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Nutritive Value Assessment of Ensiling or Mixing Acacia and Atriplex Using *In Vitro* Gas Production Technique

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Abstract: An *in vitro* gas production technique was used in the current study to evaluate berseem (*Trifolum alexandrinum*) hay (Hay), fresh acacia (*Acacia saligna,* F. Acacia), acacia silage (Acacia S), fresh atriplex (*Atriplex halimus,* F. Atriplex), atriplex silage (Atriplex S), mixture of fresh acacia and atriplex (1:1, FAA) and mixture of silages of acacia and atriplex (1:1, AAS). A cumulative gas production, kinetics of gas production, metabolizable energy (ME), net energy (NE), organic matter digestibility (OMD), microbial protein (MP), ammonia nitrogen (NH_3-N) and volatile fatty acids (VFA) concentrations were determined. A gas production was continuously measured by incubating samples in buffered rumen fluid from cannulated sheep for 96 h. Cumulative gas production was recorded at 3, 6, 9, 12, 24, 48, 72 and 96 h of incubation periods and kinetics of gas production was described. The crude protein (CP) was ranged on average from 126 to 144.5 g/kg DM. The highest value of CP was recorded with Hay and the lowest value was recorded with Acacia S. The highest value of crude fibre (CF) was 277.3 g/kg DM for Hay and the lowest value was almost similar for Atriplex S, the mixture of acacia and atriplex silages (AAS) and Acacia S (202.1, 202.3 and 202.4 g/kg DM, respectively). As for ash content, the highest value was observed with F. Atriplex (272.7 g/kg DM) and the lowest value was 140.7 g/kg DM for Hay. The value of nitrogen free extract (NFE) was ranged on average between 345.9 and 519.7 g/kg DM. The same manner of chemical composition was observed with the results of fibre fractionations. Ensiling processing of roughages and mixing of them as well increased the degradation of neutral detergent fibre (NDF), acid detergent fibre (ADF), acid detergent lignin (ADL) and hemicelluloses. Silage roughages had lower total phenols (TP), total tannins (TT) and condensed tannins (CT) than fresh roughages; as well of mixing of them. The cumulative gas production values during 96 h of incubation were 24.0, 25.5, 26.0, 29.0, 30.0 31.0 and 32.0 ml/200mg DM for Acacia S, F. Acacia, AAS, Atriplex S, Hay, FAA and F. Atriplex, respectively. The highest ($P<0.05$) value of potential degradability (a+b) was estimated with F. Atriplex and followed by FAA and Hay. The low ($P<0.05$) value of a+b was observed by Acacia S followed by F. Acacia and AAS. Production rate constant for insoluble fraction b (c) were 0.066, 0.065, 0.065, 0.061, 0.059, 0.042 and 0.044 ml/h for AAS, FAA, F. Atriplex, Atriplex S, Hay, Acacia S and F. Acacia, respectively. The fast rate was observed with AAS, FAA, F. Atriplex and Atriplex S meanwhile, AAS and FAA were recorded the fast c with shorter lag time than other roughages. There was no significant ($P>0.05$) difference among all roughages for VFA concentration after 6 h incubation. The NH_3-N concentration was ranged on average from 10.97 to 15.83 mg/100ml R.L. after 6 h incubation. ME (MJ/kg DM), OMD % and MP (g/kg OMD) values were significantly ($P<0.05$) difference among all roughages, but there was no significantly ($P>0.05$) difference for NE among all roughages. The present study concluded that time spend during ensiling processing of shrubs could be saved by mixing high tannins shrubs (acacia) with low tannins shrubs (atriplex) in fresh form as good quality roughages and an alternative animal feed resources for ruminant feeding especially in dry season or when roughage sources availability is low.

Keywords: Nutritive value, roughages, acacia, atriplex, silage, gas production, fermentation, energy contents, microbial protein.

INTRODUCTION

Many leguminous fodder trees and shrubs have high protein levels and are potentially promising supplements to overcome nutrient deficiencies provided anti-nutritional factors such as tannins and other

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secondary compounds can be controlled. Tannins are polyphenolic substances that occur widely in plants and have the ability to bind proteins. This effect of tannins can be either adverse or beneficial for the animal depending on their concentration and chemical structure[45,36]. Tannin-protein complex on one hand reduces the availability and digestibility of proteins in the ruminant, but on the other hand it allows more dietary protein escape rumen degradation to reach the lower digestive tract[50,45].

Acacia (*Acacia saligna*), a leguminous shrub species available in numerous countries, like many other plant species is high in phenolic compounds such as tannins (condensed tannins and hydrolyzable tannins), which in addition to lignin reduce the nutritive value of its foliage. Atriplex is saltbush characterized by moderate digestible crude protein (DCP) and high oxalate and mineral concentrations, also digestible ether extract and soluble carbohydrates are low[9]. Efforts have been made to improve the utilization of these plants for ruminants by reducing the tannin level through chemical, physical and biological approaches[36]. The most widely studied technique is the addition of polyethylene glycol (PEG) which can bind tannin and reduce its negative effect[4,49,61,36]. Other treatments were used to brake down these complexes under conditions of high acidity or high alkalinity. Studies by several authors[54,38] showed that treatment of tanniniferous feed sources with alkalis (urea, sodium hydroxide, potassium hydroxide, etc.) and oxidizing agents (potassium dichromate, potassium permanganate, etc.) decreased their total extractable phenols and tannins and/or condensed tannins contents. However, the major disadvantage of these chemical treatments is the loss of soluble nutrients. Unfortunately, deactivating techniques, mainly senescence processes have been tested on a limited number of feed sources and it would be difficult to generalize their advantages and disadvantages on an array of tanniniferous feedstuffs. Ensiling process improved the quality of halophytes, enhanced their acceptability for sheep and goats, appeared to be the most nutritious diets, they can successfully provide sufficient digested nutrients (TDN and DCP) and characterized by reasonable DM intake and it appeared to be the most convenient processing method under the prevailing conditions of Egypt[21,2]. Franclet and Le Houerou[25] demonstrated the validity of a ration consisting of *Opuntia ficus*, *Atriplex halimus* (saltbush) and *Acacia ligulata*, that for good synergic action of the latter in order to the best utilization of the nutritive value of atriplex. Mixing shrubs such as *Atriplex species* and *Acacia sp.* together as fresh (air-dried) or silage materials could enhance their palatability and consumption. Therefore, the objectives of the current study were to evaluate atriplex (*Atriplex halimus*, high ash) and acacia (*Acacia saligna*, high tannins content) as either fresh or silage form, also the pervious forms as individual or mixture will be tested using an in vitro gas production technique. Moreover, rumen fermentation, estimated energy and microbial protein synthesis were studied.

MATERIALS AND METHODS

Silage Procedures: Ensilage of acacia and atriplex were prepared as described by Khattab (2007).

Feed Samples: Berseem (*Trifolum alexandrinum*) hay (Hay), fresh acacia (*Acacia saligna,* F. Acacia), acacia silage (Acacia S), fresh atriplex (*Atriplex halimus,* F. Atriplex), atriplex silage (Atriplex S), mixture of fresh acacia and atriplex (1:1, FAA) and mixture of silages of acacia and atriplex (1:1, AAS) were ground in mills to pass a 1 mm sieve prior to chemical analysis, in vitro gas production measurements and rumen fermentation. The chemical composition of the roughages used for in vitro gas production is presented in Table 1.

Chemical Analysis: Dry matter (DM), crude fibre (CF), ether extract (EE), ash and crude protein (CP) of roughage samples were analyzed according to AOAC[3]. Neutral detergent fibre (NDF), Acid detergent fibre (ADF) and acid detergent lignin (ADL) were determined as described by Van Seost *et al.,*[60]. Cellulose and hemicelluloses were calculated by difference. Total phenols (TP), total tannins (TT) and condensed tannins (CT) were analyzed[36].

In Vitro Gas Production: In vitro gas production was carried out using the method described by Menke and Steingass[41]. Buffer and mineral solution were prepared and placed in a water bath at 39 ºC under continuous flushing with CO_2. Both solid and liquid rumen fractions (50 % solid: 50 % liquid) were collected before the morning feeding from three rumen-cannulated sheep fed on berseem hay and commercial concentrate mixture diet. Rumen fractions were collected into pre-warmed insulted bottles, combined among sheep, homogenized in a laboratory blender, filtered through three layers of cheese-cloth and purged with CO_2. The well mixed and CO_2 flushed rumen fluid was added to the buffered rumen fluid solution (1:2 v/v), which was maintained in a water bath at 39 ºC, and mixed.

Samples (200±10 mg) of air-dry feedstuffs were accurately weighted into syringe fitted with plungers. Buffered rumen fluid (30 ml) was pipetted into each syringe, containing the feed samples, and the syringes were immediately placed into the water bath at 39 ºC[11]. Three syringes with only buffered rumen fluid were incubated and considered as the blanks. The syringes were 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 96 h of incubation. Total gas values were corrected for the blank incubation, and reported gas values are expressed in ml per 200 mg of DM.

Cumulative gas production was fitted iteratively to the exponential model proposed by Ørskov and McDonald[48].

Rumen Fermentation, **Energy Contents, Organic Matter Digestibility and Microbial Protein Estimation:** Two bottles containing 300 mg roughages samples and 45ml Buffered rumen fluid were incubated for determination ammonia nitrogen (NH_3-N) and volatile fatty acids (VFA) concentrations at 6h of incubation. Ruminal NH_3-N concentration was determined according to Gips and Wibbens-Alberts[27] and volatile fatty acids were determined according to Warner[64]. The energy value of forages can be calculated from the amount of gas produced at 24 h of incubation with supplementary analysis of crude protein, ash and ether extract. This approach was developed by the research group in Hohenheim (Germany) and is based upon extensive in vitro incubation of forages[42,41].

- ME (MJ/kg DM) = 2.2 + 0.136GP + 0.057CP+ 0.0029CF
- 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/200 mg DM);
- CP is crude protein (% DM);
- CF is crude fibre.
- XA is ash (% DM)

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

where:
- Gas is 24 h net gas production (ml/g DM)
- CP is crude protein (% DM)
- EE is Ether extract (% DM)

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

Microbial protein synthesis was estimated according to Czerkawski[16].

Statistical Analysis: Data were subjected to analysis of variance (ANOVA) using the General Linear Model. Significant differences between individual means were identified using Tueky test[55].

RESULTS AND DISCUSSIONS

Chemical Analysis: The chemical composition, fibre fractionations and phenol compounds of berseem (*Trifolum alexandrinum*) hay (Hay), fresh acacia (F. Acacia), acacia silage (Acacia S), fresh atriplex (F. Atriplex), atriplex silage (Atriplex S) and their mixture are shown in Table 1. The crude protein (CP) was ranged on average from 126 to 144.5 g/kg DM. The highest value of CP was recorded with Hay and the lowest value was recorded with Acacia S. The highest value of crude fibre (CF) was 277.3 g/kg DM for Hay and the lowest value was almost similar for Atriplex S, the mixture of acacia and atriplex silages (AAS) and Acacia S (202.1, 202.3 and 202.4 g/kg DM, respectively). As for ash content, the highest value was observed with F. Atriplex (272.7 g/kg DM) and the lowest value was 140.7 g/kg DM for Hay. The value of nitrogen free extract (NFE) was ranged on average between 345.9 and 519.7 g/kg DM. The results of chemical analysis indicated that ensiling procedure increased the breakdown of CF as a result of decreasing it chemical composition values (Table 1). Also, the results showed decreas in CP and ash and it may be due to the soluble parts of them that lost during ensiling processing. Interestingly, the results showed an increasing in NFE during ensiling processing of shrubs and their mixture as well and this is due to the increasing of CF breakdown (Table 1). The same manner of chemical composition was observed with the results of fibre fractionations (Table 1). Ensiling processing of roughages and mixing of them as well increased the degradation of neutral detergent fibre (NDF), acid detergent fibre (ADF), acid detergent lignin (ADL) and hemicelluloses. Rubanza *et al.,*[53] reported that all Acacia species leaves which used in their study had high CP values and were relatively low in fibre compared to most other forages. *Acacia spp.* foliages could therefore be used as CP supplement for ruminants fed low quality forages, especially during the dry season. The high CP contents in these *Acacia spp.* were comparable to those reported by Topps[58], Abdulrazak *et al.,*[1] and Rubanza *et al.,*[52]. Variations in chemical composition among different studies on species of acacia foliages may be partly due to genotypic factors that control accumulation of forage nutrients. Accumulation of nutrients in plants is a property of species[46] and varies among species and genera. Differences in CP contents among species could be partly due to stage of growth

Table 1: Chemical analysis, Fibre fractionations and phenol compounds (g/kg DM) of berseem hay (Hay), fresh acacia (F. Acacia), acacia silage (Acacia S), fresh atriplex (F. Atriplex), atriplex silage (Atriplex S), mixture fresh of acacia and atriplex (FAA) and mixture silage of acacia and atriplex (AAS)

	Hay	F. Acacia	Acacia S	F. Atriplex	Atriplex S	FAA	AAS
Chemical analysis							
OM	859.3	830.9	885.3	727.3	772.3	779.1	828.8
CP	144.5	131.1	126.0	135.3	134.0	133.2	130.0
CF	277.3	228.9	202.4	215.8	202.1	222.2	202.3
EE	38.4	21.2	37.2	30.3	40.0	25.8	38.6
Ash	140.7	169.1	114.7	272.7	227.7	220.9	171.2
NFE	399.1	449.7	519.7	345.9	396.2	397.9	457.9
Fibre fractionations							
NDF	431.0	387.0	340.0	381.0	338.0	384.0	339.0
ADF	251.0	247.0	201.0	216.0	198.0	231.5	199.5
ADL	120.0	75.0	70.0	85.0	80.0	80.0	75.0
Hemicelluloses	180.0	140.0	139.0	165.0	140.0	152.5	138.5
Cellulose	131.0	172.0	131.0	131.0	118.0	151.5	124.5
NFC	245.4	291.6	382.1	180.7	260.3	236.1	321.2
Phenols compounds							
TP	25.7	84.1	48.3	23.5	13.2	53.8	30.8
TT	2.8	44.8	19.7	0.3	0.1	22.6	9.9
CT	2.5	40.7	17.7	0.27	0.09	20.5	8.9

OM, organic matter; CP, crude protein; CF, crude fibre; EE, ether extract; NFE, nitrogen free extract; NDF, neutral detergent fibre; ADF, acid detergent fibre; ADL, acid detergent lignin; NFC, Non fibre carbohydrates; TP, total phenols; TT, total tannins; CT, condensed tannins.
NFC = 100 – (% CP+ % EE + % Ash + % NDF).

Table 2: Cumulative gas production (ml/200 mg DM) for berseem hay (Hay), fresh acacia (F. Acacia), acacia silage (Acacia S), fresh atriplex (F. Atriplex), atriplex silage (Atriplex S), mixture fresh of acacia and atriplex (FAA) and mixture silage of acacia and atriplex (AAS) (Mean±S.D)

Feedstuffs	Incubation time (h)							
	3	6	9	12	24	48	72	96
Hay	5.0±0.9	7.0±0.4	10.5±0.4	15.0±0.9	22.5±1.0	26.0±1.5	28.0±0.0	30.0±0.0
F. Acacia	4.0±0.0	8.0±0.0	9.5±0.0	12.0±0.0	16.5±0.5	21.5±0.5	22.5±0.5	25.5±0.5
Acacia S	6.0±0.0	9.0±0.5	11.0±0.0	13.0±1.0	16.0±0.0	20.5±0.5	22.0±0	24.0±0.0
F. Atriplex	4.0±0.0	7.0±0.0	11.5±0.5	17.5±0.5	24.0±0.0	28.5±0.5	29.5±0.5	32.0±1.0
Atriplex S	6.0±0.5	10.0±0.5	12.0±0.0	16.0±0.0	22.0±0.0	26.0±0.0	26.5±0.5	29.0±0.0
FAA	5.0±0.0	9.0±0.0	11.5±0.0	17.0±1.0	24.0±0.0	28.5±0.5	28.5±0.5	31.5±0.5
AAS	6.0±0.0	10.0±0.0	12.0±0.5	15.0±0.0	20.0±0.0	23.0±0.0	23.5±0.5	26.0±0.0

and proportion of leaf samples collected for analysis. Differences in NDF, ADF and ADL could similarly be due to species genotypic differences in factors that control fibre accumulation in the plant, and stage of growth. Fibre contents increase with advanced foliage maturity due to lignification[46]. Fibre contents in this study were comparable to those reported by Rubanza *et al.,*[53], Reed[51] and Topps[58] in other Acacia species.

Ash content, which is characteristically high in desert shrubs such as *Atriplex spp*.[13,10,53], was ranged between 15.0 and 32.3% DM. Ash content in Atriplex which tested in the current study is in agreement with the their results. CP content differed among plant species, indicating different strategies of adaptation to salinity[28] and/or different growth rates of shrubs. Stage of maturity influenced ash, NDF and ADF[30], and this is

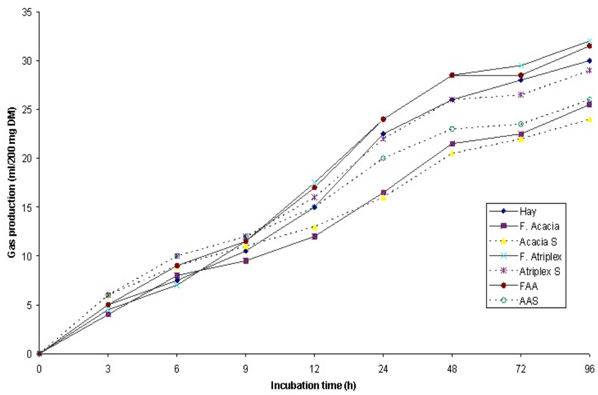

Fig. 1: Cumulative gas production for berseem hay (Hay), fresh acacia (F. Acacia), acacia silage (Acacia S), fresh atriplex (F. Atriplex), atriplex silage (Atriplex S), mixture fresh of acacia and atriplex (FAA) and mixture silage of acacia and atriplex (AAS)

in agreement with the present results of atriplex. Silage roughages had lower total phenols (TP), total tannins (TT) and condensed tannins (CT) than fresh roughages, as well of mixing of them (Table 1). The low nutritive value of *Acacia spp*. foliage is due to the presence of phenolic compounds, especially tannins[18,40,6]. Tannins can reduce the nutritive value of feedstuffs more than lignin and they can also affect the health of the animal. Ben Salem *et al.,*[6,7,8,9,5] mentioned that, the level of tannins varied substantially with season and area of plantation. The present results indicated the ensiling decreased the tannins content which might be leaded to an increment of bacterial activity during ensiling as a result of decreasing of chemical composition of CP, CF and fibre fractionations and increasing in NFE and non fibre carbohydrates (NFC).

***In vitro* Gas Production:** Cumulative gas production profiles, corrected for blank are shown in Figure 1 and Table 2. The cumulative gas production values during 96 h of incubation were 24.0, 25.5, 26.0, 29.0, 30.0 31.0 and 32.0 ml/200mg DM for Acacia S, F. Acacia, AAS, Atriplex S, Hay, FAA and F. Atriplex, respectively. The high cumulative gas production was observed in F. Atriplex which followed by FAA and Hay. The lowest value of total tannins in F. Atriplex could be a positive factor for increasing the microbial activity, resulted in enhancing the gas production. The same trend was observed when atriplex was mixed with acacia in fresh form and total tannins was decreased from 44.8 to 22.6 mg/kg DM, thus the gas production increased from 25.5 to 31.5 ml/200 mg DM at 96h of incubation for F. Acacia and FAA, respectively. The low values of gas production were observed with Acacia S and F. Acacia which is due to

Table 3: Degradation kinetics of berseem hay (Hay), fresh acacia (F. Acacia), acacia silage (Acacia S), fresh atriplex (F. Atriplex), atriplex silage (Atriplex S), mixture fresh of acacia and atriplex (FAA) and mixture silage of acacia and atriplex (AAS) using gas production technique (Mean±S.D)

Feedstuffs	a+b (ml)	c (ml/h %)	L (h)
Hay	29.20 ± 4.31^{ab}	0.059 ± 0.012^{b}	0.16 ± 0.75^{c}
F. Acacia	24.60 ± 0.45^{b}	0.044 ± 0.001^{c}	-
Acacia S	23.53 ± 0.29^{d}	0.042 ± 0.005^{d}	-
F. Atriplex	30.74 ± 0.74^{a}	0.065 ± 0.003^{a}	1.04 ± 0.53^{a}
Atriplex S	27.84 ± 0.21^{c}	0.061 ± 0.001^{a}	-
FAA	30.18 ± 0.40^{a}	0.065 ± 0.001^{a}	0.44 ± 0.18^{b}
AAS	24.63 ± 0.21^{d}	0.066 ± 0.002^{a}	-

$^{a-d}$ Means in the same column with different letters in their superscripts differ (P<0.05).

-Cumulative gas production data were fitted to the model of Ørskov and McDonald (1979), where, a+b= potential degradability (a= gas production from immediately soluble fraction and b= gas production from insoluble fraction); c= gas production rate constant for the insoluble fraction b; L, lag time.

the high tannins content in acacia, although the tannins was decreased during ensiling processing of acacia. The *in vitro* gas production technique has proved to be a potentially useful technique for feed evaluation[41,11], as it is capable of measuring rate and extent of nutrient degradation[29,15]. In addition, *in vitro* gas production technique provide less expensive, easily to determine[33] and suitable for use in developing countries[12]. Gas is produced mainly when substrate is fermented to acetate and butyrate. Substrate fermentation to propionate yields gas only from buffering of the acid and, therefore, relatively lower gas production is associated with propionate production. Rapidly fermentable carbohydrates yield relatively higher propionate as compared to acetate, and the reverse takes place when slowly fermentable carbohydrates are incubated. Many workers found more propionate and thus lower acetate to propionate ratio in the ruminal fluid of cows fed a high grain diet. If fermentation of feeds leads to a higher proportion of acetate, there will be a concomitant increase in gas production compared with a feed with a higher proportion of propionate[12,11,26,35,39]. The present results of gas production are in agreement with the previous finding, although, the proportion of individuals VFA (acetic, butyric and propionic acids) were not determined. The data of degradation kinetics of roughages using gas production confirmed the results of gas production (Table 3). The highest (P<0.05) value of potential degradability (a+b) was estimated with F. Atriplex and followed by FAA and Hay. The low (P<0.05) value of a+b was observed by Acacia S followed by F. Acacia and AAS (Table 3). Production rate constant for insoluble fraction b (c) were 0.066, 0.065, 0.065, 0.061, 0.059, 0.042 and 0.044 ml/h for AAS, FAA, F. Atriplex, Atriplex S, Hay, Acacia S and F. Acacia, respectively. The fast rate was observed with AAS, FAA, F. Atriplex and Atriplex S

Table 4: Ammonia N (NH_3-N), Volatile fatty acids (VFA) concentrations, metabolizable energy (ME), net energy (NE), organic matter digestibility (OMD) and Microbial protein (MP) for berseem hay (Hay), fresh acacia (F. Acacia), acacia silage (Acacia S), fresh atriplex (F. Atriplex), atriplex silage (Atriplex S), mixture fresh of acacia and atriplex (FAA) and mixture silage of acacia and atriplex (AAS) using gas production technique (Mean±S.D)

Feedstuffs	NH_3-N (mg /100 ml R.L.)	VFA (m.eq/100ml R.L.)	ME (MJ/kg DM)	NE (MJ/kg DM)	OMD %	MP (g/kg OMD)
Hay	$12.97±0.12^{c}$	10.41±0.39	$6.25±0.21^{b}$	3.64±1.95	$42.75±1.54^{a}$	$82.50±2.97^{a}$
F. Acacia	$10.29±0.14^{d}$	10.88±0.31	$5.26±0.20^{ef}$	5.20±0.09	$36.55±0.63^{d}$	$70.52±0.61^{b}$
Acacia S	$12.97±0.11^{c}$	10.87±0.61	$5.15±0.00^{e}$	5.12±0.00	$35.52±0.00^{e}$	$68.56±0.00^{e}$
F. Atriplex	$14.25±0.21^{b}$	10.86±0.08	$8.01±0.00^{a}$	5.84±0.57	$40.15±5.56^{b}$	$77.49±6.71^{ab}$
Atriplex S	$15.40±0.15^{a}$	10.88±0.23	$5.60±0.58^{ec}$	5.57±0.57	$38.19±5.31^{c}$	$73.72±6.41^{c}$
FAA	$15.83±0.11^{a}$	10.99±0.16	$5.88±0.58^{bdf}$	5.83±0.56	$39.93±5.26^{b}$	$77.06±5.26^{ad}$
AAS	$14.90±0.12^{a}$	11.09±0.33	$5.32±0.57^{d}$	5.29±0.55	$23.46±4.93^{f}$	$45.28±4.93^{f}$

a-f Means in the same column with different letters in their superscripts differ (P<0.05).

meanwhile, AAS and FAA were recorded the fast c with shorter lag time than other roughages. The L (time from incubation to start of gas production) is a very important digestibility parameter. Therefore, these results suggest that gas production profiles are not necessarily linearly related to degradation or fermentation of feedstuffs according to the results of the chemical composition of feedstuffs. 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[31]. Moreover, kinetics of gas production is dependent on the relative proportion of soluble and 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[12,11,26,35,39], which is in agreement and confirmed by the current study.

Rumen Fermentation, Energy Contents, Organic Matter Digestibility and Microbial Protein Estimation: The concentrations of ammonia (NH_3-N) were ranged from 10.29 to 15.83 mg/100 ml R.L. after 6 h incubation (Table 4). The values were significantly (P<0.05) increased when acacia or atriplex in silage form, and this is due to the enhancement of breakdown of crude protein of them and also due to the reduction of tannins content during ensiling processing especially with acacia. The present results of NH_3-N concentration are in agreement with the results of Khattab[32]. He found that high NH_3-N concentration and for diets containing atriplex compared to those fed acacia could be due low tannin content, high non protein nitrogen and soluble nitrogen. Leroy *et al.,*[34] reported that addition of aqueous solutions of hydrolysable tannins (tannic acid) to groundnut and soybean meals (SBM) markedly reduced *in vitro* ammonia production. Driedger and Hatfield[20] reported that pelleting SBM with tannic acid significantly increased N-retention, weight gain, and feed efficiency in growing lambs. El-Waziry *et al.,*[22,23] found that when SBM was treated with tannic acid or quebracho (condensed tannins) the concentrations of NH_3-N were significantly decreased. The studies of Dinius *et al.,*[19],Van Neval and Demeyer[59], Wallace *et al.,*[63], Whetstone *et al.,*[65], Newbold *et al.,*[47] have concluded that the lower ammonia concentrations were mainly due to reduce proteolysis, degradation of peptides and deamination of amino acids in the rumen. As for the present study, acacia could suggest that acacia protein was not being broken down rapidly in the rumen due to its bound with tannins in complex compound, which make it less susceptible to deamination and proteolysis. The concentrations of volatile fatty acids (VFA) were not significantly differences among all roughages (Table 4). The mean values of VFA concentrations of sampling times were 10.41, 10.88, 10.87, 10.86, 10.88, 10.99 and 11.09 m.eq/100 ml R.L. for Hay, F. Acacia, Acacia S, F. Atriplex, Atriplex S, FAA and AAS, respectively. The formation of undegradable complexes[37,62,24] between condensed tannins (CT) and protein and/or carbohydrates may have reduced the amount of available substrate for fermentation. The escape of these undegradable complexes from the rumen to the abomasum[50,66] could also explain the low concentrations of protein in the rumen fluid. A low concentration of protein in the rumen fluid disfavors microbial growth and activity, and this partly explains the low degradation of DM of acacia incubated in the rumen of animals fed acacia basal diets. Woodward and Reed[67], indicated that the low and steady production of VFA and ruminal NH_3-N may resulted from high fiber content in acacia and from inhibition of rumen microbes by tannins which lead to low rate of deamination.

The predicted metabolizable energy (ME, MJ/kg DM), net energy(NE, MJ/kg DM), organic matter digestibility (OMD) and microbial protein (MP) from gas production after 24 h incubation for Hay, F. Acacia, Acacia S, F. Atriplex, Atriplex S, FAA and AAS are presented in Table 4. In the method of Menke *et al.,*[42], the gas produced on incubation of 200 mg feed dry matter after 24 h of incubation together with the levels of other chemical constituents are used to predict OMD determined in vivo and ME. There were ($P<0.05$) significantly differences among all roughages in ME and OMD. These results suggest that there are positive correlation between gas production and ME and also between gas production and OMD. Deaville and Givens[17] suggested that it is preferable to use pre-dried silages to avoid interference from any indirect gas produced. The result indicates that fermentation acids in silage do not yield direct gas and therefore provide little energy for rumen microbial growth, and the contribution of fermentation acids to diet/feed ME content should be subtracted during the calculation of fermented ME. The present results of ME and OMD in silages forms are in agreement of the finding of Deaville and Givens[17]. There were no significantly differences among all roughages in NE. There were significantly ($P<0.05$) differences among roughages. The highest value was 82.5 g/kg OMD for Hay and which followed by 77.49 and 77.06 g/kg OMD for F. Atriplex and FAA, respectively, but there were no significantly differences between them. These results may due to the highest values of them for CP, VFA, NH_3-N, and ME. 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*[41]. Menke *et al.,*[42,43,44], Steingass and Menke[56], Menke and Steingass[41] and Chenost *et al.,*[14] 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. Also, there are significant correlation between *in vitro* gas measurement and *in vivo* digestibility. 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*[41].

The present study found that ensiling processing decreased tannins and ash content in acacia and atriplex, respectively. When acacia and atriplex were mixed in fresh form, the results indicated that tannins and ash content were lower in the mixture than that of individual form. In addition, the gas production of FAA was higher than that of fresh acacia, acacia silage, atriplex silage and mixture silages of acacia and atriplex. Also, FAA has the best potential degradability and a faster degradability rate with shorter lag time than of shrubs forms which tested in the present study. Therefore, the current study concluded that time spend during ensiling processing of shrubs could be saved by mixing high tannins shrubs (acacia) with low tannins shrubs (atriplex) in fresh form as good quality roughages and an alternative animal feed resources for ruminant feeding especially in dry season or when roughage sources availability is low.

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