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PASTURE AND FORAGE UTILIZATION

In vitro evaluation of palm fronds as feedstuff on ruminal digestibility and gas production

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ABSTRACT. This study was carried out to evaluate using palm fronds only or supplemented with fibrolytic enzymes as alternative roughage on the ruminal nutrients digestibility and gas production. Treatments were: clover hay (*Trifolium alexandrinum*), palm fronds only and palm fronds plus fibrolytic enzymes (enzymes were mixed with palm fronds in rate of 4 g kg^{-1} of dry matter (DM). The results showed a reduction ($p < 0.05$) in gas production, ammoniac nitrogen (NH_3-N, DM and organic matter (OM) digestibility in palm fronds compared with clover hay. While supplementing palm fronds with fibrolytic enzymes improved DM and OM digestibility compared with palm fronds only without ($p < 0.05$) differences with clover hay. Also, supplementing palm fronds with fibrolytic enzymes reduced ($p < 0.05$) gas production compared with other treatments. It could be concluded that adding fibrolytic enzymes improved the utilization of palm fronds as alternative roughage without negative effect on nutrients digestibility and reduced gas production which improve the environmental aspects of feeding ruminant animals.

Keywords: fibrolytic enzymes; gas production; rumen; nutrients digestibility.

Avaliação *in vitro* das folhas de palmeiras como alimento de digestibilidade de ruminais e produção de gás

RESUMO. Este estudo teve como objetivo avaliar as folhas de palmeiras sozinhas ou suplementadas com enzimas fibrolíticas, como uma alternativa de degradação da fibra na digestibilidade de nutrientes de ruminantes e produção de gás. Os tratamentos foram: Feno (*Trifolium alexandrinum*), folhas de palmeiras, e folhas de palmeiras com adição de enzimas fibrolíticas (as enzimas foram misturadas com a folha com taxa de 4g kg^{-1} matéria seca (MS). Os resultados mostraram uma redução da produção de gás ($p < 0.05$), nitrogênio amoniacal (NH_3-N), MS e matéria orgânica (MO) na digestibilidade das folhas da palmeira comparadas com o feno. Enquanto a suplementação de folhas com as enzimas fibrolíticas, aumentou a digestibilidade DM e OM comparado com as folhas de palmeira, que não teve diferenças com o feno ($p < 0.05$). Também, suplementando as folhas de palmeiras com as enzimas fibrolíticas reduziram em ($p < 0.05$) a produção de gás comparado com os outros tratamentos. Pode ser concluído que a adição das enzimas fibrolíticas aumenta a utilização da folha da palmeira como uma alternativa de forragem a qual não possui efeitos negativos na digestibilidade de nutrientes e reduz a produção de gás, a qual melhora os aspectos dos meios da alimentação de animais ruminantes.

Palavras-chave: enzimas fibrolíticas; produção de gás; rúmen; digestibilidade de nutrientes.

Introduction

In arid and semi-arid regions, dates production is commonly planted and considered one of the main food sources. Food and Agriculture Organization of the United Nations (FAO, 2014) estimated universal date production by 7.6 million tons, Egypt is considered the highest producing country of dates by 19% of global production. Palm fronds are one of the main by-products of date cultivation which could be used as alternative roughage in ruminant feeding (El Hag & Ekhanjari, 2000). Different studies were carried out to investigate the efficiency of including date palm fronds in ruminant diets; results showed that chopped date palm fronds palatable for ruminants (El Hag & Al Shargi, 1998; Mahgoub et al., 2004; Mahgoub et al., 2005).

One of the greatest disadvantages of ruminal fermentation is the losing of energy as gas production (CH_4 and CO_2) as final products off utilizing of hydrogen produced from anaerobic metabolism of carbohydrates in the rumen. The CH_4 produced from ruminal fermentation represents 16% of gross energy intake in ruminant

animals. This may cause a limited productive performance (Ahmed et al., 2016; Kholif et al., 2014) and contribute as environmental pollutant which contributes to global warming (Calsamiglia, Busquet, Cardozo, Castillejos, & Ferret, 2007; Johnson & Johnson, 1995).

Palm frond is characterized as a feed with high content of cellulolytic components, low nutrients digestibility coefficients and high content of crude fiber such as cellulose and anti-nutrients factors such as tannins (87.7% DM, 4.1% CP, 72.4% NDF, 52.3% ADF and 14.6% ADL (Abd El Tawab, Khattab, El-Zaiat, Matloup, & Hassan, 2016) and some anti-nutritional factors (Khattab, Sooud, Salem, Mansour, & Younan, 2008; Kholif et al., 2005) that make it difficult to digested. Different methods used to improve the nutritional value of agricultural by-products especially cellulolytic feeds such as cellulase enzyme that can be effective additive with agricultural by-product to produce simple glucose units (Smith, 1996). Increasing the nutritive value of highly fiber feedstuffs through adding fibrolytic enzymes especially cellulase enzyme were highly investigated. Sujani and Seresinhe (2015) stated that fibrolytic enzymes play a direct role in animals feeding by improving digestion in ruminants. Feng, Hunt, Pritchard, and Julien (1996) reported that fibrolytic enzymes could improve digestion in ruminants. Moreover, Nussio et al. (1997) found that supplementing diet with enzymes just before feeding was effective in improving nutrients digestion. On the other hand, Tannase enzyme can hydrolyzes tannins substrates such as tannic acid, propyl gallate, methyl gallate, epicatechin gallate, digallic acid and epigallocatechin gallate releasing gallic acid (Curiel et al., 2009; Lu & Chen, 2007). Abd El Tawab et al. (2015) suggested that using tannase enzyme as a feed additive for lactating goats' diets decreased tannins contents and improved nutrients digestibility; and milk yield.

The aim of the present study was to evaluate palm fronds only or supplemented with fibrolytic enzymes as alternative roughage on the ruminal nutrients digestibility and gas production.

Material and methods

Substrates preparation and enzyme production

Egyptian clover hay (*Trifolium alexandrinum*) and Palm fronds (Table 1) were used as incubation substrates. Clover hay and palm fronds samples were oven-dried at 60°C for 48h until constant weight; and ground through a 1-mm screen and stored in plastic bags for subsequent determination of chemical composition and *in vitro* gas production experiment. Investigated cellulase and tannase enzymes produced from anaerobic bacteria (*Clostridium butyricum*). Each g of enzyme mixture contains 5179 IU g^{-1} of cellulase and 866 IU g^{-1} of tannase (Khattab, El Tawab, & Fouad, 2017).

In-vitro procedures

In-vitro incubation procedures were carried out according to Menke and Steingass (1988). As described by Khattab, Ebeid, Abd El Tawab, El-Nor, and Aboamer (2016), rumen fluid was collected from 3 ruminally cannulated Holstein dairy cows (mean weight 680 ± 30 kg). The rumen fluid was collected before morning feeding, mixed and squeezed through a 4-layers cheesecloth under continuous flushing with CO_2 and immediately transported to laboratory at 39°C (used as a source of *inoculum*). Treatments were: clover hay (*Trifolium alexandrinum*), palm fronds only and palm fronds plus fibrolytic enzymes (enzymes were mixed with palm fronds in rate of 4 g kg^{-1} DM**)**. Each treatment was tested in eight replicates accompanied by blank bottles (no substrate). the experiment run were repelicated in different weeks. Substrate (400 mg) was added to the incubation bottles of 100 mL capacity. Each bottle was filled with 40 mL of the incubation medium (292 mg K_2HPO_4, 240 mg KH_2PO_4, 480 mg $(NH_4)_2SO_4$, 480 mg NaCl, 100 mg $MgSO_4.7H_2O$, 64 mg $CaCl_2.{}_2H_2O$, 4 mg Na_2CO_3 and 600 mg cysteine hydrochloride) per 1 liter of double distilled water (ddH_2O) and dispensed anaerobically in the 1:4 (v/v) ratio. Then the bootles were incubated at 39°C for 48h.

Substrates sampling and gas production recording

After 48h of incubation, gas production (GP) was recorded using the pressure reading technique as described by Theodorou, Williams, Dhanoa, McAllan, and France (1994) then bottles were uncapped, pH was measured using a pH meter, and the contents of each bottle were filtered to obtain the non-fermented residue for determination of degraded substrate

Chemical analysis

The non-fermented residues were dried, weighed and digestibility calculated using the equations as follows:

$$In-vitro\ d\text{ry matter disappearance (\%)} = \left[\frac{\text{initial DM input} - \text{DM residue} - \text{Blank}}{\text{initial DM input}}\right] * 100$$

$$In-vitro\ \text{organic matter disappearance (\%)} = \left[\frac{\text{initial OM input} - \text{OM residue} - \text{Blank}}{\text{initial OM input}}\right] * 100$$

Neutral Detergent Fiber (NDF) and Acid Detergent Fiber (ADF) were analyzed by Ankom200 Fiber Analyzer (Ankom Technology Corporation, Fairport, NY) according to Van Soest, Robertson, and Lewis (1991). Microbial protein production was calculated as 19.3 g microbial nitrogen per kg OMD according to Czerkawski and Cheng (1988). The NH_3-N concentration was determined as described by Khattab, Abd-El-Gawad, El-Nor, and El-Sherbiny (2015).

Statistical analysis

Data were statistically analysed using GLM procedure of Statistical Analysis System (SAS, 2009), version 9.2. Significant differences between means of treatments were carried out by the Duncan's test, and the significance threshold was set at $p < 0.05$.

Results and discussion

Chemical composition

Table 1 shows the chemical composition of clover hay and palm fronds. The results indicated that content of clover hay and palm fronds are similar in DM and OM. While, the differences between clover hay and date fronds are showed in crude protein and fiber contents, palm fronds showed higher values in fiber contents but lower in CP than found in clover hay.

Table 1. Chemical composition of Egyptian clover hay and palm fronds.

Nutrients	Clover hay	Palm frond
	% on DM basis	
Dry matter	88.34	87.74
Organic matter	94.69	94.05
Crude protein	12.75	4.12
Crude fiber	24.81	38.77
Ether extract	1.17	1.89
Nitrogen free extract	55.96	49.27
Natural detergent fiber	42.73	72.38
Acid detergent fiber	25.37	52.31
Acid detergent lignin	6.85	14.55
Hemicellulose	17.36	20.07
Cellulose	18.52	37.76
Ash	5.31	5.95

Gas production

Table 2 shows gas production. The results showed that palm fronds and supplemented palm fronds with fibrolytic enzymes decreased ($p < 0.05$) total gas production, adding fibrolytic enzymes recorded the lowest values of total gas production, GP g^{-1} DM and GP g^{-1} OM (86.7, 226.6 and 218.9 mL respectively) followed by palm fronds (90.0, 254.4 and 245.7 mL, respectively) compared with clover hay (115.8, 322.6 and 315.9 mL, respectively). The current finding showed a reduction in gas production and GP per each gram of DM or OM as using palm fronds only or with adding fibrolytic enzymes. It is well known that there is negative correlation between gas production and cell wall contents (NDF and ADF) which tend to reduce the microbial activity (De Boever, Aerts, Vanacker, & De Brabander, 2005). Different studies stated that addition of fibrolytic enzymes reduced methane production (Hernández et al., 2017; Ahmed et al., 2016; Salem et al., 2015).

Addition of fibrolytic enzymes stimulate of reductive acetogens in the rumen that alters hydrogen (H_2) metabolism and its utilization by methanogens in a manner that reduces CH_4 formation and emissions (Stewart, Flint, & Bryant, 1997).

Table 2. Effect of feedstuffs on gas production.

Parameters	Clover hay	Palm fronds	Palm fronds + enzymes	SEM	P > F
Total GP	115.8 a	90.0 b	86.7 b	3.92	0.001
GP g^{-1} DM	322.6 a	254.4 b	226.6 b	14.70	0.001
GP g^{-1} OM	315.9 a	245.7 b	218.9 b	16.97	0.001

Means in the same row with different superscripts differ, $p < 0.05$. SEM = standard error of the mean.

Ruminal pH and NH_3-N concentrations

The effect of different treatments on rumen pH and NH_3-N are presented in Table 3. The results showed that even palm fronds only or supplemented with fibrolytic enzymes decreased ($p < 0.05$) rumen NH_3-N concentrations compared with clover hay. These reductions of NH_3-N concentrations are reflecting of the low contents of crude protein as shown in chemical composition (Table 1). Also, might reflect that using palm fronds only or with adding fibrolytic enzymes affected on microbial biomass of rumen microbes which clear as microbial protein (Table 4).

Table 3. Effect of feedstuffs on pH and ammonia nitrogen concentration.

Parameters	Clover hay	Palm fronds	Palm fronds + enzymes	SEM	Pr. > F
pH	6.50	6.92	6.83	0.078	0.039
NH_3-N (mM)	11.4 a	7.4 b	9.1 ab	0.79	0.092

Means in the same row with different superscripts differ, $p < 0.05$. SEM = standard error of the mean.

Dry matter and organic matter digestibility

Evaluating palm frond only or supplemented with fibrolytic enzymes on ruminal in vitro digestibility are shown in table (4). Results showed that clover hay and adding fibrolytic enzymes to palm fronds decreased ($p < 0.05$) the DM digestibility as compared with palm fronds only. But there were no differences between palm fronds only or palm fronds supplemented with fibrolytic enzymes on OM digestibility and microbial protein, but there was a decrease in OM digestibility and

microbial protein in palm fronds compared with control. The results of dry matter and organic matter digestibility showed a reduction in palm fronds either with or without fibrolytic enzymes compared with clover hay. The low digestibility coefficients of DM and OM are most probably due to the high fiber content of palm fronds (Mahgoub, Kadim, Al-Busaidi, Annamalai, & Al-Saqri, 2007). Results showed that palm fronds with or without fibrolytic enzymes reduced ($p < 0.05$) microbial protein compared with clover hay. These findings might due to reduction of palm fronds CP contents and high contents of fibers which reflected on ruminal fermentation and releasing ammonia which is necessary component to microbial biomass growth.

Table 4. Effect of experimental diets on nutrients digestibility.

	Clover hay	Palm fronds	Palm fronds + enzymes	SEM	P < Value
DM digestibility (g kg^{-1})	592.6 a	464.4 b	533.4 b	3.63	0.002
OM digestibility (g kg^{-1})	516.1 a	431.9 b	491.2 ab	1.25	0.001
Microbial protein (mg g^{-1} DM)	2545.2 a	1978.2 b	1904.5 ab	10.12	0.001

Means in the same row with different superscripts differ, $p < 0.05$. SEM = standard error of the mean.

Conclusion

The results of the study showed that potential of using palm fronds as alternative roughage especially, if supplemented with fibrolytic enzymes to improve its digestibility and reducing gas produced through ruminal fermentation which could improve the potential of reducing gases emission from ruminant animals.

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