



Effects of Synchronicity of Carbohydrate and Protein Degradation on Rumen Fermentation Characteristics and Microbial Protein Synthesis

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ABSTRACT: A series of *in vitro* studies were carried out to determine i) the effects of enzyme and formaldehyde treatment on the degradation characteristics of carbohydrate and protein sources and on the synchronicity of these processes, and ii) the effects of synchronizing carbohydrate and protein supply on rumen fermentation and microbial protein synthesis (MPS) in *in vitro* experiments. Untreated corn (C) and enzyme-treated corn (EC) were combined with soy bean meal with (ES) and without (S) enzyme treatment or formaldehyde treatment (FS). Six experimental feeds (CS, CES, CFS, ECS, ECES and ECFS) with different synchrony indices were prepared. Highly synchronous diets had the greatest dry matter (DM) digestibility when untreated corn was used. However, the degree of synchronicity did not influence DM digestibility when EC was mixed with various soybean meals. At time points of 12 h and 24 h of incubation, EC-containing diets showed lower ammonia-N concentrations than those of C-containing diets, irrespective of the degree of synchronicity, indicating that more efficient utilization of ammonia-N for MPS was achieved by ruminal microorganisms when EC was offered as a carbohydrate source. Within C-containing treatments, the purine base concentration increased as the diets were more synchronized. This effect was not observed when EC was offered. There were significant effects on VFA concentration of both C and S treatments and their interactions. Similar to purine concentrations, total VFA production and individual VFA concentration in the groups containing EC as an energy source was higher than those of other groups (CS, CES and CFS). The results of the present study suggested that the availability of energy or the protein source are the most limiting factors for rumen fermentation and MPS, rather than the degree of synchronicity. (**Key Words:** Enzyme, Formaldehyde, Synchronicity, Rumen Fermentation, Microbial Protein Synthesis)

INTRODUCTION

Ruminal microbial protein synthesis (MPS) is the most important process in ruminant nitrogen metabolism since MPS not only contribute more than 50% of the amino acids absorbed in the small intestine, but also have an amino acid composition similar to that of the proteins required for milk synthesis and meat production (NRC, 2000). Ruminal MPS is largely dependent upon the supply of degradable carbohydrates and proteins. When energy from fermentable

carbohydrate is supplied in sufficient quantities in the rumen, ruminal microorganisms can capture nitrogen (N) sources such as amino acids or ammonia and convert them to MP (Nocek and Russell, 1988). If, however, the available carbohydrate sources are insufficient, ammonia may accumulate in the rumen and be absorbed into the body and excreted in urine, resulting in inefficient utilization of nitrogen sources. Synchronization is the provision of both rumen-degradable proteins (non-protein nitrogen and rumen degradable true protein) and energy (ruminally fermentable carbohydrate) simultaneously to the rumen (Seo et al., 2010), and has been suggested as one possible solution for improving MPS. Cole et al. (2008) reported that use of synchronized feed has the potential to improve ruminal MPS, N usage, rumen fermentation and animal performance, and to decrease urinary N excretion.

A number of previous experiments have evaluated the effects of synchronized feeds on rumen MPS and nitrogen utilization. However, their results have been equivocal. Rotger et al. (2006) used two non-structural carbohydrate

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sources, barley and C, and two protein sources, soy bean (S) and sunflower meal, each having different degradability in the rumen. Synchronized diets containing both rapid (barley-sunflower meal) and slow-fermenting components (C-S meal) had higher OM digestibility, VFA concentration, and MPS than asynchronous diets *in vitro*. However, in an *in vivo* trial, synchronization did not seem to affect rumen fermentation characteristics or digestibility (Rotger et al., 2006). A study of the exchange of feed ingredients was intended to differentiate the effects of synchronicity between treatments (Aldrich et al., 1993; Shabi et al., 1998; Rotger et al., 2006; Ichinohe and Fujihara, 2008), but this method could not separate the influence of synchronization from that of the individual feed characteristics (Dewhurst et al., 2000). Therefore, we used the same carbohydrate (C) and protein (S) source to control for the dietary characteristics of each feed component and examine the effects of synchronization in this experiment. The rate of degradation of each feed was adjusted by treating feeds with enzymes and formaldehyde. The objectives of this study were to examine i) the effects of enzyme and formaldehyde treatment on degradation characteristics of carbohydrate and protein sources and synchronicity and ii) the effects of synchronizing carbohydrate and protein supply on rumen fermentation and MPS under *in vitro* condition.

MATERIAL AND METHODS

Preparation of experimental diets

Corn and soybean meal were used as a carbohydrate and a protein source, respectively. All feedstuffs were dried at 60°C and ground in a Wiley mill (Arthur H. Thomas, Philadelphia, PA, USA) through a 1 mm screen prior to enzyme and formaldehyde treatment. Enzymes and formaldehyde were purchased from Sigma Co. Ltd. (Sigma & Aldrich, St. Louis, USA). Corn was consecutively treated with alcalase (0.2%/wt) and α -amylase (1%/wt) and incubated for 4 h at 55°C to produce enzyme-treated corn (EC). Soybean meal was also treated with alcalase (0.2%/wt) and formaldehyde (0.5%/wt) to make enzyme-treated meal (ES) and formaldehyde-treated meal (FS). The incubation time and temperature of the S treatment was same as in the C process. In total, five treated experimental diets were prepared.

In situ experiment

An *in vivo* trial was conducted to examine the degradation kinetics of each experimental diet. Ruminal dry matter (DM), organic matter (OM) and crude protein (CP) disappearance rates of each treatment feed were measured using the nylon bag technique as described by Ørskov et al. (1980). Three rumen-cannulated Holstein steers, weighing

350±18.77 kg, were used for the *in situ* experiment. Experimental animals were offered Timothy hay and a commercial concentrate feed (Cargill Agribrand Purina Korea Co. Ltd.) at a ratio of 6:4 twice daily (at 8 am and pm). The total amount of feed offered to animals was 2% of their body weight. The chemical composition of the commercial concentrate was 87.7% DM, 14.8% CP, 4.13% ether extract (EE), 5.22% ash and 71.5% total digestible nutrient DM basis. Fresh water and a mineral mixture were available to each animal at all times.

A total of 15 g of each experimental diet (C, EC, S, ES, FS) was weighed and allotted to nylon bags with a 53±10 µm (Bar Diamond, Idaho, USA) pore size. The bags were placed into the ventral sac of the rumen of each Holstein steer through the rumen cannula. The incubation was started at 8:30 am after feeding. All feed samples were incubated for 0, 3, 6, 9, 12, 24 and 48 h. After incubation, the bags were removed from the rumen and washed by hand under cold tap water to remove the feed particle attached to the surface of the bags until the rinse water ran clear. Control bags which were not incubated in the rumen (0 h) were washed in the same way. After washing, all nylon bags were dried at 65°C for 48 h and weighed to estimate the DM residues. The contents of the bags were stored at 4°C until chemical analysis.

Evaluation of the characteristics of rumen degradation

The disappearance rates of DM, OM and CP were calculated as the difference between the weight of the bag contents before and after incubation. The degradability data obtained for each treatment were fitted using PROC NLIN (SAS, 1996) to the following equation (Ørskov and McDonald, 1979):

$$P = a + b(1 - e^{-ct})$$

where P is the degradation rate at time 't'; a is the rapidly soluble fraction; b is the insoluble but potentially degradable fraction; c is the rate of degradation of fraction b; t is the incubation time.

The effective degradabilities of DM, OM and CP were calculated using the above parameters (a, b, c) and a ruminal outflow rate of 0.02, 0.05 and 0.08%/h by:

$$P = a + ((b \times c) / (c + k))$$

Where P is the effective degradability of DM, OM and CP, and k is the estimated rumen outflow rate (%/h).

Diet formulation and calculation of synchrony index (SI)

Two carbohydrate samples (C, EC) and three protein samples (S, ES and FS) were prepared as described previously. Carbohydrate and protein samples were mixed

in a ratio of 0.85:0.15 for a total of six treatment diets (CS, CES, CFS, ECS, ECES and ECFS) for the *in vitro* experiment to get chemical composition which is similar with commercial concentrated feed. The SI of the diets in the *in vitro* study was calculated from the degradation kinetics of OM and CP of the diet components from the *in situ* experiments. The SI of N to OM was calculated as described by Sinclair et al. (1993).

***In vitro* experiment**

For the *in vitro* experiment, rumen fluid was collected and mixed two to three hours after morning feeding from three Holstein steers that were fed with the same feeds as used in *in situ* experiment. The strained rumen fluid was filtered through eight layers of cheesecloth and transported to the laboratory in a pre-warmed thermo-flask. It was then mixed with artificial saliva (McDougall, 1948) at a ratio of 1:2 under a CO₂ stream. Twenty-five milliliters of rumen fluid and buffer mixture was dispensed anaerobically into 50 ml serum bottles, each containing 1 g of experimental diet. The bottles were then filled with CO₂ gas and capped with a rubber stopper. The bottles were incubated for 0, 3, 6, 12 and 24 h in a 39°C incubator.

Chemical analysis

After fermentation, bottle contents were filtered through Whatman No. 541 paper (Fisher Scientific Company, Pittsburgh, PA, USA), and the residues were dried at 65°C for 48 h to measure DM digestibility. The filtrate was centrifuged at 10,000 g for 20 min at 4°C, and the supernatant was collected to determine NH₃-N and VFA concentration. The pH of the filtrate was measured immediately after centrifugation using a pH meter. After filtering the supernatant through a Millipore filter (0.22 µm pore size), VFA concentration was measured by gas chromatography using a Hewlett Packard 5880A gas chromatograph (Hewlett Packard, Palo Alto, Ca, USA) employing a method described by Erwin (1961). The concentration of NH₃-N was determined using a modified colorimetric method (Chaney and Marbach, 1962). The *in vitro* digesta samples were analyzed for purine content (Zinn and Owens, 1986) using HPLC (HP1100, Hewlett Packard, Palo Alto, Ca, USA) according to the modified method (Makkar and Becker, 1999; George et al., 2006) to estimate MPS. All feed components and *in situ* and *in vitro* residues were ground in a Wiley mill (Arther H. Thomas, Philadelphia, PA, USA) and analyzed for dry matter (DM), ash, fiber, and nitrogen contents using the Kjeldahl procedure with a Kjeltex (KjeltexTM 2200, Foss Tecator, Sweden) and for EE using diethyl ether with a Soxhlet as described in AOAC (1990). Neutral detergent fiber (NDF) and acid detergent fiber (ADF) were analyzed by the method described by Van Soest et al. (1991) with an Ankom

200 fiber analyzer (Ankom Technology, NY, USA).

Statistical analysis

The data for the *in situ* disappearance characteristics of the experimental diets were analyzed using PROC GLM (SAS, 1996). Differences between treatments were considered significant at p<0.05. The data for DM digestibility, pH variation, NH₃-N, VFA, and purine concentrations obtained from *in vitro* experiments were analyzed using a 2×3 factorial design using the MIXED procedure (SAS, 1996) with the following model:

$$Y_{ij} = \mu + \alpha_i + \beta_j + (\alpha\beta)_{ij} + \varepsilon_{ij}$$

Where:

Y_{ij} = the measured variable

μ = the overall mean

α_i = the effect of carbohydrate sources

β_j = the effect of protein sources

(αβ)_{ij} = the effect of interaction between carbohydrate and protein sources

ε_{ij} = the random error

The effects of C treatment, S treatment and their interaction were considered fixed. Significant differences (p<0.05) in treatment least square means were reported only if the Tukey-test (SAS, 1996) was also significant (p<0.05).

RESULTS AND DISCUSSION

***In situ* degradation characteristics of feeds**

The chemical compositions of the test feeds are listed in Table 1. Although EC was treated with α-amylase and alcalase, the chemical composition of this feed was not influenced by the enzyme treatment. Similarly, treatment of soybean meal with enzyme or formaldehyde did not alter its chemical composition. However, the ruminal degradation characteristics of C and S were greatly influenced by enzyme and formaldehyde treatment (Table 2). ES showed higher (p<0.05) effective degradability of DM, CP, and OM than S, but FS had the lowest (p<0.05) effective degradability of DM, OM, and CP. When C and S were

Table 1. Chemical composition of feed ingredients

	C ¹	EC ¹	S ¹	ES ¹	FS ¹
DM (%)	88.56	87.93	90.59	85.45	82.75
OM (% DM)	99.16	99.24	93.85	93.62	93.97
CP (% DM)	8.47	9.15	54.04	54.92	54.47
EE (% DM)	3.97	4.15	1.49	1.47	1.78
NDF (% DM)	12.99	13.11	14.29	14.55	14.83
ADF (% DM)	4.65	4.77	10.17	10.11	10.54

¹ C = Corn, EC = Enzyme treated corn, S = Soybean meal, ES = Enzyme treated soybean meal, FS = Formaldehyde treated soybean meal.

Table 2. Dry matter (DM), crude protein (CP) and organic matter degradation kinetics of the feeds used in the *in situ* study

Items	Feeds	a ¹	b ¹	c ¹	a+b ¹	ED (k = 0.05) ²
DM	C	11.47 ^e	78.57 ^a	0.04 ^{ab}	90.05 ^a	45.54 ^c
	EC	15.74 ^d	67.63 ^a	0.06 ^{ab}	83.36 ^a	51.04 ^{bc}
	S	25.47 ^b	53.41 ^a	0.07 ^a	78.88 ^a	56.47 ^b
	ES	31.86 ^a	60.65 ^a	0.08 ^a	92.51 ^a	68.90 ^a
	FS	17.74 ^c	82.26 ^a	0.02 ^b	100.00 ^a	37.53 ^d
	SEM ³	1.9518	3.8519	0.0075	3.2533	2.8909
CP	C	8.72 ^b	69.12 ^{ab}	0.06 ^{ab}	77.84 ^a	40.22 ^b
	EC	17.31 ^a	59.29 ^b	0.06 ^{ab}	76.60 ^a	45.95 ^b
	S	11.56 ^b	55.38 ^b	0.08 ^a	66.94 ^a	44.29 ^b
	ES	18.75 ^a	64.22 ^{ab}	0.12 ^a	82.97 ^a	63.36 ^a
	FS	0.59 ^c	99.41 ^a	0.01 ^b	100.00 ^a	14.46 ^c
	SEM ³	1.8033	5.2152	0.011	4.4575	4.2494
OM	C	11.19 ^d	78.74 ^a	0.04 ^{bc}	89.93 ^a	45.16 ^c
	EC	16.17 ^c	68.15 ^a	0.05 ^{abc}	84.32 ^a	51.00 ^{bc}
	S	23.68 ^b	55.82 ^a	0.07 ^{ab}	79.49 ^a	54.84 ^b
	ES	30.19 ^a	63.74 ^a	0.08 ^a	93.93 ^a	67.81 ^a
	FS	15.57 ^c	84.43 ^a	0.02 ^c	100.00 ^a	35.19 ^d
	SEM ³	1.8071	3.7936	0.0068	3.2646	2.9499

¹ a = Readily soluble fraction, b = Insoluble but potentially degradable fraction, c = Constant rate of degradation of b (%/h).

² ED = Effective degradability. Rumen passage rate (k) was considered to 0.05. ³ SEM means standard error for means.

a,b,c,d,e Indicates that the means are significantly different within different feeds (p<0.05).

treated with enzyme, their 'a' fraction of DM, OM and CP increased, while the treatment of S with formaldehyde resulted in a lower (p<0.05) immediately soluble fraction 'a' and a lower degradation rate of the 'b' fraction compared with untreated S (Table 2). The potentially degradable P fraction (a+b) varied between 66.94% for S and 100% for FS. In a number of previously described experiments, the protein source was treated with formaldehyde to protect dietary protein from proteolysis by rumen microorganisms in an effort to increase N utilization efficiency in the rumen. For instance, Witt et al. (1999b) reported that formaldehyde treatment of S reduced not only the soluble N content but also the rate of degradation of the 'b' fraction. In our experiment, formaldehyde treatment increased the portion of 'b' fraction but the degradation rate of 'b' was decreased by chemical treatment. Therefore the ED value of DM, CP and OM in FS were lower than those of S significantly (p<0.05). Eghbali et al. (2011) examined the effects of different treatment methods for canola meal on rumen degradability of CP and DM in *in situ* studies. The protein fraction was measured based on Cornell Net Carbohydrate and Protein system. Treating with formaldehyde also reduced the protein degradation rate in the rumen in this study. Our results are also consistent with those of Eghbali et al. (2011), who suggested that aldehydes could crosslink with free amino acid groups in feeds so that protein

degradability in the rumen might be reduced, but these complexes could be hydrolyzed in the abomasum and small intestine thus enabling protein digestion and absorption in the hindgut.

***In vitro* experiments**

Exchange of feed ingredients (Herrera-Saldana et al., 1990; Shabi et al., 1998; Rotger et al., 2006) and adjustment of ingredient proportions (Sinclair et al., 1993; Witt et al., 1999a,b; Witt et al., 2000; Richardson et al., 2003; Ichinohe and Fujihara, 2008; Seo et al., 2010) are methods to manipulate the degree of synchronization of carbohydrate and nitrogen release in the rumen. In present experiment, we used the same carbohydrate and protein source treated with enzymes or formaldehyde and mixed at the same ratio to exclude the influence of the characteristics of different feeds on synchronization effects. As mentioned above, 0.85 g of carbohydrate source (C, EC) and 0.15 g of protein source (S, ES, FS) were mixed in order to vary synchronicity in the rumen and were used in *in vitro* experiment. In total, six kinds of experimental feeds were prepared, and the results of their proximate analysis and their fiber contents are presented in Table 3. All six diets had similar OM, CP, EE, NDF and ADF contents; the average proportion of each was 98.17%, 17.55%, 3.59%, 13.34% and 5.77% of DM, respectively. However, the SI of

Table 3. Chemical composition and synchrony index of formulated experimental feeds

	CS	CES	CFS	ECS	ECES	ECFS
DM (%)	88.95	87.96	87.45	88.44	87.46	86.94
OM (%DM)	98.14	98.10	98.17	98.21	98.16	98.23
CP (%DM)	17.19	17.36	17.27	17.74	17.91	17.82
EE (%DM)	3.50	3.49	3.55	3.64	3.64	3.70
NDF (%DM)	13.24	13.29	13.34	13.34	13.39	13.44
ADF (%DM)	5.71	5.69	5.78	5.80	5.79	5.87
SI ¹	0.85	0.95	0.71	0.93	0.80	0.72

¹ SI = synchrony index. SI close to 1.0 means that ruminal release of protein and energy sources is balanced appropriately compared to those having lower SI.

each experimental diet varied between 0.71 in the CFS group and 0.95 in the CES group. Feeds mixed with enzyme-treated carbohydrate or protein source (ECS, CES) had the highest SI, while the groups including formaldehyde treatment group (CFS, ECFS) showed lower SI, which may have been due insufficient N release of FS in the rumen.

All six experimental diets were used in the *in vitro* experiment to evaluate the effects of synchronicity on

rumen fermentation characteristics and MPS. The *in vitro* DM digestibility, pH, and ammonia-N concentration as influenced by different SI are presented in Table 4. The DM digestibility of all treatments increased as incubation time progressed from 0 h to 24 h. A significant interaction between C and S treatment was detected at 24 h incubation for DM digestibility ($p < 0.01$). CFS and ECFS with lower SI resulted in lower ($p < 0.01$) digestibility than those of high and intermediate SI groups at all incubation times, and highly synchronous diets had the greatest ($p < 0.01$) DM digestibility within the C groups. However, the degree of synchronicity did not influence digestibility within the EC groups. The results of the present study suggest that the rapidly fermentable fraction of the diet in the rumen is more important for ruminal digestion than the degree of synchronicity. Herrera-Saldana et al. (1990) also observed that barley-containing diets, which are more rapidly degraded than corn, had higher DM, OM, CP and starch digestibility than corn-based diets, regardless of the synchronicity of the carbohydrate and protein degradation rates. Since we used highly fermentable carbohydrate and protein sources in our experimental diets, the pH was rapidly dropped after 6 h of incubation in all treatments. Both treatments of C and S influenced the medium pH at

Table 4. *In vitro* DM digestibility, pH variation and NH₃-N concentration as influenced by different feed treatments

Item	Treatment ¹						SEM ²	significance		
	C			EC				CT ⁴	ST ⁴	CT×ST ⁴
	S	ES	FS	S	ES	FS				
SI ³	0.85	0.95	0.71	0.93	0.8	0.72				
DM digestibility (%)										
0 h	13.32 ^a	13.78 ^a	11.53 ^b	10.32 ^b	10.57 ^b	8.67 ^c	0.2048	**	**	NS
3 h	16.44 ^a	16.73 ^a	13.53 ^b	16.47 ^a	16.77 ^a	13.67 ^b	0.0793	NS	**	NS
6 h	20.93 ^c	21.94 ^b	17.26 ^e	22.49 ^b	24.01 ^a	18.39 ^d	0.1354	**	**	NS
12 h	31.00 ^b	32.27 ^{ab}	21.84 ^d	32.37 ^{ab}	33.73 ^a	24.31 ^c	0.2783	**	**	NS
24 h	47.78 ^{ab}	49.01 ^a	36.85 ^d	47.43 ^b	48.48 ^{ab}	40.35 ^c	0.2892	**	**	**
pH										
0 h	7.17 ^a	7.15 ^{ab}	7.11 ^b	7.15 ^{ab}	7.14 ^{ab}	7.14 ^{ab}	0.0122	NS	*	NS
3 h	7.08 ^a	7.04 ^{ab}	7.03 ^{ab}	6.91 ^{bc}	6.82 ^c	6.89 ^{bc}	0.0182	**	NS	NS
6 h	6.72 ^a	6.63 ^a	6.72 ^a	6.38 ^b	6.26 ^b	6.38 ^b	0.0215	**	**	NS
12 h	6.21 ^b	6.14 ^b	6.59 ^a	5.82 ^c	5.70 ^c	6.17 ^b	0.021	**	**	NS
24 h	5.39 ^c	5.35 ^c	5.74 ^a	5.37 ^c	5.37 ^c	5.47 ^b	0.0146	**	**	**
NH ₃ -N concentration (mg/100ml)										
0 h	3.69 ^b	3.83 ^{ab}	4.49 ^a	3.76 ^{ab}	4.13 ^{ab}	4.10 ^{ab}	0.1184	NS	**	NS
3 h	5.17 ^b	6.36 ^a	1.86 ^c	5.41 ^b	5.79 ^{ab}	2.09 ^c	0.0952	NS	**	*
6 h	6.13 ^a	8.23 ^a	0.51 ^b	7.43 ^a	8.23 ^a	1.81 ^b	0.3132	*	**	NS
12 h	11.27 ^a	11.96 ^a	2.66 ^c	6.20 ^b	5.14 ^b	1.84 ^c	0.4907	**	**	**
24 h	27.09 ^a	27.81 ^a	4.38 ^c	22.53 ^b	20.39 ^b	5.36 ^c	0.7257	**	**	**

¹ C = Corn, EC = Enzyme treated corn, S = Soybean meal, ES = Enzyme treated soybean meal, FS = Formaldehyde treated soybean meal.

² SEM = Standard error of the mean.

³ SI = Synchrony index. SI close to 1.0 means that ruminal release of protein and energy sources is balanced appropriately compared to those having lower SI.

* Significant at $p < 0.05$. ** Significant at $p < 0.01$. NS = Not significant ($p > 0.05$).

⁴ CT = The effect of C treatment, ST = The effect of S treatment and CT×ST = The interaction between C and S treatment.

^{a, b, c} Indicates significant difference within the same row ($p < 0.05$).

most incubation times, with a more noticeable effect after 6 h, but the interaction between the treatments was observed only at 24 h of incubation. EC-containing diets had a lower ($p < 0.05$) ruminal pH than those with untreated C as the carbohydrate source at 6 and 12 h incubation, indicating that rumen microbes degrade EC more efficiently than C. FS containing diets showed higher ($p < 0.01$) ruminal pH irrespective of carbohydrate source after 6 h incubation.

The ammonia-N concentrations of CS, CES, ECS, and ECES diets increased as incubation progressed. Ammonia-N concentration has been reported to increase immediately after feeding for 2 to 3 h and decrease until the next feeding in other *in vivo* studies (Chumpawadee et al., 2006; Seo et al., 2010). Our data showed the opposite result, with the above-mentioned *in vivo* ammonia-N concentration increasing consistently, which may be an indication of poor utilization of ammonia for MPS or may be due to decreased absorption of ammonia from the rumen as no absorption into the body is possible under *in vitro* conditions. We used a batch culture *in vitro* system in which the end product of fermentation could not flow out, and thus ammonia-N was overproduced and accumulated continuously. FS-containing diets had lower ($p < 0.01$) ammonia-N content than S- and ES-containing diets at all incubation times. This suggests that FS was not degraded to a significant extent by ruminal microbes. At 12 h and 24 h of incubation, EC diets showed a lower ($p < 0.05$) ammonia-N concentration than C-containing diets, regardless the degree of synchronicity,

indicating that efficient utilization of ammonia-N for MPS was achieved by ruminal microorganisms only when EC was offered as a carbohydrate source. The purine contents of all diets at 12 h of incubation were also confirmed the relationship between ammonia-N utilization and MPS, since the measurement of purine base production is an indirect method for evaluating MPS in the rumen (Makkar and Becker, 1999) (Table 5). Our data support the theory that lower ruminal $\text{NH}_3\text{-N}$ concentration correlates with higher utilization of $\text{NH}_3\text{-N}$ for MPS. There were no dietary treatment effects on purine base contents at 0 h of incubation. However, treatment effects of C and S and their interaction were significant ($p < 0.01$) at 12 h of incubation (Table 5). When C was used as an energy source, purine base concentration increased as the diets were more synchronized; this effect was not observed when EC was an energy source. ECES had the highest ($p < 0.01$) purine base content of all the diets, although it had only intermediate synchronicity of energy and protein supply in the rumen. These data imply that the supply of a highly fermentable energy source, rather than the synchronicity of energy and protein supply, was a more influential factor for stimulating MPS from ammonia-N (Hoover and Stokes, 1991). Rotger et al. (2006) have also concluded that rumen fermentation may be more limited by carbohydrate or protein availability than by the degree of synchronicity. A sufficient energy supply from a rapidly fermentable carbohydrate source may allow for the efficient incorporation of peptides, free amino

Table 5. VFA and purine concentration as influenced by different feed treatments at 12 h incubation

Item	Treatment ¹						SEM ²	significance		
	C			EC				CT ⁴	ST ⁴	CT×ST ⁴
	S	ES	FS	S	ES	FS				
SI ³	0.85	0.95	0.71	0.93	0.8	0.72				
VFA concentration (mM)										
Total	81.77 ^b	87.22 ^b	63.78 ^c	97.21 ^a	103.20 ^a	85.44 ^b	1.506	**	**	**
Acetate	41.06 ^{cd}	42.99 ^{bc}	33.59 ^e	44.61 ^{ab}	45.79 ^a	39.10 ^d	0.5146	**	**	**
Propionate	25.48 ^c	28.04 ^{bc}	18.69 ^d	35.40 ^a	38.52 ^a	30.65 ^b	0.7316	**	**	**
Iso-butyrate	0.93 ^{cd}	1.06 ^{bc}	0.69 ^d	1.34 ^{ab}	1.52 ^a	0.92 ^{cd}	0.0761	**	**	**
Butyrate	12.05 ^b	12.77 ^b	9.14 ^c	13.46 ^{ab}	14.59 ^a	12.55 ^b	0.3423	**	**	**
Iso-Valerate	1.24 ^b	1.30 ^{ab}	0.88 ^d	1.23 ^b	1.41 ^a	1.04 ^c	0.0295	**	**	**
Valerate	1.01 ^{bc}	1.06 ^b	0.79 ^c	1.16 ^{ab}	1.33 ^a	1.18 ^{ab}	0.052	**	**	**
A:P ratio	1.61 ^b	1.53 ^b	1.8 ^a	1.26 ^c	1.19 ^c	1.28 ^c	0.0304	**	**	**
Purine base (µg/ml)										
0 h	9.13	9.17	8.94	8.92	8.88	8.82	0.1366	NS	NS	NS
12 h	30.09 ^c	33.98 ^b	19.52 ^e	38.89 ^a	40.15 ^a	27.67 ^d	0.2923	**	**	**
24 h	33.92 ^a	33.42 ^a	29.11 ^b	27.86 ^b	27.09 ^b	26.70 ^b	0.7118	**	**	*

¹ C = Corn, EC = Enzyme treated corn, S = Soybean meal, ES = Enzyme treated soybean meal, FS = Formaldehyde treated soybean meal.

² SEM means standard error of the mean.

³ SI = Synchrony index. SI close to 1.0 means that ruminal release of protein and energy sources is balanced appropriately compared to those having lower SI.

* Significant at $p < 0.05$. ** Significant at $p < 0.01$. NS = Not significant ($p > 0.05$).

⁴ CT = The effect of C treatment, ST = The effect of S treatment and CT×ST = The interaction between C and S treatment.

^{a, b, c, d, e} Indicates significant difference within the same row ($p < 0.05$).

acids, and/or ammonia-N into microbial cells, thereby reducing ammonia-N concentration in the rumen and increasing MP. Hristov et al. (1997) confirmed the effects of different levels of carbohydrate and ammonia availability on the utilization of ammonia and alpha-amino N by ruminal mixed microorganisms *in vitro*. They observed an increased uptake and incorporation of ammonia into microbial cells with increasing carbohydrate level regardless of the type of N compounds. Theoretically, synchronization of energy and N release in the rumen can increase MPS, N utilization, and rumen fermentation efficiency. However, rumen microorganisms can respond to a deficiency of nutrients by synthesizing intracellular storage polysaccharides when energy sources are abundant, or by utilizing cellular storage and recycled urea N when carbohydrate and N sources are scarce (Valkeners et al., 2004; Ichinohe and Fujihara, 2008).

The VFA concentrations for each treatment after 12 h of incubation are shown in Table 5. There were significant C and S treatment effects, and the interaction of effects on VFA concentrations was also significant. Similar to purine concentrations, total VFA production and individual VFA concentrations of the groups using EC as an energy source was higher ($p < 0.01$) than in other groups (CS, CES and CFS). These parameters were not affected by the degree of synchronicity, as was seen with the purine base concentrations. All mixtures containing ECES had the highest values than any other treatments, in spite of having an intermediate SI value.

In summary, synchronization had positive effects on rumen fermentation, DM digestibility, and MPS when these were compared between low synchronous (CFS, ECFS) versus intermediate (CS, ECES) or high synchronous feeds (CES, ECS). However, there were no consistent results when we compared rumen fermentation characteristics between intermediate and high synchronous feeds. Meanwhile, rapidly fermentable energy and protein sources showed higher DM digestibility, VFA production, ammonia-N utilization, and purine base contents, indicating that the availability of energy or the protein source should be also considered as the other important factor for improving rumen fermentation and MPS.

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