Effects of additives with antioxidant activity on \textit{in vitro} rumen fermentations of two feeds

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**INTRODUCTION**

- Manipulation of rumen fermentation using bioactive compounds extracted from vegetables is a developing research area.
- Few information is available about the possibility to influence rumen fermentation by addition of vitamins and/or natural compounds extracted from agricultural products.
- Wine marc extracts contain large amount of polyphenols with antioxidant and antimicrobial properties.

**AIM**

- To evaluate the effects of ascorbic acid (AA), \( \alpha \)-tocopherol (\( \alpha-T \)) and wine marc extract (WME) \textit{in vitro} rumen fermentations.

**MATERIAL AND METHODS**

- Meadow hay (MH) and corn meal (CM) were incubated \textit{in vitro} with 25 ml of rumen fluid and 30 ml of Menke buffer, using an automatic gas production (GP) system (RF, Ankrom Technology®).
- In the 1ª incubation each feed (0.5 g), milled at 1 mm, was analyzed in quadruplicate for 4 h at 39°C without any additive (C) or with 0.01 g of ascorbic acid (AA), \( \alpha \)-tocopherol (\( \alpha-T \)) or wine marc extract (WME).
- A 2ª incubation, performed using the same experimental design described above, was stopped at the time at which half of asympotic GP was produced (\( T/2 \)), which were 16 and 9 h respectively for MH and CM.
- Data of GP were fitted with the model: GP(t) = \( A/(1+e^{c(t-b)}) \), where A is the asymptotic GP, c is the time at which half of A is produced and c is the sharpness of the curve profile.
- Residual fluids of the 2ª incubation were analyzed for VFA, \( N\text{-NH}_3 \), and for \( N\text{-N} \) balance.
- To determine \( N\text{-N} \) balance residual fluids were treated with methyl-cellulose and centrifuged 6 times at 150 g for 6 min to separate microbres (supermatant) from feed particles (pellet).
- Microbial \( N\text{-MO} \) (N-MO) was computed as: N-MO (mg/gar) = N supernatant – N\text{-NH}_3 \), where N supernatant is the N content of the supernatant and N\text{-NH}_3 \) is the ammonia content of supernatant.
- Efficiency of microbial production (EMP) was computed as: EMP = N-MO / GP\( T/2 \); where GP\( T/2 \) is the GP measured at \( T/2 \). EMP was expressed as mg of N-MO apparently retained / 100 ml of gas.
- All the data were submitted to ANOVA, considering the feed, the additive and their interaction as sources of variation.

**RESULTS**

- The two feeds showed significant differences for kinetic of GP, VFA profile, \( N\text{-NH}_3 \) content and efficiency of microbial production (Table 1; Figure 1-2).
- The three additives did not affect significantly the molar proportions of VFA at \( T/2 \).
- Ascorbic acid increased significantly GP but did not affected the end product of fermentation and the \( N\text{-N} \) balance.
- \( \alpha \)-tocopherol increase significantly the amount of \( N\text{-NH}_3 \) at the end of the fermentation only when incubated with MH.
- Wine marc extract slowed down kinetics of the GP and reduced total GP in incubation with MH. At \( T/2 \) this extract changed VFA profile and improved significantly EMP.

**Table 1. Volatile fatty acids (VFA) profile and nitrogen balance**

<table>
<thead>
<tr>
<th></th>
<th>Meadow hay (MH)</th>
<th>Corn meal (CM)</th>
<th>SE</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>C</td>
<td>AA</td>
<td>( \alpha-T )</td>
</tr>
<tr>
<td>Acetate, %</td>
<td>63.5&lt;sup&gt;a&lt;/sup&gt;</td>
<td>63.2&lt;sup&gt;b&lt;/sup&gt;</td>
<td>62.7&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Propionate, %</td>
<td>19.5&lt;sup&gt;a&lt;/sup&gt;</td>
<td>20.2&lt;sup&gt;b&lt;/sup&gt;</td>
<td>20.0&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>( n )-butyrate, %</td>
<td>10.7</td>
<td>11.3</td>
<td>11.9</td>
</tr>
<tr>
<td>Others VFA, %</td>
<td>6.3</td>
<td>5.3</td>
<td>5.4</td>
</tr>
<tr>
<td>Total ( N\text{-N} ), (mg)</td>
<td>20.3</td>
<td>20.2</td>
<td>20.5</td>
</tr>
<tr>
<td>N-pellet (mg)</td>
<td>3.4</td>
<td>4.0</td>
<td>3.7</td>
</tr>
<tr>
<td>N\text{-NH}_3 ), (mg)</td>
<td>5.6&lt;sup&gt;a&lt;/sup&gt;</td>
<td>5.4&lt;sup&gt;b&lt;/sup&gt;</td>
<td>7.7&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>N-MO (mg)</td>
<td>11.3&lt;sup&gt;a&lt;/sup&gt;</td>
<td>10.8&lt;sup&gt;b&lt;/sup&gt;</td>
<td>9.2</td>
</tr>
</tbody>
</table>

Within each feed means with different letters in the same row are different: a,b p<0.05; A,B p<0.01

C= Control; AA= Ascorbic Acid; \( \alpha-T \)= \( \alpha \)-Tocopherol; WME= Wine Marc Extract

**Figure 1. Kinetics of gas production (GP)**

**Figure 2. Efficiency of microbial production (EMP)**