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Determining Nutritive Values of Alfalfa Cuts Using *in situ* and Gas Production Techniques

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Abstract: In order to determine of nutritive value of alfalfa in different cuts using in situ and gas production technique, this study was carried out. Three wethers (49±2.6 kg) were used in *in situ* method. The gas production was measured at 2,4,6,8,12,16,24,36,48,72 and 96 h and ruminal dry matter and crude protein disappearance were measured at 0,4,8,12,16,24,36,48,72 and 96 h. Dry matter degradability's in first, second and third cuts of alfalfa at 96 h were 60.47, 64.71 and 64.36%, respectively. Crude protein degradability's of mentioned cuts were 60.47, 63.08 and 58.07%, respectively. The gas productions of them at 96 h were 322.54, 295.21 and 300.32 mL g^{-1} DM respectively. The relationship between dry matter and gas production values for alfalfa cuts obtained about 0.89, 0.85 and 0.84 and for crude protein and gas production data achieved 0.87, 0.88 and 0.84, respectively. High correlation between *in situ* and gas production techniques indicated that *in situ* degradability's values can be predicted from gas production data.

Key words: Alfalfa cuts, nylon bag, gas production

INTRODUCTION

Balancing rations for ruminants requires knowledge of the proportion of feed protein that escapes ruminal degradation[25]. Fermentation characteristics of feedstuffs in rumen fluid can be studied using *in vivo*, *in situ* and *in vitro* techniques[7]. The Dacron polyester or nylon bag technique has been used widely for estimating ruminal nutrient degradation because it is a relatively simple, low-cost method compared with methods in volving intestinally annulated animal[19]. The *in situ* nylon-bag technique is widely used to characterize the disappearance of feeds from the rumen. Nylon-bag technique provides a useful means to estimate rates of disappearance and potential degradability of feedstuffs and feed constituents[30].The *in vitro* gas production system helps to better quantify nutrient utilization and its accuracy in describing digestibility in animals has been validated in numerous experiments. Based on the strong relationship between measured digestibility and that predicted from gas production, regression equations have been developed and the method has been standardized[27,31]. Ruminants require adequate dietary fiber intake for normal rumen function and dairy animals, in particular, need fiber to maintain a normal milk fat content[32].Primary factors in the conversion of forage to animal product are intake of dry matter (DMI) or energy (IE), digestibility, efficiency of converting digested energy to metabolizable energy and efficiency of converting metabolizable energy to net energy in animal product[39].

Alfalfa (*Medicago sativa* L.) is considered good quality forage because of its high protein content and digestibility compared to many other forages[10]. Alfalfa is variable in digestibility and intake, even if harvest is aimed for uniform maturity[15]. The chemical and physical changes in alfalfa resulting from increased maturity and method of preservation may affect rumen digestion and passage[23].

Many factors influence the ruminal degradability of forage CP content including: stage of maturity[4,35], forage species[12,21], contents of different specially leaves[14,17,21,35] and climate condition[11,21,37] affect hay quality. Decreases in soluble DM and rate of digestion were observed with increasing maturity of alfalfa[23]. Mehrdad *et al*.[21] showed high degradability for third cut alfalfa compared to first and second cut. Mesgaran[22] reported the DM and CP degradability of alfalfa hay about 44 and 55%, respectively.

The objective of this study was to determine CP and DM disappearances of alfalfa different cuts in the rumen using *in situ* and to measure of their gas production.

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MATERIALS AND METHODS

Animals and feeding: Three yearling (Gizil) wethers (49±2/6 kg) were used. At least 30 d before initiation of the experiment, each wether was surgically fitted with a ruminal canola. The wethers were housed in tie stalls under controlled environmental conditions with continuous lighting and constant temperature (24-26°C). All whether were fed a diet containing of 60% hay and 40% concentrate[25].The feed was fed in equal portions every 8 h to maintain a relatively stable rumen environment.

Sample collection: Three cuts of alfalfa were collected from at least 10 different areas whiten each field. All 10 samples were thoroughly mixed and a composite sample (100 g) was taken. All samples were dried in an oven at 100°C until a constant weight was achieved. All cuts of alfalfa were then ground to pass thought a 2 mm screen in 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[3]. Neutral detergent fiber and ADF were measured according to the method of[38].

***In situ* degradation:** *In situ* methods procedures was determined using Nocek[24] and reviewed by[34], the ground samples (5 g) were placed in Dacron bags (5.5×10 cm, 47 μm pore size) and were closed using glue. Each feed sample was incubated in 6 replicates (2 replicates for each whether) in the rumen. The incubation times for alfalfa samples were 0, 4, 8, 12, 16, 24, 48, 72 and 96 h. Nylon bags were suspended in the rumen in a polyester mesh bag (25×40 cm, 3 mm 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[28]:

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

Where:

P = 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 = 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 McDougall[20] 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_2. 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 Orskov and McDonald[28]:

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

Where:

a = 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)
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[33], 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: The chemical composition of feeds was shown in Table 1. The obtained data for alfalfa different cut (13.63-15.44%) was lower than compared to NRC[26] (19.2%), AFRC[1] (19.9%), Kleinchmit *et al.*[16] (18.2%) and Trater *et al.*[36] (18.8%).

The obtained ADF and NDF values in this study were more than Kleinchmit *et al*.[16] (44.7 and 32.6) and

Broderick *et al*.[5] (43.5 and 34.7%). The difference between chemical can be resulted from the variance in variety, climate condition, soil, cut and maturity.

***In situ* ruminal degradability:** The degradability parameters of DM and CP are shown in Tables 2 and 3 and the DM and CP degradation characteristics are shown in Table 4. Second cut alfalfa showed high value for soluble fraction of DM compared to other cuts, whereas third cut alfalfa indicated high value for insoluble fraction (b) compared to other cuts of alfalfa.

The achieved data for soluble and insoluble of alfalfa DM in this study was lower than of reported data by Hoseinkhani[13]. However, the obtained results for insoluble fraction in consistent with Coblentz[6] (45.9) and pawelek[29] (43%). Andrighetto *et al*.[2] showed the values of soluble and insoluble fraction for DM of alfalfa about 17.9 and 45.1%, respectively that is consistent with the obtained data in this experiment. The difference values for degradability's parameters of different cuts of alfalfa hay can be resulted from the variance of growth rate, NDF content, soluble and insoluble fractions and environment temperature. Regarding to increasing of environmental temperature, the lignin content can be enhanced, then low degradability is expected. The CP soluble fraction for first cut was more than the others, but the CP insoluble fraction of second cut alfalfa was higher than the other cuts. The found data in this experiment showed high values for insoluble fraction compared to that the reported by[34], but the its soluble fraction agrees with finding of mentioned study. The obtained data for CP soluble fraction was lower than that reported by[9], but the CP insoluble fraction was consistent with by their data. The achieved differences can be depended on the differences in alfalfa variety, drying processing, climate conditions, soil, maturity, sample size: square area in used nylon bag and microbial contamination.

The gas production study: The gas production data are shown in Table 5. There were not significant differences between different cuts of alfalfa. Although first alfalfa cut showed numerically high gas production at incubation times compared to the other cuts due to high soluble carbohydrate fraction, but these values were not significant differences. Datt and Sinjh[8] showed more gas production in feedstuffs can be correlated with high metabolically energy, high

Table 1: The chemical composition of feedstuffs

Feeds	%DM[1]	%CP[2]	%NDF[3]	%ADF[4]	%ADIN[5]
AA1	91.56	15.20	53.68	44.26	0.655
AA2	93.38	13.63	48.28	40.81	0.481
AA3	93.62	15.44	51.64	41.97	0.682

1: Dry matter, 2: Crude protein, 3: Neutral detergent fiber, 4: Acid detergent fiber, 5: Acid detergent insoluble nitrogen

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

	Incubation time (h)									
Feeds	0	4	8	12	16	24	36	48	72	96
AA1	9.48^{a}	9.82^{a}	46.43^{a}	18.87^{a}	32.46^{a}	32.69^{a}	34.17^{b}	44.21^{a}	55.98^{a}	60.48^{a}
AA2	5.02^{b}	6.32^{b}	10.93^{b}	16.08^{b}	25.64^{b}	29.83^{a}	39.33^{a}	42.46^{a}	50.77^{a}	63.08^{a}
AA3	8.21^{a}	10.36^{a}	13.76^{ab}	16.90^{a}	27.30^{b}	31.69^{a}	33.34^{b}	35.94^{b}	48.82^{a}	58.07^{a}
SEM	0.449	0.728	0.845	1.335	1.050	0.970	0.771	1.645	2.337	2.823

^{a,b,c}: Means within a column with different subscripts differ ($p<0.05$). AA1: 1st cut alfalfa, AA2: 2nd cut alfalfa, AA3: 3rd cut alfalfa

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

	Incubation time (h)									
Feeds	0	4	8	12	16	24	36	48	72	96
AA1	23.35^{a}	24.19^{b}	29.61^{a}	31.42^{b}	35.36^{a}	38.65^{a}	50.37^{b}	52.72^{a}	55.57^{a}	60.47^{a}
AA2	22.83^{a}	28.76^{a}	31.18^{a}	35.58^{a}	37.12^{a}	41.17^{a}	54.28^{a}	55.57^{a}	60.47^{a}	64.71^{a}
AA3	22.54^{a}	23.42^{b}	25.62^{b}	28.83^{b}	31.08^{b}	39.27^{a}	50.85^{b}	52.94^{a}	58.73^{a}	64.36^{a}
SEM	1.018	0.844	0.815	0.957	1.085	1.710	0.577	1.140	1.883	2.866

^{a,b,c}: Means within a column with different subscripts differ ($p<0.05$). AA1: 1st cut alfalfa, AA2: 2nd cut alfalfa, AA3: 3rd cut alfalfa

Table 4: *In situ* DM and CP degradation characteristics

	CP degradation characteristics			DM degradation characteristics		
Feeds	A	B	C	A	B	C
AA1	8.56^{a}	62.31^{b}	0.019^{b}	21.44^{a}	42.64^{b}	0.025^{b}
AA2	3.38^{b}	67.33^{a}	0.020^{a}	2299^{a}	45.46^{b}	0.026^{a}
AA3	8.43^{a}	63.03^{b}	0.015^{c}	19.47^{b}	53.30^{a}	0.020^{c}
SEM	0.84	0.710	0.00006	0.95	0.85	0.00006

^{a,b,c}: Means within a column with different subscripts differ ($p<0.05$)

Table 5: *In vitro* gas production (mL g^{-1} DM)

	Incubation time (h)											Gas production Constants	
Feeds	2	4	6	8	12	16	24	36	48	72	96	(a+b)[1]	c[2]
AA1	13[b]	43[a]	88[a]	125[a]	168[a]	205[a]	245[a]	272[a]	290[a]	312[a]	322[a]	311[a]	0.070
AA2	18[b]	53[a]	99[a]	129[a]	168[a]	198[a]	229[a]	254[a]	270[a]	288[a]	295[a]	285[c]	0.077
AA3	23[a]	60[a]	100[a]	130[a]	172[a]	202[a]	236[a]	259[a]	273[a]	292[a]	300[a]	288[b]	0.077
SEM	2.32	5.03	4.87	4.95	6.17	7.27	7.98	9.37	10.44	11.35	11.68	0.1	0.00003

[a,b,c]: Means within a column with different subscripts differ ($p<0.05$)

fermentable nitrogen for microbial activity, resulting high growth rate and enhanced ruminal biomasses. Mansoori *et al.*[18] reported the gas yielded for alfalfa at 24 h about 41.63 mL 200 mg^{-1} DM that was lower than that the obtained value (49.15 mL 200 mg^{-1} DM) in our study. The high gas yield in first cut alfalfa probably resulted from high soluble CP, supply of N for growth of microorganism and high ruminal fermentation capacity for structural and nonstructural carbohydrate.

CONCLUSION

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.

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