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# Effects of Addition of *Pinus eldarica* Methanolic Extract on Ruminal Dry Matter Degradation of Canola Meal Using *In sacco* Technique

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**Abstract:** Modern high producing and rapidly growing ruminants require protein in excess of ruminal microbial synthesis. This protein can be supplied by increasing the amount of dietary protein escaping degradation in the rumen. *Pinus eldarica* extract prepared by with methanol. This experiment in nylon bag technique was performed. Treated samples of soybean meal for 0, 2, 4, 8, 16, 24 and 48 hrs in the rumen of three male Ghezel male sheep were incubated. Usages of *Pinus eldarica* for processing of canola meal approximately alter ruminal dry matter degradation.

**Key words:** *Pinus eldarica* · Canola meal · Disappearance

## INTRODUCTION

Protein requirements for ruminants are meet from microbial protein, synthesized in the rumen from degradable protein (RDP) and from rumen undegradable dietary protein (UDP) which is unaffected by the rumen microorganisms prior to entering the abomasum and small intestine. The acknowledgement that the ruminant has a requirement for both RDP and UDP has given rise to the importance of investigating the degradability of feedstuffs in the rumen. Hence, considerable attention has been placed in recent years on determining the degradability of feedstuffs. The in situ DM and CP degradabilities will be useful for that's determine and the best materials for use in practical ruminant diets [1]. Attempts to decrease the rumen degradability of proteins have involved treatment with heat [2, 3], formaldehyde [2, 4-6], acetic acid [7], tannic acid [8], lignosulfonate [9, 10], xylose [9-12]. Canola meal is a commonly used protein supplement for ruminants. Proteins of this supplement are extensively degraded in the rumen. Some attempts to decrease the rate and extent of ruminal degradation of canola meal proteins have used treatment with physical factors and chemical agents [10, 13-16]. *Pinus eldarica* is one of plant source of xylose and deference resin and at be seem could application for safe and economic agent of decrease degradability of plan source protein. Therefore, the objectives were to investigate the effects of different levels of *Pinus eldarica* extract on dry matter degradability of canola meal in the rumen.

## MATERIALS AND METHODS

**Sample Preparation and Treatment:** The canola meal samples treated with 6, 8 and 10 percent of methanol *Pinus eldarica* extract, with 20% additional water to solvent-extracted soybean. The mixture was then dried in room temperature and air dried to approximately 10% moisture.

**Procedure of *Pinus Eldarica* Extract Preparation:** The *Pinus eldarica* methanolic extracts were prepared with some modifications [17, 18]. The *Pinus eldarica* leaves fresh ground in and 100 g placed in 1000 ml of methanol solvent. The flasks of all the solvents were agitated with a magnetic stirrer for 24 hrs 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 hrs stirring at room temperature and centrifuged again at 3000 g for 10 min. extract concentrate at approximately 65°C using a rotary-evaporator.

**In Situ Ruminal Degradability:** Three ruminally cannulated Ghezel rams weighing approximately 50 kg were used. Nylon bags (8cm × 16 cm) with a pore size of 46 mm were filled with approximately 5 g (sample size: bag surface area of 13 mg/cm$^2$) of the samples ground to pass a 2mm screen according to Nocek [19]. Duplicate bags filled with treated canola meal were incubated in the rumen for periods of 0, 2, 4, 6, 8, 12, 16, 24 and 36 hrs. Two series of incubations were completed for each feed and sheep.

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After retrieval from the rumen, bags were washed with tap water and stored at -20°C. After thawing, bags were washed three times for 5 min in a turbine washing machine. The same procedure was applied to two series of two bags to obtain the 0 h value. The residues were analyzed for DM establishes degradation kinetics of canola meal. Digestion kinetics of DM, OM and CP were determined according to the equation of Ørskov and McDonald [20]:

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

Where p is the amount degraded at a time, a the rapidly soluble fraction (g/kg), b the potentially degradable fraction (g/kg), c the constant rate of disappearance of b, t the time of incubation (h), effective rumen degradability of DM, OM and CP was estimated using the equation of Ørskov and McDonald [20]:

$$P_e = a + \frac{bc}{K + C}$$

Where Pe is the effective degradation, k the fractional ruminal outflow rate, a, b and c are as defined above. Effective degradability was calculated with an estimated solid outflow rate from the rumen (k) of 0.02, 0.05 and 0.08 h-1 [21].

**Statistical Analyses:** Disappearances of DM were fitted to the exponential model of Ørskov and McDonald [20] as:

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

Where, p is the amount degraded at a time; (a) is the rapidly soluble fraction (g/kg); b is the potentially degradable fraction (g/kg); c is the constant rate of disappearance of b and t is the time of incubation (h).

The effective degradability (ED) was calculated using $ED = \frac{a + bc}{C + K}$, estimated outflow rates (k) of 0.02, 0.05 and 0.08 $h^{-1}$ [22].

Data were analyzed using the general linear models procedure of SAS [24] with the following statistical model of

$$Y_{ij} = \mu + T_i + e_{ij}$$

Where $Y_{ij}$ is dependent variable, μ is overall mean, $T_i$ is *Pinus eldarica levels* effect and $e_{ij}$ is residual error. Least squares means of each sample by the Duncan test were compared.

## RESULTS AND DISCUSSION

Least square means of deferent *Pinus eldarica* leave methanol extracts on the canola meal dry matter degradability shown in Table 1. According to results washing loss of dry matter in zero time of incubation from 37.04 percent in control group, was significantly affected by addition of *Pinus eldarica* extract and decreased and reached to 29.24 and 28.23 percent in treatments, respectively. Application of extract of *Pinus eldarica* could deceased soluble fraction of dry matter, this rate of disappearance continue to 2 and 4 hours of incubation

Table 1: Least square means of deferent *Pinus eldarica* leave methanol extract on the canola meal dry matter degradability

| | Incubation times | | | | | | |
|---|---|---|---|---|---|---|---|
| | Washing loss | 2 | 4 | 8 | 16 | 24 | 36 |
| Control | 37.04[a] | 41.16[a] | 48.30[ab] | 56.45[a] | 62.86[a] | 69.13[b] | 81.35[b] |
| 6 percent | 29.24[b] | 37.83[ab] | 46.15[bc] | 55.44[a] | 62.97[a] | 68.58[b] | 80.85[b] |
| 8 percent | 34.57[a] | 40.10[a] | 48.97[a] | 55.95[a] | 61.77[a] | 73.89[a] | 83.34[a] |
| 10 percent | 28.23[b] | 36.38[b] | 45.02[c] | 54.27[a] | 61.36[a] | 66.70[c] | 84.15[a] |
| *P value* | 0.0004 | 0.0478 | 0.021 | 0.2276 | 0.2463 | <.0001 | 0.0045 |
| SEM | 0.9494 | 1.0825 | 0.7801 | 0.7133 | 0.6237 | 0.4552 | 0.5101 |
| | Degradation characteristics (g/kg) | | | Effective degradability of DM Pe (g/kg) | | | |
| | a | b | a+b | c | 2 | 5 | 8 |
| Control | 38.26[a] | 53.16[c] | 91.42[a] | 0.0403[b] | 74.23[b] | 62.50[a] | 56.53[a] |
| 6 percent | 31.42[b] | 50.83[c] | 82.26[b] | 0.0711[a] | 70.70[c] | 60.73[b] | 54.80[b] |
| 8 percent | 36.43[a] | 58.44[b] | 94.86[a] | 0.0403[b] | 76.27[a] | 63.43[a] | 56.83[a] |
| 10 percent | 31.52[b] | 61.74[a] | 93.26[a] | 0.0501[b] | 74.13[b] | 60.67[b] | 53.67[b] |
| *P value* | 0.0003 | 0.0002 | 0.0004 | 0.0005 | 0.0002 | 0.0019 | 0.0016 |
| SEM | 0.7476 | 0.9739 | 1.2414 | 0.0029 | 0.4660 | 0.3846 | 0.4097 |

and only 10 percent of extract significantly decrease degradability and other levels of extract numerically decrease disappearance of dry matter. Increasing *Pinus eldarica* levels decreased the (a) fraction and increased the (b) fraction of DM (linear effect, P<0.0001). The degradation rate of the b fraction of DM decreased as *Pinus eldarica* levels increased (linear effect, P<0.0001). ED (2 %) of DM linearly (P<0.0001) decreased as *Pinus eldarica* levels increased. The cones and leafs contained large amounts of glucose (46%) and mannose (25%) and minor quantities of galactose and xylose. The cones also contained significant levels of Klason Lignin (24%) but only barely detectable quantities of acid-soluble lignin (0.7%). Ethanol/toluene extractives made up 6% of the sample. And different resins include Myrcecommunic acid, Secodehydroabietic acid, Pimaric acid, Sandaracopimaric acid, Isopimaric acid, Levopimaric acid, Palustric acid, Lambertianic acid, Dehydroebietic acid, Imbiicataloaic acid, Abietic acid, Neoabietic acid, ImbrictoIoaic acid, Isocupressaic acid, Acetylimbricatoloaic acid, Acetoxyisocupressaic acids [23] and probably this composition decrease microorganism colonization and consequence decrease degradability.

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