

Assessing the nutritive value of waterweeds to livestock production via *in vitro*

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Abstract

The predominant increase in the price of feedstuffs and the consistent shift in climate have increased the cost of producing animal protein and an attendant increase in the search of available and valuable plant options in combating this problem. The study aimed to evaluate the nutritive value of ten identified water weeds (Polygonum lanigerum, Nymphaea lotus, Paspalum scrobiculatum, Ascroceras zizanioides, Ipomea aquatica, Panicum sulbabidum, Sacciolepis africana, Leersia hexandra, Heteranthera callifolia and Dicksonia antartica) via in vitro digestibility studies. The gas production was measured by incubating samples in buffered ruminal fluid from goats for 96hr. Cumulative gas production was recorded at 3, 6, 9, 12, 15, 18, 21, 24, 27, 30, 33, 36, 39, 42, 45, 48, 51, 54, 57, 60, 66, 72, 84 and 96 hour of incubation periods and the organic matter digestibility (OMD), short chain fatty acid (SCFA) and metabolizable energy (ME) were also estimated. Results indicated that the total gas production varied significantly (P<0.05) at 24 and 48 hours incubation. Gas production ranged between 4.67 ml and 14.00 ml with least and highest obtained in Sacciolepis Africana and Ipomea aquatic, respectively. The Metabolizable energy (MJ/KgDM) recorded in all weeds differed (P<0.05) in all hours of incubation. Paspalum scrobiculatum and Dicksonia antartica recorded the least value at 24 and 48 hours incubation while Nymphaea lotus and Dicksonia antartica had the least value at 72 and 96 hours incubation. Heteranthera callifolia had the highest organic matter digestibility (%) while the least short chain fatty acid (µmol) was observed in Dicksonia antartica at 24, 48 and 96 hours incubation. Based on the results of this study, these plants had the potentials of being fed to livestock especially during the dry season when forage feeds are generally scarce.

Keywords: Waterweeds, ruminal fluid, Metabolizable energy, organic matter digestibility and short chain fatty acids.

Introduction

A major constraint to livestock production in developing countries is the scarcity and fluctuating quantity and quality of the year-round feed supply (Akinfemi *et al*., 2009; Ekunseitan *et al.,* 2013). Providing adequate good-quality feed to livestock to raise and maintain their productivity is and will be a major challenge to agricultural scientists and policy makers all over the world. Increase in population and rapid growth in world economies will lead to increase in demand for animal products; an increase of approximately 30 percent in both meat and milk production is expected in the coming 20 years. At the same time, the demand for food crops will also

increase. Future hopes of feeding the millions and safeguarding their food security will depend on the enhanced and efficient utilization of unconventional resources, which cannot be used as food for humans but as feed for livestock.

Geographically, the natural grassland occupies the larger percentage of the land mass which allows for the vast growing and cultivation of upland forages with little or no attention to the waterweeds. Nevertheless, literature revealed that in recent years, attention is now drawing to the exploring of the potential in waterweeds (Babayemi, 2007). While agro industrial products as well as leguminous plants have been extensively utilized in ruminant's nutrition with relative success in animal's maintenance and production, the use of potentially nutritive waterweeds is yet to be given much attention. Water plants are potential as forages in countries with rivers, streams, brooks, oasis, swamps and dams (Babayemi *et al*., 2006).

It has been observed that during the dry season, availability of nutritive forages for ruminant production is scarce due to long period of drought (Humphreys, 1991), resulting into unfavorable growth responses and reduced productive performances of the animals. Therefore, the need to supplement the available low quality feed resources with other nutritive sources, as well as exploration of alternative feed resources of good nutritional quality and availability in ruminant nutrition particularly during the dry season becomes absolutely imperative.

In vitro methods for laboratory estimations of degraded feeds are important for ruminant nutritionists since feed particles are retained selectively in the rumen compartment and virtually impossible to predict precise *in vivo* digestion of consumable fractions of feed (Huhtanen *et al*., 2006). An efficient laboratory method should be reproducible and should correlate well with actually measured *in vivo* parameters. *In vitro* methods have the advantage not only of being less expensive and less time-consuming (Fievez *et al.,* 2005), but allow maintaining experimental conditions more precisely than do *in vivo* trials. Hence it was employed in this present study to access the nutritive value of waterweeds via *in vitro*.

Materials and Methods

Sample collection

Fresh samples of waterweeds were obtained from Asero pond and a river in Camp area along Obantoko-Osiele road, Abeokuta, Nigeria. The samples were taken to College of Plant Science and Crop Protection of the Federal University of Agriculture, Abeokuta for proper identification and classification.

Chemical Analysis

The AOAC (1990) procedures of determining the crude protein, crude fibre, ether extract, ash content and the nitrogen free-extract was followed to determine the proximate composition of the waterweeds.

In Vitro Digestibility Study

Ruminal fluid was obtained from three West African dwarf female goats through suction tube before the morning feed. The animals were fed with 40% concentrate feed (40% corn, 10% wheat offal, 10% palm kernel cake, 20% groundnut cake, 5% soybean meal, 10% dried brewers grain, 1% common salt, 3.75% oyster shell and 0.25% fish meal) and 60% Guinea grass. Incubation was as reported by Menke and Steingass (1988) using 120 ml calibrated syringes at 39°C. To 200 mg of dried sample in the syringe was added 30 ml inoculum containing cheese cloth strained rumen liquor and buffer (9.8 g $NaHCO_3$ + 2.77g

Na_2HPO_4 + 0.57 g KCl + 0.47 g NaCl + 0.12 g $MgSO_4.7H_2O$ + 0.16 g/liter $CaCl_2.2H_2O$) (1:4, v/v) under continuous flushing with CO_2.

The gas production was measured at 3, 6, 9, 12, 15, 18, 21, 24, 27, 30, 33, 36, 39, 42, 45, 48, 51, 54, 57, 60, 66, 72, 84 and 96 h. After 24, 48, 72 and 96 h of incubation, 4 ml of NaOH (10 M) was introduced to estimate the amount of methane produced. The average volume of gas produced from the blanks was deducted from the volume of gas produced per sample. The volume of the gas produced was plotted against the incubation time, and the gas production characteristics were estimated using the equation $Y = a + b (1 – e^{-ct})$ described by Qrskov and McDonald (1979), where Y = volume of gas produced at 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.

Metabolizable energy (ME, MJ/Kg DM) and organic matter digestibility (OMD %) were estimated as established by Menke and Steingass (1988) and short chain fatty acids (SCFA) was calculated as reported by Getachew *et al*. (1999) : ME = 2.20 + 0.136*Gv + 0.057*CP + 0.0029*CF; OMD = 14.88 + 0.889Gv + 0.45CP + 0.651 XA; SCFA = 0.0239*Gv – 0.0601; where Gv, CP, CF and XA are net gas production (ml/200 mg DM), crude protein, crude fibre and ash of the incubated samples respectively.

Statistical analysis

Data obtained were subjected to one-way Analysis of Variance (ANOVA) while significantly different means were separated using the New Duncan Multiple Range test as contained in Statistical Analysis System (SAS, 1995).

Results and Discussion

The chemical composition (g/100g DM) of water weeds is presented in Table 1. The percentage dry matter (DM) in the water weeds ranged from 11.67 to 26.67 %, the least and highest DM value was obtained in *Heteranthera callifolia* and *Polygonum lanigerum* respectively. Dry matter content of aquatic plants obtained is small compared to terrestrial plants, these values were higher than that reported for some waterweeds (Khan et al., 2002) and submerge hydrophytes (Banergee and Matai, 1990). All plant species considered had higher protein content except *Paspalum scrobiculatum* which recorded the lowest value. Floating plants have been reported to contain higher protein than emergent plants (Banergee and Matai, 1990) but the variation in protein levels may be probably due to environmental differences since nutrient contents in aquatic environment are known to affect crude protein content. The crude fibre ranged from 4.00 in *Ipomea aquatic* to 26.00 in *Acroceras zizanioides.* These values may be a strength response by plants to support aerial vegetation and could serve as potential substitutes as roughages for ruminant plants. Ash component of materials details the mineral levels nonetheless adequate level of ash (mineral nutrients) are important aspect of nutritive quality; the least ash value was obtained in *Dicksonia antartica* while the highest was obtained in *Nymphaea lotus.* Although, ash component of most of the plants examined was less than 16.00 % except *Nymphaea lotus, it* compares favourably with some green roughages commonly utilized in livestock feed (Banergee and Matai, 1990).

Table 2 shows the gas production characteristics which includes; the net volume of gas produced, the gas produced from soluble fraction, the gas produced

from the insoluble fraction, gas production rate constant for the insoluble fraction and the incubation time. The *in vitro* gas production characteristics varied significantly (P<0.05) among the waterweeds. The net gas produced in the 24 and 48 hours incubation ranged between 4.67(mL g-1 DM) in *Sacciolepis africana and 14.00* (mL g-1 DM) *in Ipomea aquatic* while at 72 hours incubation the highest and the lowest gas produced were obtained in *Leersia hexandra and Nymphaea lotus,* respectively. Although gasses produced during rumen fermentation are waste products and of no beneficial value to the ruminants but gas production tests are used routinely in feed research as gas volume are used relative to both the extent and rate of substrate degradation (Blummel and Becker, 1997) since gas produced during *in vitro* digestion of whole forage comes from both the soluble and the fibre fractions. The result also showed that the cumulative gas volume (Y) at 24, 48, 72 and 96 h after incubation differed significantly (P<0.05). The rates of gas production (c) were statistically similar (P>0.05) in all plants at 24 and 96 hours of incubation while at 48 and 72 hours of incubation, it differed significantly (P<0.05) with the lowest rate of gas production observed in *Sacciolepis africana and Acroceras zizanioides, respectively* indicating that residues were less readily available to the microbes present in the rumen. There are many factors that may determine the amount of gas produced during fermentation depending on the nature and level of fibre, the presence of secondary factors (Babayemi *et al.,* 2004) and potency of the rumen liquor. It is possible to attain potential gas production of a feedstuff if the donor animals from which rumen liquor for incubation was collected possessed the recommended nutrients requirement. Generally, gas production is a function and a reflection of the degradable carbohydrate and therefore the amount depends on the nature of the carbohydrate (Blummel and Beker, 1997).

Figure 1 shows the chart for the gas produced by the weeds for 96 hours of incubation and it could be deduced that gas production increased with increase in time and decreased after the 60th hour. Gas production test is used regularly in feed research as gas volumes are related to both the extent and rate of substrates degradation (Blummel and Becker, 1997). The low net gas production observed for all waterweeds

Table 1: Chemical composition (g/100g DM) of water weeds

	Waterweeds										
Parameter	**PL**	**NL**	**PSc**	**AZ**	**IA**	**PSu**	**SA**	**LH**	**HC**	**DA**	**SEM**
Dry Matter (%)	26.67	16.67	22.67	14.67	12.67	17.67	14.67	23.33	11.67	17.67	0.38
Crude Protein (%)	27.00	25.00	13.67	20.33	31.33	15.67	25.67	19.67	32.67	20.33	0.30
Crude Fibre (%)	12.00	9.33	25.33	26.00	4.00	13.33	20.00	22.00	12.00	11.33	0.21
Ether Extract (%)	11.33	9.33	8.00	8.00	11.33	8.00	10.00	9.33	10.00	10.00	0.21
Ash (%)	7.33	26.00	8.00	13.33	12.00	7.33	11.33	12.00	15.33	7.33	0.26

PL-*Polygonum lanigerum;* NL-*Nymphaea lotus*
PSc-*Paspalum scrobiculatum;* AZ-*Acroceras zizanioides*
IA-*Ipomea aquatic;* PSu-*Panicum subalbidum*
SA-*Sacciolepis Africana;* LH-*Leersia hexandra*
HC-*Heteranthera callifolia;* DA-*Dicksonia antartica*

Table 2: *In vitro* gas production characteristics of water weeds

	PL	NL	PSc	AZ	IA	PSu	SA	LH	HC	DA	SEM
24 hour incubation											
a	1.67^{b}	1.33^{b}	1.67^{b}	1.33^{b}	5.33^{a}	2.33^{b}	2.67^{ab}	1.67^{b}	2.00^{b}	1.67^{b}	0.54
b	11.33^{cbde}	8.67^{de}	9.33^{cde}	15.00^{abc}	16.00^{ab}	10.00^{cde}	7.00e	14.00^{abcd}	19.00^{a}	7.33^{e}	1.05
a+b	13.00^{cd}	10.00^{d}	11.00^{cd}	16.33^{bc}	22.00^{a}	12.33^{cd}	9.67^{d}	15.67^{c}	21.00^{ab}	9.00^{d}	0.97
c	0.052	0.036	0.052	0.054	0.055	0.059	0.045	0.057	0.048	0.065	0.009
t	7.00^{b}	12.00^{ab}	13.00^{ab}	14.00^{ab}	13.00^{ab}	16.00^{ab}	9.00^{ab}	10.00^{ab}	11.00^{ab}	11.00^{ab}	1.29
Y	5.00^{bc}	5.67^{bc}	6.00^{bc}	9.00^{bc}	14.00^{a}	8.33^{bc}	4.67^{c}	7.00^{bc}	9.33^{b}	5.33^{bc}	0.78
48 hour incubation											
a	2.33	2.67	3.33	1.33	4.00	3.00	2.00	2.00	4.33	2.00	0.66
b	17.67^{abcd}	16.00^{cd}	17.00^{bcd}	21.33^{abc}	22.00^{ab}	22.67^{a}	15.67^{d}	21.33^{abc}	20.00^{abcd}	10.67^{e}	0.94
a+b	20.00^{bcd}	18.67^{cd}	20.33^{bc}	22.67^{abc}	26.00^{a}	25.67^{a}	17.67^{d}	23.33^{ab}	24.33^{ab}	12.67^{e}	0.79
c	0.044^{ab}	0.044^{ab}	0.020^{bc}	0.041^{abc}	0.043^{abc}	0.028^{abc}	0.016^{c}	0.032^{abc}	0.043^{abc}	0.050^{a}	0.047
t	10.00	11.00	13.00	13..00	13.00	13.00	15.00	17.00	14.00	15.00	1.75
Y	8.00^{bc}	8.67^{bc}	7.33^{bc}	9.33^{abc}	13.33^{a}	9.67^{abc}	5.33^{c}	10.67^{ab}	13.33^{a}	6.67^{bc}	0.78
72 hour incubation											
a	2.00^{ab}	1.67^{b}	2.00^{ab}	3.33^{a}	1.33^{b}	1.33^{b}	1.00^{b}	1.67^{b}	2.33^{ab}	2.33^{ab}	0.27
b	19.33^{ab}	14.33^{b}	20.67^{ab}	25.67^{a}	25.33^{a}	20.67^{ab}	21.67^{ab}	23.33^{ab}	25.00^{a}	25.00^{a}	1.65
a+b	21.33^{ab}	16.00^{ab}	22.67^{b}	29.00^{a}	26.67^{a}	22.00^{ab}	22.67^{ab}	25.00^{b}	27.33^{a}	27.33^{a}	1.65
c	0.037^{ab}	0.037^{ab}	0.043^{ab}	0.024^{b}	0.033^{ab}	0.031^{ab}	0.032^{ab}	0.056^{a}	0.036^{ab}	0.037^{ab}	0.005
t	16.00^{ab}	22.00^{ab}	23.00^{ab}	19.00^{abc}	19.00^{abc}	22.00^{abc}	22.00^{abc}	28.00^{a}	13.00^{bc}	10.00^{c}	2.18
Y	10.67^{ab}	9.00^{b}	13.33^{ab}	13.33^{ab}	11.00^{ab}	12.33^{ab}	10.67^{ab}	19.33^{a}	11.67^{b}	10.00^{b}	1.73
96 hour incubation											
a	3.00^{ab}	3.00^{ab}	2.67^{ab}	2.33^{ab}	5.33^{a}	2.33^{ab}	2.33^{ab}	1.33^{c}	3.67^{ab}	2.67^{ab}	0.64
b	20.33^{ab}	20.00^{ab}	23.67^{ab}	27.33^{a}	22.67^{ab}	24.67^{a}	26.00^{a}	25.67^{a}	26.67^{a}	13.33^{b}	1.97
a+b	23.33^{ab}	20.00^{ab}	26.33^{ab}	29.67^{a}	28.00^{a}	28.00^{a}	28.33^{a}	26.00^{ab}	30.33^{a}	16.00^{b}	1.87
c	0.034	0.051	0.029	0.066	0.041	0.026	0.021	0.024	0.054	0.055	0.009
t	9.00^{b}	11.00^{b}	11.00^{b}	11.00^{b}	11.00^{b}	21.00^{a}	12.00^{b}	11.00^{b}	12.00^{b}	13.00^{b}	1.26
Y	8.33^{cbd}	11.00^{abcd}	8.33^{bcd}	10.67^{bcd}	13.67^{ab}	13.33^{abc}	7.33^{d}	8.00^{cd}	16.33^{a}	9.33^{bcd}	0.97

$^{a,b,c,d,e;}$ means on the same row with different superscripts are significantly different (P<0.005).

PL-*Polygonum lanigerum,* NL-*Nymphaea lotus,* PSc-*Paspalum scrobiculatum,* AZ-*Acroceras zizanioides* IA-*Ipomea aquatica,* PSu-*Panicum subalbidum,* SA-*Sacciolepis africana,* LH-*Leersia hexandra,* HC-*Heteranthera callifolia,* DA-*Dicksonia antartica.*

Y = volume of gas produced at 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

may be due to their low crude protein content which varied from 13.67 (*Paspalum scrobiculatum*) to 32.67 (*Heteranthera callifolia*). This was also affirmed by the report of Akinfemi *et al.* (2009) that gas production from protein fermentation is relatively small when compared to carbohydrate fermentation with no insignificant input of fat to gas production.

Results on metabolizable energy (ME), organic matter digestibility (OMD) and short chain fatty acid (SCFA) of the waterweeds is presented in Table 2. The values for ME, OMD and SCFA ranged from 4.53 (MJ/kg DM) in *Paspalum* to 6.96 (MJ/kg DM) in *Ipomea,* 35.87 % also in *Paspalum* to 57.79 % in *Heteranthera callifolia* and 0.28 µmol in *Dicksonia* to 0.59 µmol in *Ipomea,* respectively from the 24 hour incubation. *Dicksonia antartica* had the lowest values for the ME, OMD and SCFA from the 48, 72 and 96 hours incubation while the highest values were found to be in *Ipomea aquatic and Heteranthera callifolia,* respectively. There were significant ($P<0.05$) differences among the waterweeds for ME, OMD, and SCFA at 24, 48, 72 and 96 hours of incubation. The least SCFA estimated for *Dicksonia antartica* is due to a lower gas production which suffice at 24 hour incubation since gas produced is closely related to SCFA base on carbohydrate fermentation (Blummel and Orskov 1994) and supported by Getachew et al. (2002) who also reported a synergy between SCFA and gas production *in vitro.* Though gas production is nutritionally wasteful Product but has direct influence and prediction of on

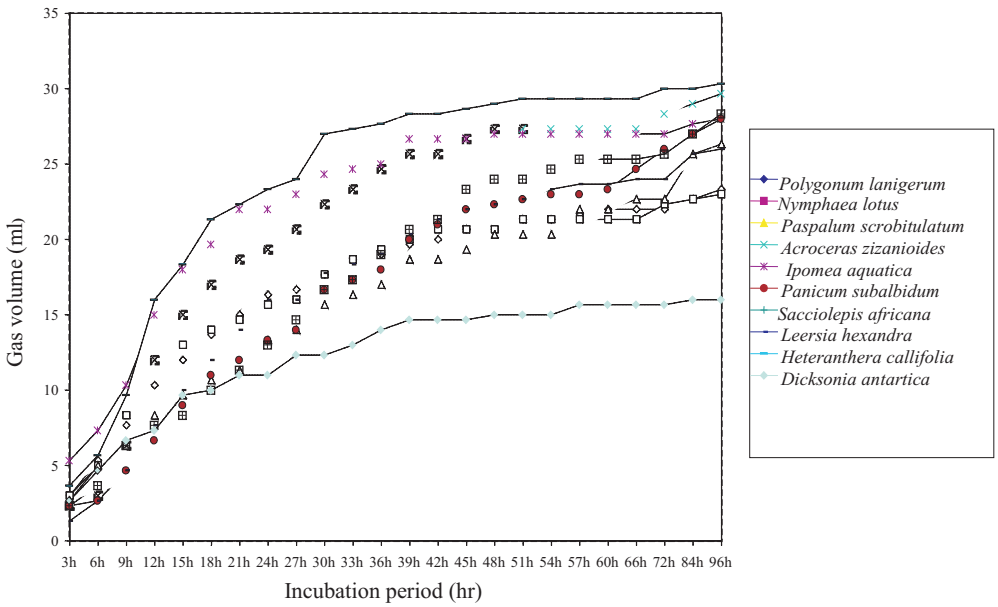

Figure 1: *In vitro* gas production of water weeds incubated for 96 hours.

ME, OMD and SCFA (Mauricio *et. al.,* 1999; Babayemi, 2006). *Heteranthera callifolia* had the highest estimated SCFA though statistically comparable with *Sacciolepis Africana, Panicum subalbidum, Ipomea aquatic and Acroceras zizanioides* suggesting their potential as source to make accessible energy to ruminants. The values obtained in the present study were higher than those reported for agro-industries (Aregheore and Abdulrazak, 2005), *Tephrosia candida and* guinea grass mixtures (Babayemi and Bamikole, 2006a) and spent tea leaf (Babayemi et al., 2006). Feedstuffs that are inherent with certain anti-nutritive factor had been reported to be low in Metabolisable energy and organic matter digestibility (Aregheore and Abdulrazak, 2005).

The methane (mL/200mg DM) production (fig. 2) ranged from 10.00 (mL/200mg DM) to 22.50 (mL/200mg DM) among the water weeds. The enteric fermentation and subsequent production of methane is influenced by the level of intake, type and quality of feed material and environmental temperature. The least and the highest methane production was obtained in *Acroceras zizanioides* and *Ipomea aquatic,* respectively. The higher methane production in *Ipomea aquatic* maybe a resultant effect of the higher gas produced at 24 and 48 hours incubation. In most cases, feedstuffs that show high capacity for gas production are also observed to be synonymous for high methane production (Babayemi, 2007). Methane production indicates an energy loss to the ruminant and many tropical feedstuffs have been implicated to increase methanogenesis (Babayemi *et al.*, 2004; Babayemi and Bamikole, 2006a) as an integrated part of carbohydrates metabolism (Demeyer and Van Nevel, 1975).

Conclusion

*T*he nutritive value depicted in the in vitro gas production showed lower volume of gas production which is a reflection of low fermentable substrate in the plant materials. The in vitro digestibility is very useful tool in the estimation of OMD, SCFA and ME of feed material therefore the result of present

Table 3: Metabolizable energy (ME) (MJ/kg DM), organic matter digestibility (OMD) (%) and short chain fatty acids (SCFA) (µmol) of some water weeds.

	PL	NL	PSc	AZ	IA	PSu	SA	LH	HC	DA	SEM
24 hour incubation											
ME	5.52^{b}	5.00^{bcd}	4.53^{d}	5.63^{b}	6.96^{a}	4.78^{cd}	5.02^{bcd}	5.48^{bc}	6.92^{a}	4.59^{d}	0.13
OMD	43.01^{c}	51.82^{b}	35.87^{d}	46.82^{c}	56.13^{ab}	37.19^{d}	42.06^{c}	45.18^{c}	57.79^{a}	36.40^{d}	0.87
SCFA	0.37^{cd}	0.30^{d}	0.32^{cd}	0.45^{bc}	0.59^{a}	0.35^{cd}	0.29^{d}	0.43^{c}	0.56^{ab}	0.28^{d}	0.02
48 hour incubation											
ME	6.48^{b}	6.17^{bc}	5.80^{c}	6.49^{b}	7.51^{a}	6.59^{b}	6.11^{bc}	6.52^{b}	7.38^{a}	5.09^{d}	0.11
OMD	49.23^{b}	59.53^{a}	44.17^{c}	52.45^{b}	59.68^{a}	49.05^{b}	59.17^{b}	52.00^{b}	60.74^{a}	39.66^{d}	0.71
SCFA	0.54^{bcd}	0.51^{cd}	0.55^{bcd}	0.60^{abc}	0.68^{a}	0.67^{a}	0.48^{d}	0.62^{ab}	0.64^{ab}	0.36^{e}	0.02
72 hour incubation											
ME	6.66^{abc}	5.81^{c}	6.12^{bc}	7.35^{ab}	7.60^{a}	6.09^{bc}	6.79^{abc}	6.75^{abc}	7.79^{a}	7.08^{abc}	0.22
OMD	50.41^{cd}	57.15^{abc}	46.24^{d}	58.08^{abc}	60.28^{ab}	45.79^{d}	53.61^{bcd}	53.48^{bcd}	63.42^{a}	52.70^{bcd}	1.47
SCFA	0.57^{ab}	0.44^{b}	0.60^{ab}	0.75^{a}	0.70^{a}	0.59^{ab}	0.60^{ab}	0.66^{ab}	0.71^{a}	0.71^{a}	0.04
96 hour incubation											
ME	6.93^{abc}	6.76^{abc}	6.61^{cb}	7.44^{ab}	7.78^{ab}	6.91^{abc}	7.56^{ab}	6.88^{abc}	8.19^{a}	5.54^{c}	0.25
OMD	52.19^{cd}	63.38^{ab}	59.50^{de}	58.67^{abcd}	61.46^{abc}	51.12^{de}	58.65^{abcd}	54.37^{bcd}	66.08^{a}	42.62^{e}	1.66
SCFA	0.62^{ab}	0.61^{ab}	0.69^{ab}	0.77^{a}	0.73^{a}	0.73^{a}	0.74^{a}	0.68^{ab}	0.79^{a}	0.44^{b}	0.04

^{a,b,c,d,e} : Means on the same row with different superscripts are significantly different (P<0.005).

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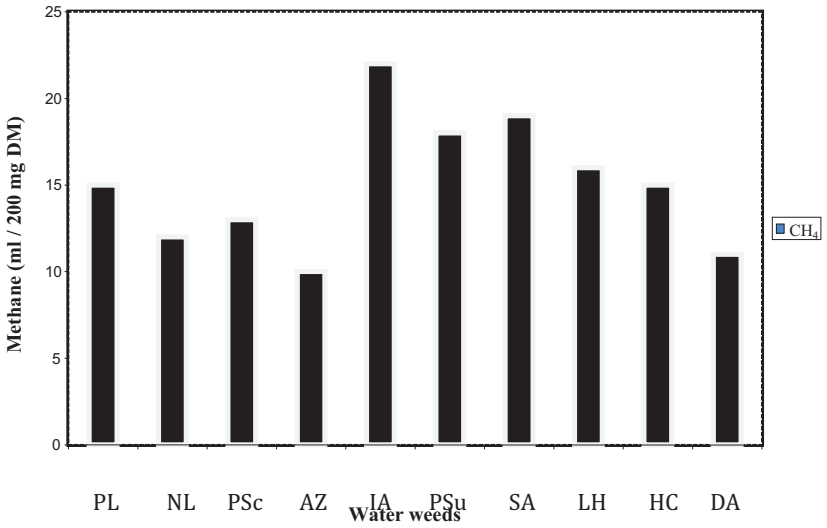

Figure 2: Methane production of water weeds after 96h incubation.
PL-*Polygonum lanigerum,* NL-*Nymphaea lotus,* PSc-*Paspalum scrobiculatum,* AZ-*Acroceras zizanioides,* IA-*Ipomea aquatica,* PSu-*Panicum subalbidum,* SA-*Sacciolepis africana,* LH-*Leersia hexandra,* HC-*Heteranthera callifolia,* DA-*Dicksonia antartica*

study revealed that these plants possess the potentials of being included in the diet of ruminant animals. Additional investigation into cyclical variation and age as it affects chemical components of plants with its attendant influence on gas production should be studied.

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