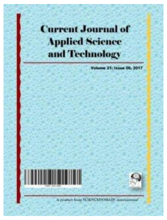

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In vitro Fermentation Characteristics and Acceptability by West African Dwarf Sheep of Ensiled Maize Stover

A. J. Amuda[1*] and K. J. Onaleye[1]

[1]*Department of Animal Production and Health, Federal University Wukari, Taraba State, Nigeria.*

Authors' contributions

This work was carried out in collaboration between both authors. Author AJA designed the study, performed the statistical analysis, wrote the protocol, and wrote the first draft of the manuscript. Authors KJO and AJA managed the analyses of the study. Author AJA managed the literature searches. Both authors read and approved the final manuscript.

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ABSTRACT

Fermentation is an important tool for upgrading crop residues for adequate utilisation by the microbes in the rumen. In a bid to actualise the mandate of better utilisation of unconventional feed sources, the potential of ensiled maize stover as a source of feed for ruminants, especially during the dry season, was investigated. The design of the experiment was complete randomized design (CRD). Half a tonne each of maize stovers (MS) was ensiled for 30 days using three energy additives (Molasses, Honey, and Sugar) at the rate of 50g/kg to obtain the following silages: MS only (control silage), MS and Molasses (M&M), MS and Honey (MSH), MS and sugar (MSS) which served as the treatments. Ten West African Dwarf Sheep (WAD sheep) were used to assess the silage acceptability using coefficient of preference (CoP) procedure. The nutritive value of the silages was determined using *in vitro* fermentation technique. MS only (control) and MSH silages were acceptable as the CoP was above unity. The organic matter digestibility (OMD %),

**Corresponding author: E-mail: aademolajoseph@gmail.com;*

metabolisable energy (ME) (MJ/kg DM), short chair fatty acids (SCFA) (µml) and dry matter degradability (DMD %) ranged from 51.44 – 59.66, 7.07 – 8.36, 0.70 – 0.92 and 40.00 – 70.00, respectively. The OMD, ME and SCFA were highest (P<0.05) in MSS silage. Total gas volume (TGV) (ml/200mg DM) and methane production (ml/200mg DM) ranged from 31.67 - 41.00 and 16.09–28.36%, the highest being from MSS silage and the least from MS silage (control). Soluble fraction ('a') (ml) and potential gas production (a+b) (ml) ranged from 8.00 – 10.67ml and 31.67 – 41.00 ml, respectively. The result showed that *in vitro* gas fermentation of the ensiled maize stover without additives compares favourably with ensiled maize stover with additives and that the CoP is above unity with low level of methane production and a higher percentage of dry matter degradability.

Keywords: Coefficient of preference; gas production; maize stover; sheep; silage.

1. INTRODUCTION

Ruminant animals live almost entirely on forages, which they convert to meat, milk, hide and wool for man's life. Forages are well known to contribute immensely to the nutrition of ruminant animals and most of these are the cheapest form of feed for this class of livestock [1]. In the early wet season, grasses are just coming up, being tender and with a lot of water in it and could result to distention of stomach orchestrated by gas accumulation. Also, ruminants benefit little from over matured grass due to lignification. The shortage of good quality forage needed to sustain livestock growth especially during the dry season has been a perennial problem in Nigeria. In an effort to alleviate the problem of animal feed, looking for an alternative means of feeding, one of which is feeding crop residues after ensiling with or without additives [2] has become necessary. Crop residues have been estimated to account for about 25 percent of the total feed energy sustainable for ruminant livestock in both developed and developing countries [3]. Two third of these crop residues are from cereal crops and form potential resources for ruminant animals. They are characterised by low digestibility, metabolisable energy, nitrogen, minerals and vitamins contents [4]. However, the feeding value of crop residues can be improved by chemical and biological (fermentation) treatments [2]. Silage in the tropics is a sustainable means of supplementing feed for ruminants in the dry season. Ensiling is a potent general for forage preservation and also a form of treatment to occasionally salvage the underutilised crop residues, and pastures for better acceptability and degradability [2].

Acceptability or free choice intake attributes of a feed connotes the actual response of an animal to a particular feed and the possible visual effects of the feed to the animal. This invariably depicts the efficiency of the feed in the rumen [5]. Among the various ways of assessing the nutritive value of feeds for ruminants, the direct intake by the animals is the best method. Free choice intake [6] and *in vitro* fermentation techniques [7] are quick means of evaluating and revalidating the nutritive value of feedstuffs.

The *in vitro* gas method is a laboratory technique to determine degradation of feed which is important in ruminant nutrition. It is a method that is reproducible and parameters obtained correlate well with *in vivo* method. The *in vitro* gas production method is accurate and predicts feed intake, digestibility, microbial nitrogen supply and animal performance [8]. For the past two decades, the technique had been used in advanced countries as an instrument to determine the amount of short chain fatty acids, carbon dioxide and metabolisable energy of feed for ruminants [9,10]. Methane is an important gas among gases produced by ruminants at fermentation, and has been reported [7] to be energy loss to the animals and when emitted, it contributes to the destruction of the ozone layer. The *in vitro* fermentation technique is capable of quantifying the amount of methane (energy loss) production [11]. The present study was designed to investigate the free choice intake and *in vitro* gas production kinetics of ensiled maize stover with or without additives.

2. MATERIALS AND METHODS

The experiment was carried out at the Small Ruminant unit of the Teaching and Research Farm, University of Ibadan, Nigeria, located within 7^o 27^1N and 3^o45^1E at altitude 200 – 300 m above sea level and with a mean temperature of 25 – 29ºC. The annual rainfall is about 1250 mm and the soils are well drained belonging to the alfisol (Rhodic kandiustalf) [12]. The fresh green maize stovers were collected from the

Practical Year Training Programme (PYTP) farm of the University of Ibadan immediately the fresh cobs were harvested for roasting as common practice in most parts of Nigeria. The harvested maize stovers were chopped into 3 – 5 cm pieces size (for easy compaction). Three energy additives were used which were: molasses, honey and sugar; and resulted in four treatments as: A = maize stover (MS) only (control); B = maize stover + molasses, (MSM); C = maize stover + honey (MSH) and D = maize stover + sugar (MSS). The additives were used at the rate of 50g kg^{-1}. The molasses was a by-product of sugarcane processing to sugar. The honey was from honey bee (*Apis* spp) while the sugar is the normal edible sugar from sugarcane or sugar beet. They were added to supply soluble carbohydrates that encourage the growth of lactic acid bacteria. Each of the treatment was ensiled in polythene bags, each capable of holding a 30 kg of wilted maize stover were used as silos. Each polythene bag was placed inside a 65 litres capacity plastic basing for reinforcement and ease of fermentation. Ensiling was done by rapid compaction of the material (to eliminate air) into the silos. Sealing of the silos was done by placing a 25 kg sandbag on top of the polythene bags after tying carefully and firmly. Fermentation was done for 30 days.

2.1 Acceptability Study

Ten West African Dwarf Female Sheep weighing 12-14 kg and about 9-12months old were used to evaluate the voluntary intake of the four silage treatments: MS only (control), MSM, MSH and MSS. The Sheep were purchased from Iwo in Osun State, Nigeria. The animals were immediately placed on prophylactic treatment through the administration of antibiotic (long acting Oxytetracycline HCl). Animals were also treated against ecto-parasites and endo-parasites using 10% diasuntol and levamisole, respectively. During the adaptation period, which lasted for two weeks, the Sheep were fed only with the feedstuffs they were served from where were purchased, including cassava peels, wheat bran, corn glutten and corn offals. An open pen that had been designed to accommodate 20-25 mature Sheep was used. The pen wall was 4 m high and raised by strong wood to 10 m height. The floor was made of concrete and the top covered with wood shavings to a depth 3cm for the absorption of the urine and faeces. In triplicates, 5 kg each of the silages were placed in strategic locations in feeding troughs measuring 150 cm by 60 cm. The Sheep were allowed to feed from 10:00 to 18:00 h daily and for upward of 5 days. Consumption was measured by deduction of remnants from the amount offered and the co-efficient of preference (CoP) value calculated as the ratio of the intakes for the individual silage to the average intake of all the silages [7,13,14]. Forage was inferred to be relatively acceptable if the CoP was greater than unity.

Coefficient of preference (CoP) =

$$\frac{\text{Intake of individual silage}}{\text{Mean intake of the four silage types}}$$

If CoP is < 1, the material is poorly accepted and when > 1, the material is well accepted. (Karbo et al., 1993, Bamikole et al., 2004).

2.2 *In vitro Gas* Production Study

Two hundred milligrams (200 mg) of dried and ground samples of the four silage types (MSM, MSH, MSS and MS only) and unensiled maize stover were weighed into 100 ml calibrated syringes with pistons lubricated with vaseline. A buffered mineral solution was prepared consisting of ($NaHCO_3$ + Na_2HPO_4 + KCl + NaCl + Mg SO_4. $7H_2O$ + $CaCl_2.2H_2O$) and stirred at 39ºC. Rumen fluid was obtained from three West African Dwarfs female goats under the same feeding regime using suction tube method [5] prior to morning feeding. The animals were previously fed with (60%) *Panicum maximum* and (40%) concentrate feed at 5% body weight twice daily for 7days.The rumen liquor was collected into a thermos flask that had been pre-warmed to a temperature of 39ºC and was later filtered through layers of cheese cloth and flushed continuously with CO_2. The post incubator parameters such as metabolisable energy (ME), organic matter digestibility (OMD) and short chain fatty acids (SCFA) were estimated from the volume of gas produced after 24 hours with CO_2. The incubation procedure was as described by [15] using 100 ml calibrated transparent plastics syringes fitted with silicon tube. 30 ml of inoculum containing strained buffered rumen fluid was introduced into the syringes containing (200mg sample) feeds. The incubation was done in triplicate at 39 ±1ºC. The gas production was measured at 3, 6, 9, 12, 15, 21 and 24hrs and after 24hours of incubation, 4 ml of NaOH (10M) was introduced to estimate methane production as reported by Fievez et al. [11]. The average volume of gas produced from the blanks was deducted from volume of gas produced per sample. Rates and extent of gas production were determined for each substrate from linear equation: $Y= a+b (1 - e^{ct})$ as described by Ørskov and McDonald [16] where Y = volume of gas produced at time (t), a = intercept (gas produced from the soluble fraction), b = gas production from the insoluble fraction (b), e = natural logarithm, c = degradation rate constant and t = incubation time. Metabolisable energy (ME) was calculated as ME = 2.20 + 0.136GV + 0.057CP + 0.0029CF [16]. Organic matter digestibility (OMD %) was calculated as OMD = 14.88 + 0.889GV + 0.45CP + 0.651XA [15]. Short chain fatty acids (SCFA) as SCFA = 0.0239GV - 0.0601 [10] was also obtained, where GV, CP, CF and XA are total gas volume (ml/200 mg DM), crude protein, crude fibre and ash contents, respectively. Data obtained were subjected to analysis of variance using SAS package of [17] and where significant differences occurred, the means were separated by the Duncan multiple range test.

3. RESULTS AND DISCUSSION

3.1 Acceptability of Silage

The mean dry matter and coefficient of preference (CoP) by Sheep placed on ensiled maize stover (MS) with and without additives are indicated in Table 1. The CoP varied from 0.95 to 1.03 and mean daily consumption (g/ DM/day) with the order of preference as; MSS > MS (control) > MSM > MSH. Among the silages, MSS and MS (control) were the most preferred, followed by ensiled maize stover with molasses (MSM). The acceptability of MSS and MS only (control) silages could be attributed to good qualities characteristics exhibited as reported by Amuda et al. [18]. In order words, MSS silage and MS (Control) silage with CoP above unity were accepted or preferred. Conversely, MSM and MSH i.e. silages with molasses and honey additives with CoP values of 0.998 and 0.95 were not accepted. Plant physical structure and chemical composition are the most important factors that influence preference [5,19].

The reason for less preference for MSM and MSH silages might be due to fermentation characteristic such as pH, fermentation acids and NH_3-N. In studies to predict voluntary intake from silage fermentation characteristics, some workers [20,21,22] found moderate correlation between fermentation acids and voluntary intake. Seglar [22] reported that it is the combination of high acetic acid levels along with the presence of alcohol and methyl-acetate that probably cause feed refusal.

3.2. *In vitro* Fermentation Characteristics of Ensiled Maize Stover at 24hrs Incubation Period

The *In vitro* gas production characteristics of ensiled maize stover at 24 hours incubation period is presented in Table 2. The *In vitro* gas production characteristics (a, b and a + b) varied significantly (P = 0.05) among the fermented silages. The intercept value (a) for all the silages including the un-ensiled/ fresh maize stover (T_1) ranged from 8.67 to 12.67 at 24hours.The intercept values for the silages were similar across the treatments except for the un-ensiled maize stover that was significantly higher than treatments T_3 and T_4. The extent of gas production ‘b’ T_3 was significantly (P = 0.05) lower than other treatments. Potential gas production (a+ b) was significantly (P = 0.05) different among the treatments with T_1 higher than T_3 and T_4 but similar to T_2 and T_5.

There were no significant (P > 0.05) differences in gas production rate (‘c’) and Y values of the incubated samples. Incubation time (‘t’) and Y values followed a similar trend. The rate of gas production ‘c’ ranged from 0.028 to 0.047 ml h^{-1} for all the treatments while the volume of gas produced at time (‘t') ranged from 18.00 to 23.33 for all the treatments. The time of most rapid increase in gas produced ‘t’ ranged from 11.00 in T_2 and T_5 to 14.0hrs in T_1.

The variations in a, b and a + b values may be due to the differences in the additives used to prepare the silages. Getachew et al. [23] reported that gas production can be attributed to the nature of carbohydrate fractions contained in the substrates. The intercept value ‘a’ for all the silages (treatments) at 24hrs ranged from 8.00 in T_4 (maize stover and Honey) to 12.67 in T_1 (unensiled/fresh MS). The similarity of T_1 (Fresh MS), T_2 (control) and T_5 (MSS) may imply that there was minimal loss of water soluble carbohydrate during fermentation and storage of the original material. The soluble fraction (a) encourages the attachment of rumen microbes to the substrate with resultant improvement in gas production. This perhaps might be the case in this study.

The values of ‘b’ obtained in this study (23.00 – 32.00) are consistent with those reported for dry matter (DM) degradation of some tropical legumes and grasses [24] and the values of 9.5 – 32.0 ml/200 mg DM reported for some crop residues [25]. At 24hrs, there were significant variations in ‘a+b’ among the treatments such that it was highest in the T_1 (Fresh MS) and lowest in the T_3 (MSM). However, T_1 values for ‘a + b’ was similar to T_2 (control) and T_5 (MSS) silages. The high value of the potential extent of gas production recorded for T_1, T_2 and T_5 may be attributed to the abundance of carbohydrate fraction embedded in the fresh MS (T_1), MS only (control, T_2) and MS and sugar (T_5). It is well known that gas production is basically the result of fermentation of carbohydrate to volatile fatty acid (acetate, butyrate and propionate) [23,26].

The volume of gas ‘Y’ at time” t”is the peak of gas production for each sample at 24hrs incubation period. Since rate ‘’c” of gas production at time’ ‘t” and volume of gas “Y” of the incubated samples were similar across the treatments, it may imply that the additives had no effect on MS silages regarding the “c”.t and “Y“ characteristics of the gas production. Factors that may determine the amount of gas to be produced during fermentation include the nature and level of fibre, the presence of secondary metabolites [14] and potency of the rumen liquor for incubation. Since gas production is dependent on the relative proportion of soluble, insoluble but degradable and undegradable particle of feed; mathematical description of gas production profiles allows evaluation of substrate and fermentability of soluble and slowly fermentable component of feeds [23].

Based on the above, it could be adduced that among the MS silages studied, MSM (T_3) silage and fresh MS (T_1) would provide a minimal proportion of residue that would take up space if utilised in *in vivo* studies and persists as indigestible residue. Saliu and Ososanya [27] reported that the rate (c) determines digestion time and consequently how long a potentially digestible material would occupy space. Therefore the potential extent of digestion (‘b’) values obtained for treatments 1, 2, 4 and 5 demonstrated that they possess more potentially degradable carbohydrates than T_3. Also, the results presented in Table 2 showed that digestion rates (‘c’) and potential extent (‘b’) of gas production provided a more meaningful index of nutritional value than ultimate digestibility. However, the conversion of true fermented organic matter into gas varied with the type of additive used to prepare the silage.

3.3 *In vitro* Fermentation Parameters of Ensiled Maize Stover at 24hrs Incubation Period

Table 3 shows *in vitro* fermentation parameters of ensiled maize stover with or without additives. All the parameters observed in this study showed that the treatments had significant effects on the nutritive value of maize stover silage with or without additives. The highest gas production was obtained in un-ensiled/fresh MS (T_1) while T_3 was lowest. However, the total gas volume (TGV) of T_2 (control) and T_5 compared well with T_1 (fresh MS). Methane (CH_4) production was equally highest in T_1 than other treatments except for T_5 which was similar. In most cases, feedstuffs that show high capacity for gas production are known to be synonymous for high methane production. Methane (CH_4) production in the rumen is an energetically wasteful process, since the portion of the animal’s feed, which is converted to $CH_{4,}$ is eructated as gas. The organic matter digestibility (OMD) which could be said to be a measure of degradability (potentials) of the microbes on the substrates especially in the presence of sufficient ammonia nitrogen (NH_3-N) which has influence on bacterial fermentation was highest in T_1 and in the order 1>5>2>4>3. The range values (51.44 – 59.66%) obtained among the silage treatments were higher than the range of 35.16 - 36.32% reported by [28] for ensiled maize stover with cassava top and Albizia saman pods. This suggest that T_3 (MSM) was the best followed by T_4 in terms of gradual release of gas because quick release of gas from fermentation processes can quickly accumulate and cause distention of the rumen which subsequently causes the diaphragm to be under pressure which may lead to suffocation as a result of difficulty in breathing especially when the animal has difficulty in expelling the gas (bloat).

The mean value for methane production was lower ($P < 0.05$) in $T_{2,}$ T_3 and T_4 than T_1. Research on rumen methanogenesis and its inhibition was initiated with aim of increasing feed efficiency. This means that reduced methane production will lead to greater efficiency in feed utilisation. Depending on the level of feed composition of the diet and digestibility, 6-7% of the gross energy in the feed is lost through methane production [29,30]. This also implied that there will be more energy for the animals on treatments 2 (control) and 3 (maize stover and molasses) silages.

However, for economical reason, treatment 2 (control silage) may be more adoptable to the farmers since molasses is costly and not readily available.

Table 1. Dry matter intake and coefficient preference of WAD sheep fed ensiled maize Stover

Silage type	Mean daily consumption of animals (g DM)	Coefficient of preference (CoP)
MS(Control)	553.35	1.02
MSM	541.32	0.998
MSH	517.13	0.95
MSS	558.21	1.03

MS - Maize stover, MSM - Maize stover + Molasses, MSH - Maize stover + Honey, MSS - Maize stover + Sugar

Table 2. *In vitro* fermentation characteristics of ensiled maize stover at 24hrs incubation period

Fermetation characteristics	Treatments					SEM
	T_1	T_2	T_3	T_4	T_5	
a(ml)	12.67[a]	10.67[ab]	8.67[b]	8.00[b]	10.67[ab]	0.62
b (ml)	32.00[a]	28.67[a]	23.00[b]	28.00[a]	30.33[a]	0.71
a+b(ml)	44.67[a]	39.33[ab]	31.67[c]	36.00[bc]	41.00[ab]	1.07
c(mlh^{-1})	0.046	0.036	0.047	0.037	0.028	0.01
t (hrs)	14.00	11.00	13.00	12.00	11.00	0.82
y (mL)	23.33	20.00	18.67	18.00	18.67	0.99

a b c = Means on the same row with different superscripts differ significantly (P < 0.05)
a = zero time which idealy reflects the fermentation of soluble fraction
b = extent of gas production from insoluble but degradable fraction
a+b = potential extent of gas production, c = rate of gas production at time (t)
Y = volume of gas produce at time (t), T_1 = Unensiled/Fresh Maize Stover
T_2 = Maize Stover Only (Control), T_3 = Maize Stover and Molasses (MSM), T_4 = Maize Stover and Honey (MSH), T_5 = Maize Stover and Sugar (MSS), SEM = Standard Error of Means

Table 3. *In vitro* fermentation parameters of maize stover at 24hrs incubation period

Parameters	Treatments					SEM
	T1	T2	T3	T4	T5	
TGV (ml)	44.68[a]	39.33[ab]	31.67[c]	36.00[bc]	41.00[ab]	1.75
CH_4 (%)	28.36[a]	16.09[c]	17.90[c]	22.22[b]	25.20[ab]	0.43
OMD (%)	63.55[a]	58.18[ab]	51.44[c]	54.83[bc]	59.66[ab]	3.2
ME (MJ/Kg DM)	8.90[a]	8.11[ab]	7.07[c]	7.64[bc]	8.36[ab]	0.18
SCFA (µml)	1.01[a]	0.88[ab]	0.88[ab]	0.70[bc]	0.92[ab]	0.03
DMD (%)	53.33[ab]	70.00[a]	60.00[ab]	40.00[c]	46.67[bc]	3.29

a,b c Means along the same row with different superscripts are significantly (P < 0.05) different
TVG = Total Volume Gas, CH_4 = Methane, ME = Metabolisable Energy, OMD = Organic Matter Digestibility, SCFA = Short Fatty Acid, DMD = Dry Matter Degradability. T_1 = Unensiled Maize Stover, T_2 = Ensiled Maize Stover Only (Control), T_3 = Ensiled Maize Stover and Molasses (MSM), T_4 = Ensiled Maize Stover and Honey (MSH) T_5 = Ensiled Maize Stover and Sugar (MSS) SEM = Standard Error of Means

Table 4. The proximate composition of ensiled maize stover

Nutrient	Unensiled	Control	MSM	MSH	MSS	SEM
Dry matter	31.1[b]	31.6[b]	31.3[b]	32.7[ab]	35.3[a]	0.50
Crude protein	9.3[a]	8.4[a]	8.3[a]	7.9[a]	8.6[a]	0.46
Crude fibre	32.3[a]	31.9[a]	30.0[a]	30.0[a]	31.4[a]	0.55
Ash	7.4[a]	7.1[a]	7.2[a]	6.3[b]	6.9[a]	0.11
Ether extract	1.8[a]	1.4[a]	1.7[a]	1.5[a]	1.8[a]	0.69
Nitrogen free extract	49.4[b]	51.2[ab]	52.9[a]	53.7[a]	51.5[ab]	0.58

a,b = Means on the same row with different superscripts are signinficantly (P < 0.05) different
Control = Ensiled Maize stover without additives, MSM = Ensiled Maize stover + Molasses, MSH = Ensiled Maize stover + Honey, MSS = Ensiled Maize stover + Sugar, SEM = Standard Error of Means

Metabolisable energy (ME), short chain fatty acid (SCFA) and dry matter degradability (DMD) production all differed significantly among the treatments. The value for the ME, SCFA and DMD ranged from 7.07 to 8.90, 0.70 to 1.01 and 40.00 to 70.00, respectively. A correlation between ME values measured *in vivo* and predicted from 24hr *in vitro* gas production and chemical composition of feed was reported by Ørskov and McDonald [16]. The result obtained in this study agrees with that reported for forage legumes and crop residues by [19,31,32,33,18]. Furthermore, the ME values of the silage (7.07-8.90 MJ/Kg) are within the 4.5 to15 $MJkg^{-1}$ DM range reported by [15] for various European feeds.

The non significant result obtained for unensiled/fresh MS (T_1), control (T_2) and MSS (T_5) silages in SCFA levels was in line with other reports [18,33]. The direct proportionality of gas production to SCFA reported by Beuvink and Spoelstra [34] was observed in this study. Short chain fatty acid level indicates the energy that is available to the animal and it contributes up to 80% of animal daily energy requirement [35]. In this study, short chain fatty acid (SCFA) is directly proportional to metabolisable energy (ME) and agrees with the report of [36].

The chemical composition of the experimental silages is presented in Table 4.

4. CONCLUSION AND RECOMMENDATION

It is concluded that maize stover could be ensiled without additives to provide acceptable and highly degradable dry season feed for sheep. Therefore, sheep farmers are enjoined to ensile maize stover for dry season feeding.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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