Research Article
Utilizing of Celery and Thyme as Ruminal Fermentation and Digestibility Modifier and Reducing Gas Production

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Abstract
Background and Objectives: Improving ruminant environmental impacts and productivity get a great interest in last years, herbal plants were investigated as feed additive for decreasing gas production from rumen and enhancing nutrients digestibility. So, in the current study was carried out to investigate the effect of supplementing ruminant diets with different levels of thyme or celery on rumen fermentation, digestibility and gas production. Materials and Methods: Seven experimental treatments were done using rumen in vitro batch culture technique. Treatments were: 60% CFM, 40% clover hay (control), control diet+5 g thyme kg⁻¹ DM (T1), control diet+10 g thyme kg⁻¹ DM (T2), control diet+20 g thyme kg⁻¹ DM (T3), control diet+5 g⁻¹ celery kg⁻¹ DM (T4), control diet+10 g⁻¹ celery kg⁻¹ DM (T5), control diet+20 g celery kg⁻¹ DM (T6), control diet+0.4 g salinomycin kg⁻¹ DM (T7). Results: The obtained results showed no significant (p>0.05) change in ruminal pH, neutral detergent fiber (NDF) and acid detergent fiber (ADF) degradation. Thyme addition to diet (T1 and T2) significantly lowered (p<0.05) total gas production (TGP) compared with control (152, 152 vs. 157 mL, respectively). Dry matter and organic matter disappearance (DMd, OMd) appeared showed no significant difference (p<0.05) between control and thyme treatments (T1, T2 and T3) and celery treatments (T4, T5 and T6). Conclusion: It could be concluded the adding thyme or celery at low levels to ruminant diets could improve ruminal fermentation and reducing gas production without adverse effect on nutrients digestibility.

Key words: Thyme, celery, rumen fermentation, gas production, nutrients digestibility, ionophores


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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.
INTRODUCTION

The term "sustainability" has gained a strong attention in recent years from both scientific and productive point of view. Interestingly, agriculture contribute in all three pillars of sustainability in both positive way, by triggering the worldwide economy and social acceptability and negative way; being a source of environmental pollution and high concentration of greenhouse gasses (GHG)\(^1\). Hobson and Stewart\(^1\) highlighted that methane is one of the rumen byproduct released due to the inability of the ruminants to benefit from hydrogen and carbon dioxide production during fermentation, which is later converted to \(CH\(_4\)\) by the methanogenic population. It was estimated that dairy farms contribute in no less than 3% of the total GHG\(^4\) that is in addition to a loss in feed energy by up to 12% in the form of emitted methane\(^5\). Developing strategies to mitigate methane from dairy animals represents an inevitable challenge in both environmental and economic perspectives. Antibiotics were widely used as a supplement in ruminant diet for their ability to modulate the rumen fermentation, mitigate methane emission and improve animal productivity has been proven for years\(^6\). However, the excess of using antibiotic would lead animals to develop a resistance against different drugs, additionally, antibiotics transfer to ruminant product (milk or meat) which could threaten human health\(^7\). Since European Union (EU) banned using antibiotics as feed additive, this radical change in laws resulted in an intensive development of research that relates to find effective natural compounds that could inhibit GHG and modulate the rumen fermentation and fatty acid composition in the produced milk\(^8\). Development of antibiotics resistant bacteria cannot be ignored; therefore, it is essential to find more desirable alternatives\(^9\) from natural and safe sources.

Recently, many attempts were carried out to evaluate plant secondary metabolites (saponins, tannins and essential oils) as natural rumen modifers\(^10\). Generally, the antimicrobial activity of essential oils, is mainly a result of its content of terpenoid and phenolic compounds, these compounds have noted as antimicrobial activity by inactivation of some microbial enzymes. Many herbs contains essential oils have been investigated (such as lemongrass, galangal, rosemary, cinnamonum, etc.)\(^11\) on modifying rumen fermentation and positivity affected volatile fatty acids, methane production, starch, protein degradation and decrease ruminal bio-hydrogenation. So, the current study was aimed to evaluate the effect of two herbs (thyme and celery) at different supplementing levels on rumen fermentation and gas production, dry matter and fiber degradation and ammonia production in vitro.

MATERIALS AND METHODS

The experiment was carried out at the laboratory of dairy animal production, National Research Centre (Egypt) during spring of 2019 (March-May, 2019).

Experimental treatments: In vitro incubation procedures were carried out as described by Khattab \textit{et al.}\(^11\), rumen fluid was collected before morning feeding from 3 ruminally cannulated Holstein dairy cows (mean weight 680±30 kg), mixed and squeezed through 4 layers cheese cloth under continuous flushing with CO\(_2\) and immediately transported to laboratory at 39°C (used as a source of inoculum)\(^11\). Treatments were: 60% CFM, 40% clover hay (control), control diet+5 g thyme kg\(^{-1}\) DM (T1), control diet+10 g thyme kg\(^{-1}\) DM (T2), control diet+20 g thyme kg\(^{-1}\) DM (T3), control diet+5 g celery kg\(^{-1}\) DM (T4), control diet+10 g celery kg\(^{-1}\) DM (T5), control diet+20 g celery kg\(^{-1}\) DM (T6) and control diet+0.4 g salinomycin kg\(^{-1}\) DM (T7) (Table 1). Each treatment was tested in 8 replicates accompanied by blank bottles (no substrate). The experiment run were replicated twice 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\(_2\)HPO\(_4\), 240 mg KH\(_2\)PO\(_4\), 480 mg (NH\(_4\))\(_2\)SO\(_4\), 480 mg NaCl, 100 mg MgSO\(_4\), 7H\(_2\)O, 64 mg CaCl\(_2\).2H\(_2\)O, 4 mg Na\(_2\)CO\(_3\), and 600 mg cysteine hydrochloride)/1 L of double distilled water (ddH\(_2\)O) and dispensed anaerobically in the 1:4 (v/v) ratio\(^12\). Then the bootles were incubated at 39°C for 48 h.

<table>
<thead>
<tr>
<th>Items</th>
<th>DM (%)</th>
<th>OM (%)</th>
<th>CP (%)</th>
<th>EE (%)</th>
<th>NDF (%)</th>
<th>ADF (%)</th>
<th>Ash (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CFM</td>
<td>93.09</td>
<td>93.98</td>
<td>12.73</td>
<td>6.23</td>
<td>49.98</td>
<td>18.09</td>
<td>6.02</td>
</tr>
<tr>
<td>Clover hay</td>
<td>93.86</td>
<td>88.31</td>
<td>11.97</td>
<td>6.10</td>
<td>43.49</td>
<td>33.06</td>
<td>11.69</td>
</tr>
<tr>
<td>Experimental diet composition</td>
<td>93.40</td>
<td>91.71</td>
<td>12.43</td>
<td>6.18</td>
<td>47.38</td>
<td>24.08</td>
<td>8.29</td>
</tr>
</tbody>
</table>

CFM: Concentrate feed mixture, consisted of 20% yellow corn, 20% wheat bran, 32% sugar beet pulp, 5% soybean meal, 20% cottonseed meal, 0.1% sodium bicarbonate, 1.5% limestone, 1% NaCl, 0.1% vitamins and 0.3% minerals, DM: Dry matter, OM: Organic matter, CP: Crude protein, EE: Ether extract, NDF: Neutral detergent fiber, ADF: Acid detergent fiber
Substrates sampling and gas production recording: After 48 h of incubation, gas production (GP) was recorded using the pressure reading technique according to Khattab and Abd El Tawab, bottles were uncapped, pH was measured using a pH meter and then contents were filtered of each bottle to obtain the non-digested residue for determination of degradation percent.

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

\[
\text{In vitro dry matter disappearance (\%)} = \frac{\text{Initial DM input-DM residue-Blank}}{\text{Initial DM input}} \times 100
\]

\[
\text{In vitro organic matter disappearance (\%)} = \frac{\text{Initial OM input-OM residue-Blank}}{\text{Initial OM input}} \times 100
\]

Neutral detergent fiber (NDF) and acid detergent fiber (ADF) were analyzed by Ankam200 Fiber Analyzer (Ankom Technology Corporation, Fairport, NY) according to Van Soest et al. Microbial protein production was calculated as 19.3 g microbial nitrogen per kg OMD according to Khattab et al.. The NH3-N concentration was determined as described by Khattab et al.

Statistical analysis: Data were statistically analyzed using GLM procedure of SAS software (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.

Table 2: Effect of supplementing diet with different level of thyme or celery on some ruminal parameters

<table>
<thead>
<tr>
<th>Items</th>
<th>Control</th>
<th>T1</th>
<th>T2</th>
<th>T3</th>
<th>T4</th>
<th>T5</th>
<th>T6</th>
<th>T7</th>
<th>Pr&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
<td>6.92</td>
<td>6.89</td>
<td>6.88</td>
<td>6.86</td>
<td>6.87</td>
<td>6.87</td>
<td>6.88</td>
<td>7.00</td>
<td>0.07</td>
</tr>
<tr>
<td>Ammonia-N (mg/100 mL)</td>
<td>37.10a</td>
<td>35.70a</td>
<td>32.60a</td>
<td>30.50a</td>
<td>31.40a</td>
<td>24.00f</td>
<td>34.00a</td>
<td>37.00a</td>
<td>0.0016</td>
</tr>
<tr>
<td>SCFA (mmol)</td>
<td>6.97b</td>
<td>6.73b</td>
<td>6.74b</td>
<td>6.96b</td>
<td>6.78b</td>
<td>6.80b</td>
<td>7.06b</td>
<td>6.07b</td>
<td>0.0001</td>
</tr>
<tr>
<td>Microbial protein (mg g(^{-1}) DM)</td>
<td>281.06c</td>
<td>280.12c</td>
<td>280.90c</td>
<td>290.35c</td>
<td>281.76c</td>
<td>283.65c</td>
<td>295.81a</td>
<td>259.22a</td>
<td>0.0073</td>
</tr>
</tbody>
</table>

Control: 60% CFM, 40% clover hay, T1: Control diet + 5 g thyme kg\(^{-1}\) DM, T2: Control diet + 10 g thyme kg\(^{-1}\) DM, T3: Control diet + 20 g thyme kg\(^{-1}\) DM, T4: Control diet + 5 g celery kg\(^{-1}\) DM, T5: Control diet + 10 g celery kg\(^{-1}\) DM, T6: Control diet + 20 g celery kg\(^{-1}\) DM, T7: Control diet + 0.4 g salinomycin kg\(^{-1}\) DM, SCFA: Short chain fatty acids, different superscript letters in a the same row differ significantly (p<0.05).

Table 3: Effect of supplementing diet with different level of thyme or celery on ruminal gas production

<table>
<thead>
<tr>
<th>Items</th>
<th>Control</th>
<th>T1</th>
<th>T2</th>
<th>T3</th>
<th>T4</th>
<th>T5</th>
<th>T6</th>
<th>T7</th>
<th>Pr&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total GP (mL)</td>
<td>157a</td>
<td>152a</td>
<td>152a</td>
<td>157a</td>
<td>153c</td>
<td>153c</td>
<td>159c</td>
<td>137d</td>
<td>0.0001</td>
</tr>
<tr>
<td>GP/g DM (mL)</td>
<td>344a</td>
<td>333a</td>
<td>333a</td>
<td>345a</td>
<td>335c</td>
<td>336c</td>
<td>351c*</td>
<td>302a</td>
<td>0.0001</td>
</tr>
<tr>
<td>GP/g NDF (mL)</td>
<td>721a</td>
<td>698a</td>
<td>697a</td>
<td>722a</td>
<td>701c</td>
<td>704e</td>
<td>735s</td>
<td>632a</td>
<td>0.0001</td>
</tr>
<tr>
<td>GP/g ADF (mL)</td>
<td>1485a</td>
<td>1438b</td>
<td>1436a</td>
<td>1487a</td>
<td>1444c</td>
<td>1451c</td>
<td>1515c</td>
<td>1302c</td>
<td>0.0001</td>
</tr>
</tbody>
</table>

Control: 60% CFM, 40% clover hay, T1: Control diet + 5 g thyme kg\(^{-1}\) DM, T2: Control diet + 10 g thyme kg\(^{-1}\) DM, T3: Control diet + 20 g thyme kg\(^{-1}\) DM, T4: Control diet + 5 g celery kg\(^{-1}\) DM, T5: Control diet + 10 g celery kg\(^{-1}\) DM, T6: Control diet + 20 g celery kg\(^{-1}\) DM, T7: Control diet + 0.4 g salinomycin kg\(^{-1}\) DM, Total GP: Total gas production after 48 h, different superscript letters in a the same row differ significantly (p<0.05), GP: Gas production, NDF: Natural detergent fiber, ADF: Acid detergent fiber
Table 4: Effect of supplementing diet with different level of thyme or celery on ruminal nutrients disappearance (%)  

| Items | Control T   | Thyme T1 | Thyme T2 | Thyme T3 | Celery T4 | Celery T5 | Celery T6 | Celery T7 | Pr>  
|-------|------------|----------|----------|----------|------------|-----------|-----------|-----------|--------  
| DMd   | 54.71a     | 53.73ab  | 53.50bc  | 55.05c   | 54.85a     | 53.50ab   | 54.54b    | 42.18b    | 0.052  
| OMd   | 55.90ab    | 54.19a   | 54.27b   | 55.83ab  | 54.58bc    | 54.66bc   | 56.45c    | 49.61d    | 0.0001  
| NDFd  | 30.39      | 30.59    | 30.48    | 30.31    | 29.32      | 31.14     | 30.55     | 26.96     | 0.09   
| ADFd  | 20.32      | 20.71    | 20.84    | 21.03    | 19.86      | 20.88     | 21.57     | 18.04     | 0.085  

Control: 60% CPM, 40% clover hay, T1: Control diet + 5 g thyme kg⁻¹ DM, T2: Control diet + 10 g thyme kg⁻¹ DM, T3: Control diet + 20 g thyme kg⁻¹ DM, T4: Control diet + 5 g celery kg⁻¹ DM, T5: Control diet + 10 g celery kg⁻¹ DM, T6: Control diet + 20 g celery kg⁻¹ DM, T7: Control diet + 0.4 g salinomycin kg⁻¹ DM, DMd: Dry matter disappearance, OMd: Organic matter disappearance, NDFd: Natural detergent fiber disappearance, ADFd: Acid detergent fiber disappearance, different superscript letters in the same row differ significantly (p<0.05)

Nutrients degradability: Nutrients degradability are listed in Table 4. The data of dry matter disappearance (DMd) appeared that there were no significant variance (p<0.05) between control and thyme treatments (T1, T2 and T3) and celery treatments (T4, T5 and T6) but salinomycin (T7) actually depressed DMd (54.71, 53.73, 53.5, 55.05, 54.85, 53.5, 54.54 and 42.18%, respectively).

Organic matter disappearance (OMd) significantly (p<0.05) affected by supplementing diets with thyme (T1 and T2) compared with control (54.19, 54.27 vs. 55.9%, respectively), while, high level of thyme (T3) and celery supplementation (T4, T5 and T6) were not significantly differ with control. It well noted that salinomycin (T7) recorded the lowest (p<0.05) value of OMd by 49.61%.

Fiber fraction degradability (NDFd and ADFd) results showed non-significant (p>0.05) change between control and other treatments.

DISCUSSION

The main active compounds of thyme and celery are thymol, carvacrol, eugenol and γ-terpinene. Overall decrease in rumen fermentation in supplementing diets with thyme and celery may be due to their effective antimicrobials components (thymol, eugenol and γ-terpinene) because of the presence of a hydroxyl group in the phenolic structure and results into loss of solidity of bacterial cell membrane which ultimately resulted in reduction in glucose uptake by bacteria.

Reduction in concentration of NH₃-N suggested the potentiality of celery for inhibiting deamination. Evans and Martin in their study in pure culture reported that thymol affected the energy metabolism of two major rumen deaminating bacteria, *Streptococcus bovis* and *Selenomonas ruminantium*.

The current results by NH₃-N reduction was agreed with results of previous studies Wanapat et al., Macheboeuf et al. and Cobellis et al., this reduction might be due to effect of the active essential oils especially cinnamaldehyde and cinnamon which noted as ammonia concentrations reducer in the rumen. It proposed that essential oils act on inhibiting attachment of bacteria on feed particles and deamination activity of amino acids. Another study concluded that number and diversity of Hyper-NH₃ producing bacteria was reduced by supplementing diets with essential oils which reflect as a decrease in NH₃ production from amino acids. It was proposed that essential oil inhibit proteolysis, peptidolysis and deamination of amino acids different studies reported that the effective dose of essential oils which needed to decrease ammonia production was lower than that needed to decrease gas production.

Corroborating the study of Busquet et al., present experiment also reported reduction of short chain fatty acids (SCFA) in low and medium dose level of thyme which could be a good indicator of simultaneous methane reduction in the rumen. Finally, under the conditions of the present study, using celery and/or thyme (as natural feed additive) in the diet of ruminants had potential positive impacts on ruminal fermentation and digestion.

CONCLUSION

The present study indicated that supplementing ruminant diet with herbal plants (thyme and celery) had potential positive effect on rumen fermentation and gas production and reduced NH₃-N concentration with no negative effect on DM, OM, NDF and ADP digestibility. Because of the high variability of the compounds and dosages used, the results found so far are inconsistent. Currently, further
in vivo experiments are carrying out to elucidate topics such as the effect of those plants on voluntary intake, rumen fermentation and methane emission.

**SIGNIFICANCE STATEMENT**

The current study cleared that using natural herbal plants could play an effective role on improving ruminal activity and fermentation and digestion, in addition to reduce ruminal gas production which help in decreasing negative environmental impacts (gas emission from ruminant) and improve diet energy availability for animal performance. The current study could contribute in improving the knowledge of reducing the negative impacts of ruminant animal due its digestion performance and help in the new trend and theory of using natural products to improve ruminant production and decline its environmental negative effects.

**REFERENCES**