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Incorporation of [^{15}N]Ammonia by the Cellulolytic Ruminal Bacteria *Fibrobacter succinogenes* BL2, *Ruminococcus albus* SY3, and *Ruminococcus flavefaciens* 17

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The origin of cell nitrogen and amino acid nitrogen during growth of ruminal cellulolytic bacteria in different growth media was investigated by using $^{15}NH_3$. At high concentrations of peptides (Trypticase, 10 g/liter) and amino acids (15.5 g/liter), significant amounts of cell nitrogen of *Fibrobacter succinogenes* BL2 (51%), *Ruminococcus flavefaciens* 17 (43%), and *Ruminococcus albus* SY3 (46%) were derived from non-NH_3-N. With peptides at 1 g/liter, a mean of 80% of cell nitrogen was from NH_3. More cell nitrogen was formed from NH_3 during growth on cellobiose compared with growth on cellulose in all media. Phenylalanine was essential for *F. succinogenes*, and its ^{15}N enrichment declined more than that of other amino acids in all species when amino acids were added to the medium.

Knowledge of the nitrogen compounds required for growth of ruminal bacteria is important in understanding the protein nutrition of ruminants and factors affecting ruminal fermentation, particularly fiber digestion. There is a long-held belief that cellulolytic ruminal bacteria use NH_3 as their sole source of N. Some recently published results are not consistent with this conclusion, however. Bryant (7), in summarizing the nutrient requirements of ruminal bacteria, concluded that cellulolytic bacteria used only NH_3 as an N source for growth. They were unable to grow on other nitrogen sources in the absence of NH_3 (9), and the incorporation of preformed amino acids was minimal, based on the low level of uptake of ^{14}C-labeled amino acids found with *Fibrobacter succinogenes* (3, 10, 11) and *Ruminococcus flavefaciens* (2, 3, 10) and in other studies with pure cultures of ruminal bacteria which had indicated that disappearance of NH_3-N from the growth medium was equal to N incorporation into bacterial protein (8). The uptake of ^{14}C from labeled amino acids was, however, significant in the early experiments of Bryant and Robinson (10) and Allison et al. (2, 3), though less than that found with *Escherichia coli* and noncellulolytic ruminal bacteria. The amino acid transport experiments of Ling and Armstead (29) also indicated that *F. succinogenes* accumulated radioactivity from ^{14}C-labeled peptides and amino acids. The stimulation of cellulolytic species by precursors of various amino acids (1, 31, 36) also suggests a quantitative dependence on amino acids for optimum growth. Furthermore, there is experimental evidence that preformed amino acids stimulate microbial growth and increase fiber digestion in vivo and in vitro (13, 15, 23, 30), and pure cellulolytic species grow faster on cellobiose when peptides are added to the medium (16). In addition, bacteria most closely associated with solids derived a substantial proportion of their cell N from sources other than ammonia (13, 19, 28). All of these observations indicate that amino acids are significant nutrients for cellulolytic bacteria. The present experiments were therefore undertaken to determine the extent to which preformed amino acids affect amino acid and cell N synthesis from $^{15}NH_3$ in the three main species of cellulolytic ruminal bacteria.

The main bacteria used in these studies were *F. succinogenes* BL2 (NCFB 2576), *Ruminococcus albus* SY3 (37), and *R. flavefaciens* 17 (21). Other strains are held in the culture collection of the Rowett Research Institute. The bacteria were maintained on the liquid form of medium M2 (24). ^{15}N uptake experiments were carried out with the basal medium of Hungate and Stack (25) with either 0.6% (wt/vol) cellulose (Avicel pH 101; Honeywill and Stein Ltd., London, United Kingdom) added before autoclaving or 0.6% (wt/vol) cellobiose (Sigma, Poole, Dorset, United Kingdom) added as a filter-sterilized solution after autoclaving and with 0.05 mg of vitamins B_1 and B_2 per ml. Part (40%) of the NH_4Cl in the minerals solution was replaced by $^{15}NH_4Cl$ (Sigma; 98% ^{15}N). Peptides (Trypticase; Becton Dickinson Microbiology Systems, Cockeysville, Md.) or amino acids (4) were added at various concentrations before autoclaving.

Bacteria were inoculated (5% by volume) from stock cultures into cellobiose-containing defined medium and incubated at 39°C for 24 h. These cultures were used to inoculate cellobiose- and cellulose-containing media. Cultures were analyzed once stationary phase was reached in the cellobiose cultures (24 to 48 h) or after 6 days of incubation for cellulose cultures. The bacteria were harvested by centrifugation (15,000 × *g*, 15 min), pellets were washed once with ice-cold water, and then the resuspended cells and supernatants were freeze-dried. ^{15}N enrichment was measured by isotope ratio mass spectrometry as described by Barrie and Workman (6). Total cell N was measured by a Kjeldahl procedure (17). Samples were processed and analyzed for ^{15}N enrichment in amino acids by gas chromatography-mass spectrometry (GC-MS) of derivatized amino acids (12) as described previously (4). Ammonia and ^{15}N enrichment in ammonia were measured as described by Whitehead et al. (39) and Nieto et al. (32), respectively. Calculations were described previously (4). Protein

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TABLE 1. Influence of Trypticase and amino acid addition on incorporation of $^{15}NH_3$ by three predominant cellulolytic ruminal bacteria grown on cellobiose[a]

Organism and medium[b]	NH_3 concn (g of N/liter)		Enrichment in NH_3 (atom %)		Microbial N formed (g/liter)	Proportion of microbial N derived from ammonia	Mean proportion of amino acid N derived from ammonia	Mean proportion of phenylalamine N derived from ammonia
	Initial	Final	Initial	Final				
F. succinogenes BL2								
A	NG[c]							
B	0.134	0.128	31.1	33.7	0.025	0.87	0.81	0.02
C	0.257	0.142	29.8	28.3	0.083	0.57	0.61	0.02
D	0.306	0.167	28.1	26.0	0.131	0.52	0.51	0.01
R. flavefaciens 17								
A	0.129	0.094	32.1	32.3	0.021	1.01	1.02	0.96
B	0.134	0.079	31.3	30.8	0.032	0.93	0.89	0.77
C	0.257	0.090	29.8	26.9	0.078	0.72	0.62	0.59
D	0.306	0.125	28.1	26.1	0.160	0.58	0.51	0.52
R. albus SY3								
A	0.129	0.057	32.1	32.3	0.063	1.09	1.02	0.74
B	0.134	0.042	31.1	30.2	0.076	0.93	0.92	0.65
C	0.257	0.096	29.8	26.9	0.122	0.75	0.74	0.56
D	0.306	0.124	28.1	24.1	0.141	0.68	0.67	0.71
SED[d]	0.006	0.012	0.4	0.6	0.003	0.04	0.01	0.04

[a] Results are the means of triplicate cultures.
[b] A, basal medium; B, basal medium plus 1 g of Trypticase per liter; C, basal medium plus 10 g of Trypticase per liter; D, basal medium plus 15.5 g of amino acids per liter.
[c] NG, no growth.
[d] $P < 0.001$.

was hydrolyzed by using HCl, which results in the breakdown of glutamine, asparagine, and tryptophan, and the GC-MS method did not detect lysine or cysteine adequately. Thus, the enrichment of these amino acids was not determined.

Results are all means derived from the analysis of triplicate cultures. The data were compared by analysis of variance with different cultures used as a blocking factor. To compare the effects of treatments on ammonia uptake into amino acids, individual amino acids were considered as a subplot within the design. All analysis was carried out with the GENSTAT 5 statistical program (Lawes Agricultural Trust, Rothamsted Experimental Station, Harpenden, Herts, England).

F. succinogenes did not grow on cellobiose without peptides or amino acids (Table 1) but grew in the cellulose-containing medium (Table 2). The cellulose was impure, containing sufficient N to contribute 11 μg of N per ml of medium, perhaps explaining the growth of *F. succinogenes* on cellulose. Also as a consequence, the proportion of cell N derived from NH_3 in the basal medium with no added amino acids was less than 100% (Table 2).

The growth of all species was stimulated by peptides or amino acids, except for *R. albus* on cellulose. Final NH_3 concentrations indicated that NH_3 did not limit growth. Net NH_3 utilization occurred in all cultures, and the ^{15}N enrichment in ammonia in the spent medium fell only slightly at high peptide concentrations, indicating that little breakdown of peptides and amino acids to NH_3 had occurred. This growth stimulation occurred even though the higher and branched-chain volatile fatty acids found by Allison et al. (1) had been added to the medium. Several experiments with the mixed population carried out in vivo and in vitro appear to be consistent with cellulolytic bacteria being stimulated by peptides or amino acids. Providing preformed amino acids in addition to ammonia to mixed ruminal microorganisms resulted in stimulation of microbial growth and increased fiber digestion (13, 15, 23, 30). However, the benefit does not appear to be consistent, because no benefit of adding preformed amino acids on microbial growth or fiber digestion was found in other studies (16, 21, 26, 27).

In all three bacteria, the proportion of cell N derived from ammonia fell as the concentration of peptides increased in the medium. At the highest peptide concentration tested (10 g/liter), between 42 and 75% of the cell N and amino acid N was derived from NH_3. The experiment was repeated in the 10-g/liter peptide-cellobiose medium with different strains of the bacteria used in Tables 1 and 2. The proportions of cell N derived from NH_3 in *F. succinogenes* strains S85, Bac10, Bac12, Bac20, and SD35, *R. albus* strains 8 and J6, and *R. flavefaciens* strains FD1 and 007 were 0.45, 0.44, 0.53, 0.51, 0.52, 0.71, 0.45, 0.62, and 0.55 (standard error of the difference [SED], 0.04), respectively, similar to the results obtained with the strains used here.

At a lower peptide concentration, 1 g/liter, an average of 80% of the cell N was derived from ammonia (Tables 1 and 2). The average ^{15}N enrichment in individual amino acids responded to additions to the medium in a way similar to that of cell N (Tables 1 and 2). The 1-g/liter concentration of peptides and amino acids is among the highest concentrations that would be found in the liquid phase of ruminal digesta in vivo (14, 38, 40). At ruminal peptide and NH_3 concentrations, therefore, it might be expected that 80% or more of the cell N of cellulolytic bacteria would be derived from NH_3, in comparison with lower proportions for noncellulolytic bacteria (4). Quantitatively, therefore, this conclusion differs little from the general conclusion made by Bryant (7) and incorporated into the CNCPS model (34). Yet, in the mixed population, Carro

TABLE 2. Influence of Trypticase and amino acid addition on incorporation of $^{15}NH_3$ by three predominant cellulolytic ruminal bacteria grown on cellulose

Organism and medium[a]	Final NH_3 concn (g of N/liter)	Enrichment in NH_3 (atom %)		Microbial N formed (g/liter)	Proportion of microbial N derived from ammonia	Mean proportion of amino acid N derived from ammonia	Mean proportion of phenylalanine N derived from ammonia
		Initial	Final				
F. succinogenes BL2							
A	0.086	33.2	30.0	0.035	0.91	0.90	0.89
B	0.136	31.4	30.4	0.033	0.52	0.73	0.15
C	0.198	29.1	26.6	0.061	0.42	0.50	0.10
D	0.256	38.1	24.2	0.075	0.47	0.40	0.08
R. flavefaciens 17							
A	0.113	33.2	30.5	0.016	0.75	0.79	0.62
B	0.090	31.4	28.6	0.025	0.80	0.76	0.74
C	0.140	29.1	26.4	0.040	0.55	0.52	0.53
D	0.209	28.1	26.6	0.045	0.44	0.40	0.40
R. albus SY3							
A	0.108	33.2	32.0	0.029	0.63	0.70	0.60
B	0.122	31.4	30.7	0.031	0.61	0.66	0.43
C	0.189	29.1	24.8	0.032	0.49	0.46	0.36
D	0.280	28.1	24.7	0.030	0.25	0.27	0.38
SED[b]	0.004	0.36	0.34	0.003	0.033	0.018	0.07

[a] A, basal medium; B, basal medium plus 1 g of Trypticase per liter; C, basal medium plus 10 g of Trypticase per liter; D, basal medium plus 15.5 g of amino acids per liter.
[b] Results are the means of triplicate cultures ($P < 0.001$).

and Miller (13) and Komisarczuk et al. (28) found that only about half of the microbial amino acids most closely associated with fiber was derived from exogenous NH_3. Incomplete equilibration of NH_3 pools in the microenvironment of the consortium digesting plant tissue may have contributed to these findings. It is also possible that amino acid incorporation occurred predominantly in the secondary fiber digesters associated with the consortium. However, it is also possible that local peptide and amino acids concentrations in the fiber-associated microenvironment are much higher than in the rest of the digesta, leading to an extent of amino acid incorporation similar to that observed here at the higher peptide and amino acid concentrations.

The present study used $^{15}NH_3$, which gives information different from that of earlier, ^{14}C, studies (2, 3, 10). Amino acid N is subject to transformation by aminotransferase activity and by deamination followed by reincorporation by, for example, glutamate dehydrogenase activity (20), as well as direct incorporation of intact amino acid. The main factor which might lead to misleading calculated incorporation values is failure of bacterial intracellular NH_3 pools to equilibrate with the measured extracellular pools, in which case amino acid N could be released intracellularly by deamination without the resultant NH_3 equilibrating with the measured NH_3 pool, which was extracellular. This would lead to a low estimate of cell N derived from NH_3. Intracellular NH_3 pools in ruminal bacteria are greater than extracellular NH_3 concentrations, implying that an accumulation mechanism is present (33). It is not known how rapidly exchange between intra- and extracellular NH_3 pools occurs. Even if equilibration is not complete, however, there is little doubt that the results are consistent with the conclusion that substantial incorporation of amino acid N can occur with the cellulolytic bacteria.

The amino acid treatment was included to compare the effects of isonitrogenous mixtures of peptides and amino acids as additions to the growth medium. A concentration of a complete amino acid mixture at 15.5 g/liter was compared with isonitrogenous peptides at 10 g/liter. Amino acids decreased NH_3 incorporation into amino acids in all media more than peptides, suggesting that these bacteria have a preference for amino acids over peptides (Tables 1 and 2). The incorporation of amino acids, which dilutes the cell N derived from $^{15}NH_3$, is therefore compatible with the early ^{14}C incorporation data (2, 3, 10), the later amino acid transport experiments of Ling and Armstead (30), and incorporation experiments with fiber-associated mixed populations (13, 19, 28).

Among individual bacterial amino acids, all enrichments decreased as the concentration of peptides or amino acids in the medium increased (data not shown), roughly in line with cell N, with the exception of phenylalanine (Tables 1 and 2). Phenylalanine synthesis was insignificant in *F. succinogenes*, irrespective of peptide concentration, and was generally lower than that of the other amino acids in the ruminococci. The incorporation of ^{15}N into all of the other amino acids measured responded in a much less abrupt way, apparently falling into two groups: Ala, Gly, Ser, Thr, Asp, Glu, and Tyr were generally more highly enriched than Val, Leu, Ile, and Pro (data not shown). Stimulation of growth by phenylalanine or its precursors, phenylacetic acid and phenylpropionic acid, and the implications for the competitiveness of *Ruminococcus* spp. have been well established (3, 31, 36). However, although there have been observations of a phenylalanine requirement (11) and incorporation of phenylacetic acid (3) in *F. succinogenes*, the response to added amino acids was surprising, because it was much more abrupt than that to any other amino acids with the cellulolytic bacteria examined here and the noncellulolytic species examined previously (4). Furthermore, unlike with the ruminococci, no growth occurred in the basal medium, which

lacked phenylalanine or its precursors. *F. succinogenes* may therefore have an even greater requirement for phenylalanine than *R. albus* or *R. flavefaciens.* Tyrosine, which like phenylalanine is derived from prephenic acid (35), did not respond in the same way (data not shown), suggesting that prephenate dehydratase may be lacking in *F. succinogenes*, while prephenate dehydrogenase remains active. Allison (3) suggested that it may be more economical for microorganisms in the rumen to use phenylacetic acid in Phe synthesis than to synthesize the carbon skeleton from other sources, since phenylacetic acid would always be present as a precursor in ruminal digesta.

The sensitivity of proline to added amino acids was similar to that of the other amino acids (data not shown) and therefore was much less than that in noncellulolytic species (4) or in the mixed population (5). There was no indication that, with the exception of Phe in *F. succinogenes*, any single amino acid might be limiting the growth of cellulolytic ruminal bacteria significantly more than any other.

These results therefore indicate (i) that cellulolytic ruminal bacteria incorporate preformed amino acids, (ii) that preformed amino acids stimulate the growth of cellulolytic ruminal bacteria, (iii) that amino acids are preferred over peptides, and (iv) that phenylalanine biosynthesis may be a limiting factor in some species. While some of these conclusions might at first appear to conflict with accepted dogma, they are in fact fairly consistent with most published data and may help us to resolve some apparent contradictions which appear in the literature.

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REFERENCES

1. **Allison, J. M., M. P. Bryant, and R. N. Doetsch.** 1958. Volatile fatty acid growth factor for cellulolytic cocci of bovine rumen. Science **128:**474–475.
2. **Allison, J. M., M. P. Bryant, and R. N. Doetsch.** 1962. Studies on the metabolic function of branched-chain, volatile fatty acids, growth factors for ruminococci. I. Incorporation of isovalerate into leucine. J. Bacteriol. **83:** 523–532.
3. **Allison, M. J.** 1965. Phenylalanine biosynthesis from phenylacetic acid by anaerobic bacteria from the rumen. Biochem. Biophys. Res. Commun. **18:** 30–35.
4. **Atasoglu, C., C. Valdés, N. D. Walker, C. J. Newbold, and R. J. Wallace.** 1998. *De novo* synthesis of amino acids by the ruminal bacteria *Prevotella bryantii* $B_1$4, *Selenomonas ruminantium* HD4, and *Streptococcus bovis* ES1. Appl. Environ. Microbiol. **64:**2836–2843.
5. **Atasoglu, C., C. Valdés, C. J. Newbold, and R. J. Wallace.** 1999. Influence of peptides and amino acids on fermentation rate and *de novo* synthesis of amino acids by mixed rumen micro-organisms from the sheep rumen. Br. J. Nutr. **81:**307–314.
6. **Barrie, S., and C. T. Workman.** 1984. An automated analytical system for nutritional investigations using 15-N tracers. Spectrosc. Int. J. **3:**439–447.
7. **Bryant, M. P.** 1973. Nutritional requirements of the predominant rumen cellulolytic bacteria. Fed. Proc. **32:**1809–1813.
8. **Bryant, P. M., and I. M. Robinson.** 1961. Studies on the nitrogen requirements of some ruminal cellulolytic bacteria. Appl. Microbiol. **9:**96–103.
9. **Bryant, P. M., and I. M. Robinson.** 1962. Some nutritional characteristics of predominant culturable ruminal bacteria. J. Bacteriol. **84:**605–614.
10. **Bryant, P. M., and I. M. Robinson.** 1963. Apparent incorporation of ammonia and amino acid carbon during growth of selected species of ruminal bacteria. J. Dairy Sci. **46:**150–154.
11. **Bryant, P. M., I. M. Robinson, and H. Chu.** 1959. Observations on the nutrition of *Bacteroides succinogenes*—a ruminal cellulolytic bacterium. J. Dairy Sci. **42:**1831–1847.
12. **Calder, A. G., and A. Smith.** 1988. Stable isotope ratio analysis of leucine and ketoisocaproic acid in blood plasma by gas chromatography/mass spectrometry. Use of tertiary butyldimethylsilyl derivatives. Rapid Commun. Mass Spectrom. **2:**14–16.
13. **Carro, D. M., and E. L. Miller.** 1999. Effect of supplementing a fibre basal diet with different nitrogen forms on ruminal fermentation and microbial growth in an *in vitro* semi-continuous culture system (RUSITEC). Br. J. Nutr. **82:**149–157.
14. **Chen, G., J. B. Russell, and C. J. Sniffen.** 1987. A procedure for measuring peptides in rumen fluid and evidence that peptide uptake can be a rate-limiting step in ruminal protein degradation. J. Dairy Sci. **70:**1211–1219.
15. **Chikunya, S., C. J. Newbold, L. Rode, X. B. Chen, and R. J. Wallace.** 1996. Influence of dietary rumen-degradable protein on bacterial growth in the rumen of sheep receiving different energy sources. Anim. Feed Sci. Technol. **63:**333–340.
16. **Cruz Soto, R., S. A. Muhammed, C. J. Newbold, C. S. Stewart, and R. J. Wallace.** 1994. Influence of peptides, amino acids and urea on microbial activity in the rumen of sheep receiving grass hay on the growth of rumen bacteria *in vitro*. Anim. Feed Sci. Technol. **49:**151–161.
17. **Davidson, J., J. Mathieson, and A. W. Boyne.** 1970. The use of automation in determining nitrogen by the Kjeldahl method, with final calculation by computer. Analyst **95:**181–193.
18. **Dehority, B. A., H. W. Scott, and P. Kowaluk.** 1967. Volatile fatty acid requirements of cellulolytic rumen bacteria. J. Bacteriol. **95:**537–543.
19. **Dixon, R. M., and S. Chanchai.** 2000. Colonization of source of N substrates used by microorganisms digesting forages incubated in synthetic fibre bags in the rumen. Anim. Feed Sci. Technol. **83:**261–272.
20. **Duncan, P. A., B. A. White, and R. I. Mackie.** 1992. Purification and properties of NADP-dependent glutamate dehydrogenase from *Ruminococcus flavefaciens* FD-1. Appl. Environ. Microbiol. **58:**4032–4037.
21. **Flint, H. J., C. A. McPherson, and J. Bisset.** 1989. Molecular cloning of genes from *Ruminococcus flavefaciens* encoding xylanase and β(1-3,1-4)glucanase activities. Appl. Environ. Microbiol. **55:**1230–1234.
22. **Fujimaki, T., Y. Kobayashi, M. Wakita, and S. Hoshino.** 1989. Influence of amino acid supplementation on cellulolysis and microbial yield in sheep rumen. J. Anim. Phys. Nutr. **62:**119–124.
23. **Griswold, K. E., W. H. Hoover, T. K. Miller, and W. V. Thayne.** 1996. Effect of form of nitrogen on growth of ruminal microbes in continuous culture. J. Anim. Sci. **74:**483–491.
24. **Hobson, P. N.** 1969. Rumen bacteria. Methods Microbiol. **3B:**133–149.
25. **Hungate, R. E., and R. J. Stack.** 1982. Phenylpropanoic acid: growth factor for *Ruminococcus albus*. Appl. Environ. Microbiol. **44:**79–83.
26. **Kanjanapruthipong, J., and R. A. Leng.** 1998. A comparison of ammonia and preformed protein as a source of nitrogen for microbial growth in the rumen of sheep given oaten chaff. Aust. J. Anim. Sci. **11:**351–362.
27. **Kernick, B. L.** 1991. The effect of form of nitrogen on the efficiency of protein synthesis by rumen bacteria in continuous culture. Ph.D. thesis. University of Natal, Pietermaritzburg, South Africa.
28. **Komisarczuk, S., M. Durand, P. Beaumatin, and G. Hannequart.** 1987. Utilisation de l'azote 15 pour la mesure la protéosynthèse microbienne dans les phases solide et liqide d'un fermenteur semi-continu (Rusitec). Reprod. Nutr. Dev. **27:**261–262.
29. **Ling, J. R., and I. P. Armstead.** 1995. The *in vitro* uptake and metabolism of peptides and amino acids by five species of rumen bacteria. J. Appl. Bacteriol. **78:**116–124.
30. **Merry, R. J., A. B. McAllan, and R. H. Smith.** 1990. *In vitro* continuous culture studies on the effect of nitrogen source on microbial growth and fibre digestion. Anim. Feed Sci. Technol. **31:**55–64.
31. **Morrison, M., R. I. Mackie, and A. Kistner.** 1990. 3-Phenylpropanoic acid improves the affinity of *Ruminococcus albus* for cellulose in continuous culture. Appl. Environ. Microbiol. **56:**3220–3222.
32. **Nieto, R., A. G. Calder, S. E. Anderson, and G. E. Lobley.** 1996. Method for the determination of $^{15}NH_3$ enrichment in biological samples by gas chromatography/electron impact mass spectrometry. J. Mass Spectrom. **31:**289–294.
33. **Russell, J. B., and H. J. Strobel.** 1987. Concentration of ammonia across cell membranes of mixed rumen bacteria. J. Dairy Sci. **70:**970–976.
34. **Russell, J. B., J. D. O'Connor, D. G. Fox, P. J. Van Soest, and C. J. Sniffen.** 1992. A net carbohydrate and protein system for evaluating cattle diets. 1. Ruminal fermentation. J. Anim. Sci. **70:**3551–3561.
35. **Sauer, F. D., J. D. Erfle, and S. Mahadevan.** 1975. Amino acid biosynthesis in the mixed rumen cultures. Biochem. J. **150:**357–372.
36. **Stack, R. J., R. E. Hungate, and W. P. Opsahl.** 1983. Phenylacetic acid stimulation of cellulose digestion by *Ruminococcus albus* 8. Appl. Environ. Microbiol. **46:**539–544.
37. **Varel, V. H., and B. A. Dehority.** 1989. Cellulolytic bacteria and protozoa from bison, cattle-bison hybrids, and cattle fed three alfalfa-corn diets. Appl. Environ. Microbiol. **55:**148–153.
38. **Wallace, R. J., and N. McKain.** 1990. A comparison of methods for determining the concentration of extracellular peptides in rumen fluid of sheep. J. Agric. Sci. (Cambridge) **114:**101–105.
39. **Whitehead, R., G. H. Cooke, and B. T. Chapman.** 1967. Problems associated with the continuous monitoring of ammoniacal nitrogen in river water. Automat. Anal. Chem. **2:**377–380.
40. **Williams, A. P., and J. E. Cockburn.** 1991. Effect of slowly and rapidly degraded protein sources on the concentration of amino acids and peptides in the rumen of steers. J. Sci. Food Agric. **56:**303–314.