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Nitrogen Utilization in Bacterial Isolates from the Equine Cecum†

ANNE E. MACZULAK,‡* KARL A. DAWSON, AND JOHN P. BAKER

Department of Animal Sciences, University of Kentucky, Lexington, Kentucky 40546-0215

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A total of 114 bacterial isolates were obtained from the cecal contents of two mature cecally fistulated horses on a habitat-simulating medium containing 40% energy-depleted cecal fluid. Of these isolates, 108 were maintained in pure cultures and were tentatively grouped on the basis of cell morphology and physiological characteristics. Gram-negative rods (50.9%), gram-positive rods (22.8%), and gram-positive cocci (21.9%) represented the largest groups isolated from these animals. Fifty isolates were tested for their ability to grow in media containing urea, ammonia, peptones, or amino acids as sole nitrogen sources. None of the isolates had a unique requirement for urea or ammonia since nitrogen derived from peptones, amino acids, or both supported growth as well as did ammonia or urea in a low nitrogen medium. Of the cecal isolates, 18% were able to use urea for growth, and 20.5% were able to grow with ammonia as the sole nitrogen source. All organisms grew in the experimental media containing peptones as the sole nitrogen source. Urease activity was detected in only 2 of 114 isolates tested. The inability of isolates to use urea or ammonia as nitrogen sources may have been a reflection of growth conditions in the habitat-simulating medium used for isolation, but it could also suggest that many cecal bacteria require nitrogen sources other then ammonia or urea for growth.

The contributions of the microbial population inhabiting the equine gastrointestinal tract to the overall nutrition of the host animal are undetermined. Based on the total numbers of microorganisms and the diversity in the types present in pony cecum, it seems likely that the microbial population has multiple roles in digestion (16, 17). Although it is known that equine animals rely on the hindgut population to digest dietary fiber (10, 24), the extent to which the intestinal bacteria utilize protein and nonprotein nitrogen and supply amino acids to the horse is not known. The site of protein digestion and absorption, the contribution of the microbial populations in the cecum and colon to nitrogen utilization, and the recycling of urea remain as controversial problems in equine nutrition. For instance, opinions vary regarding the extent to which urea nitrogen is used by the horse. The ability of horses to use urea nitrogen would depend on the hydrolysis of urea by bacterial urease and incorporation of the liberated ammonia nitrogen into microbial amino acids (13, 27). This type of microbial utilization of urea has been suggested by various nitrogen balance studies which demonstrate that urea is recycled in the equine gastrointestinal tract (19, 22). However, other studies indicate that urea causes decreases in nitrogen retention when added to low-protein diets (15, 20). There is a need to evaluate the role of cecal and colonic bacteria in protein and nonprotein nitrogen utilization, especially urea utilization, in the horse.

The objectives of the present study were to develop a habitat-simulating medium for the quantitative recovery of viable bacteria from the horse cecum, to determine the taxonomic distribution of the predominant culturable cecal bacteria, and to examine the patterns of nitrogen utilization by pure cultures of these bacteria.

MATERIALS AND METHODS

Recovery of viable bacteria. Several habitat-simulating media were tested for their ability to support the growth of bacteria from the ceca of horses. One group of media was prepared in a manner similar to medium 98-5 (5) and contained glucose, soluble starch, cellobiose and xylose (0.05% [wt/vol] each), and 0.05% Trypticase peptones (BBL Microbiology Systems, Cockeysville, Md.). This medium was modified to contain either 40% clarified rumen fluid, 40% clarified cecal fluid, or a mixture of rumen fluid and cecal fluid (32 and 8%, respectively). The second group of media (CCA type) was prepared in a manner similar to that described by Allison et al. (2) and contained either clarified cecal fluid, energy-depleted (9) cecal fluid, or a mixture of rumen fluid and cecal fluid. All media were prepared under a CO_2 gas phase by using a modification of the Hungate technique (3). Digesta samples were collected from two cecally fistulated horses through cannulae which had been surgically implanted at a point adjacent to the cecocolic orifice. Horses were maintained on a bluegrass pasture (12.4% digestible protein [18]). Samples were collected during the morning hours (9:00 to 10:00 a.m.) when the horses were actively grazing. Cecal contents were withdrawn by using a 1-m Tygon tube (Norton Co.) attached to a stainless steel strainer (pore size, 1.2 mm) and were maintained under anaerobic conditions during transport to the laboratory. Samples were diluted in an anaerobic dilution solution (4) and agitated in a blender jar. Serial dilutions were prepared and were used to inoculate five replicate roll tubes of each habitat-simulating medium. The total viable bacteria were enumerated after incubation of the roll tubes for 5 days at 37°C. The three replicates in each dilution sequence containing the highest number of CFU (between 30 and 300 CFU per tube) were used to calculate the concentrations of viable bacteria. Isolates were picked from the roll tubes, checked for purity and motility, and maintained on medium 98-5 modified to contain 40% cecal fluid (5).

Direct microscopic counts of total bacteria were determined by using a Petroff-Hauser counting chamber (Arthur H. Thomas Co., Philadelphia, Pa.) and a microscope equipped with a condenser for dark-field illumination.

Taxonomic distribution of isolates. Bacterial isolates were tentatively identified to the genus level by using the procedures described by Holdeman et al. (11). The end products

* Corresponding author.

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‡ Present address: New York State Department of Health, Empire State Plaza, Albany, NY 12201.

produced by each isolate were determined after 5 days of incubation at 38°C in peptone-yeast extract medium (11) containing 1.0% glucose. The volatile acids and the methyl esters of nonvolatile acids were quantitatively separated by gas chromatography (11) on an Aerograph model 2100 gas chromatograph (Varian Instrument Division, Palo Alto, Calif.). A sample of the gas over each culture was analyzed for hydrogen by using a dual-column gas chromatography system equipped with a 10-port Valco switching valve (Valco Instruments, Houston, Tex.), a chamber maintained at 70°C, and a thermal conductivity detector. Column 1 was a stainless steel column (inside diameter, 3.2 mm; length, 152.4 cm) containing 60/80 Chromosorb 102, and the second column (inside diameter, 3.2 mm; length, 137.2 cm) contained molecular sieve 5A. Nitrogen was used as a carrier gas (flow rate, 25 ml/min). During the analysis, the flow of gas was directed through column 1 and into column 2 for 30 s. The flow was then diverted with the switch valve to allow gases to flow from column 2 back into column 1. When hydrogen was present, a peak was observed within 10 s after flow was diverted through column 2.

Nitrogen utilization. Urease production was determined by using a modification of the urease detection medium of Wozny et al. (28). The modified medium contained (per 100 ml): glucose, maltose, cellobiose, and sodium lactate, 0.1 g each; Trypticase peptones, 0.05 g; yeast extract, 0.05 g; mineral solution 3 (25), 5.0 ml; B vitamin solution (25) without calcium D-pantothenate, 0.5 ml; hemin solution (4 mg/100 ml), 0.5 ml; vitamin K1 solution (11), 0.1 ml; VFA mixture (25), 0.45 ml; ferrous sulfate solution (0.22%), 0.2 ml; resazurin solution (0.1%), 0.2 ml; dithiothreitol solution (1.0%), 1.0 ml; sodium sulfide solution (5.0%), 2.0 ml; sodium carbonate solution (8.0%), 5.0 ml; and urea solution (1.5 M urea nitrogen), 3.3 ml. This medium was prepared under a CO_2 gas phase. The presence of urease was determined by inoculating each culture into duplicate tubes of the urease detection medium and into duplicate tubes of a control medium (prepared without urea). After incubation for 24 h at 38°C, the optical density at 600 nm (OD_{600}) was determined on a Spectronic 70 spectrophotometer (Bausch & Lomb, Inc., Rochester, N.Y.). Those cultures with an OD_{600} at least 0.05 U higher in the urea-containing medium than in the control medium and with a minimum OD_{600} of 0.1 were tested for ammonia production from urea by using a qualitative spot test (28). Growth on the urea-containing medium and ammonia production from urea were considered evidence for urease activity. No ammonia was detected in cultures of isolates which did not grow in the urea-containing medium or which were not stimulated by the addition of urea to the control medium. *Selenomonas ruminantium* D (14) served as a urease-positive control organism.

The ability of isolates to use various nitrogen sources for growth was tested by using the following additions to this urease detection medium: ammonium sulfate, Trypticase peptones, Casamino Acids (Difco Laboratories, Detroit, Mich.), or urea, at concentrations of 0.01 or 0.10% in place of the urea; peptones; and yeast extract. The ability of isolates to use each nitrogen source was determined by first growing each isolate in the urease detection medium and then inoculating 0.01 ml/5 ml of medium into duplicate tubes of the various nitrogen source media and comparing growth (measured by OD_{600}) in cultures incubated for 24, 48, and 72 h with the growth in similar cultures incubated in a control medium (without added nitrogen sources). Growth stimulation due to the added nitrogen source was used as evidence of nitrogen utilization and was recorded as an increase of at least 0.05 U at the 0.01% concentration and at least 0.10 U at the 0.10% concentration of added nitrogen source over the maximum OD_{600} attained in the nitrogen-free control medium. Similar tests were conducted with media prepared with 0.01% yeast extract to evaluate the use of the various nitrogen sources in the presence of cofactors supplied in yeast extract.

RESULTS AND DISCUSSION

The direct microscopic counts of cecal bacteria from the two horses ranged from 2.37×10^9 to 4.72×10^9 cells per ml of cecal contents on the days that these animals were sampled for enumeration of viable bacteria. The greatest numbers of viable cells were recovered on a habitat-simulating medium containing 40% energy-depleted rumen fluid, 0.125% total carbohydrates, Trypticase peptones, glycerol, and hemin. The range of counts on this medium was 2.62×10^8 to 4.12×10^8 CFU/ml of cecal contents, with a mean count of $3.6 \pm 0.44 \times 10^8$ CFU/ml. The cecal-fluid-containing medium allowed for the recovery of more bacteria than did similar media containing either rumen fluid or a mixture of rumen fluid and cecal fluid (Table 1). These data suggested that some cecal organisms required growth factors provided by cecal fluid but not provided by rumen fluid.

The 114 organisms isolated from the habitat-simulating media were distributed as follows: gram-negative rods, 50.9%; gram-positive rods, 22.8%; gram-positive cocci, 21.9%; and gram-negative cocci, 4.4%. This distribution is consistent with values reported by Kern et al. (17) for ponies consuming timothy or clover hay diets. However, Kern et al. (17) also detected differences in the morphological distribution of cecal bacteria in ponies fed various diets. It can be expected that similar changes in microbial distribution might have taken place in the horses in this study if the diets had been altered.

A total of 108 bacterial isolates were presumptively grouped on the basis of cell morphology, motility, oxygen tolerance, gas production, and fermentation end products (Table 2). Butyrivibrio, bacteroid, and selenomonad strains represented the largest groups of gram-negative rods, and streptococci and lactobacilli represented the largest groups of gram-positive cocci and rods, respectively. These isolates appear to be representative of the populations previously described for the equine cecum (17).

Of 114 isolates tested for urease activity, only 2 isolates were urease positive. These organisms were identified as members of the genus *Staphylococcus*. Additional urease-

TABLE 1. Colony counts on various media inoculated with horse cecal contents

Base medium	Addition to medium (%)	CFU/10^{-8} ml of cecal contents[a]
98-5	Cecal fluid (40)	2.51 ± 0.37
98-5	Rumen fluid (40)	1.96 ± 0.30
98-5	Rumen fluid (32) + cecal fluid (8)	1.86 ± 0.29
CCA	Energy-depleted cecal fluid (40)	3.65 ± 0.44[b]
CCA	Energy-depleted rumen fluid (32) + energy-depleted cecal fluid (8)	2.01 ± 0.34
CCA	Cecal fluid (40)	2.83 ± 0.41[b]

[a] Means and standard errors were calculated from six values (three replicate values from each of two horses).
[b] Mean differs significantly from other values ($P < 0.10$).

TABLE 2. Presumptive identification of predominant groups of bacteria from the horse cecum

Tentative bacterial group	Gram reaction	Form	Oxygen tolerance[a]	Products from glucose[b]		Growth-stimulatory nitrogen sources[c]	No. (%) of isolates[d]
				Major	Minor		
Butyrivibrios	−	Motile rod	−	B	(A,L,P)	T(C,A,U)	24 (22.2)
Streptococci	+	Coccus	+	L(A)	(A)	T(C,U)	18 (16.7)
Bacteroids	−	Rod	−	A,S	(L,P)	T(C)	11 (10.2)
Lactobacilli	+	Rod	−(+)	L	(P,S)	T(U)	11 (10.2)
Selenomonads	−	Motile curved rod	−	A,P	(L)	T,C(A,U)	7 (6.5)
Eubacteria	+	Rod	−	A	(B,L)	T,A,U	7 (6.5)
Propionibacteria	+	Rod	−	P	S(A,L)	T,U(C,A)	4 (3.7)
Staphylococci	+	Coccus	+	L		T,C,U	2 (1.8)
Unclassified	−	Rod	−		(A,B,F,L,S)		13 (12.0)
	+	Rod	+		(A,B,L)		5 (4.6)
	−	Coccus	−		(A,B,L,S)		4 (3.7)
	+	Coccus	−(+)		A(P,B,L)		2 (1.8)

[a] Method described by Holdeman et al. (11). Symbol in parentheses indicates a reaction produced by only a few strains in each bacterial group.
[b] Abbreviations: A, acetic acid; B, butyric acid; F, formic acid; L, lactic acid; P, propionic acid; S, succinic acid. Major products have a concentration of ≥20 μmol/ml. Minor products have a concentration of <20 μmol/ml. Products in parentheses were produced by only a few strains in each bacterial group.
[c] Abbreviations: A, ammonia; C, Casamino Acids; T, Trypticase peptones; U, urea. Sources in parentheses were utilized by only a few strains in each bacterial group.
[d] Data from 108 isolates from two horses.

positive staphylococci were isolated on Baird-Parker medium (Difco) to confirm the presence of these organisms in the ceca of the experimental animals. Fifty isolates were used to compare the ability of representative pure cultures to use urea, ammonia, Trypticase peptones, and Casamino Acids as sole nitrogen sources in a low-nitrogen basal medium. Of the isolates tested, 11 did not respond to any of the nitrogen sources tested when growth was compared with that in media without added nitrogen sources (Table 3). A total of 32 of the remaining 39 isolates (82.0%) did not show increased growth when urea was the sole nitrogen source. Similarly, the growth of 31 isolates (79.5%) was not stimulated when the medium contained ammonia as the sole nitrogen source. Growth of most (71.8%) of the 39 isolates was supported by peptones and amino acids. None of the organisms had a unique requirement for urea or ammonia since nitrogen derived from peptones could stimulate growth as well as either of these nitrogen sources.

The growth of seven (17.9%) of the isolates was stimulated in urea-containing media. However, lactobacillus, seleno-

TABLE 3. Compounds serving as sole nitrogen sources for equine cecal bacteria[a]

Growth in following compounds[b]:				No. (%) of isolates demonstrating growth in medium:	
Urea	Ammonia	Trypticase	Casamino Acids	Without yeast extract[c]	With 0.01% yeast extract
−	−	+	−	19 (48.7)	20 (45.4)
−	−	+	+	9 (23.1)	10 (22.7)
+	+	+	+	3 (7.7)	5 (11.4)
+	−	+	−	3 (7.7)	3 (6.8)
−	+	+	+	2 (5.1)	2 (4.5)
−	+	+	−	2 (5.1)	2 (4.5)
+	+	+	−	1 (2.6)	1 (2.3)
+	−	+	+	0 (0.0)	1 (2.3)

[a] Data from 50 isolates.
[b] +, Stimulated growth due to nitrogen source as interpreted by an increase of at least 0.05 OD_{600} U at the 0.01% nitrogen source concentration and an increase of at least 0.10 OD_{600} U at the 0.10% concentration when compared with an inoculated control medium without a nitrogen source; −, no stimulated growth due to nitrogen source.
[c] Includes results from isolates which could grow on nitrogen source media to which only 0.01% (wt/vol) yeast extract was added. Six organisms failed to grow on nitrogen source media with or without yeast extract added.

TABLE 4. Ability of equine cecal isolates to use various sole nitrogen sources

Compound	% of isolates demonstrating growth in low-nitrogen medium with:	
	No yeast extract[a]	0.01% Yeast extract[b]
Trypticase peptones	100.0	100.0
Casamino Acids	35.9	40.9
Ammonia	20.5	22.7
Urea	17.9	22.7

[a] Data from 39 isolates.
[b] Data from 44 isolates, including 39 which did not require yeast extract.

monad, and butyrivibrio strains in this group of organisms did not produce ammonia at levels which could be detected by the qualitative spot test (28). Similar growth responses have been seen with other known urease-positive organisms in our laboratory. Therefore, it is unlikely that the seven strains of cecal bacteria stimulated by urea were using nitrogen sources such as residual nitrogen from lysed cells in this basal medium. It is also possible that further transfer of these isolates in urea-containing media would be required to eliminate carry-over of nutrients from previous media. The question as to why some cecal isolates tested negative for urease activity by the ammonia spot test and yet had stimulated growth in urea-containing media remains unresolved, but these data suggest that simple detection of ammonia production from urea may not be a conclusive test for urease production. Other investigators have suggested that this test is not reliable and that urease activity may be altered during storage of cultures (21, 28).

Of the 11 isolates which did not grow with any of the added nitrogen sources 5 did grow when yeast extract (0.01%) was added to the medium. With the addition of one or more growth factors provided by the yeast extract, these 5 isolates responded to individual nitrogen sources in a manner similar to that observed for the original 39 isolates. Six isolates failed to grow in the experimental medium, even with added yeast extract. These organisms most likely had an absolute requirement for additional growth factors supplied in cecal fluid.

All organisms which grew on the experimental media had the ability to utilize Trypticase peptones (Table 4). Fewer bacteria had the ability to use amino acids, ammonia, or urea as sole nitrogen sources. Addition of 0.01% yeast extract slightly increased percentages of isolates which could grow in the presence of amino acids, ammonia, or urea. However, even with the added growth factors supplied by yeast extract, few cecal isolates had the ability to use ammonia or urea as nitrogen sources for growth. The nitrogen requirements observed in this study may be a reflection of the medium used to isolate these organisms or may reflect the form of nitrogen entering the horse cecum; that is, endogenous and partially digested proteins.

The distribution of cecal organisms isolated from horses in this study was consistent with that determined by Kern et al. (17) and permitted the assumption that a representative bacterial population was recovered by the use of the experimental habitat-simulating medium, even though the numbers of viable cells recovered were low relative to the direct microscopic counts. Only a small proportion of this population could use urea or ammonia as a nitrogen source for growth, an observation which differs from that of rumen bacteria in which ammonia serves as an important, and often essential, nitrogen source (6). The low numbers of ureolytic bacteria isolated in this study may be explained in two ways. First, generally low numbers of ureolytic bacteria may exist in the equine cecum so that relatively few bacteria provide the urease activity in that organ. This hypothesis is supported by Alexander and Davies (1), who suggested that bacteria in the equine large intestine did not contribute greatly to ureolysis. However, later studies support models for urea nitrogen recycling in horses involving bacterial urease and active ureolytic populations in the hindgut (13, 27). Second, urease activity in the equine cecum may be related to a distinct population which associates with the cecal wall. In ruminant animals, ureolytic activity has been demonstrated in only a small number of rumen species (8, 23, 28). Some studies suggest that the major rumen ureolytic bacteria are facultative organisms closely associated with the rumen wall epithelium (7, 12, 26). Although there is no evidence to show how this model applies to ureolysis in the equine large intestine, this association may provide an explanation for the low number of ureolytic bacteria isolated in this study. The sampling procedures used in this study may not have been adequate to allow for the isolation of ureolytic bacteria associated with the cecal wall. Further pursuit of this question may aid in clarifying the theories on urea recycling and the role of equine cecal bacteria in converting urea nitrogen to protein in the horse.

LITERATURE CITED

1. **Alexander, F., and M. E. Davies.** 1963. Production and fermentation of lactate by bacteria in the alimentary canal of the horse and pig. J. Comp. Pathol. **73**:1–8.
2. **Allison, M. J., I. M. Robinson, J. A. Bucklin, and G. D. Booth.** 1979. Comparison of bacterial populations of the pig cecum and colon based upon enumeration with specific energy sources. Appl. Environ. Microbiol. **37**:1142–1151.
3. **Bryant, M. P.** 1972. Commentary on the Hungate technique for culture of anaerobic bacteria. Am. J. Clin. Nutr. **25**:1324–1328.
4. **Bryant, M. P., and L. A. Burkey.** 1953. Cultural methods and some characteristics of some of the more numerous groups of bacteria in the bovine rumen. J. Dairy Sci. **36**:205–217.
5. **Bryant, M. P., and I. M. Robinson.** 1961. An improved nonselective culture medium for ruminal bacteria and its use in determining diurnal variation in numbers of bacteria in the rumen. J. Dairy Sci. **44**:1446–1456.
6. **Bryant, M. P., and I. M. Robinson.** 1962. Some nutritional characteristics of predominant culturable ruminal bacteria. J. Bacteriol. **84**:605–614.
7. **Cheng, K. J., and R. J. Wallace.** 1979. The mechanism of passage of endogenous urea through the rumen wall and the role of ureolytic epithelial bacteria in the urea flux. Br. J. Nutr. **43**:553–557.
8. **Cook, A. R.** 1976. Urease activity in the rumen of sheep and the isolation of ureolytic bacteria. J. Gen. Microbiol. **92**:32–48.
9. **Dehority, B. A., and J. A. Grubb.** 1976. Basal medium for the selective enumeration of rumen bacteria utilizing specific energy sources. Appl. Environ. Microbiol. **32**:703–710.
10. **Fonnesbeck, P. V.** 1968. Digestion of soluble and fibrous carbohydrate of forage by horses. J. Anim. Sci. **27**:1336–1344.
11. **Holdeman, L. V., E. P. Cato, and W. E. C. Moore (ed.).** 1977. Anaerobe laboratory manual, 4th ed. Virginia Polytechnic Institute and State University, Blacksburg.
12. **Houpt, T. R., and K. A. Houpt.** 1968. Transfer of urea nitrogen across the rumen wall. Am. J. Physiol. **214**:1296–1303.
13. **Houpt, T. R., and K. A. Houpt.** 1971. Nitrogen conservation by ponies fed a low-protein ration. Am. J. Vet. Res. **32**:579–588.
14. **John, A., H. R. Issacson, and M. P. Bryant.** 1974. Isolation and characteristics of ureolytic strains of *Selenomonas ruminantium*. J. Dairy Sci. **57**:1003–1014.
15. **Johnson, R. J., and J. W. Hart.** 1974. Utilization of nitrogen

from soybean mean, biuret, and urea by equine. Nutr. Rep. Int. **9**:209–216.

16. **Kern, D. L., L. L. Slyter, E. C. Leffel, J. M. Weaver, and R. R. Oltjen.** 1974. Ponies vs. steers: microbial and chemical characteristics of intestinal ingesta. J. Anim. Sci. **38**:559–564.
17. **Kern, D. L., L. L. Slyter, J. M. Weaver, E. C. Leffel, and G. Samuelson.** 1973. Pony cecum vs. steer rumen: the effect of oats and hay on the microbial ecosystem. J. Anim. Sci. **37**:463–469.
18. **National Research Council.** 1978. Nutrient requirements of domestic animals. No. 6. Nutrient requirements of horses. National Research Council, Washington, D.C.
19. **Prior, R. L., H. F. Hintz, J. E. Lowe, and W. J. Visek.** 1974. Urea recycling and metabolism of ponies. J. Anim. Sci. **38**:565–571.
20. **Reitnour, C. M., and J. M. Treece.** 1971. Relationship of nitrogen source to certain blood components and nitrogen balance in the equine. J. Anim. Sci. **32**:487–490.
21. **Robinson, I. M., M. J. Allison, and J. A. Bucklin.** 1981. Characterization of the cecal bacteria of normal pigs. Appl. Environ. Microbiol. **41**:950–955.
22. **Slade, L. M., D. W. Robinson, and K. E. Casey.** 1970. Nitrogen metabolism in nonruminant herbivores. I. The influence of nonprotein nitrogen and protein quality on the nitrogen retention of adult mares. J. Anim. Sci. **30**:753–760.
23. **Tillman, A. D., and K. S. Sidhu.** 1969. Nitrogen metabolism in ruminants: rate of ruminal ammonia production and nitrogen utilization by ruminants—a review. J. Anim. Sci. **28**:689–697.
24. **Vander Noot, G. W., and E. B. Gilbreath.** 1970. Comparative digestibility of components of forage by geldings and steers. J. Anim. Sci. **31**:351–355.
25. **Varel, V. H., M. P. Bryant, L. V. Holdeman, and W. E. C. Moore.** 1974. Isolation of ureolytic *Peptostreptococcus productus* from feces using defined medium; failure of common urease tests. Appl. Microbiol. **28**:594–599.
26. **Wallace, R. J., K. J. Cheng, D. Dinsdale, and E. R. Orskov.** 1979. An independent microbial flora of the epithelium and its role in the ecomicrobiology of the rumen. Nature (London) **279**:424–426.
27. **Wooton, J. F., and R. A. Argenzio.** 1975. Nitrogen utilization within equine large intestines. Am. J. Physiol. **229**:1062–1067.
28. **Wozny, M. A., M. P. Bryant, L. V. Holdeman, and W. E. C. Moore.** 1977. Urease assay and urease-producing species of anaerobes in the bovine rumen and human feces. Appl. Microbiol. **33**:1097–1104.