Effect of Protein Sources on Rumen Microbial Protein Synthesis Using Rumen Simulated Continuous Culture System

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ABSTRACT : A rumen simulated continuous culture (RSCC) system was used to study the influence of supplementation of the three different types of protein sources such as urea, casein and soy protein on rumen microbial synthesis in terms of rumen microbial synchronization. The urea treatment showed the highest pH value. Ammonia nitrogen concentration was rapidly increased after feeding and not significantly different in the urea treatment (13.53 mg/100 ml). Protozoa numbers were not significantly different for soy protein and casein treatment compared to urea treatments during incubation. The average concentration of total VFA (mMol) was not detected with significant difference among treatments, but *iso*-butyrate production showed the highest for soy protein treatment among treatments ($p<0.001$). The lowest concentration in total *iso*-acids (*iso*-butyrate and *iso*-valerate) production was observed in urea treatment. The soy protein treatment showed no significantly change in acetate/propionate. The amounts of dry matter (DM) out flow showed no significant difference among treatments. Organic matter (OM) flow was the highest for urea treatments and the lowest for casein treatment ($p<0.03$). The nitrogen flow for casein treatment was not significantly different from other treatments. The efficiency of microbial protein synthesis in terms of microbial nitrogen (MN) synthesis (g MN/kg ADOM) digested in the rumen was highest for casein treatment (58.53 g MN/kg ADOM) compared to soy protein and urea ($p<0.05$). This result suggests that rumen ammonia releasing rate may influence on microbial protein synthesis in the rumen. ***(Asian-Aust. J. Anim. Sci. 2005. Vol 18, No. 3 : 326-331)***

Key Words : RSCC, Urea, Casein, Soy Protein, Microbial Protein Synthesis, N Flow

INTRODUCTION

The synchronization of energy and nitrogen sources in rumen can improve microbial protein synthesis and the efficiency of their utilization can improve ruminant productivity. However, the different degradability of the two sources will disorder the efficient utilization in practices. The conversion rate can be varying in the different nitrogen sources into ammonia in rumen. For instance, urea can be degraded much faster than other nitrogen sources such as amino acid, peptide, and feed protein; therefore, it would be hard to match with the volatile fatty acid (VFA) production rate, which is an indicator of ATP synthesis rate from the carbohydrate degradation. VFA production rate can vary in the different carbohydrate sources, and the microbial protein production rate from feed nitrogen sources can be dependent on the ruminal degradation rate and the range. In addition, digestion rate of feed nitrogen can affect the microbial protein synthesis (Crooker et al., 1978) and can be associated with some energy supply (Russell et al., 1981). The high activity of degradation in nitrogen sources compared to its in energy sources allows the access nitrogen to flow into liver where urea produces in, and rest of them mainly excrete to urine (Church, 1988). In contrast, the high degradation rate of energy sources can not permit ATP produced to be recruited for microbial protein synthesis, instead of the accumulation of carbohydrate in body.

Protein digestibility in rumen may affect on the flux of amino acids into small intestine (Stern et al., 1994), and it can also affect on production rate and quantity of ammonia, peptide, branched-chain VFA and free amino acid (Broderick et al., 1988). According to the National Research Council (NRC, 1994), microbial protein synthesis in rumen is important for the demand of the protein in small intestine. The absorbable protein in small intestine is the key for the demand and it will be decided by undegradable protein (UDP) contents of feed protein (Koeln and Paterson, 1986).

In summary, this study was conducted to elucidate the method and effect of synchronization of protein and carbohydrate digestibility in rumen. We used the modified RSCC (rumen simulated continuous culture) system, the dual phase dilution system in study of the ammonia production rate, rumen fermentation and microbial protein synthesis by using different protein sources with various rates of ammonia production such as urea, casein and soy protein. Therefore, it will be important to apply the feed formula in practice.

MATERIAL AND METHOD

Experimental design

The rumen simulated continuous culture (RSCC)

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Table 1. The experimental diets of protein source[1]

Items	Treatments		
	Urea	Casein	Soy protein
	---------- g DM/day ----------		
Urea[2]	0.99	-	-
Casein[3]	-	3.64	-
Soy protein[4]	-	-	4.29
Cellulose	10.08	10.08	10.08
Starch	6.72	6.72	6.72
Total	17.79	20.44	21.09

[1] *iso*-nitrogenous base (17%). [2] Urea: 46% TN.
[3] Casein: 14.4% TN. [4] Soy protein: 12.6% TN.

system with a dual phase dilution was used to assess the rumen synchronization of protein from feeds. This RSCC system was modified by Merry and McAllan (1987), using the dual phase system the flow rate of effluent and filtered effluent (FE) of pore size of filter 50±15 μm (#510, ANKOM Inc. Australia) in fermenter was fixed in 60:40, McDougall buffer (1948) was supplied in the system in the rate of 70 ml/h. One litter of this stained rumen fluid was used in the 1,500 ml capacity fermenter of RSCC system.

Dilution rate of FE was 31 ml/h and dilution rate of effluent was 4.8%/h. Collection of FE and supply of buffer solution were controlled by the peristaltic pump (MP-3A, Eyela, Japan), feed infusion was made in the powder form of mixture in each treatments (2% of rumen fluid in fermenter) and 09:00 and 21:00 h, 12 h interval everyday. Sufficient of this material was freeze-dried for analysis of purine bases, DM, OM and TN. Rumen microbial in mixed effluent was used to calculate daily microbial N flow and apparently digestibility organic matter (ADOM) from the vessel contents.

Daily flows of digesta components and digestibility were calculated using the data for mixed effluent (ME) and the corresponding dietary component inputs. Daily incubation time was 23 h and 30 min before the feed infusion was considered as preparation period. The ME, 200 ml/day were stored in -20°C.

The treatments of feed were urea, casein and soy protein in 3×3 Latin Square, and samples were collected from every 1h during the 12h incubation at the 5th day after 4 days for the adaptation period. The samples were either measured immediately or stored in -20°C for the later analysis.

The rumen fluid was obtained from a Hostein lactating cow with cannulae (Bar Diamond Inc., USA), and it was filtered through 8-layers of cheesecloth to remove any remain feed particles. And exceptions to this were the urea, casein and soy protein diets which were obtained from a donor cow fed *ad libitum* on corn silage only.

Experimental feeds

For 100% of protein sources were from urea, casein, or soy protein in each treatment, and the ratio of forage and concentrate was 60:40 with the same quantity of protein supplied. The experimental feeds were consisting of 17% total nitrogen (TN) in basis of *iso*-nitrogen and three sources contained 46% for urea, 14.4% for casein and 12.6% for soy protein (Table 1). Forage source was cellulose (Fibrous, long, Sigma Co., USA) and carbohydrate source was soluble starch (Sigma Co., USA).

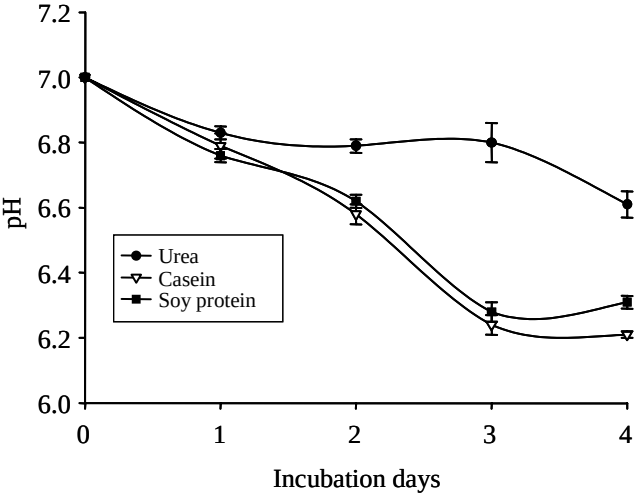

Figure 1. The Change in pH of continuous culture system provided different protein sources during adaptation period (0 hr=just before feeding, n=3).

Experimental analysis

The sample pH was immediately measured after incubation with the pH meter (Model 420A, Orion, USA), after incubation protozoa of FE samples was counted with optical microscopy (Model CHS, Olympus, Japan) by the method of Ogimoto and Soichi (1981) method. After FE was centrifuged (20,000×g for 20 min), ammonia nitrogen (NH_3-*N*) concentration was measured by spectrophotometer (UVKON 860, Kontron, Swiss). The VFA concentration was detected by gas chromatography (HP 5890, Hewlett Packard, USA) according to the Erwin (1961) method. Microbial protein indicator in rumen was used for DNA and RNA analysis of the ME and bacteria samples. And the level of adenine, guanine, thymine, cytosine and uracil were detected by HPLC (Gilson 305 system, Gilson, France) by the method of Cozzi and Polan (1993). Efficiency of microbial protein synthesis was presented in nitrogen amount for organic digestion (kg) in rumen according to McAllan et al. (1994).

Statistical analysis

Data were analyzed of ANOVA using GLM (General Linear Model) of SAS package program (2,000, release. 8.1a), treatment differences were declare at $p<0.05$ using the Duncan's multiple range test (Steel and Torrie, 1981) was applied for the statically significance.

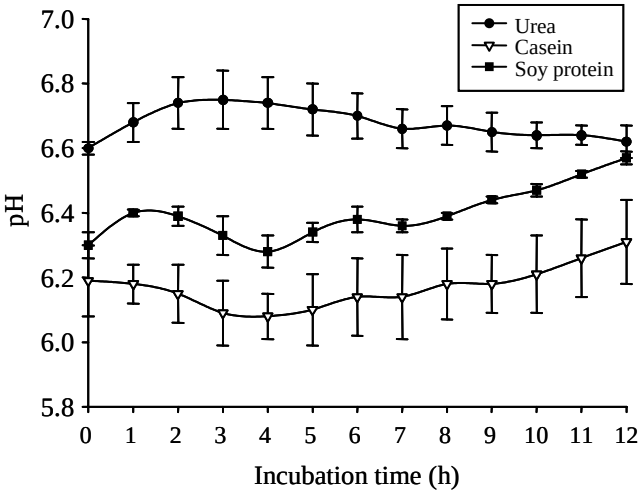

Figure 2. The change in pH of continuous culture system provided different protein sources during incubation period (0 hr=just before feeding, n=3).

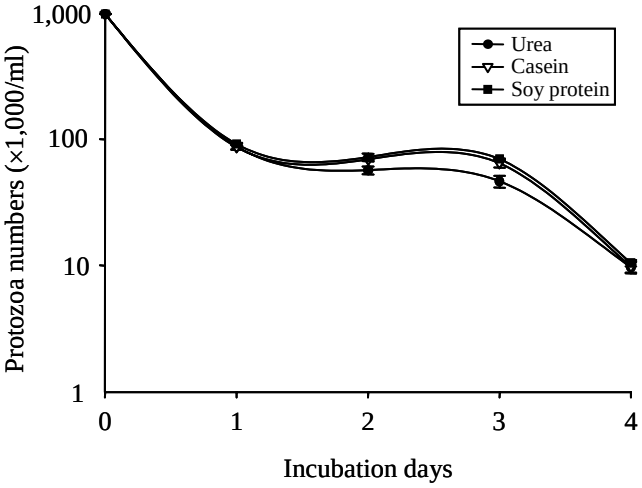

Figure 3. The change in protozoa numbers of continuous culture system provided different protein sources during adaptation period (0 hr=just before feeding, n=3).

RESULTS AND DISCUSSION

During the adaptation period of day 1 to 4, the pH of the samples within the optimum range of 6.2-7.0 for the microbial protein synthesis, including the urea treatment, in particular, showed increased values (Hazlewood et al., 1981) (Figure 1). After feeding in the day 5, pH was maintained in average of 6.44 between 7-12 h. It was gradually increased in urea treatment by 3 h, and then tends to be decreased. In casein treatment, pH was sustained in average of 6.20 until 7 h, and then tend to go up slightly afterward. In soy protein treatment, pH 6.35 was averagely maintained until 8 h, and then tend to increase afterward pH values in the different treatments at 9 h ($p<0.03$). However, there was no difference afterward (Figure 2). This pH changes might be due to the volatile fatty acid (VFA) and

Table 2. Variation in NH_3-*N* concentration of continuous culture system provided different protein sources[1]

Incubation time (h)	Treatments			Significance
	Urea	Casein	Soy protein	
	---------- mg/100 ml ----------			
0	20.72±2.13	12.29±1.50^{b}	20.52±0.29	0.05
1	34.41±4.64^{a}	16.68±1.36^{ab}	26.54±2.07^{b}	0.04
2	34.03±4.56	12.90±0.33	25.36±0.04	0.05
3	35.34±3.22	15.33±0.67^{b}	30.43±1.65	0.01
4	35.88±4.43	14.75±1.82^{b}	30.71±1.43	0.03
5	26.74±8.31	14.67±1.67^{b}	26.14±1.04	0.02
6	30.42±4.37^{a}	16.96±2.75^{ab}	22.02±3.69^{b}	0.04
7	25.62±2.57	13.67±1.22^{a}	26.57±0.36	0.03
8	23.51±8.30^{a}	12.29±0.12	18.31±1.32	NS[2]
9	21.00±5.32	11.74±0.38	20.30±1.96	NS
10	22.80±4.57	11.35±1.90	17.14±4.98	NS
11	21.62±5.12	11.65±1.39	20.48±0.32	NS
12	22.21±5.60	11.65±0.40	17.91±1.15	NS

[1] Mean of triplicates±SE.
[a, b, ab] Means with different superscripts in the same row are significantly different.
[2] NS: not significant.

lactic acid production during the incubation (Mould et al., 1983). In addition, the low pH may be considered to be a complex of substrates supplying specificity, quantity of feeding and VFA production by microbial activity. The pH was increased in the urea treatment due to the high level of ammonia nitrogen (NH_3-N) production.

Protozoa numbers showed no differences among the treatments (Figure 3). The variations of the protozoa numbers was not sufficiently changed to affect the microbial protein synthesis in this study as it was described in the previous study (Jouany and Ushida, 1999; Shabi et al., 2000). And it is generally considered that a steady number of protozoa reflects a steady state of fermentation in a fermenter (Merry et al., 1987) although a criticism (Blake and Sterm, 1988) in that a rapid turnover rate of a fermenter would not maintain a proper number of protozoal poulations exists.

In urea treatment, NH_3-N concentration was significantly higher than other treatments until the 7 h incubation, and no differences afterward (Table 2). This high production with urea addition may be due to the low efficiency of microbial protein synthesis compared to other treatments, and this fast NH_3-N production may be resulted from the delay of ATP conversion to microbial protein (Russell et al., 1992). NH_3-N concentration, of average 1-72 mg/ml in rumen, is an important factor of microbial protein synthesis efficiency and this can be varies in quantity of digestible carbohydrate, the level of grain feeds can be utilized by protein degradable bacteria (Siddons and Paradine, 1981; Chanjula et. al., 2004). In addition, NH_3-N concentration at the level of above 5 mg/100 ml did not affect to the microbial protein synthesis (Roffler and Satter,

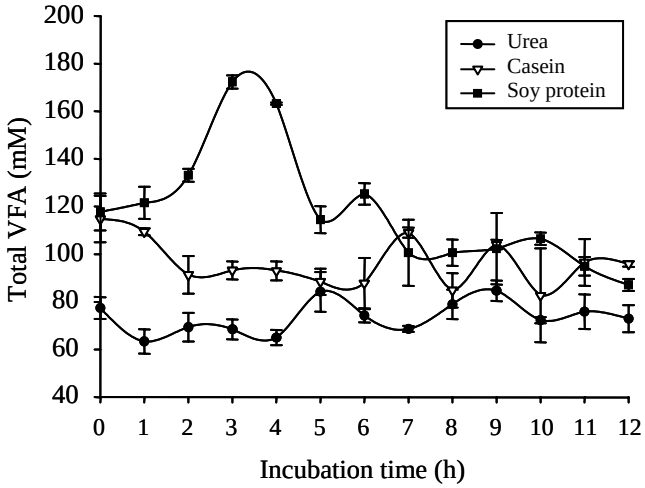

Figure 4. The change in total VFA concentration of continuous culture system provided different protein sources during adaptation period (0 h=just before feeding, n=3).

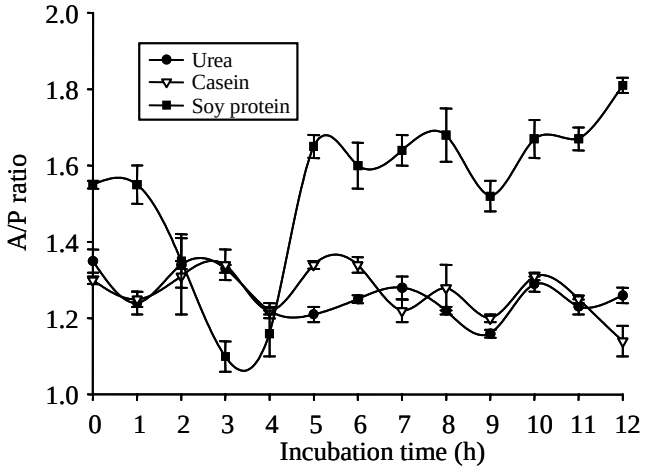

Figure 5. The change in acetate/propionate ratio concentration of continuous culture system provided different protein sources during adaptation period (0 h=just before feeding, n=3).

1975). In this study, the NH_3-N concentration was well above the level for the no effects on microbial protein synthesis throughout the incubation (Table 2).

Total VFA production tend to be down regulated in urea treatment, whereas soy protein treatment showed a trend to increase rapidly up to 172.4 mM at the 3 h incubation time, and then it was dropped afterward. This may be resulted from the higher supply amount of substrates in soy protein treatment compared to other treatments (Figure 4). The ratio of acetate/propionate (A/P) in soy protein treatment was rapidly dropped at 3 h incubation time, but showed, in contrast, a higher ratio than other treatments after 5 h incubation (Figure 5). At the 0 h of sampling time, VFA concentration; acetate, propionate, butyrate showed no significant differences, whereas *iso*-butyrate was the highest in the soy protein treatment of 1.90 mM, and was the lowest in the Urea treatment of 0.76 mM ($p<0.001$). The A/P ratio in soy protein treatment was the highest of 1.55, and urea treatment of 1.35 and casein treatment of 1.30 showed no changes in A/P ratio ($p<0.007$). For the individual VFA production, urea treatment demonstrated no changes in all the time of incubation, casein treatment showed a decreased amount at 3 h incubation, and soy protein treatment tended to increase. The concentration of *iso*-valerate and valerate was similar in casein and soy protein treatments, but urea treatment was slightly higher. This higher concentration of branched-chain VFA in casein and soy protein treatments was due to the higher amount of branched-chain amino acids compared to the urea treatment (Orias et al., 2002).

Flow rates of DM, OM, N and microbial protein synthesis efficiency in 4-5 days incubation were presented in gram of microbial nitrogen (MN) synthesis measured in adenine basis per kilogram of organic matter digested (OMD) (Table 3). DM flow and N flow trend of high in casein treatment, and OM flow were low. Efficiency of microbial protein synthesis (EMPS, g MN/kg ADOM) was 30.02, 58.58 and 46.49 in urea, casein and soy protein treatments respectively. Even though the same amount of 3.8 g of N was added to all treatments at the day 1 incubation, urea treatment showed a low in nitrogen flow because the NH_3-N concentration in fermenter were thought to be sustained in high during the incubation. From the microbial protein flow data, most of N in ME was the microbial protein in soy protein and casein treatments, and this can be considered that nitrogen utilization rate can be very high if purified diets were fed. These results can be referred from studies in uptake of peptides by rumen bacteria, and importance of peptides can positively correlate

Table 3. The results of digesta flow and efficiency of microbial protein synthesis in continuous culture system at last incubation day[1]

Items	Treatments			Significance
	Urea	Casein	Soy protein	
	---------- g DM/day ----------			
DM[2] flow	17.41±0.32	18.31±0.72	17.51±0.54	NS
OM[3] flow	8.02±0.39[a]	6.64±1.01[b]	7.42±0.87[c]	0.03
TN[4] flow	3.18±0.30	3.73±0.73	3.54±0.16	NS
	---------- g MN/kg ADOM ----------			
EMPS[5]	30.02±12.76[b]	58.53±12.70[a]	46.49±7.84[ab]	0.05

[1] Mean of triplicates±SE. [2] DM: dry mater. [3] OM: organic matter.
[4] TN: total nitrogen. [5] EMPS: efficiency of microbial protein synthesis. [6] NS: not significant.

with the rumen microbial protein synthesis rather than amino acids (Griswold et al., 1996). Chen et al. (1987) demonstrated that peptide uptake can be limited by microbial growth rate over the protein degradation, and it can be even more increased if the protein source can be degradable in the rapid rate. The differences in microbial protein synthesis and microbial growth rate of three treatments used in this study were proportionally correlated between peptide concentration and microbial protein synthesis in rumen (Ha et al. 1991). However, increased amount of NH_3-N flow in urea treatment resulting of providing 100% nitrogen from ammonia was due to the shortage of peptides converted into microbial protein, and microbial protein flow into the next digestive organs is consequently lower compared to the protein quantity.

In the work of Merry et al. (1987), types of nitrogen supply can affect the substrate utilization, in particular, fiber utilization. From this study, the reason of low substrate utilization in urea treatment may be due to that nitrogen source affects microbial growth and microbial specificity and the changes in microbial ecology toward the substrates. When amino acid was fed as nitrogen source, cellulose digestibility was higher than ammonia. However, no difference was observed between amino acid and peptide (Thomas et al., 1985). The level of peptide is an efficiency parameter of energy utilization from nitrogen source. Peptide synthesis was the highest in casein treatment at the 4-5 days incubation in this study and this result is similar to the previous works (Broderick and Wallace, 1988). In addition, for the VFA production amount and rate, *iso*-acids production was higher in casein and soy protein treatments than in urea treatment, and this was resulted from high degradation amount of branched chain amino acid and high rate of microbial protein degradation. The level of *iso*-acids production in urea treatment was resulted from the lysis of microbial protein and this nitrogen recycling pool during microbial fermentation needs a further investigation.

From the results above, microbial protein synthesis efficiency can be differ from NPN, free amino acid, peptide rather than the protein quantity. The synchronization of carbohydrate and protein digestibility to maximize the rumen microbial protein synthesis efficiency may be optimized by peptide type feeding for the different protein digestibility when the same carbohydrate source was fed. However, it required more study on changes in ammonia and peptide concentration, production rates, and the relative fields.

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