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DEGRADATION CHARACTERISTICS OF SOME SUDANESE GRASSES AND GAS PRODUCTION TECHNIQUES

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ABSTRACT: *Eighteen plant species, three ingredients, and six diets were studied for their degradation characteristics, using gas production techniques. The palatable grasses were selected during the rainy season from the range land of Kordofan, Sudan. The ingredients were Roselle seeds, Sorghum grain and Groundnut cake. The samples were incubated for 4, 8, 12, 24, 48, 72 and 96 h, using rumen inoculum of three of the sheep used for the nylon bag. The results showed a large variation between the different plant species in the gas volume. The potential gas volume reflected the presence of anti-nutritional factors. Gas production from the ingredients indicated that sorghum grain recorded the highest gas production volume. The gas production at different time intervals showed increased degradability in the grasses, diets and the ingredients. Eragrostis tremula could be used as reference forage in evaluating the organic matter digestibility and energy density of grasses and Farsefia longisiliqua as a reference for crude protein.*

ORIGINAL ARTICLE

Key words: *In vitro, Gas production, Grasses degradability, Rangeland of Kordofan, Sudan*

INTRODUCTION

In vitro gas production from a grass sample incubated with a rumen fluid inoculums has been successfully used by Menke et al. (1979) and Steingass and Menke (1986) to predict the nutritive value of the substrate fermented. In their feed evaluation systems, the gas produced in 24 h is one parameter others are crude protein, crude fat, crude fibre and crude ash, which are used to predict metabolisable energy (ME).

There are relationships between digestibility in vivo and gas production (carbon dioxide and methane) in vitro, when the feeding stuff is incubated with rumen liquor for 24 hours. It can be used for estimation of digestibility of organic matter and metabolizable energy of grass (Steingass and Menke 1986; Manke and Steingass, 1987); Manke and Steingass, 1988. The present study was conducted with the objective of testing the degradation characteristics of some Sudanese grasses using gas production techniques to rank grasses according to their nutritive value.

MATERIAL AND METHODS

Preparation of the sample

In vitro digestibility method (the gas test, Close and Menke, 1986) was used for three ingredients, experimental diets (experiment1 and 2 rations) and 20 palatable range grasses from rangeland of Kordofan, Sudan (Tables 1 and 2). The samples were crushed to pass through 1 mm screen, and about 230 mg air dry material was placed into the bottom of a glass syringe. The feeding stuff samples were incubated in triplicates in two different days (with different batches of sheep rumen liquor) yielding 6 parallel measurements. The medium composed of micro-mineral solution, rumen buffer solution, macro-mineral solution, rezuine solution and reduction solution were prepared immediately

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before collection of rumen liquor. Rumen samples were obtained from two crossbred sheep fed on freshly cut forage and concentrate. It was collected before the morning feeding, placed in a container, sealed immediately and transported to the laboratory that needed one hour. Preparation of N rich media and rumen liquor was as described by Menke and Steingass (1988). The method used for gas production measurements was as described by Menke et al., (1979).

Rumen liquor was collected from two sheep fed on a roughage diet, homogenized, strained and filtered through glass wool to prevent N deficiency in the syringes, the liquor was mixed with the medium. Thirty ml of rumen liquor medium-mixture was pumped with automatic pipette in to each syringe. The samples were incubated in 100 ml calibrated glass syringes in two automatic incubators, basically by the procedure of Menke et al. (1979) and Steingass and Menke (1988). As a modification the syringes were incubated in an incubator (Electrically heated isothermal oven set at 39 ± 0.5°C) with holes to hold the syringes upright in it. Incubations were started in the mooring, thus the second readings were done 6 hours later. The syringes were shaken automatically during the runs. Readings were made at 4, 8, 12, 24, 48 and 72 h post-infusion. All readings were taken quickly to avoid a change in temperature. The gas production was also compared with a standard hay sample and concentrate which had been used by Steingass and Menke (1986). Calculation of gas volume results were estimated by Fit curve exile programme (Chen, 1997). The IVGP profiles were fitted to the monophasic equation of Groot et al. (1996).

The digestibility of organic matter (do %) was calculated from the gas production (Gb) and content of crude protein (XP, g/kg DM) and crude ash (XA, g/kg DM):

do =14.88 + 0.889 Gb + 0.045 XP + 0.065 XA

Similarly the content of the metabolizable energy (ME, MJ/kg DM) was calculated from the gas production (Gb) and content of crude protein (XP) and crude lipids (XL, g/kg DM) for concentrate compounds test samples:

ME =1.06 + 0.157 Gb + 0.0084 X P + 0.022 X L – 0.0081 X A

The equation for roughages test samples is:

ME = 2.20 + 0.136 Gb + 0.0057 X P + 0.00029 X L 2

Statistical analysis

The results of gas volume recordings were fitted to the exponential equation $P = a + b\,(1 - e^{-ct})$, where p is the gas volume at time t and a, b, and c are constants describing gas production with time: the constants 99 are based on gas volume recordings at 4, 8, 12, 24, 48 and 72 h. Significant differences between means with respect to gas volume readings were tested using Duncan's multiple range test (Duncan, 1955).

Table 1 - Ingredients used in the experimental rations (%) Experiment 1

Ingredients	Diet		
	A	B	C
Molasses	10	–	10
Ground nut cake	89	99	–
Roselle seeds	–	–	89
Common salt	0.75	0.75	0.75
Salt lick	0.25	0.25	0.25
Total	100	100	100

Table 2 - Ingredient used in the experimental diets (%) Experiment 2

Ingredients	Diet		
	1	2	3
Molasses	5	–	7.5
Ground nut cake	40	40	40
Roselle seeds	25	25	25
Sorghum	29	34	26.5
Common salt	0.75	0.75	0.75
Salt lick	0.25	0.25	0.25
Total	100	100	100

The results

Chemical compositions (%) of the diets were shown in table 3 and 4. The gas production at different time intervals showed increased degradability in the samples of diets and the ingredients (Table 5). In experiment1, diet A reflected the greater gas volume at different time intervals. However, diet B showed a decrease in gas volume at 4 h up to 8 h incubation, while in diet C a slight decrease was observed between 12 and 48 h incubation (Table 5). For the experiment 11 diets, diet 3 showed highest level of gas volume in intervals, 4, 8, 12 and 24 h incubation, while diet 2 was recorded lowest levels of gas volume in intervals, 4, 8, 12 and 24 h incubation, but diet1 recoded higher gas

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volume only at 48 h incubation (Table 5). Gas production from experimental ingredients was indicated that Hibiscus sabdarifa recorded lower gas production volume in the intervals, 8, 12, 24 and 48 h incubation, while grain sorghum was recorded highest gas production volume in the same intervals (Figure 1).

Gas volumes which could be attributed to rapidly fermentable fraction of the feed, a, for experiment 1 (diet A, diet B, and diet C), experiment 11 (diet 1, diet 2, and diet 3) and ingredient samples ranged from - 81.0818 to -4.9398, from - 67.6603 to 65.30115 and from -92.0139 to -5.7504 ml, respectively. Negative values pointed to the general occurrence of a lag phase in these plant species. The volume produced from the insoluble but potentially degradable, b, part of the feed ranged from 48.16 to 105.5625, 104.6856 to 111.3883 and from 48.16 to 116.25 ml for experiment 1 rations, experiment11 rations and ingredients, respectively. The potential gas volume (a + b) ranged from 24.4807 to 45.1202, 39.38445 to 43.728 and 24.2361 to 82.2783 ml for the experiment 1, experiment11 and ingredient samples, respectively.

Table 6 represents the gas production of grasses at different time intervals. The results showed increased degradability in some grasses as was reflected in the greater gas volume. However, the grass *Zornia glochidiata* showed the lowest gas volume at 4 h up to 24 h incubation, they were 3.7, 7.0, 13.2 and 21.0 ml, but *Caltropis procera* recorded higher volume at 4, 8 and 48 h .While *Caltropis procera*(flowers) and *Eragrostis termila* revealed an increase in gas at 24 h incubation. *Farsefia longisiliqua* was recorded highest gas volume at 48 h incubation (57.6 ml). The gas production of the grasses at 24 h incubation was ranged from 20.9 to 49.5 ml.

The fermentable fraction (a) for grasses ranged from −10.0468 to7.2818 ml. Also the negative values pointed to the general occurrence of a lag phase in these plant species. The volume produced from the insoluble but potentially degradable, (b), part of the feed ranged from 29.98 to 66.99 ml. The minimum and maximum potential gas volume (a + b) was ranged from21.2885 to 65.3228 ml, respectively.

Table 3 - Chemical composition (%) of the supplementations on dry matter basis (experiment 1)

Nutrient	Diet A	Diet B	Diet C
Dry matter (DM)	93.99	94.13	94.22
Crude protein (CP)	54.20	57.32	23.91
Crude fibre (CF)	5.22	5.14	16.2
Ether extract (EE)	5.05	6.18	14.47
Neutral detergent fibre (NDF)	10.53	10.71	28.48
Acid detergent fibre (ADF)	6.82	7.09	22.02
Acid detergent lignin (ADL)	1.13	1.32	7.06
Hemicelluloses HEMI	3.71	3.62	6.46
Cellulose(CELLU)	5.69	5.77	14.96
Energy density (ME, MJ/Kg DM)	13.67	12.76	9.87
In vitro OM digestibility (%)	87.74	80.78	62.17

Table 4 - Chemical composition (%) of supplementations on dry matter basis. (Experiment 2)

Nutrient	Diet 1	Diet 2	Diet 3
Dry matter (DM)	92.31	92.63	93.42
Crude protein (CP)	38.06	36.93	33.03
Crude fibre (CF)	8.95	9.79	8.77
Ether extract (EE)	5.3	6.5	8.77
Neutral detergent fibre (NDF)	19.13	19.24	20.46
Acid detergent fibre (ADF)	14.08	14.86	12.98
Acid detergent lignin (ADL)	4.51	4.43	3.95
Hemicelluloses HEMI	5.05	4.38	7.48
Cellulose (CELLU)	9.57	10.43	9.03
Energy density (ME, MJ/Kg DM)	11.25	11.29	11.71
In vitro OM digestibility (%)	73.38	71.53	71.21

DISCUSSION

Table 5 shows the gas production of the experimental diets and the ingredients. In experiment 1, diet A recorded greater gas volume at different time intervals. Diet B had lowest gas volume at 4 h incubation time, while diet C recorded lowest gas volume at 12, 24 and 48 h incubation. It was observed that diet A was superior from the other diets, diet A had high percentage of groundnut cake which represent source of energy (Table 1). Also diet A had highest in vitro OM digestibility followed by diet B and C. This may be due to the crude protein content which increased in diet A and then increased the digestibly (Table 3). These results were in line with the findings of Bahatta et al. (2002) and Guimaraes-Beelen et al. (2006), who reported that, the increase in gas production may be attributable to both protein and carbohydrate fermentation.

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Table 5 - Gas production (mean ±S.E) ml per 200 mg dry sample from incubation at different time intervals

Diets and ingredients	Incubation time (h)					Constants			
	4	8	12	24	48	a	b	c	a+b
Diet A	23.8±1.13	37.1±1.17	43.1±1.10	48.5±0.98	51.3±1.51	–4.9398	50.0600	0.1093	45.1202
Diet B	16.4±1.20	29.1±1.15	35.3±1.23	39.3±0.99	43.8±1.41	–5.7504	48.16	0.1565	42.4096
Diet C	22.2±1.10	29.4±1.19	30.9±1.20	30.5±1.00	37.6±1.34	–81.0818	105.5625	0.5611	24.4807
Diet 1	18.5±0.78	30.4±0.99	37.1±1.10	41.0±0.99	44.4±0.99	–66.1004	107.2198	0.4603	41.1194
Diet 2	15.5±0.98	28.2±0.65	36.0±0.64	39.9±0.65	43.0±1.02	–67.6603	111.3883	0.4812	43.728
Diet 3	20.1±0.34	31.4±0.97	37.7±1.23	41.5±0.89	42.2±1.14	65.30115	104.6856	0.49664	39.38445

a, b and c represent constants in the equation $P = a + b(1 - e^{-ct})$ describing gas production with time: the constants are based on gas volume recordings at 4 ,8 ,12 ,24 , 48 and 72 h)

Table 6 - Gas Production (mean ± S.E) (ml) from incubation of different types grasses at different time intervals

Grasses species	Incubation time (h)					Constans			
	4	8	12	24	48	a	b	c	a+b
Accaacia monifera	12.7±2.21	23.8±2.91	32.8±3.56	40.1±3.55	45.6±3.59	-4.9398	50.06	0.1093	45.1202
Eragrostis tremula	21.0±1.40	36.1±1.31	42.9±0.38	49.5±1.24	53.0±1.28	-4.8117	56.93	0.1534	52.1183
Farsefia longisiliqua	10.3±0.80	17.8±1.04	26.5±1.82	43.7±1.75	57.6±2.18	-1.6672	66.99	0.0457	65.3228
Chascanum marrubifolum	14.1±0.94	25.2±1.36	36.2±0.94	44.7±1.21	50.8±1.97	-4.8606	55.47	0.1031	50.6094
Euphorbia aegyptiaca	14.9±0.27	25.1±0.27	32.6±0.66	38.6±0.98	40.6±1.74	-3.9911	44.57	0.1366	40.5789
Ipomoea cordofana	17.0±1.49	27.3±3.68	31.9±4.56	42.5±2.64	51.2±1.81	7.2818	45.47	0.0657	52.7518
Echinochloa colonum	4.9±0.85	13.0±0.88	21.5±0.89	37.8±0.64	46.7±1.59	-8.5713	58.95	0.0607	50.3787
Zornia glochidiata	3.7±0.60	7.0±1.63	13.2±1.61	21.0±0.88	24.6±2.16	-4.4773	30.53	0.0695	26.0527
Trebulus terrestris	11.0±0.33	21.6±0.69	29.2±1.06	36.1±0.92	40.0±1.59	-6.2986	46.01	0.1181	39.7114
Gisekia Pharnacoides	12.0±0.14	21.2±0.49	25.0±0.70	28.6±0.90	31.2±3.33	-3.3325	33.74	0.1544	30.4075
Polygala erioptera	18.0±0.45	30.4±0.64	38.7±0.94	47.3±1.25	52.1±1.80	-1.0154	52.78	0.1129	51.7646
Abadaib spp	18.5±0.76	31.7±0.91	35.9±0.50	43.0±0.13	47.7±0.73	1.2589	45.45	0.1250	46.7089
Indigofera spp	8.8±1.151	19.0±1.90	25.1±1.97	32.1±2.70	32.3±3.90	-10.0468	42.85	0.1437	32.8032
Sesamum alatum	8.2±0.86	14.6±1.03	19.7±1.28	20.9±2.51	21.1±3.19	-8.6915	29.98	0.2037	21.2885
Zaleya pentandra	17.1±0.81	30.2±0.88	36.7±0.53	41.3±0.54	44.8±1.01	-6.8842	50.68	0.1618	43.7958
Belpharis ciliaris	13.9±0.42	24.7±0.44	32.1±0.33	40.1±0.52	45.0±0.91	-2.0652	46.89	0.1053	44.8248
Amaranthus viridis	19.3±1.51	34.0±1.51	41.0±1.68	46.4±1.98	51.3±1.96	-5.5519	55.41	0.1511	49.8581
Caltropis procera(flowers)	26.1±1.05	41.4±0.91	45.9±0.96	49.2±0.83	53.3±0.64	-5.5316	57.02	0.2046	51.4884

a,b and c represent constants in equation $P = a+b(1-e^{-ct})$ describing gas production with time: the constants are based on gas volume recordings at 4 ,8 ,12 ,24 , 48 and 72 h).

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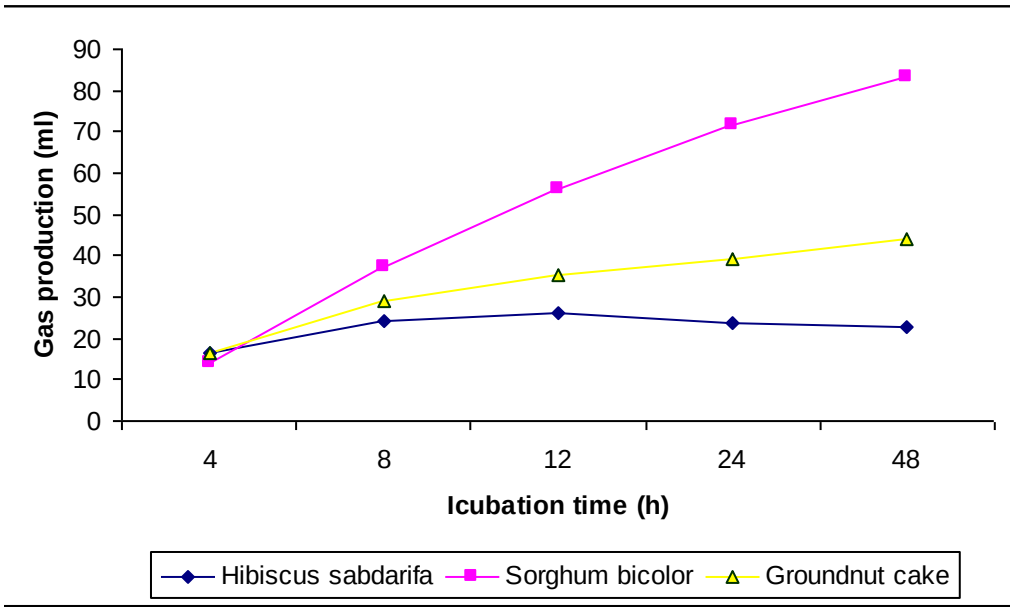

Figure 1 - Gas production from experimental ingredients

Diet C was composed of 89% of Roselle seeds (*Hibiscus sabdarifa*), pure Roselle seeds recorded lowest gas volume, when compared with the other ingredients. The lowest volume of the gas in diet C due to Roselle seeds that, contain lower crude protein (Table 3). The chemical composition of diet C showed that, it has highest Hemicelluloses and Cellulose content than the other diets (Table 3). Both Hemicelluloses and Cellulose represent anti-nutritional factors, this result is similar to that reported by Ahmed and El-Hag (2004) and Khazaal et al., (1993), who recorded that, anti-nutritional factor decreased the digestibly of the dry matter.

High hemicelluloses and cellulose content of Roselle seeds might explain the lower organic mater digestibility observed in diet C, through a decrease in rumen microbial activity .This explanation is in line with findings of Carvalho et al. (2005). This explanation is not acceptable for groundnut cake. In this case, the response observed could be eventually attributed to its lower gas volume in late incubation time (Figure 1) additionally; the relatively high content of cell wall structures (hemicelluloses and cellulose) might restrict microorganism activity, and then lowered gas volume.

The estimated metabolizable energy (ME) content of the supplements reflects their gas production level, crude protein and crude lipids content. Diet A and diet C, have the highest and the lowest energy content, respectively. It was observed that, diet A recorded highest gas at24 h, followed by diet B and diet C which had lowest gas level. Energy density is affected mainly by gas level. In experiment II, the results show that energy densities in the three diets were similar, because the differences in gas volume at 24 h were 41, 39.5 and 41.5 ml for diet 1, diet2 and diet 3, respectively (Table 5).

Diet 3 showed highest gas volume in 4, 8, 12 and24 h incubation, while diet 2 recorded lowest gas volumes in the same incubation intervals, but diet1 recoded higher gas volume only at 48 h incubation. Diet 3 showed the best gas volume, because it has higher molasses than diet 1 (Table 2). The gas production at 8, 12, 24 and 48 h showed highest degradability in grain sorghum that was reflected in the greater gas volume. While *Hibiscus sabdarifa* showed lowest gas production amongst the ingredients, which could be due to the presence of certain anti-nutritional factors (Figure 1). Sorghum is rich in structural carbohydrates that contain high energy (Molina Alcaide et al., 2003), so it produced more gas than other ingredients (protein sources).

The gas production of grasses at different time intervals is shown in Table 6. The results showed increased degradability in some grasses as was reflected in the greater gas volume. However, *Zornia glochidiata* showed the lowest gas volume at 4 h up to 24 h incubation, this explains that *Zornia glochidiata* had lowest energy content. *Eragrostis termila* revealed an increase in gas at 24 h incubation, so it recorded the highest energy. *Farsefia longisiliqua* recorded highest gas volume at 48 h incubation. This result may be due to the maximum potential gas volume (a + b). It may also be explained by a high solubility, or degradability of *Farsefia longisiliqua*. Other studies have shown similar explanation Ahmed and El-Hag, (2004). *Sesamum alatum* had lowest gas volume at24 h. This is due to minimum potential gas of the grass. In fact, the gas level at 24 h had direct effect to energy content of the grasses (Table 5). Some samples recorded higher potential gas volume they were reflecting the presence of less anti-nutritional factors. These findings are in general agreement with Ahmed and El-Hag (2004) and Bahatta et al. (2002) , they found that, the potential gas volume (a + b) was higher for the samples treated with polyethylene glycol, reflecting the presence of anti-nutritional factors. These were higher for legumes than tree pods or grasses.

In this study, the gas production of grasses at different time intervals recorded higher levels compared with the study carried by Ahmed and El-Hag (2004). The variation between the two studies may be due to many factors, such as grasses species, varieties, season and rain fed, all these factors may affect the plant components. The gas

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production results of grasses are in agreement with Kubuga and Darko, 1993) and Blümmel and Ørskov (1993).They used both the nylon bag and in vitro techniques and found valuable assistance in evaluating the quality of a large number of forage samples.

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