Forage Maturity Effects on Rumen Fermentation, Fluid Flow, and Intake in Grazing Steers

D.C. ADAMS, R.C. COCHRAN, AND P.O. CURRIE

Abstract

Eight ruminally fistulated steers were observed on native range from 4 May 1981 to 5 Nov. 1981 to determine effects of advancing forage maturity on rumen fermentation, fluid passage, fluid volume, and forage intake. Effects of these factors are poorly defined for cattle on the Northern Great Plains but are essential for developing management strategies for optimum animal production. On 6 different dates, the steers were given an intraruminal dose of cobalt ethylenediaminetetraacetate (CoEDTA), and samples of rumen fluid were drawn at 4-hour intervals over a 24-hour time period. Rumen fluid samples were analyzed for volatile fatty acid, ammonia-N, cobalt concentration, and pH. CoEDTA was used as a marker to estimate rumen fluid passage and volume. Forage intake was determined by total fecal collection and in vitro digestibility of the forage. Total ruminal volatile fatty acid, molar proportions of individual volatile fatty acid, pH, and ammonia-N concentrations varied ($P<0.01$) within each of the six 24-hour periods, but the changes were dependent on date. Advancing forage maturity was associated with reduction in individual and total ruminal volatile fatty acid, ammonia-N, pH, and fluid dilution rate. Rumen fluid volume increased with increasing forage maturity. Variation in organic matter intake was small ($P>0.05$) over the range of forage maturities studied. We concluded that variation in rumen fluid passage, volume, and fermentation depended on forage maturity, and protein supplementation may be beneficial during late summer-early fall to increase or sustain animal production.

Key Words: forage quality, ammonia-nitrogen, volatile fatty acid, liquid dilution rate, liquid volume, intake, native range

Adams and Currie are animal and range research scientists, USDA, Agricultural Research Service, Fort Keogh Livestock and Range Research Laboratory, Route 1, Box 2021, Miles City, Mont. 59301; Cochran is animal research scientist, Department of Animal Science and Industry, Kansas State University, Manhattan 66506.

This study is a contribution from the U.S. Department of Agriculture, Agricultural Research Service, and Montana Agriculture Experiment Station, Miles City, Mont., Journal Series No. J-1888.
Manuscript accepted 31 March 1987.

Rumen fermentation in cattle is the primary source of energy (Annison and Armstrong 1970) and protein (Bull et al. 1979, Owens and Bergen 1983) metabolites. Rumen fermentation is influenced by rumen fluid dilution and pH and by forage maturity (Harrison et al. 1975, Russell et al. 1979, McCollum et al. 1985). Also, rumen fluid dilution rate and pH are influenced by factors such as forage maturity (McCollum and Galyean 1985) and level of intake (Adams and Kartchner 1984). Therefore, an understanding of forage intake and quality, rumen fermentation, and liquid flow kinetics may provide insight for development of management strategies to optimize animal production on rangeland, e.g., for supplementation and season of use of rangeland for various classes of livestock. A few studies (McCollum and Galyean 1985, McCollum et al. 1985) have evaluated these variables on rangelands but not for the Northern Great Plains. Therefore, objectives of our study were to test the hypothesis that rumen fermentation and liquid flow in cattle change with plant growth during spring and summer and with plant dormancy in summer and fall and to characterize changes that occur in rumen variables for cattle grazing Northern Great Plains rangeland.

Materials and Methods

Eight ruminally fistulated Angus × Hereford steers with an average initial weight of 251 kg and 5 esophageal-fistulated steers with an average weight of 350 kg were allowed to graze freely on native range forage from 4 May 1981 to 5 November 1981. The site was on a deep, well-drained soil formed from alluvial sediments located on the Fort Keogh Livestock and Range Research Station, Miles City, Mont. The site is nearly level to moderately sloping, and the soils are primarily Borollic Camborthids of the Kobar series. The Kobar series is a silty clay loam and represent a highly productive range site. Major forage species are western wheatgrass *(Pascopyrum smithii)*, blue grama *(Bouteloua gracilis)*, needle-

Table 1. Chemical composition and digestibility of the forage diet consumed by esophageal-fistulated steers.

Chemical component[1], (%)[2]	Date					
	4 May	20 May	17 June	27 July	8 Sept.	28 Oct.
Organic matter	87.8	88.2	87.5	90.9	91.8	90.2
Crude protein	14.3	15.1	13.1	9.0	6.7	7.0
Acid-detergent fiber	38.1	41.3	38.5	42.5	42.6	47.7
Acid-detergent lignin	3.7	4.4	4.3	4.2	3.4	4.0
In vitro organic matter digestibility	71.6	72.1	74.2	66.9	62.6	63.8

[1]Average of 3 esophageal collections from 5 steers.
[2]Dry matter basis.

and-thread grass *(Stipa comata)* and threadleaf sedge *(Carex filifolia)*. Perennial forbs are rare. Most rapid growth of the grasses generally occurs in late April, May, and early June with completion of growth in late June or early July (Reed and Peterson 1961). Six 8-day trials were conducted in a single 52.8-ha pasture. In order to maintain a common forage supply for all trials, steers were moved at the conclusion of each 8-day trial to an adjacent 68.8-ha pasture with similar forage. Steers grazed in the study pasture for 15 days, which provided a 7-day adaptation period followed by an 8-day grazing trial.

Trials began on 4 May, 20 May, 17 June, 27 July, 8 September, and 28 October. Three trial dates were used in May and June to evaluate the periods of most active plant growth. At 0600 hour (0 hour) on day 1 of each trial, each ruminally fistulated steer received an intraruminal dose of 150 ml of cobalt ethylenediaminetetraacetate (CoEDTA; Uden et al. 1980) containing 429 mg of Co to estimate rumen fluid dilution rate and volume. Rumen contents were hand-mixed to increase marker equilibration. Immediately before dosing (0 hour) with CoEDTA and again at 4, 8, 12, 16, and 20 hour postdosing, 100 ml samples of rumen fluid were withdrawn from the ventral sac of the rumen of each steer. The pH was immediately determined with a combination electrode. Samples were then strained through 4 layers of cheesecloth, acidified with 1 ml of 20% H_2SO_4, frozen in plastic bags and stored for future analysis.

Rumen fluid samples from the 0, 4, 8, 12, 16, and 20 hour collections were thawed at room temperature and centrifuged at 10,000 × g for 10 min. The supernatant was analyzed for ammonia N by the procedures of Broderick and Kang (1980) and for Co by atomic absorption spectrophotometry with an air-acetylene flame. After addition of 2-ethylbutyric acid as an internal standard and a second centrifugation at 10,000 × g for 10 min, volatile fatty acid concentrations were determined by gas chromatography using the techniques described by Supelco (1975). Rumen fluid volume and fluid outflow rates were calculated by regression of the natural logarithm of Co concentration with time. Dilution rate is the slope of the line (percent/hour) and volume is calculated by relating the concentration of Co at time zero to the original dose.

Table 2. Least squares means, error mean squares and orthogonal contrasts for rumen fluid flow characteristics, rumen fluid volume and organic matter intake in steers grazing native range of advancing maturity.

Item	4 May (1)	20 May (2)	17 June (3)	27 July (4)	8 Sept. (5)	28 Oct. (6)	Error Mean Square	Orthogonal contrast
Number of steers	8	6	8	8	7	8		
Average body weight of steers, kg	251	253	282	331	354	370		
Organic matter intake, kg/100 kg body weight	2.1	1.9	1.9	2.0	1.9	1.9	.034	All constrasts NS
Dilution rate, %/hour	18.3	17.1	15.2	12.1	9.0	10.9	4.83	1 vs. 2 NS 1 & 2 vs. 3* 1 & 2 vs. 4** 3 vs. 4* 4 vs. 5 & 6*
Turnover time, hour	5.5	6.2	6.7	8.5	11.5	9.6	1.39	1 vs. 2 NS 1 & 2 vs. 3+ 1 & 2 vs. 4 3 vs. 4* 4 vs. 5 & 6**
Flow rate, liter/hour	4.1	5.6	8.0	7.7	7.3	6.6	1.86	1 vs. 2* 1 & 2 vs. 3** 1 & 2 vs. 4** 3 vs. 4 NS 4 vs. 5 & 6 NS
Volume, liter	22.8	34.9	53.6	63.4	83.4	62.6	207	1 vs. 2+ 1 & 2 vs. 3** 1 & 2 vs. 4** 3 vs. 4 NS 4 vs. 5 & 6+

*$P<0.05$
**$P<0.01$
+$P<0.1$
NS = Nonsignificant ($P>0.1$)

At 0700 hour on day 2 of each trial, each ruminally fistulated steer was fitted with a fecal bag and total fecal collections were made on day 2 through 8 of each trial. Esophageal-fistulated steers were used to collect diet samples on days 2, 4, and 6 of each trial; each collection began at about 0800 hour and lasted for 45 to 60 min. Fistula forage samples from individual steers were composited within each trial. Fecal and fistula-diet samples were dried at 45° C and ground to pass through a 1-mm screen. Fistula diet samples were analyzed for crude protein, dry matter, and ash by standard methods (AOAC 1980), and in vitro organic matter digestibility (IVOMD) by the Tilley and Terry (1963) 2-stage technique. Inoculum for in vitro digestibility was obtained from a ruminally fistulated steer maintained on an adlibitum diet of prairie hay fed once daily in the morning. On days when rumen fluid was collected, feed was withheld until after the collection was made. Acid detergent fiber and acid detergent lignin of fistula diet samples were determined by the procedures of Goering and Van Soest (1970; sodium sulfite excluded). Organic matter intake was determined from in vitro organic matter digestibility and fecal output by the procedure of Schneider and Flatt (1975).

Rumen fluid volume, fluid outflow, and intake data were analyzed with a 1-way analysis of variance with trial date as a main effect in the model. Orthogonal contrasts were used to separate means. Orthogonal contrasts were (1) 4 May vs. 20 May; (2) 4 May and 20 May vs. 17 June; (3) 4 May and 20 May vs. 27 July; (4) 17 June vs. 27 July; (5) 27 July vs. 8 September and 28 October (Steel and Torrie 1960). Orthogonal contrasts were selected at the outset of the study to compare key periods of plant growth and dormancy. Ruminal pH, ammonia-N, and volatile fatty acids were analyzed by analysis of variance procedures (Harvey 1979). Trial date and sample time were considered fixed effects and steer a random effect in the model. Steer/trial date/sample time was used as the error term to test trial date, sample time, and steer effects. Effects of steer/trial date and steer/sample time were considered trivial; if steer/trial date and steer/sample time were not trivial, the error term would be more conservative. Orthogonal contrasts were used to separate means and orthogonal contrasts were the same as described for rumen fluid outflow data.

Table 3. Least squares means, error mean square and orthogonal contrasts for molar proportion of individual ruminal volatile fatty acids (VFA), total VFA concentration acetate to propionte ratio, ammonia-n concentration in pH in steers grazing native range of advancing maturity.

Item	4 May (1)	20 May (2)	17 June (3)	27 July (4)	11 Sept. (5)	28 Oct. (6)	Error Mean Square	Orthogonal contrast
Acetate, moles/100 moles	67.1	67.0	67.2	74.3	73.0	73.0	2.43	1 vs. 2 NS 1 & 2 vs. 3 NS 1 & 2 vs. 4** 3 vs. 4** 4 vs. 5 & 6**
Propionate, moles/100 moles	19.2	18.6	18.4	15.7	16.7	17.2	2.15	1 vs. 2* 1 & 2 vs. 3* 1 & 2 vs. 4** 3 vs. 4** 4 vs. 5 & 6**
Butyrate, moles/100 moles	9.81	10.2	10.7	8.65	7.97	7.81	.718	1 vs. 2* 1 & 2 vs. 3** 1 & 2 vs. 4** 3 vs. 4** 4 vs. 5 & 6**
Minor VFA[1] moles/100 moles	3.89	4.19	3.68	1.31	2.26	1.94	.105	1 vs. 2* 1 & 2 vs. 3** 1 & 2 vs. 4** 3 vs. 4** 4 vs. 5 & 6**
Total VFA moles/liter	131	122	127	107	109	111	156.7	1 vs. 2** 1 & 2 vs. 3 NS 1 & 2 vs. 4** 3 vs. 4** 4 vs. 5 & 6 NS
Acetate/propionate ratio	3.51	3.61	3.71	4.75	4.39	4.27	.137	1 vs. 2 NS 1 & 2 vs. 3* 1 & 2 vs. 4** 3 vs. 4** 4 vs. 5 & 6**
Ammonia-N mg/100 ml	146	194	184	78	56	58	1614	1 vs. 2** 1 & 2 vs. 3* 1 & 2 vs. 4** 3 vs. 4** 4 vs. 5 & 6**
pH	6.23	6.24	6.34	6.59	6.07	5.85	.038	1 vs. 2 NS 1 & 2 vs. 3** 1 & 2 vs. 4** 3 vs. 4** 4 vs. 5 & 6**

*$P<0.05$
**$P<0.01$
NS = Nonsignificant ($P>0.1$)
[1]Minor VFA = isobutyrate, valerate and isovalerate

Results and Discussion

Chemical composition and in vitro organic matter digestibility (IVOMD) of forage in the diet consumed by esophageal-fistulated steers during the 6 trial periods is presented in Table 1. Organic matter intake of ruminally fistulated steers was similar ($P>0.10$) for each of the 6 sample periods (Table 2), whereas McCollum and Galyean (1985) in studies on other range types found organic matter intake declined with advancing forage maturity.

Through September, rumen fluid dilution rate (FDR) declined and turnover time (FT), flow rate (FFR), and volume (FV) increased with advancing forage maturity. By October, the values for rumen fluid flow characteristics and FV deviated from this pattern. This deviation probably resulted from intake of forage regrowth resulting from fall rains. This observation tends to be supported by results from the chemical composition of fistula forage samples.

Rumen fluid dilution rate ranged from a high of 18.3%/hour in May to a low of 9.0%/hour in September and was greater ($P<0.05$ to $P<0.01$) for early sample dates than for later sample dates for all but the 4 May vs. 20 May contrast, which was nonsignificant ($P>0.1$). In early May when range grasses were immature, dilution rates were extremely rapid for cattle on an all-forage diet. McCollum and Galyean (1985) also reported very rapid dilution rates (14.9%/hour) for steers grazing range grasses in the early vegetative stage. From our data, we surmise that forage quality significantly affects rumen FDR. The decline in dilution rate with advancing maturity in our study does not appear to be fully explained by intake effects. Forage intake relative to body weight was similar over trial dates. Body weights and rumen capacity have been shown to have a high, positive correlation (Purser and Moir 1966). This suggests that intake relative to rumen capacity was also similar over trial dates in our study. Therefore, the modifying influence of intake on dilution rate should have been minimal. It also appears unlikely that higher water content in less mature forage would significantly influence FDR. Rogers et al. (1979) ruminally infused steers consuming high forage diets with 9 liters of water/day and found no effect on FDR. However, osmotically active agents such as mineral salts will increase FDR (Harrison et al. 1975, Rogers et al. 1979). If immature forage could contribute a more osmotically active fluid (e.g., forage fluids carrying cell organelles, etc.), an increased FDR would be expected. This factor needs further research.

Fluid turnover was greater ($P<0.1$ to $P<0.01$) for later sample dates than earlier sample dates for all orthogonal contrasts except for the 4 May vs. 23 May contrast which was not significant. The fluid flow rate was lower ($P<0.05$ to $P<0.01$) in May than at later sample dates, but June through October contrasts were not significant. Fluid volume increased 60.5 liters from May to September. Orthogonal contrasts revealed that FV was greater ($P<0.1$ to $P<0.5$) at the later sample dates for all but the 17 June vs. 27 July contrast which was nonsignificant. The increased FV observed with the mature forage diets is attributable to some degree to increased rumen volume resulting from growth of the steers. However, FV differences in the present study may be greater than can be explained by growth of the steers alone. One explanation for small FV observed for steers consuming immature forage (May–June) might be rapid FT and FDR which would result in decreased fluid volume.

Effect of sample date did not depend on time of sampling ($P>0.5$) for ruminal propionate, butyrate and the acetate to propionate ratio but was significant ($P<0.5$ to $P<0.1$) for ruminal acetate, minor VFA, ammonia-N and pH. The sample data × time of sampling interaction was generally found to be one of magnitude rather than ranking. Variables varied at a given time across date but the ranking of low to high values were constant across dates. Based on this observation, we concluded that sample date × time of sampling had no meaningful effect on interpretation of the data; therefore, a least squares mean of all sample times for each sample date is presented and discussed for each rumen fermentation trait (Table 3).

The molar proportion of ruminal acetate was similar ($P>0.1$) for May and June sample dates but was lower ($P<0.01$) in May and June than for later dates. The molar proportion of propionate was greater ($P<0.05$ to $P<0.1$) for earlier than later sample dates for all contrasts except the 27 July vs. 8 September and 28 October contrast. The molar proportion of butyrate increased ($P<0.05$) from May to June and then declined ($P<0.01$) through October. The molar proportion of minor VFA in the rumen fluid was less than 4 moles/100 moles at all dates and was highest in May ($P<0.05$ to 0.01) and lowest ($P<0.01$) in July. The concentration of total VFA was greater ($P<0.01$) for 4 May than 20 May, greater ($P<0.01$) for the average of 4 May and 20 May than 27 July and greater ($P<0.01$) for 17 June than 27 July; all other contrasts were not significant ($P<0.05$). These findings for acetate, propionate and total VFA are in general agreement with other reports for foraging cattle and sheep (Topps et al. 1965, Langlands and Sanson 1976, McCollum et al. 1985). However, Topps et al. (1965) reported that proportions of butyrate remained relatively constant with advancing forage maturity. The acetate to propionate ratio ranged from a low of 3.51 to a high of 4.75. Ratios were lower ($P<0.01$) for May and June than later sample dates, but the highest ($P<0.01$) ratio was associated with the July sample date. The smaller ratios of acetate to propionate would be expected to support greater beef production. High propionate fermentations are considered to be more efficient energetically than high acetate fermentations (Hungate 1966). Increased production of propionate will enhance utilization of acetate at the tissue level and increase nitrogen retention (Eskeland et al. 1974).

Rumen ammonia-N concentration increased ($P<0.01$) from 4 May to 20 May then declined with the largest reduction noted between 17 June and 27 July. Concentrations of ruminal ammonia-N were greater ($P<0.01$) for May and June dates than for July, September and October dates. The lowest ($P<0.01$) concentration of rumen ammonia-N was observed on September and October dates. The greater concentrations of ammonia-N for May and June compared to other dates reflects the higher concentration of crude protein in the diet (Roffler and Satter 1975) and small rumen fluid volume (Harrop 1974) observed at these dates. Low levels of ammonia-N in September and October are near the level where they might begin adversely affecting microbial protein synthesis (Satter and Slyter 1974, Okorie et al. 1977); however, this warrants further investigation because rumen volume was greatest in September and October diluting the ammonia-N concentrations.

Ruminal pH increased ($P<0.01$) from May through July, then declined ($P<0.01$) with the lowest pH value occurring in October. Similar pH values have been reported from immature and mature forages in cattle (McCollum et al. 1985). Cellulose digestion has been found to be markedly reduced at low pH values (Terry et al. 1969, Stewart 1977). Most pH values observed during the present study are below the suggested optimum for fiber digestion in ruminants (Mertens 1979). The low ruminal pH observed in September and October could have significantly reduced ruminal fiber digestion. The low ruminal pH's observed in the present study may have also been low enough to adversely affect bacterial cell yields and, therefore, microbial protein synthesis (Russell and Dombrowski 1980).

Conclusions

Organic matter intake relative to body weight was rather constant across the range of forage maturities. We concluded that rumen fluid passage, volume and fermentation depended on forage maturity. Rumen fluid dilution rate was lower at advanced stages of forage maturity whereas FV and FT were greater. The molar proportion of priopionate was greater and the acetate to propionate ratio smaller for earlier than later dates. Rumen fermentation and fluid flow characteristics were more favorable for production

from diets of immature than mature forage. Ruminal pH and ammonia-N may have been limiting for optimal fiber digestion and bacterial cell yields at some trial dates, particularly in September and October. Modification of ruminal pH with buffers or protein supplementation may be beneficial during the late summer-early fall period to increase or sustain animal production.

Literature Cited

Adams, D.C., and R.J. Kartchner. 1984. Effect of level of forage intake on rumen ammonia, pH, liquid volume and liquid dilution rate in beef cattle. J. Anim. Sci. 58:708-713.

AOAC. 1980. Official methods of analysis (13th Ed.). Association of Official Analytical Chemists, Washington, D.C.

Annison, E.F., and D.G. Armstrong. 1970. Volatile fatty acid metabolism and energy supply. P. 422-438. *In:* A.T. Phillipson (Ed.) Physiology of digestion and metabolism in the ruminant. Orrel Press Limited, Newcastle, England.

Broderick, G.A., and J.H. Kang. 1980. Automated simultaneous determinations of ammonia and total amino acids in ruminal fluid and in vitro media. J. Dairy Sci. 63:64-75.

Bull, L.S., W.V. Rumpler, T.F. Sweeney, and R.A. Zinn. 1979. Influence of ruminal turnover on site and extent of digestion. Fed. Proc. 38:2713-2719.

Eskeland, B., W.H. Pfander, and R.L. Preston. 1974. Intravenous energy infusion in lambs: effects on nitrogen retention, plasma free amino acids, and plasma urea nitrogen. Brit. J. Nutr. 31:201-211.

Goering, H.K., and P.J. Van Soest. 1970. Forage fiber analysis. ARS, USDA Agr. Handb. 379.

Harrison, D.G., D.E. Beever, D.J. Thompson, and D.F. Osbourn. 1975. Manipulation of rumen fermentation in sheep by increasing the rate of flow of water from rumen. J. Agr. Sci. (Camb.) 85:93-101.

Harrop, C.J.F. 1974. Nitrogen metabolism in the bovine stomach. 2. Factors influencing ruminal ammonia levels in sheep maintained on different diets. J. Agr. Sci. (Camb.) 82:409-417.

Harvey, W.R. 1979. Least-squares analysis of data with unequal subclass numbers. USDA-SEA, AR. 0-310-945/SEA-5.

Hungate, R.E. 1966. The rumen and its microbes. Academic Press, New York.

Langlands, J.P., and J. Sanson. 1976. Factors affecting the nutritive value of the diet and composition of rumen fluid of grazing sheep and cattle. J. Aust. Agr. Res. 27:691-707.

McCollum, F.T., and M.L. Galyean. 1985. Cattle grazing blue grama rangeland II. Seasonal forage intake and digesta kinetics. J. Range Manage. 38:543-546.

McCollum, F.T., M.L. Galyean, L.J. Krysl, and J.D. Wallace. 1985. Cattle grazing blue grama rangeland I. Seasonal diets and rumen fermentation. J. Range Manage. 38:539-542.

Mertens, D.R. 1979. Effects of buffers upon fiber digestion. p. 65-76. *In:* W.H. Hale and P. Meinhardt (Ed.) Regulation of acid-base balance. Church and Dwight Co. Inc., New York, NY.

Okorie, A.U., P.J. Buttery, and D. Lewis. 1977. Ammonia concentration and protein synthesis in the rumen. Proc. Nutr. Soc. 36:38A.

Owens, F.N., and W.G. Bergen. 1983. Nitrogen metabolism of ruminant animals: historical perspective, current understanding, and future implications. J. Anim. Sci. 57(Suppl. 2):498-518.

Purser, D.B., and R.J. Moir. 1966. Rumen volume as a factor involved in individual sheep differences. J. Anim. Sci. 25:509-515.

Reed, M.J., and R.A. Peterson. 1961. Vegetation, soil and cattle responses to grazing on Northern Great Plains Range. USDA Tech. Bull. No. 1252. p. 1-79.

Roffler, R.E., and L.D. Satter. 1975. Relationship between ruminal ammonia and nonprotein nitrogen utilization by ruminants. 1. Development of a model for predicting nonprotein nitrogen utilization by cattle. J. Dairy Sci. 58:1880-1888.

Rogers, J.A., B.C. Marks, C.L. Davis, and J.H. Clark. 1979. Alteration of rumen fermentation in steers by increasing rumen fluid dilution rate with mineral salts. J. Dairy Sci. 62:1599-1605.

Russell, J.B., and D.B. Dombrowski. 1980. Effect of pH on the efficiency of growth by pure cultures of rumen bacteria in continuous culture. Appl. Environ. Microbiol. 39:604-610.

Russell, J.B., W.M. Sharp, and R.L. Baldwin. 1979. The effect of pH on maximum bacteria growth rate and its possible role as a determinant of bacterial competition in the rumen. J. Anim. Sci. 48:251-255.

Satter, L.D., and L.L. Slyter. 1974. Effect of ammonia concentration on rumen microbial protein production in vitro. Brit. J. Nutr. 32:199-204.

Schneider, B.H., and W.P. Flatt. 1975. The evaluation of feeds through digestibility experiments. Univ. of Georgia Press, Athens. pp. 178-179.

Steel, R.G.D., and J.H. Torrie. 1960. Principles and procedures of statistics. McGraw-Hill Book Co., New York.

Stewart, C.S. 1977. Factors affecting the cellulolytic activity of rumen contents. Appl. Environ. Microbiol. 33:497-502.

Supelco, Inc. 1975. GC separation of UFA C2-C5. Bull. 749 E. Bellefonte, Penn. 16823-0048. p. 1-3.

Terry, R.A., J.M.A. Tilley, and G.E. Outen. 1969. Effect of pH on cellulose digestion under in vitro conditions. J. Sci. Food Agr. 20:317-320.

Tilley, J.M.A., and R.A. Terry. 1963. A two-stage technique for the in vitro digestion of forage crops. J. Brit. Grassl. Soc. 18:104-111.

Topps, J.H., W.D.C. Reed, and R.C. Elliott. 1965. The effect of season and of supplementary feeding on the rumen contents of African cattle grazing subtropical herbage. J. Agr. Sci.(Camb.) 64:397-402.

Uden, P., D.E. Colucci, and P.J. Van Soest. 1980. Investigation of chromium, cerium, and cobalt as markers in digesta rate of passage studies. J. Sci. Food Agr. 31:625-632.