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EVALUATION OF CONOCARPUS (*CONOCARPUS ERECTUS*) LEAVES AND BERMUDA GRASS (*CYNODON DACTYLON* L.) USING CHEMICAL ANALYSIS AND *IN VITRO* GAS PRODUCTION TECHNIQUE

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Abstract

AL KOAIK, F., A. M. EL-WAZIRY, A. I. KHALIL, H. METWALLY and M. A. AL-MAHASNEH, 2014. Evaluation of conocarpus (*Conocarpus erectus*) leaves and Bermuda grass (*Cynodon dactylon* L.) using chemical analysis and *in vitro* gas production technique. *Bulg. J. Agric. Sci.*, 20: 824-829

The present study was conducted to assess of Conocarpus (*Conocarpus erectus*) Leaves and Bermuda Grass (*Cynodon dactylon L.*) fresh or silage using chemical analysis and *in vitro* gas production technique. Rumen liquor was obtained from four slaughtered Naimey sheep fed on barely and alfalfa hay. The mixture of rumen fluid with buffer (1:2 v/v, 30 ml) were placed into each syringe, containing the samples. The gas production was recorded after 3, 6, 9, 12, 24, 48 and 72 h of incubation. There were no significant differences between fresh and silage of conocarpus leaves in organic matter, crude protein, crude fat and ash. Crude fiber was higher in silage form than that in fresh form of conocarpus leaves. The potential degradability (a+b) in fresh conocarpus leaves was significantly ($P<0.05$) increased compared to conocarpus leaves silage. Crude fiber decreased in Bermuda grass silage compared to fresh form. The potential degradability (a+b) in silage form higher ($P<0.05$) than that in fresh form. There was no significant difference ($P>0.05$) in pH between conocarpus leaves in both forms. The pH of Bermuda grass silage was lower ($P<0.05$) than that of fresh. The energy, organic matter digestibility and microbial protein from gas production were determined in conocarpus leaves and Bermuda grass in two forms (fresh and silage). The current study conclude that the fresh form of conocarpus leaves and Bermuda grass appears better than that of silage, therefore it can be used in fresh form to preserve ensiling time as alternative feeds for ruminants.

Key words: Conocarpus leaves, Bermuda grass, silage, gas production, alternative feeds, ruminant

Introduction

There is a shortage of green fodder in Saudi Arabia because of the lack of groundwater and low rainfall. The livestock and small holders in the Kingdom mainly used alfalfa as a traditional green fodder to feed their animals. This crop consumes large amounts of water when planted. However, the nutritionists should find alternative solutions for green fodder and it must be cheaper than alfalfa and when grown consume less water. Under these environments, it is convenient to use shrubs (acacia or atriplex), grasses (Bermuda grass or Rhodes grass) and tree leaves (conocarpus or palm). Leaves of ornamental plants can be used in mixed diet for ruminants as alternative green fodder. Conocarpus *(Conocarpus erectus*) is an ornamental plant. Ornamental trees leaves used in ruminant diets in Africa as green fodder but not used in Asia where it is available in large quantities in

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some arid and semi-arid countries, such as Saudi Arabia. Therefore, it must be to test the leaves of these trees as alternative green fodder for ruminant feeds. On the other hand, Bermuda grass also has been an important green fodder for ruminant feeds. It is widely used in USA and Europe, has high yield potential, and can be used as grazing, hay and silage for ruminants. Bermuda grass was established as high-yielding, high-quality forage for cattle (Hill et al., 2001). However, Saudi Arabia has a lot amounts of Bermuda grass and it can use as green fodder for ruminants. Therefore, the objective of this study was conducted to assess of conocarpus leaves and Bermuda grass in two forms (fresh or silage) using chemical analysis and *in vitro* gas production technique.

Materials and Methods

Silage of conocarpus leaves and Bermuda grass procedure

Conocarpus leaves and Bermuda grass were collected, cleaned to remove any foreign substances and cut to 5-10 cm pieces, and then mixed with 5% palm molasses (v/w). The mixtures of Conocarpus leaves or Bermuda grass with palm molasses were placed in three-liter plastic bucket by hand, firmly compressed, closed and strapped to prevent air ingress. Each group was prepared as three replicates and plastic bucket were left for fermentation at room temperature for 30 days. Samples of silage were opened after fermentation and used for determining of pH, further analyses.

***In vitro* trial**

In vitro gas production technique was conducted according to Menke and Steingass (1988). Rumen liquor was obtained from four sheep fed on barley and alfalfa hay (slaughterhouse). Buffer solution was prepared according to Onodera and Henderson (1980) and placed in a shaker water bath at 39°C under continuous flushing with CO_2. Approximately 200 mg air dry of fresh or silage of conocarpus leaves and Bermuda grass samples were placed into syringe (100 ml, three syringes per sample). The mixture of rumen fluid with buffer (1:2 v/v, 30 ml) was placed into each syringe, containing the samples according to Blümmel and Ørskov (1993). The incubation procedure was repeated three times. The gas production was recorded after 3, 6, 9, 12, 24, 48 and 72 h of incubation. Cumulative gas production values was fitted to the potential equation, Gas (Y) = a + b $(1-\exp^{-ct})$, where; a = the gas production from the immediately soluble fraction, b = the gas production from the insoluble fraction, a+b = potential degradability, c the gas production rate constant for the insoluble fraction (b), t = incubation time, according to the model of Ørskov and McDonald (1979).

Energy and microbial protein estimation

The energy values of fresh and silage of conocarpus leaves and Bermuda grass were calculated from the amount of gas produced at 24 h of incubation with supplementary analysis of crude protein, ash, crud fiber and ether extract (Menke et al., 1979; Menke and Steingass, 1988).

$$\text{ME (MJ/kg DM)} = 2.2 + 0.136GP + 0.057CP + 0.0029CF$$

$$\text{OMD (\%)} = 14.88 + 0.889\,GP + 0.45CP + 0.0651XA,$$

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 (% DM); XA is ash (% DM).

$$\text{NE (Mcal/lb)} = (2.2 + (0.0272*Gas) + (0.057*CP) + (0.149*EE))\ /14.64,$$

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 (MP) was calculated as g/kg OMD according to Czerkawski (1986).

pH measurement

Seventy ml of distilled water were added to 35 g of each sample (fresh or silage) in glass conical flask soaked at 4ºC. The fresh and silage extracts were filtered through 2 layers of gauze and filter paper. The filtrate was stored at -20ºC prior to chemical analysis (Shao et al., 2007 as described by Li et al., 2012). pH values were measured using a digital pH-meter.

Chemical analysis

The fresh and silage samples of conocarpus leaves and Bermuda grass were analyzed for dry matter (DM), crude protein (CP), ether extract (EE), and ash according to AOAC (1995). Neutral detergent fiber (NDF) and acid detergent fiber (ADF) were determined according to Van Soest et al. (1991).

Statistical analysis

All data were analyzed using SPSS packet software was used (SPSS, 2002).

Results

The chemical composition, fiber fraction and pH of conocarpus leaves and Bermuda grass in two forms (fresh and silage) are shown in Table 1. There were no significant differences between fresh and silage of conocarpus leaves in OM, crude protein, ether extract and ash. The values were 86.67, 9.69, 5.27 and 13.33 % for OM, crude protein, ether extract and ash, respectively, in fresh form and 86.66, 10.30, 5.38 and 13.34 % for OM, crude protein, ether extract and ash, respectively, in silage form of conocarpus leaves. Crude fiber was higher in silage form than that in fresh form of conocarpus leaves, and the values were 24.14 and 13.47%, respectively. The crude protein in Bermuda grass silage was decreased compared to fresh Bermuda grass. The values were 14.28 and 18.63% for Bermuda grass silage and fresh Bermuda grass, respectively. Crude fiber decreased in silage form compared to fresh grass, and the values were 23.94 and 17.49% for fresh Bermuda grass and silage of Bermuda grass, respectively. There was no significant (P>0.05) difference in pH between conocarpus leaves in both forms (fresh and silage), and the values were 5.58 and 5.51 for fresh and silage of conocarpus leaves, respectively. The pH of Bermuda grass silage was lower (P<0.05) than that of fresh form and the values were 5.41 and 7.36, respectively.

Figure 1 shows the cumulative gas produced at different incubation times of conocarpus leaves and Bermuda grass in both forms (fresh and silage). The values of gas production extent at 72 h were 26.50 and 31.83 ml/ 200 mg DM of conocarpus leaves in both forms (fresh and silage), and 40.50 40.00 83 ml/ 200 mg DM for Bermuda grass in two forms (fresh and silage), respectively. The potential degradability (a+b) in fresh conocarpus leaves was significantly (P<0.05) increased compared to conocarpus leaves silage (Table 2), and the values were 34.77 and 29.58 ml for fresh and silage of conocarpus leaves, respectively. There was no significant (P>0.05) difference between two forms in gas production rate (c) (Table 2). The values were 0.03 and 0.03 ml/h, for fresh and silage of conocarpus leaves, respectively. For Bermuda grass, potential degradability (a+b) in silage form was higher (P<0.05) than that in fresh form (Table 2). There was no significant (P>0.05) difference in (c) between two forms of Bermuda grass (fresh and silage), and the values were shown in Table 2.

The predicted metabolizable energy (ME, MJ/kg DM), net energy (NE, MJ/kg DM), organic matter digestibility (OMD, %) from gas production, and microbial protein (MP, g/kg OMD) are presented in Table 3. The predicted ME, which calculated from gas production at 24 h incubation, was 4.34 and 5.53 MJ/kg DM for conocarpus leaves si-

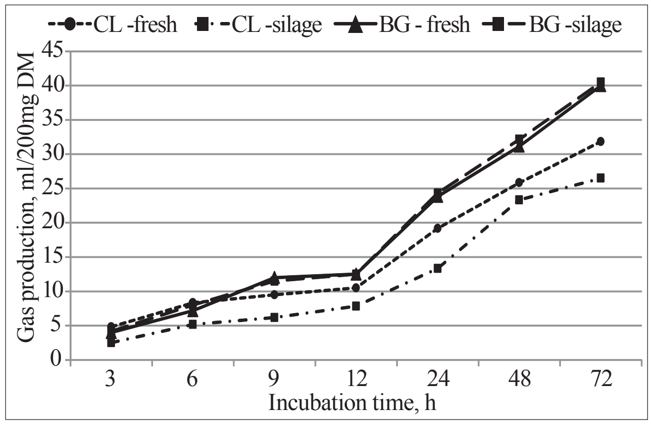

Fig. 1. The cumulative gas produced at different incubation times of conocarpus leaves (CL) and Bermuda grass (BG) in both forms

Table 1
Chemical Composition, Fiber fraction (% of DM basis) and pH of Conocarpus (*Conocarpus erectus*) Leaves and Bermuda grass (*Cynodon dactylon* L.)

	Conocarpus leaves		Bermuda grass	
	Fresh	Silage	Fresh	Silage
Ash	13.33	13.34	14.1	15.77
Organic matter	86.67	86.66	85.9	84.23
Crude protein	9.69	10.3	18.63	14.28
Ether Extract	5.27	5.38	1.96	2.44
Crude fiber	13.47	24.14	23.94	17.49
NFE	58.24	46.84	41.37	50.02
NDF	26.78	41.36	67.44	63.06
ADF	21.87	36.6	34.25	37.39
pH	5.58±0.01^{b}	5.51±0.08^{b}	7.36±0.01^{b}	5.41±0.27^{b}

NDF, Neutral detergent fiber; ADF, Acid detergent fiber; NFE, Nitrogen free extract.
^{ab} Means within a column bearing different superscripts differ (P<0.05).
Mean ± standard deviation.

Table 2
Parameters of gas production produced from Conocarpus (*Conocarpus erectus*) leaves and Bermuda grass (*Cynodon dactylon* L.) during 72h incubation

Items		a+b	c
Conocarpus leaves	Fresh	34.77±11.58^{b}	0.03±0.01^{a}
	Silage	29.58±2.57^{c}	0.03±0.003^{a}
Bermuda Grass	Fresh	34.77±3.43^{b}	0.03±0.01^{a}
	Silage	43.46±0.48^{a}	0.03±0.004^{a}

Cumulative gas production data were fitted to the model of Ørskov and McDonald (1979), Gas (Y) = a + b (1-exp^{-ct}), where; a = the gas production from the immediately soluble fraction, b = the gas production from the insoluble fraction, a+b = potential degradability, c the gas production rate constant for the insoluble fraction (b), t = incubation time.
Mean ± standard deviation.
$^{a-c}$ Means within a column bearing different superscripts differ (P<0.05).

lage and fresh conocarpus leaves (Table 3), respectively. The value of NE was higher in fresh form compared to silage form of conocarpus leaves although there was no significant (P>0.05) between two forms (3.87 and 3.10 MJ/kg DM for fresh and silage of conocarpus leaves, respectively). The OMD was higher (P<0.05) in fresh conocarpus leaves than that of silage. The values were 29.44 and 38.54% for conocarpus leaves silage and fresh conocarpus leaves, respectively (Table 3). The same manner of ME, NE and OMD was found with MP, the values were 35.52 and 46.49 g/kg OMD for silage and fresh forms of conocarpus leaves, respectively (Table 3). For Bermuda grass, the fresh form of grass appears better than that of silage form in ME, NE, OMD and MP (Table 3).

Discussion

As the results of the chemical composition, fiber fraction and pH of conocarpus leaves and Bermuda grass, there were no significant differences between fresh and silage of conocarpus leaves in OM, crude protein, crude fat and ash. Ziaei and Sharifi Hosseini (2009) found that supplementation of palm leaves with energy supplementary had no significant effect on crude protein content of silages. They results are in agreement with the current results of crude protein in both forms of conocarpus leaves although the type of leaves was different. The higher crude fiber in ensiled conocarpus leaves was probably due to the decrease of soluble carbohydrate or nitrogen free extract (NFE) (Table 1). Fiber fractions (NDF and ADF) were increased in ensiled of conocarpus leaves with increasing of crude fiber and decreasing of NFE may be due to probably lignin (undetermined component) content, because hemicelluloses was not changed in both forms (about 5%). The decrease of crude protein in silage compared to fresh Bermuda grass may be due to some crude protein in grass silage was converted into ammonia during ensiling (Abarghoei et al., 2011), and may be also due to the increase of ash and decrease of organic matter in silage form (Table 1). Crude fiber decreased in silage form compared to fresh grass, and the lower crude fiber in ensiled Bermuda grass was probably due to the degradation of cell wall by cellulolytic clostridia or acid hydrolysis (McDonald et al., 1991; Baytok et al., 2005). There was no significant difference in pH between conocarpus leaves in both forms (fresh and silage). The pH of Bermuda grass silage was lower (P<0.05) than that of fresh form. The grade of good silage, the pH must be lower than 4.0, with the exception of DM content is higher than 30% (Dulphy and Demarquilly, 1981; Vanbelle et al., 1981; Demarquilly and Andrieu, 1988).

Figure 1 shows the cumulative gas produced at different incubation times of conocarpus leaves and Bermuda grass in both forms (fresh and silage). The values of gas production extent at 72 h were 26.50 and 31.83 ml/ 200 mg DM of conocarpus leaves in both forms (fresh and silage), and 40.50 40.00 83 ml/ 200 mg DM for Bermuda grass in two forms (fresh and silage), respectively. The potential degradability (a+b) in fresh conocarpus leaves was significantly (P<0.05) increased compared to conocarpus leaves silage (Table 2), may be due to the decreasing of crude fiber and increasing of soluble carbohydrates in fresh forms. There was no significant difference between two forms in gas production rate (c) (Table 2). For Bermuda grass, potential degradability (a+b) in silage form higher (P<0.05) than that in fresh form may be due to the decreasing of crude fiber and increasing of soluble carbohydrates in silage forms (Table 2). There was no significant difference in (c) between two forms of Bermuda grass (fresh and silage). Gas production procedure has been widely used to assess the nutritive value of feedstuffs, plant products/ by-products, legumes, grasses and tropical plants (El-Waziry et al., 2005; El-Waziry, 2007; El-Waziry et al., 2007; Razligi et al., 2011; Getachew et al., 1998).

The predicted ME, which calculated from gas production at 24 h incubation, was 4.34 and 5.53 MJ/kg DM for conocar-

Table 3
Predicted of metabolizable energy (ME), net energy (NE), organic matter digestibility (OMD) and microbial protein (MP*) in vitro* from Conocarpus *(Conocarpus erectus*) leaves and Bermuda grass (*Cynodon dactylon* L.) during 72h incubation

Items		ME (MJ/kg DM)	NE (MJ/kg DM)	OMD %	MP g/kg OMD*
Conocarpus leaves	Fresh	5.53±0.45^{b}	3.87±0.19^{b}	38.54±2.63^{b}	46.49±3.17^{b}
	Silage	4.34±0.54^{c}	3.10±0.43^{b}	29.44±4.18^{d}	35.52±5.04^{d}
Bermuda Grass	Fresh	6.22±0.43^{a}	4.68±0.86^{a}	43.14±3.55^{a}	52.04±4.28^{a}
	Silage	5.65±0.52^{b}	3.55±0.32^{b}	37.75±4.09^{c}	45.54±4.93^{c}

Mean ± standard deviation.
$^{a-c}$ Means within a column bearing different superscripts differ (P<0.05).
* Calculated according to Czerkawski (1986).

pus leaves silage and fresh conocarpus leaves (Table 3), respectively. The value of NE was higher in fresh form compared to silage form of conocarpus leaves although there was no significant between two forms (3.87 and 3.10 MJ/kg DM for fresh and silage of conocarpus leaves, respectively). The energy obtained from fresh form of conocarpus leaves compared to silage form may probably due to the high content of soluble carbohydrates and ether extract. The OMD was higher (P<0.05) in fresh conocarpus leaves than that of silage. The increase of OMD in fresh form of conocarpus leaves may probably due to the increase of soluble carbohydrates and decreased of crude fiber content compared to silage form of conocarpus leaves. The same manner of ME, NE and OMD was found with MP, the values were 35.52 and 46.49 g/kg OMD for silage and fresh forms of conocarpus leaves, respectively (Table 3). The high value of MP for fresh form compared to silage form may due to the high values of OMD, ME and NE. For Bermuda grass, the fresh form of grass appears better than that of silage form in ME, NE, OMD and MP (Table 3) may due to probably high content of organic matter, crude protein, NDF and low content of ash (Table 3). The result of Reyes et al. (2009) is agreement with the present study for ME and the values were 6.4 and 6.22 MJ/kg DM, respectively.

Conclusion

The present study conclude that conocarpus leaves and Bermuda grass could be suitable for ruminants as a source of green fodders which used as an energy source for host animal and microbes in the rumen. The fresh form of both appears better than that of silage, therefore it can be used in fresh form to preserve ensiling time as alternative feeds for ruminants.

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