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Effects of Synchronization of Carbohydrate and Protein Supply on Ruminal Fermentation, Nitrogen Metabolism and Microbial Protein Synthesis in Holstein Steers

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ABSTRACT : Three rumen-cannulated Holstein steers were fed three diets, each with a different synchrony index (SI) (LS: 0.77, MS: 0.81, and HS: 0.83), in order to examine the effect of diet on rumen fermentation, nitrogen balance, and microbial protein synthesis. Synchrony index was calculated based on the carbohydrate and crude protein fractions of each ingredient and their degradation rates. Feeding the steers diets with different SIs did not influence dry matter, crude protein, NDF, or ADF digestibility. The concentrations of total and individual VFA in the rumens of steers that were fed the two higher-SI diets were higher than in those fed the low-SI diet ($p<0.05$), but there was no significant difference between the two higher-SI diets. One hour after feeding, steers on the LS diet had lower ruminal pHs than did those fed the MS or HS diets ($p<0.05$), and animals on the LS diet generally showed higher ruminal NH_3-N levels than did animals on the other diets, with the 4-h post-feeding difference being significant ($p<0.05$). Steers receiving the LS diet excreted more nitrogen (N) in their urine than did those on the two higher-SI diets ($p<0.05$), and the total N excretion of those on the LS diet was also higher ($p<0.05$). Microbial N levels calculated from the concentration of urinary purine derivatives were generally higher when the SI was higher, with the highest microbial protein synthesis being produced by steers on the HS diet ($p<0.05$). In conclusion, in the current study, ingestion of a synchronous diet by Holstein steers improved microbial protein synthesis and VFA production and decreased total N output. (**Key Words :** Synchrony Index, Carbohydrate and Protein Fraction, Nitrogen Utilization, Purine Derivatives, Ruminal pH, Ammonia, Volatile Fatty Acids)

INTRODUCTION

It is commonly accepted that microbial protein synthesis (MPS) in the rumen is the most important process in maximizing the amino acid supply to a ruminant. Microbial proteins are known to be of excellent quality with good amino acid composition for ruminants (Clark et al., 1992) and may account for more than 50% of the amino acids absorbed in the small intestine (NRC, 2000). Due to the importance of this process, extensive research efforts have been focused on understanding and improving MPS, and the delivery of a balanced or synchronized supply of feed protein and energy has been proposed as a primary factor influencing MPS (Nocek and Russell, 1988). Synchronization is the provision of both rumen-degradable protein (non-protein nitrogen (N) and rumen-degradable true protein) and energy (ruminally fermentable carbohydrate (CHO)) to the rumen, so that microorganisms can utilize both simultaneously.

It has been reported that a synchronous supply of energy and N to the rumen enhance the efficiency of microbes in capturing N and in utilizing ATP for microbial growth (Johnson, 1976; Herrera-Saldana et al., 1990; Sinclair et al., 1991, 1993; Richardson et al., 2003), which may mean that synchronized feeds increase microbial protein production in the rumen and enhance rumen fermentation efficiency, thereby improving nutrient utilization and animal performance. Although there is supportive evidence for the beneficial effects of a synchronous supply of energy and N in the rumen with regard to the efficiency of nutrient utilization in ruminants (Herrera-Saldana et al., 1990; Witt et al., 1999a, b), conflicting results have also been reported.

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Additionally, Kim et al. (2000) showed no significant effect of nutrient synchrony on the efficiency of microbial growth or VFA production. Ichinohe and Fujihara (2008) reported that the microbial N supply was greater for an asynchronous diet than it was for a synchronous diet. Kaswari et al. (2007) and Richardson et al. (2003) also showed that there were no differences in the efficiencies of MPS or N deposition between diets that were formulated to have different synchronization characteristics.

The objective of this study was to confirm the effects of synchronization of carbohydrate and protein supply on ruminal fermentation, nitrogen metabolism, and MPS in Holstein steers. We formulated diets having different synchrony indices (SI) with minimum variation in feed ingredients in order to minimize confounding variables.

MATERIALS AND METHODS

Preparation of experimental diets

Three TMRs were formulated to have similar protein contents with different SIs, as shown in Table 1. The synchrony indices of the three experimental diets were calculated from the CHO and crude protein (CP) fractions of each feed ingredient, values which were determined in our previous study, and the degradation rates of the CHO and CP fractions were acquired from NRC (2000). The amount of CHO digested in the rumen was calculated using the equation

$$P \text{ of CHO} = cA+cB1\times(1-e^{-KdB1t})+cB2\times(1-e^{-KdB2t}),$$

where P of CHO is the cumulative amount of degraded CHO at time t, cA is the amount of the rapidly-soluble fraction, cB1 is the amount of the starch fraction, and cB2 is the amount of the available fiber fraction. KdB1 and KdB2 are the digestion constants of CHO, as suggested by NRC (2000).

The amount of CP digested in the rumen was calculated using the following equation:

$$P \text{ of CP} = pA+pB1\times(1-e^{-KdB1t}) +pB2\times(1-e^{-KdB2t})+pB3\times(1-e^{-KdB3t})$$

where P of CP is the cumulative amount of degraded CP at time t, pA is the concentration of the non-protein N fraction, pB1 was the concentration of rapidly-degraded true protein, pB2 is the concentration of intermediately-degraded true protein, and pB3 is the concentration of slowly-degraded protein. KdB1, KdB2, and KdB3 are the digestion constants of CP, as suggested by NRC (2000).

The synchrony index of N:CHO was calculated using the equation proposed by Sinclair et al. (1993):

Table 1. Feed ingredients and compositions of the experimental diets

Item	Treatment[1]		
	LS	MS	HS
Ingredient (%/DM)			
Timothy hay	40.00	40.00	40.00
Corn	10.47	8.08	13.59
Corn gluten feed	3.24	2.84	2.86
Corn germ meal	5.08	4.79	4.83
Corn bran	0.00	0.73	0.74
Wheat hard	6.98	4.79	0.00
Wheat flour	8.25	7.78	6.04
Wheat bran	0.00	1.80	1.81
Rape seed meal	4.44	4.19	4.23
Coconut meal	4.44	4.19	4.23
Sesame meal	1.59	1.80	1.81
Palm meal	4.44	4.19	4.23
Soy bean meal	6.67	3.29	3.99
Roasted soybean meal	0.00	2.99	3.02
Molasses	1.81	1.80	1.81
Protected fat	0.59	0.59	0.60
DDGS	0.00	4.19	4.22
Lime stone	0.88	0.88	0.88
Salts	0.36	0.36	0.36
Mineral mixture	0.12	0.12	0.12
Vitamin mixture	0.12	0.12	0.12
Chemicalcomposition (%/DM)			
DM	10.49	11.12	10.72
CP	16.80	16.88	17.23
Ether extract	4.27	3.87	4.31
Ash	7.19	7.74	7.20
NDF	43.78	44.40	42.73
ADF	22.42	22.69	22.05

[1] LS = low synchronous, MS = middle synchronous, HS = high synchronous
[2] DDGS = distillers grains soluble

$$SI = \frac{32 - \frac{\sum_{1-24}\sqrt{\left(32 - \frac{\text{hourly released N (g)}}{\text{CHO (kg)}}\right)^2}}{24}}{32}$$

Animals and experimental procedure

Three Holstein steers, weighing 385±20.95 kg, were fitted with a rumen cannula and were fed three different diets in order to evaluate the effects of synchronization of N and CHO release in the rumen on rumen fermentation, N metabolism, and MPS. Steers were allocated to one of three dietary treatment regimens and were housed in individual pens. All diets were formulated to have similar nutrient contents but different synchrony indices (Table 1). The

animals were fed amounts that were 2.0% of their body weight in two equal portions, one at 8:00 am and another at 8:00 pm. A clean water and mineral mixture was available to each animal at all times in unlimited amounts. The experimental feeding scheme was based on a 3×3 Latin square, and each of the three feeding periods was 18 days long, with 14 days allocated to adaptation and four days allocated to sampling.

Sample collection and chemical analysis

Ruminal fluid was removed via the cannula on the last day of each period at 0, 1, 2, 3, 4, 6, 8, and 10 h after the 8:00 a.m. feeding. Ruminal pH was measured immediately after sampling using a Mettler®Delta 340 pH meter. The ruminal fluid was centrifuged at 8,000 rpm for 10 min, and the supernatant withdrawn from each tube was transferred to a 50 ml Corning centrifuge tube. Each supernatant was acidified with 5 ml 6 N HCl and stored at -20°C for subsequent determination of ammonia and volatile fatty acid concentrations. Ammonia-N concentration was determined using a modified colorimetric method (Chaney and Marbach, 1962), and VFA concentration was analyzed by gas chromatography using a Hewlett Packard 5880A gas chromatograph (Hewlett Packard, Palo Alto, Ca, USA), employing a method described by Erwin in 1961.

Urine was collected for three consecutive days (day 15 to day 17) of each period using a plastic receptacle, which contained 500 ml 4 N H_2SO_4 to prevent N losses. Total urine output was determined by weight each day, prior to the morning feeding. The urine was sampled at a output rate of 10% and was frozen at -20°C after collection. Total N concentration was determined using the Kjeldalh method (AOAC, 1990). Urine was analyzed for purine derivatives (PD) according to the modified method (George et al., 2006) of Chen et al. (1990) for allantoin and uric acid. The daily output of purine derivatives in the urine was calculated, and this value was used to estimate the amount of microbial N absorbed, using the method of Chen et al. (1992).

Total feces were collected at 7:30 am during each sampling period. A 1/10 aliquot of total feces was sampled and pooled for each animal. These samples were dried in a 60°C dry oven for 72 h and ground to pass through a 2 mm screen. Feed and feces samples were analyzed for DM, OM, ether extract, and N according to AOAC (1990), and NDF and ADF were analyzed by the method of Van Soest et al. (1991).

Statistical analysis

Data were analyzed using the GLM procedure of the Statistical Analysis System Institute, Inc. (SAS) (1996). Differences between means were analyzed using the least significant difference (Tukey) test of SAS (1996).

Table 2. Apparent total tract digestibility (%), VFA concentration (mM), pH, and NH_3-N concentration (mg/100 ml) in the rumen according to dietary synchrony index

Item	Treatment			SEM
	LS	MS	HS	
SI	0.77	0.81	0.83	
Apparent total tract digestibility[1]				
DM	73.53	75.01	73.66	0.56
CP	74.90	73.14	72.97	0.60
NDF	62.18	64.79	60.90	0.88
ADF	52.64	51.83	50.82	1.06
Ruminal fermentation				
VFA				
Total	51.10^{b}	97.27^{a}	81.07^{a}	5.98
Acetic acid	31.78^{b}	60.03^{a}	48.48^{a}	3.51
Propionic acid	9.27^{b}	18.43^{a}	16.74^{a}	1.30
Butyric acid	7.78^{b}	14.58^{a}	12.03^{ab}	0.93
Isobutyric acid	0.56^{b}	1.00^{a}	0.89^{a}	0.06
Valeric acid	0.74^{b}	1.46^{a}	1.26^{ab}	0.13
Isovaleric acid	0.97^{b}	1.77^{a}	1.66^{a}	0.11
A:P ratio	3.45^{a}	3.38^{a}	2.93^{b}	0.08
pH	6.21	6.21	6.23	0.02
NH_3-N	16.18	14.34	13.26	0.62

[a, b] Within the same row, means without a common superscript are significantly different ($p<0.05$).

[1] DM = Dry matter, CP = Crude protein, NDF = Neutral detergent fiber, ADF = Acid detergent fiber

RESULTS

Nutrient digestibility and ruminal fermentation characteristics

The apparent total tract digestibility values of DM, CP, NDF, and ADF, thought to be affected by dietary SI, are presented in Table 2. However, diets having different SIs did not influence DM, CP, NDF, or ADF digestibility ($p>0.05$). The concentrations of total and individual VFA in the rumens of steers fed MS and HS diets were higher than those fed the LS diet ($p<0.05$). However, VFA composition was not different between the two steer groups receiving the MS or HS diet.

Diurnal variations in ruminal pH of steers on different synchronous diets are presented in Figure 1. Consumption of the LS diet resulted in a lower pH than did that of the MS or HS diet 1 h after feeding. This difference was maintained until 3 h after feeding. However, there was no significant effect of diet SI on daily mean pH (Table 2).

Ruminal NH_3-N concentrations at different times after feeding are presented in Figure 2. There was no significant effect of synchronous treatment on mean daily ruminal

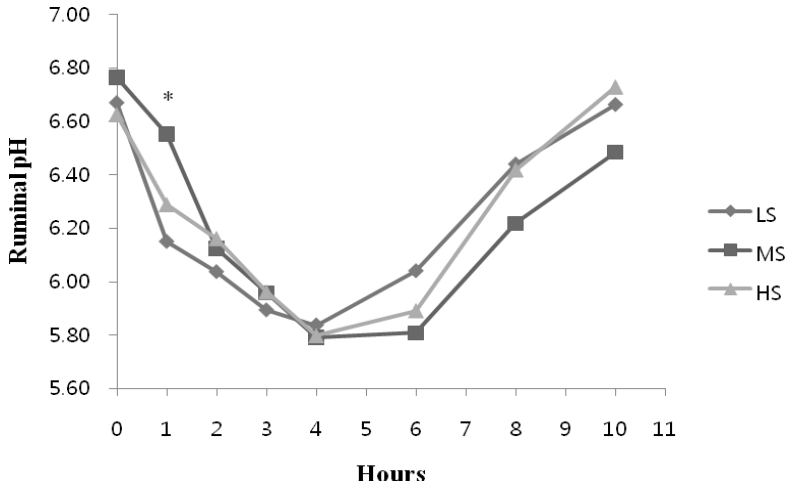

Figure 1. Ruminal pH in steers fed three different synchronous diets. Asterisk indicates significantly different means ($p<0.05$).

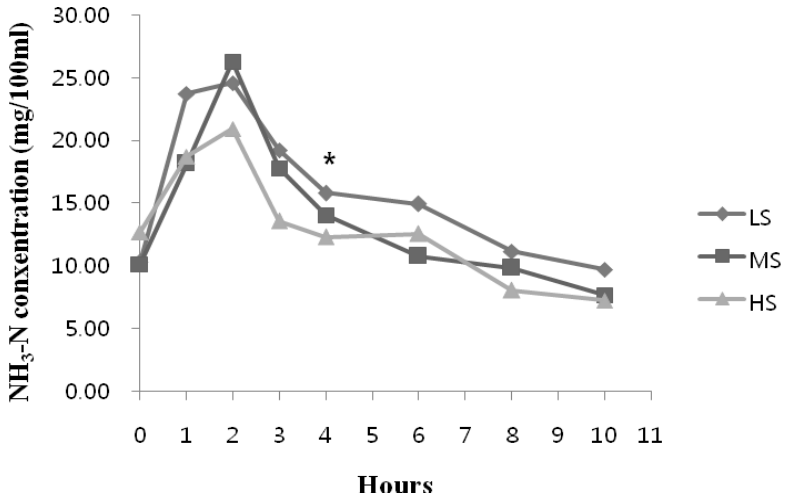

Figure 2. Ruminal NH_3-N concentration in steers fed three different synchronous diets. Asterisk indicates significantly different means ($p<0.05$).

NH_3-N concentration (Table 2). The patterns of ruminal NH_3-N concentration were similar among treatments, with peak concentrations being obtained 2 h after feeding. Steers on the LS diet generally showed higher ruminal NH_3-N levels than did those on higher SI diets, and the difference 4 h after feeding was significant ($p<0.05$).

Nitrogen excretion and MPS

The urinary and fecal N excretions and daily urinary output of purine derivatives of steers according to the three different synchronous diets are presented in Table 3. Dietary SI did not influence the amount of N excreted through feces. However, steers receiving the LS diet excreted significantly more N in their urine and had a greater total N excretion than did those on the two higher SI diets ($p<0.05$), and animals given the HS diet had greater excretion of urinary

Table 3. Daily outputs of total nitrogen in feces and urine, PD excretions in urine, and microbial Ns in steers according to diet

Item	Treatment			SEM
	LS	MS	HS	
SI	0.77	0.81	0.83	
N output (g/d)				
Total	141.99[a]	129.84[b]	136.02[ab]	1.61
Urine	92.49[a]	81.34[b]	84.79[b]	1.11
Fecal	49.50[a]	48.50[a]	51.23[a]	1.47
Urinary PD(g/d)				
Total	9.76[b]	11.07[ab]	12.46[a]	0.42
Allantoin	9.22[b]	10.59[ab]	11.57[a]	0.4
Uric acid	0.54[a]	0.48[a]	0.89[a]	0.09
Microbial N (g/d)[1]	22.63[b]	31.52[ab]	38.51[a]	2.32

[a, b] Within the same row, means without a common superscript are significantly different ($p<0.05$).

[1] Microbial N was calculated using the method of Chen et al. (1992).

allantoin and total purine derivatives ($p<0.05$). Microbial N calculated from PD excretion was generally higher when SI was higher, with the highest MPS being produced by steers on the HS diet ($p<0.05$).

DISCUSSION

The present experiment was conducted to evaluate the hypothesis that synchronization of dietary N and energy will improve N metabolism, rumen fermentation, and MPS in ruminants. To test this hypothesis, experimental diets were formulated to have different SIs but to have minimum differences in the types of ingredients. All animals were fed the same amounts at the same times in order to avoid the influences of ingredient characteristics (Dewhurst et al., 2000) and intake level (Witt et al., 1999b) on the effects of synchronization. Previous studies have manipulated protein degradability (Sinclair et al., 1995; Joo et al., 2005; Jetana et al., 2010) to achieve different synchronicities; however, we formulated three experimental diets with similar chemical compositions (Table 1) and degradable protein concentrations, but with different non-structural carbohydrate (NSC) contents. The concentrations of NSC in the LS, MS, and HS diets were 338.7, 300.1, and 299.7 g/kg DM, respectively.

Dietary SI did not influence the apparent total tract digestibilities of DM, CP, NDF, or ADF, although SI significantly influenced rumen fermentation characteristics and MPS. Why digestibility was not affected is not readily apparent from our results. The impact of dietary SI on the lower digestive tract may have diluted the influence on rumen fermentation, or the magnitude of the differences in the SIs between the three diets may have not been large enough to produce measurable differences in digestibility. In fact, a number of studies (Aldrich et al., 1993; Kolver et al., 1998; Chanjula et al., 2004; Rotger et al., 2006; Khezri et al., 2009) showed results similar to ours. Valkeners et al. (2008) reported that the total tract digestibilities of OM, NDF, and starch were not negatively affected by lack of ruminally available N. Kolver et al. (1998) also reported that the apparent total tract digestibilities of DM, OM, CP, and NDF were not improved by supplementation of carbohydrate-rich food with pasture N in the rumen. Hall and Huntington (2008) suggested that the whole digestive tract should be considered instead of solely the rumen in such experiments because ruminant animals tend to maintain homeostasis in terms of nutrition and physiology.

Consuming the LS diet resulted in the lowest mean VFA concentration of all three groups, and the ruminal pHs of steers on the LS diet were lower than those of the other groups, a situation which contradicts the commonly accepted VFA-pH relationship of the rumen. We postulate that the LS diet may produce more VFA during the first few hours after feeding, even though daily mean VFA is low, consistent with the low ruminal pH of the LS group during the early phase of digestion. However, it is possible that the low pH due to fermentation of the abundance of rapidly soluble CHO (Khezri et al., 2009) in the LS diet decreased the activity of cellulolytic bacteria, thereby reducing the growth of microorganisms in the rumen; this reduction would be consistent with the results of a previous study (Khalili and Huhtanen, 1991). Joo et al. (2005) changed protein sources to differentiate synchronicity between energy and N release in the rumen. Their soy protein-source group had increased VFA concentrations, and they concluded that protein sources that complement OM degradability may improve rumen fermentation. Although daily mean ruminal NH_3-N concentrations were not significantly different between groups ($p>0.05$), there was a trend for higher ruminal NH_3-N concentrations with the LS diet throughout the day and significantly higher ruminal NH_3-N concentration 4 h after feeding, which may be indicative of less efficient utilization of N for MPS in the LS diet. The present study also showed significantly lower levels of MPS in steers on the LS diet. This may be explained by the high amount of NSC in that diet, which could lead to increased lactic acid production (Kim et al., 2000). Since lactate may produce less ATP per unit of glucose fermented than does VFA (Strobel and Russell, 1986), the LS diet may afford lower MPS levels than those of the two higher SI diets. Our results are supported by previous studies (Sinclair et al., 1993; Chumpawadee et al., 2005; Valkeners et al., 2006; Kaswari et al., 2007); lower ruminal NH_3-N concentration correlated with higher usage of NH_3-N for MPS (Kaswari et al., 2007; Mahr-un-Nisa et al., 2008), and synchronization between N and CHO release in the rumen improved N utilization by rumen microorganisms through the more efficient supplication of ATP (Nocek and Russell, 1988; Chamberlain and Choung, 1995). There are several previously reported results that have demonstrated that MPS increases as SI increases (Sinclair et al., 1993, 1995; Casper et al., 1999; Kim et al., 1999; Elseed, 2005; Rotger et al., 2006). In this experiment, microbial N calculated by the amount of PD excretion in the urine was lower than those in other experiments (Chumpawadee et al., 2005; Valkeners et al., 2006), despite using animals with similar body weights. Perez et al. (1997) reported that the urinary PD excretion method of calculating microbial N consistently underestimated microbial N flow to the small intestine when compared to the method using duodenal flow of a microbial marker, such as ^{15}N.

In our study, a synchronous diet improved microbial protein synthesis, VFA production, and decreased total N output. However, contradictory results on the effect of dietary SI on MPS are also present in the literature. Some challenges to succeeding in the nutrient synchrony concept

proposed in this study may be feed characteristics (Hersom, 2007), N recycling, type of N source, and availability of other nutrients (Sniffen et al., 1987). Therefore, the nutrient synchrony concept needs to be investigated further prior to its having a practical application in the field (Yang et al., 2010). Furthermore, better understandings of the complex ruminal ecosystem and physiological effects, such as N recycling, are required.

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