Manipulation of the Rumen Microbial Environment with Thyme Extracts in Ruminants Using the Nylon Bags Technique

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Abstract: The objective of this study was to investigate the effects of thyme methanolic extract on ruminal Dry Matter (DM) degradation parameters of sunflower meal. Treatments were: Sunflower meal (no additive), thyme methanolic extract (0.15 ml/30 ml buffered rumen fluid). In situ rumen degradability was performed with three Gezel rams rumen fistulaed in times at 0, 2, 4, 8, 16, 24 and 48 h. Potential degradation (a+b) for sunflower meal and thyme methanolic extract (0.15 ml/30 ml buffered rumen fluid) were estimated, 76.82 and 80.47%, respectively. Effective rumen degradable dry matter for sunflower meal and thyme methanolic extract (0.15 ml/30 ml buffered rumen fluid) at a rate of 0.08/h, 45.33 and 50.03%, respectively were estimated. Thyme methanolic extract (0.15 ml/30 ml buffered rumen fluid) treatments significantly decreased dry matter gradualty of sunflower meal on different incubation times. Although thyme methanolic extract (0.15 ml/30 ml buffered rumen fluid) decreased (p<0.05) the water soluble fraction (a) and Effective Rumen Degradability of Dry Matter (ERDM) at a rate of 0.02/h but increased (p<0.05) the potentially degradable fraction (b) of DM, constant rate of degradation (c), total degradability (a+b) and Effective Rumen Degradability of Dry Matter (ERDM) at a rate of 0.05 and 0.08/h.

Key words: Gezel rams, sunflower meal, potential degradation, incubation, methanolic, degradability, thyme

INTRODUCTION
Modification of ruminal fermentation using feed additives, such as antibiotics, has proved to be a useful strategy to improve production efficiency in ruminants (Kongmun et al., 2010). The use of antibiotics as feed additives has proved to be a useful tool to reduce energy and nitrogen losses from the diet (McGuffey et al., 2001; Kongmun et al., 2010). However, the use of antibiotics as feed additives in ruminants has been of increasing concern due to the potential appearance of residues in milk and meat (Kongmun et al., 2010). Furthermore, the use of antibiotics as a feed additive has been banned in the European Union (Russell and Houlihan, 2003; Kongmun et al., 2010). For this reason, scientists are interested in evaluating the potential use of natural antimicrobials such as herbs and plant extracts. Currently, the use of plant herbs has resulted in improving rumen ecology (Kamra, 2005; Wanapat and Cherdthong, 2008; Kongmun et al., 2010). Compounds with phenolic structures, such as thymol (active compound of thyme), are more effective as antimicrobials in comparison with other nonphenolic secondary plant metabolites because of the presence of a hydroxyl group in the phenolic structure (Calsamiglia et al., 2006; Ullte et al., 2002; Helander et al., 1996). Furthermore, the small molecular weight of thymol (active compound of thyme) allows it to gain access to the cell membrane through the pores of the external wall (Calsamiglia et al., 2007). Chumpawadee et al. (2007) stated that nutritive value of ruminant feeds is determined by the concentration of its chemical compositions, as well as rate and extent of digestion in the rumen. Three common methods including: in situ, in vivo and in vitro techniques have been used in order to evaluate the nutritive value of feedstuffs (Maheri-Sis et al., 2007, 2008). The nylon bag (in situ) technique provides a powerful tool for the initial evaluation of feedstuffs and for improving our understanding of the processes of degradation which occur within the rumen. It is the more efficient method for measuring rate and extent of digestion in the rumen (Orskov et al., 1980; Maheri-Sis et al., 2011). Therefore, the objective of this in situ study was to study the effect of thyme extract on improving ruminal dry matter degradation in Gezel rams using nylon bags technique.

MATERIALS AND METHODS
Preparation of extracts: For the preparation of sample extracts, the method reported by Patra et al. (2006) was used. For this purpose, 1000 ml of methanol solvent was added into 100 g of thyme materials and the mixture was left for 24 h. afterwards, it was filtered and the methanol was vaporized in an evaporator (45°C). The sample extracts were kept in the refrigerator (4°C).

In situ degradation procedures: Three ruminally cannulated Gezel rams (about 55 kg BW) were used to determine in situ degradation characteristics. Rams were housed in individual tie stalls bedded with sawdust. Rams fed diets containing alfalfa hay (70%) and concentrate mixture (30%) at the maintenance levels. Dacron bags (18°9 cm, 40-45 micron pore size)
were filled with 5 g dried and ground samples and then incubated in the rumen of rams for the periods of 0, 2, 4, 8, 16, 24 and 48 h. After the removal of bags from the rumen, bags were washed in cold water until rinse water were clear and dried at 80°C for 48 h (Karsli and Russell, 2002). Then rumen degradation kinetics of sunflower meal (no additive) and thyme methanolic extract (0.15 ml/30 ml buffered rumen fluid), was calculated using the nonlinear models proposed by Orskov and McDonald (1979):

\[ P = a + b (1 - e^{-ct}) \]

Where:
- \( P \) = Percentage of degradability for response variables at \( t \)
- \( t \) = Time relative to incubation (h)
- \( a \) = Highly soluble and readily degradable fraction (%)
- \( b \) = Insoluble and slowly degradable fraction (%)
- \( c \) = Rate constant for degradation (h\(^{-1}\))
- \( e \) = 2.7182 (Natural logarithm base)

Following determination of these parameters, the effective degradability of DM in sunflower meal (no additive), thyme methanolic extract (0.15 ml/30 ml buffered rumen fluid) was calculated using equation described by Orskov and McDonald (1979):

\[ ED = a + (b + c)/(c + k) \]

Where:
- \( ED \) = Effective degradability for response variables (%)
- \( a \) = Highly soluble and readily degradable fraction (%)
- \( b \) = Insoluble and slowly degradable fraction (%)
- \( c \) = Rate constant for degradation (h\(^{-1}\))
- \( k \) = Rate constant of passage (h\(^{-1}\))

When calculating effective degradability, rate constant of passage was assumed to be 0.02, 0.05 and 0.08 per hour (Bhargava and Orskov, 1987) so that the results could be extrapolated to other ruminants that differ in rumen capacity.

Statistical analysis: All of the data were subjected to one-way analysis of variance using the analysis of variation model ANOVA of SAS (2000). Multiple comparison tests used Duncan’s multiple-t-test (1980). All data obtained from three replicates \( n = 3 \).

RESULTS AND DISCUSSION
Ruminal DM degradation of sunflower meal (Control) and thyme methanolic extract (0.15 ml/30 ml buffered rumen fluid) at different incubation times were shown in Table 1. As shown in the Table increasing incubation time lead to increase in degradability of nutrients. The zero hours incubation time degradability (as index of solubility) for DM of sunflower meal (Control) and thyme methanolic extract (0.15 ml/30 ml buffered rumen fluid) was similar but the 2, 4 and 8 hours incubation time degradability of sunflower meal (Control) considerably was higher than that of thyme methanolic extract (0.15 ml/30 ml buffered rumen fluid).

Table 1: Ruminal degradation (%) of sunflower meal (Control) and thyme methanolic extract (0.15 ml/30 ml buffered rumen fluid) at different incubation times

| Incubation time (h) | Control | Thyme extract
<table>
<thead>
<tr>
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<tbody>
<tr>
<td>0</td>
<td>17.27</td>
<td>17.27</td>
</tr>
<tr>
<td>2</td>
<td>31.58</td>
<td>24.30</td>
</tr>
<tr>
<td>4</td>
<td>36.79</td>
<td>31.94</td>
</tr>
<tr>
<td>8</td>
<td>52.25</td>
<td>41.92</td>
</tr>
<tr>
<td>16</td>
<td>61.96</td>
<td>60.23</td>
</tr>
<tr>
<td>24</td>
<td>65.89</td>
<td>64.59</td>
</tr>
<tr>
<td>48</td>
<td>79.16</td>
<td>79.15</td>
</tr>
</tbody>
</table>

Table 2: Ruminal degradation parameters and effective degradability of sunflower meal (Control) and thyme methanolic extract (0.15 ml/30 ml buffered rumen fluid)

| Items         | Control | Thyme extract
<table>
<thead>
<tr>
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<tbody>
<tr>
<td>a (%)</td>
<td>19.76</td>
<td>17.26</td>
</tr>
<tr>
<td>b (%)</td>
<td>57.05</td>
<td>63.33</td>
</tr>
<tr>
<td>a + b (%)</td>
<td>76.82</td>
<td>80.47</td>
</tr>
<tr>
<td>c (h(^{-1}))</td>
<td>0.09</td>
<td>0.64</td>
</tr>
<tr>
<td>Lag time (h)</td>
<td>0.00</td>
<td>0.10</td>
</tr>
<tr>
<td>ED (%) Out flow rate 0.02 h(^{-1})</td>
<td>65.40</td>
<td></td>
</tr>
<tr>
<td>ED (%) Out flow rate 0.05 h(^{-1})</td>
<td>56.50</td>
<td></td>
</tr>
<tr>
<td>ED (%) Out flow rate 0.08 h(^{-1})</td>
<td>50.03</td>
<td></td>
</tr>
</tbody>
</table>

a: Washout fraction as measured by washing loss from nylon bags.
b: Potentially degradable fraction; c: Rate of degradation of fraction b (h); ED: Effective Degradability

Ruminal DM degradation of sunflower meal (Control) and thyme methanolic extract (0.15 ml/30 ml buffered rumen fluid) at different incubation times were shown in Fig. 1 and 2. Ruminal degradation parameters and effective degradability of sunflower meal (Control) and thyme methanolic extract (0.15 ml/30 ml buffered rumen fluid) were presented in Table 2. As illustrated in the Table 2, sunflower meal have a low immediately degradable fraction (a) higher than of thyme methanolic extract (0.15 ml/30 ml buffered rumen fluid). Solubility of DM (19.76%) in sunflower meal was higher than that of thyme methanolic extract (0.15 ml/30 ml buffered rumen fluid) (17.26%). Potential degradability (a + b) of thyme methanolic extract (0.15 ml/30 ml buffered rumen fluid) (80.47%) also was higher than that of sunflower meal (76.82%). Effective degradability of sunflower meal (Control) and thyme methanolic extract (0.15 ml/30 ml buffered rumen fluid) did decreased by increasing out flow rate. Higher effective degradability obtained for sunflower meal. In case of maintenance level feeding (Out flow rate 0.02 h\(^{-1}\)) effective degradability of sunflower meal (Control) and thyme methanolic extract (0.15 ml/30 ml buffered rumen fluid) were 66.45 and 65.4%, respectively.
Borchers observed that the addition of thymol (active compound of thyme and oregano) to rumen fluid in vitro resulted in the accumulation of ammonia acid and the reduction of ammonia nitrogen concentrations, suggesting that thymol inhibited deamination (Calsamiglia et al., 2007). In general, rumen microbial activity was affected by the use of thyme extracts. These results agree with the observations of (Oh et al., 1967; Busquet et al., 2005; Salamatarz et al., 2011; Rezaei et al., 2011), those reported that high doses of different plant secondary metabolites, tested on in vitro fermentation of mixed ruminal microorganisms, resulted in an inhibition of rumen microbial fermentation.

**Abbreviations:** BEO, blend of essential oil compounds; DM, dry matter; ED, Effective degredability for response variables (%); a, highly soluble and readily degradable fraction (%); b, Insoluble and slowly degradable fraction (%); c, Rate constant for degradation (h⁻¹); k, Rate constant of passage (h⁻¹); h, hours; EO, essential oil.

**Conclusion:** Implications these experiments with thyme extract demonstrate that it is possible to use natural plant extract to manipulate ruminal dry matter degradation by selective suppression of certain microbial species.

**ACKNOWLEDGMENTS**

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**REFERENCES**


