

Effects of *Thyme methanolic* Extract on Ruminant Protein Degradation of Soybean Meal using Nylon Bag Technique

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Abstract: This study was conducted to evaluate effects of *Thyme methanolic* extract (0 and 0.15 mL/30 mL buffered rumen fluid) on ruminal Crude Protein (CP) degradation parameters of Soybean Meal (SBM). Nylon bags filled with 5 g of each of untreated or *Thyme methanolic* extract treated soybean meal, were suspended in the rumen of three fistulated Gezel rams for 0, 2, 4, 8, 16, 24 and 48 h, and obtained data were fitted to a non-linear degradation model to calculate ruminal degradation characteristics. *Thyme methanolic* extract treatments significantly decreased protein degradability of soybean meal on different incubation times. Effective rumen degradable crude protein at a rate of 0.02/h, for untreated and *Thyme methanolic* extract treated soybean meal, 82.43 and 77.80%, respectively were estimated. Although *Thyme methanolic* extract decreased ($p < 0.05$) the water soluble fraction (a), constant rate of degradation (c) and total degradability (a+b) of CP degradation but potentially degradable fraction (b), were not significantly affected by *Thyme methanolic* extract.

Key words: Degradability, gezel rams, *Methanolic*, nylon bags, soybean meal

INTRODUCTION

Today, to provide protein requirements of lactating cows and fattening calves with high growth rate usage slowly degradable protein in the rumen, but digestible in the small intestine is essential (Van Soest, 1994; Waltz and Stern, 1989). Microorganisms in the rumen degrade nutrients to produce volatile fatty acids and synthesize microbial protein as an energy and protein supply for the ruminant, respectively. However, this fermentation process has energy, losses of methane and protein, losses of ammonia nitrogen inefficiencies that may limit production performance and contribute to the release of pollutants to the environment (Calsamiglia *et al.*, 2007). Antibiotic ionophores have been very successful in reducing this energy and protein losses in the rumen, but the use of antibiotics in animal feeds is facing reduced social acceptance, and their use has been banned in the European Union since January 2006 (Calsamiglia *et al.*, 2007). For this reason, scientists have become interested in evaluating other alternatives to control specific microbial populations to modulate rumen fermentation. Essential oils can interact with microbial cell membranes and inhibit the growth of some gram-positive and gram-negative bacteria. As a result of such inhibition, the addition of some plant extracts to the rumen results in an inhibition of deamination and methanogenesis, resulting in lower ammonia nitrogen, methane, and acetate, and in higher propionate and butyrate concentrations (Calsamiglia *et al.*, 2007). Several methods such as *in vivo*, *in situ* and *in vitro* techniques have been used in

order to evaluate the nutritive value of feedstuffs (Maheri-Sis *et al.*, 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. However, it must be remembered that the technique has limitations as well as advantages. There are three important limitations. Firstly, since the sample is confined within the bag it is not exposed to any breakdown due to chewing and rumination. Secondly food would normally be able to leave the rumen once broken down to a suitable particle size. Thirdly, it must be remembered that, strictly speaking, what is actually measured is the breakdown of material to a size small enough to leave the bag and not necessarily a complete degradation to simple chemical compounds. The results must therefore be treated with due caution, and, in general, be used as qualitative indicators of general principles (Ørskov *et al.*, 1980; Maheri-Sis *et al.*, 2011b). In spite of these limitations, it is the best method for simulation of real ruminal condition (Maheri-Sis *et al.*, 2011a). The aim of this study was to determine the effects of *Thyme methanolic* extract on ruminal protein degradation of soybean meal using nylon bags technique.

MATERIALS AND METHODS

Thyme and soybean meal samples: Soybean meal samples were obtained from commercial sources in Iran. During summer season *thyme* samples were collected from different parts of Esfahan province. Next, there were

drying for one week, and homogeneous mixture were papered for nutritive chemical analyzes. For determination of (*Thyme* extract) effects, we added *Thyme methanolic* extract with two doses (0 and 0.15 mL/30 mL buffered rumen fluid) into gas test syringes. All samples were then ground in a laboratory mill through a 1 mm screen.

Procedure of plant extracts preparation: The *Thyme methanolic* extract were prepared according to (Patra *et al.*, 2006; Sallam *et al.*, 2009) 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 65°C by using a rotary-evaporator (Patra *et al.*, 2006; Sallam *et al.*, 2009).

Treatments and experimental design: The different doses of *Thyme methanolic* extract were added to the diet sample. Two doses (0 and 0.15 mL/30 mL buffered rumen fluid) of *Thyme methanolic* extract were investigated as follow:

- Untreated SBM
- *Thyme methanolic* extract treated soybean meal

In situ evaluation of crude protein: Nylon bag technique was used to measure disappearance in the rumen of untreated and treated SBM. Nylon bags (45 µm pore size; 6 cm×16 cm bag size) containing 5 g of SBM samples were incubated in the rumen of each rams. In a completely randomized design with three treatments and sex replications for each animal were performed, two bags of each type of treated SBM were removed after 0, 2, 4,

8, 16, 24 and 48 h of incubation in the rumen. Then individual bags with contents were washed in running tap water until the bags were free of rumen content. To reach constant weight, bags were dried at 60°C for 48 h. The solubility or washing loss was determined by soaking samples of each material in water at 37-40°C for 1 h followed by the washing procedure above. Digestion kinetics of CP was determined according to the equation of Ørskov and McDonald (1979):

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

where, p is the amount degraded at a time, the rapidly soluble fraction (g/kg), b the potentially degradable fraction (g/kg), c the constant rate of disappearance of b, and t the time of incubation (h). The effective rumen degradability of CP was estimated using the equation of Ørskov and McDonald (1979):

Statistical analysis: Data on apparent gas production parameters 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). Significance between individual means was identified using the Duncan's multiple range tests. Mean differences were considered significant at (p<0.05). Standard errors of means were calculated from the residual mean square in the analysis of variance. All data obtained from three replicates n = 3.

RESULTS AND DISCUSSION

The crude protein degradation (%) of untreated and treated soybean meal in different incubation times was shown in Table 1. The results showed that crude protein degradation at 24 h incubation time, were 88.49 and 81.85 for soybean meal (control) and *Thyme methanolic extract* (0.15 mL/30 mL buffered rumen fluid), respectively. *In situ* crude protein degradability characteristic of untreated and treated soybean meal was shown in Table 2. The crude protein degradability from the soluble fraction (a)

Table 1: *In situ* degradability of crude protein in untreated and treated soybean meal (%)

Doses	Incubation times							
	0	2	4	8	16	24	48	
Untreated SBM (i)	-	18.60	36.23	39.723	53.09	74.72	88.49	95.79
<i>Thyme</i> extract (ii)	0.15	18.60	30.09	33.663	48.47	69.03	81.85	91.46

Table 2: The rumen degradation characteristics of crude protein in untreated and treated soybean meal

Doses	Degradation characteristics				Effective degradability			
	a	b	a+b	c	0.02	0.05	0.08	
Untreated SBM (i)	-	20.28 ^a	78.82 ^a	99.16 ^a	0.0744 ^a	82.43 ^a	67.33 ^a	57.60 ^a
<i>Thyme</i> extract (ii)	0.15	18.32 ^b	77.63 ^a	95.95 ^b	0.0653 ^b	77.80 ^b	62.36 ^b	53.30 ^b

a = washing losses, soluble or rapidly degradable; This value is the intercept of the degradation curve at time 0 h (%); b = the insoluble but potentially fermentable (%); c = degradability rate (h); a+b = Potential degradability (%)

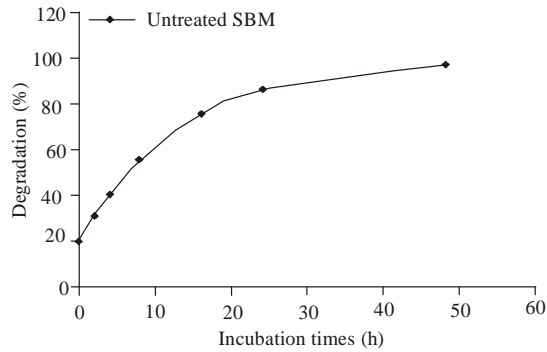


Fig. 1: Crude protein degradation (%) of untreated soybean meal in different incubation times

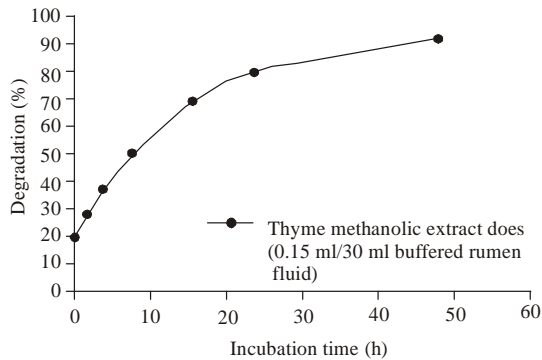


Fig. 2: Crude protein degradation (%) of treated soybean meal in different incubation times

and Potential degradability (a+b) were, 20.28, 99.16 and 18.32, 95.95%, and the rate degradability (c) was 0.0744 and 0.0653/h for Untreated SBM and *Thyme methanolic* extract treated soybean meal respectively estimated. The total degradability of the sample is given by a+b which obviously cannot exceed 100. It follows that 100-(a+b) represents the fraction which will appear to be undegradable in the rumen. If 'a' is positive, then there is a component which is degraded rapidly and/or a component which is soluble, or fine enough to escape from the bags simply by soaking and washing. Whether 'a' represents rapid degradation, or simply washing losses, can be determined with control bags which are simply soaked in water and then washed and dried in the normal way. When a negative value for 'a' is obtained this means that there has to be an initiation period for degradation to start (termed the lag phase). Effective Degradability (ED) of the examined nutrient components were calculated using the outflow rates of 0.02, 0.05 and 0.08/h, according to Ørskov *et al.* (1980), model: $ED = a + [bc/(c+k)]$ where ED is effective degradability and 'a', 'b' and 'c' are the constants as described earlier in the non-linear equation above and 'k' the rumen fractional outflow rates. Effective rumen degradable crude protein at a different rate were determine, Effective Degradability (ED) of

crude protein decreased with increase in outflow rates crude protein decreased of 82.43% (k = 0.02) to 57.60% (k = 0.08) in the untreated soybean meal and treated soybean meal decreased of 77.80% (k = 0.02) to 53.30% (k = 0.08). Mupangwa *et al.* (1997) observed ED of DM to decrease as the outflow rate. The wide range of variation in CP of by-products can be due to different original materials, growing conditions (geographic, seasonal variations, climatic conditions and soil characteristics), extent of foreign materials, impurities and different rocessing and measuring methods. Different chemical composition leads to different nutritive value, because chemical composition is one of the most important indices of nutritive value of feeds (Maheri-Sis *et al.*, 2008; Aghajanzadeh-Golshani *et al.*, 2010). The rumen bag technique has been used for many years to study the degradation of forages However, the digestibility of a feedstuff is defined by the potential degradability of the material, the rate of degradation of this potentially degradable fraction, and its residence time in the rumen (i.e., its effective degradation) plus digestion after the rumen fermentation in the hind gut (Kiyani-Nahand *et al.*, 2011). Salamat azar *et al.* (2011) estimation effect of tree doses *Thyme* water extract (0, 0.15 and 0.3 mL/30 mL buffered rumen fluid) on degradability kinetics, of canola meal and report Gas volume at 24 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 canola were 47.32, 2.15, 54.96, 57.12 mL/200 mg DM and 0.113 mL/h, Gas volume at 24 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* water extract (0.3 mL/30 mL buffered rumen fluid) were 47.12, 1.73, 54.64, 56.37 mL/200 mg DM and 0.112 mL/h, respectively. Gas volume at 48 and 72 h incubation (for 200 mg dry samples), of canola were 51.41 and 53.79 mL/200 mg DM, while for *Thyme* water extract (0.3 mL/30 mL buffered rumen fluid) were 50.77 and 52.82 mL/200 mg DM, respectively. These results are in agreement with the findings of (Salamat azar *et al.*, 2011).

The crude protein degradation (%) of untreated and treated soybean meal in different incubation times was shown in Fig. 1 and 2.

CONCLUSION

Results of current study indicated that *Thyme methanolic* extract (0.15 mL/30 mL buffered rumen fluid) resulted in decreasing soluble fraction and potentially degradable fraction of soybean meal protein. This study suggested that the *Thyme methanolic* extract (0.15 mL/30 mL buffered rumen fluid) have the potential to affect ruminal protein degradation efficiency.

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