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IN-VITRO RUMEN GAS PRODUCTION VERSUS IN-SITU (*IN-VIVO*) RUMEN KINETICS OF SORGHUM STOVER

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Target Audience: Ruminant nutrition researchers and experts

ABSTRACT

Rumen kinetics of urea-treated sorghum stover was determined using the *in-vitro* rumen gas production and the *in-situ (in-vivo)* rumen degradability techniques, with a view to comparing their efficiency as tools for predicting nutritive value of roughages. Five, ten and fifteen-day urea-treated and ensiled sorghum stover samples were rumen-incubated in both methods side by side with untreated sorghum stover samples. The *in-vitro* rumen gas production curves which were typically centripetal in progression, did not however, statistically differ (P>0,05). In the case of *in-situ* rumen degradability of the sorghum stover treatments, the curves did not only statistically differ (P>0.05), they were curvilinear in progression. The results generally showed a high correlation (P<0.05) between the chemical composition of the sorghum stover samples and their degradability rate constants, C and particulate and outflow rate, K. While the *in-vitro* gas technique might have been less precise than the *in-situ* rumen technique, it nevertheless proved more effective in establishing that much longer anaerobic urea fermentation may not bestow any significant nutritive advantage on ensiled sorghum stover. Furthermore, since *in-vitro* methane (CH_4) produced has been found to correspond to stoichiometric prediction of *in-vivo* production, it is practicable to estimate the amount of CH_4 produced by sheep under feeding conditions. Also, despite its limitations like higher cost, the in-vitro gas technique is commercially viable where large samples need to be nutritionally predicted. It would, thus appear that the combination of both *in-vitro* and *in-situ (in-vivo)* dry matter Kinetic elevation of roughages (especially where some have anti-nutritional compounds), may be the most accomplished way of predicting their nutritive value.

Key words: In-vitro, gas production, in-situ, rumen kinetics, sorghum stover

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DESCRIPTION OF PROBLEM

The use of rumen-cannulated farm animals for nutritional studies, especially in developing countries, is now a major research tool. Furthermore, as predictive models for estimating the nutrient requirements of ruminant livestock have become increasingly complex in their approach, they have highlighted the need for an accurate characterization of the degradation kinetics of different feed fractions (1,2,3). Digestibility measured *in-vivo* has historically been used to assess apparent digestibility. However, this technique is limited to describing the digestive effect of the whole gut and does not permit differentiation between what is degraded in the rumen and what is digested post-ruminally (4). *In vitro* technique has, however, been used to evaluate the degradation kinetics of rumen fermentation previously through the method (5) or the more recent gas production technology (6). Although the comparative advantages of *in-vivo* and *in-vitro* fermentation are still in debate, the *in-vitro* systems are said to have the potentials of being more accurate since micro-organisms are sensitive to undetermined factors that influence rate and extent of digestion.

The objectives of the following study were to:

(1) Employ the *in-vitro* rumen gas production technique in determining the degradation kinetics of sorghum stover;
(2) Compare the *in-vitro* gas production technique with *in-situ (in-vivo)* rumen degradation technique of estimating feedstuff fermentation pattern in the rumen;
(3) Use the two results to predict the nutritive value for sorghum stover if fed to sheep.

MATERIALS AND METHODS

Pre-incubation treatment of sorghum stover

Sorghum stover (mixed varieties) were anaerobically treated (ensiled) with urea using adopted procedures with modifications (7,8,9). Chopped sorghum stovers (4cm long) were treated with 4% fertilizer grade urea and incubated in air-tight pits for five, ten and fifteen days. At the end of ensilage, they were sun-dried and stored ready for rumen studies. Chemical analyses were done according to the method of Van Soest (10).

In-vitro gas Production by sorghum stover

The sorghum stover samples ensiled for five, ten and fifteen days with urea and one sample of untreated sorghum stover were evaluated for their *in-vitro* rumen gas production capacity using a modified Menke technique (9, 11).

In-situ (in-vivo) rumen degradability of rumen stover

The same four sorghum stover samples were subjected to *in-situ (in-vivo)* rumen degradability evaluation (12).

Calculation of rumen kinetics

Using sodium chromate modanted protein (which equally applies to dry matter or other dry matter (DM) components of feeds), ruminal outflow rate can be calculated using the following (12) formula:

$$P=a+bc/c+k$$

Here, **P** is the percent disappearance from the rumen (nylon) bag at time, t. Furthermore, **a,b** and **c** are the constants from the equation $P=a-b(1-\exp^{-ct})$ describing degradation; **a** is Y axis intercept at time 0, and it represents soluble and completely degradable substrate that is rapidly washed out of the bag; **b** is the difference between the intercept **(a)** and the asymptote, it represents the insoluble but potentially degradable substrate which is degraded by the micro-organisms according to the first order kinetics; **c** is the rate constant; **k** is the fractional outflow rate.

Statistically analysis

Graphical presentation, analysis of variance (ANOVA) and other statistical analyses were carried out (13, 14).

RESULTS AND DISCUSSION

The chemical composition of urea-treated dorghum stovers and the results of *in-vitro* rumen gas production by sorghum stover are shown in Tables 1 and 2. The 24 and 96-hour gas production (millitres) values were 19.40 and 37.20 respectively for untreated sorghum stover (u-SS); 20.33 and 42.50 respectively for 5-day treated sorghum stover (t-SS) 5d); 19.87 and 38.33 respectively for 10-day treated sorghum stover (t-SS 10d); 18.33 and 37.17 respectively for 15-day treated sorghum stover (t-SS 15d). In all, there was no significant difference (P>0.05) in the *in-vitro* gas production capacity of the variously treated sorghum stover samples.

Table 1: Chemical Composition of Urea-Treated Sorghum Stover (SS), g/Kg DM:

Nutrient	U-SS	t-SS(5d)	t-SS(10d)	t-SS(15d)	S.E.D.	F(P < 0.05)
DM	931.11	920.00	949.40	937.50	6.14	NS
CP	48.80	101.10	138.10	137.90	21.28	S
NDF	674.50	681.60	650.30	698.80	10.02	NS
ADF	682.30	670.60	692.40	698.80	6.16	NS
OM	898.10	913.10	906.20	911.40	3.37	NS
Ash	69.20	68.40	69.00	60.80	2.02	NS
Ca	4.40	4.10	3.80	4.20	0.13	NS
P	1.50	1.60	1.20	1.90	0.14	NS
CF	342.20	303.30	354.00	316.60	11.60	NS
HC	290.00	265.40	240.20	234.20	11.86	NS
CLL	443.60	347.70	358.40	316.70	27.15	S
LG	63.80	63.80	67.60	60.10	4.02	NS

Where: DM = Dry matter, CP = Crude Protein, NDF = Neutral detergent fibre. ADF = Acid detergent figure, OM = Organic Matter, CF = Crude Fibre, HB = Hemicellulose, CLL = Cellulose, LG = Lignin

NS = Not Significant, S = Significant

u-SS = Untreated sorghum stover

t=SS(5d) = Treated sorghum stover (5 days)

t-SS(10d) = Treated sorghum stover (10 days)

t-SS(15d) = Treated sorghum stover (15days)

Table 2: ***In-vitro* rumen gas production (mls) by urea-treated sorghum stover (ss)**

Incubation time (Hours)	u-SS	t-SS(5d)	t-SS(10d)	t-SS (15d)	S.E.M.
3	3.16	2.83	2.60	2.20	0.20
6	5.50	5.00	5.16	4.50	0.21
12	11.00	10.37	11.33	10.50	10.22
24	19.40	20.33	19.87	18.33	0.43
48	28.77	32.07	29.40	29.87	0.72
72	33.43	38.83	35.23	34.20	1.90
96	37.20	42.50	38.33	37.17	1.26
RSD	4.37	4.84	4.98	5.20	
Curve Deviation Constants (CDc)	Y=6.1107: 0.3661x; R-0.96018	Y=5.3146: 0.43957X; R=0.9670	Y=5.961: 0.3861X; R-0.9591	Y=5.2533: 0.38313X; R=0.95808	

Correlation coefficient (r) for untreated SS and the mean for urea-treated SS = 0.9998 (P=0.001)

Where R = Deviation constant of the curve to the linear equivalent.
u-SS = Untreated sorghum stover
t-SS(5d) = Treated sorghum stover (5 days)
t-SS(10d) = Treated sorghum stover (10 days)
t-SS(5d) = Treated sorghum stover (15 days)

The pattern of *in-situ* rumen degradability of sorghum stover as shown in Table 3 may best be appreciated by comparing the 2,48 and 96-hour degradability values for the urea-treated matrials with the rate constant, C and asymptote (a+b) values. Table 2,48 and 96 hour percent degradability values for u-sorghum stover, t-sorghum stover(5d), t-sorghum stover(10d) and t-sorghum stover(15d) were 9.63, 22.10, 18.26 and 18.01 respectively (for 2-hour degradability), 43.83, 51.71, 54.37 and 53.93 respectively (for 48-hours degradability) and 54.79, 58.32, 60.91 and 59.69 respectively (for 96-hour degradability). The corresponding C and (a+b) values as shown in Table 3 were 0.023, 0.028, 0.032 and 0.038 (for C) and 56.29, 77.32, 60.91 and 75.69 for the asymptote. In all cases there were significant differences ($p<0.05$) between the sorghum stover treatments in the specified rumen degradability indices. They were also curvilinear progression. The relation between chemical relationship of the sorghum stover and their degradability rate constants were highly correlated. The correlation coefficients (r) between acid detergent fibre (ADF) and crude protein (CP) was 0.98 while those for ADF: C and CP: C were 0.96 and 0.91 respectively (Table 4). The ruminal outflow rates (K) for the experimental sorghum stover were calculated and the values set against their corresponding C values as shown in Table 5.

Table 3: Percent degradability of Sorghum stover: a, b, and c calculated using the formula, $P = a + b\ (1 - {}^{ct})$

Ingredients	2	4	8	16	24	48	72	96	WL(%)	a	b	c	(a+b)	RSD
u-SS	9.63	15.63	19.08	23.81	33.25	43.83	49.70	54.79	13.09	1.50	54.79	0.023	56.29	3.72
t-SS (5d)	22.10	25.53	25.57	28.04	40.52	51.71	53.69	58.32	10.59	19.00	58.32	0.028	77.32	4.03
t-SS(10d)	8.26	19.14	19.88	32.70	43.87	54.37	58.34	60.91	15.65	0.00	60.91	0.032	60.91	8.48
t-SS(15)	18.01	18.48	21.79	33.69	41.69	53.93	59.84	59.69	16.03	16.00	59.69	0.038	75.69	6.20

Where: a = Rapidly disappearing fraction; b = Proportion of feed which gradually degrades with time; c = Rate Constant for degradation of b; (a+b) = final degradable portion; RSD = Residual standard deviation;
r(c/b) = 0.92; WL = washing loss;
u-SS = untreated sorghum stover; t-SS (5d) = treated sorghum stover (5 day); t=SS (10d) = treated sorghum stover (10 days); t-SS (15 d) = treated sorghum stover (15 days).

Table 4: Relationship between chemical composition (g/kg) and degradability rate constant (C') of Sorghum stover (SS)

	Acid detergent fibre (ADF)	Crude Protein (CP)	"C"
u-SS	625.30	48.00	0.023
t-SS(5d)	670.60	101.10	0.028
t-SS(10	692.40	138.10	0.032
t-SS(15d)	698.80	137.90	0.038
SED	10.62	21.28	0.003

Where:
r(ADF:CP) = 0.9775
r(ADF:`C') = 0.9629
r(CP :`C') = 0.9071
u-SS = Untreated sorghum stover
t-SS(5d) = Treated sorghum stover (5 days)
t-SS (10d) = Treated sorghum stover (10days)
t-SS (15d) = Treated sorghum stover (15 days)

While there was no significant difference ($P > 0.05$) between *in-vitro* rumen gas production curves of untreated and urea-treated sorghum stover, there was however, a significant difference ($P < 0.05$) between the rates of *in-situ* degradability for the same materials. Thus, in the circumstances of this experiment the *in-situ* rumen degradability evaluation proved to be a more sensitive parameter for predicting and fermentation characteristics of sorghum stover.

Although the 5-day treated sorghum stover sample demonstrated a consistently non-significant ($P>0.5$) higher *in-vitro* gas production capacity than the other treatments, the total gas production was 12% higher than any of the rest. This suggested that there was treatment effect on microbial activity especially for shorter urea fermentation period.

Table 5: Comparative Degradability Rate (`C') and Outflow Rate (`k') of Urea-Treated Sorghum Stover.

Ingredient	48-hour k value (% hour)	C' Value
t-SS (15)	19.76	0.038
t-SS (10d)	16.54	0.032
t-SS (5d)	15.61	0.028
u-SS	12.46	0.023

where
u-SS = Untreated sorghum stover
t-SS(5d) = Treated sorghum stover (5 days)
t-SS (10d) = Treated sorghum stover (10days)
t-SS (15d) = Treated sorghum stover (15 days)

Thus, while *in-situ* rumen degradability rate demonstrated urea treatment effect in general on sorghum stover, the *in-vitro* gas production technique seemed to reflect the chemical composition of these sorghum stover samples to the effect that much longer anaerobic urea fermentation may not bestow any significant nutritive advantage on sorghum stover. This observation is in agreement with another study (15) which concluded that methane gross energy (GE) loss is either unchanged or reduced through alkalichemic fermentation. Compared with other techniques, the *in-vitro* gas production technique allows the monitoring of the kinetics of fermentation of the feed over a long incubation period without the need of using a large number of tubes to terminate treatment after different incubation periods (17). This particular point allows for the observation of the increase in gas production over time, giving an idea of the speed and value of biological response.

Also, since recovered energy as methane (CH_4) has been found to agree with predicted loss values (17), and the *in-vitro* CH_4 produced has also been found to correspond to stoichiometry predictions of *in-vitro* production (17), it is practicable to estimate the amount of CH_4 produced by sheep, as well as the nutritional and environmental implications of same.

An important limitation of the *in-vitro* gas production technique is the high cost of the set up. Fortunately, this disadvantage is made up by the technique's ability to handle more samples at a time than the *in-situ* technique.

The high correlation ($P<0.05$) between major chemical components of sorghum stover (ADF and CP) and degradability rate constant (Table 4) demonstrates the extent to which chemical composition (which is a good indicator of nutritive value of feedstuffs, especially those without anti-nutritional compounds) could be related with rates of rumen degradability and by implication, the nutritive value of ruminant feedstuff. The results, therefore appear consistent with observations made by other workers (12,18).

It has been established (12) that the extent of degradability and degradability rate constant (C value) have close relationship with rumen outflow rate, K. The K values of sorghum stover (Table 4) were, therefore, in compliance. These values are used in comparing digestion kinetics with those of kinetics related to particulate outflow from the rumen to the lower gut in ruminant animals. Depending on the feedstuff, degradability ranking may include correcting for outflow rate which occurs with proteins having a large **b** fraction and a low value for **c**. According to Orskov (12), the adjustment of degradability for difference in outflow sometimes also has the effect of changing the ranking order so that a listing of degradability at one particular outflow rate can be misleading

CONCLUSION AND APPLICATIONS

(1) The *in-situ* rumen degradability technique proved to be a good tool in predicting chemical composition and nutritive value of sorghum stover;

(2) The *in-vitro* rumen gas technique demonstrated good prediction of the chemical composition and nutritive values;

(3) It was also a good parameter for estimating appropriate length of ensilage as well as fermentation characteristics of sorghum stover;

(4) Despite its limitations like higher cost, the *in-vitro* gas technique is commercially viable where large samples need to be nutritionally predicted;

(5) It would appear that the combination of both *in-vitro* and *in-situ* (in-vivo) dry matter kinetic evaluation of roughages (especially where some have anti nutritional compounds), may be the most accomplished way of predicting their nutritive values.

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