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Full Length Research Paper

Determination of processed soybean meal degradability by *Pinus eldarica* methanol extract

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Protected soybean meal is an important part of high producing dairy cow diet and many methods are used for its safe and economic processing. *Pinus eldarica* contains xylose and resins and results show that these components could affect dry matter and crude protein degradability. This is the first time a study is being carried out to investigate the effect of this plant extract on protein source. This experiment was performed using the nylon bag technique. Samples for treating soybean meal for 0, 2, 4, 8, 16, 24 and 48 h in the rumen of three male Ghezel male sheep were incubated. Results indicated that the addition of this plant extract to soybean meal samples could affect dry matter and crude protein degradability and thus protect proteins from rumen degradation.

Key words: *Pinus eldarica*, soybean meal, degradability, protection.

INTRODUCTION

Various parts of some pine species, such as bark, needle, cone and resin have been used as folk medicine for rheumatism or as anti-inflammatory, antioxidant and antiseptic. In the last decades, modern science has shown increasing interest in folk medicine for a better understanding of the chemical composition of natural products and in finding alternative usages (Ahmed et al., 1969; Packer et al., 1999; Kähkönen et al., 1999; Devaraja et al., 2002; Villagomez et al., 2005; Willför et al., 2009). Pine cone extracts is one area of such interest. One of the way to optimize the amount of absorbable amino acid (AA) for high producing dairy cows is to increase and provide adequate amounts of rumen un-degradable protein (RUP) (Schwab, 1995). Research has been conducted on rumen degradability of both plant and animal protein supplements (Erasmus et al., 1994; Cozzi et al., 1995; Ceresna´kova´ et al., 2002). Soybean meal (SBM) is the most commonly used protein supplement for dairy cattle. It has the highest content of essential AA (NRC, 2001). For these reasons, SBM has continued to be extensively studied as a source of AA for high-producing dairy cattle (Ipharraguerre and Clark, 2005).

The rumen microorganism could rapidly degrade protein and amino acid of protein supplement. The ruminant dietary proteins are degraded by microbial protease in the rumen to amino acids which are degraded by deamination to organic acids, ammonia and carbon dioxide. Since protein sources vary in their solubility, the degree of degradation in the rumen is variable. High degradability of protein leads to excessive amount of ammonia which is lost in urine in form of urea. *Pinus eldarica* is one of the plant sources of xylose and resin and could be applied as a safe and economic agent for decreasing protein degradability.

MATERIALS AND METHODS

Sample preparation and treatment

The SBM samples (soybean meal imported from Brazil) were obtained from commercial sources in Iran. SBM was treated with 6, 8 and 10% of methanolic *P. 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. The remainder of each sample was ground to pass a 2 mm screen for the ruminal *in situ* study and preserved as described earlier.

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Table 1. Least square means of different *Pinus eldarica* leave methanol extract on the soybean meal dry matter degradability.

Experimental group	Incubation time (h)						
	Washing loss	2	4	8	16	24	36
Control	19.78^{a}	27.64^{ab}	33.61^{a}	53.94^{a}	75.57^{a}	88.43^{a}	90.90^{a}
6% Extract	16.16^{b}	29.46^{a}	31.33^{a}	36.28^{c}	51.02^{d}	59.82^{c}	68.49^{d}
8% Extract	16.02^{b}	25.52^{b}	31.35^{a}	48.05^{b}	63.80^{c}	73.02^{b}	85.99^{b}
10% Extract	16.02^{b}	28.26^{a}	33.67^{a}	37.95^{c}	67.63^{b}	72.74^{b}	83.53^{c}
P value	0.0002	0.0173	0.0787	<.0001	<.0001	<.0001	<.0001
SEM	0.3680	0.6735	0.7453	0.6238	0.7502	1.9067	0.5075

Experimental group	Degradation characteristic (g/kg)				Effective degradability of DM (g/kg)		
	a	b	a + b	c	2	5	8
Control	16.91^{b}	82.41^{a}	99.32^{a}	0.0739^{a}	81.70^{a}	66.00^{a}	56.47^{a}
6% Extract	19.69^{a}	59.11^{b}	78.81^{b}	0.4787^{c}	61.33^{c}	48.57^{c}	41.77^{c}
8% Extract	16.38^{b}	77.74^{a}	94.12^{a}	0.0592^{b}	74.47^{b}	58.50^{b}	49.43^{b}
10% Extract	17.21^{b}	78.06^{a}	95.28^{a}	0.0544^{bc}	74.13^{b}	57.73^{b}	48.70^{b}
P value	0.0008	<.0001	<.0001	0.0006	<.0001	<.0001	<.0001
SEM	0.3656	1.7967	1.6349	0.0026	0.6335	0.4803	0.5086

Preparation of *P. eldarica* extract

The *P. eldarica* methanolic extracts were prepared with some modifications (Patra et al., 2006; Sallam et al., 2009). The fresh leaves of *P. eldarica* were ground and 100 g was placed in 1000 ml of methanol solvent. The flasks of all the solvents were 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. Finally, the extracts were concentrated at approximately 65°C using a rotary-evaporator.

In situ determination of dry matter degradability

Nylon bag technique was used to measure the disappearance in the rumen of untreated and treated SBM. Nylon bags (45 µM pore size; 10 × 15 cm bag size) containing 5 g of SBM samples were incubated in the rumen of each cow. The experiment was performed in a completely randomized design with three treatments and sex replications for each animal. Two bags of each type of treated SBM were removed after 2, 4, 8, 16, 24 and 36 h of incubation in the rumen and 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 to 40°C for 1 h, followed by the washing procedure above. Digestion kinetics of crude protein (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; 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 rumen degradability of CP was estimated using the equation of Ørskov and McDonald (1979):

EDDM or EDCP = a + (bc)/(c + k)

Where, k is the estimated rate of out flow from the rumen, and a, b and c are the same parameters as described earlier. Effective degradability of dry matter (DM) or crude protein (CP) was estimated for each ingredient.

Statistical analysis

Data were analyzed in a complete randomized design using the GLM procedure of SAS version 8.2 (SAS Inst. Inc., Cary, NC). For statistical analysis of data, Neway and SAS software package was used. After analysis of variance, least squares means of each sample were compared by the Duncan's test.

RESULTS AND DISCUSSION

Dry matter degradability

The least square means of *P. eldarica* leave methanolic extract on the soybean meal dry matter degradability are shown in Table 1. Results show that washing loss of dry matter of SBM in the control group was 19.78%, but with the usage of *P. eldarica* extract, it significantly decreased to 16% range of soluble fraction. The condition in the 2 and 4 h incubation period have no logical rate and was probably due to the delay of colonization of micro-organism on the feed particles. For 8 h incubation time, compared to the control group with 53.94, 36.28, 48.05 and 37.95% were obtained in the experimental treatments with 6, 8 and 10% of extract, respectively. Critical time of incubation was 16 h and it is important for passage to pot ruminal and inter to small intestine.

For 16 h of incubation, the 75.57% found in the control

Table 2. Least square means of different *Pinus eldarica* leave methanol extract on the soybean meal crude protein degradability.

Experimental group	Incubation time (h)						
	Washing loss	2	4	8	16	24	36
Control	18.28[a]	19.64[a]	25.61[a]	45.94[a]	67.57[a]	80.43[a]	84.24[a]
6% Extract	10.66[b]	17.46[b]	19.33[b]	24.28[b]	39.02[c]	47.82[c]	56.49[c]
8% Extract	10.52[b]	13.52[c]	19.35[b]	26.05[b]	40.80[c]	47.69[c]	54.99[c]
10% Extract	10.52[b]	16.26[b]	21.67[b]	25.95[b]	59.31[b]	57.07[b]	72.87[b]
P value	<.0001	0.0012	0.0009	<.0001	<.0001	<.0001	<.0001
SEM	0.3680	0.6735	0.7453	0.6238	1.6018	0.7981	1.1070

Experimental group	Degradation characteristic (g/kg)				Effective degradability of DM (g/kg)		
	a	b	a+b	c	2	5	8
Control	12.92[a]	83.15[a]	96.07[a]	0.0643[a]	75.87[a]	59.13[a]	49.47[a]
6% extract	11.35[b]	66.16[b]	77.51[b]	0.0323[c]	52.30[c]	37.40[c]	30.47[c]
8% extract	9.43[c]	56.03[c]	65.46[c]	0.0511[b]	48.90[d]	36.87[c]	30.47[c]
10% extract	8.88[c]	79.87[a]	88.75[a]	0.0501[b]	64.00[b]	46.63[b]	37.60[b]
P value	0.0002	0.0005	0.0003	0.0009	<.0001	<.0001	<.0001
SEM	0.3662	2.8802	2.8507	0.0031	1.0211	0.5351	0.5351

significantly reached 51.02, 63.80 and 67.63% in the group processed with 6, 8 and 10% of *P. eldarica* extract and the result show that this extract could protect soybean and significantly decreased degradability of dry matter.

Degradation characteristics including a, b, a + b and c significantly affected extract levels and results show that soy bean was protected from degradability so that a fraction of dry matter of soluble dry matter of soy bean meal was not affected compared to the control group. For a + b, the fraction or slowly degradable dry matter of soybean meal significantly changed from 82.41% in the control group to 78.81, 94.12 and 95.28%, respectively, for different levels of extract. The level of 6% *P. eldarica* extract highly protected slowly degradable fraction of soybean meal as it significantly decreased the degradability of soy bean meal compared with the control and other treatment groups. Effective degradability of dry matter was reported in three conditions; maintenance (2%), to time of maintenance (5%) and production (8%). According to the result obtained the use of extract of *P. eldarica* changed from 81.70% in the control group to 61.33, 74.47 and 74.13% in the experimental treatments, respectively.

Crude protein degradability

The least square means of different *P. eldarica* leave methanol extract on the soybean meal crude protein degradability are shown in Table 2. Soluble fraction of crude protein in zero time of incubation was affected with different level of *P. eldarica* extract and significantly decreased from 18.28% in the control group to 10% ranges in the experimental treatments. Protection in experimental treatments was shown in 2, 4 and 8 h of incubation, which significantly decreased degradability, while for 16 h of incubation, the result show the value from 67.57% in the control group which significantly reached 39.02, 40.80 and 59.31%, respectively, in 6, 8 and 10% extract. Decreased protein degradability was also seen in 24 and 36 h of incubation and was from 80.43 and 84.24% in control group; the value reached 47 to 57% and 54 to 72% range, respectively.

Degradation characteristics including a, b, a+b and c significantly affected extract levels and soluble fraction from 12.92% in control group to 11.35, 9.43 and 8.88% in experimental treatments. Same condition was noticed for slowly degradable protein (b) which from 83.15% decreased and was slowly degraded to 66.16, 56.03 and 79.87%. Sum of a and b constitute potential of degradability (a+b) and was significantly affected as the value changed from 96.07 to 77.51% and 65.46 for 6 and 8% of *P. eldarica*, respectively. Although 10% of extract had numerical decrease, no significant difference was observed with the control group. Effective degradability for maintenance condition (2%) affected different levels of extract and from 75.87% in the control group significantly decreased and reaches to 52.30, 48.90 and 64.00% in the experimental treatments.

In addition, 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 to 6% of the sample, and different resins including myrcecommunic acid, secodehydroabietic acid, pimaric acid,

sandaracopimaric acid, isopimaric acid, levopimaric acid, palustric acid, lambertianic acid, dehydroabietic acid, imbiicataloaic acid, abietic acid, neoabietic acid, imbrictoloaic acid, isocupressaic acid, acetylimbricatoloaic acid, acetoxyisocupressaic acid (Micales and Davis, 1994) and probably these composition decrease microorganism colonization and consequently decrease protein degradability.

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REFERENCES

Ahmed Z, Siddiqui M, Khan I (1969). Combined effects of diphenyliodonium chloride, pine oils and mustard oil soaps on certain microorganisms. Appl. Environ. Microbiol., 17(6): 857–860.

Ceresna´kova´ A, Sommer A, Chrenkova´ M, and Dolesova´ P (2002). Amino acid profile of escaped feed protein after rumen incubation and their intestinal digestibility. Arch. Anim. Nutr., 56: 409–418.

Cozzi G, Andrighetto I, Berzaghi P (1995). *In situ* ruminal disappearance of essential amino acids in protein feedstuffs. J. Dairy Sci., 78: 161–171.

Devaraja S, Vega-Lópeza S, Kaula N, Schönlaub F, Rohdewaldb P, Jialala I (2002). Supplementation with a pine bark extract rich in polyphenols increases plasma antioxidant capacity and alters the plasma lipoprotein profile. Lipids, 37(10): 931–934.

Erasmus LJ, Botha PM, Cruywagen CW, Meissner HH (1994). Amino acid profile and intestinal digestibility in dairy cows of rumen-undegradable protein from various feedstuffs. J. Dairy Sci., 77: 541–551.

Ipharraguerre IR, Clark JH (2005). Impacts of the source and amount of crude protein on the intestinal supply of nitrogen fractions and performance of dairy cows. J. Dairy Sci., 88: 22–37.

Kähkönen MP, Hopia AI, Vuorela HJ, Rauha JP, Pihlaja K, Kujala TS, Heinonen M (1999). Antioxidant activity of plant extracts containing phenolic compounds. J. Agric. Food Chem., 47(10):3954–3962.

Micales JA, Davis JL (1994). Chemical Composition and Fungitoxic Activities of Pine Cone Extractives. Proceedings of 4th meeting of the Pan American Biodeterioration Society; 1991 August 20–25; as an electronic symposium. New York: Plenum Press: 317-332.

NRC (National Research Council) (2001). Nutrient Requirements of Dairy Cattle. 7th rev. ed. National Academy Press, Washington, DC.

Ørskov ER, McDonald I (1979). The estimation of protein degradability in the rumen from incubation measurements weighted according to rate of passage. J. Agric. Sci., 92:499–503.

Packer L, Rimbach G, Virgili F (1999). Antioxidant activity and biological properties of a procyanidin rich extract from Pine. Free Radic. Biol. Med., 27:704–724.

Patra AK, Kamra DN, Agarwal N (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.

Sallam SMA, Bueno ICS, Brigide P, Godoy PB, Vitti DMSS, Abdalla AL (2009). Investigation of potential new opportunities for plant xtracts on rumen microbial fermentation *in vitro*. Nutritional and foraging ecology of sheep and goats, 303: 255-260.

Schwab CG (1995) Protected proteins and amino acids for ruminants. Pages 115–141 in Biotechnology in Animal Feeds and Animal Feeding. R. J. Wallance and A. Chesson, ed. VCH, Wenheim, Germany.

Villagomez HZ, Peterson DM, Herrin L, Young RA (2005). Antioxidant activity of different components of pine species. Holzforschung, 59:156–162.

Willför S, Ali M, Karonen M, Reunanen M, Arfan M, Harlamow R (2009). Extractives in bark of different conifer species growing in Pakistan. Holzforschung, 63: 551–558.