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Effect of Different Rumen-degradable Carbohydrates on Rumen Fermentation, Nitrogen Metabolism and Lactation Performance of Holstein Dairy Cows

A. Khezri*, K. Rezayazdi, M. Danesh. Mesgaran[1] and M. Moradi-Sharbabk

Department of Animal Science, Faculty of Agriculture, University of Tehran, Karaj, P.O. Box 31587-77871, Iran

ABSTRACT : Four multiparous lactating Holstein cows fitted with rumen cannulae were fed diets varying in the amount and source of rumen-degradable carbohydrates (starch vs. sucrose) to examine their effects on rumen fermentation, nitrogen metabolism and lactation performance. A 4×4 Latin square with four diets and four periods of 28 days each was employed. Corn starch and sucrose were added to diets and corn starch was replaced with sucrose at 0 (0 S), 2.5 (2.5 S), 5.0 (5.0 S) 7.5% (7.5 S) of diet dry matter in a total mixed ration (TMR) containing 60% concentrate and 40% forage (DM basis). Replacing corn starch with sucrose did not affect ($p>0.05$) ruminal pH which averaged 6.41, but the ruminal pH for 7.5 S decreased more rapidly at 2 h after morning feeding compared with other treatments. Sucrose reduced ($p\leq0.05$) ruminal NH_3-N concentration (13.90 vs. 17.09 mg/dl) but did not affect peptide-N concentration. There was no dietary effect on total volatile fatty acids (110.53 mmol/L) or the acetate to propionate ratio (2.72). No differences ($p>0.05$) in molar proportion of most of the individual VFA were found among diets, except for the molar proportion of butyrate that was increased ($p\leq0.05$) with the inclusion of sucrose. Total branched chain volatile fatty acids tended to increase ($p\geq0.051$) for the control treatment (0 S) compared with the 7.5 S treatment. Dry matter intake, body weight changes and digestibility of DM, OM, CP, NDF and ADF were not affected by treatments. Sucrose inclusion in the total mixed ration did not affect milk yield, but increased milk fat and total solid percentage ($p\leq0.05$). Sucrose tended ($p\geq0.063$) to increase milk protein percentage (3.28 vs. 3.05) and reduced ($p\leq0.05$) milk urea nitrogen concentration (12.75 vs. 15.48 mg/dl), suggesting a more efficient utilization of the rapidly available nitrogen components in the diet and hence improving nitrogen metabolism in the rumen. (**Key Words :** Sucrose, Rumen Degradable Carbohydrates, Rumen Fermentation, Nitrogen Metabolism, Lactation Performance)

INTRODUCTION

The efficiency of utilization of dietary N for milk protein synthesis in high- producing dairy cows is relatively low (19-21%) and loss of NH_3-N in the rumen is the main reason for this low efficiency (Tamminga, 1992). Rumen ammonia concentration is inversely related to the rate of energy fermentation and different studies (Hristov and Jouany, 2005) indicated that the efficiency of dietary N utilization will be improved when synchronization of carbohydrate and protein fermentation happened in the rumen. Starch and sugars are the two dietary sources of energy for the high- producing dairy cows (Varga, 2003). Although these sources of carbohydrates besides other non-fiber carbohydrates are considered equal regarding fermentation characteristics, their fermentation produces different volatile fatty acid (VFA) profiles (Strobel and Russell, 1986; Ariza et al., 2001) and has varied effects on ruminal pH (Strobel and Russell, 1986; Khalili and Huhtanen, 1991), microbial product yield (Hall and Herejk, 2001; Sannes et al., 2002) and fiber digestion (Heldt et al., 1999; Miron et al., 2002). As documented in the Cornell Net Carbohydrate and Protein (CNCPS) model, sugars are considered to have a fast degradation rate, and starch an intermediate rate (Sniffen et al., 1992). Moreover, the CNCPS indicated that the organisms that ferment soluble sugars could contribute approximately 18% more microbial protein than the organisms that ferment starches in high moisture corn. This would imply that supplementation of dairy cows diets with sugars would result in more effective capture of rapidly available rumen degradable protein and improved supply of metabolizable protein to the dairy cow. Results of some studies showed that sucrose stimulates dry

* Corresponding Author: A. Khezri. Tel: +98-9133977126, Fax: +98-261-2246752, E-mail: akhezri@mail.uk.ac.ir

[1] Department of Animal Science, Faculty of Agriculture, Ferdowsi University of Mashhad, Mashhad, P.O. Box 91775-1163, Iran.

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Table 1. Ingredients and chemical composition of diets fed to animals (% of DM)

Item	Treatments[1]			
	0 S	2.5 S	5.0 S	7.5 S
Ingredients				
Alfalfa hay	30.00	30.00	30.00	30.00
Corn silage	10.00	10.00	10.00	10.00
Barley grain	25.00	25.00	25.00	25.00
Wheat bran	6.50	6.50	6.50	6.50
Soybean meal	19.00	19.00	19.00	19.00
Sodium bicarbonate	0.62	0.62	0.62	0.62
Calcium carbonate	0.33	0.33	0.33	0.33
Vitamins and minerals	0.86	0.86	0.86	0.86
MagOx	0.19	0.19	0.19	0.19
Sucrose	0.00	2.50	5.00	7.50
Corn starch	7.50	5.00	2.50	0.00
Chemical composition				
DM	70.24	70.31	70.63	70.19
OM	92.13	92.75	92.88	92.47
CP	17.01	17.19	17.36	17.28
RDP (% of CP)[3]	68.12	68.12	68.12	68.12
RUP (% of CP)[3]	31.88	31.88	31.88	31.88
NDF	32.92	33.06	32.28	32.46
ADF	19.24	19.67	19.32	19.75
NFC[4]	40.05	40.32	41.23	40.56
NEL[3] (Mcal/kg)	1.67	1.67	1.67	1.67

[1] 0 S = 0% Sucrose, 2.5 S = 2.5% sucrose, 5.0 S = 5.0% sucrose, and 7.5 S = 7.5% sucrose of diet dry matter substituted for corn starch.
[2] Provided 65 mg of Zn, 51 mg of Mn, 22 mg of Fe, 15 mg of Cu, 0.9 mg of I, 0.5 mg of Co, 0.4 mg of Se, 6,640 IU of vitamin A, 2,500 IU of vitamin D, and 17 IU of vitamin E/kg of DM.
[3] Estimated using the NRC (2001) models.
[4] NFC = Non-fiber carbohydrate (organic mater-CP-ether extract-NDF).

matter intake (Broderick et al., 2000) and is effective in reducing ruminal ammonia concentration and increasing milk protein yield (Sannes et al., 2002). However, there is limited information about the effects of different sources of rumen degradable carbohydrates (starch vs. sucrose) on ruminal nitrogen metabolism, peptide nitrogen and animal performance The objective of this study was to determine the effects of two sources of carbohydrates with different rates of fermentation (starch vs. sucrose) on rumen nitrogen metabolism, peptide nitrogen, rumen fermentation and lactation performance of Holstein dairy cows, when corn starch was replaced by sucrose in the total mixed rations.

MATERIAL AND METHIODS

Cows, experimental design and diets

Four multiparous lactating Holstein cows (4 years of age), previously fitted with rumen cannulae (10 cm i.d.; Bar-Diamond Inc., Parma, ID), that averaged 665±45 kg in body weight (BW) and 170±22 days in milk (DIM) were used in this experiment. The surgery on animals was done according to procedures approved by the University of Tehran, Laboratory Animal Care Advisory Committee. Cows were housed in individual stanchions equipped with water bowls and bedded with rubber mats and straw. Cows had free access to salt stone. With the exception of the last day of each period when samples were being collected, cows were allowed to exercise in a dry lot from 1200 to 1300 h. Cows were fed a TMR at 0800 and 1900 h for *ad libitum* intake to allow 10% orts with half of the daily feed allotment offered at each feeding and were milked twice daily at the same time. The experimental design was a 4×4 Latin square with four periods of 28 days each. The first 21 d of each period were used to adapt the cows to treatments, and the remaining 7 d were used to collect data. Each cow was randomly assigned to one of 4 diets. Corn starch and sucrose were added to diets and corn starch were replaced with sucrose. The four experimental diets were: i) 7.5% corn starch+0.0% sucrose (0 S), ii) 5.0% corn starch+2.5% sucrose (2.5 S), iii) 2.5% corn starch+5.0% sucrose (5 S), and iv) 0.0% corn starch+7.5% sucrose (7.5 S) of diet dry matter in a total mixed ration (TMR) containing alfalfa hay, corn silage, barley, soybean meal and Min-Vitamin mixture. The ingredients and chemical composition of the diets are shown in Table 1.

Measurements and analytical methods

Body weight was measured at the beginning of period 1 (d 1) and at the end of each of the four periods (d 28) at the same time on each day. Dry matter intake and orts were measured and recorded daily. Samples of individual feed ingredients, TMR, and orts were collected for the last 5 d of each period. Samples of individual feedstuffs, TMR and orts (composited per cow and period) were dried at 55°C in an oven for 72 h, ground through a 1 mm screen in a Wiley Mill (Arthur Hill Thomas Co., Philadelphia, PA). Grab fecal samples (200 g per sampling) were collected from the rectum at 0800 and 1900 during the 5 days of each period. Samples were then composited per cow and period and oven-dried at 55°C for 72 h and then ground through a 1 mm sieve. Samples of individual feedstuffs, TMR and orts were analyzed for DM, OM, ether extract, Kjeldahl N (AOAC, 1999), NDF and ADF (Van Soest et al., 1991). Total-tract apparent digestibility of DM, OM, CP, NDF and ADF were determined using acid-insoluble ash (AIA; Van Keulen and Young, 1977).

Samples of ruminal fluid were collected from multiple sites in the rumen at 0, 1, 2, 4, 6, and 8 h post-a.m. feeding on the last two days of each experimental period. Samples of ruminal fluid were strained through two layers of cheesecloth and immediately the pH was measured using a portable pH meter with a combination electrode. Ruminal fluid (8 ml) from each collection at 0, 2, 4, 6 and 8 h was combined with 2 ml of 25% (wt/vol) metaphosphoric acid and frozen for VFA analysis and 20 ml was combined with 20 ml 0.2 N HCl (Robles and et al., 2007) and frozen for

Table 2. Least square means for ruminal fermentation parameters of lactating dairy cows fed diets with increasing levels of sucrose

Item	Treatments[1]				SE
	0 S	2.5 S	5.0 S	7.5 S	
pH	6.46	6.49	6.38	6.31	0.04
Ammonia N (mg/dl)	17.09 [a]	16.37 [a]	13.90 [b]	14.36 [b]	0.37
Peptide N (mg/L)	149.22	160.50	195.57	184.65	17.91
Total VFA (mmol/L)	105.38	112.35	113.24	111.17	3.89
VFA (mol/100 mol)					
Acetate (A)	61.01	61.15	62.11	61.28	1.41
Propionate (P)	23.38	23.22	22.44	21.86	0.44
Butyrate	11.76 [b]	11.94 [b]	12.35 [ab]	13.57 [a]	0.31
BCVFA [2]	3.85	3.69	3.10	3.29	0.17
A:P ratio	2.62	2.65	2.79	2.83	0.07

[1] 0 S = 0% sucrose, 2.5 S = 2.5% sucrose, 5.0 S = 5.0% sucrose, and 7.5 S = 7.5% sucrose of diet dry matter substituted for corn starch.
[2] Branched chain fatty acids. [a, b] Least squares means within the same row without a common superscript differ ($p<0.05$).

ammonia analysis. Thirty milliliters of ruminal fluid at 0, 2, 4, 6 and 8 h post-a.m. feeding, were collected for low molecular peptide-N concentration and prepared for analysis according to Chen et al. (1987). These samples were immediately centrifuged at 1,000×g for 10 min to remove protozoa and feed particles. The supernatant was then centrifuged at 30,000×g for 25 min to remove bacteria and then the supernatant was frozen for subsequent analysis. After thawing, ruminal fluid samples for VFA were centrifuged at 30,000×g for 20 min. Ruminal VFA concentration was measured in the supernatant by 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). Nitrogen was the carrier gas and the temperature of the injector port and column was 175°C and 125°C, respectively. Ruminal NH_3-N was determined according to the procedures outlined by Crooke and Simpson (1971). Peptide-N concentration of rumen fluid samples (mg/L) was determined using the Kjeldahl method according to the procedures of Chen et al. (1987). Milk yields were calculated for the last week of each period. For compositional analyses, milk samples were collected from the a.m. and p.m. milking on two consecutive days (days 25 and 26), and analyzed for fat, protein, lactose, total solids (TS), solids not fat (SNF) and milk urea nitrogen (MUN) at the Central Milk testing Laboratory of Tehran.

Statistical analysis

Data were analyzed using the PROC MIXED procedure of 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:

$$Y_{jkl} = \mu + P_j + C_k + T_l + \varepsilon_{jkl}$$

Where Y_{jkl} is the dependent variable, μ is the overall mean, P_j is the effect of period j, C_k is the effect of cow k, T_l is the effect of treatment l, and ε_{jkl} is the residual error.

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

$$Y_{jklm} = \mu + P_j + C_k + T_l + Z_m + ZT_{ml} + \varepsilon_{jklm}$$

where Y_{jklm} is the dependent variable, μ is the overall mean, P_j is the effect of period j, C_k is the effect of cow k, T_l is the effect of treatment l, Z_m is the effect of time m, ZT_{ml} is the interaction between time m and treatment l, and ε_{jklm} is the residual error. Various variance-covariance error structures were used, depending on which error structure produced the lowest Akaike's information criterion and Bayesian information criterion values for each variable. Considering this, the heterogeneous autoregressive structure ARH (1) was selected as the appropriate covariance structure. Differences between least squares means were considered significant at $p<0.05$, using PDIFF in the LSMEANS statement.

RESULTS AND DISCUSSION

Ruminal pH, nitrogen metabolism and volatile fatty acid concentration

In the present study, replacing corn starch with sucrose in TMR did not affect ($p>0.05$) mean ruminal pH and averaged 6.41 (Table 2), but the ruminal pH for 7.5 S diet decreased more rapidly at 2 h after morning feeding compared with other treatments (Figure 1). These results are consistent with some studies (Broderick et al., 2000), where ruminal pH of dairy cows was similar among treatments and averaged 6.19 and the results obtained by Sannes et al. (2002) in which ruminal pH was not affected by including 3.2% sucrose in the diet and averaged 6.02 .Our results were inconsistent with others (Khalili and Huhtanen, 1991; Lee et al., 2003) who reported a significant effect of sucrose inclusion in the diet on ruminal pH. Lee et