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.

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; Khollif et al., 2014) and contribute to environmental pollutants which contributes to global warming (Calsamiglia, Busquet, Cardozo, Castillejos, & Ferret, 2007; Johnson & Johnson, 1995).

Palm frond is characterized as a feed high with content of cellulosic 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, Khollab, El-Zaiat, Matloup, & Hassan, 2016) and some anti-nutritional factors (Khattab, Sooud, Salem, Mansour, & Younan, 2008; Khollif et al., 2005) that make it difficult to digested. Different methods used to improve the nutritional value of agricultural by-products especially cellulosic 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 was 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, Nussios 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 Khollab, 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 CO₂ 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 replicated 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 K₂HPO₄, 240 mg KH₂PO₄, 480 mg (NH₄)₂SO₄, 480 mg NaCl, 100 mg MgSO₄.7H₂O, 64 mg CaCl₂.2H₂O, 4 mg Na₂CO₃ and 600 mg cysteine hydrochloride) per 1 liter of double distilled water (ddH₂O) and dispensed anaerobically in the 1:4 (v/v) ratio. Then the bottles 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:

\[
\text{In – vitro dry matter disappearance} = \frac{\text{initial DM input} - \text{DM residue - Blank}}{\text{initial DM input}} \times 100
\]

\[
\text{In – vitro organic matter disappearance} = \frac{\text{initial OM input} - \text{OM residue - Blank}}{\text{initial OM input}} \times 100
\]

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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.

<table>
<thead>
<tr>
<th>Nutrients</th>
<th>Clover hay</th>
<th>Palm frond</th>
</tr>
</thead>
<tbody>
<tr>
<td>% on DM basis</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dry matter</td>
<td>88.34</td>
<td>87.74</td>
</tr>
<tr>
<td>Organic matter</td>
<td>94.69</td>
<td>94.05</td>
</tr>
<tr>
<td>Crude protein</td>
<td>12.75</td>
<td>4.12</td>
</tr>
<tr>
<td>Crude fiber</td>
<td>24.81</td>
<td>38.77</td>
</tr>
<tr>
<td>Ether extract</td>
<td>1.17</td>
<td>1.89</td>
</tr>
<tr>
<td>Nitrogen free extract</td>
<td>53.96</td>
<td>49.27</td>
</tr>
<tr>
<td>Natural detergent fiber</td>
<td>42.73</td>
<td>72.38</td>
</tr>
<tr>
<td>Acid detergent fiber</td>
<td>25.37</td>
<td>52.31</td>
</tr>
<tr>
<td>Acid detergent lignin</td>
<td>6.85</td>
<td>14.55</td>
</tr>
<tr>
<td>Hemicellulose</td>
<td>17.36</td>
<td>20.07</td>
</tr>
<tr>
<td>Cellulose</td>
<td>18.52</td>
<td>37.76</td>
</tr>
<tr>
<td>Ash</td>
<td>5.31</td>
<td>5.95</td>
</tr>
</tbody>
</table>

**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.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Clover hay</th>
<th>Palm fronds</th>
<th>Palm fronds + enzymes</th>
<th>SEM</th>
<th>P &gt; F</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total GP</td>
<td>115.8 a</td>
<td>90.0 b</td>
<td>86.7 b</td>
<td>3.92</td>
<td>0.001</td>
</tr>
<tr>
<td>GP g$^{-1}$ DM</td>
<td>322.6 a</td>
<td>254.4 b</td>
<td>226.6 b</td>
<td>14.79</td>
<td>0.001</td>
</tr>
<tr>
<td>GP g$^{-1}$ OM</td>
<td>315.9 a</td>
<td>245.7 b</td>
<td>218.9 b</td>
<td>16.97</td>
<td>0.001</td>
</tr>
</tbody>
</table>

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.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Clover hay</th>
<th>Palm fronds</th>
<th>Palm fronds + enzymes</th>
<th>SEM</th>
<th>Pr. &gt; F</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
<td>6.50</td>
<td>6.92</td>
<td>7.14</td>
<td>0.078</td>
<td>0.039</td>
</tr>
<tr>
<td>NH$_3$-N (nM)</td>
<td>11.4a</td>
<td>7.4b</td>
<td>9.1ab</td>
<td>0.79</td>
<td>0.092</td>
</tr>
</tbody>
</table>

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-Saqqi, 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.

<table>
<thead>
<tr>
<th></th>
<th>Clover hay</th>
<th>Palm fronds</th>
<th>Palm fronds + enzymes</th>
<th>SEM</th>
<th>P &lt; Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>DM digestibility (g kg$^{-1}$)</td>
<td>592.6 a</td>
<td>464.4 b</td>
<td>533.4 b</td>
<td>3.63</td>
<td>0.002</td>
</tr>
<tr>
<td>OM digestibility (g kg$^{-1}$)</td>
<td>516.1 a</td>
<td>431.9 b</td>
<td>491.2 ab</td>
<td>1.25</td>
<td>0.001</td>
</tr>
<tr>
<td>Microbial protein (mg g$^{-1}$)</td>
<td>2545.2 a</td>
<td>1978.2 b</td>
<td>1904.5 ab</td>
<td>10.12</td>
<td>0.001</td>
</tr>
</tbody>
</table>

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.

References


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