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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 of 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 gradability 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 Mattre (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*., 2007; Ultee *et al*., 2002; Helander *et al*., 1998). 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 were clear and dried at 60°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 model 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$_{0.15}$
0	17.27	17.273
2	31.58	24.30
4	36.79	31.94
8	52.25	41.92
16	61.96	60.23
24	65.89	64.59
48	79.16	78.15

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$_{0.15}$
a (%)	19.76	17.26
b (%)	57.05	63.33
a + b (%)	76.82	80.47
c (h^{-1})	0.09	0.64
Lag time (h)	0.00	0.10
ED (%) Out flow rate 0.02 h^{-1}	66.46	65.40
ED (%) Out flow rate 0.05 h^{-1}	52.70	56.50
ED (%) Out flow rate 0.08 h^{-1}	45.33	50.03

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 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.

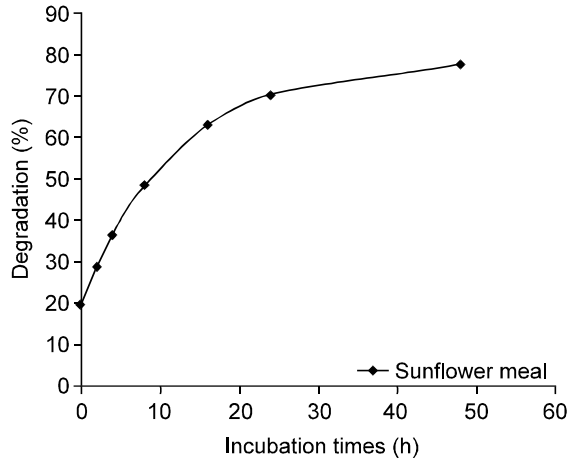

Fig. 1: Ruminal DM degradation of sunflower meal (Control) was at different incubation times

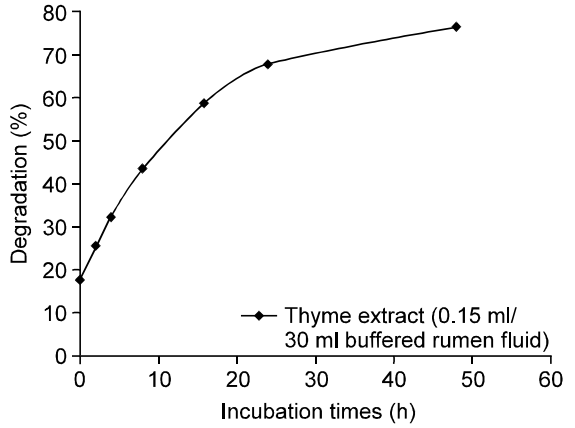

Fig. 2: Ruminal DM degradation of thyme methanolic extract (0.15 ml/30 ml buffered rumen fluid) was at different incubation times

Compounds with phenolic structures have a broad spectrum of activity against a variety of both Gram-positive and Gram-negative bacteria (Helander *et al*., 1998; Lambert *et al*., 2001; Kim *et al*., 1995; Dorman and Deans, 2000; Benchaar *et al*., 2007). The mechanism of action by which phenolic compounds are thought to exert their antimicrobial activity is through the disturbance of the cytoplasmic membrane, disrupting the proton motive force, electron flow active transport and coagulation of cell contents (Burt, 2004; Benchaar *et al*., 2007). Helander *et al*. (1998) showed that thymol is a monoterpene [5-methyl-2-(1methylethyl) phenol; $C_{10}H_{14}O$] decreased the intracellular ATP pool and increased the extracellular ATP concentration of *E. coli*, an observation indicative of disruption of the cytoplasmic membrane (Benchaar *et al*., 2007). Borchers (1965) was the first to report the potential benefit of essential oils on rumen microbial fermentation (Calsamiglia *et al*., 2007). 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; Salamatazar *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 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}); 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.

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