Effects of thymus vulgar extract (0 and 0.15 ml/30 ml buffered rumen fluid) on organic matter ruminal degradability of canola meal using nylon bag technique

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Abstract: This study were to evaluate of effects tow doses (0 and 0.15 ml/30 ml buffered rumen fluid) of Thymus vulgar extract on organic matter ruminal degradability of canola meal using nylon bag technique. Samples were collected from commercial sources in Iran. Nylon bags filled with 5 g of each of untreated or Thymus vulgar extract treated canola 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. The results showed that Organic matter disappearance at 8 h incubation, were 57.47 and 50.78 percent for canola meal and Thymus vulgar extract (0.15 ml/30 ml buffered rumen fluid) respectively. Organic matter disappearance at 16 h incubation, were 63.403 and 62.15 percent for canola meal and Thymus vulgar extract (0.15 ml/30 ml buffered rumen fluid) respectively. Organic matter disappearance at 24 h incubation, were 72.59 and 72.14 percent for canola meal and Thymus vulgar extract (0.15 ml/30 ml buffered rumen fluid) respectively. Organic matter disappearance at 48 h incubation, were 78.21 and 77.92 percent for canola meal and Thymus vulgar extract (0.15 ml/30 ml buffered rumen fluid) respectively.
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Keywords: Canola meal; Thymus vulgar; nylon bag technique; rumen; organic matter degradation; dry matter; degradability.

Abbreviations: **NAD**, nicotinamide adenine dinucleotide; **NADH**, reduced form of NAD; **CM,** canola meal; **VFA**, volatile fatty acids; H_2, hydrogen; CO_2, carbon dioxide; CH_4, methane; **N**, nitrogen; **DM**, dry matter.

1. Introduction

Rumen microbes consisting of protozoa, fungi and bacteria play a pivotal role in rumen fermentation including fiber digestion (Yasuo Kobayashi, 2010). Fermentation results in the synthesis of various products, some of which are not entirely beneficial. One such non-beneficial product is methane (Yasuo Kobayashi, 2010). This gaseous compound is the most prominent hydrogen sink product synthesized in the rumen. Since methane contains energy, its emission during rumen fermentation is considered to be a loss of feed energy that is equivalent to 2-12% of the gross energy of animal feed (Yasuo Kobayashi, 2010; Johnson and Johnson, 1995).

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 (McGuffey et al., 2001; Busquet et al., 2006). 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 (Sallam et al., 2009). 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 (Calsamiglia et al., 2007; Sallam et al., 2009; Calsamiglia et al., 2006). Many plants produce secondary metabolites such as phenolic compounds, essential oils, and sarsaponins (Calsamiglia et al., 2007; Sallam et al., 2009; Calsamiglia et al., 2006).

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 (Maheri-Sis et al., 2011; Ørskov et al., 1980).

The objective of this study was to evaluate effects of Thymus vulgar extract (0.15 ml/30ml buffered rumen fluid) on organic matter disappearance, of canola meal using the nylon bags technique.

2. Material and Methods

2.1. Sample Collection

Canola meal samples were obtained from commercial sources in Iran.

2.2. Thymus vulgar

During summer season Thymus vulgar 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 (Thymus vulgar extract) effects, we added Thymus vulgar water extract with tow doses (0, 0.15 mL: 200 mg sample) into gas test syringes. All samples were then ground in a laboratory mill through a 1 mm screen.

2.3. Procedure of Thymus vulgar extracts preparation

The Thymus vulgar water extract were prepared according to (Patra et al., 2006; Sallam et al 2009) with some modifications. The Thymus vulgar materials were dried at 50°C and ground in mills to pass a 1 mm sieve and 100 g placed in 1000 ml of distilled water 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 Thymus vulgar water extract were combined. Distilled water was evaporated from the solution at approximately 85°C using a rotary-evaporator (Patra et al., 2006; Sallam et al 2009).

***2.4. 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 60oC for 48 h. Then rumen degradation kinetics of sunflower meal (no additive) and Iranian zataria multiflora extract (0.15 ml/30 ml buffered rumen fluid), was calculated using the nonlinear model proposed by Ørskov 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), Iranian zataria multiflora extract (0.15 ml/30 ml buffered rumen fluid) was calculated using equation described by Ørskov 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 were 0.02, 0.05 and 0.08 per hour (Bhargava and Ørskov, 1987) so that the results could be extrapolated to other ruminants that differ in rumen capacity.

2.5. 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.

3. RESULTS AND DISCUSSION

***In situ* degradation procedures**

Organic matter disappearance of canola meal (Control) and Thymus vulgar extract (0.15 ml/30 ml buffered rumen fluid) at different incubation times were shown in Figure 1 and 2.

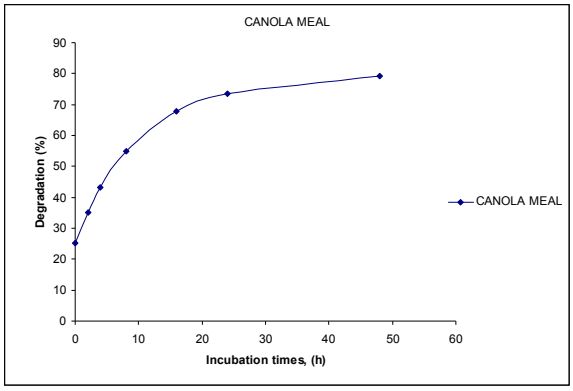

Figure.1. organic matter disappearance of canola meal (Control) was at different incubation times.

Organic matter disappearance of canola meal (Control) and Thymus vulgar extract (0.15 ml/30 ml buffered rumen fluid) at different incubation times were shown in Table 1.

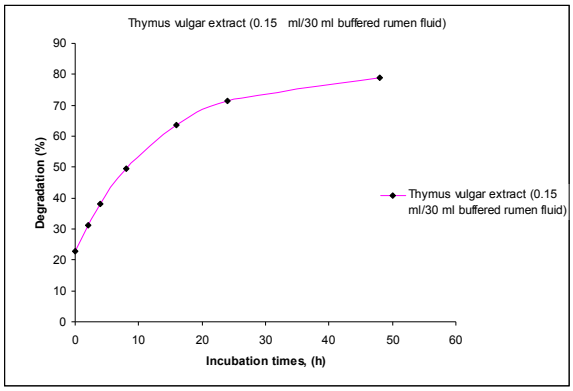

Figure.2. organic matter disappearance of Thymus vulgar extract (0.3 ml/30 ml buffered rumen fluid) was at different incubation times.

The results showed that organic matter disappearance at 0 h incubation, were 24.297 and 24.9 percent for canola meal and Thymus vulgar extract (0.15 ml/30 ml buffered rumen fluid) respectively. Organic matter disappearance at 2 h incubation, were 35.52 and 29.043 percent for canola meal and Thymus vulgar extract (0.15 ml/30 ml buffered rumen fluid) respectively. Organic matter disappearance at 4 h incubation, were 40.684 and 38.913 percent for canola meal and Thymus vulgar extract (0.15 ml/30 ml buffered rumen fluid) respectively. Organic matter disappearance at 8 h incubation, were 57.47 and 50.78 percent for canola meal and Thymus vulgar extract (0.15 ml/30 ml buffered rumen fluid) respectively. Organic matter disappearance at 16 h incubation, were 63.403 and 62.15 percent for canola meal and Thymus vulgar extract (0.15 ml/30 ml buffered rumen fluid) respectively. Organic matter disappearance at 24 h incubation, were 72.59 and 72.14 percent for canola meal and Thymus vulgar extract (0.15 ml/30 ml buffered rumen fluid) respectively. Organic matter disappearance at 48 h incubation, were 78.21 and 77.92 percent for canola meal and Thymus vulgar extract (0.15 ml/30 ml buffered rumen fluid) respectively. High levels of animal productivity cannot be sustained by forage alone (Bunthoeun, 2007; Nocek and Russel, 1988) and ruminant nutritionists have sought methods for decreasing fermentation losses (e.g., methane and ammonia) or increasing the rate of fermentation acid formation and increasing microbial protein synthesis in the rumen (Bunthoeun, 2007). Additives that modify ruminal fermentation, such as organic acids, yeasts, enzymes and antibiotics, have been used to optimize performance in dairy and beef cattle production (Bunthoeun, 2007; Martin et al., 1999; Russell and Houlihan, 2003). The increased concentration of greenhouse gases (carbon dioxide, methane and nitrous oxide) in the troposphere has been implicated in the consistent increase in atmospheric temperature and global warming over the last few decades (IPCC, 2001; Bunthoeun, 2007). The rising concentration of CH_4 is correlated with increasing world populations and currently about 70% of CH_4 production arises from anthropogenic sources and the remainder from natural sources (Bunthoeun, 2007; Moss et al., 2000).

In the rumen, H_2 is produced during the anaerobic fermentation of glucose. This H_2 can be used during the synthesis of volatile fatty acids (VFA) and microbial organic matter. The excess of H_2 from NADH (reduced form of nicotinamide adenine dinucleotide) is eliminated primarily by the formation of CH_4 by methanogens, which are microorganisms from the Archea group (Bunthoeun, 2007) that are normally found in the rumen ecosystem (Bunthoeun, 2007; Baker, 1999). The stoichiometric balance of VFA, CO_2 and CH_4 indicates that acetate and butyrate promote CH_4 production whereas propionate formation conserves H_2, thereby reducing CH_4 production (Bunthoeun, 2007; Wolin, 1960).

Decreasing the retention time of feed in the rumen may reduce CH_4 production. Okine et al., (1986) indicated that a 30% decline in CH_4 production was observed when the ruminal passage rate was increased by 50% or more. When expressed as a proportion of digestible energy, CH_4 losses decreased 1.6 percentage units for each unit of increase in feed intake above the maintenance requirement (Bunthoeun, 2007; Johnson et al., 1995). The addition of grain or soluble carbohydrates to the diet also changes the fermentation pattern in the rumen to a more competitive environment for the methanogens (Bunthoeun, 2007; Van Soest, 1994).

Table 1: organic matter disappearance (%) of canola

meal (Control) and Thymus vulgar extract (0.15 ml/30 ml buffered rumen fluid) at different incubation times		
Incubation time (h)	Control	TVE_{015}
0	24.297	24.9
2	35.52	29.043
4	40.684	38.913
8	57.47	50.78
16	63.403	62.15
24	72.59	72.14
48	78.21	77.92

Rezaei et al, (2011) evaluation effect of tree doses clove methanolic extract (0, 0.5 and 1 ml/30 ml buffered rumen fluid) on degradability, of soybean 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 soybean meal were 71.240, 1.767, 70.880, 72.647 ml/200 mg DM and 0.100 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 clove methanolic extract (1 ml/30 ml buffered rumen fluid) were 22.717, 8.914, 19.516, 28.429 ml/ 200 mg DM and 0.051 ml/h, respectively. Gas volume at 72 and 96 h incubation (for 200 mg dry samples), of soybean meal were 72.24 and 74.360 ml/200 mg DM, while for clove methanolic extract (15 ml/30ml buffered rumen fluid) were 25.383 and 29.130 ml/200 mg DM, respectively.

Salamat azar et al, (2011) 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.

4. Discussions

The results of this study showed that the addition Thymus vulgar extract doses (0.3 ml/30 ml buffered rumen fluid), decreased the organic matter degradation of canola meal. This study suggested that the doses (0.3 ml/30 ml buffered rumen fluid) of Thymus vulgar extract have the potential to affect ruminal fermentation efficiency.

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References

Baker, S.K., 1999. Rumen methanogens and inhibition of methanogenesis. Aust. J. Agric. Res. 50, 1293-1298.

Bunthoeun, P.E.N., J. Takhashi, S. Matsouka, T. Takhashi and S. Salga., 2007. Major Chair of Animal Production the United Graduate School of Agricultural Sciences, Iwate University. Obihiro University of Agriculture and Veterinary Medicine.

Busquet, M., S. Calsamiglia, A. Ferret and C. Kamel. 2006. Plant extracts affect in vitro rumen microbial fermentation. J. Dairy. Sci. 89: 761-771.

Calsamiglia S., M. Busquet, P.W. Cardozo, L. Castillejos and A. Ferret. Invited review: essential oils as modifiers of rumen microbial fermentation. 2007. J. Dairy. Sci. 90:2580–2595.

Calsamiglia, S., L. Castillejos, and M. Busquet, 2006. Alternatives to antimicrobial growth promoters in cattle. Pages 129–167 in Recent Advances in Animal Nutrition. P. C. Garnsworthy, and J. Wiseman, ed. Nottingham University Press, Nottingham, UK.

IPCC, 2001. Climate Change 2001: Synthesis Report. A Contribution of working groups I, II, and III to the third assessment report of the Intergovernmental Panel on Climate Change. Watson, R.T., Core Writing Team, (Eds.), Cambridge University Press, Cambridge, UK, and New York, USA.

Johnson, K.A., Johnson, D.E., 1995. Methane emission from cattle. J. Anim. Sci. 73, 2483-2492.

Martin, S.A., Streeter, M.N., Nisbet, D.J., Hill, G.M., Williams, S.E., 1999. Effects of DL-malate on ruminal metabolism and performance of cattle fed a high-concentrate diet. J. Anim. Sci. 77, 1008–1015.

McGuffey, R. K., L. F. Richardson, and J. I. D. Wilkinson. 2001. Ionophore for dairy cattle: current

Moss, A.R., Jouany, J.P., Newbold, J., 2000. Methane production by ruminants: its contribution to global warming. Ann. Zootech. 49, 231253.

Nocek, J.E., Russel, J.B., 1988. Protein and energy as an integrated system. Relationship of ruminal protein and carbohydrate available availability to microbial synthesis and milk production. J. Dairy Sci. 71, 2070-2107.

Okine, E.K., Mathison, G.W., Hardin, R.T., 1989. Effects of change in frequency of reticular concentrations on fluid and particulate passage rates in cattle. J. Anim. Sci. 67, 3388-3396.

Ørskov, E.R. and I. McDonald, 1979. The estimation of protein degradability in the rumen from incubation measurements weighted according to rate of passage. J. Agric. Sci., 92: 499-503.

Patra, A.K., D.N. Kamra and N. Agarwal 2006. Effect of plant extracts on in vitro methanogenesis, enzyme activities and fermentation of feed in rumen liquor of buffalo. Anim Feed Sci. Technol., 128: 276–291.

Rezaei, N., R. Salamat doust-Nobar, N. Maheri Sis, M. Salamatazar, M. Namvari, S. Goli and H. Aminipour, 2011. Evaluation effect of some plant extracts on degradibility of soybean meal with gas product technique. Annals Biol Res., 2 (4): 224-228.

Russell, J.B., Houlihan, A.J., 2003. The ionophore resistance of ruminal bacteria and its potential impact on human health. FEMS Microbiol. Rev. 27, 65-74.

Salamatazar, M., R. Salamatdoustnobar, Y. Asadi, N. Maheri Sis, S. Najafyar, B. Khodaparast, H. Aminipour, N. Rezayi, M. Maleki, 2011. Effect of thyme methanolic extract on degradability kinetics, of Sunflower meal. Annals Biol Res., 2 (4):104-109.

Sallam, S.M.A., Bueno, I.C.S., Brigide, P., Godoy, P.B., Vitti, D.M.S.S. and Abdalla, A.L. 2009. Investigation of potential new opportunities for plant extracts on rumen microbial fermentation in vitro. Nutritional and foraging ecology of sheep and goats. 85:255-260.

SAS, 2000. SAS Users Guide. Cary, USA: Statistical Analysis Systems Institute.

Snedecor, G.W. and W.G. Cochran, 1980. Statistical Methods. Iowa State University Press, Ames, IA, USA.

Van Soest, P.J., 1994. Nutritional ecology of the ruminant. 2nd ed. Comstock Publ. Assoc., Ithaca, New York, USA.

Wolin, M.J., 1960. A theoretical rumen fermentation balance. J. Dairy Sci. 43, 1452-1459.

Maheri-Sis, N., B. Abdollahi-Ziveh, R. Salamatdoustnobar, A. Ahmadzadeh, A. Aghajanzadeh-Golshani and M. Mohebbizadeh, 2011. Determining Nutritive Value of Soybean Straw for Ruminants Using Nylon Bags TechniquePak. J. Nut., 10 (9): 838-841.

Ørskov, E.R., F.D. DeB Hovell and F. Mould, 1980. The use of the nylon bag technique for the evaluation of feedstuffs.Trop. Anim. Prod., 5: 195-213.

Bhargava, P.K. and E.R. Ørskov, 1987. Manual for the use of nylon bag technique in the evaluation of feedstuffs. Rowett Research Institute, Aberdeen,Scotland,UK.

Yasuo Kobayashi. 2010. Abatement of Methane Production from Ruminants: Trends in the Manipulation of Rumen Fermentation. Asian-Aust. J. Anim. Sci. 23, (3):410-416

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