The Requirement of Ruminal Degradable Protein for Non-Structural Carbohydrate-Fermenting Microbes and Its Reaction with Dilution Rate in Continuous Culture[a]

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ABSTRACT : A continuous culture study was conducted to determine the impact of ruminal degradable soy protein (S-RDP) level and dilution rate (D) on growth of ruminal non-structural carbohydrate-fermenting microbes. Corn starch, urea and isolated soy protein (ISP) were used to formulate three diets with S-RDP levels of 0, 35 and 70% of total dietary CP. Two Ds were 0.03 and 0.06 h^{-1} of the fermenter volume in a single-effluent continuous culture system. As S-RDP levels increased, digestibilities of dietary dry matter (DM), organic matter (OM) and crude protein (CP) linearly (p=0.001) decreased, whereas digestion of dietary starch linearly (p=0.001) increased. Increasing D from 0.03 to 0.06 h^{-1} resulted in decreased digestibilities of dietary DM and OM, but had no effect on digestibilities of dietary starch (p=0.77) and CP (p=0.103). Fermenter pH, the concentration of volatile fatty acids (VFA) and daily VFA production were unaffected (p=0.159-0.517) by S-RDP levels. Molar percentages of acetate, propionate and butyrate were greatly affected by S-RDP levels (p=0.016-0.091), but unaffected by D (p=0.331-0.442). With increasing S-RDP levels and D, daily bacterial counts, daily microbial N production (DMNP) and microbial efficiency (MOEFF; grams of microbial N produced per kilogram of OM truly digested) were enhanced (p=0.001). The increased microbial efficiency with increasing S-RDP levels is probably the result of peptides or amino acids that served as a stimulus for optimal protein synthesis. The quantity of ruminal degradable protein from soy proteins required for optimum protein synthesis of non-structural carbohydrate-fermenting microbes appears to be equivalent to 9.5% of dietary fermented OM. (*Asian-Aus. J. Anim. Sci. 2000. Vol. 13, No. 10 : 1399-1406*)

Key Words : Rumen Microbial Growth, Ruminal Degradable Protein, Dilution Rate, Continuous Culture

INTRODUCTION

Microbial proteins synthesized within the rumen provide a major source of amino acids to ruminant animals. The ruminal microbial ecosystem can be divided into two groups, microbes that ferment structural carbohydrate and those that ferment non-structural carbohydrate (NSC, Russell et al., 1992). NSC-fermenting microorganisms usually represent a predominant population of rumen microbial flora in high-producing ruminant animals, such as lactating dairy cows and feedlot beef cattle. The nitrogen requirement of NSC-fermenting microbes can be met by either ammonia or peptides and amino acids (Russell et al., 1992). When NSC-fermenting microorganisms grow, they derive 66% of their N from peptides or amino acids and 34% of the N from ammonia (Russell et al., 1983). Jones et al. (1998) reported that growth of rumen microbes achieved a highest value on the diet containing 10% peptides. Peptides or amino acids in the rumen are derived mainly from degradation of dietary proteins. Therefore, in practice, the requirement of peptides or amino acids for ruminal microbes can be simply met by ruminal degradable protein (RDP) from true proteins such as soybean meal. Fu et al. (1999) demonstrated that ruminal microbial efficiency was maximized at 9.4% RDP of dietary fermented OM.

Dilution rate influences rumen fermentation. Isaacson et al. (1975) demonstrated that increasing D in a continuous culture considerably altered ruminal fermentation parameters, and enhanced microbial efficiency. Similar results were also obtained from other studies *in vitro* (Crawford et al., 1980; Maeng et al., 1989; Meng et al., 1999) and *in vivo* (Kennedy et al., 1976). Little information is available on the effect of reaction of D with dietary RDP levels on growth and fermentation of NSC-fermenting microbes in the rumen. Therefore, an experiment was conducted to evaluate effects of S-RDP levels and dilution rate on growth of ruminal microbes incubated with non-structural carbohydrates in continuous culture.

EXPERIMENTAL PROCEDURES

Continuous culture system

A single-effluent continuous culture system was used in this experiment. The basic apparatus consisted of 12 independent 2000-ml fermenter flasks which are

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immersed in a waterbath heated by a thermostatically controlled heater (Model 730, Fisher Scientific, Pittsburgh, PA) and magnetically stirred. The fermenter was a polycarbonate jar (Fisher) which was equipped on the top cover with several input ports for buffer, gas, pH electrode and feed, and also with an effluent outlet at a height allowing a liquid volume of 1460 ml within the fermenter before overflow. Peristaltic pumps (Masterflex model 7520-10, Cole Parmer Instrument Co., Chicago IL) were used for delivering buffer solution from buffer reservoirs into fermenters. An automated feeding device, mounted above each of the fermenters, was used to deliver pelleted diets into fermenter.

Fermentation conditions

Three to four donor cows were fed a ration formulated to meet their requirements (NRC, 1989), consisting of 20% alfalfa haylage, 20% corn silage and 60% mixed concentrate including mineral-vitamin premix. Ruminal contents were taken via ruminal fistulas and strained through one layer of cheesecloth into a prewarmed thermos. The strained ruminal fluid was added to the fermenters up to the level of the overflow port. Fermenters were continuously infused with a high buffer capacity solution (Slyter, 1990) with urea plus ammonium sulfate (87.5 mg NH_3-N/l and 30 mg S/l) added at a rate of 0.73 and 1.46 ml/min to provide the dilution rates of 0.03 and 0.06 h^{-1}. Carbon dioxide was flushed constantly to fermenters at a rate of about 15-30 ml/min to preserve anaerobiosis. Temperature was kept constant at 39℃ under a continuously stirring condition. Fermenters were supplied daily with about 31 g of DM of pelleted diets by the automated feeding device in 12 equal portions over a 24-hr period. Fermenters were incubated for a total of 8 days that included 5 days for adaptation and 3 days for sampling.

Diets

Three experimental diets consisting of corn starch, urea and isolated soy protein (ISP, 90.4% CP, ICN Biomedicals, Costa Mesa, CA) were formulated to provide ruminal degradable soy protein (S-RDP) levels of 0, 35 and 70% of total dietary CP. The ingredients and chemical analyses of the diets are presented in table 1. All ingredients were mixed well and then extruded (120℃) to form pellets (6 mm diameter × 12 mm long). The diets were dried at room temperature until use.

Sample collection and processing procedures

During a 3-day sampling period, effluent fluid and fermenter contents were sampled once daily. When sampling started, 37% formaldehyde solution (Fisher) was added at a rate of 2.5% of estimated volume to each effluent reservoir immersed in ice-cooled water to inactivate microbial activity. The total effluent fluid collected and recorded for volume over 3 sampling days was pooled and stored at 4℃. Subsamples of formalinized effluent fluid (approximately 1200 ml) were weighed and centrifuged at 30,000 × g for 30 min. The pellet, containing undigested feed fractions, microorganisms and a small amount of buffer salt contamination, was washed two times with distilled water, then frozen and lyophilized (10℃, shelf; Model Unitop 800 L and Freezemobile 24, Virtis, Gardiner, NY). Samples were then placed at room temperature to balance moisture for 3 days, then weighed and ground (1-mm screen). Samples for bacterial and protozoal counting were taken from fermenters into 50% formalin-saline solution at a ratio of 1:1 and stored at 4℃. For determination of VFA and ammonia concentration, fermenter content samples were taken and mixed with 20% (v/v) sulfuric acid solution (1:99), then stored at -20℃ until analyses. To isolate pure bacterial samples, at the termination of each period 37 ml of 37% formaldehyde solution was added to the fermenter content (1460 ml) and blended for 1 min to promote release of attached microorganisms from feed particles. After being strained through 4 layers of cheesecloth, the fluid fraction was centrifuged at 1,000 × g for 5 min to remove feed particles and probably most protozoa. The supernatant fluid was recentrifuged at 30,000 × g for 30 min at 4℃. The pellet, containing bacteria and some protozoa, was washed three times with 0.9% (w/v) saline solution (first time) and distilled water (second and third time) by centrifugation (30,000 × g, 30 min, 4℃). The resulting pellet was frozen and lyophilized.

Table 1. Ingredients and chemical composition of the experimental diets

Item	S-RDP (% of total CP)		
	0	35	70
Ingredients, % DM			
Isolated soy protein (90.4% CP)	0	7.8	15.8
Corn starch	94.9	89.6	84.2
Urea	5.1	2.6	0
Chemical analyses			
CP (% of dietary DM)	14.3	14.4	14.3
S-RDP[a] (% of total dietary CP)	0	34.3	70.1
Starch (% of dietary DM)	94.9	89.9	85.1
Ash (% of dietary DM)	1.2	1.5	2.1

[a] Ruminal degradable protein from soy proteins. The RDP percentage of ISP, 70.1%, was determined by the nylon bag method with 36-hour incubation in the rumen of the experimental cows used in the experiment.

Analyses

The samples of diets, isolated microorganisms and effluent residues were analyzed for DM and ash (AOAC, 1984), N content (Leco Model FP-428, Leco Co., St. Joseph, MI) and starch (Smith, 1969). Isolated microorganisms and effluent residues were used to determine RNA contents according to the procedure of Zinn and Owens (1986). Microbial N of effluent residues was calculated from the RNA content of the effluent residues. True digestibility of DM, OM, starch and CP was computed as the difference of DM, OM, starch and CP between diets and effluent residues corrected for microbial contributions. Microbial growth efficiency was expressed as grams of microbial N per kilogram of OM truly digested. The pH of fermenter contents was measured with a glass electrode pH meter. Ammonia concentration of fermenter contents was analyzed using a DU-50 spectrophotometer (Beckman, Palo Alto, CA) according to the procedure of Broderick and Kang (1980). Samples for VFA analysis were prepared as described by Grigsby et al. (1992) and VFA concentration was determined by gas chromatography (Varian model 3400, Varian Instrument Group, Walnut Greek, CA). Total bacterial and protozoal numbers were counted by a counting chamber method. The formalin-fixed samples were diluted with 10% formalin solution 25-50 fold depending on the bacterial concentration. One volume of the diluted sample was mixed with an equal volume of Grams crystal violate solution and stained for several minutes. Bacterial numbers were counted with a Petroff-Hausser counting chamber under oil immersion. Counting protozoa with a Hausser-Hemacytometer (Hausser Scientific, Horsham, PA) was based on the procedure of Dehority (1993). Briefly, the formalin-fixed sample was diluted with 10% formalin solution 2 to 20 fold, depending on the concentration of protozoa in samples. For this study, samples were diluted 2 fold. Then, 1 ml of the diluted sample was added to 0.1 ml of 2.0% (w/v) brilliant green solution and allowed to stain for at least 4 hours. The stained sample was further diluted with 30% glycerol-saline solution to give 50-100 protozoa in a counting chamber area ($10 \times 10 \times 0.5$ mm). The protozoal number was counted by a microscope at a magnification of 100x.

Statistical analysis

Data were analyzed as a randomized complete block design with 2 D and 3 S-RDP levels as treatments arranged as a 2×3 factorial and 2 runs as a block using the GLM procedures of SAS (1991). On each run, treatments were randomly allocated to fermenters, giving two replications for each treatment. Because block effects were not statistically significant in either item, the sums of squares and degrees of freedom due to block were pooled with error. Sums of squares for treatments were separated into effects of S-RDP levels and D, and the interaction. Statistical analysis also included comparison of the linear and quadratic patterns among three S-RDP levels using the CONTRAST statement of SAS (1991). Because the interaction of S-RDP levels and D was not significant, main effect means are presented in table 2, 3, 4 and 5.

RESULTS AND DISCUSSION

Digestion of dietary DM, OM, starch and CP

Results of true digestibilities of DM, OM, starch and CP are presented in table 2. Digestibilities of DM, OM, starch and CP were significantly influenced by S-RDP levels (p=0.001-0.027). As S-RDP levels increased from 0 to 70% in the diet, digestibilities of DM, OM and CP linearly (p=0.001-0.002) decreased, whereas digestibility of starch was linearly (p=0.003) increased. The decreased digestibilities of dietary DM and OM with increasing S-RDP levels seemed to be related to lower digestion for ISP than for urea, which is reflected in the higher digestibility of CP with the 0% S-RDP level (100% urea N) than with the 70% S-RDP level. Although CP digestion was depressed, the digestibility of starch linearly increased as S-RDP levels increased in the diet. This result is in agreement with the study that showed increased digestibility of NSC and OM when the dietary protein level increased from 17 to 20% (Christensen et al., 1993), but contrasts with the result of Griswold et al. (1996) in

Table 2. Effect of S-RDP level and dilution rate on digestibilities of DM, OM, starch and CP of the purified diet in continuous culture[a]

	S-RDP (% of total CP)				D (h^{-1})			p= S-RDP		
Digestibility	0	35	70	SEM	0.03	0.06	SEM	L	Q	D
DM (%)	78.5	76.6	74.3	0.6	79.2	73.8	0.5	0.001	0.743	0.001
OM (%)	79.1	77.0	74.7	0.6	79.6	74.2	0.5	0.002	0.876	0.001
Starch (%)	77.6	80.1	82.7	0.5	80.1	80.2	0.4	0.003	0.937	0.770
CP (%)	98.5	77.8	64.7	2.0	82.3	78.3	1.7	0.001	0.148	0.103

[a] S-RDP=ruminal degradable protein from soy proteins; D=dilution rate; L=linear effect; Q=quadratic effect.

which the digestibility of NSC decreased when S-RDP replaced urea N in continuous culture.

When D increased from 0.03 to 0.06 h^{-1}, digestibilities of DM and OM were remarkably reduced (p=0.001) whereas digestibilities of starch and CP were unaffected by D (p=0.103-0.770; table 2). The reduced digestibilities of DM and OM obtained from this study may be the result of shortening of residence time of dietary substrates in the fermenters due to increasing D as suggested by Owens and Goetsch (1986). The decreased digestion of DM and OM as a result of increasing D was reported in other *in vitro* (Meng et al., 1999; Maeng et al., 1989; Crawford et al., 1980) and *in vivo* studies (Kennedy and Milligan, 1978). Unaltered digestion of starch and CP owing to increasing D may be mainly associated with starch and urea being sensible to digestion by ruminal microbes in the fermenters.

Fermentation parameters

Table 3 shows the effects of S-RDP levels and D on microbial fermentation parameters. Fermenter pH values increased (p=0.001) with increasing D, but were unaffected by S-RDP levels (p=0.159). *In vivo*, ruminal pH is a function of the amount of saliva entering the rumen and the amounts of organic acids, particularly lactic acids and VFA that are produced and accumulated in rumen contents (Church, 1976). In the current study, two Ds were made by varying amounts of buffer solution or artificial saliva flowing into fermenters. Therefore, increased buffer capacity of the system can explain such increased pH with increasing D. In agreement, other studies (Crawford et al., 1980; Isaacson et al., 1975; Meng et al., 1999) also showed that ruminal or fermenter pH was affected by D.

Although increasing D significantly (p=0.001) decreased fermenter VFA concentration (molar/l), increasing dietary S-RDP levels had no impact on VFA concentration (p=0.517) and daily VFA production (mol/d, p=0.308; table 3). The higher fermenter VFA concentrations at slow D can be ascribed to extensive fermentation occurring in the fermenters. The higher digestibilities of DM and OM with the slow D than with the fast D shown in table 2 are an indicator of such extensive fermentation. As dietary S-RDP levels increased, molar percentages of acetate increased (linear, p=0.016), but the percentages of propionate and butyrate decreased (linear, p=0.017-0.091). The molar percentages of acetate, propionate and butyrate were unaffected by D (p=0.331-0.442). The altered proportions of major individual VFAs with increasing dietary S-RDP levels may reflect a shift in microbial species or alteration of microbial metabolism in the fermenters. In contrast with the molar percentages of major individual VFAs, molar percentages of isobutyrate, valerate and isovalerate increased (linear, p=0.001-0.007) with increasing S-RDP levels, but decreased with increasing D (p=0.004-0.022). Higher molar proportions of isobutyrate, valerate and isovalerate occurring in the higher S-RDP diets would be expected, because these three VFAs are major end products of amino acid fermentation (Barnett and Reid, 1961). The three VFAs have been recognized to be essential for many species of bacteria for synthesis of their branched chain amino acids or microbial proteins (Yokoyama and Johnson, 1988). The decreased concentration of isobutyrate, valerate and isovalerate with increasing D may be a reflection that either production of these acids was reduced, or utilization of the acids by microbes increased, or both. In another continuous culture study using ground corn as non-fibrous carbohydrate substrates and ISP as a protein source,

Table 3. Microbial fermentation parameters in continuous culture fermenters as affected by soy RDP levels and dilution rate[a]

	S-RDP (% of total CP)				D (h^{-1})			p= S-RDP		
	0	35	70	SEM	0.03	0.06	SEM	L	Q	D
pH	6.32	6.22	6.21	0.04	5.77	6.72	0.03	0.109	0.118	0.001
Total VFA										
mM/L	106.3	101.4	100.3	3.9	133.6	71.7	3.2	0.286	0.695	0.001
mM/day	157.9	151.2	146.2	5.2	148.8	154.8	4.3	0.132	0.887	0.335
Individual VFA	---------- mol/100 mol ----------									
Acetic	47.2	49.2	50.9	1.0	49.5	48.6	0.8	0.016	0.922	0.442
Propionic	33.7	33.5	30.7	1.3	31.9	33.3	1.0	0.091	0.407	0.331
Butyric	18.5	15.5	15.0	0.9	15.8	16.9	0.8	0.017	0.272	0.334
Isobutyric	0.0	0.1	0.2	0.0	0.2	0.1	0.0	0.001	0.858	0.004
Valeric	0.5	1.3	2.3	0.4	2.0	0.8	0.3	0.007	0.931	0.022
Isovaleric	0.1	0.5	1.0	0.1	0.7	0.3	0.1	0.001	0.839	0.004

[a] S-RDP=ruminal degradable protein from soy proteins; D=dilution rate; L=linear effect; Q=quadratic effect.

Meng et al. (1999) also found a decreased concentration of isobutyrate, valerate and isovalerate with increasing D.

Bacterial and protozoal counts

Results of microscopic counting of bacteria and protozoa are shown in table 4. As dietary S-RDP levels increased, bacterial counts (cells/ml) and bacterial production (cells/day) linearly (p=0.001) increased. When S-RDP levels increased, amounts of peptides or amino acids derived from degradation of ISP must be enhanced, which may stimulate bacterial growth and protein synthesis as suggested by Argyle and Baldwin (1989). When D increased from 0.03 to 0.06 h^{-1}, bacterial counts had no change (p=0.3), but daily bacterial production significantly increased (p=0.001). It is interesting that increasing D to 0.06 h^{-1} resulted in an abolition of protozoa in the fermenter contents. Both a washout effect that may select fast growth bacterial flora to be maintained in fermenters and a reduction in engulfment of bacteria by protozoa appeared to be a direct reason for the increased bacterial counts and bacterial production with increasing D. For the disappearance of protozoa with increasing D from 0.03 to 0.06 h^{-1}, although washout effect seems to be partly responsible, other reasons such as lower pH (see table 3) and lack of microbial crossfeeding, may also be involved.

Microbial growth and efficiency

Data on nitrogen partitioning in the fermenter effluents are presented in table 5. Fermenter nitrogen input came from both dietary addition and buffer infusion. Daily nitrogen loss as ammonia decreased linearly (p=0.001) as dietary S-RDP levels increased. There was a decrease in ferment ammonia-N concentration with increasing S-RDP levels (linear, p=0.001) and with increasing D (p=0.001). As S-RDP levels increased, the declined ammonia-N concentration was expected because the proportion of urea, which is highly hydrolyzed by ruminal microorganisms, decreased in the diets. Although average ammonia-N concentrations in those three diets across two Ds were greater than the 5.0 mg/100 ml required for maximum microbial protein synthesis (Satter and Slyter, 1974), the ammonia concentrations for the diets with 0, 35 and 70% S-RDP levels at faster D (0.06 h^{-1}) were 21.3, 10.0 and 4.3 mg/dl, respectively. Ammonia-N in the fermenter was derived from buffer solution infused as urea and ammonium sulfate-N (added at 87.5 mg/l) and from degradation of dietary urea and soy proteins. If complete hydrolysis of urea into ammonia was assumed, the net ammonia-N from dietary soy protein can be computed (data not shown). The net ammonia-N concentration derived from degradation of ISP was negative in the diet with 70% S-RDP level, implying that ruminal degradable protein from soy proteins alone was limiting for maximum microbial protein synthesis, in particular at a relatively high dilution rate.

Daily microbial nitrogen production (DMNP), and microbial efficiency (MOEFF) expressed as grams of microbial N produced per kilogram of OM truly digested, responded linearly (p=0.001) to S-RDP levels. Several studies have shown improved microbial growth and efficiency when peptide or amino acids replaced urea or ammonia as the sole or major source of N (Russell and Sniffen, 1984; Argyle and Baldwin, 1989; Griswold et al., 1996). In the current study, elevating S-RDP levels in the diet should result in an increased accumulation of peptides or amino acids in the fermenter contents. Thus, our study supported the previous results.

As D increased from 0.03 to 0.06 h^{-1}, DMNP and MOEFF were enhanced (p=0.001; table 5). At a high-dilution rate, fast-growing bacterial species may be selected to be maintained in the fermenters and microbial autolysis would be reduced, which can further reduce recycling of energy and N and provide more nutrients for microbial growth. Reducing engulfment of bacteria by protozoa owing to protozoal disappearance at the fast D which has shown in table

Table 4. Bacterial and protozoal numbers in continuous culture fermenters as affected by S-RDP level and dilution rate

	S-RDP (% of total CP)				D (h^{-1})			p= S-RDP		
	0	35	70	SEM	0.03	0.06	SEM	L	Q	D
Bacterial number										
Cells/ml ($\times 10^9$)	7.5	10.4	11.2	0.4	9.9	9.5	0.4	0.001	0.645	0.352
Cells/day ($\times 10^{12}$)	1.2	1.7	1.9	0.1	1.1	2.1	0.1	0.001	0.206	0.001
Protozoal number										
Cells/ml ($\times 10^3$)	0.4	3.0	4.8	0.5	5.4	0.0	0.4	0.001	0.001	0.520
Cells/day ($\times 10^5$)	0.5	3.3	5.3	0.6	6.1	0.0	0.5	0.556	0.001	0.001

[a] S-RDP=ruminal degradable protein from soy proteins; D=dilution rate; L=linear effect; Q=quadratic effect.

Table 5. Nitrogen metabolism and microbial yield and efficiency[a]

	S-RDP (% of total CP)				D (h^{-1})			p= S-RDP		
Item	0	35	70	SEM	0.03	0.06	SEM	L	Q	D
N intake (g/d)	0.855	0.865	0.833	0.003	0.806	0.896	0.002	-	-	-
Dietary N	0.713	0.723	0.689	0.001	0.708	0.708	0.001	-	-	-
Buffer N	0.142	0.142	0.144	0.002	0.098	0.188	0.002	-	-	-
Effluent N										
NH_3 N (g/d)	0.482	0.260	0.120	0.012	0.321	0.254	0.010	0.001	0.052	0.001
NH_3 N(mg/dl)	33.4	18.6	9.0	0.9	28.8	11.8	0.8	0.001	0.136	0.001
NAN[b] (g/d)	0.373	0.605	0.713	0.012	0.485	0.642	0.010	0.001	0.002	0.001
DMNP[c] (g/d)	0.362	0.445	0.469	0.015	0.361	0.490	0.012	0.001	0.135	0.001
NANMN[d](g/d)	0.011	0.161	0.244	0.014	0.124	0.152	0.012	0.001	0.075	0.109
MOEFF[e]	14.1	17.8	19.4	0.6	13.9	20.3	0.5	0.001	0.155	0.001

[a] S-RDP=ruminal degradable protein from soy proteins; D=dilution rate; L=linear effect; Q=quadratic effect.

[b] Non-ammonia nitrogen.

[c] Daily microbial nitrogen production.

[d] Non-ammonia non-microbial N.

[e] Microbial efficiency expressed as grams of microbial N/kg of organic matter truly digested.

4 appeared to be another reason for the increased DMNP and MOEFF with increasing D. Increased microbial yield and efficiency resulting from increasing dilution rate were reported in other studies (Isaacson et al., 1975; Kennedy et al., 1976; Kennedy and Milligan, 1978; Maeng et al., 1989; Meng et al., 1999).

Rumen NSC-fermenting microbes require peptide-amino acid N for their growth (Russell et al., 1992). Maeng and Baldwin (1976) reported that microbial growth and efficiency were maximized when the diet contained 25% of the N from urea and 75% from amino acids. Russell et al. (1992) indicated that NSC-fermenting microbes required peptides at 14% of the combined feed NSC plus peptides. If the end products of digestion from ISP were mainly peptides or amino acids as assumed by Jones et al. (1998), we calculated that the maximum growth of NSC-fermenting microbes was achieved when the ratio of peptide-amino acid N to ammonia N was 75.6: 24.4, or the level of RDP from ISP was 9.5% of dietary digestible OM. This observation is comparable to the results of Maeng and Baldwin (1976) and Fu et al. (1999). In the later study, Fu et al. (1999) reported that the theoretical RDP (from casein and cracked corn) requirement to maximize the growth of NSC-fermenting microbes was 9.4% RDP on an OM fermented basis. Although increasing S-RDP level in the diet increased microbial growth and microbial efficiency, the magnitude of the increase was less at slower D than at faster D (figure 1). At 0.03 h^{-1} D, increasing S-RDP level from 0 to 35% and from 35% to 70% of total dietary CP resulted in significantly (p=0.001-0.042) enhanced microbial yield (DMNP, figure 1a) and efficiency (MOEFF, figure 1b). At 0.06

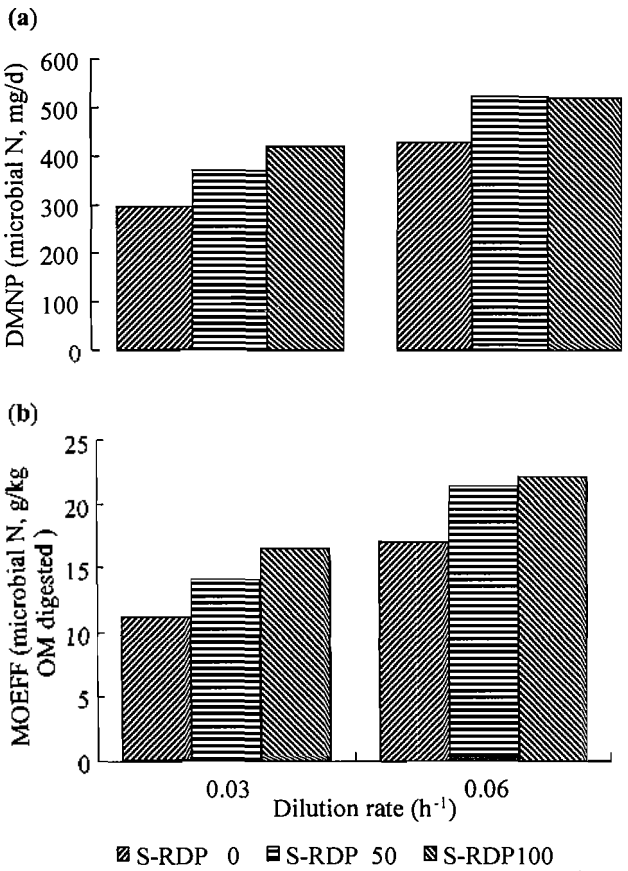

Figure 1. Daily microbial nitrogen production (a) and microbial efficiency (b) in response to dietary S-RDP level and dilution rate in continuous culture

h^{-1} D, however, increasing dietary S-RDP level from 0 to 35% of total dietary CP led to significant increase

(P=0.015) in microbial yield and efficiency, whereas further increasing S-RDP level from 35% to 70% of total dietary CP had no influence (p>0.5) on microbial growth and efficiency. A part of the explanations for this discrepancy in microbial growth between different Ds may be a lower ammonia N concentration (4.3 mg/dl) at the highest S-RDP level with faster D than the requirement for maximum growth of ruminal microbes (>5 mg/dl) as suggested by Satter and Slyter (1974). This result further suggested that, in practice, soy proteins alone involved in ruminant diets may not provide sufficient degradable N for maximum microbial growth, espically under higher dilution rate conditions, such as feeding lactation dairy cows.

CONCLUSIONS

As soy RDP levels in the diet increased, digestibilities of dietary DM, OM and CP decreased, whereas digestion of dietary starch increased. At a fast-dilution rate, soy protein used as a sole protein source may provide insufficient degradable protein for maximum microbial protein synthesis. Increasing dilution rate from 0.03 to 0.06 h^{-1} decreased digestibilities of dietary DM and OM, but had no effect on digestion of dietary starch and crude protein. With increasing levels of dietary RDP from soy proteins and dilution rate, rumen microbial growth and efficiency were enhanced. The quantity of ruminal degradable protein from soy proteins required for optimum protein synthesis of non-structural carbohydrate-fermenting microbes appears to be equivalent to 9.5% of dietary fermented OM.

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