PJN
ISSN 1680-5194

PAKISTAN JOURNAL OF NUTRITION

ANSInet
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Pakistan Journal of Nutrition 9 (2): 141-145, 2010
ISSN 1680-5194

Nutritive Value of Oak Leaves in Sheep

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Abstract: Oak (*Quercus* sp.) leaves and branches is an important source of forage in the north-west of Iran, during winter season, when the pasture herbages are not available, but the nutritive value of this forage is not well known. In this study nutritive value of three species of oak tree leaves: *Quercus persica, Q. infectoria* and *Q. libani* were assessed by chemical analysis and *in situ* method. The chemical composition (g/kg DM basis) of the above species, respectively were as follow; 951, 927, 946, Organic Matter (OM); 115, 92, 123, Crude Protein (CP); 532, 540, 512, Neutral Detergent Fiber (NDF); 317, 300, 331, Acid Detergent Fiber (ADF); 98, 103, 95, lignin (ADL); 78, 115, 104, total phenols; 73, 109, 100, Total Tannins (TT); 14, 15, 12, Condensed Tannin (CT) and 46, 87, 62, Hydrolysable Tannin (HT). Protein Precipitable Phenolics (PPP) were respectively 160, 190 and 230 (g/kg total phenols). Rumen liquor taken from three male Ghezel sheep was used to measure the *in situ* degradability characteristics of oak leaf. The soluble component (a), insoluble but fermentable fraction (b), the potential degradability (a+b) and the Effective Degradability (ED) were higher in *Q. persica* ($p<0.01$). There was a strong negative correlation between TT, HT and ED in sheep. The rank order of nutritive value, in terms of chemical composition and *in situ* degradability were as follows: *Q. persica* > *Q. libani* > *Q. infectoria*.

Key words: Oak leaves, chemical composition, *in situ* degradability, sheep

INTRODUCTION

Oak leaves and twigs are often grazed by ruminants or harvested for use as livestock feed during feed shortages (Singh *et al*., 1996). Approximately 3 million ha of forest are covered by various oak species, mainly dominated by *Quercus persica*, *Quercus infectoria* and *Quercus libani*, in the north-west of Iran (Fatahi, 1995). In this region, oak leaves are an important source of forage for small ruminant during periods of the year when quality and quantity of pasture herbages is limited. However, *Quercus* species have been reported to contain high levels of tannins in both hydrolysable (Makkar, 2003) and condensed (Makkar *et al*., 1991) forms. Therefore, the value of these leaves as feeds for ruminants is offset by their potentially negative effects on protein utilization, and the risk of toxicity when intake is high (Garg *et al*., 1992). *In situ* rumen disappearance technique is useful for rapid screening of feeds to assess their potential as feed energy sources for ruminants (Preston, 1995). There is little information available on the nutritive value of *Quercus* Spp. in Iran. The present study was, therefore, carried out to determine the chemical composition, phenolic composition and degradation of *Quercus* leaves.

MATERIALS AND METHODS

***Quercus* species:** Samples consisted of three indigenous *Quercus* species, being *Q. persica*, *Q. infectoria* and *Q. libani*. Samples were harvested by hand during the summer at several locations in the NW of Iran. Branches were randomly sampled from at least 10 plants per species. leaves were removed from branches, pooled to five samples per species and air dried in the shade to minimize changes in tannin content and activity (Makkar and Singh, 1991b). Water Soluble Carbohydrate (WSC) was Measured using the anthrone method (MAFF, 1982).

Chemical analysis: Standard methods as described in AOAC (1990) were used for determination of Dry Matter (DM), ash and CP (N x 6.25). Ash-free Neutral Detergent Fiber (NDF) was determined using sodium sulfate according to the method of Van Soest *et al*. (1991) and ash-free Acid Detergent Fiber (ADF) was determined based on AOAC (1990). Lignin (ADL) was determined by solubilization of cellulose with sulphuric acid as described by Robertson and Van Soest (1981).
Samples were analyzed for Total Phenols (TPH), Total Extractable Tannin (TT), Condensed Tannins (CT), Hydrolysable Tannins (HT) and Protein Precipitible Phenolics (PPP), as described below.

Total phenols: Dried plant material (200 mg) was extracted with acetone: water (10 ml; 70:30, v/v) in ultrasonic bath for 20 min. Contents were centrifuged (4°C, 10 min, 3000 g) and the supernatant was kept on ice until analysis. Total phenols were determined with the Folin-Ciocalteau reangent and detected at 725 nm (Makkar, 2000). A calibration curve was prepared using tannic acid (Merck Gmbh, Darmstadt, Germany). Total phenols were calculated as tannic acid equivalents and expressed as equiv. g/kg DM.

Tannins: Condensed tannins were measured by the HCL-butanol method (Makkar, 2000). An aliquot from the above acetone: water extract (0.5 ml; although this extract occasionally needed diluting with the extractant, acetone: water, if final absorbance at 550 nm exceeded 5.6 absorbance units) plus HCL-butanol (3 ml) and ferric ammonium sulphate (0.1 ml) regents were heated in a boiling water bath for 60 min. Absorbance was read at 550 nm. The colorimetric data (in absorbance units) were converted to leucocyanidin equivalents based on the assumption that the color yield of condensed tannins, E 1%, 550 nm, is 460 (Porter *et al*., 1986).

Non-Tannin Phenols (NTP) were determined using absorption to insoluble polyvinylpyrrolidone. The insoluble Polyvinylpyrrolidone (PVPP; 100 mg) was weighted into 100 x 12 mm test tubes. Distilled water, 1 ml and then 1 ml tannin containing extract were added and vortexed. The tube was kept at 4°C for 15 min, vortexed again, then centrifuged (3000x g) for 10 min and supernatant collected. The phenolic content of the supernatant was measured by the Folin-Ciocalteau reaction and this was accepted as the NTP (Makkar, 2000).

Total tannins were calculated as the difference between TPH and NTP. Hydrolysable tannins were analyzed using Rhodanin assay according to Makkar (2000). The results were expressed as gallotannin. Protein precipitable phenolics were determined according to Makkar (2000) and results were expressed as tannic acid equivalent.

***In situ* degradability:** Three remun-fistulated Ghezel sheep (live weight 64±2.5 kg) were used to determine the rate of degradability of DM (AFRC, 1992) from oak leaves. Sheep were fed a ration consisting of Lucerne hay, oak leaves (mix of three oak spices), wheat bran and barley grain (50:50) with a ratio of forage to concentrate of 60:40 (DM basis), which was calculated to ME at their maintenance level. Sheep were adapted to the diet for 10 days.

In situ bags were made from a Dacron material (21 x 10 cm) with a pore size of 45 μm (AFRC, 1992). All samples of feeds were dried and milled through a 4.0 mm sieve. Then 5 g of each sample was put in the *in situ* bags and incubated at the same time in the rumen for 3, 6, 12, 24, 48, 72 and 96 h. In each sheep, one bag was used for time interval. Bags were attached on semi-rigid stalks to ensure immediate insetion within the liquid of the rumen contents while allowing free movement. After withdrawing the bags from the rumen, they were washed in a washing machine for 1 h using cold water and dried for 48 h at 50°C. The degradability value at t = 0 was obtained by washing two bags in a washing machine for 1 h using cold water. For each bag, the residue was analyzed for DM. Degradability at each incubation time was calculated by taking the values obtained from the three bags (i.e., n = 3). The ruminal degradability (Y) of DM at time (t) was obtained from an exponential curve of the type:

$$Y = a + b\,(1-e^{(-ct)})$$

This was fitted to the experimental data by iterative regression analysis (Ørskov and McDonald, 1979). In this equation, the constant a represents the soluble and very rapidly degradable component and b represents the insoluble but potentially degradable component which degrades at a constant fractional rate (c) per unit time. The effective degradability of DM in each species was then estimated (Ørskov and McDonald, 1979) by the equation: effective degradability (g/kg DM) = a + bc/c+ k. In this equation, k refers to the fractional outflow rate of small particles from the rumen. A value of 0.05 fraction/h was used for k.

Statistical analysis: Data on chemical and tannin composition and *in situ* degradability were subjected to analysis using the General Linear Model (GLM) procedure of SAS (2001), based on the statistical model:

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

Where Y_{ij} is the general observation on chemical composition and tannin composition and *in situ* degradability, μ the general mean, S_i the ith effect of oak species on the observed parameters and e_{ij} the standard error term. Means were tested using Duncan test.

RESULTS AND DISCUSSION

Chemical composition and phenolics: *Quercus infectoria* leaves had the lowest OM, CP and ADF (Table 1), but contained the highest NDF and ADL versus other species. In all *Quercus* spp., the level of HT was high (Table 2). Among the oak leaves, *Q. infectoria* had the highest TPH, TT, CT and HT content ($p<0.05$) in *Quercus libani* versus other species.

The variation in chemical composition among these species of *Quercus* may be partly due to the genotypic factors that control accumulation of forage nutrients (Minson, 1990). In our oak leaves, the CP content was more than 80 g/kg DM (range: 92-123 g/kg DM) which, according to Norton (1998), should provide ruminal ammonia levels above the minimum required by rumen microorganism to support optimum growth. It seem to be likely the *Quercus* leaves studied in this experiment will meet the CP requirements of small ruminants for maintenance. Makkar and Singh (1991b) reported a similar range of CP content in mature *Q. incana*, *Q. semecarpifolia*, *Q. serraata*, *Q. ilex* and *Q. glauca*. However, Kamalak *et al*. (2004) obtained lower CP content in *Q. branti*, *Q. coccifera*, *Q. ceris*, *Q. libari* and *Q. infectoria* than our *Qercus* sp. The ADF and ADL content

Table 1: Chemical composition (g/kg DM) of oak species

	Q. persica	*Q. infectoria*	*Q. libani*	SEM
DM	939b	943a	946a	0.4
OM	951a	927c	946b	0.9
CP	115b	92c	123a	0.1
ADF	317ab	300b	331a	0.7
NDF	532a	540a	512b	0.4
ADL	98b	103a	95b	1.3
WSC	8.9c	11.7b	19.2a	0.51

Means in the same row with different letters differ (p<0.05)

Table 2: Phenolic composition (g/kg DM) of oak species

	Q. persica	*Q. infectoria*	*Q. libani*	SEM
TPH	78c	115a	104b	0. 2
TT	73c	109a	100b	0.2
CT	14b	15a	12c	0.05
HT	46c	87a	62b	0.1
PPP	160c	190b	230a	0.4

Means in the same row with different letters differ (p<0.05)

of our oak species were lower than in *Q. incana*, *Q. semecarpifolia*, *Q. serraata*, *Q. ilex* and *Q. glauca* (Makkar and Singh, 1991b), but higher than in *Q. libari* (Kamalak *et al*., 2004). The level of NDF in our experiment was lower than in *Q. incana*, *Q. semecarpifolia* and *Q. serraata* than *Q. libari* (Kamalak *et al*., 2004). There was a relationship between TPH and TT which is similar to findings of Makkar *et al*. (1993) who noted a high positive correlation between content of total tannins and total phenolics. Levels of TPH and total tannins in the experimental species were higher than in *Q. incana* (Singh *et al*., 2005), *Q. hartwissiana* (Yildiz *et al*., 2005), *Q. coccifera* (Ben Salem *et al*., 2003;2005), similar to *Q. rotundifolia* (Khazaal *et al*., 1994), but lower than *Q. coccifera* (Khazaal *et al*., 1994). Condensed tannins in our oak leaves were similar to *Q. hartwissiana* (Yildiz *et al*., 2005), *Q. coccifera* (Khazaal *et al*., 1994) and *Q. suber* (Gasmi-Boubaker *et al*., 2005), but lower than *Q. coccifera* (Ben Salem *et al*., 2003;2005); *Q. incana* (Singh *et al*., 2005), *Q. cercis* (Canbolat *et al*., 2005). The level of HT is high in *Quercus* sp. Similarly, some groups have reported that oak leaves are rich in HT (Garg *et al*., 1992; Makkar, 2003).

However, others noted low levels of HT in oak leaves (Yildiz *et al*., 2005; Singh *et al*., 2005). The variations between our oak leaves and other oak species in the chemical composition and phenolics contents is probably due to any or all of the vegetative stage (Makkar and Singh, 1993), method of storage (Makkar and Singh, 1993), drying conditions (Makkar and Singh, 1991a), species (Makkar and Singh, 1991b; Makkar *et al*., 1991) and habitat (Goncalves-Alvim *et al*., 2004).

***In situ* DM disappearance and estimated parameters:** The *in situ* dry matter constants are given in Table 3. The soluble component (a), insoluble but fermentable component (b), the degradation rate of b (c), the potential degradability (a+b) and the Effective Degradability (ED)

Table 3: *In situ* dry matter disappearance in oak species

	Q. persica	*Q. infectoria*	*Q. libani*	SEM
a	20.66a	18.14b	20.14a	0.33
b	16.50a	13.16c	15.37b	0.15
a+b	37.17a	31.29c	35.52b	0.32
c	0.026a	0.028a	0.018b	0.001
ED	30.00a	25.73c	27.47b	0.16

a: Water-soluble fraction (g/kg DM); b: Insoluble but fermentable fraction (g/kg DM); c: The degradation rate of b (/h); a+b: The potential degradability (g/kg DM); ED: The effective degradability of dry matter calculated for an outflow rate of 0.05/h (g/kg DM). Means with different letters within species differ (p<0.01)

of the oak leaves were influenced (p<0.01) by species. In the samples, the DM disappearance characteristics (a, b, a+b, ED) of *Q. persica* were higher (p<0.01) than those in other species.

These variable values of *in situ* degradability among our *Quercus* sp. could be due to variations in tannin activity (PPP, Table 1) between *Quercus* spp. Values obtained using a protein precipitation assay better relate to the nutritional values of tannin-rich feeds (Hagerman and Butler, 1989; Makkar, 1989). Tannin activity has been reported to vary among forage species due to the in functionality of tannin and chemical structure (Dalzell and Kerven, 1998), degree of polymerization (Schofield *et al*., 2001), biochemical processes (Wong, 1973) and to the tannin structure-biological activity relationship (Haslam, 1998).

Conclusion: This study has shown that the *in situ* rumen degradability measurement appear to be suitable for assessing the potential nutritive value of *Quercus* leaves. On the basis of TT, TPH, HT, PPP and DM degradation parameters, oak leaves of *Q. persica* species were judged to be nutritionally best. However, further research is needed to assess their impacts on animal performance.

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