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RESEARCH ARTICLE

The Influence of the Plant Tannins on *in vitro* Ruminal Degradation and Improving Nutritive Value of Sunflower Meal in Ruminants

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ABSTRACT

The objective of this study was to evaluate the effect of 30 g/kg dry matter (DM) from the tannins of oak leaves and fruit (OL, OF), pistachio hull and leaves (PH, PL) on *in vitro* ruminal degradation, gas production parameters and nutritive value of sunflower meal (SM) in ruminants. *In vitro* gas production, organic matter digestibility (OMD), metabolizable energy (ME) and fermentative parameters of samples were measured. Kinetics of gas production was fitted to an exponential model. The results showed that tannin of oak leaves and pistachio hull did not influence the fermentable fraction (b) and gas production rate constant (c), but tannin of oak fruit and pistachio leaves reduced these parameters ($P<0.05$), and the lowest (b) and (c) obtained for SM treated by 30 g/kg oak fruit tannin. The OMD and ME values of sunflower meal were decreased by tannin of oak fruit and pistachio leaves ($P<0.05$). The lowest OMD and ME was for sunflower meal treated with oak fruit tannin, however, tannin of oak leaves and pistachio hull did not influence these parameters. It means that using of some plant tannins with protein meals caused to decrease of OMD and ME of meal ($P>0.05$). The ammonia-N (NH_3-N) concentrations of culture fluid decreased ($P<0.05$) when SM was treated with all tannins sources used in this experiment. Concentration of NH_3-N and short chain fatty acid (SCFA) was lowest for SM treated by oak fruit tannin. The results showed that *in vitro* degradation; fermentation and nutritive value of sunflower meal are decreased by 30 g/kg DM tannin of oak fruit and pistachio leaves. Therefore, tannin of oak leaves and pistachio hull was proper than the other tannin sources to improving ruminal degradation and nutritive value of sunflower meal.

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INTRODUCTION

Selection of the proper source of supplemental crude protein (CP) for feeding offers an excellent opportunity for influencing the supply of amino acids to dairy cows (Clark *et al.,* 1992). Sunflower meal is used as supplemental protein in dairy rations and is classified as highly degradable (Economides, 1998). There are some methods for decreasing protein degradation in rumen such as using tannins. Tannins have high affinity for proteins and protect them from ruminal microbial degradation (Cortes *et al.,* 2009; El-Waziry *et al.,* 2007).

About 3 million hectare of forests is covered by various oak species, in the north-west of Iran. Oak leaves are often grazed by ruminants or harvested for use as livestock feed during feed shortage. However, Oak (Quercus) species have been reported to contain high levels of tannin. It is also reported that about 150,000 tons of pistachio by-product is produced from dehulling process in Iran, annually, that is a natural source of phenolic contents and the most important anti-nutritional factor is tannin.

At normal pH of the rumen, proteins remain bound to the tannin, but at the acidic pH in the abomasum the tannin-protein complexes may be cleaved resulting in an increase in the amount of dietary protein available for digestion in the intestine (Lascano *et al.,* 2003). Therefore, the objective of the current study was to determine the effects of protecting sunflower meal (SM) from ruminal degradation with the different sources of tannin; 30 g/kg tannin of oak (*Quercus persica*) leaves and fruit and tannin of pistachio (*Pistachio vera*) hull and leaves and the effect on *in vitro* rumen fermentation, degradation and improved nutritive value.

MATERIALS AND METHODS

Experimental samples and analysis: Experimental samples were including: untreated SM (USM), SM treated with 30 g/kg DM oak leaves tannin (OLSM), oak fruit tannin (OFSM), pistachio hull tannin (PHSM) and pistachio leaves tannin (PLSM). Tannin contents of oak leaves and fruit, pistachio hull and leaves were determined by using Folin-Ciocalteu reagent in calorimetric method (Makkar and Singh, 1992).

***In vitro* gas production:** Rumen fluid was supplied from two fistulated Baluchi sheep fed a 40:60 concentrate: forage (3 kg concentrate: 2 kg alfalfa hay + 2.5 kg corn silage) prior to the morning meal, homogenized in a laboratory blender, filtered through three layers of cheese-cloth and purged with CO_2 and was added to the anaerobic mineral buffer solution (1:2 v/v). Buffer medium composition, per litre was $NaHCO_3$, 70.0 g; NH_4HCO_3, 4.00 g; Na_2HPO_4, 5.7g; KH_2PO_4, 6.2g; $MgSO_4{\cdot}7H_2O$, 0.6 g; Na_2S, 0.52 g; $CaCl_2{\cdot}H_2O$, 13.2 g; $MnCl_2{\cdot}4H_2O$, 10.00 g; $CoCl_2{\cdot}6H2O$, 1.00 g; sodium resazurin, 0.01 g and 60 ml freshly prepared reduction solution containing 580 mg $Na_2S{\cdot}9H_2O$ and 3.7 ml 1 M NaOH. The mixture was kept stirred, under CO_2 flushing at 39°C using a magnetic stirrer fitted on a hot plate. Effect of tannins on protecting of SM was assessed by incubating approximately 200 mg experimental sample (1.0 mm screen, triplicate) with 30 ml of rumen buffer mixture in 100 ml glass syringes based on Menke and Steingass (1988) procedures. Gas production (ml) was recorded at 2, 4, 6, 8, 10, 12, 16, 24, 48, 72 and 96 h. Total gas values were corrected for blank with a known gas production.

Calculations and statistical analyses: After 96 hours of incubation with rumen buffer mixture, the culture fluid of each syringe was used for determination of NH_3-N concentration using distillation method (Kjeltec 2300 Autoanalyzer, Foss Tecator AB, Hoganas, Sweden). Cumulative gas production data were fitted to the exponential equation $Y=b\ (1-e^{-ct})$, where b=gas production from the fermentable fraction (ml), c; the gas production rate constant (ml/h), t=the incubation time (h) and Y=gas produced in time t. The values of organic matter digestibility (OMD) and metabolisable energy (ME) of experimental samples were calculated by the equation of Menke and Steingass (1988), OMD (g/kg OM) = 148.8 + 8.89 GP + 4.5 CP + 0.651 XA and ME (MJ/kg DM) = 2.20 + 0.136 GP + 0.057 CP + 0.0029 CP^2. Short chain fatty acids (SCFA) were determined by the equation reported by (Getachew *et al.,* 1999). SCFA (µmol) = 0.0239 GP-0.0601. CP and XA were crude protein and ash in g/100 g DM, and GP was the net gas production (ml/200 mg DM) after 24 h incubation.

Data of *in vitro* gas production, ME, OMD, NH_3-N and SCFA were subjected to analysis as a completely randomized design using the General Linear Model (GLM). Duncan's multiple range test was used to compare treatment means at $P<0.05$.

RESULTS AND DISCUSSION

The result of this experiment showed that tannin contents of OL, OF. PH and PL were 53, 79, 48, and 65 g/kg DM, respectively. Tannin of oak leaves and pistachio hull did not reduce the fermentable fraction (b) and gas production rate constant (c), but tannin of oak fruit and pistachio leaves reduced these parameters ($P<0.05$), and the lowest (b) and (c) were obtained for SM treated by 30 g/kg oak fruit tannin (Tables 1 and 2). Earlier, researchers have also reported that fractions b and c of soybean meal significantly decreased when it was treated by querbracho tannin (El-Waziry *et al.* 2007). It may be that the contents of condensed tannin of oak fruit and pistachio leaves had been more than the other tannins. Bach *et al.* (2005) reported that addition of tannin increased the fermentation lag time, possibly due to inhibition of microbial enzyme activity. Comparable results were obtained by Del Pino *et al.* (2005), who also found a positive relationship between condensed tannin content and gas production lag time.

Table 1: Ruminal degradation, gas production and estimated parameters of sunflower meal treated with various tannins

Item	Treatments					S.E.M
	USM	OLSM	OFSM	PHSM	PLSM	
b (ml)	149.8[a]	124.2[b]	108.2[d]	126.2[b]	119.2[c]	1.5
c (ml/h)	0.13[a]	0.06[b]	0.03[d]	0.06[b]	0.05[c]	0.01
OMD (g/kg OM)	182.6[a]	165.8[b]	151.3[d]	168.2[b]	158.8[c]	0.4
ME (MJ/kg DM)	12.30[a]	10.2 [b]	8.1[d]	10.4[b]	9.2[c]	0.02

b-gas production from fermentable fraction; c-gas production rate constant; USM-Untreated sunflower meal; SM treated with 30 g/kg DM oak leaves tannin (OLSM), SM treated with 30 g/kg DM oak fruit tannin (OFSM), SM treated with 30 g/kg DM pistachio hull tannin (PHSM) and SM treated with 30 g/kg DM pistachio leaves tannin (PLSM).

Table 2: Gas production of sunflower meal treated with various tannins after different incubation times

Incubation time (h)	Treatments				
	USM	OLSM	OFSM	PHSM	PLSM
0	0	0	0	0	0
2	12	8	4	10	6
4	21	15	10	16	13
8	62	23	20	31	21
12	81	55	45	65	50
24	92	71	65	75	69
48	118	86	79	91	80
72	134	104	95	110	101
96	145	120	104	125	116

Untreated SM (USM), SM treated with 30 g/kg DM oak leaves tannin (OLSM), SM treated with 30 g/kg DM oak fruit tannin (OFSM), SM treated with 30 g/kg DM pistachio hull tannin (PHSM) and SM treated with 30 g/kg DM pistachio leaves tannin (PLSM).

It has been reported that tannins decrease cumulative gas production, probably by formation of tannin–macromolecule complexes which inhibit microbial enzymes (McSweeney *et al*., 2001) and/or nutrient utilization by ruminal anaerobes (Makkar, 2003). The reduced gas production by adding tannins might be due to a decline in attachment of microbes to feed particles (Alipour and Rouzbehan, 2010). The studies of Tabacco *et al.* (2007) showed that tannins significantly depressed gas production, probably by hampering rumen microorganisms. The reduced gas production from mimosa tannin reflects inhibition of degradation of bacterial cell walls (Bento *et al.,* 2005). Bueno *et al.* (2008) suggested that binding of mimosa tannin to microorganisms or their enzymes caused to deprive of substrate and reduced microbial degradation of carbohydrates and subsequently gas production (Muhammed *et al.,* 1994; Frutos *et al.,* 2004). Makkar (2003) reported that if tannin concentration in the diet

becomes too high, microbial enzyme activities including cellulase and intestinal digestion may be depressed. But McMahon *et al.* (2000) concluded that tannins do not simply inhibit cellulose digestion by rumen fluid, but the inhibitory effects of tannins involved the bacterial cells themselves. The researchers showed condensed tannins for having effects on microbial adhesion, penetration, colonization and consortium formation processes which are essential for the ruminal digestion of feed (Sinclair *et al.,* 2009).

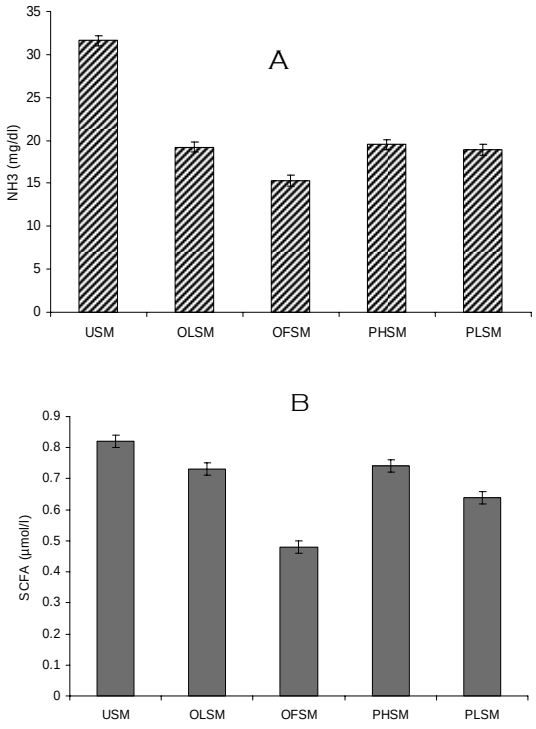

Fig. I: The effect of various tannins on in vitro NH_3 (upper) and SCFA (lower) of sunflower meal. Untreated SM (USM), SM treated with 30 g/kg DM oak leaves tannin (OLSM), SM treated with 30 g/kg DM oak fruit tannin (OFSM), SM treated with 30 g/kg DM pistachio hull tannin (PHSM) and SM treated with 30 g/kg DM pistachio leaves tannin (PLSM).

The values of ME were 12.3, 11.2, 8.1, 10.9 and 9.2 MJ/kg DM for USM, OLSM, OFSM, PHSM and PLSM, respectively. The value of OMD and ME of sunflower meal were decreased by tannin of oak fruit and pistachio leaves and the lowest OMD and ME were for sunflower meal treated with oak fruit tannin ($P<0.05$), but oak leaves and pistachio hull did not influence these parameters ($P>0.05$). Also the other researchers observed decrease of ME for soybean meal treated with quebracho tannin (El-Waziry *et al*., 2007), and reduce OM digestibility by about 5.1% (Tabacco *et al.,* 2007). The decrease in OMD and ME was probably due to decreased rumen degradability and formation of complexes between tannins and dietary proteins and carbohydrates, as well as reducing rumen microbial proteolytic cellulolytic and general fermentative activities (Muhammed *et al.,* 1994).

The concentration of SCFA and NH_3-N decreased ($P<0.05$) when SM was treated with tannin sources used in this experiment (Fig. 1). The mean values of SCFA concentrations were 0.82, 0.73, 0.48, 0.74 and 0.64 µmol/L for untreated SM, SM treated with 30 g/kg tannin of oak leaves, oak fruit, pistachio hull and pistachio leaves, respectively. This result proved the findings of other researchers who reported that volatile fatty acid concentrations were significantly decreased when soybean meal was treated by querbracho tannin (EL-Waziry *et al.*, 2007). Tannins caused to reduce enzyme production from the microbes available to ferment substrate (Shanmugavelu *et al.,* 2004) and carbohydrate (Hess *et al.,* 2003).

Content of NH_3-N was lowest for SM treated by oak fruit tannin ($P<0.05$). The mean values of NH^3-N concentrations were 31.6, 19.2, 15.3, 19.5 and 18.2 mg/dL for USM, OFSM, OLSM, PHSM and PLSM, respectively (Fig. 1A). The same manner of reducing ruminal NH_3-N concentrations was observed in researches of Sliwinski *et al.* (2002) and El-Waziry *et al.* (2005). The lower ammonia concentrations were mainly due to reduce proteolysis, degradation of peptides and deamination of amino acids in the rumen (Frutos *et al.*, 2004). Tannins have been shown to protect dietary protein from ruminal degradation and could be used advantageously to increase bypass protein to improve ruminant performance (Makkar, 2003).

The results showed that *in vitro* ruminal degradation and nutritive value of sunflower meal are decreased by 30 g/kg DM tannin from oak fruit and pistachio leaves. Therefore, tannin of oak leaves and pistachio hull were more suitable than the other tannin sources to improve nutritive value of sunflower meal.

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