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Full Length Research Paper

The effects of different levels of sodium caseinate on rumen fermentation pattern, digestibility and microbial protein synthesis of Holstein dairy cows

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This study was conducted to investigate the effects of different levels of peptide supplementation on rumen fermentation pattern, digestibility and microbial protein synthesis. Three rumen-cannulated Holstein dairy cows were used in a 3 × 3 Latin square experiment within 21 days period. The ruminal infusion of sodium caseinate (CN) was 0 (control), 50 and 100 g/d. Dry matter intake, milk yield and composition , total tract apparent digestibility of nutrient, rumen parameters and purine derivatives in urine of cows were measured. Results showed that dairy cows received sodium caseinate, had significantly increased microbial protein synthesis, milk fat yield, acetate and branched chain fatty acids concentrations in rumen fluid and fiber digestibility compared with the control treatment ($P < 0.05$). CN significantly affected the concentrations of rumen ammonia nitrogen (NH_3-N), rumen peptide nitrogen (Pep-N) and the ratio of rumen ammonia nitrogen/ rumen peptide nitrogen ($P < 0.05$) and consequently blood urea nitrogen, milk urea nitrogen and urinary urea nitrogen concentrations. However digestibility of dry matter and crude protein did not differ among treatments. In conclusion, if the optimum level of NH_3-N/Pep-N was the best compromise among the need for rumen fermentation, microbial protein synthesis and nitrogen excretion through urine in animal, the recommended level from this study would be 0.86 in rumen fluid.

Key words: Sodium caseinate, digestibility, microbial protein synthesis, rumen fermentation, dairy cows.

INTRODUCTION

Protein degradation by rumen microbes results in the formation of ammonia in rumen fluid and peptides and amino acids are intermediates in this process (Reynal et al., 2007). Ammonia is the preferred N source for fiber-digesting bacteria (Hungate, 1966) and required bystarch, sugar and secondary fermenters for protein synthesis (Cotta and Russell, 1982). However, previous works reported enhanced growth and efficiency of rumen microbes (Russell and sniffen, 1984) or improvement in digestion of organic matter, dry matter and fiber (Griswold et al., 2003) with peptide increase in rumen fluid. In a controversial study, Jones et al. (1998) reported that by increasing the level of peptide supplementation in continuous culture, a linear decrease of ammonia nitrogen concentration caused depression in fiber digestion and microbial protein production.

The results of the conducted studies showed that alteration of both ammonia nitrogen and peptide nitrogen in rumen fluid could affect rumen fermentation characterristics and microbial protein synthesis (Broderick and Wallace, 1988; Jones et al., 1998). Although peptide nitrogen could stimulate microbial growth, a basal

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Abbreviations: CN, Sodium caseinate; **NH_3-N,** ammonia nitrogen; **NH_3-N/ Pep-N,** the ratio of ammonia nitrogen to peptide nitrogen; **Pep-N,** peptide nitrogen; **BW,** body weight; **SD,** standard deviation; **DM,** dry matter; **TMR,** total mixed ration; **NDF,** neutral detergent fiber; **ADF,** acid detergent fiber; **SNF,** solids non fat; **MUN,** milk urea nitrogen; **PD,** total purine derivatives; **RDP,** rumen degradable protein; **VFA,** volatile fatty acids; **BCS,** Body condition score; **NHA,** ninhydrin colorimetric assay.

concentration of ammonia nitrogen in rumen fluid is necessary to prevent depression in rumen fermentation and fiber digestibility (Jones et al., 1998). There have been clarified different recommendations for ammonia nitrogen concentrations in rumen fluid for maximal *in vitro* microbial growth (Satter and Slyter, 1974) or *in situ* dry matter and crude protein degradation (Wallace, 1979) and for *in vivo* rumen fermentation (Song and Kennelly, 1989). However, there is little knowledge about quantitative relationships between ammonia nitrogen and peptide nitrogen in rumen fluid and the optimum level of ammonia nitrogen to peptide nitrogen (NH_3-N/Pep-N) in rumen fluid of dairy cows need to be investigated through more studies.

Sodium caseinate (CN) is a well known source for peptide nitrogen that extensively has been used in previous works (Fu et al., 2001; Khalili and Huhtanen, 2002) and also was a rapidly degradable protein in rumen that could positively affect ruminal ammonia concentration (Broderick and Wallace, 1988). In the present study, CN was infused in the rumen of dairy cows to induce different levels of NH_3-N/Pep-N in rumen environment. Consequently, the effects of different levels of peptide nitrogen source in the diet of dairy cows on rumen fermentation, total tract digestibility of nutrients, microbial protein production and blood urea nitrogen were investigated.

MATERIALS AND METHODS

Cows, management and diets

Three non pregnant multiparous late-lactating Holstein dairy cows fitted with rumen cannulae averaging BW 682 ± 13 and DIM 260 ± 17 (mean ± SD) kg at the beginning of the study were allocated to a 3 × 3 Latin square design within 21 days period such that the first 14 days were considered as adaptation period and the last 7 days as collection period. Basal diet was formulated with CPM-Dairy (CPM Dairy v. 2.0.23; University of Pennsylvania, Kennett Square; Cornell University, Ithaca, NY; and William H. Miner Agric. Res. Inst., Chazy, NY). Diet ingredients and chemical composition of the diet are presented in Table 1.

The peptide supplementation was given as sodium caseinate (Iran Caseinate Company, ICC Tehran, Iran) that 0, 50 and 100 g/d CN as treatments 1, 2 and 3, respectively. The CN was dissolved in 0.3 L water and then infused in the rumen through the cannulae just before morning feeding (0.3 L water was infused for control treatment). The cows were kept in individual stanchions and were fed twice daily at 08:00 and 16:00 h and milked twice daily at 10:00 and 18:00 h. The cows had free access to water and salt block. Orts were collected and weights recorded once daily at 07:00 h and the feeding rate were adjusted daily to yield orts of about 5 - 10% intake.

Experimental procedures and chemical analyses

The dry matter (DM) was determined for weekly composites of feed and ort by drying at 60°C for 48 h (AOAC, 1980). Intake of DM was computed based on the 60°C DM determinations for total mixed ration (TMR) and orts. After drying, ingredients and TMR were ground through a 1 mm screen (Wiley mill, Arthur H. Thomas, Philadelphia, PA) and period composites were prepared by mixing equal DM. Composite samples were analyzed for total nitrogen, DM, ash and organic matter (AOAC, 1980), sequentially for neutral detergent fiber (NDF) and acid detergent fiber (ADF) (Van Soest et al., 1991). Milk was sampled at two consecutive milking in a day on days 15 and 20 of each period and analyzed for fat, protein, lactose, solids non fat (SNF) and milk urea nitrogen (MUN). Fecal samples were collected each day from the rectum at 07:00 and 19:00 h during the first five days of collection period in each experimental period. Samples were composite per cow per period and then oven-dried at 55°C for 72 h and then ground through a 1 mm sieve. After analyzing the fecal samples for nutrients, total tract apparent digestibility of nutrients was determined by using acid insoluble ash as an internal marker (Van Keulen and Young, 1977). The body weight (BW) changes were measured at the first and the last day of each period to compute BW change. Body condition score (BCS) was measured based on the Wildman et al. (1982) at the first and the last day of each experimental period.

Blood was sampled at 0 (before CN infusion) and 4 h after feeding from the coccygeal vein of the cows on day 15 of each

Table 1. Ingredients and chemical composition of basal diet.

Item	Basal diet
Ingredients, % of DM	
Alfalfa hay, Chopped	18.5
Corn silage	31.2
Wheat straw	6.50
Barely, ground	7.80
Corn, ground	6.50
Wheat, ground	5.50
Wheat bran	4.70
Soybean meal, 44% CP	4.00
Canola meal	6.80
Beet pulp	7.50
Vitamin-mineral mix*	0.40
Calcium carbonate	0.20
Sodium bicarbonate	0.25
Salt	0.15
Chemical composition	
CP, % of DM	13.5
RDP, % of DM	8.60
NE_L**, Mcal/kg	1.52
NDF, % of DM	40.20
NFC***, % of DM	34.60
Ca, % of DM	0.45
P, % of DM	0.27
Na, % of DM	0.16

Composition: 1.8% Mn; 1.8% Zn; 0.85% Fe; 0.40% Cu; 0.03% I; 0.03% Co; 0.01% Se; 2,200,000 IU/kg of vitamin A; 860,000 IU/kg of vitamin D; 8,000 IU/kg of vitamin E.
** Estimated using the NRC (2001) model.
*** NFC = 100 - (%NDF + %CP + %fat + %ash) according to the NRC (2001) model.

experimental period. Blood samples were heparinized and held at 2℃ for about 6h. Thereafter, samples were centrifuged (3,000 × g, 4℃, 20 min) and the plasma stored at -20℃ until analyzed for urea nitrogen (Teco No B551-132, Teco Diagnostics). Samples of rumen fluid were collected at 0 (i.e., before CN infusion), 1.5, 3, 4.5 and 6 h after feeding on days 16 and 17 in each experimental period. Samples of rumen fluid were strained through four layers of cheesecloth and pH measured immediately. For determination of ammonia nitrogen and volatile fatty acids (VFA) in rumen fluid, 10 ml subsamples of strained rumen fluid were preserved by addition of 0.2 ml sulfuric acid 50% and stored at - 20℃. Just before analysis, samples were thawed, centrifuged (10,000 × g, 4℃, 20 min) and analyzed for ammonia (Crooke and Simpson, 1971) and VFA (Gas chromatography; model 5890, Hewlett-Packard, Avondale PA) using a 1.8 m glass column packed with 10% SP 1,200/1% H_3PO_4 on 80/100 chromosorb WAW (Supelco, 1975). For isolation and measurement of peptide nitrogen concentration in rumen fluid, 20 ml subsample of strained rumen fluid for each sampling interval was acidified with 2 ml sulfuric acid 10% immediately to prevent peptide hydrolysis and stored at -20℃. Later, peptide nitrogen in rumen fluid was isolated and measured using acid hydrolysis and assessed using ninhydrin colorimetric assay (NHA) full details described by Choi et al. (2002).

Total urine collection was performed for 24 h with urine collection bag tightly attached over the vulva (Eriksson et al., 2004) which was inserted on day 19 of each period. The 0.5 l of 40% sulfuric acid was in each container to keep the pH of collected urine under 3 (Valadares et al., 1999). Weight of acidified urine was recorded and 15 ml aliquots were diluted immediately with 40 ml 0.036 N sulfuric acid and stored at - 20℃ for analysis. Later, urine samples were thawed at room temperature and were analyzed for uric acid and allantoin (Chen and Gomes, 1992) and urea nitrogen with the colorimetric method (Broderick and Clayton, 1997). Total excretion of allantoin and acid uric (mmol/d) was computed as the product of the urine volume obtained during 24 h and metabolite concentration (mmol/l). Total purine derivatives (PD) excretion was the sum of allantoin and uric acid excreted in urine.

Calculations and statistical analysis

Total absorption of microbial purines was calculated as: purine absorption (mmol/d) = (PD excretion - 0.385 × $BW^{0.75}$)/0.85, where 0.385 is the endogenous PD excretion (mmol/d) was estimated from BW of individual cows as: 0.385 mmol/$BW^{0.75}$ per day and 0.85 is the absorptive efficiency of purines. Ruminal synthesis of microbial N was computed as: microbial N (g/d) = (purine absorption × 70) / (0.134 × 0.83 × 1000), where 70 is the N content of purines (mg N/mmol), 13.4: 100 is the mean ratio of purine-N: total-N measured for mixed rumen microbes (Valadares et al., 1999) and 0.83 is the assumed digestibility of microbial purines (Chen and Gomes, 1992). Data were analyzed using Proc Mixed in SAS (version 8.1; SAS institute Inc., Cary, NC). The following model was fitted to all variables that did not have repeated measurements over time;

$$Y_{ijk} = \mu + P_i + C_j + T_k + \varepsilon_{ijk}$$

where Y_{ijk} is the dependent variable, μ is the overall mean, P_i is the effect of period i, C_j is the effect of cow j, T_k is the effect of treatment k and ε_{ijk} is the residual error.

The following model was used for ruminal variables for which there were repeated measurements over time (pH, NH_3-N, Pep-N, NH_3-N/Pep-N and VFA):

$$Y_{ijkl} = \mu + P_i + C_j + T_k + Z_l + ZT_{kl} + \varepsilon_{ijkl}$$

Where Y_{ijkl} is the dependent variable, μ is the overall mean, P_i is the effect of period i, C_j is the effect of cow j, T_k is the effect of treatment k, Z_l is the effect of time l, ZT_{kl} is the interaction between time l and treatment k and ε_{ijkl} is the residual error. All terms were considered fixed except for ε_{ijkl} which was considered random. Differences between least square means were considered significant at $P < 0.05$ and differences were considered to indicate a trend toward significance at $0.05 < P < 0.10$ using PDIFF in the LSMEANS statement.

RESULTS

Rumen fermentation and the level of ammonia nitrogen to peptide nitrogen

The results for the effect of CN infusion on ruminal fermentation parameters are presented in Table 2. CN increased ammonia nitrogen and peptide nitrogen concentrations ($P < 0.01$). Also level of NH_3-N/Pep-N increased significantly with CN infusion ($P < 0.05$). The greatest levels of peptide nitrogen (Figure 1), ammonia nitrogen (Figure 2) and NH_3-N/Pep-N (Figure 3) resulted at 1.5, 3 and 3 h after morning feeding, respectively. Alteration of the level of CN had no effect on ruminal pH ($P > 0.05$). Ruminal concentration of total VFA tended to rise ($P = 0.07$) by peptide supplementation treatment compared with the control treatment. Ruminal concentrations of branched chain VFA ($P < 0.05$) and acetate ($P < 0.01$) significantly increased with use of CN.

Dry matter intake, performance and digestibility of nutrients

The data for dry matter intake, performance, milk yield and composition and total tract apparent digestibility of nutrients are given in Table 3. In this study, neither DMI ($P = 0.49$) nor milk yield ($P = 0.43$) were affected by sodium caseinate. Also, CN did not affect milk lactose yield ($P = 0.75$), SNF yield ($P = 0.29$) and milk protein yield ($P = 0.24$). However, milk fat yield significantly increased ($P < 0.05$) with infusion of CN. When the level of NH_3-N/Pep-N increased, the MUN concentration significantly increased ($P < 0.05$). CN did not affect body weight changes ($P = 0.37$) and body condition score changes of the cows ($P = 0.61$). Total tract apparent digestibility of NDF and ADF were affected significantly by infusion of CN ($P < 0.05$). However, there was no significant affect of CN infusion on crude protein and dry matter digestibility ($P > 0.05$) and digestibility of OM has tended to rise in this study ($P = 0.09$).

Table 2. Least square means for ruminal fermentation parameters in dairy cows infused with sodium caseinate in the rumen.

Item	Treatments*			SEM
	1	2	3	
pH	6.57	6.61	6.60	0.2
NH_3-N, mM/l	6.86^{c}	8.81^{b}	10.63^{a}	0.86
Pep-N, mM/l	8.94^{c}	10.19^{b}	11.53^{a}	0.94
NH_3-N/Pep-N	0.77^{b}	0.86^{ab}	0.94^{a}	0.02
Total VFA, mM/l	82.94	88.45	91.29	3.16
Propionate, mM/l	16.17	17.40	17.18	1.13
Butyrate, mM/l	10.89	11.29	11.04	1.05
Acetate, mM/l	52.53^{b}	55.98^{ab}	59.12^{a}	0.94
Acetate/Propionate	3.24	3.21	3.44	0.38
BCVFA **, mM/l	3.35^{b}	3.78^{ab}	3.95^{a}	0.06

$^{a, b, c}$ Least squares means within the same row without a common superscript differ ($P < 0.05$).
* Treatments 1, 2 and 3 were 0, 50 and 100 g/d sodium caseinate infused in the rumen, respectively.
** Branched-chain volatile fatty acids.

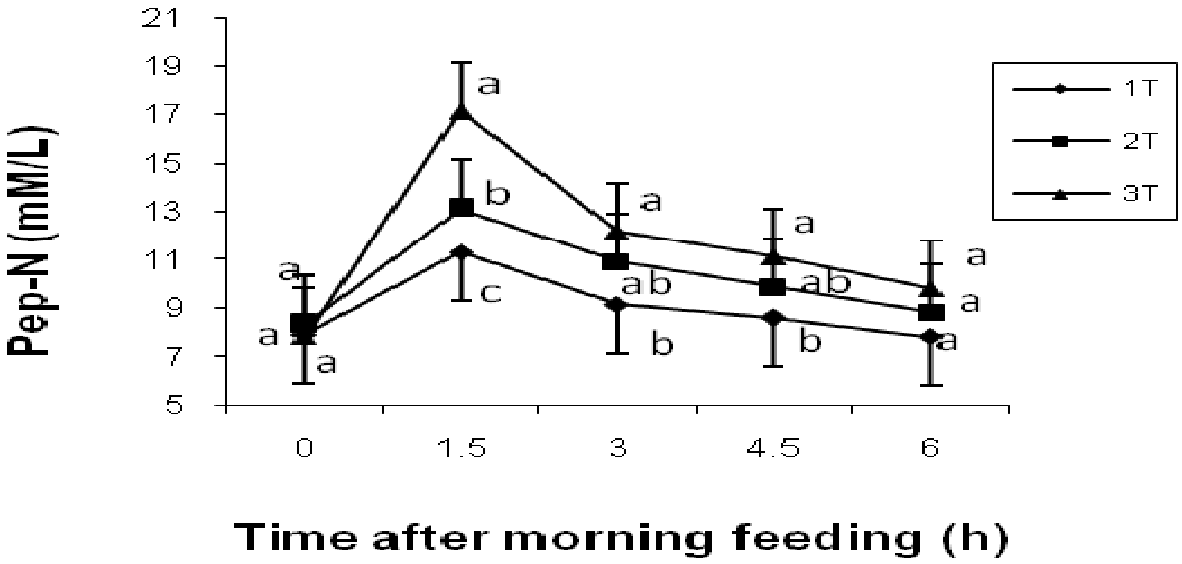

Figure 1. The peptide nitrogen concentration in rumen fluid of dairy cows received sodium caseinate†.
†Treatments 1, 2 and 3 were 0, 50 and 100 g/d sodium caseinate infused in the rumen, respectively.

Urinary parameters, microbial protein synthesis and blood urea nitrogen

The results for urine volume, urinary excretions of purine derivatives (urinary allantoin and uric acid) and urea nitrogen excretion and microbial protein synthesis are presented in Table 4. The use of CN in the rumen increased total urine excretion compared with the control treatment ($P < 0.05$). Urinary excretion of allantoin, uric acid and urinary purine derivatives were affected significantly with CN infusion in the rumen ($P < 0.05$). Urea nitrogen excreted through urine increased with peptide source and control treatment showed the lowest and treatment with 100 g sodium caseinate showed the highest urinary excretion of urea nitrogen. Increasing the level of CN infusion increased microbial protein synthesis and blood urea nitrogen concentration ($P < 0.05$).

DISCUSSION

Rumen fermentation pattern and the level of ammonia nitrogen to peptide nitrogen

Caseinate was infused in the rumen of dairy cows to change the peptide nitrogen and ammonia nitrogen

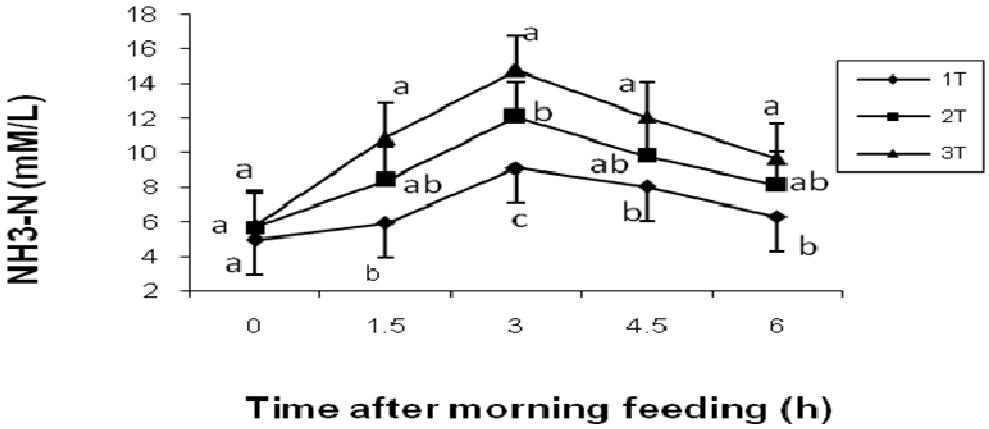

Figure 2. The ammonia nitrogen concentration in rumen fluid of dairy cows ruminally infused with sodium caseinate†. †Treatments 1, 2 and 3 were 0, 50 and 100 g/d sodium caseinate infused in the rumen, respectively.

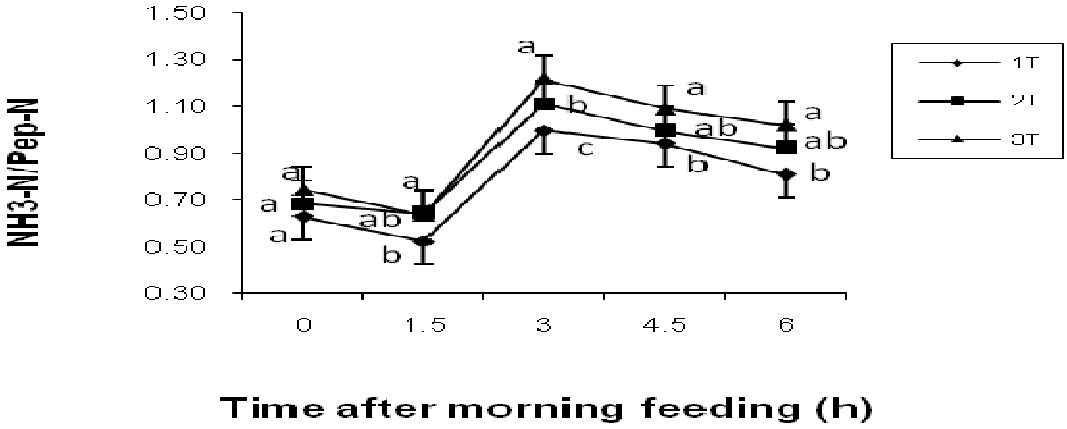

Figure 3. The ratio of ammonia nitrogen to peptide nitrogen in rumen fluid of dairy cows received sodium caseinate†. †Treatments 1, 2 and 3 were 0, 50 and 100 g/d sodium caseinate infused in the rumen, respectively.

concentrations and induce different levels of NH_3-N/Pep-N in rumen environment to find out the optimum NH_3-N to Pep-N ratio in rumen fluid. Compared with the control treatment, infusion of CN in the rumen had no effect on ruminal pH. Feeding the rumen degradable protein (RDP) from the range of 10.6 to 13.2% to dairy cows, Reynal and Broderick (2005) reported no significant effect of high degradable protein content in diet on ruminal pH. In the present study, infusion of CN increased peptide nitrogen (Figure 1) and ammonia nitrogen (Figure 2) concentrations in rumen fluid. The concentrations of ammonia nitrogen and peptide nitrogen in rumen fluid depend on the source and amount of the degradable protein in rumen (Reynal et al., 2007). Broderick and Wallace (1988) reported transient accumulation of peptide concentration in rumen fluid of sheep supplemented with casein. However, Vanhatalo et al. (2003) found a numerical increase in NH_3-N concentration in diet supplemented with caseinate in comparison with control diet. Casein is more rapidly degradable protein than most other proteins in rumen (Broderick and Wallace, 1988), therefore increased ammonia nitrogen concentration with CN infusion could be the result of the greater amount of caseinate degradation. In the present study, the level of NH_3-N/Pep-N was 0.77, 0.86 and 0.94 for treatments 1, 2 and 3, respectively. The results of the present study suggest that accumulated peptide in treatment 3 converted to ammonia nitrogen and the level of NH_3-N/Pep-N dramatically increased (up to 1.22 at 3 h after feeding) and even 6 h after feeding, was significantly higher among treatments (0.81, 0.92 and 1.02 for treatments 1, 2 and 3, respectively) (Figure 3).

The concentrations of acetate and branched chain VFA in rumen fluid increased in this study. Using different forms of nitrogen (peptides, amino acids and urea) in continuous culture, Griswold et al. (1996) found that the

Table 3. Least square means for DMI, performance and total tract apparent digestibility of nutrients in dairy cows received sodium caseinate.

Item	Treatments*			SEM
	1	2	3	
DMI, kg/d		16.4	15.3	1.43
Milk yield, kg/d	11.5	12.3	11.9	1.03
Milk fat, kg/d	0.38^{b}	0.41^{ab}	0.42^{a}	0.02
Milk protein, kg/d	0.34	0.38	0.36	0.09
Milk lactose, kg/d	15.7	0.60	0.58	0.07
Solids non fat, kg/d	0.79	0.83	0.81	0.11
Milk urea nitrogen, mg/dl	13.8^{b}	14.5^{ab}	16.1^{a}	0.33
Total tract apparent digestibility, %				
Dry matter	64.4	66.2	66.9	2.29
Organic matter	68.8	70.6	71.4	1.08
Crude protein	71.5	69.5	72.3	3.17
Neutral detergent fiber	50.6^{b}	54.1^{ab}	55.7^{a}	0.82
Acid detergent fiber	44.2^{b}	47.2^{ab}	49.3^{a}	0.97

$^{a, b, c}$ Least squares means within the same row without a common superscript differ ($P < 0.05$).
* Treatments 1, 2 and 3 were 0, 50 and 100 g/d sodium caseinate infused in the rumen, respectively.

Table 4. Least square means for urine volume, urinary purine derivatives excretion and microbial protein production in dairy cows infused with sodium caseinate in the rumen.

Item	Treatments*			SEM
	1	2	3	
Total urine volume, L/d	21.4^{b}	21.8^{ab}	22.7^{a}	0.96
Allantoin, g/d	51.52^{b}	54.28^{ab}	54.93^{a}	0.73
Uric acid, g/d	1.54	1.44	1.70	0.08
Allantoin, mM/d	326^{c}	342^{b}	347^{a}	2.36
Uric acid, mM/d	8.4^{b}	9.3^{ab}	11.4^{a}	0.88
Urea nitrogen, g/d	89.3^{b}	94.2^{ab}	102.9^{a}	1.16
PD**, mM/d	334.4^{b}	351.3^{ab}	358.4^{a}	4.13
Microbial crude protein, g/d	1315.9^{c}	1394.3^{b}	1409.1^{a}	8.43

^{a,b,c} Least squares means within the same row without a common superscript differ ($P < 0.05$).
* Treatments 1, 2 and 3 were 0, 50 and 100 g/d sodium caseinate infused in the rumen, respectively.
** PD = Purine derivatives excreted through urine calculated as; urine allantoin + urine uric acid.

greatest amounts of isobutyrate were produced by using 100% peptide nitrogen form. Hristov et al. (2004) found no effect of different rumen degradable protein levels on concentrations of total VFA, acetate, butyrate and propionate in rumen fluid and only they found an increase in the amount of isobutyrate with high RDP level. Although limited documents have been clarified on the increase of propionate by addition of degradable protein in the diets of dairy cows (Reynal and Broderick, 2005), most of the studies frequently have been shown the increase of acetate, butyrate and branched chain VFA by adding different amounts and sources of rumen degradable protein (Griswold et al., 1999; Yang, 2002; Griswold et al., 2003). It could be suggested that increased acetate and branched chain VFA concentrations in rumen fluid in this study probably was due to the positive effects of degradable protein on fiber digestibility.

Dry matter intake, performance and digestibility of nutrients

Dry matter intake of the cannulated cows did not differ by infusion of CN in the rumen. The study conducted by Reynal and Broderick (2005) showed that increasing rumen degradable protein from 10.6 to 13.2% did not have any significant effect on dry matter intake in dairy cows. Milk fat yield increased in this study probably due to increased NDF and ADF digestibility and also because acetate concentration was affected significantly by infusion

of CN that could affect milk fat production (Reynal and Broderick, 2005). Previous works clarified that increasing protein content of the dairy cow's diets, milk protein and milk urea nitrogen increased (Vanhatalo et al., 2003). In this study, milk protein yield did not differ among treatments but milk protein yield was increased in treatment 2 and MUN concentration increased in treatment 3. The results suggested that greater nitrogen source in rumen fluid have the potential to improve milk protein production but from the other hand, higher ammonia nitrogen concentration resulted by degradation of greater amount of CN in rumen increased urea concentration in milk.

Digestibility of organic matter has tended to be risen with infusion of CN in the rumen. Postruminally infusion of caseinate by Vanhatalo et al. (2003) clarified that organic matter (P = 0.06) and crude protein (P = 0.07) digestibility were tended to increase but they found no effect of postruminally caseinate infusion on NDF digestibility (P=0.21). Although digestibility of dry matter and crude protein were not affected significantly in the present study, digestibility of organic matter tended to increase and NDF and ADF digestibility increased significantly by infusion of CN in the rumen. In comparison with the results reported by Vanhatalo et al. (2003), it could be suggested that infusion site of the nitrogen source probably affect nutrients digestibility in a different manner. The reports by Yang (2002) clarified that increase in peptide nitrogen concentration in rumen fluid could directly affect fiber digestibility. Compared with the control treatment, infusion of CN increased branched chain VFA concentration; that probably also have potential to affect fiber digestibility. The work by Gorosito et al. (1985) showed that fiber digestion by ruminal bacteria was improved by branched-chain carbon skeleton supplementation. In addition, Griswold et al. (1996) reported that peptide addition in continuous culture increased branched chain VFA production that consequently could increase fiber digestion. On the other hand, Bryant (1973) stated that ammonia nitrogen in rumen fluid could stimulate the activity of cellulolytic bacteria. Therefore, increasing the level of ammonia nitrogen by infusion of CN probably increased the NDF and ADF digestibility. Fiber digestibility increased in this study probably either due to direct effects of increased peptide nitrogen concentration in rumen fluid or the effect of increased branched chain VFA and ammonia in rumen fluid and further studies are needed to demonstrate the unknown mechanisms of peptide and ammonia effects on fiber digestion.

Urinary parameters and microbial protein synthesis

Urine volume increased in this study probably due to the higher concentrations of ammonia nitrogen in rumen fluid. Extra nitrogen has to be excreted from the body via converting to urea in liver and then excrete via urine (Broderick et al., 1991). Urea nitrogen excreted through urine significantly increased when the level of CN infusion was increased suggesting that higher ammonia nitrogen concentration in rumen fluid increased urinary nitrogen excretion. The results of the present study showed that urine allantoin, urine uric acid and total urinary purine derivatives were affected by the infusion of CN in the rumen and subsequently estimated microbial protein synthesis in the rumen increased with infusion of CN. Griswold et al. (1996) stated that produced branched chain VFA would be utilized along with ammonia nitrogen to synthesize amino acids and form microbial protein. In this study, both ammonia nitrogen and branched chain VFA concentration were increased with infusion of CN in the rumen and had potential to increase microbial protein synthesis. Increased microbial protein production by addition of peptide nitrogen or amino acid nitrogen could also be due to the reason explained by Erfle et al. (1997). They stated that when peptides or amino acids are available, the anabolic and catabolic rates were more closely matched and less energy was spilled out as heat and this implies that improved growth rates of microbes with peptides may be due to energetic savings from reduced amino acid synthesis. Previous evidences indicated that peptides and free amino acids stimulate microbial yield and fermentation (Argyle and Baldwin, 1989; Chikunya et al., 1996). The results of the present study confirmed that infusion of CN in the rumen positively affected microbial protein synthesis, but whether the direct effects (peptide nitrogen) or indirect effects (branched chain VFA and ammonia nitrogen) influenced the microbial protein synthesis need confirmation by more studies. It could be suggested that extra ammonia nitrogen concentration is not a useful source of nitrogen for rumen microbes and therefore increasing the level of NH_3-N/Pep-N from 0.86 to 0.94 did not affect microbial protein production extensively (1409.1 versus 1394.3 g/d microbial protein production for the levels of 0.94 and 0.86, respectively).

Blood urea nitrogen and nitrogen efficiency

Urea nitrogen concentration in blood increased with infusion of CN in the rumen and consequently urea nitrogen excretion through urine was influenced when the blood urea nitrogen level increased. Urea nitrogen excretion through urine was considered by Broderick (2003) as an indicator of nitrogen efficiency. According to Kohn et al. (2005), blood urea nitrogen is also useful as an indicator of protein status and there is a strong linear relationship between blood urea nitrogen and urinary nitrogen excretion for cattle. In the present study, the highest blood urea nitrogen concentration and the highest urinary urea nitrogen were obtained when the level of NH_3-N/Pep-N was greater than 0.86. In ruminants,

excessive breakdown of protein to ammonia in the rumen can lead to inefficient utilization of dietary protein (Tamminga, 1979). Although infusion of CN in the rumen increased peptide nitrogen concentration that affected fiber digestibility and microbial protein synthesis, increased ammonia nitrogen in rumen caused increase in blood concentration of urea nitrogen and subsequently increased urea nitrogen excretion through urine to the environment. These results suggest that there is limitation of peptide utilization by the rumen microbes and accumulated peptide after feeding which is more than rumen microbes' requirements will be more degraded and converted to ammonia; this increases the urea nitrogen excretion through urine. Hristov et al. (2004) concluded that an excess of ruminally degradable protein in the diet of low-producing lactating dairy cows cannot be utilized efficiently for microbial protein synthesis and will be lost through urinary nitrogen excretion.

Conclusion

If the optimum level of NH_3-N/Pep-N was the best compromise among the need for rumen fermentation, microbial protein synthesis and nitrogen excretion through urine, the recommended level from this study would be 0.86 in rumen fluid.

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