum. Various approaches to optimize postpartum nutrient supply and, thus, minimize the mobilization of body reserves, have been investigated. These include altering dietary protein (Komaragiri and Erdman, 1997; Phillips et al., 2003) and amino acid (Phillips et al., 2003) supply, and feeding supplemental fat (Komaragiri et al., 1998; DeFrain et al., 2005), or gluconeogenic precursors (DeFrain et al., 2005).

Nitrogen balance probably changes the most during the period from late gestation into the first few weeks of lactation. The splanchnic tissues [Portal Drained Viscera (PDV; the gastrointestinal tract, pancreas, spleen and associated adipose) plus liver], mammary gland and fetus increase protein synthesis at a time when DMI might be insufficient to meet protein requirements (Bell et al., 2000).

Therefore, with proper balancing of rumen degradable protein (RDP) and rumen un-degradable protein (RUP), some mobilization and repletion of body protein seems to help transition of the cow to lactation. Ruminal microbial protein synthesis alone is insufficient to meet the protein needs of high producing cows (NRC, 2001) and therefore, it is important to include feeds in diets that have low protein degradabilities. Feeds such as fish meal and corn gluten meal, are low in ruminal degradability (NRC, 2001). To optimize the amount of absorbable amino acids (AA) for high producing dairy cows, one of the diet formulation objectives is to provide adequate amounts of RUP (Schwab, 1995).

The objective of this study was to investigate whether consuming of different levels of MP, would affect performance of Holstein cows at the early stage of lactation and could improved rumen function.

Materials and Methods

Diets and Cow Management

Fifty eight Holstein cows were assigned to 3 dietary treatments randomly within blocks. The cows (Treatment1 = 17, Treatment 2 = 21, Treatment 3 = 20) were blocked by parity (16 primiparous, 11 at the second calving and 31 at the third or higher lactation). At calving, they were randomly assigned in a completely randomized block design with unequal repeats, for 21 days of lactation to the three experimental diets: 1) LMP: contained 17.1% CP with 1893 g/d of MP, 2) MMP: contained 19% CP with 2023 g/d of MP, and 3) HMP: contained 20.1% CP with 2149 g/d of MP). RDP was constant between diets (11.3%, based on NRC recommendations). They received supplemental CGM and fish meal, partially substituted with SBM and barley, during early postpartum period (wk 1 to 3). The amount of CGM and fish meal fed was designed to raise ration CP by 1.1 to 2.2 percentage units.

Experimental diets are shown in Table 1. The daily total mixed ration (TMR) was offered throughout the trial *ad libitum* to achieve 5-10% orts at 0830 and 1530. Cows were milked at 05:00, 13:00 and 21:00.

Table 1: Ingredients of the experimental Diets (% DM)[1]

Feedstuffs	LMP	MMP	HMP
Alfalfa hay	26.30	26.30	26.30
Corn silage	12.60	12.60	12.60
Beet pulp	9.50	9.50	9.50
Steam rolled Barley grains	13.90	12.30	11.0
Ground corn grains	9.70	9.70	9.70
Soybean meal	7.70	6.20	4.60
Roasted soybean	3.60	3.60	3.60
Whole cottonseed	6.70	6.70	6.70
Canola meal	0.51	0.51	0.51
Fish meal	2.0	3.60	5.15
Corn gluten meal	2.0	3.60	5.15
Fat	0.51	0.51	0.51
Salt	0.25	0.25	0.25
Sodium bicarbonate	1.0	1.0	0.92
Calcium carbonate	0.61	0.56	0.51
Magnesium oxide	0.15	0.15	0.13
Di-calcium phosphate	0.2	0.15	0.13
Min-Vit supplement[2]	0.825	0.825	0.825
Vitamin A[3]	0.05	0.05	0.05
Vitamin E[4]	0.5	0.5	0.5
Toxin binder	0.07	0.07	0.07
Glycoline[5]	1.29	1.29	1.29
Monensin	0.01	0.01	0.01
Availa 4[6]	0.01	0.01	0.01

1. LMP = low metabolizable protein, MMP = medium metabolizable protein, HMP = high metabolizable protein; 2. Contained 196 gr Ca; 96 gr P; 71 gr Na; 19 gr Mg; 3 gr Fe; 0.3 gr Cu; 2 gr Mn; 3 gr Zn; 0.1 gr Co; 0.1 gr I; 0.001 gr Se; 3 gr antioxidant; 5000 IU vit A; 100000IU vit D_3; and 100mg vit E; 3. Contained: 5000000IU vit A; 4. Contained: 4400 IU vit E; 5. Net energy=1500kcal; Ca 1.45%; EE 0.8%; CF 0.3%; 6. Zn not less than 5.15% ; Mn not less than 2.88% ; Cu not less than 1.08% ; Co not less than 0.18%

Sample Collection

Orts were measured daily and feed offered was adjusted to allow for 5 to 10% orts. Because cows were housed in pens, it was not possible to measure individual feed intakes. Instead, the intake of each pen was recorded daily. Weekly samples of rations and orts were taken to determine DM content. These DM

percentages were then used to calculate the pen average daily DM intakes (DMI).

Milk yield was recorded daily throughout the trial. Milk samples were collected from milking of the 3 sampling days. The Milko-Scan B-133 (Foss, Denmark) was used to determine milk fat, protein, lactose and SNF (AOAC, 2000). Fecal samples were grasped on days 19 to 21. Duplicate samples were taken at each sampling time. One sample was dried at 60°C for 36 h and the other was frozen at -20°C for later analysis.

At the day 20 after calving, about three hours after PM meal, ruminal fluid was collected by esophageal tube, and rumen pH immediately was determined after squeezing the fluid, by the pH meter. In order to measure the ammonia nitrogen and volatile fatty acids, two samples each of 10-ml of rumen fluid + 0.2 ml of 50% sulfuric acid were frozen at the - 20° C.

Table 2: Chemical composition of the experimental diets[1]

Items	LMP	MMP	HMP
NE_L (Mcal/kg)	1.65	1.67	1.68
CP (%)	17.9	19	20.1
RDP (% of CP)	11.31	11.28	11.25
RUP (% of CP)	6.65	7.72	8.79
Soluble protein (%)	23	22.3	21.4
Metabolizable protein (g/d)	1893	2023	2149
Methionine (g/d)	39	43	46
Lysine (g/d)	121	128	135
NDF (%)	33.2	32.7	32.2
PeNDF (%)[2]	24	23	23
NFC (%)[3]	36.5	35.7	35
Ether Extract (%)	4.7	4.9	5.1

1. LMP = low metabolizable protein, MMP = medium metabolizable protein, HMP = high metabolizable protein; 2. PeNDF = predicted neutral detergent fiber; 3. NFC = non fibrous carbohydrates, NFC (%) = 100- (%CP+ %NDF + %EE +%Ash)

Sample Analysis

Orts samples, alfalfa hay, corn silage, beet pulp, concentrate mix (n = 3 for each mix) were dried at 105°C for 24h (except for corn silage that were dried at 60°C for 72 h) and ground to pass a 1-mm screen (Wiley mill). Dry matter content of TMR was determined by drying at 60°C for 72 h; Crude protein was determined by micro-Kjeldahl (AOAC, 2000). Total tract digestibility of dry matter and crude protein were calculated by acid insoluble ash method (Van Keulen and Young, 1977).

Ruminal parameters

One of the two samples was used to determine ammonia nitrogen by titration method (Crooke and Simpson, 1971). The following formula was used to calculate the ammonia nitrogen:

Ammonia nitrogen in ml per 100 ml of rumen fluid = 100 × 1.4 × (ml of sulfuric acid used for titration) / 20**.** The other sample was used to measure the rumen volatile fatty acids by chromatography method (Ottensin and Butler, 1971).

Statistical Analyses

The Data measured over time (DMI, Milk yield and components) within the period of interest were subjected to 2-way ANOVA (3 treatment × 3 blocks) using the REPEATED statement MIXED procedure of SAS (2003). Ruminal parameters data and digestibility data were analyzed using GLM procedure of SAS. When treatment is significant, means were separated by Tukey test.

Results and Discussion

Dry matter intake

Least square means of DMI during experimental period for LMP, MMP, and HMP were 14.15, 14.40 and 15.04 kg/d, respectively (Table 3). In comparison with LMP, dry matter intake showed a trend of insignificant (P=0.054) increase in MMP and HMP groups.

The dry matter intake has special importance to meet nutrient requirements of cows to maintain their health and production. The cows in first days of lactation period, specially immediately after parturition, show loss of appetite, because of the increased level of estrogen in plasma (Ingvartsen, 2006). NRC (2001) recommended a high concentration of CP for the high levels of milk yield, so because of low DMI in fresh cows, this amount of CP, must be given in the form of high concentrate of RDP and RUP in diets (Khorasani et al., 1996). Decreasing DMI in the early postpartum period causes declining in passage rate and consequently protein degradability in the rumen increases; thus, ruminal outflow of non ammonia nitrogen (NAN), non ammonia non microbial nitrogen (NANMN) and essential amino acids (EAA) into the small intestine will decrease (Ipharraguerre and Clark, 2005). Therefore, ratio of RUP supplements (corn gluten meal and fish meal) could be increased. The current findings were in agreement with Law et al. (2009), Broderick (2003), who reported higher DMI using RUP. It was reported that cows received higher amounts of RUP (10%) than NRC (2001) recommendations have 2.1 kg higher DMI per day (Flis and Wattiaux, 2005).

Milk Production and Composition

The least square means of whole milk production and fat corrected milk (FCM) 4% were shown in Table 3. Increasing level of MP is accompanied by supply of EAA in the small intestine (Chen et al., 2009; Flis and Wattiaux, 2005). Schwab and Foster (2009) suggested that the limiting factor for milk production in the first weeks of lactation is MP but not net energy for lactation

Table 3: Least square means± SE of DMI and milk yield and composition[1] of cows fed the experimental diets

Items	LMP	MMP	HMP	p-value		
				Treatment	Block	Treat×Period
Number of animals	17	21	20			
DMI (kg/d)	14.15±0.17	14.40 ±0.17	15.04±0.17	0.0542	0.0002	0.6180
Milk yield (kg/d)	35.42 ± 0.92^{b}	35.81 ± 0.85^{ab}	38.54 ± 0.89^{a}	0.028	0.0001	0.0001
FCM 4 % (kg/d)[2]	29.89 ± 0.9^{b}	31.24 ± 0.83^{ab}	33.0 ± 0.87^{a}	0.0477	0.0001	0.0001
FCM 3.5% (kg/d)[3]	32.20 ± 0.98^{b}	33.68 ± 0.91^{ab}	35.57 ± 0.95^{a}	0.0477	0.0001	0.0001
Milk fat (%)	3.01± 0.14	3.22 ± 0.13	3.17 ± 0.14	0.5464	0.0011	0.0001
Milk fat (kg/d)	1.048 ± 0.04	1.12 ± 0.04	1.17 ± 0.04	0.1995	0.0241	0.0001
Milk protein (%)	3.41 ± 0.02^{b}	3.53 ± 0.02^{a}	3.53 ± 0.02^{a}	0.0008	0.0841	0.0001
Milk protein (kg/d)	1.20 ± 0.03^{b}	1.26 ± 0.03^{ab}	1.36 ± 0.03^{a}	0.0072	0.0001	0.0001
Milk lactose (%)	5.18 ± 0.03	5.18 ± 0.03	5.09 ± 0.03	0.1282	0.4262	0.0001
Milk lactose (kg/d)	1.83 ± 0.04	1.85 ± 0.04	1.95 ± 0.04	0.0913	0.0001	0.0001
Milk SNF(%)	9.29 ± 0.06	9.41 ± 0.06	9.39 ± 0.06	0.1172	0.4430	0.0001
Milk SNF(kg/d)	3.29 ± 0.08	3.36 ± 0.07	3.61 ± 0.07	0.0901	0.0001	0.0001
ECM (kg/d)[4]	31.95 ± 0.85^{b}	33.47 ± 0.82^{ab}	35.35 ± 0.83^{a}	0.0242	0.0001	0.0001
Milk Energy value (kg/d)[5]	0.67 ± 0.01	0.69 ± 0.01	0.68 ± 0.01	0.3898	0.0001	0.0001
SCC (× 1000/ml)[6]	293 ± 2.01^{a}	150 ± 2.31^{b}	142 ± 2.41^{b}	0.030	0.001	0.0001
Future Milk yield[7] (kg/d)	42.94± 1.47	41.99± 1.36	43.97 ± 1.42	0.8435	0.0001	0.0001

1. a, b, c: means in the same row of different superscripts are significantly different ($p< 0.05$); 2. Fat corrected milk (FCM) 4%=[0.4×milk(kg)]+[15×milk fat(kg)]; 3. FCM 3.5%=[0.4324×milk(kg)]+[16.216×milk fat(kg)]; 4. Energy corrected milk (ECM)=milk(kg)×[383×fat(%)+242×protein(%)+165.4×lactose(%)+20.7]/3140; 5. Milk energy (Mcal/kg) = (0.0929×fat%) +(0.0547×protein%)+(0.0395×lactose%) (NRC, 2001); 6. Somatic cell counts; 7. Milk production from day 21 to day 120 of lactation.

(NEL); therefore, enhancing RUP has a beneficial effect on milk production. A quadratic relationship between milk production and dietary CP at the range of 16 to 21% was reported (NRC, 2001); however, this CP enhance- ment using RDP had less benefit. Flis and Wattiaux (2005) indicated that diets contained over 10% of CP than the NRC recommendation, permits 1.5 kg more milk per day and this increase in milk production is due to RUP enhancement. In agreement with the present study findings, Broderick (2003) reported an increment of 2.8 kg/d, whereas, Cunningham et al. (1996) reported an increment of 2.7 kg/d.

The least square means of milk protein content and yield of the treatments LMP, MMP and HMP were illustratedin Table 3. Milk protein significantly increased with enhancing RUP ($P<0.05$). This increase, was probably due to the providing of good profiles of amino acids that were similar to the milk amino acids profile, and enhancing RUP specially with FM could have led to optimal levels of Lys to Met ratios in the small intestine (Schwab and Foster, 2009; Van Amburgh et al., 2009). Since Lys and Met are limiting amino acids for milk production and milk protein, the high levels of RUP cause an increment of milk protein. In agreement with the current study findings, Broderick (2003) found that milk protein yield was improved by enhancing dietary CP from 15.3 to 16.7%, but no any changes were found with 18.4% of CP.

Nutrient digestibility

The least square means of Digestibility of DM and CP were presented in table 4 that indicated that treatment effects on these data were statistically significant ($P<0.05$).

Apparent digestibility of DM and N increased linearly as RUP supplement in diet, increased (Wright et al. 1998) and this agreed with Grummer et al. (1996), Flis and Wattiaux (2005). This increase in digestibility was due to the high post-ruminal digestibility of RUP supplements.

Ruminal parameters

The least square means of rumen ammonia nitrogen concentration were showed that the treatment had no significant effect on this parameter (Table 5). The rumen ammonia nitrogen was measured in many nutritional researches and is a crude predictor of converting efficiency of dietary nitrogen to microbial nitrogen, But as soon as the concentration decreased to below 5 mg/ dL (For optimal microbial protein synthesis that is often recommended), blood urea nitrogen is transferred into the rumen and provides an increased buffer against the low concentrations of NH3-N, and supply amine nitrogen which have important roles in improving microbial functions of dairy cow (Firkins et al., 2007). Klusmeyer et al. (1990) noted that the mean concentration of rumen NH3-N, for a diet containing 14.5% protein, were 10.5 and 5.4 mg/dL for soybean meal and corn gluten meal, respectively. Further reduction of CP to 11% of DM, caused the CP to be more restrictive than NEL and reduced the average of NH3-N to 2.5 and 1.9 mg/dL, respectively. The study of Klusmeyer et al. (1990) and Firkins et al. (2007) showed that the importance of blood urea

Table 4: Least square means ± SE of digestibility of DM and CP of the experimental diets

Items	LMP	MMP	HMP	p-value		
				Treatment	Block	Treat×block
DCP(%)	74.59 ± 0.47[b]	76.58±0.65[a]	77.68±0.91[a]	0.0006	0.1439	0.5944
DDM(%)	67.94±0.78[b]	70.63±0.86[ab]	72.10±0.86[a]	0.0052	0.0140	0.1184

Table 5: Least square means ±SE of Ruminal parameters of the cows fed the experimental diets

Items	LMP	MMP	HMP	p-value		
				Treat	Block	Treat×Block
N-NH_3 (mg/dL)	12.62±0.79	12.49±0.85	13.26±0.89	0.85	0.35	0.69
pH	6.41±0.07	6.42±0.07	6.31±0.1	0.69	0.15	0.16
Acetic acid (mM/L)	65.71 ± 4.0[b]	62.76 ± 4.3[ab]	70.56 ± 5.6[a]	0.004	0.43	0.03
Propionic+isoButyric (mM/L)	18.34 ±1.48	16.80±1.59	20.46±2.09	0.072	0.06	0.09
Butyric (mM/L)	8.94 ± 0.74-b	10.82 ± 0.79[b]	12.78 ± 1.04[a]	0.018	0.017	0.09
IsoValeric acid (mM/L)	0.73±0.08	0.71±0.09	1.05±0.12	0.078	0.67	0.33
Valeric acid (mM/L)	1.05 ± 0.09[b]	1.14 ± 0.10[b]	1.72 ± 0.13[a]	0.001	0.072	0.12
Total VFA(mM/L)	94.80 ± 6.1[b]	92.26 ± 6.55[b]	106.58 ±8.62[a]	0.01	0.32	0.039

a, b, c: means in the same row of different superscripts are significantly different

nitrogen (BUN) returned to the rumen. In Cattle and sheep, 40% and 80% of the blood urea nitrogen synthesized by the liver, returns into the digestive tract, respectively (Lapierre and Labley, 2001).

The ruminal pH is an indicator of a net balance between carbohydrate digestion, absorption and utilization of VFA and buffer (Ipharraguerre et al., 2005). Balance between acid and buffer secretion is a key determinant of rumen pH. The least square means of ruminal pH, shown Table 5 revealed that diets had no significant effect on pH. For the cows of the three experimental groups, rumen environment was satisfactory for the fermentation of nutrients, especially fiber (Colmenero and Broderick, 2006b). The least square means of acetate concentration (Table 5) showed that the effect of treatments was significant ($P<0.05$). Similarly, Henson (1997) noted that with increasing RUP by use of animal resources and corn gluten, acetate increased significantly ($P<0.05$), also the ammonia concentration and pH of rumen fluid and blood urea had no significant difference between treatments. Yang (2002) observed that addition of amino acids and peptides will lead to improvements in fiber digestibility.. Due to the significant increase in digestibility of dry matter, dietary fiber degradation was likely to be improved and had caused the ratio of acetate and total volatile fatty acids to increase significantly with increasing levels of MP and CP.

Cunningham et al. (1996) reported only increase of acetate concentration in response to the increase in crude protein in the diet, while Colmenero and Broderick (2006a) found that when dietary protein increased from 13.5 to 19.4 percent, acetate linearly and propionate quadratic increased.

The relationship between rumen butyrate and glucose is largely unknown. It was proven that beta-hydroxy butyrate, increases activity of hepatic gluconeogenesis via its metabolism to acetyl coenzyme - A which is an allosteric activator of pyruvate carboxylase (Utter and Keech, 1963). In support of these findings, Black et al (1966) showed that the increase of concentrations of ruminal butyrate, was metabolized by ruminal epithelium and then transferred into the blood in the form of beta-hydroxy butyrate and this cause it to be spared in the oxidation of pyruvate, and to increase pure conversion of pyrovate to oxaloacetate. Experiments of Anand and Black (1970) showed that intravenous injection of butyrate stimulates gluconeogenesis in the cow. So we can conclude that butyrate produced in the rumen can be useful for the cows during the transition period. This occurs because of the following reasons: 1) sparing in hepatic oxidation of pyruvate and increasing its conversion to oxaloacetate, 2) sparing the use of glucose by extra mammary tissues (Holtenius and Holtenius, 1996), 3) providing precursors of fatty acid synthesis (Palmquist et al., 1969).

The significant increase in the molar concentrations of isovalerate and isobutyrate probably related to deamination of amino acids in the rumen. The carbon skeleton of these amino acids also has participated in producing of propionate, acetate and butyrate (leucine and lysine). In addition, the portion of the amine caused the raising ammonia level.

Conclusions

The study concluded that increasing the amounts of MP in the diets of cows at early stage of lactation, increased milk yield and milk protein content. Furthermore, ruminal VFA concentrations increased significantly and we suggest that high needs for MP in this period (0 - 3 wks of lactation) have improved cow performance.

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