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Nutritive Value Assessment of the Alternative Feed Resources by Gas Production and Rumen Fermentation *In vitro*

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Abstract: *In vitro* gas production and rumen fermentation characteristics were measured to evaluate some alternative feed resources e.g. rice straw, tropical shrubs (Artiplex semibacata, Artiplex halimus, Leucaena), Artichoke leaves and Artichoke stems in comparison with berseem hay. A gas test technique was performed using fistulated sheep rumen fluid. Cumulative gas production was recorded at 3, 6, 9, 12, 24, 48, 72 and 96 h of incubation and gas production constants (*b, c, L*) were described using the equation Gas(t)= $b(1\text{-exp}^{-c(t-L)})$. Ammonia-N and volatile fatty acids (VFA) concentration were determined at 0,1,3 and 6 hr. of incubation. The NDF, ADF and ADL were significantly different among roughages used in this study. The rice straw contain highest level of NDF and ADF, while Leucaena contain the highest level of ADL. The lowest fiber constitutes were observed in artichoke leaves or stems. The maximum gas volume (*b*) was highest for Artichoke stems followed by artichoke leaves, bereem hay, rice straw, Atriplex semibacata and Leucaena and lowest for Atriplex halimus. The maximum rate of gas production (*c*) was highest for both artichoke stems and leaves and berseem hay and lowest for rice straw. The fermentation end products either ammonia-N or total volatile fatty acids varied significantly among the roughages. There were significant ($P<0.05$) differences between forages in terms of metabolizable energy (ME), net energy (NE) (MJ/kg DM) and organic matter digestibility (OMD). The organic matter digestibility values ranged from 41.48-67.57% and the highest value was that of artichoke stems and the lowest value was that of rice straw. The leaves or stems of artichoke showed the highest ($P<0.05$) values for ME, NE and OMD than all of the other roughages used in this study. All tropical shrub plants and rice straw had lower *in vitro* fermentation characteristics, cumulative gas production and kinetics parameters than berseem hay used as standard hay. The *in vitro* gas production technique can be used to determine the nutritive value of the roughages and to identify differences among their potential digestibility and energy contents. This study concluded that artichoke was considered as high quality roughage in comparison with berseem hay according to nutritive quality and gas production parameters.

Key word: Nutritive value, roughages, gas production, fermentation, *in vitro*

INTRODUCTION

The appellation; Alternative feed resources; has become commonly used to design those local feeds, which could replace partially or totally conventional feedstuffs either grass forages or concentrate feeds without reducing livestock performance but should decrease the feeding cost.

Tropical plants and agricultural by products represent an important fodder reserve for livestocks in harsh conditions that can be used by grazing ruminant in periods of feed scarcity. Leucaena leucocephala is a leguminous plant and it has high content of protein, energy, carotens and minerals[1]. Atriplex species contain a moderate level of crude protein, high level of fiber fractions and ash. These forages should be salt tolerant or / and drought resistant to be adaptable for propagation under arid conditions and can considerably provide low-cost fodder for animals[18]. Use of these forages in animal diets could participate in reducing the shortage of animal feed resources and subsequently increase milk and meat production in Egypt. The knowledge about their potential feeding value is insufficient. The nutrient composition of feeds is commonly determined primarily by chemical analyses. However, this does not provide sufficient information to determine the feed's true nutritive value. The efficiency by which an animal utilizes feed nutrients has a significant impact on its productive performance and waste production. In the search to improve and optimize this "trophic chain", it is therefore crucial to further develop methods to evaluate fibrous feeds, so that ruminant production can be maximized. Therefore, *in vitro* methods of feed evaluation have numerous advantages over *in vivo* methods. They are less expensive, less time-consuming and allow incubation to be maintained more precisely than *in vivo*. In addition, *in vitro* techniques

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Table 1: Chemical composition and fiber fractionations of berseem hay, rice straw, Atriplex semibacata, Artiplex halimus, Leucaena, Artichoke leaves and Artichoke stems

Item	Hay	Rice straw	Artiplex semibacata	Artiplex halimus	Leucaena	Artichoke leaves	Artichoke stems
Ash	11.2	17.75	0.4321	23.2	8.59	7.91	11.71
Organic matter	88.8	82.25	78.67	76.8	91.41	92.09	88.29
Crude protein	15.32	4.85	14.28	12.07	19.93	16.62	14.34
Crude fiber	29.82	36.51	15.32	18.02	11.06	22.09	16.5
Ether extract	1.03	0.47	1.28	1.9	0.97	0.95	1.35
Nitrogen free extract	42.63	40.42	47.69	44.81	59.45	52.43	56.1
Fiber fractionation (%)							
NDF	54.92	77.5	53.91	44.07	42.21	43.88	33.28
ADF	38.31	47.48	20.58	20.7	21.63	28.57	21.32
ADL	12.27	12.75	9.05	8.33	15.74	8.94	6.21
Cellulose	26.04	34.73	11.53	12.37	5.89	19.63	15.11
Hemicellulose	16.61	30.02	33.33	23.37	20.58	15.31	11.96

utilize small amounts of test feeds making them applicable to screening feeds that are not available in sufficient quantity for *in vivo* experiments. The *in vitro* methods of Tilley and Terry[41], in sacco method of Mehrez and Ørskov[30], and enzymatic method of Jones and Hayward[25] have all been widely used to predict digestibility of feeds, and used as a selection tool for screening feeds for nutritional quality.

The *in vitro* gas production system helps to better quantify nutrient utilization, and its accuracy in describing digestibility in animals has been validated in numerous experiments. Animal experiments will continue to add information to our understanding of nutrient metabolism. However, where applicable the *in vitro* gas production system can be used to predict animal performance at a much lower cost. Based on the strong relationship between measured digestibility and that predicted from gas production, regression equations have been developed and the method has been standardized.

The association between rumen fermentation and gas production has long been known, in an early attempt in 1939 was tried to record the gas production directly via the rumen cannula in sheep, the technique was too difficult to execute and of poor reproducibility[9]. In 1974 Menke and Ehrensvärd (cited by Theodorou *et al*.[40]) studying the stoichiometry of rumen fermentation with a syringe closed system noticed a very reproducible gas production data when the same substrate was incubated in the same quantity in different runs. The main innovation in such method was that the gas production is recorded rather than degradation. The same postulate continues until today, even when the method had been simplified and improved. The kinetics of gas production during ruminal fermentation may provide valuable information about feeds that can be used to formulate rations and model animal responses.

Menke and Steingass[31] reported a strong correlation between metabolizable energy (ME) values measured *in vivo* and predicted from 24 h *in vitro* gas production and chemical composition of feeds. The *in vitro* gas production method has also been widely used to evaluate the energy value of several classes of feeds[15], particularly straws[28], agro-industrial by-products[21], compound feeds[2] and various tropical feeds[24]. The main objective of this study is assessing the nutritive value of tropical shrubs (Artiplex semibacata, Artiplex halimus, Leucaena leucocephala), Artichoke leaves, Artichoke stems, rice straw in comparison with berseem hay by chemical analysis, rumen fermentation characteristics and gas production technique *in vitro*.

MATERIALS AND METHODS

Feedstuffs: Rice straw, Artiplex semibacata, Artiplex halimus, Leucaena leucocephala, Artichoke leaves, Artichoke stems (*Cynara scolymus*) and berseem hay were ground in mills to pass a 1 mm sieve prior to chemical analyses, *in vitro* gas production measurements and rumen fermentation. The chemical composition of the roughages used for *in vitro* gas production and rumen fermentation are presented in Table 1. Only one sample of each feed was utilized, as feeds were selected to represent a range in fermentability, rather than to categorize the feeds

Chemical Analyses: All feedstuff samples were analyzed according to AOAC,[4] for crude protein (CP), Crude fat (CF), ash, Nitrogen free extract (NFE), organic matter (OM), crude fiber. Neutral detergent fiber(NDF), acid detergent fiber (ADF), acid detergent lignin (ADL), cellulose and hemicellulose were determined according to Van Soest *et al*[43].

***in vitro* gas production and rumen fermentation:** *in vitro* gas production was undertaken according to the procedure described by Menke and Steingass[31]. Buffer and mineral solution are prepared and placed in a water bath at 39°C under continuous flushing with CO_2. Rumen fluid is collected from fistulated sheep fed on berseem hay and commercial concentrate mixture diet into a pre-warmed thermos flask. The rumen fluid is filtered and flushed with CO_2, and the mixed and CO_2 flushed rumen fluid is added to the buffered mineral solution (1:2 v/v), which is maintained in a water bath at 39°C, and combined.

Samples (200 ± 10mg) of the air-dry feedstuffs were accurately weighted into syringe fitted with plungers. Buffered rumen fluid (30 ml) is pipetted into each syringe, containing the feed samples, and the syringes are immediately placed into the water bath at 39°C[6]. Three syringes with only buffered rumen fluid are incubated and considered as the blank. The syringes are gently shaken every 2 h, and the incubation terminated after recording the 96 h gas volume. The gas production was recorded after 3, 6, 9, 12, 24, 48, 72, and 96h of incubation. Total gas values are corrected for the blank incubation, and reported gas values are expressed per 200mg of DM. Another two bottles containing 300mg feedstuffs samples and 45ml Buffered rumen fluid were incubated for the determination volatile fatty acids (VFA) and ammonia concentration at 0, 1, 3 and 6h from incubation. Ammonia and VFA concentration were determined according to Gips and Wibbens-Alberts[16] and Warner[46] respectively.

Cumulative gas was expressed as milliliter of gas produced per 200mg of dry matter and corrected for blanks. Berseem hay was used as standard hay. Cumulative gas production GAS(t) at time t was fitted to the modified exponential model[11]

$Gas(t) = b(1-\exp^{-c(t-L)})$, with the exclusion of the intercept.

Where
b = The gas production from the insoluble fraction (ml)
c = The gas production rate constant for the insoluble fraction (b)
t = Incubation time(h)
L = Lag time (h)

Energy estimation: The energy value of forages can be calculated from the amount of gas produced at 24 h of incubation with supplementary analyses of crude protein, ash and crude fat. This approach was developed by the research group in Hohenheim (Germany) and is based upon extensive *in vitro* incubation of forages[32], Menke and Steingass[31].

$$ME(MJ/Kg\ DM) = 2.2 + 0.136Gp + 0.057CP$$

$$OMD(\%) = 14.88 + 0.889\ Gp + 0.45CP + 0.0651\ XA$$

Where
ME is the metabolizable energy
OMD is organic matter digestibility
Gp is 24 h net gas production (ml/200DM)
CP is crude protein (% of DM)
XA is ash (%of DM)

$$NE(Mcal/lb) = (2.2 + (0.0272*Gas) + (0.057*CP) + (0.149*CF))/14.64$$

Where
Gas is 24 h net gas production (ml/gDM)
CP is crude protein (%of DM)
CF id crude fat (%of DM)

Then net energy unit converted to be MJ /Kg DM.

Statistical analyses: Data were subjected to analysis of variance (ANOVA) using the General Linear Model. Significant differences between individual means were identified using least significance difference (LSD) multiple range test[36].

RESULTS AND DISCUSSION

Chemical composition and gas production: The chemical composition and fiber fractionations of rice straw, Atriplex semibacata, Atriplex halimus, Leucaena, Artichoke leaves, Artichoke stems and berseem hay are presented in Table 1. Forages had variable organic matter content with lowest in A. halimus and semibacata and highest in artichoke and Luecaena. The crude protein content varied from 4.85% in rice straw to 19.93 % in Leucaena. The ether extract content of forages was lowest in rice straw and it does not differ significantly among other forages. The fiber fractions differ significantly among forages. The highest value for NDF, ADF, ADL, cellulose and hemicellulose were observed in rice straw and the lowest values in artichoke stems. Leucaena had a higher ADL content and lower cellulose content than other roughages. Artichoke either leaves or stems had a lower ADL content.

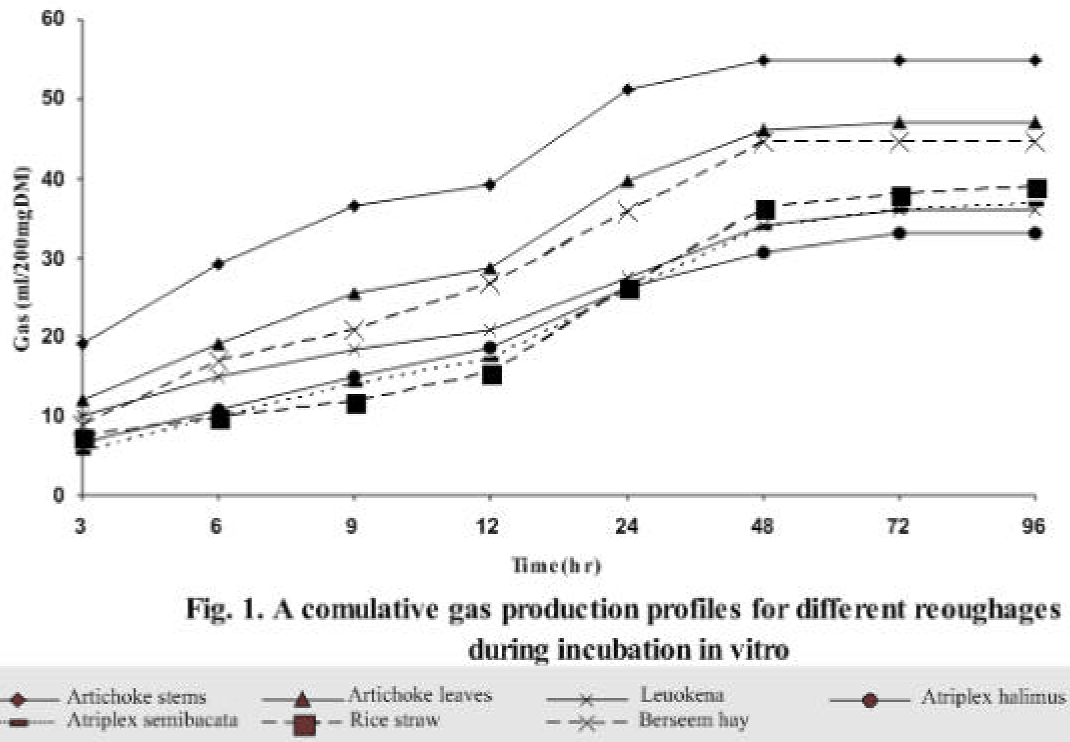

Fig. 1. A comulative gas production profiles for different reoughages during incubation in vitro

Figure 1 showed the cumulative gas production profiles, corrected for blank fermentation, of the seven different roughage samples that were incubated in buffered rumen fluid from sheep fed the ration with berseem hay and concentrate. There was a fast initial gas production, without lag time, after which gas production diminished little but stayed at a high level. After 15 to 24 h of incubation, gas production decreased further but did not stop fully. Incubation of feedstuff with buffered rumen fluid *in vitro*, the carbohydrates are fermented to short chain fatty acids (SCFA) , gases mainly CO_2 and CH_4 and microbial cells. Gas production is basically the result of fermentation of carbohydrates to acetate, propionate and butyrate[47,5,8]. Gas production from protein fermentation is relatively small as compared to carbohydrate fermentation while, contribution of fat to gas production is negligible[47].

The fast initial gas production was highly significant ($P<0.01$) in Artichoke stems in comparison with other roughages. The highest cumulative gas production was observed in artichoke either stems or leaves which, was followed by sample of berseem hay and the lowest was obtained by the sample from A.halimus. This is due to high content of the artichoke from NFE which, have a positive correlation with gas production. On the other hand, Cell wall content (NDF and ADF) were negatively correlated with gas production at all incubation times and estimated parameters. This may tend to reduce the microbial activity through increasing the adverse environmental conditions as incubation time progress. Also, The relatively high level of ADL in rice straw and Leucaena as shown in Table 1 explains in part the limited *in vitro* degradation and therefore the lower amount of gas produced.

Gas production can be regarded as an indicator of carbohydrate degradation and the low gas production in Atriplex are explained by condensed tannin's binding to the carbohydrate and then by the inhibition of enzymes or microorganisms[17], complexing with lignocellulose, and preventing the microbial digestion. Tropical feeds contain considerable amounts of phenolic compounds that reduced *in vitro* gas production. The correlation between

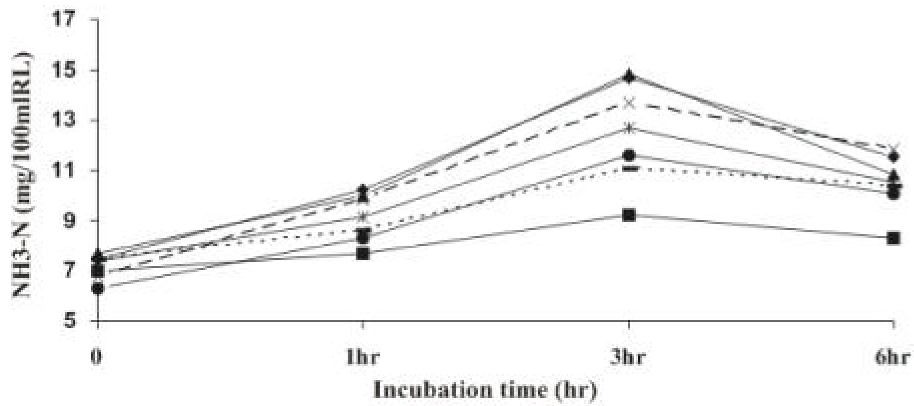

Fig. 2. Ammonia-N concentration for some alternative feed resources at different times of incubation *in vitro*

the change in gas production in the presence of tannin binding agent and phenolic contents of browses was consistent with those of Khazaal *et al*[22] and Tolera *et al*[42]. However, since gas production on incubation of feeds in buffered rumen fluid is associated with feed fermentation, the low gas production from rice straw, Artiplex semibacata, Artiplex halimus, Leucaena, could be related to low feeding value of these feeds. In addition, Leucaena contains toxin compounds eg. mimosine which, has a negative correlation with gas production. The variation in the *in vitro* gas production on incubation of these roughages were explained by the differences in total VFA production as observed in Fig. 3, which mainly comes from carbohydrate fermentation. These results are consistent with the finding of Kamalak *et al*[20] and Abdulrazak *et al*[3].

Values for the estimated parameters obtained from the kinetics exponential model and cumulative gas production at 24 and 48 hr (GAS24 and GAS48) are given in Table2. GAS 24 and GAS 48 ranged from 25.7to 51.15 and from 30.59 to 54.8 ml/200mg DM, respectively. Estimated lag varied from –3.26h in Leucaena to 0.32h in A.semibacata. Estimated constant rate of gas production (c) differ significantly between roughages. The highest rate of gas production was observed with artichoke stems followed by artichoke leaves and berseem hay. The lowest rate was found with A.halimus which, did not differ significantly from rice straw, A.semibacata and Leucaena. There are similarity between observed gas production and predicted gas production by exponential model (data not shown). The gas yield of artichoke stems is higher than artichoke leaves. There is no significant difference between rice straw, A.halimus, A.semibacata and Leucaena in cumulative gas production.

The lag time is (time from incubation to start of gas production) very important digestibility parameters. The shorter lag time period for Leucaena could be due to high whole structure carbohydrates content. Since the utilization of roughages is largely dependent upon microbial degradation within the rumen, description of roughages in terms of their degradation characteristics would provide a useful basis for their evaluation (anonymous). Kinetics of fermentation of feedstuffs can be determined from fermentative gas and the gas released from buffering of the SCFAs. Kinetics of gas production is dependent on the relative proportions of soluble, insoluble particles of the feed. Mathematical descriptions of gas production profiles allows analysis of data, evaluation of substrate and media-related differences, and fermentability of soluble and slowly fermentable components of feeds. Various models have been used to describe gas production profiles. The exponential model[33] is widely used in ruminant feed evaluation to describe degradation kinetics as measured with the nylon bag

Table 2: Accumulative gas production (Gas24h, Gas 48h) and estimated kinetic parameters for variety of roughages incubated with rumen fluid *in vitro*.

	Gas 24h (ml/200mgDM)	Gas48h (ml/200mgDM)	b (ml/200mgDM)	c(percentage of b per hour)	Lag(h)
Hay	35.69	44.58	45.04[bc]	0.070[c]	-0.34[bc]
Rice straw	26.16	36.13	40.43[ab]	0.040[a]	-0.89[bc]
A.semibacata	25.71	33.89	37.01[ab]	0.052[ab]	0.32[c]
A.halimus	26.31	30.59	33.04[a]	0.065[bc]	-0.36[c]
Leucaena	27.34	34.12	35.10[ab]	0.050[ab]	-3.26[a]
Artichokeleaves	39.77	46.07	47.07[bc]	0.075[c]	-0.96[bc]
Artichoke stems	51.15	54.81	54.97[c]	0.101[d]	-1.34[b]

a, b and c means within same column with differing superscript are significantly different (P<0.05).

Table 3: Metabolizable energy (ME MJ/KgDM), net energy (NE MJ/KgDM) and organic matter digestibility (OMD) prediction for different roughages *in vitro*.

	ME MJ/KgDM	NE MJ/kgDM	OMD %
Hay	7.94[ab]	5.19[b]	54.34[b]
Rice straw	6.03[a]	3.92[a]	41.48[a]
Artiplex semibacata	6.50[a]	4.30[a]	45.57[a]
Artiplex halimus	6.47[a]	4.34[a]	45.20[a]
Leucaena	6.78[a]	4.45[a]	46.89[a]
Artichoke leaves	8.56b[c]	5.59[b]	58.24[bc]
Artichoke stems	9.99[c]	6.54[c]	67.57[c]

a, b and c means within same column with differing superscript are significantly different (P<0.05).

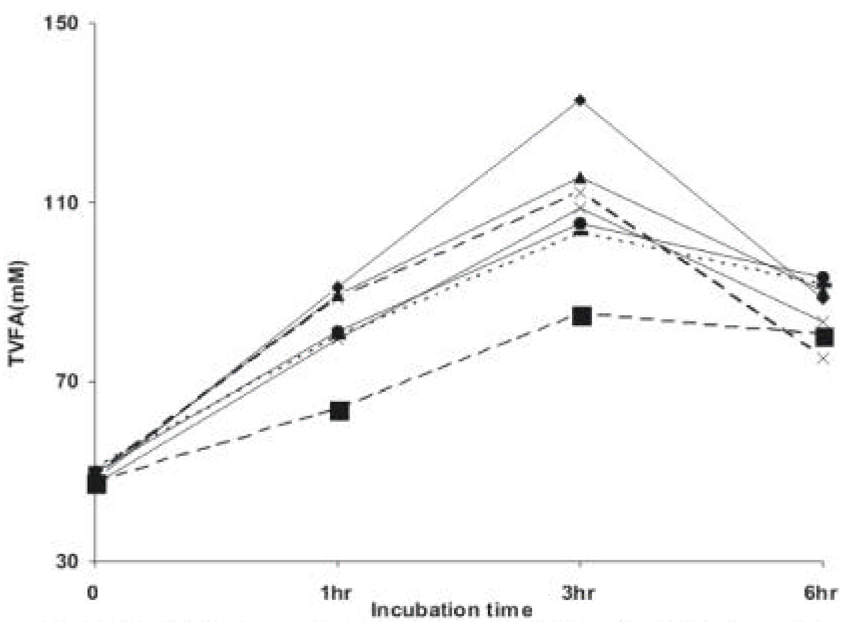

Fig.3. Total Volatile fatty acids concentration (mM) at 0, 1, 3 1nd 6 hr incubation for different alternative feed resources *in vitro*

technique, but the model has also been used to describe kinetics of gas production data as describing by Blummel *et al*[7], Siaw *et al*[37] and Khazaal *et al*[23].

Rumen fermentation: NH_3-N concentration (mg/100ml Rumen fluid) profile at 0, 1, 3 and 6 hr of incubation *in vitro* for rice straw, A. semibacata, A. halimus, Leucaena, Artichoke leaves, Artichoke stem and berseem hay are presented in Table 3. The highest NH_3-N concentration was observed after 3hr of incubation particularly in artichoke and berseem hay. The lowest NH_3-N concentration was observed in rice straw and significantly ($P<0.05$) different from all of the other roughages. There is no significant ($P<0.05$) difference between artichoke either leaves or stems and berseem hay in NH_3-N concentration. While, the ammonia concentration in artichoke leaves or stems and berseem hay is significantly ($P<0.05$) higher than rice straw, A. semibacata, A. halimus or Leucaena. This may be due to high N solubility in berseem hay and artichoke. The profile of NH_3-N concentration is similar and there is no significant difference ($P<0.05$) among A. semibacata, A. halimus or Leucaena. The reduction of NH_3-N concentration in Atriplex species may be attributed to anti-nutritional factors like condensed tannins which form a complex compound with protein which adversely affect N digestion[34].

Total volatile fatty acids (TVFA, mM) concentration profile at 0, 1, 3 and 6 hr of incubation *in vitro* for rice straw, A. semibacata, A. halimus, Leucaena, Artichoke leaves, Artichoke stem and berseem hay are presented in Figure 2. VFA produced varied significantly ($P<0.05$) between rice straw and all of other roughages. There is no significant difference in VFA production among A. semibacata, A. halimus or Leucaena but the VFA concentration in Artichoke leaves or stems and berseem hay was significantly higher ($P<0.05$) in comparison with A. semibacata, A. halimus or Leucaena. The variation in VFA production among the roughages used in this study may be due to the differences in amount, nature and fermentability of carbohydrates available to microorganisms. Doane *et al*[12] and Kamalak *et al*[19] found a significant correlation between SCFA and gas production.

Energy contents and organic matter digestibility: The predicted metabolizable energy (ME, MJ/kgDM), net energy(NE, MJ/kgDM) and organic matter digestibility (OMD) from gas production for rice straw, A. semibacata, A. halimus, Leucaena, Artichoke leaves, Artichoke stems and berseem hay are presented in Table 3. The predicted ME and NE profile are similar in seven forages and varied widely being particularly high in artichoke stems, leaves and berseem hay. While the other four forages had significantly lower values of ME and NE. The data showed that there was no significant difference among rice straw, A. semibacata, A. halimus, Leucaena, or berseem hay. The artichoke stems and leaves had the higher content of ME and NE in comparison with breseem.. There was a positive correlation between metabolizable energy calculated from *in vitro* gas production together with CP and fat content with metabolizable energy value of conventional feeds measured *in vivo*[31].

The organic matter digestibility (OMD)% was higher ($P<0.05$) in artichoke stems and leaves and lowest in rice straw. The OMD differs significantly among artichoke leaves and stems and berseem hay in comparison with rice straw, A.halimus, A.semibacata, and Leucaena. The OMD of artichoke stems is significantly higher than berseem hay but it was not significant with artichoke leaves. There are no significant differences between berseem hay, rice straw, A.halimus, A.semibacata, or Leucaena in OMD. However, the tropical feeds contained considerable amounts of secondary plant metabolites, particularly tannins which, is associated with reduction in OMD[38,45,14]. Using the *in vitro* gas measurement and chemical composition in multiple regression equation, Menke *et al*[32], McLeod and Minson[29] and Van Soest[44] found a high precision in prediction of *in vivo* OMD. This group further used a correlative approach to predict the ME content of feed by *in vitro* gas volume measurements and chemical constituents and concluded that the prediction of ME is more accurate when based on gas and chemical constituents measurements as compared to calculations based on chemical constituents only. These studies have been reviewed by Steingass and Menke[39] and Menke and Steingass[31]. Other workers Chenost *et al*[10], Fernandez-Rivera[13], Macheboeuf *et al*[27] and Romney *et al*[35] have also reported significant correlation between *in vitro* gas measurement and *in vivo* digestibility. Inclusion of crude protein content to gas volume improved precision of prediction of *in vivo* OMD Chenost *et al*[10], Macheboeuf and Jestin[26], Macheboeuf *et al*[27]. There was a positive correlation between ME calculated from *in vitro* gas production together with CP and fat content with ME value of conventional feeds measured *in vivo*[31].

In conclusion, there are considerable differences in the fermentability of carbohydrates between different roughages. This study suggested that artichoke have a potential fermentation efficiency better than berseem hay and therefore, artichoke could be incorporated in feed mixtures to replace conventional roughage sources (e.g. berseem hay, silage) in ruminant diets. In addition, due to the high digestibility of artichoke, it can be incorporated into the diet without major problem.

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