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NUTRITIONAL EVALUATION AND METHANE PRODUCTION OF SOME FODDER PLANTS USING IN VITRO GAS PRODUCTION TECHNIQUE

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ABSTRACT

The aim of this study was to evaluate the nutritive value of four salt tolerant plants (*Pennisetum americanum, Acacia saligna, Leucaena leucocephala and kochia indica*) based on their chemical composition, in vitro gas production and fermentation kinetics. The chemical composition of *A. saligna* and *L. leucocephala* showed the higher content of crude protein and non-protein nitrogen (NPN) as well. Total tannins, phenols and saponin contents in *A. saligna* and *L. leucocephala* was higher compared to *K indica*. While *P americanum* was free of total tannins and saponin. A significant differences were observed in short-chain fatty acids and acetic acid for the tested plants. Cumulative gas production for *A. saligna*, *L. leucocephala* and *K indica* showed pronounced methane inhibition compared to *P americanum*. The results indicated that the salt-tolerant plants used in the experiment could be promising feed resources to decrease energy loss as methane in ruminant diets. However, the presence of secondary metabolites and protein nitrogen (NPN) should be taken into consideration when formulating diets containing salt-tolerant forages for small ruminants.

Keywords: salt-tolerant plants, digestibility, rumen fermentation, methane production

INTRODUCTION

In Egypt, the availability of forage feeds is more restricted particularly in areas with dry to semi-dry climate. Halophytes and other salt-tolerant plants have the advantage of tolerating high salt levels in the saline lands and drought conditions (Helal *et al.,* 2013). These plants can provide great potentialities particularly as sources of livestock fodders and can fill up the feed gaps in the summer (Aderao *et al.* 2018). In the current study four fodder plants, *Acacia saligna, Leucaena leucocephala, Kochia indica and Pennisetum americanum* will present as salt-tolerant plants which could be used for feeding ruminants.

Acacia and Leucaena species are belonging to family Fabacea, and characterized as a drought resistant, moderately salinity tolerant, have high production of green biomass and high crude protein content (El Shaer 2010 & Shaker *et al.,* 2014). *K indica* are annual shrubs which belong to the family Chenopodiaceae, these plants are adapted to be grown under drought and /or salt-affected lands (El Shereef 2016). Besides, *P americanum* is a salt and drought tolerant grass that could be used successfully and safely for feeding ruminants in semi-arid regions (Fahmy *et al.,* 2010). Because of that, there is a need to use the available forages from such plants (shrubs, trees, and grasses) for feeding livestock with low feed costs under desert conditions. So, the purpose of this study is to assess the nutritional values of these plants by evaluate nutrient digestibility, ruminal fermentation profiles and methane production at in vitro level.

Material and methods

Sample collection and preparation

Plant samples *(P americanum, A saligna, L leucocephala* and *K indica*) were collected from six different sites randomly selected in South Sinai of Egypt, Sinai Peninsula (200 km South East of Cairo), Egypt. Laboratory work was conducted at the Laboratory of Climate Change and Livestock Production of FMVZ-UADY, Mexico. The experimental procedures were approved and complied with the ethical standards set by the faculty. For each species, the plants were cut into small pieces (3-5 cm). Samples were then dried at 50°C for 48 h using a forced air oven to prevent enzymatic degradation of the phenolic compounds present in the plant matter (Makkar *et al.* 1993b). Once dry, 400 g of plant matter was ground in a Lab-Willey Grinder (code MSW-342- IN; 10122740) and sieved through a 2-mm screen. The grounded material was mixed well and then 100 g were sub-sampled, reground and passed through a 0.5-mm screen sieve. These finely ground subsamples were used in tannin analysis while the rest of the material was used for in vitro gas production analysis.

The proximate analysis

Dry matter (DM), crude protein (CP), crude fiber (CF) and ash of feed ingredients were determined according to AOAC (2007). Neutral detergent fiber (NDF) and acid detergent fiber (ADF) were determined using the ANKOM fiber technology technique (Robinson *et al.* 1999) without using alpha-amylase. The non-protein nitrogen (NPN) was obtained by precipitation of true protein in the filtrate with tungstic acid (10% sodium tungstate solution) and determined as the difference between total N and the N content of the residue after filtration. Ammonia nitrogen were determined by Warner (1964). Determine of saponins was according to the method of Segal *et al.* (1966). Measurement of microbial protein was analyzed using the spectrophotometric method of Zinn & Owens (1986) after incorporating suggested modifications of Makkar & Becker (1991) and Obispo & Dehority (1999). Microbial counts as bacteria and protozoa of ruminal fluid were determined using a counting cell (Hawskley, UK) as described by Demeyer (1981). Alkaloids concentration was determined according to Shamsa *et al.* (2007). Total oxalate was determined by HPLC method (Savage *et al.*, 2000). 1 mL of 50% sulphuric acid was added and they were frozen at –20 °C until analysis fractionation of SCFA's according to Erwin *et al*. (1961). Calcium was determined by spectrophotometer (Gindler and King, 1972). Inorganic phosphorus was determined by atomic absorption spectrophotometer according to Chapman and Pratt (1961). Sodium (Na) and potassium (K) were determined by using the standard flame photometry (Jackson, 1958) while copper (Cu), and zinc (Zn) concentrations were tested using atomic absorption techniques.

In vitro gas production

Gas production (GP) was determined following Theodorou *et al.* (1994) technique. Rumen fluid was collected from Pelibuey hair sheep in pre-warmed insulted bottle. Samples weighing 0.999 g of each plant then placed in 125 ml serum glass bottles, and approximately 90 ml of buffered rumen fluid was added to each bottle. Once closed, the bottles were gently shaken and placed in a water bath at 39 °C. Gas production measurements, for the samples incubated 72 h, were taken hourly up to 8 hours after incubation, then every four (from 12- to 28), eight (from 36-to 60) and 72 h post incubation. After 24 and 72 h, the incubation residue, respectively, was analyzed for digestibility of dry matter (DMD), organic matter (OMD) and the digestibility of neutral detergent fiber (DNDF) content using the ANKOM fiber technology technique (Robinson *et al.* 1999).

Phenols content was determined using the Folin-Ciocalteu method and tannins were measured using polyvinylpolypyrrolidone (PVPP) as described by Makkar *et al.* (1993b).

Methane measurement

Using a gas-tight syringe, gas samples were collected from each bottle at 24 h post-incubation as in Bhatta *et al.* (2015) and Kaya *et al.* (2016). After the volume of gas

was recorded, and the sample removed for methane analysis, the remaining gas was released. The CH4 content was determined by injecting 1 ml of gas into a Perkin Elmer gas chromatograph (model: Clarus 500 series) equipped with a flame ionized detector (FID). Separation was achieved using an Elite-Q Plot Capillary Column (Perkin Elmer) packed with a 60/80 mesh carboxenTM-1000 stationary phase. Nitrogen was used as the carrier gas with a flow rate of 30 mL−1min, an isothermal oven temperature of 50 o C, and an injector temperature of 250 o C. The calibration curve using a regression equation was completed with standard CH4 (99.99 % from ALTECH).

Statistical analysis

The data obtained from each plant were analyzed for variance using an ANOVA procedure according to SAS (2000) using the following model: $Yij = \mu + ai + \varepsilon ij$

where Yij is observation, μ is overall mean, ai is plant species (i = 1 to 4), and εij is error. Tukey's test was used for the multiple comparisons among mean values for the four plants and the significance level was set at $p < 0.05$.

Results and discussion

Proximate analysis

The chemical composition is the first step to evaluate the nutritive value of such plants to be a feed for animals. Although *A saligna* and *L leucocephala* were high in CP (113 and 147 g/kg), the nitrogen richness of such plants may not be fully used by ruminants since non-protein nitrogen (NPN) represents 46 and 52 % of CP content, respectively (Table 1). The NPN could not be metabolized and converted to protein in the rumen if there is not sufficient energy source or some of these compounds would be converted to ammonia in the rumen, which is absorbed and converted to urea then excreted in the urine (SCA, 2007). Otherwise *K indica* had moderate contents of CP and NPN %. While *P americanum* had the lowest CP, NPN and ADL contents compared with the other plants. In this regards, forages with CP content of less than 70 g /kg DM require protein supplementation to offset limitations on voluntary feed intake as recommended by Melaku *et al.* (2003). The present study suggest that crude protein (CP) is inadequate estimation for salt tolerant plants of true protein because it is based on the assumption (certainly untrue) that all nitrogen in the biomass will become protein; i.e. CP (%) = nitrogen (%) * 6.25.

The differences in cell wall constituents as NDF, ADF and ADL could be due to species genotypic differences for the tested plants and their values were agreed with Shawket *et al.* (2010), Shaker *et al.* (2014) and El Shereef (2016).

Plant secondary metabolites (PSM) and minerals

PSM is a group of chemical bioactive compounds such as tannins, saponins, alkaloids, flavonoids, glucosides, etc., that are not involved in the primary biochemical processes of growth and reproduction, but play a vital role in the interaction between plants and the

environment (Kliebenstein 2013). *A saligna* and *L leucocephala* showed higher alkaloids, saponin, and phenols components compared to the other plants (Table 2). Both plants had alkaloids content above 40 g/ kg DM. Ventura *et al.* (2000) recorded the negative correlation between feed intake and alkaloids content. Besides, Abd El-Rahman (2003) reported that *Halocnemum strobilaceum* and *Hammada elegans* which consider non palatable plants was related to high level of alkaloids (31.1 and 61.6 g/kg DM). Likewise, saponins are characterized by a bitter taste and foaming properties (Kumar, 2011). Thus the present results of alkaloids and saponins concentrations could explain the lower dry matter intake from *A saligna* and *L leucocephala* in different studies (Shawket *et al.,* 2010, Helal *et al.* 2013 and Hassan *et al.* 2015) compared to traditional rations. Concerning condensed tannins (CT), it was reported that moderate levels (30 to 40 g/kg DM) of CT may result in nutritional advantages by increased bypass protein availability and bloat suppression in cattle. The mode of action of condensed tannins (at low concentrations) was noticed by bind with plant protein at nearly neutral pH in the mouth and rumen to form tannin-protein complexes which are stable and insoluble at pH 3.6 -7.0, but dissociate and release protein at pH <3.5 in the abomasums (Soltan *et al.,* 2013). Therefore, the presence of high contents of CT should be taken into consideration when formulating diets containing the tested plants for feeding ruminants.

Total oxalate was higher in *K indica* compared to the other plant species. El Shereef *et al.* (2016) reported a significant inhibition for Ca concentration in blasma for sheep fed *K indica* silage, the authors suggests that Ca bioavailability may decrease as result of the binding with Ca to form calcium oxalate, a non-soluble and non-digestible compound. *P americanum* was free of total tannins, condensed tannins and saponin. In general, the concentrations of alkaloids, saponin, total tannins, total oxalate and condensed tannins contents for the experimental plants were comparable to that obtained by El Shereef *et al.* (2016) and Fahmy & Ibrahim (2005) for *K indica* and *P americanum* while these values was above than that reported by Bueno *et al*. (2005) and Soltan *et al.* (2012) for *A saligna* and *L leucocephala*. This variation could be due to many factors like temperature, drought, salinity, seasonality, altitude and light, metal ions, wounding and nutrient deficiencies can affect their concentration and these are also dependent on the growing conditions and metabolic pathways of related PSM (Gouvea *et al.* 2012).

In respect to minerals values (Table 2), it seems that *A saligna* and *L leucocephala* could be good resource of Ca for feeding animals. *K indica* and *A saligna* surpassed the other plants in Na contents while *P americanum* had the highest P concentration. This finding was agreed with Helal *et al.,* (2013). A wide variation of the other minerals concentrations are recorded for the experimental plants, these was in harmony with the finding by

El Shaer (2016) when he set that salt tolerant plants are characterized by moderate digestible crude protein, soluble carbohydrates and high mineral contents, particularly Na, K, Cl and Ca concentrations.

In vitro fermentation profiles

As expected, in vitro fermentation parameters differed (P<0.05) among plant species (Table 3). *A saligna* and *L leucocephala* releasing a lower ruminal NH3-N concentration resulting a significant decreasing in pH values compared to *K indica* plant. This may be associated with the protection of dietary protein from microbial activity by binding tannin-protein complexes which are stable and insoluble at pH 3.6 -7.0 as mention in Table 2. While *P americanum* releasing the lowest ruminal NH3-N and lower pH which may be attributed to their lower nitrogen components (CP and NPN) as shown in Table 1.

Total short chain fatty acid (SCFA's) and acetic acid was higher (P<0.05) for *K indica* and *P americanum* compared to the other plants. Otherwise the results showed that, *A saligna, L leucocephala* and *K indica* presented a pronounced CH4 inhibition while *P americanum* significantly decreased ruminal bacteria. The major challenge of utilize salt-tolerant plants is that their high cell wall contents, phenolic compounds, with variable mineral concentration. As indicated in the present study for the experimental plants species which have a considerable proportion of condensed tannins that may form complexes with proteins and carbohydrates resulting in reduction of their ruminal fermentation. Even though, this generally increases efficiency of ruminal N utilization and intestinal input of N, it can restrict fiber digestion in the rumen, resulting in unsynchronized availability of N and energy to microbes for synthesis VFA (Attia *et al.* 2018) . So, it could be explain the lower SCFA's and acetic acid for rich phenolic plants (Table 2). The variable responses of in vitro gas production among plants could be due to variable levels of PSM. It is noticeable the higher tannins and saponins contents for *A saligna* and *L leucocephala* plants (Table 2) that reflect in reduction of total gas, methane production and total number of ruminal Bacteria as well (Table 3). In this regards, the anti-methanogenic activity of tannins has recently been reported by many studies (Goal and Makkar, 2012 and Liu and Zhou, 2011) the mechanism of tannins may be due to inhibit ruminal microorganisms through bactericidal or bacteriostatic activities, the growth or activity of rumen methanogens and protozoa. However, Saponins have a potent antimicrobial activity and limit the H2 availability for methanogenesis in the rumen, thereby could reduce CH4 production (Bodas *et al*. 2012 and Patra & Saxena, 2009).

In vitro nutrients digestibility

The variations of *in vitro* digestibility values (Table 4) could be due to variable levels of phenolic, tannin activity and cell wall content among the experimental plants. The high IVDMD, IVOMD and IVNDFD for *P americanum* could be due to the lower

phenolic components, absence of condensed tannin and saponin. The lower digestibility of *A. saligna, L leucocephala* could be attributed to the higher concentration of condensed tannins through formation of complexes with dietary carbohydrates which is associated with reduction in organic matter digestibility. Moreover, other researchers (Min *et al*. 2003 and Ammar *et al.* 2005) reported that concentrations of condensed tannins are negatively correlated with *in vitro* dry matter degradability. The result of all nutrients digestibility of the experimental plants was lower than that recorded by Hassan *et al.* (2015) and Shawket *et al.* (2010) at in vivo level, this mainly attributed to supplemented the salt-tolerant plants with different energy resources that could improve ruminal microbes for better utilization of their nutrients.

Table 1. Chemical composition and fiber fraction of the experimental plants

parameters	Plants			
	P americanum	*A saligna*	*L leucocephala*	*K indica*
DM (g/kg)	427	435	383	337
CP (g/kg DM)	54	113	147	87
NPN * (CP %)	13	46	52	43
CF (g/kg DM)	287	268	240	276
NDF (g/kg DM)	560	460	410	584
ADF (g/kg DM)	361	350	260	392
ADL (g/kg DM)	64	108	147	95
Ash (g/kg DM)	115	95	75	141

DM = dry matter, CP = crud protein, CF = crud fiber, NDF = nutrient detergent fiber, ADF = acid detergent fiber, ADL = acid detergent lignin, * The percentage of crude protein of the plant that is non-protein nitrogen x 6.25.

Table 2. Secondary metabolites and some minerals contents of the experimental plants

parameters	Plants			
	P americanum	*A saligna*	*L leucocephala*	*K indica*
Alkaloids (g/kg DM)	11.9	77.9	42.4	21.0
Total oxalate (g/kg DM)	12	18.0	26.0	34.0
Saponin (g/kg DM)	0	105.6	64.8	38.1
Total phenols (g/kg DM)	5.6	95.1	103.0	34.7
Total tannins (g/kg DM)	0	75.0	89.0	48.3
Condensed Tannins (g/kg DM)	0	68.0	59.0	32.0
Mineral contents				
Cu (mg/kg)	35.8	17.0	7.8	6.5
Zn (mg/kg)	60.1	41.8	30.8	29.4
K (g/kg DM)	15.0	21.5	17.5	6.5
Na (g/kg DM)	10.5	15.0	2.0	15.5
Ca (g/kg DM)	7.8	13.4	11.9	5.5
P (g/kg DM)	3.3	1.2	2.0	1.4

Cu = copper, Zn = zinc, K = potassium, Na = sodium, Ca = calcium, P = phosphorus.

Table 3. Comparative in vitro evaluation of the experimental plants on ruminal fermentation

	plants			
Items	*P americanum*	*A saligna*	*L leucocephala*	*K indica*
pH	6.05^{b} ±0.13	6.08^{b} ±0.04	6.06^{b} ±0.01	6.59^{a} ±0.04
NH3-N	16.7 ±0.5	19.1 ±1.0	19.3 ±0.9	20.8 ±0.3
Total SCFA's	82.7^{a} ±1.7	78.5^{b} ±1.74	78.4^{b} ±0.5	83.9^{a} ±1.7
Acetic	52.1^{a} ±1.7	42.2^{b} ±1.9	45.8^{b} ±1.0	48.7^{ab} ±0.6
Propionic	19.0 ±0.6	19.4 ±0.5	19.4 ±0.6	19.7 ±0.3
But.	9.9 ±0.7	10.8 ±0.23	10.0 ±0.4	11.2 ±0.4
Iso- But.	1.5 ±0.1	1.6 ±0.14	1.4 ±0.02	1.4 ±0.03
Val.	1.2 ±0.03	1.3 ±0.03	1.2 ±0.01	1.3 ±0.04
Iso-Val.	0.8±0.07	0.7 ±0.03	0.7 ±0.04	0.6 ±0.04
AC: Pr	2.7 ±0.1	2.2 ±0.14	2.4 ±0.13	2.5 ±0.1
TGP	175.1^{a} ±18.3	121.7^{b} ±3.9	122.1^{b} ±8.8	159.2^{a} ±4.2
CH4	10.54^{a} ±0.22	6.41^{b} ±0.35	7.97^{b} ±0.17	6.81^{b} ±0.32
Bact *10^{6}	7.5^{b} ±0.7	8.8^{ab} ±0.5	8.6^{ab} ±0.4	9.7^{a} ±0.8
Prot *10^{3}	4.7 ±0.4	4.9 ±0.4	4.2 ±0.8	5.1 ±0.1
MP	11.0 ±1.6	12.5 ±1.1	13.5 ±1.7	12.6 ±0.8

a,b,c Means having different superscripts within the same row differed significantly($P < 0.05$), otherwise no significant differences were detected. Total SCFA´s = total short chain fatty acid, But. = Butyric acid, Val. = Valeric acid, AC = acetic acid, TGP = total gas production, CH4 = methane, Bact. = Bacteria, Prot. =protozoa, MP = microbial Protein.

Table 4. in vitro nutrients digestibility of the experimental plants

Items	*P americanum*	*A saligna*	*L leucocephala*	*K indica*
IVDMD	488.3[a] ±7.4	408.6[c] ±6.6	366.7[d] ±5.9	`453.5[b] ±5.6
IVOMD	493.7[a] ±6.9	397.3[c] ±6.2	355.5[d] ±5.6	459.1[b] ±6.9
IVNDFD	443.7[a] ±13.8	363.3[b] ±10.5	329.6[c] ±11.0	426.5[a] ±5.9
IVCPD	109.8 ±12.6	116.6 ±6.0	117.6 ±9.5	127.9 ±10.5
b	135.4 ±4.4	118.5 ±4.0	116.8 ±2.3	164.5 ±8.1
C	0.09 ±0.01	0.11 ±0.02	0.11 ±0.01	0.09 ±0.01

IVDMD = in vitro dry matter digestibility, IVOMD = in vitro organic matter digestibility, IVNDFD = in vitro nutrient detergent fiber digestibility, IVCPD = in vitro crud protein digestibility, b, c = fermentation kinetics.

Conclusion

In conclusion, the results indicated that the salt-tolerant plants used in the experiment could be promising feed resources to decrease energy loss as methane in ruminant diets. However, the presence of secondary metabolites and protein nitrogen (NPN) should be taken into consideration when formulating diets containing salt-tolerant forages for feeding small ruminants.

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