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Effects of *Thyme Methanolic* Extract on Dry Matter Degradation of Sunflower Meal Using Nylon Bag 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 (0.3 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. The results showed that dry matter disappearance at 24 h incubation, were 65.89 and 54.14 percent for sunflower meal and thyme methanolic extract (0.3 ml/30 ml buffered rumen fluid) respectively. Dry matter disappearance at 48 h incubation, were 79.16 and 73.98 percent for sunflower meal and thyme methanolic extract (0.3 ml/30 ml buffered rumen fluid) respectively. Water soluble fraction (a) for sunflower meal and thyme methanolic extract (0.3 ml/30 ml buffered rumen fluid) were estimated, 19.76 and 17.14%, respectively. Effective rumen degradable dry matter for sunflower meal and thyme methanolic extract (0.3 ml/30 ml buffered rumen fluid) at a rate of 0.02/h, 61.53 and 66.46%, respectively were estimated. Potentially degradable fraction (b) for sunflower meal and thyme methanolic extract (0.3 ml/30 ml buffered rumen fluid) were estimated, 57.05 and 72.6%, respectively. Thyme methanolic extract (0.3 ml/30 ml buffered rumen fluid) treatments significantly decreased dry matter degradability of sunflower meal on different incubation times. Although thyme methanolic extract (0.3 ml/30 ml buffered rumen fluid) decreased ($p<0.05$) the water soluble fraction (a) 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 0.02, 0.05 and 0.08/h.

Keywords: Dry Matter; Gezel Rams; Potential Degradation; Incubation; Thyme Methanolic Extract; Degradability; Disappearance; Sunflower Meal.

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

INTRODUCTION

Chumpawadee et al. [5] 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 [9, 10 and 8]. 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 [13, 8]. Modification of rumen microbial fermentation to decrease methane and ammonia nitrogen production using feed additives, such as antibiotics, has proved to be a useful strategy to improve production efficiency in dairy cattle [12, 2]. The public concern over the routine use of antibiotics and growth promoters in livestock production has increased recently because of the risk of the antibiotic residues presence in milk and meat and its effect on human health [18]. These led to its prohibition in the European Union in 2006 in animal feeding. Accordingly, there is greater interest in using plants and plant extracts as alternatives to feed antibiotics to manipulate ruminal fermentation, improve feed efficiency and animal productivity [3, 4 and 18]. Many plants produce secondary metabolites such as phenolic compounds, essential oils, and sarsaponins [3, 4 and 18]. That affect was microbial activity [18]. The objective of this study was to evaluate effects of thyme methanolic extract (0.3 ml/30ml buffered rumen fluid) on dry matter disappearance, of sunflower meal using the nylon bags technique.

MATERIALS AND METHODS

2.1. Preparation of extracts

The thyme methanolic extract were prepared according to Patra et al [15]; Sallam et al [18] with some modifications. The thyme materials were dried at 50°C and ground in mills to pass a 1 mm sieve and 100 g placed in 1000 ml of methanol solvent. The flasks of all the solvents were stoppered and agitated with a magnetic stirrer for 24 h at room temperature. Then the solutions were centrifuged at 3000 g for 10 min. The residue was re-extracted with 500 ml of methanol for 24 h stirring at room temperature and centrifuged again at 3000 g for 10 min. The thyme methanolic extract were combined. Distilled water was evaporated from the solution at approximately 45°C using a rotary-evaporator [18]. The sample extracts were kept in the refrigerator (4°C).

2.2. *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. 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 was clear and dried at 60^{oC} for 48 h, [7]. Then rumen degradation kinetics of sunflower meal (no additive) and thyme methanolic extract (0.3 ml/30 ml buffered rumen fluid), was calculated using the nonlinear model proposed by Ørskov and McDonald [14]:

$$P = a + b\ (1\text{-}e^{-ct})$$

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 (0.3 ml/30 ml buffered rumen fluid) was calculated using equation described by Ørskov and McDonald [14]:

$$ED = a + (b*c)/(c + k)$$

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 were 0.02, 0.05 and 0.08 per hour (Bhargava and Ørskov), [1] so that the results could be extrapolated to other ruminants that differ in rumen capacity.

2.3. Statistical Analysis

All of the data were subjected to one-way analysis of variance using the analysis of variation model ANOVA of SAS [19]. Multiple comparison tests used Duncan's multiple- t-test [20]. All data obtained from three replicates n=3.

RESULTS AND DISCUSSION

Dry matter disappearance of sunflower meal (Control) and thyme methanolic extract (0.3 ml/30 ml buffered rumen fluid) at different incubation times were shown in Table 1.

Table 1: dry matter disappearance (%) of sunflower meal (Control) and thyme methanolic extract (0.3 ml/30 ml buffered rumen fluid) at different incubation times

Incubation time (h)	Control	Thyme extract$_{0.3}$
0	17.27	17.2
2	31.58	20.69
4	36.79	24.38
8	52.25	38.04
16	61.96	43.74
24	65.89	54.14
48	79.16	73.98

The results showed that dry matter disappearance at 4 h incubation, were 36.79 and 24.38 percent for sunflower meal and thyme methanolic extract (0.3 ml/30 ml buffered rumen fluid) respectively. Dry matter disappearance at 8 h incubation, were 52.25 and 38.04 percent for sunflower meal and thyme methanolic extract (0.3 ml/30 ml buffered rumen fluid) respectively. Dry matter disappearance at 16 h incubation, were 61.96 and 43.74 percent for sunflower meal and thyme methanolic extract (0.3 ml/30 ml buffered rumen fluid) respectively. Dry matter disappearance at 24 h incubation, were 65.89 and 54.14 percent for sunflower meal and thyme methanolic extract (0.3 ml/30 ml buffered rumen fluid) respectively. Dry matter disappearance at 48 h incubation, were 79.16 and 73.98 percent for sunflower meal and thyme methanolic extract (0.3 ml/30 ml buffered rumen fluid) respectively. Thyme methanolic extract (0.3 ml/30 ml

buffered rumen fluid) treatments significantly decreased dry matter degradability of sunflower meal on different incubation times.

Ruminal degradation parameters and effective degradability of sunflower meal (Control) and thyme methanolic extract (0.3 ml/30 ml buffered rumen fluid) were presented in Table 2.

Table 2: Ruminal degradation parameters and effective degradability of sunflower meal (Control) and thyme methanolic extract (0.3 ml/30 ml buffered rumen fluid)

Items	Control	Thyme extract $_{0.3}$
a (%)	19.76	17.14
b (%)	57.05	72.6
a + b (%)	76.82	89. 74
c (h^{-1})	0.09	0.031
Lag time (h)	0.000	0.100
ED (%) Out flow rate 0.02 h^{-1}	61.53	66.46
ED (%) Out flow rate 0.05 h^{-1}	45.26	56.05
ED (%) Out flow rate 0.08 h^{-1}	37.7	50.3

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; a + b: Potential degradability.

The results showed that water soluble fraction (a) for sunflower meal and thyme methanolic extract (0.3 ml/30 ml buffered rumen fluid) were estimated, 19.76 and 17.14%, respectively. Effective rumen degradable dry matter for sunflower meal and thyme methanolic extract (0.3 ml/30 ml buffered rumen fluid) at a rate of 0.02/h, 61.53 and 66.46%, respectively were estimated. The results showed that Potential degradability (a + b) for sunflower meal and thyme methanolic extract (0.3 ml/30 ml buffered rumen fluid) were estimated, 76.82 and 89.74%, respectively. Potentially degradable fraction (b) for sunflower meal and thyme methanolic extract (0.3 ml/30 ml buffered rumen fluid) were estimated, 57.05 and 72.6%, respectively. Thyme methanolic extract (0.3 ml/30 ml buffered rumen fluid) decreased ($p<0.05$) the water soluble fraction (a) 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 0.02, 0.05 and 0.08/h.

Dry matter disappearance of sunflower meal (Control) and thyme methanolic extract (0.3 ml/30 ml buffered rumen fluid) at different incubation times were shown in Figure 1 and 2.

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). Salamat azar et al., [17] estimation effect of tree doses *thyme methanolic extract* (0, 0.15 and 0.3 ml/30 ml buffered rumen fluid) on degradability kinetics, of sunflower meal and report gas volume at 48 h incubation (for 200 mg dry samples), soluble fraction (a), insoluble but fermentable fraction (b), potential gas production (a + b) and rate constant of gas production (c) of sunflower meal were 44.99, 3.60, 49.32, 52.92 ml/200 mg DM and 0.135 ml/h, gas volume at 48 h incubation (for 200 mg dry samples), soluble fraction (a), insoluble but fermentable fraction (b), potential gas production (a+ b) and rate constant of gas production (c) of *thyme methanolic extract* (0.15 ml/30 ml buffered rumen fluid) were 29.91, 0.53, 36.25, 36.79 ml/200 mg DM and 0.049 ml/h, respectively.

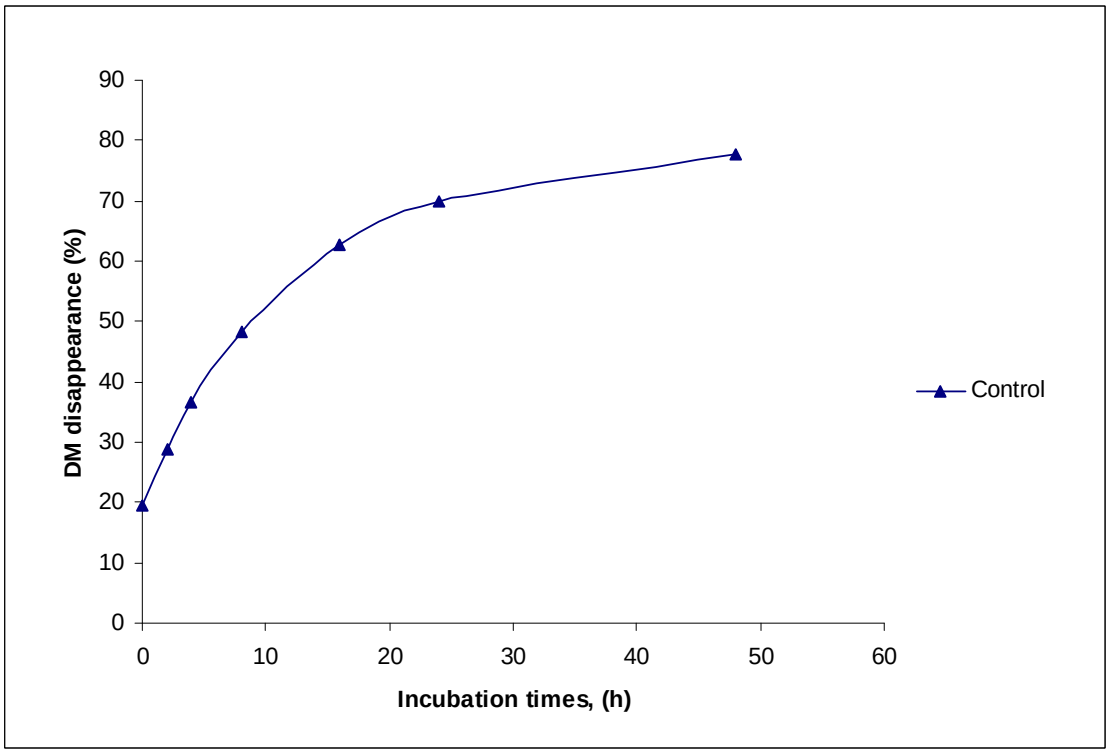

Figure.1. dry matter disappearance of sunflower meal (Control) was at different incubation times.

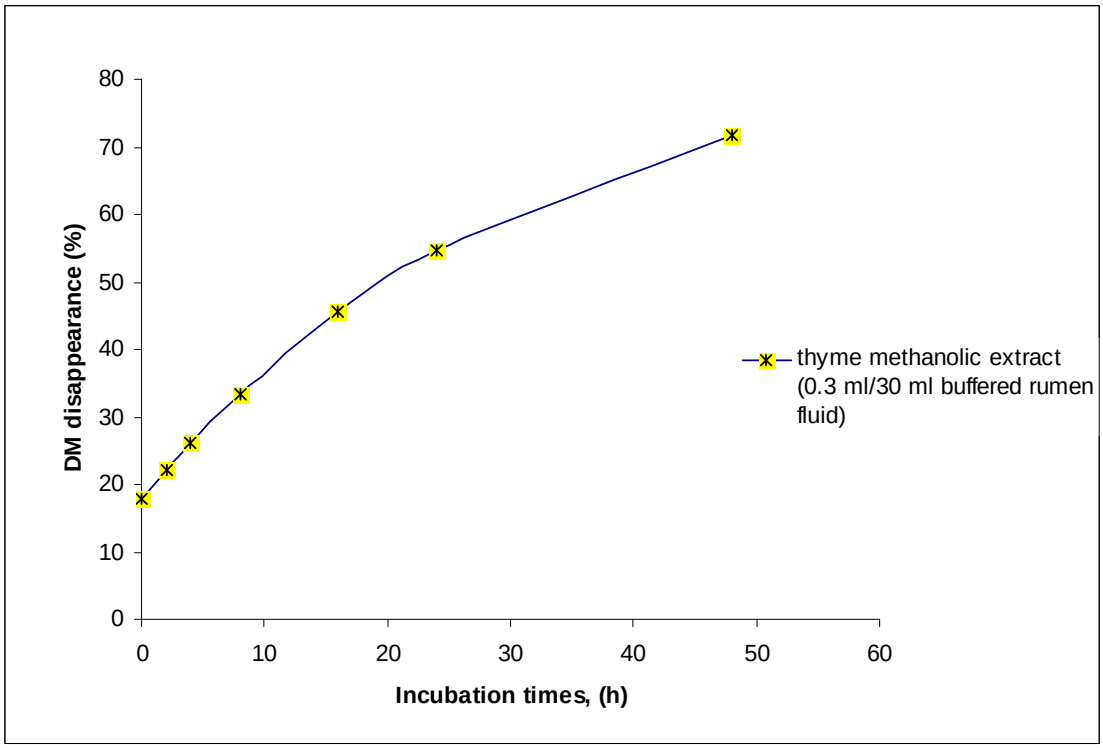

Figure.1. dry matter disappearance of thyme methanolic extract (0.3 ml/30 ml buffered rumen fluid) was at different incubation times.

Rezaei et al., [16] evaluation effect of tree doses *fennel methanolic extract* (0, 0.5 and 1 ml/30 ml buffered rumen fluid) on degradability, of soybean meal and report gas volume at 12 h incubation (for 200 mg dry samples), soluble fraction (a), insoluble but fermentable fraction (b), potential gas production (a + b) and rate constant of gas production (c) of soybean meal were 51.620, 1.767, 70.880, 72.647 ml/200 mg DM and 0.100 ml/h, gas volume at 12 h incubation

(for 200 mg dry samples), soluble fraction (a), insoluble but fermentable fraction (b), potential gas production (a + b) and rate constant of gas production (c) of *fennel methanolic extract* (0.5 ml/30 ml buffered rumen fluid) were 54.970, 4.302, 70.919, 75.221 ml/ 200 mg DM and 0.088 ml/h, respectively. Gas volume at 24 and 48 h incubation (for 200 mg dry samples), of soybean meal were 65.370 and 71.240 ml/200 mg DM, while for *fennel methanolic extract* (0.5 ml/30ml buffered rumen fluid) were 65.470 and 71.883 ml/200 mg DM, respectively.

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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