

## The Determining of Nutritive Value of Sallow and Service Leaves Using Nylon Bags and Gas Production Techniques

*Valiollah Palangi and Peyman Eivazi*

Islamic Azad University, Department of Animal Science,  
Faculty of Agriculture, Parsabad Moghan Branch, Iran

**Abstract:** This study was carried out to the determination of nutritive value of sallow and service leaves using nylon bag and gas production techniques in Gizel sheep. Two fistulated Gizel sheep with average BW  $35 \pm 1.8$  kg are used in a complete randomized design. The gas production was measured at 2, 4, 6, 8, 12, 16, 24, 36, 48, 72 and 96 h and ruminal DM and CP disappearance were measured at 0, 2, 4, 6, 8, 12, 16, 24, 36, 48, 72 and 96 h. Dry matter degrade abilities of service and sallow leaves at 96 h were 67.92 and 73.40%, respectively. Crude protein degradabilities of service and sallow leaves at 96 h were 79.91 and 82.02%, respectively. The gas production of service and sallow leaves at 96 h were 138.324 and 212.566 ml/g DM. The relationship between dry matter and gas production values for service and sallow leaves obtained about 0.99652 and 0.99039 and this parameter for crude protein and gas production achieved 0.98817 and 0.99443, respectively. High correlation between in situ and gas production techniques indicated that digestibility values can be predicted from gas production data.

**Abbreviation key:** DM: Dry Matter • CP: Crude protein • NDF: Neutral detergent fiber • ADF: Acid detergent fiber • ADIN: Acid detergent insoluble nitrogen

**Key words:** Gas production • Nylon bags • Sallow and Service leaves

### INTRODUCTION

Feeding costs are one of the major problems in the economic balance of the sheep farmers. It has been well established that ruminant animals are capable of utilizing cellulose and hemicelluloses from forages, wood and other complex fibrous carbohydrates [1]. Non-traditional by-products must search in order to decrease the relay on traditional resources to fill the gap and decrease feeding costs [2]. Use of browse species as feeds for ruminants is important in many parts of the world. However, the presence of tannins and other phenolic compounds, in a large number of nutritionally important shrubs and tree leaves hampers their utilization as animal feeds [3]. Tannins are polyphenolic substances with various molecular weights and a variable complexity. These are chemically not well-defined substances but rather a group of substances with the ability to bind proteins in aqueous solution. Their multiple phenolic hydroxyl groups lead to the formation of complexes primarily with proteins and to a lesser extent with metal ions, amino acids and polysaccharides. Tannins are tentatively classified into

two classes: hydrolysable and condensed tannins (although tannins are known which have components of both hydrolysable and condensed tannins) and are considered to have both adverse and beneficial effects depending on their concentration and nature besides other factors such as animal species, physiological state of the animal and composition of the diet [4].

Predicting the feeding value of feedstuffs as accurately as possible and with methods of low cost and easy to handle is an important economical target. This goal is of particular importance for grazing and browsing ruminants that valorize local resources often of low and variable nutritive value. Chemical composition can give an idea of the nutritive value of feeds, but it is not sufficient [5]. Biological methods involving microorganisms and enzymes that are sensitive to factors influencing the rate and extent of digestion seem more appropriate in this case than chemical methods. Among them, the most popular are the in situ dry matter degradability [6] and the gas-test method proposed by Menke *et al.* [7] which is quite reproducible [8].

In ruminants due to ruminal microorganism's activity, the protein evaluation system is based on rumen degradable protein (RDP) and rumen undegradable protein (RUP) [9]. The level of milk yield and growth performance of ruminants is limited largely by the feed quality. The objective of this work is the study of nutritive value of sawlow and service leaves using nylon bag and *in vitro* gas production techniques.

## MATERIALS AND METHODS

**Animals and Feeding:** Two yearling (Gizil) wethers (35±1.8 kg) were used. At least 30 d before initiation of the experiment, each wethers was surgically fitted with a ruminal cannula. The wethers were housed in tie stalls under controlled environmental conditions with continuous lighting and constant temperature (24 to 26°C). All whether were fed a diet containing of 60% hay and 40% concentrate (NRC, 1989). The feed was fed in equal portions every 8 h to maintain a relatively stable rumen environment.

**Sample Collection:** Leaves of sawlow (*Salix alba*) and service (*Elaeagnus angustifolia*) were hand harvested from plot established in the experimental filed. All samples were thoroughly mixed and a composite sample (100g) was taken. All samples were dried in an oven at 100°C until a constant weight was achieved. All leaves were then ground to pass through a 2-mm screen in a Wiley mill (model 4, Arthur H. Thomas Co., Philadelphia, PA) before incubation.

**Chemical Analysis:** DM was determined by drying the samples at 105°C. Nitrogen (N) content was measured by the Kjeldahl method (AOAC, 2005). Neutral detergent fiber and ADF were measured according to the method of Goering and Van Soest *et al.* [10].

**In Situ Degradation:** In situ methods procedures was determined using Nocek *et al.* [11] and reviewed by Taghizadeh *et al.* [12], the ground samples (5g) were placed in Dacron bags (5×10 cm;46-µm pore size) and were closed using glue. Each feed sample was incubated in 4 replicates (2 replicates for each whether) in the rumen. The incubation times for samples were 0,2,4,6,8,12,16,24,36,48,72 and 96 h. Nylon bags were suspended in the rumen in a polyester mesh bag (25×40 cm;3mm pore size) and were removed from the

rumen at the same time so that all bags could be washed simultaneously. The nylon bags were then removed from the mesh bag and washing until the rinse water remained clear. Samples were then dried in an oven at 55°C until a constant weight was achieved before determination of DM disappearance. The DM and CP degradation data was fitted to the exponential equation  $P = a+b(1 - e^{-ct})$  (Ørskov and McDonald, 1979) [13], where P: is the disappearance of nutrients during time t, a: the soluble nutrients fraction which is rapidly washed out of the bags and assumed to be completely degradable, b: the proportion of insoluble nutrients which is potentially degradable by microorganisms, c: is the degradation rate of fraction b per hour and t is time of incubation.

**In vitro Gas Production:** Rumen fluid was obtained from two fistulated wethers fed twice daily with a diet containing alfalfa hay (60%) and concentrate (40%). Equal volumes of ruminal fluid from each sheep collected 2 h after the morning feeding squeezed through four layers and mixed with Mc Dougall [14] buffer prewarmed to 39° C. The inoculums was dispensed (20 mL) per vial into 100 mL serum vial (containing of 300 mg sample per vial) which had been warmed to 39° C and flushed with oxygen free CO<sub>2</sub>. The vials were sealed immediately after loading and were affixed to a rotary shaker platform (lab-line instruments Inc, Iran) set at (120 rpm) housed in an incubator. Vials for each time point, as well as blanks (containing no substrate), were prepared in triplicate. Triplicate vials were removed after 2, 4, 6, 8, 12, 16, 24, 36, 48, 72 and 96 h of incubation.

Cumulative gas production data were fitted to the model of Ørskov and McDonald [13]:

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

where a: is the gas production from the immediately soluble fraction (ml), b: the gas production from the insoluble fraction (ml), c: the gas production rate constant for the insoluble fraction (b), t: the incubation time (h) and P: the gas production at the time "t".

**Calculations and Statistical Analysis:** Data were analyzed as a completely randomized design using a general linear model (GLM) procedure of SAS (SAS, 1999), with Duncan's multiple range test used for the comparison of means. Feeds were the only sources of variation considered.

**RESULTS AND DISCUSSION**

**Chemical Composition:** chemical composition of experimental samples is shown in Table 1. The obtained data for Sallow leaves showed higher DM, OM and ADF (P<0.05). Whereas CP and ADF of Service leaves was higher than Sallow leaves (P<0.05). The achieved data for DM in this study is consistent with alfalfa's DM, that reported by Taghizadeh *et al.* [12] and Besharati and Taghizadeh [15]. The obtained NDF values in this study were difference from Kostas *et al.* [16] and Afsharmirzai *et al.* [17]. This difference can be expected due to differences in environmental factors, type and variety of leaves.

**In Situ Ruminal Degradability:** disappearance of tree leaves at the incubation times are given in Table 2 and 3. There were differences among levels of disappearance for DM of leaves at the different incubation times (P<0.05).

Since disappearance of DM was little during the first 6h of fermentation, sallow leaves showed higher ruminal disappearance of DM (P<0.05), but processing of ruminal DM degradation showed that service leaves have a high ruminal degradation in other times (P<0.05). service leaves showed high value for soluble fraction of DM compared to sallow (P<0.05), whereas sallow's leaves insoluble fraction was higher than service (P<0.05). leaves DM degradability inconsistent with alfalfa that reviewed by Mesgaran [18].

Yazen Ruiz *et al.* [19] showed the values of soluble and insoluble fraction for DM of olive leave about 28.2 and 45.2, respectively. That is consistent with obtained data in this experiment. Our results for DM were higher than Yousef elahi *et al.* [20]. Sallow showed higher CP degradation at the 24h of incubation (P<0.05). CP degradation process in our study is in consist with Waghorn *et al.* (1995)'s reported data [21]. The chemical composition of feeds influenced ruminal degradation process.

Table 1: The chemical composition of tree leaves

Leaves	%DM <sup>1</sup>	%CP <sup>2</sup>	%CF <sup>3</sup>	%NDF <sup>4</sup>	%ADF <sup>5</sup>	%Ash <sup>6</sup>	%OM <sup>7</sup>	%HC <sup>8</sup>
service	90.9 <sup>b</sup>	11.27 <sup>a</sup>	1.98	33.51 <sup>b</sup>	21.93 <sup>a</sup>	3.48	87.42 <sup>b</sup>	11.58 <sup>b</sup>
sallow	92.7 <sup>a</sup>	9.67 <sup>b</sup>	2.04	37.46 <sup>a</sup>	19.72 <sup>b</sup>	3.55	89.15 <sup>a</sup>	17.74 <sup>a</sup>
SEM	0.589	0.340	0.583	0.341	0.394	0.434	0.581	0.913

1: Dry matter, 2: Crude protein, 3: Crude Fiber 4: Neutral detergent fiber, 5: Acid detergent fiber, 6: Ash 7: Organic Matter and 8: Hemi Cellulose

Table 2: In situ DM disappearance (% of DM)

Leaves	Incubation time (h)											
	0	2	4	6	8	12	16	24	36	48	72	96
Service	26.99 <sup>a</sup>	28.12 <sup>a</sup>	31.20 <sup>a</sup>	34.94	36.47 <sup>b</sup>	40.29 <sup>b</sup>	45.01 <sup>b</sup>	49.18 <sup>b</sup>	55.32 <sup>b</sup>	61.76 <sup>b</sup>	65.08 <sup>b</sup>	67.92 <sup>b</sup>
Sallow	20.09 <sup>b</sup>	22.74 <sup>b</sup>	26.97 <sup>b</sup>	34.70	40.21 <sup>a</sup>	46.82 <sup>a</sup>	49.98 <sup>a</sup>	55.01 <sup>a</sup>	61.26 <sup>a</sup>	67.22 <sup>a</sup>	71.09 <sup>a</sup>	73.4 <sup>a</sup>
SEM	0.4332	0.5296	0.6945	0.6610	0.5514	0.4734	0.5650	0.5011	0.5135	0.5629	0.4530	0.5607

<sup>a,b,c</sup>: Means within a column with different subscripts differ (p<0.05).

Table 3: In situ CP disappearance (% of DM)

Leaves	Incubation time (h)											
	0	2	4	6	8	12	16	24	36	48	72	96
Service	37.22	40.70	42.13	46.66 <sup>b</sup>	49.10	54	59.13	62.22 <sup>b</sup>	68.60	73.41	77.81	79.91
Sallow	38.03	39.47	40.22	49.78 <sup>a</sup>	50.29	56.62	61.37	68.20 <sup>a</sup>	70.27	76.50	80.98	82.02
SEM	0.5787	0.6412	0.7140	0.8537	0.4140	0.8195	0.6760	0.8902	0.7436	1.0213	0.9629	0.9644

<sup>a,b,c</sup>: Means within a column with different subscripts differ (p<0.05).

Table 4: In situ DM and CP degradation characteristics

Leaves	DM degradation characteristics			CP degradation characteristics		
	a	b	c	a	b	c
Service	26.40 <sup>a</sup>	43.36 <sup>b</sup>	0.033	37.36	43.17	0.0387
Sallow	19.53 <sup>b</sup>	52.45 <sup>a</sup>	0.052	36.56	45.57	0.0460
SEM	0.2951	0.6431	0.0017	0.3931	0.9114	0.0026

<sup>a,b,c</sup>: Means within a column with different subscripts differ (p<0.05)

Table 5: *In vitro* gas production (mL g<sup>-1</sup> DM)

Leaves	Incubation time (h)											Parameters		
	2	4	6	8	12	16	24	36	48	72	96	a	b	c
service	23.331	31.819 <sup>a</sup>	46.726 <sup>a</sup>	79.480 <sup>a</sup>	88.434 <sup>a</sup>	99.033 <sup>a</sup>	136.432 <sup>a</sup>	165.988 <sup>a</sup>	180.057 <sup>a</sup>	193.901 <sup>a</sup>	212.566 <sup>a</sup>	11.97 <sup>b</sup>	198.3 <sup>a</sup>	0.041
sallow	19.998	24.042 <sup>b</sup>	30.285 <sup>b</sup>	34.596 <sup>b</sup>	41.994 <sup>b</sup>	52.220 <sup>b</sup>	65.813 <sup>b</sup>	82.032 <sup>b</sup>	101.804 <sup>b</sup>	115.408 <sup>b</sup>	138.324 <sup>b</sup>	14.55 <sup>a</sup>	140 <sup>b</sup>	0.019
SEM	1.4655	1.5070	1.4486	1.4739	2.7123	3.8326	3.4004	3.7625	4.4462	4.1334	3.295	0.2311	1.3550	0.029

<sup>a,b,c</sup>: Means within a column with different subscripts differ (p<0.05)

**Gas Production Study:** The gas test data are shown in Table 5. Service leaves showed higher gas production in all the incubation times compared with Sallow (P<0.05). high gas production can be resulted with high Metabolic energy, high soluble and insoluble fraction, high degradable carbohydrate, high fermentable nitrogen for microbial activity, resulting high growth rate and enhanced ruminal biomasses.

Negative effects of tannins on rumen fermentation can be studied using *in vitro* techniques, including measurement of gas production. we think that the sallow had high tannins and because of it showed lower gas production.

### CONCLUSION

Service leaves showed high ruminal degradability and *in vitro* gas production as same as alfalfa and it can used instead of alfalfa. There was high positive correlation between *in vitro* and *in situ* disappearances of dry matter and crude protein so the *in vitro* technique can be suitable replacement for *in situ* method.

### ACKNOWLEDGMENT

The authors are grateful to A. Nobakht and S. Shabestani for their helping.

### REFERENCES

- Singh, M. and L. D. Kamstra, 1981. Utiliation of whole Aspen tree material as a roughage componenet in growing cattle diets. *J. Anim Sci.*, 53: N3.
- Afaf, M.F., M.A. El-Ashry and H.A. Aziz, 2009. Effect of feeding olive tree pruning by-products on sheep performance in Sinai. *world Journal of Agriculture Science*, 5(4): 430-445.
- Tolera, A., K. Khazaal and E.R. Ørskov, 1997. Nutritive evaluation of some browse species. *Anim. Feed Sci. Technol.*, 67: 181-195.
- Makkar, H.P.S., 2003(b). Effects and fate of tannins and stragies to overcome detrimental effects of feeding tannin rich-feed. *Small Rumin. Res.*, 49: 241-250. A review.
- Krishnamoorthy, U., H. Soller, H. Steingass and K.H. Menke, 1995. Energy and protein evaluation of tropical feedstuffs for whole tract and ruminal digestion by chemical analyses and rumen inoculum studies *in vitro*. *Anim. Feed Sci. Technol.*, 52: 177-188.
- Mehrez, A.Z. and E.R. Ørskov, 1977. A study of the artificial fibre bag technique for determining the digestibility of feeds in the rumen. *J. Agric. Sci.*, 88: 645-650.
- Menke, K.H., L. Rabb, A. Salewski, H. Steingass, D. Fritz and W. Schnider, 1979. The estimation of the digestibility and metabolizable energy content of ruminant feed stuffs from the gas production when they are incubated with rumen liquor *In vitro*. *J. Agr. Sci.*, 93: 217-222.
- Getachew, G., G.M. Crovetto, M. Fondevila, U. Krishnamoorthy, B. Singh, M. Spanghero, H. Steingass, P.H. Robinson and M.M. Kailas, 2002. Laboratory variation of 24 h *in vitro* gas production and estimated metabolizable energy values of ruminant feeds. *Anim. Feed Sci. Technol.*, 102: 169-180.
- Taghizadeh, A., V. Palangi and A. Safamehr, 2008. Determining Nutritive Values of Alfalfa Cuts Using *in situ* and Gas Production Techniques. *American Journal of Animal and Veterinary Sciences*, 3(3): 85-90.
- Van Soest, P.J., J.B. Robertson and B.A. Levis, 1991. Methods for dietary fiber, neutral detergent fiber and non starch polysaccharides in ration to animal nutrition. *J. Anim. Sci.*, 74: 3583-3597.
- Nocek, J.E., 1988. *In situ* and other methods to estimate ruminal protein and energy digestibility. *J. Dairy Sci.*, 71: 2051-2069.
- Taghizadeh, A., D. Mesgaran, M. Eftekhar F. Shahroodi and K. Stanford, 2005. Digestion of feed Amino Acids in rumen and intestine of Steers measured using a Mobile Nylon Bag Technique. *J. Dairy Sci.*, 88: 1714-1807.
- Ørskov, E.R.I. and I.M. Mc Donald, 1979. The estimation of protein degradability in the rumen from incubation measurements weighted according to rate of passage. *J. Agric. Sci.*, 92: 499-503.

14. Dougall Mc, E.I., 1948. The composition and output of sheep in saliva. *Bio Chem. Journal*, 43: 99-109.
15. Besharati, M. and A. Taghizadeh, 2009. Evaluation of dried grape by-product as a tanniferous tropical feedstuff. *J. Anim. Feed Sci. and Techn.*, 152: 198-203.
16. Kostas, F., G. Zervas, S. Stamouli and E. Apostolaki, 1995. Nutritive value of dried citrus pulp and its effect on milk yield and milk composition of lactating ewes. *J. Dairy. Sci.*, 78: 1116-1121.
17. Aghsaghali Afshar Mirzaei and N. Maheri-Sis, 2008. Nutritive value of some agro-industrial by-products for ruminants- a review. *World. Journal of Zoology*, 3(2): 40-46.
18. Mesgaran, M.D., 2003. Ruminant and intestinal protein disappearance of some tropical (Iranian) feeds used in dairy cow diets estimated by the mobile nylon bag technique. *Proceeding of the British Society of Animal Science*, pp: 118.
19. Yanez Ruiz, D.R., A.I. Martin Garcia, A. Moumen and E. Molina Alcaide, 2004. Ruminal fermentation and degradation patterns protozoa population and urinary purine derivatives excretion in goats and wethers fed diets based on olive leaves.
20. Yousef elahi, M. and Y. Rouzbehan, 2008. Characterization of quercus persica, quercus infectoria and quercus libani as ruminant feeds. *J. Anim. Feed Sci. And Techno.*, 140: 78-89.
21. Waghorn, G.C. and I.D. Shelton, 1995. Effect of condensed tannins in *Lotus pedunculatus* on the nutritive value of ryegrass (*Lolium perenne*) fed to sheep. *J. Agric. Sci. Cambridge*, 125: 291-297.