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Effect of a Daily Supplementation of Polyethylene Glycol on Intake and Digestion of Tannin-Containing Leaves (*Ceratonia siliqua*) by Sheep[†]

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The nutritional value of carob leaves (CL) and the effectiveness of increasing amounts (12.5, 25, 32, and 50 g/day) of polyethylene glycol 4000 (PEG) supplemented once daily in overcoming the negative effect of tannins in carob leaves, were examined in four Merino ewes. Ewes fed solely CL were not able to sustain their body weights, and excreted in feces more protein than was consumed; the digestibility of cell walls was very low (220 g/kg). Supplementation with 25 g/day PEG increased digestible organic matter intake by 2-fold. This increase was associated with a marked improvement in protein and cell wall digestibility and recovery of weight loss. The present results provide evidence for the first time that condensed tannins may induce a marked depressive effect on the intestinal activity of trypsin and amylase in ruminating animals (as indicated by their activity in fecal samples).

INTRODUCTION

Tannins are a very complex group of plant antinutritional metabolites that are distinguished from other polyphenolic compounds by their ability to precipitate proteins. Their major negative effect results from either direct inhibition of digestive enzymes, or from formation of indigestible complexes with endogenous proteins, like salivary proline-rich protein (Mehanso et al., 1987), or food proteins (Hagerman, 1989). Jones and Mangan (1977) found that condensed tannin of sainfoin form a stable complex with fraction 1 leaf protein and with submaxillary mucoprotein over a wide range of pH 3.5−7.0, but they dissociate at pH <3.5 and >8.5. Therefore, in the presence of tannins in the rumen (pH 5.5−7.0) most plant proteins are bound and protected from microbial degradation, but are released in the abomasum (pH 2.5−3.5), enabling protein digestion and absorption of amino acids in the small intestine (Barry and Manley, 1984). The presence of condensed tannins at levels of 1% to 4% in the diet of sheep increased markedly the postruminal amino acid absorption in comparison with non-tannin-containing forage (Barry and Manley, 1984; Barry et al., 1986; Waghorn et al., 1987, 1990). However, release of tannins in the abomasum may exert adverse effects on enzymes and proteins in the intestine (pH 7.5−8.5). In monogastric animals, one of the main adverse effects of tannins on nutrient utilization is depression of pancreatic trypsin and amylase activities (Ahmed et al., 1991; Longstaff and McNab, 1991).

Tannins have a higher affinity to form complexes with polyethylene glycol (PEG) with molecular weight of 4000, than with proteins (Jones and Mangan, 1977). The complex PEG−tannins is irreversible over a wide range of pH, and therefore markedly reduce the formation of protein−tannin complex (Jones and Mangan, 1977). *In vitro* studies showed that the addition of PEG to plant samples containing tannins increased the digestibility of dry matter and protein (Carrido et al., 1991). This positive effect of PEG also occurs *in vivo* [see Kumar and Vaithiyanathan (1990) for review; and Pritchard et al. (1992) and Terril et al. (1992) for more recent information]. PEG has been applied to animals by various methods which included: spraying of tannin-rich green bushes, treatment of harvested leaves, infusion into the rumen, and drenching the animals. These methods are either uneconomical or impractical under farm management.

Carob trees (*Ceratonia siliqua*) are an important browse source in countries bordering the Mediterranean sea. The pods contain 50% sucrose and were once considered an important feedstuff for domestic animals (Alumot et al., 1964; Volcani and Rodrig, 1961). However, their inclusion in ruminant and poultry diets in Israel was eventually ceased, because the presence of tannins in the pods induced deleterious effects on digestion, growth and milk production (Alumot et al., 1964; Tagari et al., 1965; Tamir and Alumot, 1969; Volcani and Rodrig, 1961).

The purposes of the present experiment were to (i) obtain information on the composition and nutritional value of carob tree leaves for ruminants, using sheep as a model, (ii) study the effect of carob leaves on the activity of pancreatic enzymes in the intestine, and (iii) examine whether PEG, given to the animals once a day, is effective in neutralizing the negative effect of tannins.

MATERIALS AND METHODS

Animals. The experiments were carried out during the winter months with four nonlactating and nonpregnant Merino ewes weighing 47 (SD ± 5) kg. The animals were stall-fed individually in a yard protected from rain and wind and equipped with troughs which enabled quantitative measurement of feed intake.

Feed. Carob leaves attached to small branches (2−3 mm wide) (CL) were harvested once a week early in the morning and stored at −20 °C. The daily allotment was removed from the freezer and fed once daily *ad libitum* at 8:00 a.m. Water was always available.

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Table 1. Effect of Once-a-Day Supplementation of PEG-4000 on Intake and Digestion of Carob Leaves in Sheep (n = 4, Mean of the Second Week of Each Treatment ± SD)[a]

			digestibility (fraction of intake)			
PEG intake, g/day	OM intake, g/day	ΔBW, g/day	OM	protein	cell wall	lignin
0^{b}	562^{a} ± 47	−150^{a} ± 27	0.507^{a} ± 0.044	−0.089^{a} ± 0.025	0.220^{a} ± 0.038	−0.031^{a} ± 0.000^{c}
	565^{a} ± 41	−155^{a} ± 33	0.510^{a} ± 0.048	−0.091^{a} ± 0.017	0.225^{a} ± 0.035	−0.025^{a} ± 0.007
12.5	760^{b} ± 46	15^{b} ± 25	0.572^{b} ± 0.039	0.253^{b} ± 0.03	0.301^{b} ± 0.035	0.005^{b} ± 0.005
25	972^{c} ± 55	100^{c} ± 30	0.651^{c} ± 0.040	0.560^{c} ± 0.048	0.455^{c} ± 0.045	0.021^{c} ± 0.006
12.5 × 2	974^{c} ± 57	104^{c} ± 41	0.653^{c} ± 0.039	0.565^{c} ± 0.052	0.460^{c} ± 0.046	0.024^{c} ± 0.008
32	1074^{c} ± 61	120^{c} ± 45	0.655^{c} ± 0.042	0.572^{c} ± 0.045	0.466^{c} ± 0.038	0.022^{c} ± 0.006
50	1116^{c} ± 82	115^{c} ± 49	0.660^{c} ± 0.049	0.579^{c} ± 0.048	0.478^{c} ± 0.042	0.020^{c} ± 0.007

[a] PEG, polyethylene glycol; OM, organic matter; ΔBW, changes in body weight. Values followed by different superscript letters are significantly different (p < 0.05). [b] First experimental period. [c] Last experimental period.

Digestibility Trial. The animals were adapted for 3 weeks to consume CL as their sole feed by gradually replacing their previous diet (wheat hay *ad libitum* + 100 g of concentrates/day) with CL. The animals were fed CL solely for a week before the commencement of the digestibility trials.

The experimental treatments were as follows: (i) CL alone; (ii–vi) CL with various amounts of PEG given once a day at 8:00 a.m. or twice a day at 8:00 a.m.and 4:00 p.m.; (ii) 12.5 g/day PEG; (iii) 25 g/day PEG; (iv) 32 g/day PEG; (v) 50 g/day PEG; (vi) 2 × 12.5 g/day PEG; (vii) CL alone (second time). PEG mixed with a small amount (3–5 g) of concentrates (16% protein in a mesh form) was given to each animal separately in the morning, before providing CL. The animals were weighed routinely twice a week at 10:00 a.m.

Each level of PEG was given for 2 weeks, daily feed intake was recorded and during the second week digestibility trial was carried out. Daily subsamples from the fresh feed offered and from total feed refusals were stored at −20°C for further analyses. Fecal grab samples were taken every 6 h over a period of 4 days and stored at −20 °C. Digestibility of organic matter, protein (N × 6.25), and cell wall (NDF) was determined by measuring their concentration and the concentration of an internal marker in the feed and in the grab samples. Acid-insoluble ash (Sunvold and Cochran, 1991) and NDF of samples incubated for 8 days in Dacron bags in the rumen of fistulated cows (Lippke et al., 1986) were used as the internal marker.

Chemical and Statistical Analyses. Feed, feed refusals, and fecal samples were dried at 40 °C (to exert minimal changes on tannin content and activity; Hagerman, 1988; Makkar and Singh, 1991) to constant weight and then ground to pass through a 1-mm screen. Dry matter, organic matter, and nitrogen (Kjeldahl) were determined by standard procedures (AOAC, 1984). The contents of cell wall (as material insoluble in neutral detergent, NDF) and lignin (as material insoluble in 72% sulfuric acid) in the cell wall were determined according to Goering and Van Soest (1970).

Samples of feed and feed refusal were extracted with 70% aqueous acetone and with 1% HCl in methanol (Hagerman, 1988). Total phenolic compounds was measured by the Folin–Denis method (Swain and Hillis, 1959), and the values were expressed as catechin equivalent. Condensed tannin was measured by the butanol/HCl method (Porter et al., 1986) using quebracho (Trask Chemical Corp., Georgia) condensed tannin as standard, after purification on Shephadex LH-20 (Pharmacia) according to Asquith and Butler (1985).

Trypsin and amylase activities were determined in the supernatant prepared by homogenizing nondried fecal samples with water (1:5) according to Nitsan and Nir (1977).

Comparisons between treatments were made by analysis of variance, with sheep, treatments, and marker type as independent terms in the model. Statistical significance was assessed by the contrast routine (Duncan test) using SAS (1982) procedures. Similar levels of digestibilities were obtained with both markers (within 5%); therefore, the results were pooled.

RESULTS

Carob Leaves Composition. CL contained 550 g/kg DM, and on a dry matter basis: 930 g/kg organic matter, 300 g/kg water-soluble materials, 330 g/kg cell wall, and 130 g/kg lignin. CL contained approximately 20 g/kg total phenolic compounds with the two extraction methods. The yield of condensed tannins was 50 g/kg (CV 6%) when extracted by aqueous acetone, whereas extraction with 1% HCl in methanol yielded only 20 g/kg (CV 5%). Feed refusals contained 335 g/kg cell wall and 50 g/kg (based on aqueous acetone extraction) condensed tannins, which was not significantly different (by t-test analysis) from the amount of tannins in the food offered, indicating that the ewes did not reject specific parts of CL because of high tannin content.

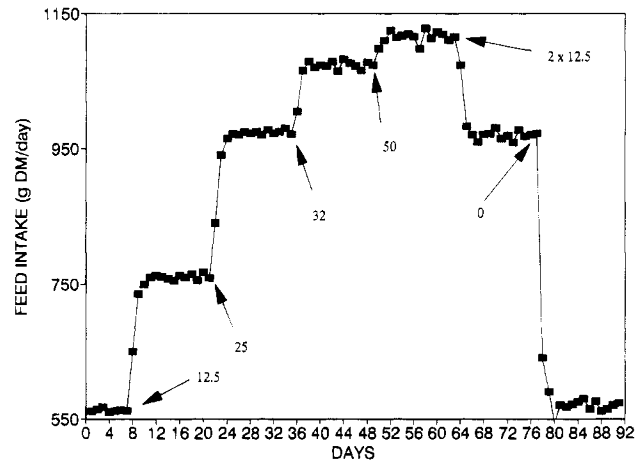

Figure 1. Effect of once-a-day supplementation of PEG-4000 on voluntary feed intake of sheep fed carob leaves. Arrows mark changes in the level of PEG supplementation. Standard error of the mean = 35 g/day. Each increase up to 32 g of PEG/day or decrease below this level was followed by significant (p < 0.05) response in feed intake.

Intake, Digestion, and Body Weight. When the ewes were fed solely CL, they were not able to sustain their body weight (Table 1). The animals excreted in feces more protein than they consumed and, therefore, a negative figure was obtained for protein digestibility. Cell-wall digestibility was also very low (22%). PEG supplementation increased DM intake (Figure 1) and digestibility of protein, cell wall, and organic matter (Table 1). Addition of PEG to goats fed tannin-free diet (wheat hay) had no effect on feed intake and digestibility (unpublished results). When supplemented with 12.5 g of PEG/day, the animals maintained their body weights. Intake of organic matter increased by 200 g/day, protein digestibility became positive, and the digestion of cell wall and organic matter increased significantly (p < 0.05). When the animals were supplemented with 25 g/day PEG, weight gain reached 100 g/day. Organic matter intake further increased by 210 g/day along with a significant increase in the digestion

Table 2. Effect of Once-a-Day Supplementation of PEG-4000 on the Activity of Amylase and Trypsin in Fecal Samples from Sheep Fed Carob Leaves ($n = 4$, Mean ± SD)[a]

PEG intake, g/day	amylase activity, unit/g DM	trypsin activity, unit/g DM
0[b]	$0.5^{a} \pm 0.3$	$0.60^{a} \pm 0.4$
12.5	$1.2^{b} \pm 0.4$	$0.98^{a} \pm 0.3$
25[c]	$3.7^{c} \pm 0.5$	$1.38^{b} \pm 0.4$
30	$4.0^{cd} \pm 0.5$	$1.89^{c} \pm 0.4$
50	$4.3^{d} \pm 0.5$	$2.07^{c} \pm 0.5$

[a] Values followed by different superscript letters are significantly different (at least $p < 0.05$). [b] Average of first and last experimental periods. [c] Average of 2 × 12.5 g PEG/day and 1 × 25 g PEG/day.

of protein, cell wall and organic matter ($p < 0.05$ *vs* 12.5 g/day PEG; $p < 0.01$ *vs* no PEG). Supplementation with 32 g/day PEG was followed by a further increase of 100 g/day in organic matter intake ($p < 0.05$) (Table 1). However, at this level of PEG supplementation, the increase in body weight gain (20 g/day), and the improvement in digestibility of protein, cell wall, and organic matter, over that found when 25 g of PEG was given daily, was not significant (Table 1). Increasing PEG supplementation to 50 g/day did not improve any of the variables examined. When the animals were supplemented with 12.5 g/day PEG twice daily, organic matter intake was similar to the level recorded for animals supplemented with the same amount of PEG once daily at 8:00 a.m. (Table 1; Figure 1).

Each change in the amount of PEG supplementation was followed by a marked increase in feed intake; 50% of the maximal response was obtained within 24 h, while maximal response was recorded within 48–72 h (Figure 1).

When PEG supplementation was stopped, feed intake and digestion of protein, cell wall, and organic matter dropped to the level recorded at the beginning of the experiment, when given CL alone (Table 1; Figure 1). This indicates that the changes in feed intake and body weight were a result of PEG supplementation, and not a result of periodic or cyclic changes.

Enzymatic Activity. Enzymatic activity of trypsin and amylase in fecal samples was the lowest when CL alone was given to the animals (Table 2). Enzymatic activity of both enzymes increased significantly ($p < 0.05$) over the previous treatment when 12.5, 25, and 32 g/day PEG were given. When the animals received 50 g/day PEG, the increase in trypsin and amylase activities over the previous level (32 g/day) was not significant (Table 2).

DISCUSSION

Characterization of Tannins in Carob Leaves. The higher yield of condensed tannins, when aqueous acetone rather than acidic methanol was used as a solvent for extraction, is a phenomenon which was previously found with different tannin-containing plants. Condensed tannins may bind proteins during homogenization with methanol, but aqueous acetone prevents this binding (Hagerman and Robins, 1987; Hagerman, 1988). Leucoanthocyanins, percursors for condensed tannin, were found in the leaves of carob tree [Beth-Smith and Lerner, 1954, as cited by Nachtomi and Alumot (1963)]. Polymeric condensed tannins are the major fraction in carob pods polyphenols (Tamir et al., 1971).

Nutritional Implications. The results of the present work confirmed the common view that the major antinutritional effect of tannins is reduction of food protein availability and depression of digestive tract enzymes activities (Kumar and Singh, 1984; Robbins et al., 1987; Hagerman, 1989). The present results also sustained findings that tannins may reduce cell-wall digestibility by binding bacterial enzymes and/or forming indigestible complexes with cell-wall carbohydrates (Barry and Manley, 1984; Barry et al., 1986; Reed, 1986). This view is supported by the negative digestibility of cell wall, in CL samples, incubated in dacron bags in cows' rumen during 24 h (Silanikove et al., 1992). Tannin–protein complexes formed in the digestive tract were determined as fecal lignin (Reed, 1986), which led to apparent negative digestibility of lignin. Thus, the change from negative to positive lignin digestibility, following supplementation with 25 g/day PEG (or more), reflects the protein-sparing effect of PEG.

When fed CL alone, the voluntary consumption of calculated metabolizable energy (ME), 70 kcal/kg$^{0.75}$ (assuming a caloric value of 4.4 kcal/g digestible organic matter and a conversion factor of 0.8 between digestible energy and metabolizable energy), was 36% below their expected maintenance requirements (110 kcal/ kg$^{0.75}$; NRC, 1985). However, when the sheep were supplemented with 25 g of PEG/day (or more), intake of digestible organic matter was increased 2-fold (166 kcal ME/kg$^{0.75}$), which is 51% above their expected maintenance requirements, allowing recovery of the weight lost during the adaptation and unsupplemented periods.

Effect of Condensed Tannins in the Lower Gut. PEG is soluble in water and is not absorbed from the gastrointestinal tract (Bauman et al., 1971). Once ingested, its content (and thus concentration) in the rumen decreases exponentially according to first-order kinetics. Typical biological half life and mean retention time of soluble marker in the rumen are 7 and 10 h, respectively [e.g., Silanikove et al. (1993)]. The fact that no significant response was recorded when PEG was supplemented twice daily *vs* once a day suggested that its tannin-binding activity was spread along the entire digestive tract. Condensed tannins induced a marked depressive effect on the intestinal activity of trypsin and amylase (as judged by their activity in fecal samples), which agrees with findings that extracts from carob pods inhibited the activity of digestive enzymes *in vitro* (Tamir and Alumot, 1969). Protein–tannin complexes formed in the rumen may dissociate in the acidic environment of the abomasum (Jones and Mangan, 1977). Thus, the presence of PEG in the intestine may prevent a depressive effect on enzymatic activity by binding the released tannins.

Potential Applications. Supplementing sheep with 25 g of PEG/day seems to be the optimal amount in terms of cost–benefit response; its cost (approximately U.S. $0.03 per day) according to Israeli prices is much lower than that of alternative feed resources which supplement 300 g of digestible organic matter (U.S. $0.06–0.08 per day). Supplementing PEG once a day to sheep and goats grazing on Mediterranean scrub land increased weight gain, birth weight of lambs and kids, and milk yield (Silanikove et al., 1992). Further work is required to verify the optimal amount of PEG for grazing animals in areas with large varieties of plants and availability of grass pasture at different seasons.

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