Utilization of Ruminal Epithelial Cells by *Ruminococcus albus*, with or without Rumen Protozoa, and Its Effect on Bacterial Growth

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ABSTRACT : Effects of supplementation with ruminal epithelial cells on fiber-degrading activity and cell growth of *Ruminococcus albus* (*R. albus*, strain 7) was tested using a basal substrate of rice straw and formulated concentrate. Cultures of *R. albus* alone and *R. albus* with rumen protozoa were grown at 39°C for 48 h with an 8.4% crude protein (CP) substrate, 33% of the CP supplemented with either ruminal epithelial cells or defatted soybean meal. The ruminal epithelial cells had lower amounts of rumen soluble and degradable protein fractions as compared to defatted soybean meal, as determined by an enzymatic method, and the same was found with amino acid composition of protein hydrolysates. Ruminal epithelial cells were directly utilized by the *R. albus*, and resulted in greater growth of cell-wall free bacteria compared to defatted soybean meal. The effect of epithelial cells on bacterial growth was enhanced by the presence of rumen protozoa. In consistency with cultures of *R. albus* and *R. albus* with rumen protozoa, fermentative parameters such as dry matter degradability and total volatile fatty acid did not differ between supplementation with ruminal epithelial cells or defatted soybean meal. ***(Asian-Aust. J. Anim. Sci. 2003. Vol 16, No. 1 : 44-49)***

Key Words : Bacterial Growth, Digestive Activity, Rumen Microorganisms, Ruminal Epithelial Cells

INTRODUCTION

Digestive activity and protein synthesis by ruminal microorganisms can be increased, by synchronizing the supply of energy and protein (Nocek and Russell, 1988). Since the nutritional properties of protein sources supplied to ruminal microorganisms varies with their chemical structure, protein supplements such as soybean meal, cottonseed meal and corn gluten meal are commonly used to balance dietary levels of ruminal soluble proteins (RSP), ruminal degraded proteins (RDP) and ruminal undegraded proteins (RUP).

Endogenous proteins from digestive secretions and epithelial cells lining the digestive tract are also available to microorganisms. Epithelial cells are normally desquamated and regenerated in the rumen, and their outflow from the rumen has been reported to be 1.3×10^{10}/d to 1.6×10^{10}/d in intragastrically infused steers (Kimura et al., 1996). Since physical abrasion from forage in ruminant diet causes epithelial cells to be shed, even more endogenous proteins should be available to rumen microorganisms. However, microbial incorporation and utilization of endogenous proteins has never been evaluated and little information is available on whether those cells can stimulate, other than as a source of protein, growth and digestive activity of ruminal non-proteolytic bacteria.

Protein metabolism in the rumen is a shuttle exchange of nitrogenous compounds between protozoa and bacteria, as protozoa ingest dietary and bacterial proteins and then transfer peptides and amino acids back to bacteria. *Ruminococcus albus*, one of the most active bacteria involved in plant cell wall digestion in the rumen, is non-proteolytic and requires ammonia as well as one or more VFA such as iso-butyric and iso-valeric acid (Stewart and Bryant, 1988). Thus, utilization of nitrogenous compounds by ruminal bacteria can vary depending upon activities of rumen protozoa. Michalowski (1988) noted that the protein content in the substrate must exceed 10% of dry matter (DM) to meet the maintenance requirements of rumen protozoa *in vitro*, when purified substance is used for culture medium, but may be lower when plant materials are used in the diets.

This study aim to evaluate direct and indirect effects of ruminal epithelial cells on digestive activity and cell growth of *Ruminococcus albus*, a comparison of ruminal epithelial cells and defatted soybean meal was made in the absence or presence of rumen protozoa. The crude protein (CP) content of substrates supplemented with protein sources was 8.4% of the basal substrate, or 5.6% with no protein supplementation.

MATERIALS AND METHODS

Culture methods and substrates

Cultures of *R. albus* alone and *R. albus* with rumen protozoa were incubated on a basal substrate of 270 mg of rice straw plus 30 mg of formulated commercial concentrate. One of three nitrogenous supplements {i.e., ruminal epithelial (RE) cells; defatted soybean meal (SM); on half each of ruminal epithelial cells and defatted soybean meal (ES)} was added. The control (CTL) received no protein supplement. The total concentration of CP in 20 ml of culture solution was adjusted to 1.26 mg/ ml for the RE, SM

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and ES treatments, approximately 33% of which was from ruminal epithelial cells and/or defatted soybean meal. The concentration of CP in the CTL treatment was 0.84 mg/ ml, 76% from rice straw and 24% from the formulated commercial concentrate.

The basal substrate was weighed into a 30 ml serum bottle, suspended in 19 ml of deoxidized buffer solution (McDougall, 1948) containing none or each of the three supplements, and then sealed with a rubber stopper and aluminum cap. The culture bottle was autoclaved at 121°C for 15 min, cooled to room temperature, and inoculated with 1.0 ml of a single inoculum of *R albus*, or 1.0 ml of the combined inoculum of *R. albus* and rumen protozoa. Eight replicates were anaerobically incubated at 39°C for 48 h, and another three containing filter paper discs (Whatman No.1, 8 mm diameter) were incubated for 20 h under the same conditions to measure the extent of bacterial plus substrates attachment to fibrous material.

Fresh ruminal epithelial cells were scraped from rumen tissues of slaughtered beef cattle with a stainless steel knife, washed thoroughly with ice water, and stored at -80°C until use. Defatted soybean meal and the formulated concentrate were obtained from Zennoh Company (Tokyo, Japan), and the rice straw was from the Experimental Farm of Mie University (Tsu, Japan). Before use the epithelial cells were all finely ground with a Motter and Pistle.

Preparation of the inoculum of *Ruminococcus albus* and rumen protozoa

The rumen bacterium, *R. albus* (strain 7) was obtained from (Dr. Mitsumori) the culture collection of the National Institute of Animal Industry, Tsukuba, Japan. Stock culture was maintained on rumen fluid-glucose-cellobiose-maltose-starch-agar (RGCMSA) medium, was prepared under 100% O_2-free CO_2 by the anaerobic method of Bryant (1972). *R. albus* for inoculation was grown on the RGCMSA medium without glucose or agar for 18 h at 39°C and adjusted to a cell count of 3.3×10^7 cell / ml by optical density at 650 nm.

Rumen protozoa were obtained from ruminal fluid of sheep fed 200 g/d of a formulated commercial concentrates, 500 g/d of alfalfa hay and rice straw *ad libitum*. Strained rumen fluid was anaerobically incubated at 39°C for 30 min, and the protozoal fraction recovered from the bottom layer was incubated again for 5 h with 100 mg/L each of streptomycin, penicillin G and chloramphenicol in 50 mM acetate buffer solution at pH 6.5 (Ushida et al., 1997). The protozoal fraction was washed with 50 mM acetate buffer solution (pH 6.5) and centrifuged at 2,300×g for 10 min, repeated three times, then filtered through a 50 μm pore size nylon mesh. The number of protozoa in the filtrate was adjusted to a 1,600 cell /ml and immediately inoculated. The protozoal fraction, observed by light microscopy, contained *Entodinium spp*. at over 85% of ruminal protozoa. Gas-liquid chromatography with molecular sieve packed column (Molecular Sieve 5A 30/60, 3 mm ID×2 m, GL Science, Tokyo, Japan).

Measurements of microbial growth and fermentation

Gas production was measured using a graduated syringe at 8 h intervals during incubation. Concentrations of VFA (i.e., formate, lactate, acetate, propionate, and iso-butyrate and iso-valerate) in the culture supernatants, sampled at the end of incubation, were determined by HPLC (Jasco LC-800, Tokyo, Japan) using an ion exchange column (Shimadzu SCR-102 (H), 12 mm ID-30 cm, Shimadzu Co., Japan) (Goto et al., 1993). The pH was determined with a glass electrode. Undigested residual particles plus attached bacteria were recovered by low speed centrifugation (2,300×g for 10 min), followed by drying for 48 h at 80°C, and were then weighed and used to calculate DM degradability (DMD). Values of DMD obtained from mixed cultures of *R. albus* and rumen protozoa were then corrected by subtracting the protozal weight. This was then calculated from the correlation between the number and dry weight of mixed protozoa obtained in the present study ($y=0.0058+5.24\times10^{-5}x$, r=0.998, n=3; y (g), dry weight; x, cell number).

Protein content of bacteria adhered to the filter paper discs 20 h after incubation was determined by the method of Lowry et al. (1951). Counts of total viable bacteria (Suto, 1973) and protozoa (Kurihara and Takechi, 1973) were done under a light microscope, using a sample of the cultures at 48 h incubation, by being mixed with 200 g/ L formaldehyde for bacteria and 100 g/L methylgreen-formaldehyde in saline solution for protozoa. Cell growth and specific growth yield of the bacteria (i.e., cells/g of DDM) were calculated.

Chemical composition of the substrate

Neutral detergent fiber (NDF) and acid detergent fiber (ADF) were determined according to methods of Van Soest et al. (1991) without the use of sodium sulfite or amylase. Acid detergent lignin (ADL) was determined using 72% H_2SO_4 solution as modefoied by Van Soest et al. (1991). The CP and organic matter (OM) contents were determined as decribed by procedures 954.01 and 954.02 respectively (AOAC, 1990). Proportions of ruminal soluble protein (RSP), ruminal degradable protein (RDP) and ruminal undegraded protein (RUP) were determined according to methods of Krishnamoorthy et al. (1983). The amino acid composition of hydrolysates recovered from treatment with 6 N HCl at 110°C for 17 h under N_2 (Spackman et al., 1958) was analyzed using an amino acid analyzer (DP-8020, Tosoh Co., Tokyo, Japan). The analyzer was equipped with an ion exchange column (TSK-GEL Aminopak, 4.6 mm ID ×12 cm, Tosoh Co., Tokyo, Japan).

Statistical analysis

Data was analyzed using an analysis of variance, and means were separated by the Turkey-Kramer test with the F-test significant at the 0.05 probability level (Steel and Torrie, 1980).

RESULTS

Chemical composition and protein solubility of dietary sources

Rice straw had more NDF and ADF than the formulated concentrate and defatted soybean meal (Table 1). Amounts of NDF, ADF and ADL in the basal substrate (i.e., rice straw plus formulated concentrate) were calculated to be 551, 331, and 31 g/kg DM, respectively. CP levels of the RE, SM and ES treatment diets were 8.4% of the basal substrate on a DM basis, and the CTL treatment was 5.6% (not shown).

There were large differences in the amount and solubility of CP among rice straw, formulated commercial concentrate, defatted soybean meal and ruminal epithelial cells. The CP content of defatted soybean meal and ruminal epithelial cells were higher, at 442 and 804 g/kg DM respectively, than in rice straw and commercial concentrate. The amount of RSP in rice straw, formulated concentrate and defatted soybean meal was similar, at 32.9 to 37.9% of CP, but ruminal epithelial cells had a much lower value of 12.9%. Defatted soybean meal had the highest RDP fraction followed by commercial concentrate. Ruminal epithelial cells were intermediate between the formulated concentrate and rice straw. The SM treatment had the highest amount of RDP (19.6 mg CP in 20 ml of culture solution), followed by ES (18.8 mg CP), RE (18.0 mg CP) and CTL (11.4 mg CP).

Table 1. Chemical composition of rice straw, formulated concentrate, defatted soybean meal and ruminal epithelial cells

	Rice straw	Formulated concentrate	Defatted soybean meal	Ruminal epithelial cells
Organic matter[1]	953	935	844	974
Crude protein[1]	47	136	442	804
RSP[2]	37.9	35.6	32.9	12.9
RDP[2]	61.7	87.6	97.4	78.6
RUP[2]	38.3	12.4	2.6	21.4
Neutral detergent fiber[1]	594	163	141	nd[3]
Acid detergent fiber[1]	360	69	92	nd
Acid detergent lignin[1]	33	10	6	nd

[1] Gross composition was determined in triplicate and expressed on a DM basis (g/ kg).

[2] The RSP, RDP and RUP indicated the fractions of ruminal soluble, degradable, and undegradable proteins, respectively, and were expressed as a proportion of crude protein.

[3] nd; no data.

Total content by weight of 17 amino acids (AA) in rice straw, formulated commercial concentrate, defatted soybean meal and ruminal epithelial cells was 17, 118, 438 and 793 g/kg DM, respectively, which was comparable to the corresponding values of CP (Table 2). The percentage of AA was similar among the four dietary sources, except for lower percentages of lysine and arginine in rice straw.

Gas production, VFA concentration, DMD and bacterial growth in the cultures

Volatile fatty acids (VFA) concentration and DMD were higher in any mixed culture of *R. albus* with rumen protozoa than in any single culture of *R. albus* (Table 3), ruminal pH values in both *R. albus* and *R. albus* plus protozoa cultures did not. Within *R. albus* cultures, the RE treatment had higher ($p<0.05$) DMD than the CTL while the ES and SM treatments had a higher ($p<0.05$) DMD than CTL and RE within the mixed cultures.

An apparent inconsistency of DMD versus total VFA concentration within single and mixed cultures was observed. In the *R. albus* cultures, the CTL treatment had a lowest DMD than RE, but had similar total VFA concentration. The CTL treatment also had a the highest proportion of iso-butyrate in VFA ($p<0.05$), while it had the lower composition of acetate ($p<0.05$). In mixed cultures, the total VFA concentration was lower in the CTL versus the SM treatment ($p<0.05$). The CTL treatment also had the highest composition of lactate and formate ($p<0.05$), and a higher composition of iso-butyrate than the ES or SM

Table 2. Amino acid composition of rice straw, formulated commercial concentrate, defatted soybean meal and ruminal epithelial cells.

Proportion of amino acid (%)	Rice straw	Formulated concentrate	Defatted soybean meal	Ruminal epithelial cells
Aspartic acid	9.2	7.1	9.6	7.1
Threonine	5.8	4.2	4.2	4.3
Serine	5.8	5.1	5.7	5.8
Glutamic acid	15.8	22.9	21.2	15.3
Glycine	8.6	5.7	5.2	11.1
Alanine	9.6	8.2	5.1	6.8
Valine	8.6	6.4	5.7	5.5
Cystine	0	1.4	1.2	1.3
Methionine	1.5	1.5	1.6	2.2
Isoleucine	6.3	5.0	5.5	4.5
Leucine	9.1	9.7	7.4	7.6
Tyrosine	4.4	4.1	4.3	3.8
Phenylalanine	6.6	5.8	5.9	4.6
Histidine	0.7	1.0	1.0	0.8
Lysine	2.3	5.3	8.2	10.4
Arginine	5.7	6.6	8.2	8.4
Proline	0	0	0	0.5
Total (g/kg DM)	17	118	438	793

[a] Amino acid composition was determined in triplicate on single samples.

Table 3. Fermentation profiles in the cultures of *R. albus* alone and *R. albus* and rumen protozoa as supplemented with ruminal epithelial cells and defatted soybean meal

	R. albus alone				*R. albus* with rumen protozoa			
	CTL	SM	RE	ES	CTL	SM	RE	ES
Ruminal pH[2]	6.86±0.06	6.83±0.05	6.86±0.05	6.87±0.05	6.73±0.06	6.66±0.07	6.67±0.06	6.67±0.07
DM degradability (g/kg)	189±45^{a}	258±39^{ab}	273±35^{ab}	230±41^{ab}	439±39^{a}	527±54^{a}	417±57^{a}	580±66^{a}
VFA concentration (μmol/ml)	6.4±1.6^{b}	5.9±1.2^{b}	5.9±1.0^{b}	4.9±0.9^{b}	15.1±1.8^{a}	20.1±2.9^{a}	18.2±4.1^{a}	17.3±5.1^{a}
VFA composition (mol %)								
Lactate	nd[3]	nd	nd	nd	31.4±7.9^{a}	11.0±7.6^{b}	16.1±6.9^{b}	nd
Propionate	nd	nd	nd	nd	13.1±3.9^{c}	33.4±11.8^{b}	29.1±12.5^{b}	46.1±15.5^{a}
Formate	4.4±0.9t	3.4±1.4^{ae}	3.1±1.3^{ae}	2.5±1.9^{a}	6.4±0.7^{a}	1.1±0.3^{a}	1.2±0.4^{a}	nd
Acetate	60.5±11.9^{c}	76.9±8.1^{b}	73.2±9.6^{b}	84.3±6.3^{a}	45.0±6.3	51.5±13^{b}	49.9±14^{b}	50.4±17^{b}
iso-Butyrate	35.1±9.6^{a}	19.7±8.5^{b}	23.7±9.3^{b}	13.2±6.0^{c}	3.7±1.2^{c}	1.9±1.2^{c}	2.9±1.4^{c}	1.8±0.9^{c}
n-Butyrate	nd	nd	nd	nd	0.4±0.2^{b}	1.1±0.4^{b}	0.8±0.3^{b}	1.7±0.4^{a}

Within rows in the same type of culture, means followed by different letters differ ($p<0.05$) by the Tukey-Kramer multiple range test.

[1] CTL, Control; SM,defatted soybean meal; RE,ruminal epithelial cells; ES, defatted soybean meal +ruminal epithelial cells.

[2] The values of ruminal pH, DMD and VFA concentration were measured after 48h of incubation.

[3] nd; not detected.

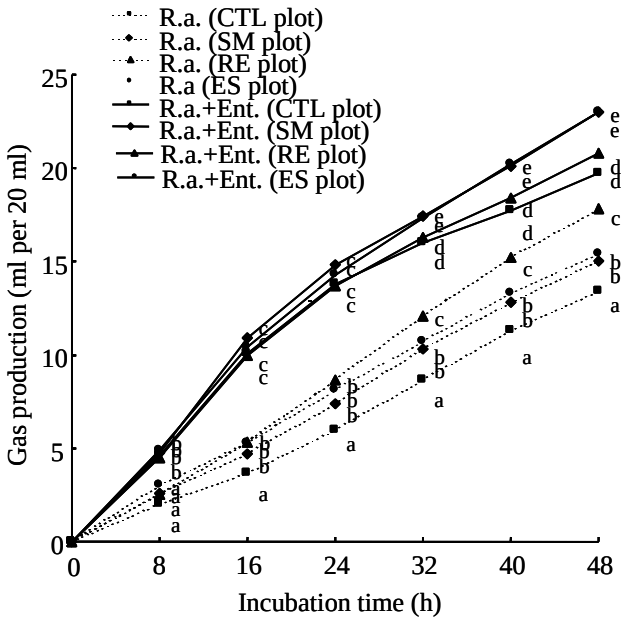

Figure 1. Gas production by cultures of *R. albus* alone, or *R. albus* and rumen protozoa, supplemented with ruminal epithelial cells and/or defatted soybean meal. Plotted points represent the mean of accumulated gas production (n=8), and means at each of incubation time followed by different letters are significantly different ($p<0.05$) by the Tukey-Kramer test.

treatments ($p<0.05$). In contrast, the CTL treatment had, the lowest proportion of propionate in VFA ($p<0.05$).

Gas production was affected by nitrogen supplements and the presence of rumen protozoa (Figure 1). Gas production in the culture of *R. albus* alone with RE, SM and ES was higher ($p<0.05$) than CTL after 16 h incubation, and but RE was highest than CTL, SM and ES treatment cultures after 32 h ($p<0.05$). Mixed cultures of *R. albus* and rumen protozoa had greater gas production compared to corresponding single cultures of *R. albus*. Total gas production of mixed cultures was higher with the ES and SM treatments than with the CTL and RE treatments after 32 h of incubation ($p<0.05$).

Bacterial cell proteins detected on filter paper discs after 20 h incubation were similar among treatments within the *R. albus* cultures while within mixed cultures they were lower ($p<0.05$) in the CTL treatment (Table 4). After 48 h incubation of the *R. albus* cultures, the RE treatment had the highest ($p<0.05$) number of bacterial cells, and the CTL, SM and ES treatments had similarly lower levels. The specific growth yield of the bacteria was higher with the RE treatment than with the SM treatment ($p<0.05$). Sharply higher bacterial cell numbers in all mixed cultures, compared to the single cultures, were also observed. Within the mixed cultures, the bacterial cell numbers of the SM, ES and RE treatments were higher than the CTL treatment ($p<0.05$), while the specific growth yield of the bacteria was highest with the RE ($p<0.05$).

DISCUSSION

Protein metabolism of ruminants is characterized by microbial protein synthesis in the rumen and absorption of microbial protein from the small intestine. In lactating cows, the amount of bacterial protein synthesized in the rumen can be up to 3.0 kg/d, which may account for over 70% of the total CP entering the small intestine (NRC, 1989). The quantity and quality of protein entering the rumen influences feed intake, digestibility (Church and Santos, 1981; Warly et al., 1992), microbial protein synthesis, and animal performance (Nocek and Russell, 1988).

In this study, digestion profiles and bacterial growth in single cultures of *R. albus* and mixed cultures of *R. albus* and rumen protozoa were improved with protein supplements. Furthermore, the supplement with ruminal epithelial cells was more effective in increasing the

Table 4. Cell number of *R. albus* and rumen protozoa in cultures as supplemented with ruminal epithelial cells and defatted soybean meal

	R. albus alone[1]				*R. albus* with rumen protozoa[1]			
	CTL	SM	RE	ES	CTL	SM	RE	ES
Bacterial protein (μg p/ FP disk)[2]	224±1[a]	229±1[a]	229±2[a]	214±2[b]	137±6[a]	216±22[b]	211±11[b]	192±21[b]
Bacterial cell number ($\times10^8$/ml)[3]	2.1±0.6[c]	2.1±0.5[c]	3.1±0.7[c]	2.2±0.6[c]	11.3±3.6[b]	18.8±3.2[b]	23.0±3.6[a]	22.0±3.0[a]
Specific growth yield ($\times10^9$ cells/g DDM)[3]	3.1±1.1[c]	2.3±0.6[c]	3.4±0.8[c]	2.7±0.8[c]	8.4±2.8[b]	11.7±2.0[b]	18.1±2.8[a]	12.4±1.7[b]
Protozoal number ($\times10^3$ cells/ ml)[3]	nd[4]	nd	nd	nd	5.4±1.7[b]	8.4±3.9[a]	5.6±2.4[b]	9.0±3.4[a]

Within rows in the same type of culture, means followed by different letters differ ($p<0.05$) by the Tukey-Kramer multiple range test.
[1] CTL, Control; SM,defatted soybean meal; RE,ruminal epithelial cells; ES, defatted soybean meal +ruminal epithelial cells.
[2] Proteins of the bacterium attached on the filter paper (Whatman No. 1, 8 mm diameter disk) after 20 h of incubation was determined.
[3] The values were measured after 48 h of the incubation.
[4] nd; not detected.

bacterial cell numbers and their specific growth yield than defatted soybean meal, in both the single and mixed cultures (Table 4). However total VFA and gas production was less altered with ruminal epithelial cells than defatted soybean meal, probably because defatted soybean meal contained higher nonstructural carbohydrates which were available to the microorganisms (Figure 1). Digestion by *R. albus* alone at 24 h of incubation, indicated by gas production, was higher with ruminal epithelial cells than with no protein supplement. The amount of bacterial protein attached to the filter paper at 20 h of incubation was similar among treatments, which could be associated with increased digestive activity of *R. albus*. Furthermore, the higher DMD in the treatment with ruminal epithelial cells (Table 3) compared to those of other treatments was probably due to greater population density observed at the end of incubation. Bacteria attached to plant cell walls are generally more important for degrading fibrous material such as rice straw, as compared to the cell-wall free bacteria. The population density of cell-wall associated bacteria would also be expected to be higher in cultures supplemented with ruminal epithelial cells than in those with defatted soybean meal, although cell number was only determined for the cell wall-free bacteria in this study.

The higher gas production and VFA concentration, as well as DMD, in the mixed cultures of *R. albus* and rumen protozoa compared to cultures of *R. albus* alone are likely due to increased fermentation of nonstructural carbohydrates of defatted soybean meal and formulated concentrates by rumen protozoa. This is supported by lactate and propionate production that only occurred in mixed cultures. Furthermore, the synergistic effect of rumen protozoa with *R. albus* was observed in the degradability of rice straw. It was because that the DMD of rice straw calculated for the mixed cultures was higher than that of whole substrates of single cultures, assuming that the formulated commercial concentrate was fully degraded in the mixed cultures. Thus, bacterial growth in the presence of rumen protozoa was ten times higher than that which occurred in the absence of the protozoa. The quantity and quality of nitrogenous sources entering the rumen can be controlling factors limiting the digestive activity and cell growth of rumen microorganisms (Church et al., 1981; Warly et al., 1992). The ammonia N requirement of cellulolytic bacteria for optimal activity is generally reported to be about 0.05 to 0.28 mg/ ml (Durand, 1989). In our study, *R. albus* in the mixed cultures supplied more nitrogenous supplements than obtained in the single cultures, due to concentrations of 0.38 mg/ ml (2.5% RSP DM basis) and 0.94 mg/ ml (6.3% RDP DM basis) of incubation solution Table 1. This suggests considerable transfer of peptides and amino acids to bacteria from rumen protozoa.

Bacterial cell numbers and specific growth yields in the mixed cultures were increased more with ruminal epithelial cells than with defatted soybean meal, in similar to results with *R. albus* alone (Table 4). The greater cell growth of *R. albus* with supplementation of ruminal epithelial cells was not however consistent with the concentration of RDP fraction, as shown by levels of 0.90 mg/ml and 0.98 mg /m of incubation solution with supplementation of ruminal epithelial cells and defatted soybean meal, respectively. There were no differences between the two nitrogenous supplements in the composition of any amino acids (Table 2). In the pathway of glucose catalysis by fiber-degrading bacteria, iso-butyrate can be produced together with formate and acetate and also be utilized for bacterial amino acid biosynthesis. Therefore, a lower composition of iso-butyrate, both in the mixed cultures compared to the single cultures and in the cultures of the two nitrogenous supplements compared to those with no supplement, could be the result of its greater utilization by bacteria. This suggests that bacteria required more ammonia N and free amino acids for growth, and the greater response of bacterial growth with supplements of ruminal epithelial cells observed in this study would provide evidence of a disparate supplement for synthesis of bacterial proteins in the rumen. Thus, the composition of amino acids, and complex role played by non-nitrogenous products formed by protozoa, needs to be fully elucidated to explain their enhancement of higher bacterial growth. Also the role of various nutritious compounds in the ruminal epithelial cells needs to be examined.

CONCLUSION

Results suggest that desquamated epithelial cells can be directly utilized by non-proteolytic bacteria in the rumen and enhance bacterial growth more than defatted soybean meal. This effect is magnified by the presence of rumen protozoa. Further research is needed to elucidate characteristics of proteins and other nutritional factors of ruminal epithelial cells in increasing bacterial synthesis in the rumen.

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