Effects of Feeding System on Rumen Fermentation Parameters and Nutrient Digestibility in Holstein Steers

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ABSTRACT : In order to compare effects of feeding systems on rumen fermentation characteristics and nutrient digestion, steers were fed either total mixed ration (TMR) or separate concentrate-roughage ration (CR). Total tract digestibility of nutrients was higher in steers receiving TMR. Especially, DM, ADF and NDF in TMR were digested to a greater extent than those in CR. Rumen pH was not influenced by the feeding systems. Holstein steers on TMR had higher ruminal NH_3-N than those on CR. Feeding system did not alter VFA production but TMR feeding resulted in lower A/P ratio. TMR feeding tended to increase the number of bacteria and protozoa in the rumen fluid. Also steers fed TMR generally had higher fiber degrading enzyme activities, which might be the result of increased number of cellulolytic microbes in the rumen of animals on TMR. Our results indicate that TMR may provide more favorable condition for nutrient digestion both in the rumen and in the total tract of steers. ***(Asian-Aust. J. Anim. Sci. 2003. Vol 16, No. 10 : 1482-1486)***

Key Words : TMR, Feeding System, Celullase, Xylanase, *in vivo* Digestibility

INTRODUCTION

Total mixed ration (TMR) has been used with a great interest by farmers because of its expected benefits in nutrition, management and production of ruminant animals by early researchers (Owen, 1979; Howard et al., 1986; Sirhi et al., 2001). Moseley et al. (1976), McGilliard et al. (1983) and Nock et al. (1985) reported that TMR system helped to maintain rumen pH and A/P ratio because TMR could provide more balanced ration with a uniform rate of roughage and concentrate and increased DM intake. For the high yielding lactating dairy cattle which require high concentrate feeds, TMR has been known to give benefits by increased meal frequency and feed intake, enhanced fiber digestion and nitrogen utilization, and increased milk yield and milk fat production (Moseley et al., 1976; Owen, 1984). TMR is a proper type of feed especially when agricultural by-products with high moisture are to be included. Nutritive value of these by-products has been reported by Givens (1987) and Njie and Reed (1995). Also, Miron et al. (2002) showed an improved feed efficiency with partial replacement of corn by citrus pulp in TMR of high producing dairy cows. Although fair amount of information is available on the merit of TMR feeding system, its effects on rumen fermentation characteristics, especially microbial population and cellulolytic enzymes have not been clearly shown. We herein report effects of TMR in comparison with conventional feeding system on ruminal fermentation characteristics and nutrient digestibility in Holstein steers.

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MATERIALS AND METHODS

Digestion trial

Four rumen fistulated Holstein steers (average body weight of 300 kg) were divided into two groups and fed either 2×2 total mixed ration (TMR) or conventional ration (separate feeding of concentrate mixture and roughage, CR) with crossover design. Rations were formulated in a way that both groups will receive the same CP and TDN. Animals were fed with each feed at the level of 2% body weights on DM basis at 9:00 AM and 6:00 PM during the experiment. The formulation and chemical analysis of experiment feeds are showed in the Table 1. Water and trace mineral salt were available free-choice. Approximate analysis was carried out by the method of AOAC (1990), and ADF and NDF were analyzed by the method of Goering and Van Soest (1970). TDN was calculated by Adams's equation (1994). Digestibility *in vivo* was expressed as an apparent digestibility.

Rumen fermentation characteristics

Rumen contents were sampled at the end of digestion study. Ruminal fluid samples were taken before feeding, and 3, 6 and 9 h after the morning feeding and strained through four layers of cheesecloth.

Roll tube methods of Hungate (1966 and 1969) was used to measure rumen bacterial population. One ml of rumen fluid was diluted to 10^{-8}, 10^{-9} and 10^{-10}, inoculated into 9 ml medium with anaerobic gassing system and incubated at 39°C for 48 or 72 h. Total bacteria count was estimated by colony forming unit (CFU). Rumen fungal counts were measured by using Lowe's medium (Lowe et al., 1985) containing 0.121% penicillin-G, 0.026% streptomycin and 0.06% chloramphenicol. Rumen fluid (1

Table 1. The formula and chemical composition of experimental feeds

Item	Total mixed ration	Conventional ration		
Ingredient (%, as fed basis)				
Beet pulp	4.8	-		
Tall fescue straw	14.3	-		
Brewers' grain	14.3	-		
Apple pomace	14.3	-		
Barley bran	9.5	-		
Wheat bran	7.7	-		
Gluten	7.4	-		
Corn grain	9.5	-		
Supplements[a]	18.2	-		
Rice straw	-	30.8		
Alfalfa cube	-	13.4		
Commercial concentrate	-	55.8		
Chemical composition (%, on dry matter basis)				
		Rice straw	Alfalfa cube	Commercial concentrate
Dry matter	55.87	89.76	86.26	86.98
Total digestible nutrients	67.53	41.70	60.37	78.10
Crude protein	15.23	3.62	18.57	17.51
Ether extract	4.37	1.68	2.84	4.95
Crude fiber	15.98	32.54	27.73	6.48
Crude ash	8.30	10.85	10.15	7.58
Neutral detergent fiber	47.66	77.27	37.88	30.32
Acid detergent fiber	25.24	50.32	32.19	14.98

[a] Contains molasses (2.9%), liquified yeast (14.0%), salts (0.3%), sodium bicarbonate (0.5%), calcium phosphate (0.3%), and vitamin and mineral mixture (0.2%).

Table 2. Influence of feeding system on *in vivo* digestibility of nutrients (%)

Item	CR feeding	TMR feeding	SEM	Significance
DM	60.87	66.23	1.300	*
CP	58.88	68.46	1.601	**
EE	78.17	80.69	2.190	NS
NDF	54.84	64.10	1.884	**
ADF	50.43	59.88	1.840	**

*, ** Significant at $p<0.05$ or 0.01. NS: Non-significant.

ml) was diluted to 10^{-2}, 10^{-3} and 10^{-4}, inoculated into 9 ml medium and was incubated at 39°C for 48 or 96 h. Total fungal population was estimated by counting thallus-forming unit (TFU). Protozoa was counted on the hematocrit under light microscope (×150-200) after stained 1 ml sample with 4 ml methylgreen formalin-saline (MSF, Ha et al., 2003). Ruminal pH was measured by pH meter (Mettler Delta 340). To determinate volatile fatty acids (VFA) and NH_3-N, 1 ml rumen fluid was treated with 0.2 ml HPO_3 for 30 min and stored at -20°C. VFA concentration of rumen fluid was analyzed with gas chromatography (HP6890, USA) by the method proposed by Erwin et al. (1961) after the samples were strained through 0.45 μm of disposable micro filter. NH_3-N contents were measured at A_{630} with UV-spectrophotometer (UV-1601) according to the method of Chaney and Marbach (1962). Activity of celullase (CMCase) and xylanase was measured by using 2% (w/v) carboxyl methylcellulose (CMC) and 1% (w/v) oat spelt xylan in 0.05 M citrate buffer (pH 5.0) as a substrate, respectively. The rumen sample and respective substrate in 0.5 ml was incubated at 39°C for 1 h for enzyme reaction, put into ice bath for 30 min to stop the reaction and centrifuged at 6.000 rpm for 15 min. To measure the concentration of reduced sugar, 0.2 ml supernatant was mixed with DNS (dinitrosalicyclic acid) reagent, placed in a boiling water bath for 5 min, cooled to room temperature, and OD was measured at A_{540} with standard calibration using glucose (Miller, 1959). One unit of enzyme activity was defined as the amount of enzyme that released 1 μmol reducing sugar per min under the above condition (μmol reducing sugar/ml/min).

Statistical analysis

Data analysis was carried out with PROC GLM of SAS (1995). Treatment means were compared by a t-test (Steel and Torrie, 1980), and the differences were considered significant when $p<0.05$ or 0.01.

RESULTS AND DISCUSSION

Total tract nutrient digestibility

Steers receiving TMR generally had higher nutrient digestibility as shown in Table 2. This trend was true especially in the digestibility of DM, CP, NDF and ADF. The extent of improvement for DM, CP, NDF and ADF was 8.8, 16.3, 16.9 and 18.7%, respectively. Effects of TMR on nutrient digestion in the literature are conflicting. For

Table 3. Effects of feeding system on ruminal pH and NH_3-N concentration

Time (h)	CR feeding	TMR feeding	SEM	Significance
	pH			
0	6.97	7.01	0.049	NS
3	6.05	6.01	0.053	NS
6	6.22	6.10	0.093	NS
9	6.74	6.69	0.044	NS
Mean	6.50	6.45	0.051	NS
	NH_3-N (mg/100 ml)			
0	6.60	5.72	0.234	NS
3	9.92	17.33	1.143	**
6	2.80	7.46	0.887	**
9	4.00	3.42	0.458	NS
Mean	5.83	8.48	0.543	**

** Significant at 0.01. NS: Non-significant.

Table 4. Ruminal VFA concentration as influenced by feeding system

Time (h)	CR feeding	TMR feeding	SEM	Significance
	Acetic acid (mM)			
0	56.59	52.21	1.840	NS
3	68.41	67.88	3.122	NS
6	67.80	63.92	2.774	NS
9	64.45	51.24	2.793	NS
Mean	64.31	58.81	2.123	NS
	Propionic acid (mM)			
0	13.39	15.75	0.898	NS
3	20.20	26.09	1.232	*
6	18.70	21.17	0.856	NS
9	16.52	15.59	0.667	NS
Mean	17.23	19.65	0.691	NS
	Butyric acid (mM)			
0	8.34	8.01	0.235	NS
3	12.27	11.80	0.416	NS
6	13.51	10.80	0.573	*
9	11.94	8.48	0.636	**
Mean	11.52	9.77	0.379	*
	Total VFA (mM)			
0	81.73	79.44	2.904	NS
3	105.23	111.61	4.700	NS
6	103.42	100.05	4.048	NS
9	96.22	77.95	4.128	*
Mean	96.65	92.26	3.018	NS
	A/P ratio			
0	4.25	3.42	0.144	**
3	3.38	2.60	0.116	**
6	3.64	3.01	0.092	**
9	3.93	3.29	0.099	**
Mean	3.80	3.08	0.105	**

*, ** Significant at $p<0.05$ or 0.01. NS: Non-significant.
A/P ratio=acetic acid (mM) per propionic acid (mM).

instance. Holter et al. (1977) reported that no differences in digestibility of energy, DM and other dietary nutrients were observed between blended and separately feeding, but others (Howard et al., 1986; Yang and Varga, 1989; McCullough, 1991) indicated that nutrient digestibility was improved with TMR compared with separate feeding. The improved digestibility might be the result of more stabilized ruminal condition and an improved ruminal function by TMR feeding system as noticed by Donald et al. (1985), and substantiated by higher fibrolytic enzyme activity (Table 6) in the present study.

Ruminal pH, NH_3-N and VFA concentration

The effects of feeding system on ruminal pH and NH_3-N are presented in Table 3. Feeding system did not significantly influence ruminal pH levels, which reached the lowest value at 3 h after feeding, and increased thereafter as has been observed in other studies (Briggs et al., 1957; Reid et al., 1957; Smith et al., 1974; Fan et al., 2002). Son et al. (1994) compared rumen pH of lactating dairy cows in farms with a separate and a TMR feeding system and found no differences in rumen pH level. Limited feed intake at 2% body weight in this study might have resulted in non-significant difference in ruminal pH between two feeding systems. Peak concentration of ruminal NH_3-N reached at 3 h after feeding and the level thereafter decreased to the level lower than pre-feeding. NH_3-N at 3 and 6 h after feeding for TMR treatment was significantly higher than CR group ($p<0.01$).

NH_3-N is regarded as the most important nitrogen source for microbial protein synthesis in the rumen (Bryant, 1974) and the level in the rumen is usually high when feeds are more digestible (Erdmen et al., 1986). Higher NH_3-N and protein digestibility (Table 2) in TMR group of current study may have been due to wet brewers' grain included in TMR (Murdock et al., 1981; Davis et al., 1983; Kim et al., 1995), or due to partial degradation of TMR protein because TMR was mixed and stored for two or three days before feeding. High concentration of NH_3-N in the rumen and protein digestion in the total digestive tract observed in this study, however, does not necessarily indicate higher utilization of TMR nitrogen. Further nitrogen balance studies and/or feeding trials are required to provide necessary information on the benefit of TMR in protein nutrition for ruminants

The influence of feeding system on ruminal VFA profile is depicted in Table 4. The concentration of acetate was slightly higher in CR than in TMR, but the difference was not statistically significant. Animals receiving TMR tended to have higher propionic acid, and the difference was significant ($p<0.05$) at 3 h after feeding. On the other hand, butyric acid concentration was higher ($p<0.05$) after 6 h post-feeding in animals on CR. Feeding TMR reduced A/P ratio throughout experiment ($p<0.01$).

It has been well documented that composition and production of VFA largely depends on a hay level in the ration and an amount of hay intake (Merchen et al., 1986; Sasaki et al., 2001) and A/P ratio increases with higher rate of dried hay fed to cows (Davis, 1979). Steers on CR

Table 5. Influence of feeding system on ruminal microbial population (number per ml)

Time (h)	CR feeding	TMR feeding	SEM	Significance
	Total bacteria counts ($\times 10^{10}$)			
0	12.44	15.86	2.219	NS
3	12.13	18.28	2.193	NS
6	22.86	21.86	5.586	NS
9	17.44	25.00	4.042	NS
Mean	16.98	20.28	2.885	NS
	Total fungi counts ($\times 10^{4}$)			
0	4.66	3.43	1.233	NS
3	9.31	5.17	1.654	NS
6	8.40	4.39	1.791	NS
9	7.47	2.38	1.736	NS
Mean	7.66	3.86	1.439	NS
	Protozoa ($\times 10^{5}$)			
0	4.44	6.06	0.423	*
3	4.31	5.19	0.428	NS
6	3.81	4.37	0.393	NS
9	3.37	5.06	0.411	*
Mean	3.99	5.17	0.245	**

*, ** Significant at $p<0.05$ or 0.01. NS: Non-significant.

Table 6. CMCase and xylanase activities of rumen fluid as influenced by feeding system (reducing sugar μmol/min/ml)

Time (h)	CR feeding	TMR feeding	SEM	Significance
	CMCase			
0	0.35	0.51	0.039	*
3	0.31	0.35	0.023	NS
6	0.30	0.35	0.030	NS
9	0.33	0.44	0.050	NS
Mean	0.33	0.41	0.026	NS
	Xylanase			
0	1.79	3.51	0.249	**
3	2.79	4.37	0.296	**
6	2.22	3.78	0.232	**
9	2.35	3.76	0.203	**
Mean	2.28	3.86	0.224	**

*, ** Significant at $p<0.05$ or 0.01. NS: Non-significant.

system received 2.57 kg/day of rice straw and afalfa cube, while those on TMR received 1.39 kg/day of tall fescue straw, and therefore lower amount of coarse roughage in TMR might have been the possible cause of lower acetate, higher propionate and lower A/P ratio. Smaller particle size of roughage sources in TMR may have been another possible contribution factor for the differences in VFA profile.

Microbial population and enzyme activity in the rumen

Feeding system did not affect microbial population to a great extent, although there were some differences at a certain incubation time (Table 5). TMR tended to increase rumen bacterial count except at 6h after feeding. TMR also tended to increase total count of protozoa and especially, the population was significantly higher at 9 h after feeding ($p< 0.05$). The influence of feeding system on fungal population was minimal.

The activities of CMCase and xylanase as influenced by feeding system is presented in Table 6. There was a tendency of higher activity of both enzymes in the rumen of steers fed TMR compared to those fed CR.

It seems that TMR can be more favorable feeding system over CR considering increased microbial population and hydrolytic enzyme activity in steers fed TMR in the present study. No direct explanation for the increased microbial numbers and enzyme activity is possible by data of this study, but TMR seems to have provided better condition for rumen fermentation and nutrient digestion, which are well documented in the literature (Hungate, 1966; Lee et al., 2000; Russell and Rychlik, 2001).

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