Effects of supplementation with sodium caseinate of early lactation diet on performance, digestibility, microbial nitrogen flow, and nitrogen efficiency in Holstein cows

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Summary

Performance, digestibility of nutrients, microbial nitrogen flow (MNF) and nitrogen efficiency (NE) in early lactating Holstein cows were investigated by diet supplementation with sodium caseinate (CN). Multiparous lactating Holstein cows (n = 15) with an average body weight of 638 kg and 21 days in milk were assigned to a completely randomized design (five cows per treatment) and fed a basal diet with different CN levels (treatments 1, 2, and 3, contained 0, 50, and 100, g/d/head CN, respectively). The study lasted 49 days (first 14 days for adaptation and the last 35 days for collection of data). There were no statistical differences in dry matter intake, milk yield, milk lactose yield, and protein yield with CN supplementation. 3.5% fat corrected milk yield was increased by CN supplementation ($P<0.05$). Digestibility of ADF was increased by CN supplementation ($P<0.05$). The higher level of CN affected MNF was estimated by spot urine sampling technique ($P<0.05$). Predicted N excretion through urine was affected ($P<0.05$); however, there was no effect of CN supply on predicted N excretion through faeces. In conclusion, the results indicated that although supplementation with CN improved MNF, negligible effects on performance of the cows were observed. Furthermore, increased milk urea nitrogen (MUN) concentration and predicted urine N excretion revealed the lower NE in early lactating dairy cows supplemented with CN compared with control treatment.

Key words: Early lactating cows, Sodium caseinate, Nitrogen efficiency

Introduction

Ammonia is the preferred nitrogen source for most rumen bacteria species (Hungate, 1966). However, enhanced growth and efficiency of rumen microbes, as a result of providing nitrogen sources such as amino acids and peptides in addition to ammonia, has been demonstrated (Cotta and Russell, 1982; Russell and Sniffen, 1984). It seems that when amino acids and peptides are available, the anabolic and catabolic rates are more closely matched and less energy is spilled out as heat and this implies that improved growth rates of microbes with peptides and amino acids may be due to energetic savings from reduced amino acid synthesis (Erfle *et al*., 1977). Reynal *et al*. (2007) clarified how manipulation of the diets, which causes the production of peptide in the rumen, may have nutritional benefits for both ruminal microbes and the host animal which could be through improving the utilization of dietary nitrogen. Furthermore, peptides may contribute to amino acid absorption from the intestines of ruminants and may have nutritional benefits for the host animal (Matthews and Webb, 1995). Most of the studies that have investigated the effects of peptide nitrogen supplements are conducted *in vitro* (Griswold *et al*., 2003), continuous culture (Griswold *et al*., 1996), or on fistulated cows (Khalili and Huhtanen, 2002). It seems that higher passage rate in early lactating cows might cause the lower accessibility of rumen microbes to nitrogen sources (Griswold *et al*., 2003) and hence could affect milk

composition (Mohebbi-Fani *et al*., 2006). In the present study, we hypothesized that due to the higher passage rate in early lactating dairy cows, the CN supplementation more than the protein requirements of the animal might have positive effects on performance, nutrients digestibility, purine derivatives excreted through urine and MNF. The peptide nitrogen source used in this study (caseinate; CN) is a well-known source for peptide and amino acid nitrogen that has been used in previous works (Fu *et al*., 2001; Khalili and Huhtanen, 2002).

Materials and Methods

Cows, management and diets

Multiparous lactating Holstein cows (n = 15) averaging body weight of 638 ± 17 kg, 21 ± 5 days in milk and 30 ± 0.8 kg/d milk yield were assigned to a completely randomized design (five cows per treatment). The cows' rations were supplied with sodium caseinate in three levels (0, 50, and 100 g/d/head as treatments 1, 2 and 3, respectively). Supplemental CN was mixed with the morning diet at 08:00 every day. The 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 Agricultural Research Institute, Chazy, NY). The ingredients and the chemical composition of the basal diet are presented in Table 1. The experiment lasted 49 days and consisted of a 14-day adjustment period and a 35-day sample collection period. The study was conducted in a moderate temperature (March and April) in Karaj, Iran. The cows were kept in individual stalls and were fed twice daily at 08:00 and 16:00 h and were milked three times daily at 02:00, 10:00, and 18:00. The cows had free access to water and salt block and also they had enough space to walk. Orts were collected and weighed once daily at 07:00 and the individual feeding rate was adjusted daily to yield orts of about 5-10% of intake.

Table 1: Ingredients and chemical composition of basal diet in study

Item	Basal diet[1]
Ingredients, % of DM	
Alfalfa hay, Chopped	21
Corn silage	23
Barely, ground	17.5
Corn, ground	6
Wheat, ground	4.5
Wheat bran	3
Soybean meal, 44% CP	10
Canola meal	5
Cottonseed meal	4
Tallow	1.5
Beet pulp	3
Vitamin-mineral mix[2]	0.4
Calcium carbonate	0.3
Sodium bicarbonate	0.5
Salt	0.3
Chemical composition	
DM %	53
CP, % of DM	17.2
RDP, % of CP	10.3
RUP, % of CP	6.9
NEL[3], Mcal/kg	1.72
NDF, % of DM	31.2
ADF, % of DM	19.5
NFC, % of DM	40.3
Ether extract, % of DM	5.6
Ca, % of DM	0.59
P, % of DM	0.33
Na, % of DM	0.2

[1] Treatments in experiment 2 were; treatment 1 (basal diet), treatment 2 (basal diet + 50 g supplemented peptide nitrogen source) and treatment 3 (basal diet + 100 g supplemented peptide nitrogen source). [2] Composition: 190 g Ca, 80 g P, 21 g Mg, 3 g Fe, 60 g Na, 0.3 g Cu, 2 g Mn, 3 g Zn, 0.1 g Co, 1 g I and 0.3 g Se; 500,000 IU/kg of vitamin A, 200,000 IU/kg of vitamin D_3, 1,000 IU/kg of vitamin E. [3] Estimated using the NRC (2001) model

Experimental procedures and chemical analyses

The weekly composites of feed ingredients were determined for DM by drying at 60°C for 48 h (AOAC, 1990). Intake of DM was computed based on 60°C DM determinations for total mixed ration and orts. After drying, the ingredients and total mixed ration were ground through a 1 mm screen (Wiley Mill, Arthur H. Thomas, Philadelphia, PA), and composites were prepared by mixing equal DM. Composite samples were analysed for total nitrogen, DM at 105°C, ash and organic matter (AOAC, 1990), sequentially for neutral detergent fiber (NDF) and acid detergent

fiber (ADF) (Van Soest *et al*., 1991). Body weights and body condition score of the cows were recorded at three stages of the study with 12 day intervals (Wildman *et al*., 1982). Milk yield was recorded daily at three consecutive milking times throughout the sample collection period. Milk was sampled three times per week at three daily consecutive milking times; and samples were analysed for fat, protein, lactose and solids-non-fat (Milkoscan; Foss Electric, Hillerod, Denmark). Milk urea content was analysed by the method of Crooke and Simpson (1971) after urease treatment. On the last five days of the sample collection period, two faecal grab samples were collected 6 and 18 h after feeding (Reynal and Broderick, 2005). Faecal samples were dried in a forced draft oven (60°C; 72 h), and then ground through a 1-mm screen (Wiley mill). Equal DM from each faecal subsample was mixed to obtain one composite sample for each cow. Faecal samples were analysed for total N, NDF and ADF as described for feed samples. Apparent digestibility of organic matter (OM), dry matter (DM) and crude protein (CP) were also measured by using acid insoluble ash as an internal marker (Van Keulen and Young, 1977). On days 10 and 35 of sample collection, two urine samples per day at approximately 09:00 and 14:00 h were collected from all cows when urinated spontaneously and 10-ml aliquots were diluted immediately with 90 ml 0.036 N sulfuric acid and stored at -20°C for later analysis. After thawing, the concentration of creatinine, allantoin and uric acid were measured in urine samples (Chen and Gomes, 1992).

Calculations and statistical analyses

The parameters of urine nitrogen, faecal nitrogen and nitrogen efficiency were predicted using the following equations (Wattiaux and Karg, 2004):

Urine N output (g/d) = 0.0283 × MUN (mg/dL) × BW (kg)

Faecal N (g/d) = intake N (g/d) – urinary N output (g/d) – milk N (g/d)

N efficiency (%) = milk N (g/d)/intake N (g/d) × 100

Milk N output was calculated using the following equation: total milk protein/6.38 (Brito and Broderick, 2007).

Daily urinary volume and purine derivatives (PD; allantoin plus uric acid) were estimated from urinary creatinine concentration assuming a creatinine excretion rate of 29 mg/kg of body weight (BW) (Valadares *et al*., 1999). The MNF was estimated by the equations described by Chen and Gomes (1992). Endogenous PD excretion (mmol/d) was estimated from body weights of individual cows as: 0.385 mmol/0.75 BW per day (Chen and Gomes, 1992). Total absorption of microbial purines was calculated as:

Purine absorption (mmol/d) = (PD excretion – 0.385 × 0.75 BW)/0.85

where,

0.85 = The absorptive efficiency of purines (Chen and Gomes, 1992).

Microbial nitrogen flow through rumen was computed as:

Microbial N (g/d) = (purine absorption × 70) / (0.134 × 0.83 × 1000)

Where,

70 = The N content of purines (mg N/mmol)

13.4:100 = The mean ratio of purine-N: total-N measured for mixed rumen microbes (Valadares *et al*., 1999)

0.83 = The assumed digestibility of microbial purines (Chen and Gomes, 1992).

Collected data were analysed using Proc Mixed in SAS (2000). The following model was fitted to all variables that did not have repeated measurements over time:

$$Y_i = \mu + T_i + \varepsilon_i$$

Where,

Y_i: The dependent variable

μ: The overall mean

T_i: The effect of treatment i

ε_i: The residual error

The following model was used for variables, which were repeated measurements over time (milk yield and composition, PD, and urinary creatinine):

$$Y_{ij} = \mu + T_i + Z_j + ZT_{ij} + \varepsilon_{ij}$$

Where,

Y_{ij}: The dependent variable

μ: The overall mean

T_i: The effect of treatment i

Z_j: The effect of time j

ZT_{ij}: The interaction between time j and

treatment i
ε_{ij}: The residual error

Differences between least squares means were considered significant at P<0.05, and differences were considered to indicate a trend toward significance at 0.05<P<0.10.

Results

Dry matter intake and lactation performance data of the cows are presented in Table 2. Supplied CN in lactating dairy cows had no significant effects on DMI, milk yield, milk protein yield and milk lactose yield. However, milk fat yield tended to increase (P=0.09) and 3.5% fat corrected milk (FCM) production was increased by CN supplementation (P<0.05). Compared with control treatment, MUN concentration increased in supplemented treatments (P<0.05). The body condition score and BW changes were not affected by treatments (P>0.05) (Considering that the initial body condition scores for the cows were 3.8, 3.7 and 3.7 for treatments 1, 2 and 3, respectively). The apparent digestibility of DM, OM, CP and NDF was not affected by CN supplementation. Digestibility of ADF was increased by the greater supply of CN (P<0.05). Urinary creatinine excretion was 88.4, 91.2 and 91.8 mg/dl for treatments 1, 2 and 3, respectively (P>0.05). Urine volume estimated through spot urine sampling technique did not differ among treatments (Table 3). In comparison with the basal diet, the concentrations of urine allantoin and urine PD increased by CN supplementation (P<0.05). Estimated MNF numerically increased with CN supply and was about 11.3 g microbial N (~70.5 g microbial protein) higher in treatment 3 compared with control treatment. Predicted nitrogen excretion through faeces and predicted milk nitrogen efficiency did not show any significant differences among treatments, however predicted nitrogen excretion through urine was affected by CN supplementation (P<0.05).

Discussion

In the present study milk fat yield tended to be increased and 3.5% FCM yield was increased by CN supplementation. In agreement with our results, previous studies demonstrated that milk fat yield improved by ruminal infusion of casein (Vanhatalo *et al*., 2003). Groff and Wu (2005) also showed similar results with increasing crude protein level of dairy cow diet (Groff and

Table 2: Least square means for DMI, performance and apparent digestibility of nutrients through total tract of early lactating dairy cows supplemented with caseinate

Item	Treatments[1]				
	1	2	3	SEM	P
DMI, kg/d	22.91	23.32	23.65	0.62	0.42
Milk yield, kg/d	38.71	39.54	39.32	0.43	0.57
3.5% FCM,[2] kg/d	36.19[b]	37.14[a]	37.65[a]	0.26	0.02
Milk fat, kg/d	1.21	1.24	1.28	0.03	0.09
Milk protein, kg/d	1.19	1.23	1.23	0.05	0.46
Milk lactose, kg/d	1.92	1.95	1.94	0.05	0.86
Milk total solids, kg/d	4.55	4.67	4.68	0.1	0.71
Milk urea nitrogen, mg/dl	16.97[b]	17.14[ab]	18.13[a]	0.12	0.01
BW change, kg/d	0.08	0.12	0.14	0.01	0.23
Body condition score	2.9	2.8	2.9	0.03	0.88
Apparent digestibility (% DM)					
Dry matter	67.3	67.8	68.5	1.07	0.33
Organic matter	71.1	72.3	72.9	0.85	0.38
Crude protein	69.5	70.9	69.3	0.82	0.40
Neutral detergent fiber	55.1	55.8	58.3	0.68	0.08
Acid detergent fiber	49.5[b]	50.1[ab]	53.4[a]	0.63	0.001

[1] Treatments 1, 2 and 3 were 0, 50, and 100 g/d sodium caseinate dietary supplemented, respectively. [2] 3.5% fat corrected milk. [a, b, c] Least square means within the same row without a common superscripts differ (P<0.05)

Table 3: Least square means for purine derivatives and microbial nitrogen flow through rumen of early lactating dairy cows supplemented with caseinate

Item	Treatments[1]				
	1	2	3	SEM	P
Urine volume, L/d	24.4	24.2	23.8	0.52	0.61
Urinary allantoin, mmol/d	464.8[c]	469.7[b]	480.8[a]	5.33	0.01
Urinary uric acid, mmol/d	23.41	24.18	23.02	0.47	0.24
PD, [2] mmol/d	488.3[b]	493.9[ab]	503.9[a]	6.12	0.02
MNF, [3] g/d	325.6	329.6	336.9	5.21	0.13

[1] Treatments 1, 2 and 3 were 0, 50, and 100 g/d sodium caseinate dietary supplemented, respectively. [2] PD = Urinary purine derivatives (urinary allantoin + urinary uric acid). [3] MNF = Microbial nitrogen flow; calculated based on Chen and Gomes (1992). [a, b, c] Least square means within the same row without a common superscripts differ (P<0.05)

Wu, 2005). Fat yield was positively affected when Groff and Wu (2005) fed dairy cows with different protein concentrations (15 to 18.75% CP). Fat yield increased in our study probably due to increased ADF digestibility. Animal feeds showed different rates of ruminal disappearance of protein (Jahani-Azizabadi *et al*., 2009) and the higher degradable protein in rumen positively affects fiber digestibility (Yang, 2002) that have the potential to increase milk fat yield (Cyriac *et al*., 2008). Our results showed that digestibility of ADF was improved by CN supplementation and NDF digestibility tended to rise (P=0.08). The results of the present study confirm that diet supplied with CN may have positive effects on fiber digesting bacteria in rumen and consequently on milk fat yield. Some studies showed that addition of peptide and amino acid nitrogen sources improved digestion of OM, DM and fiber (Cotta and Russell, 1982; Yang, 2002). Elevated MUN concentration in diets supplied with CN indicated that protein contents higher than required levels in early lactating cow's diets have no favorable effect on milk protein content.

In the present study, urine volume estimated based on creatinine excretion did not differ among treatments (P<0.05). Regarding the spot urine sampling technique, creatinine excretion and body weight are considered as two main components for estimation of urine volume. None of these factors were affected by CN supplementation and hence estimated urine volume was not different among treatments. However, urinary PD excretion increased by CN supplementation. Moreover, urine allantoin was affected by CN supply in diets. Previous reports indicated that peptides and free amino acids stimulate microbial yield and fermentation (Argyle and Baldwin, 1989; Chikunya *et al*., 1996).

Duodenal flow of nucleic acids is essentially all of microbial origin and, after intestinal digestion of the purine nucleotides, absorbed adenine and guanine is catabolized and proportionally excreted as PD (Valadares *et al*., 1999), therefore it could be concluded that increased PD excretion with CN supplementation was due to increased microbial nitrogen flow through rumen. The MNF was improved by CN supplement in early lactating dairy cows diets. As PD excretion increases, the MNF will increase (Chen and Gomes, 1992). Predicted N excretion through urine was also affected by supplementing CN in this study. However, predicted N excretion through faeces was almost similar among treatments. Nitrogen intake was affected by treatments and cows on treatment 3 were fed about 34 g/d more nitrogen compared with control (Table 4). Greater N intake in treatment 3 is related to higher N intake because of CN supplementation on the one hand, and to the greater DMI on the other hand (about 740 g in treatment 3 compared to treatment 1). Although N efficiency did not differ among treatments, the lowest N efficiency was gained in treatment 3 (28.86%). Brito and Broderick (2007) supplemented the dairy cow's diets with different nitrogen sources and found the lowest nitrogen efficiency (24.9%) for urea supplemented diet than other protein sources. Cyriac *et al*. (2008) found a linear decrease in milk nitrogen efficiency by increasing the rumen degradable protein levels. In that study, milk

Table 4: Least square means for predicted nitrogen output though faeces and urine and nitrogen efficiency in early lactating dairy cows supplemented with caseinate

Item	Treatments[1]				
	1	2	3	SEM	P
Intake N, g/d	630.3[b]	648.5[a]	664.5[a]	10.23	0.003
Milk N output, [2] g/d	186.4	192.3	192.1	4.15	0.47
Predicted urine N, [3] g/d	305.9[b]	316.1[ab]	327.1[a]	6.64	0.02
Predicted fecal N, [4] g/d	138.2	140.1	145.4	4.13	0.57
N efficiency, [5](%)	29.53	29.62	28.86	0.73	0.46

[1] Treatments 1, 2 and 3 were 0, 50, and 100 g/d sodium caseinate dietary supplemented, respectively. [2] Predicted urine N output (g/d) = 0.0283 × MUN (mg/dL) × BW (kg) (Wattiaux and Karg, 2004). [3] Predicted faecal N (g/d) = intake N (g/d) – urinary N output (g/d) – milk N (g/d) (Wattiaux and Karg, 2004). [4] Milk N output (g/d) = total milk protein/6.38 (Brito and Broderick, 2007). [5] N efficiency (%) = Milk N output (g/d)/intake N (g/d) × 100 (Brito and Broderick, 2007). [a, b, c] Least square means within the same row without a common superscripts differ (P<0.05)

nitrogen efficiencies were 38.6, 35.5, 30.9 and 27.7% for degradable protein levels of 7.6, 8.8, 10.1 and 11.3%, respectively. In the present study, although NE in diet supplemented with 50 g/d CN had no considerable difference with control treatment, 100 g/d supplemental CN numerically decreased NE, suggesting that protein supplementation more than the requirements in early lactating dairy cows has the potential to decrease NE.

The results of the present study showed that supplementation of CN increased PD excretion and improved MNF, however, the effects on performance of cows was negligible. Furthermore, elevated MUN concentration and greater urine N prediction excretion suggest that supplementation with CN in early lactating dairy cow's diets causes the lower efficiency of N. Further studies must be encouraged to investigate the replacement of the peptide nitrogen source with some part of protein in early lactation diets.

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