SOME FACTORS AFFECTING THE INVITRO GAS PRODUCTION OF SOME RUMINANTS FEEDSTUFFS

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ABSTRACT: *This study was conducted to investigate some factors affecting nutritive value using fermentation gas production technique (Pressure transducer for In-vitro cumulative gas production techniques. Four buffers: (BUF1= MENKE AND STEINGASS (1988). BUF2= GOERING AND VANSEOST (1970. BUF3= THEODORUE (1993). BUF4= STEINGASS (1983)) were used with two different ratios of rumen liquid buffers: (1:2 and 1:3), two different sizes of rumen liquid for incubation (30 ml and 60 ml), two feedstuffs samples weights (200sw1-600sw2 mg) have been investigated. Various feedstuffs were evaluated: Roughages (rice straw RS), corn stover CS), sugar cane bagasse SCB) and Alfalfa hay AA)). Concentrate (soybean meal SBM) - cottonseed meal CSM) - concentrate feed mix CFM) and corn C)). Results showed that the highest gas emitted was found with BUF2 (GOERING and VANSEOST (1970) and the lowest was with BUF3 (THEODORUE (1993). Differences were significant (P<0.05). The degradability of OM and NDF followed the same pattern. Rumen liquor size showed a significant increase for effective gas production and DM degradability with RL1 (30 ml rumen liquor size); whereas degradability of OM and NDF followed the same pattern. Sample size had no significant effect on gas emitted and the predicted gas production. Degradability of DM, OM, and NDF followed the same pattern. The higher effective gas production in concentrate was in high energy sources (corn) than that in protein sources. However, the highest effective gas production in low-quality roughages was reported with AA followed by SCB and RS, while it was least with CS. In general, data revealed that degradability was higher with concentrates than with roughages. With different testing factors.*

Keywords: In-vitro gas production, rumen liquor, buffers, degradability.

INTRODUCTION

The methodology used to measure *in-vitro* gas production has been reviewed to determine the impacts of sources of variation on resultant gas production profiles (GPP). Current methods include measurement of gas production at constant pressure (e.g., use of gas-tight syringes), a system that is inexpensive but may be less sensitive than others thereby affecting its suitability in some situations. The principle of determining the potential rumen degradability/ fermentability of a feed by measuring gas produced from a batch culture was first developed by Hungate (1966). Trei *et al.* (1970) adapted the earlier techniques by attaching a water displacement manometer to each vessel to measure the gas produced. Similarly, Jouany and Thivend, (1986); Beuvink and Spoelstra, (1992) used inverted measuring cylinders to determine the volume of water displaced. Beuvink *et al.* (1992) then automated this water displacement technique.

Although the pressure transducer technique is a simple approach to estimate the fermentation kinetics of a feed, several factors in the application of the method could potentially affect the gas production profile of any feedstuff. The objective of this review is to consider these sources of variation and assess what impact they have on the GPP, how this variation may be reduced, and how results produced from one laboratory and one experiment within a laboratory, may be compared with, and extrapolated to, data from other laboratories.

MATERIAL AND METHODS

This study was conducted at Rumen Microbiology Laboratory, Animal Production Researches Institute (APRI), Agriculture Researches Center (ARC), and Nutrition Laboratory, Animal Production Department, Faculty of Agriculture, Menoufia University to investigate some factors affecting feeding value using fermentation gas production technique. Four forages (rice straw, corn stover, alfalfa hay, and sugar cane bagasse) and four concentrates (concentrate feed mixture, corn, soya bean meal, and cottonseed meal) were used. All experimental samples were chopped to pass through a 2mm screen for proximate analysis (Table 1), with two weight feedstuffs (200mg sw1 and 600mg sw2).

Four buffers were prepared as media (BUF1= MENKE AND STEINGASS (1988). BUF2= GOERING AND VANSEOST (1970. BUF3= THEODORUE (1993). BUF4= STEINGASS (1983)) and mix with rumen liquor. The composition of solutions is shown in Table (2). Rumen liquor was collected from three rams (approximately weight 40-45kg) by stomach tube. The animals were fed on berseem hay and concentrate feed mixture (CFM 14% CP) at a ratio of 70:30 (on a DM basis). Feed was offered to animals once daily with free access to fresh water. Rumen fluid was collected from rams and transferred immediately into pre-warmed thermos bottles after strained through two layers of cheese-cloth. Rumen fluid was manipulated under continuous flushing of CO_2. Buffers were mixed with rumen liquor at the ratio of (2: 1) and (3: 1) respectively (buffer to rumen fluid, v/v). A volume of 30 ml of the mixture was added to 200mg of feedstuffs and a volume of 60 ml of the mixture was added to 600mg of feedstuffs at 100ml of dark glasses each glass saturated with CO_2 and tightly closed with a rubber stopper fitted with outlet valve and incubated at 39°C in a water bath for 72 hours. Glasses were well shaken during all incubation periods and the gas volume was recorded after 3, 6, 12, 24, 48, and 72h incubation by pressure transducer for *In-vitro* cumulative gas production techniques. Three bottles contained the buffer and rumen fluid mixture was included in each run as blank.

The complete chemical composition of the tested feeds was determined according to (AOAC, 2005). Kinetic parameters of gas production (i.e., GP) estimated by fitting GP results (mL/g OM) in the nonlinear regression models (NLIN), an option of SAS (2008), according to the model described by France *et al.*, (2000): $GP = b \times (1 - e^{-c(t-L)})$

where: GP is the volume of GP at time t; b is the asymptotic GP (mL/g OM); c is the rate of GP (ml/h) from the slowly fermentable feed fraction b, and L is the lag time before GP. The degradability of NDF was estimated according to (Van Soest *et al.*, 1991).

Data of feed intake, degradability*, in-vitro* gas production kinetics, OMD, and contents of samples were subjected to the random completely design analysis using the General Linear Model (GLM) of SAS (2002). Statistical model applied for analysis was: $Y_{ijk} = \mu + S_i + e_{ijk}$

Table 1: Chemical composition of experimental feedstuffs (on DM basis)

Item%	DM	CP	CF	NFE	EE	ASH
CFM	90.22	13.3	19.23	58.17	4.8	4.5
C	86.5	7.75	2.23	80.22	7.5	2.3
SBM	89.02	38.93	3.88	42.99	7.9	6.3
CSM	90.8	19.68	10.67	53.52	6.53	9.6
RS	89.93	3.25	35.89	40.16	6.7	14
CS	93.05	3.1	32.31	50.99	4.6	9
AA	89.25	8.31	29.14	48.95	4.6	9
SCB	89.9	3.5	30.1	62.9	2	1.5

DM: dry matter, CP: crude protein, CF: crude fiber, NFE: nitrogen-free extract, EE: either extract, CFM: concentrate feed mixture(yellow corn, soya bean meal, limestone, NACL, wheat brain, molas, undecorticated cottonseed meal "solvent extract", C: corn, SBM: soya bean meal, CSM: cottonseed meal, RS: rice straw, CS: corn Stover, AA: alfalfa hay, SCB: sugar cane bagasse.

Table 2: Composition of various buffer solutions that are used (g/l) with *in-vitro* gas production techniques

Component	Media			
	Menke and Steingass (1988)	Steingass (1983)	Goering and van Soest (1970)	Theodorou (1993)
$CaCl_2 \cdot 2H_2O$	0.013	0.017	0.016	0.015
$MnCl_2 \cdot 4H_2O$	9.7×10^{-3}	0.015	0.012	0.011
$CoCl_3 \cdot 6H_2O$	0.97×10^{-3}	0.002	1.25×10^{-3}	1.11×10^{-3}
$FeCl_3 \cdot 6H_2O$	0.77×10^{-3}	0.012	9.96×10^{-3}	8.84×10^{-3}
Na_2HPO_4	1.09	1.43	1.42	2.09
KH_2PO_4	1.19	1.55	1.55	1.37
$MgSO_4 \cdot 7H_2O$	0.12	0.15	0.15	0.13
$NaHCO_3$	6.71	8.75	8.74	7.73
$(NH_4)HCO_3$	0.77	1.00	1.00	0.88
Resazurin	9.87×10^{-4}	0.125	1.25×10^{-3}	1.11×10^{-3}
Na_2S	0.23	0.52	0.016	2.79×10^{-4}
Trypticase	1.00	--	2.50	2.21
Cysteine hydrochloride	--	--	0.016	2.79×10^{-4}

Where Y_{ijk} represents the general observation of chemical composition, *in-vitro* gas production kinetics, OMD, μ is the expected score, S_i is the subject i, S effect of tested treatments on the observed parameters, and e_{ijk} the standard error term common for all observations. Significant differences among individual means were identified using Duncan's New Multiple Range Test (1955). Mean differences were considered significant at ($P < 0.05$).

RESULTS AND DISCUSSION

Data in Table (3) present the effect of buffer source on gas production; the highest gas emitted was found with BUF2 and the lowest was with BUF3.

Table (4) show that the highest effective gas production and DM degradability were with BUF2 and the lowest values with BUF3, with significant differences ($P<0.05$). Degradability of OM and NDF followed the same pattern (Table 3), with significant differences

(P<0.05). Several media have been developed which buffer the incubation and provide relevant micro-nutrients to the microorganisms. Until now, little research has been completed on relationships between the composition of the medium and measured GP. However, comparing GP from media either rich in N or N-free allows assessment of contributions of N-containing compounds in the sample. Data in the present study revealed that buffer source affects gas production; the highest gas emitted was found with BUF2 and the lowest was with BUF3.

Wilkins (1974) described a different approach to measure fermentation kinetics *in-vitro*, whereby fermentation took place in a sealed vessel and gas produced was determined using a pressure transducer to measure the accumulation of pressure in the vessel headspace. This principle of measuring pressure with a sensor or transducer has been widely adopted as a simple, yet sensitive, method of determining fermentation kinetics. The simplest pressure measurement technique requires manual measurement of headspace pressure, as described by Theodorou *et al.* (1994). Semi- and full automation of headspace pressure recording have also been developed, as described by Pell and Schofield (1993), Cone *et al.*, (1996), Mauricio *et al.* (1998a), and Davies *et al.* (2000). Whereas Abo-Donia *et al.* (2015) reported that cumulative gas production was maximum for moth (P<0.05) fodder. The concentrations of VFAs and NH_3-N/ total N were significantly (P<0.05) higher in moth fodder compared to khejri. Also, a positive correlation was observed between gas production and either OM or NDF disappearance on incubation of moth fodder for 24h compared to khejri.

Table (3): Effect of buffer on gas emitted and gas predicted

Item	Gas emitted (ml)						Gas predicted (ml)					
	h3	h6	h12	h24	h48	h72	Ph3	Ph6	Ph12	Ph24	Ph48	Ph72
BUF1	13.61[ab]	24.76[b]	35.47[b]	48.26[b]	62.87[b]	73.48[b]	10.56[b]	22.43[b]	33.41[b]	46.61[b]	61.40[c]	72.41[b]
BUF2	14.94[a]	27.28[a]	39.39[a]	53.09[a]	68.62[a]	79.49[a]	12.37[a]	25.38[a]	37.68[a]	51.71[a]	67.36[a]	78.61[a]
BUF3	12.91[b]	24.07[b]	35.12[b]	48.09[b]	62.52[b]	72.63[b]	11.27[ab]	22.85[b]	34.02[b]	47.21[b]	61.72[c]	72.07[b]
BUF4	13.94[ab]	25.55[ab]	36.9[b]	50.10[b]	64.98[b]	75.56[b]	12.30[a]	24.34[ab]	35.83[ab]	49.22[ab]	64.18[b]	75.01[b]
±SE	0.46	0.68	0.89	0.90	0.88	1.02	0.46	0.68	0.89	0.90	0.88	1.02

*h = time, Ph =Prediction time, BUF1= MENKE AND STEINGASS (1988). BUF2= GOERING AND VANSEOST (1970), BUF3= THEODORUE (1993), BUF4= STEINGASS (1983), SE= standard error.
[a, and b,] values with different superscript letters within the same column are significant (P<0.05).

Table (4): Effect of buffer on effective gas production and degradability of DM,OM and NDF

Item	kinetics of gas production (ml)		Degradability (%)		
	b	c	DMD	OMD	NDFD
BUF1	76.71[b]	0.042[a]	45.25[b]	50.08[b]	37.44[b]
BUF2	82.22[a]	0.045[a]	49.18[a]	54.42[a]	40.69[a]
BUF3	75.83[b]	0.043[a]	45.02[b]	49.83[b]	37.26[b]
BUF4	78.90[b]	0.043[a]	46.89[ab]	51.89[b]	38.80[ab]
±SE	1.18	0.002	1.16	1.17	1.04

* c= Rate of gas production, b= total gas, DM=dry matter degradability, OM=organic matter degradability, NDF= neutral detergent fiber, BUF1= MENKE AND STEINGASS (1988). BUF2= GOERING AND VANSEOST (1970), BUF3= THEODORUE (1993), BUF4= STEINGASS (1983), SE= standard error.
[a, and b], values with different superscript letters within the same column are significant (P<0.

Automated GP techniques as an *in-vitro* technique, is a simple method, but interactions between fermentation end products, buffering system, and amount of gas produced are very complex (Beuvink and Spoelstra, 1992). This method detects and provides useful data on fermentation kinetics of both soluble cell contents and non-soluble fractions of feedstuffs (Getachew *et al.,* 1997). Another advantage of this method is that it can analyze a large number of samples at one time, making the analytical capacity high (Cone *et al.,* 1997).

It is recommended to take the rumen liquor before feeding because it is most constant in its composition and activity. It is approved to take the rumen fluid mixture from two donor animals at least as the guarantees a greater constancy of activity (Menke *et al.,* 1979; Menke and Steingass, 1988).

Table (5) show that the highest gas emitted was with RL1 and the lowest was found with RL2.

The highest effective gas production was found with RL1 (Table 6) while the lowest was reported for RL2; on the other hand, the highest DM degradability was found with RL1 and the lowest was with

RL2. The degradability of OM and NDF followed the same pattern (Table 6). With significant differences ($P < 0.05$).

Results of Rymer *et al.,* (2005) indicated that there was little effect of rumen liquid preparation on gas production. When Pell and Schofield. (1993) used the next percentage of rumen liquor in the medium (5, 10, 20, and 40%) and found that when the rumen liquor percent has increased the percent of alfalfa hay digestibility was increased with the best rate of maximum fiber digestion was 20%. Rymer *et al.,* (2005) reported that when rumen liquor was increased in the incubation medium, the lag time of gas production was reduced, but the volume or the rate of gas production was increased. It is known that the decrease in rumen liquor to the buffer will decrease the microbial activity; this will lead to reducing the digestibility of feeds.

Results of the effect of sample size on gas emitted are presented in Table (7). It was found that sample size had no significant effect on gas emitted. The predicted gas production followed the same pattern of gas emitted.

Table (5): Effect of rumen liquor size on gas emitted and gas predicted

Item	Gas emitted						Gas predicted					
	h3	h6	h12	h24	h48	h72	Ph3	Ph6	Ph12	Ph24	Ph48	Ph72
RL₁	14.71[a]	26.8[a]	38.7[a]	52.6[a]	68.5[a]	79.9[a]	12.4[a]	25.1[a]	37.2[a]	51.4[a]	67.4[a]	79.1[a]
RL₂	12.99[b]	23.95[b]	34.7[b]	47.10[b]	60.92[b]	70.63[b]	10.81[b]	22.35[b]	33.25[b]	45.9[b]	59.8[b]	69.8[b]
±SE	0.32	0.47	0.62	0.58	0.58	0.66	0.32	0.47	0.62	0.62	0.58	0.66

*h = time, Ph =Prediction time, RL1= rumen liquor1 (30ml), RL2=rumen liquor 2(60ml), SE= standard error.
[a, and b,] values with different superscript letters within the same column are significant ($P < 0.05$).

Table (6): Effect of rumen liquor size on effective gas production and degradability of DM, OM, and NDF

Item	kinetics of gas production (ml)		Degradability (%)		
	b	c	DMD	OMD	NDFD
RL1	83.67[a]	0.042[a]	49.47[a]	54.75[a]	40.93[a]
RL2	68.17[b]	0.044[a]	43.70[b]	48.36[b]	36.16[b]
±SE	0.76	0.001	0.80	0.80	0.72

*c= Rate of gas production, b= total gas, DMD=dry matter degradability, OMD=organic matter degradability, NDFD= neutral detergent fiber, RL1= rumen liquor1 (30ml), RL2=rumen liquor 2(60ml), SE= standard error.
[a, and b,] values with different superscript letters within the same column are significant (P<0.05).

Table (7): Effect of samples size on gas emitted and gas predicted

Item	Gas emitted(ml)						Gas predicted(ml)					
	h3	h6	h12	h24	h48	h72	Ph3	Ph6	Ph12	Ph24	Ph48	Ph72
SS$_1$	13.64	25.15	36.43	49.54	64.37	74.86	11.71	23.70	35.13	48.50	63.42	74.20
±SE	0.29	0.43	0.57	0.58	0.58	0.67	0.30	0.43	0.57	0.58	0.58	0.66
SS$_2$	14.20	25.86	37.22	50.47	65.38	76.00	11.50	23.84	35.40	49.01	64.07	75.08
±SE	0.38	0.56	0.74	0.75	0.74	0.86	0.38	0.56	0.74	0.75	0.74	0.86

*h = time of incubation, Ph =Prediction time, SS1= sample size 1 (200mg), SS2=sample size 2 (600mg).

Table (8) presents the effect of sample size (SS) on effective gas production. It was obvious that no differences were found between both sizes used and the degradability of DM, OM and NDF followed the same pattern (Table 8). The effect of sample preparation on GP is important whereas using a small amount of substrate is prone to experimental error in sample weighing, but further research is required to determine the optimum preparation that mimics animal chewing. Inoculum is the single largest source of variation in measuring GPP, as rumen fluid is variable and sampling schedules, diets fed to donor animals, and ratios of rumen fluid/medium must be selected such that microbial activity is sufficiently high that it does not affect the rate and extent of fermentation. Species of donor animals may also cause differences in GP.

Table (8) show that the highest effective gas production with SW2 and the lowest effective gas production with SW1 and highest degradability of dry matter with SW2 and the lowest degradability of dry matter with SW1.

Menke and Steingass (1988) observed that a close correlation between *in-vitro* gas production and digestibility which the better correlation was achieved when the equation includes crude protein, crude fat, and ash content. Blümmel and Ørskov (1993) adapted gas production technique to describe the kinetics of fermentation based on the following exponential model $[p=a+b(1-e^{-CT})]$ and to predict feed intake in cattle. The results showed that the total gas production (a+b) value as described by the equation was correlated with intake (0.88), digestible dry matter intake (0.93), and growth rate (0.95) in a multiple regression model. The use of the rate of gas production c value did not improve the precision of the correlation.

Table (8): Effect of samples weight on effective gas production and degradability of DM, OM, and NDF

Item	kinetics of gas production (ml)		Degradability (%)		
	b	c	DMD	OMD	NDFD
SW1	78.12	0.043	46.37	51.32	38.37
±SE	0.76	0.001	0.74	0.75	0.66
SW2	78.91	0.043	46.95	51.95	38.84
±SE	0.98	0.001	0.96	0.96	0.86

* b= total gas, c= Rate of gas production, DMD=dry matter degradability, OMD=organic matter degradability, NDFD= neutral detergent fiber, SW1= sample weight 1 (200mg), SW2=sample weight 2 (600mg).

Tuah *et al.* (1996) evaluating several local Ghanaian foodstuffs and found a positive and significant correlation (0.58 to 0.95) between *in-sacco* DM degradability and *in-vitro* gas production.

Table (9) show that gas emitted from corn (high energy source) at almost all times of incubation was less than that emitted from protein sources (SBM, CSM, and CFM); however, within the roughage sources alfalfa hay revealed the highest gas production. Also, data of gas predicted followed the same pattern (Table 9). The highest gas production from roughages was lower than that emitted from concentrates.

The by-products poor in fiber and rich in sugars are quickly degraded by the rumen microbes (Montcho *et al.*, 2017). Menke *et al.,* (1979) observed that there is a high correlation between the amount of substrate and the gas produced over time. It is suggested that no more than 200mg of dry matter of sample should be used, but if it is poorly digestible, it could be 300g. The *in-vitro* assay of foodstuffs in rumen fluid requires careful drying (Menke and Steingass, 1988).

Generally, gas production up to 24h of incubation was higher with concentrates than with roughages either emitted or predicted. It is well known that concentrates are fermented faster than the roughages thus that lead to more gas production early with concentrates while with roughages more gas production was obtained at the late time of incubation. Mertens, (1998) indication that the higher-fiber diets produce gas more than the lower-fiber diets which can mean that acetate production would be more with a high-fiber diet, where fermentation of fiber acetate primarily generated and gas is produced when a substrate is fermented to generate acetate or butyrate rather than propionate. The quantity of gas produced from the *in-vitro* incubation of a substrate is closely related to its digestibility and consequently to its energetic value (Menke *et al.,* 1979; Menke and Steingass 1988). The gas production data may provide a prediction of the effective organic matter degradation *in-sacco* (Deaville and Givens, 1998a). Van Larr *et al.* (1998) found differences in fermentation characteristics between hull and endosperm of full-fat Soya beans; this is possibly related to the polysaccharide composition, mainly the differences in cellulose, hemicellulose, and pectin concentration in each fraction. This work pointed out the necessity to assess the different vegetal fractions in a sample.

Table (10) show that the highest effective gas production was in concentrate with high energy (corn) than

that with protein; the highest effective gas production in low-quality roughages was reported with AA followed by SCB and RS while it was least with CS.

Table (9): Effect of feedstuffs on gas emitted and gas predicted

Item	Gas emitted						Gas predicted					
	h3	h6	h12	h24	h48	h72	Ph3	Ph6	Ph12	Ph24	Ph48	Ph72
Concentrate (ml/g DM)												
SBM	18.7ᵃ	31.7ᵇ	42.2ᵇ	53.8ᶜ	63.9ᵈ	70.8ᵈ	16.5ᵃ	30.4ᵇ	40.7ᵇ	52.6ᶜ	62.1ᵈ	70.0ᵈ
CSM	17.0ᵇᶜ	29.6ᶜ	37.7ᵈ	47.4ᵉ	55.7ᵉ	63.2ᶠ	15.bc	28.0ᶜ	36.2ᵈ	46.2ᵉ	54.6ᵉ	62.4ᶠ
CFM	17.9ᵇ	34.4ᵃ	49.2ᵃ	61.8ᵃ	72.4ᵇ	80.8ᵇ	15.7b	32.7ᵃ	47.7ᵃ	60.5ᵃ	71.4ᵇ	80.0ᵇ
C	16.9ᶜ	27.2ᵈ	40.5ᶜ	50.6ᵈ	61.3ᵈ	75.8ᶜ	14.7c	25.6ᵈ	39.0ᶜ	49.4ᵈ	60.2ᵈ	75.0ᶜ
Roughages (ml/g DM)												
AA	14.4ᵈ	26.3e	41.2ᵇᶜ	58.4ᵇ	76.5ᵃ	89.8ᵃ	12.1ᵈ	24.5ᵉ	39.7ᵇᶜ	57.2ᵇ	75.4ᵃ	89.0ᵃ
CS	7.9ᶠ	16.0h	22.5ᵍ	37.3ᵍ	57.8ᵉ	67.9ᵉ	5.7ᶠ	14.3ʰ	21.0ᵍ	36.1ᵍ	56.7ᵉ	67.1ᵉ
RS	8.4ᶠ	17.6g	27.5ᶠ	40.7ᶠ	62.3ᵈ	73.3ᶜᵈ	6.1ᶠ	16.0ᵍ	26.1ᶠ	39.5ᶠ	61.2ᵈ	72.5ᶜᵈ
SCB	9.1ᵉ	20.3f	32.7ᵉ	48.8ᵈᵉ	68.4ᶜ	80.4ᵇ	6.8ᵉ	18.6ᶠ	31.2ᵉ	47.6ᵈᵉ	67.3ᶜ	79.7ᵇ

*h = time of incubation, Ph =Prediction time, SBM, soya bean meal; CSM, cottonseed meal; CFM, concentrate feed mix; C, corn; AA, Alfalfa hay; CS, corn Stover; RS, rice straw; SCB, sugar cane bagasse.

ᵃ,ᵇ, values with different superscript letters within the same column are significant (P<0.05).

Table (10): Effect of feedstuffs on effective gas production and degradability of DM, OM, and NDF

Item	kinetics of gas production (ml)		Degradability (%)		
	b	c	DMD	OMD	NDFD
Concentrate (ml/g DM)					
SBM	68.38ᵉ	0.06ᵇ	59.97ᵃ	66.98ᵃ	52.36ᵃ
CSM	60.95ᶠ	0.06ᶜ	57.07ᵇ	61.24ᵇ	51.48ᵃ
CFM	77.05ᵈ	0.07ᵃ	54.96ᶜ	57.62ᶜ	47.49ᵇ
C	76.64ᵈ	0.04ᵈ	54.50ᶜ	59.00ᶜ	29.28ᵉ
Roughages (ml/g DM)					
AA	92.71ᵃ	0.04ᵈ	44.49ᵈ	49.57ᵈ	36.19ᶜ
CS	81.57ᶜ	0.02ᵍ	34.61ᵉ	41.71ᵉ	31.70ᵈ
RS	84.25ᵇᶜ	0.03ᶠ	33.85ᵉ	37.25ᶠ	30.96ᵈ
SCB	85.78ᵇ	0.03ᵉ	33.43ᵉ	39.06ᶠ	28.93ᵉ
±SE	1.03	0.001	0.62	0.70	0.55

SBM= soya bean meal; CSM= cottonseed meal; CFM= concentrate feed mix; C= corn; AA= Alfalfa hay; CS= corn Stover; RS= rice straw; SCB=sugar cane bagasse.

ᵃ,ᵇ, values with different superscript letters within the same column are significant (P<0.05).

In general, data revealed that degradability was higher with concentrates than with roughages. Within concentrate sources degradability of OM was highest with protein sources (SBM and CSM) while within roughages it was highest with AA than the other sources.

Khazaal *et al.* (1993) correlated chemical composition (i.e. CP, NDF, ADF, or ADL) with *in-vitro* two-stage digestibility, *in-sacco* degradability, and gas production with voluntary intake and *in-vivo* apparent DM digestibility of 10 *graminaceous* hays in sheep. Accurate prediction of intake and *in-vivo* apparent DMD was achieved using NDF, ADF, ADL, and CP in multiple regression. However, using the (a + b and c) of gas production only intake was predicted accurately. The lower performance of the gas test was attributed to the small contribution to gas production and higher buffering capacity resulting from protein fermentation.

In-vitro cumulative gas production techniques were developed to predict the fermentation of ruminant feedstuffs. A feedstuff is incubated with buffered rumen fluid as soon as gas produced is measured as an indirect indicator of fermentation kinetics. When a feedstuff is incubated with buffered rumen fluid, it is first degraded and the degraded fraction may either be fermented to produce gas and fermentation acids or incorporated into microbial biomass. When combined with measures of degradation, gas production techniques provide a measure of the proportion of feed that is fermented as opposed to that which is partitioned to microbial growth.

From a historical point of view, the *in-vivo* method has generated a lot of important knowledge about feed fermentation for different types of feeds

(Kitessa *et al.,* 1999). This method will also be important in the future because *in-vivo* trials will be needed as it represents the actual animal response to dietary treatment. In the system published by Menke *et al.* (1979) the substrate was incubated in a calibrated gas-tight glass syringe fitted with a plunger, the gas produced over 96 h period is recorded. The incubation media include rumen liquor and a buffer. The main innovation in such a method was that gas production is recorded rather than degradation. The same postulate continues until today, even when the method had been simplified and improved.

The most negative aspect of this method is that it requires donor animals to supply rumen fluid, which is materially expensive (Kitessa *et al.,* 1999). Another limitation of the GP technique is that it has a lack of standardization in the methodology, which makes it difficult to make comparisons on results between different laboratories (Williams, 2000).

Where it appeared that the dried grass negatively affects the gas production profile (Rymer and Givens, 1998). Nagadi *et al.*, (1998) observed that the gas production profile of dried *Festuca rubra* for 20 h at 65°C is more closely associated with that of the fresh sample when milling is completed through 0.4 mm Sieve as compared to 1.0 mm. Van Larr *et al.*, (1998) found differences in fermentation characteristics between hull and endosperm of full-fat Soya beans; this is possibly related to the polysaccharide composition, mainly the differences in cellulose, hemicellulose and pectin concentration in each fraction. Gas production rate was increased when particle size was decreased in fibrous feeds. Rymer *et al.*, (2005) indicated that

maize grain that was steam-flaked, rolled or left intact had the same rate and extent of gas production when it was ground through a 1-mm screen but not when it had been ground through a 4-mm screen.

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بعض العوامل المؤثرة على انتاج الغاز معمليا فى بعض اغذية المجترات

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الملخص العربى:

اجريت هذه الدراسة لتقييم بعض العوامل المؤثرة على القيمة الغذائية عن طريق قياس انتاج الغاز بالطريقة المعملية باستخدام جهاز (Pressure transducer for *In-vitro* cumulative gas production techniques)

تم استخدام المنظمات الأتية :-

1- BUF1= MENKE AND STEINGASS (1988) (المنظم الأول)

2- BUF2= GOERING AND VANSEOST (1970) (المنظم الثانى)

3- BUF3= THEODORUE (1993) (المنظم الثالث)

4- BUF4= STEINGASS (1983) (المنظم الرابع)

استخدمت نسب خلط مختلفة من سائل الكرش بالمنظمات (١:٢ و ١:٣) على الترتيب وأحجام مختلفة من سائل الكرش المعد للتحضين واختبرت أوزان مختلفة لعينات مواد العلف وكانت الاوزان (٢٠٠ – ٦٠٠ مجم على الترتيب) كذلك تم استخدام ٣٠ مل و ٦٠ مل على الترتيب (سائل الكرش بالمنظمات)

تم تقييم مواد علف مركزة ومالئة مختلفة كالتالى :-

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وأظهرت نتائج الدراسة ما يلى:

١- تأثير مصدر المنظم : سجلت أعلى كمية غاز منبعثة وكذلك اعلى معدل هضم للمادة الجافة عند استخدام المنظم Van Soest (1970) وكانت اقلها عند استخدام المنظم Theodorou (1993). وكانت الاختلافات معنوية,واتبعت معاملات هضم المادة العضوية والألياف المتعادلة نفس المنوال.

٢- بدراسة تأثير حجم سائل الكرش المعد للتحضين على الغاز المنبعث وجد أن استخدام ٣٠ مل من سائل الكرش أحدث زيادة معنوية فى انتاج الغاز مقارنة ب ٦٠ مل , كما سلكت معاملات هضم كلا من المادة الجافة والمادة العضوية والالياف المتعادلة نفس النمط.

٣- تاثير وزن العينة : لم يكن هناك تأثير معنوى لوزن العينة على كلا من حجم الغاز المنبعث او المتنبأ بانبعاثة واظهرت معاملات هضم المادة العضوية والألياف المتعادلة نفس النمط.

٤- حجم الغاز المنبعث مع الذرة (كمصدر غنى بالطاقة) فى جميع أوقات التحضين كان أقل مقارنة بمصادر البروتين (كسب فول الصويا – كسب القطن – العلف المركز).

٥- عند مقارنة مواد العلف الخشنة سجل دريس البرسيم الحجازى اكبر حجم من الغاز تبعه كلا من مصاصة القصب وقش الأرز على الترتيب فى حين كانت حطب الذرة الاقل قيمة .

٦- يمكن استنتاج من نتائج الدراسة ارتفاع معاملات هضم مواد العلف المركزة مقارنة بالمواد المالئة باختلاف العوامل المؤثرة على انتاج الغاز معمليا.