

Impact of diets containing graded level of malted sorghum sprout mixed with pineapple waste on rumen fermentation profile of West African dwarf goats using *In Vitro* gas production technique

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Target audience: Ruminant Nutritionist, farmers and Researchers

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Abstract

An experiment was conducted to determine the impact of diets containing graded levels of malted sorghum sprout mixed with pineapple waste (MSPW) on rumen fermentation profile of West African dwarf goats using *in vitro* gas production techniques. The malted sorghum sprout and pineapple waste mixture were obtained at ratio 1:2 (weight/weight) respectively and incorporated in graded levels to produce four treatment diets; T₁ (0% MSPW), T₂ (20% MSPW), T₃ (40% MSPW) and T₄ (60% MSPW). Each diet sample (200mg) was incubated in buffered rumen liquor for 48 h and *in vitro* gas parameters were determined using *in vitro* gas production technique. Gas production was measured at 3, 6, 9, 12, 15, 18, 21 and 24 h post incubation to estimate total gas volume, methane (CH₄), metabolisable energy (ME; MJ/Kg DM), organic matter digestibility (OMD; %) and short chain fatty acids (SCFA; µmol/200 mg DM) were estimated using 4mL of 10M NaOH. Results showed range of values for the compounded feed in which dry matter (87.27–92.00 %), crude protein (2.59–7.47 %), ether extract (13.20–18.44 %), ash (10.07–10.68 %), nitrogen free extract (36.42–44.14 %), neutral detergent fibre (46.75–71.85 %), acid detergent fibre (24.25–45.50 %) and acid detergent lignin (9.20–12.25 %). There was significant ($P < 0.05$) difference in all the parameters observed across the dietary treatments. Gas volume varied significantly ($P < 0.05$) from 38.00 to 54.00 ml/200mg DM. Diet containing 20% MSPW produced highest ($P < 0.05$) volume of gas at 24, 42, and 48 hrs. Volume of gas produced in time (b) recorded highest value (15.552) in 60% MSPW. Fractional rate (c) of gas production (0.810–1.118 ml/hr) and lag time (0.952–1.098 hr) varied significantly ($P < 0.05$) across the dietary treatment. The pH (5.07), temperature (26.60°C), ammonium nitrogen (15.60mg), metabolizable energy (9.54 MJ/KgDM), organic matter digestibility (62.89%), short chain fatty acids (1.23 µmol), gas volume h (54.00) and methane (7.50 ml) were significantly ($P < 0.05$) higher with inclusion level of 20% MSPW. It was therefore concluded that efficient gas production was attained in diet containing 20% MSPW which could serve as a valuable alternative animal feed source in ruminant production.

Keywords: Malted Sorghum Sprout, Pineapple Waste, *In Vitro* Gas Production

Description of problem

Inadequate nutrition is one of the major factors affecting goat productivity.

Despite the naturally endowed vegetation, there are still inadequate feeds and feedstuffs for livestock in Nigeria. Period

of dry season is always a stressful circumstance for livestock, as the environment is characterized by insufficient feed, occasioned by scarce forage and fibrous standing hays. Incidence of disease outbreak is rampant as a result of low immunity arising from malnutrition. As a result of this, many livestock farmers alternatively feed their animals with agro-industrial by-products (1). Malted sorghum sprout is a by-product from malt processing companies. It is the dried roots and shoots left after extraction of malt from germinated sorghum (2). Malted Sorghum Sprout contains dry matter (88.79%), crude protein (26.38%), ether extract (2.35%), ash (5.21%), nitrogen free extract (51.06%), neutral detergent fibre (49.57%), acid detergent fibre (31.25%), acid detergent lignin (3.92%), hemicelluloses (18.32%) and cellulose (27.33%) respectively (3). Pineapple waste is a by-product resulting from the processing of pineapple that consists of peel, core and crown. The pineapple waste is either used as animal feed or disposed to the soil as a waste that can cause environmental problems. This waste still retains a considerable amount of soluble sugars, as well as high fiber and low protein contents (4). *In vitro* gas production technique is a quick means of evaluating the nutritive value of feedstuffs for ruminants (5). In Nigeria, little research has been done to characterize the combination of malted sorghum sprout and

pineapple waste for nutrient content and evaluate them as potential feed resources in ruminant livestock. At present, attempts are therefore been made to include them in rations for ruminants. *In vitro* gas production technique is a very useful tool for the rapid screening of feeds to assess their potential as energy sources. Hence, the objective of this study was to determine the impact of diets containing graded level of malted sorghum sprout mixed with pineapple waste (MSPW) on rumen fermentation profiles of West African dwarf goats using *in vitro* gas production technique.

Materials and Methods

Preparation of Tested feed

Malted Sorghum Sprout was obtained from Live Care Sorghum Processing Industry, Sango Ota, Abeokuta, Ogun State. Fresh wet pineapple waste was obtained from Lafia Canning Factory, Factory of Fumman Agricultural Product Nigeria Limited, Apata Ibadan. Fresh wet pineapple waste was thoroughly hand mixed with malted sorghum sprout accordingly to earlier specified ratio (6). Thereafter, the mixture was spread for (5 - 7 days) on the concrete floor to sun dry. After it was properly dried, it was thereafter milled and then mixed with other ingredients at varying levels of 0%, 20%, 40% and 60% to formulate four dietary treatments: T₁, T₂, T₃ and T₄ respectively.

Table 1: Chemical Composition of diets containing graded levels of malted sorghum mixed with Pineapple waste

Parameters	Inclusion levels of MSPW			
	T ₁ (0%)	T ₂ (20%)	T ₃ (40%)	T ₄ (60%)
Maize bran	60.0	40.0	20.0	-
MSPW	-	20.0	40.0	60.0
Wheat Offal	34.25	34.25	34.25	34.25
Premix	0.25	0.25	0.25	0.25
Limestone	5.00	5.00	5.00	5.000
Salt	0.50	0.50	0.50	0.50
Total	100.0	100.0	100.0	100.0
Determined Analysis %				
Dry Matter	92.00	89.22	88.82	87.27
Crude Protein	13.20	15.28	17.84	18.44
Ether Extract	7.47	5.18	4.56	2.59
Ash	10.66	10.68	10.07	10.40
NFE	36.42	40.32	42.77	44.14
NDF	71.85	65.00	51.20	46.75
ADF	45.50	35.38	24.25	33.25
ADL	12.25	11.25	10.50	9.20
Cellulose	33.25	24.88	13.00	24.05
Hemicellulose	26.35	29.63	26.95	13.50

MSPW: Malted Sorghum Sprout mixed with Pineapple Waste. NFE: Nitrogen Free Extract, NDF: Neutral Detergent Fibre ADF: Acid Detergent Fibre, ADL: Acid Detergent Lignin

Procedure for *In Vitro* Gas Production

The gas production procedure was carried out in the laboratory of Department of Pasture and Range Management, College of Animal Science and Livestock Production, Federal University of Agriculture, Abeokuta. Rumen fluid was obtained using the procedure of (7) from three West African dwarf goats fed 40% concentrate feed and 60% guinea grass at 5% of their body weight. Incubation procedure as reported by (8) was carried out using 120ml calibrated plastic syringe with fitted silicon tube. The sample weight 200mg (n=3) was carefully drop into the syringes and thereafter 30ml inoculums was poured. The silicon tube in the strained rumen liquor and buffer (g/litre) of

(9.8NaHCO₃ + 2.77Na₂HPO₄ + 0.57Kcal + 0.47Nacl + 2.16MgSO₄ 7H₂O + 0.16CaCl₂ 2H₂O) (1:4 v/v) under continuous flushing with CO₂ was dispensed using another 50ml plastic calibrated syringes. The syringe was taped and pushed upward by the pistol in order to completely eliminate air in the inoculums. The silicon tubes in the syringes were then tightened by a metal clip so as to prevent escape of gas. Incubation was carried-out at 39 ± 1⁰ C and the volume of gas was measured at 3, 6, 9, 12, 15, 18, 21, 24, 27, 30, 33, 36, 39, 42, 45, and 48 hours. At post incubation period, 4ml of NaOH (10M) was introduced to estimate methane production as reported by (9). The average of the volume of gas produced from the blanks

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was deducted from the volume of gas produced per sample. The gas production characteristics were estimated using the equation $y = a + b(1 - e^{-ct})$ as described by (10).

Where y = volume of the gas produced at a time “ t ”, a = intercept (gas produced from the soluble fraction), b = gas production from the insoluble fraction, c = gas production rate constant for the insoluble fraction (b), t = incubation time.

Determination of *In vitro* dry matter digestibility

After 24 and 48 hours of incubation, samples were centrifuged at the speed of 2300 rpm for 10 minutes. The residue was washed with warm water until the rinsed water was clear. At the end of washing, each sample was dried at 65°C and the weights of the oven-dried residues were recorded.

The percentage dry matter degradability (DMD %) was then calculated as;

$$\text{DMD \%} = \frac{\text{Dry matter of sample input} - \text{Dry matter of residue} \times 100}{\text{Dry matter of sample incubated}}$$

Calculation

Metabolizable energy (ME, MJ/kg DM) was calculated as $ME = 2.20 + 0.136 GV + 0.057 CP + 0.0029 CF$ (8).

Organic matter digestibility (OMD %) was assessed as $OMD = 14.88 + 0.889 GV + 0.45 CP + 0.651 XA$ (8).

Short chain fatty acids (SCFA) as $SCFA = 0.0239 GV + 0.0601$ (11).

Where GV, CP, CF and XA are total gas volume, crude protein, crude fibre and ash respectively of the incubated samples.

Chemical Analysis

Formulated feed samples were oven-dried, ground and sieved through a 2-mm sieve and stored in airtight container for proximate (12) and fibre fractions (13).

Statistical Analysis

Data obtained were subjected to one way analysis of variance and the significant differences among variable were separated using Duncan multiple range test of (14).

Results and Discussion

The *in vitro* gas production of diets containing graded levels of malted sorghum sprout mixed with pineapple waste (MSPW) is presented in Table 2. The results revealed that the gas produced during different incubation time was significantly ($P < 0.05$) influenced by the different inclusion levels. Diet with 20% MSPW recorded the highest values all through the experiment while diet with 60% MSPW had the lowest values through the incubation time. The highest values recorded by 20% MSPW might be attributed to its high content of degradable carbohydrates as was reported in similar research by (15). Generally, gas production is a function and mirror of degradable carbohydrate and the volume depends on the nature of the carbohydrate in the feed (16, 17).

Table 2: *In vitro* gas production of diets containing graded levels of malted sorghum sprout mixed with pineapple waste (MSPW)

Time (hr)	Inclusion levels of MSPW (%)				SEM±
	0	20	40	60	
3	14.00 ^a	16.00 ^a	14.00 ^a	10.00 ^b	0.70
6	22.00 ^a	22.00 ^a	18.00 ^b	16.00 ^c	0.82
9	28.00 ^a	30.00 ^a	22.00 ^c	22.00 ^c	1.11
12	34.00 ^b	36.00 ^a	26.00 ^c	26.00 ^c	1.40
15	40.00 ^b	42.00 ^a	32.00 ^c	30.00 ^d	1.56
18	44.00 ^b	46.00 ^a	34.00 ^d	36.00 ^c	1.56
24	50.00 ^b	54.00 ^a	38.00 ^d	42.00 ^c	1.92
30	54.00 ^b	58.00 ^a	44.00 ^c	42.00 ^d	2.03
36	56.00 ^b	62.00 ^a	46.00 ^c	44.00 ^d	2.23
42	58.00 ^b	62.00 ^a	48.00 ^c	44.00 ^d	2.21
48	58.00 ^b	64.00 ^a	50.00 ^c	46.00 ^d	2.12

^{a,b,c,d} Means on the same row with different superscripts are significantly different (P<0.05)

MSPW=Malted sorghum sprout mixed with pineapple waste

The *in vitro* gas production characteristics of diet containing graded levels of malted sorghum sprout mixed with pineapple waste (MSPW) are presented in Table 3. The results revealed that there were significant differences (P<0.05) in all the parameters observed. Volume of gas produced (b) in time varied significantly (P< 0.05) across the dietary treatments in which diet containing 60% MSPW recorded the highest value (15.552) while 20% MSPW had the lowest value (15.47). The lower volume of the gas produced in time (b) observed on 20% MSPW based diet was expected because of the highest value of lignin it recorded when compared with other dietary treatment containing MSPW. The fractional rate of the gas (c) also varied significantly (P<0.05) across the dietary treatments in which diet containing 20% MSPW (1.118), 0% MSPW (1.055) and 40% MSPW (1.048) were statistically similar (P>0.05) but however higher (P<0.05) than diet

containing 60% MSPW (0.810). Time between incubation and gas produced (Lag) was significantly (P<0.05) influenced by the experimental diets in which diet containing 60% MSPW recorded the highest value (1.098) compared to those containing 0% MSPW (0.984), 20% MSPW (0.952) and 40% MSPW (0.983) respectively which were statistically similar. The volume of gas produced (GV) was significantly (P<0.05) influenced across the dietary treatments in which 20% MSPW (54.000) had the highest value while the lowest value was observed in diet containing 40% MSPW (38.000). Gas volume (GV) generally reflects the contents of fermentable carbohydrate or carbohydrate degradation, nitrogen and lipids (18). This was further explained by (19) that gas production from protein fermentation is relatively small as compared to carbohydrate fermentation while contribution of fat to gas production is negligible.

Table 3: *In vitro* gas production characteristics of diets containing graded levels of malted sorghum sprout mixed with pineapple waste (MSPW)

Parameter	Inclusion level of MSPW (%)				SEM±
	0	20	40	60	
b (ml/200mgDM)	15.548 ^c	15.470 ^c	15.550 ^b	15.552 ^a	0.000
c (ml/hr)	1.055 ^a	1.118 ^a	1.048 ^a	0.810 ^b	0.038
Lag (hr)	0.984 ^b	0.952 ^b	0.983 ^b	1.098 ^a	0.018
Gv (ml/mg DM)	50.000 ^b	54.000 ^a	38.000 ^d	42.000 ^c	1.924

^{a,b,c} Means along the same row with different superscripts are significantly different (P<0.05).

b =Volume of the gas produced in time (t), c =Fractional rate of gas produce. Lag = Time between incubation and gas produced, Gv = Gas volume at 24hrs

Table 4 shows the post incubation parameters of compounded ration containing graded level of Malted Sorghum Sprout mixed with Pineapple Waste. Temperature obtained in this study ranged from (26.60 – 26.70°C). The metabolizable energy (ME) value varied significantly (P<0.05) across the dietary treatment with diet containing 20% MSPW recorded the highest value while the lowest value was observed with diet containing 40% MSPW. The organic matter digestibility (OMD) value observed in this study ranged significantly from 52.22 – 62.89% but higher than the ranges of value reported by (7) for *P. maximum* (grass) and *Tephrosia candida* (browse plant) mixtures. The organic matter digestibility and metabolizable energy values of the

malted sorghum sprout mixed pineapple waste based diet are reflection of their fermentation ability (20). Short chain fatty acids are simply referred to volatile fatty acids (VFA) and their presence denote energy is available in a feedstuff. The short chain fatty acid and total gas volume observed in this study follow the same trend as observed in metabolizable energy (ME) in which diet containing 20% MSPW recorded the highest value and the lowest value was observed in diet containing 40% MSPW and gas volume range from 38.00 – 54.00ml. It was suggested that gas production from different classes of feeds incubated *in vitro* in buffered rumen fluid was closely related to the production of SCFA which was based on carbohydrate fermentation (21).

Table 4: Post incubation parameters of compounded diets containing graded levels of malted sorghum sprout mixed with pineapple waste.

Parameters	Inclusion levels of MSPW				SEM±
	0%	20%	40%	60%	
pH	4.97 ^b	5.07 ^{ab}	5.21 ^{ab}	5.26 ^a	0.05
Temperature (°C)	26.60 ^b	26.60 ^b	26.00	26.70	0.08
NH ₃ N (mg/100ml)	2.80 ^c	15.60 ^a	13.20 ^b	0.90 ^d	1.92
ME (MJ/Kg)	9.00 ^b	9.54 ^a	7.37 ^d	7.91 ^c	0.26
OMD (%)	59.33 ^b	62.89 ^a	48.66 ^d	52.22 ^c	1.72
SCFA (µmol)	1.13 ^b	1.23 ^a	0.85 ^d	0.94 ^c	0.05
GV h (ml/mgDM)	50.00 ^b	54.00 ^a	38.00 ^d	42.00 ^c	1.92

^{a,b,c} Means along the same row with different superscripts are significantly different (P<0.05)

ME: Metabolizable energy, OMD = organic matter digestibility, SCFA = short chain fatty acid, GV=gas volume, MSPW: Malted sorghum sprout mixed with pineapple waste

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Presented in Figure 1 is the *In vitro* gas production of compounded diet containing graded levels of malted sorghum sprout mixed with pineapple waste (MSPW). Gas volume produced by 20% MSPW was consistently high throughout the incubation

period. Gas production is an indication of microbial degradability of samples (22). In most cases, feedstuffs that showed high capacity for gas production were also observed to be synonymous for high methane production.

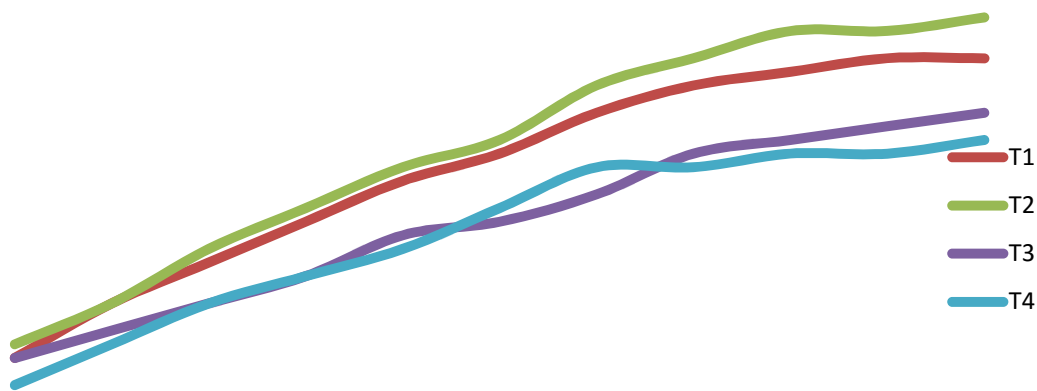


Figure 1: *In vitro* gas production of diets containing graded levels of malted sorghum sprout mixed with pineapple waste (MSPW)

Methane production of compounded ration containing graded levels of malted sorghum sprout mixed with pineapple waste (MSPW) is shown in Figure 2. Significant reduction in methane (23) can be achieved by manipulating animal diets. The *in vitro* gas method can be used to study the efficiency of feed utilization and to examine animal waste components that impact the environment in order to develop appropriate mitigation strategies (24). The significant variation observed in methane production in this present study showed a decrease in methane production as the

malted sorghum sprout mixed pineapple waste increased across the dietary treatment. The same trend was observed in the total gas production and short fatty acid estimates. This might be attributed to high fibre content of the pineapple waste derived from the malted sorghum sprout mixed pineapple waste based diet. The inclusion of 20% to 60% MSPW reduced ($P < 0.05$) *in vitro* methane production. This is noteworthy because of the practical implication of the greenhouse gas on the environment.



Figure 2: Methane gas production of diets containing graded levels of malted sorghum sprout mixed with pineapple waste (MSPW).

Conclusion and Application

1. Malted Sorghum Sprout mixed with Pineapple waste (MSPW) at inclusion level of 20% had better potential as feedstuff for animals especially during the long dry season period because of its higher capacity for gas volume production as the incubation time increased.
2. Diet containing 20% Malted Sorghum Sprout mixed with Pineapple waste (MSPW) recorded the best result in terms of metabolizable energy, organic matter digestibility and short chain fatty acids.

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