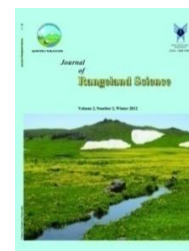


Contents available at ISC and SID

Journal homepage: [www.rangeland.ir](http://www.rangeland.ir)



### Full Length Article:

## Determination of Nutritional Value in Three Forage Species in Three Phonological Stages in Sabalan Rangelands, Ardebil, Iran

Abazar Ghanbari<sup>A</sup>, Mahmood Sahraei<sup>B</sup>

<sup>A</sup>MSc. of Animal Nutrition in Research Center of Agriculture and Natural Resources of Ardabil Province (Moghan).

<sup>B</sup>Scientific Board in Research Center of Agriculture and Natural Resources in Ardabil Province (Moghan) and Ph.D Student in Animal Nutrition of Tabriz University. Email: m.sahraei2009@gmail.com. (Corresponding author).

Manuscript Received: 21/08/2011

Manuscript Accepted: 25/01/2012

**Abstract.** In order to estimate the nutrient value of three dominant range species; *Festuca ovina*, *Trifolium montanum* and *Alopecurus textilis* via proximate analyses and gas production technique, samples were collected in three stages during grazing period from 26<sup>th</sup> May to 1<sup>th</sup> September in six areas of Sabalan Mountain in North West of Iran. Results showed that *Trifolium montanum* as a legume had higher values for Crude Protein (CP), total ASH, Organic Matter Digestibility (OMD), Metabolizable Energy (ME) and gas production at 24 hours and showed lower values for ADF (Acid Detergent Fiber) and NDF (Neutral Detergent Fiber) as compared to two other species that were considered as gramineae. Variation of quality traits in *Trifolium montanum* was lower than two other species. The obtained values of CP, NDF, ADF, ASH, EE, OMD, gas production and Dry Matter (DM) for *Trifolium montanum* were 18.65%, 41.83%, 26.97%, 8.95%, 0.26%, 64.50% g/kg and 50.96 ml/200mg, respectively. For *Festuca ovina*, they were 9.37%, 68.63%, 36.87%, 6.77%, 0.58%, 53.82% and 40.04 ml/200mg DM and for *Alopecurus textilis*, they obtained 5.01%, 73.03%, 42.18%, 5.97%, 0.56%, 55.63 % and 35.46 ml/200mg DM, respectively. The values of ME prediction that were calculated via Menke equation for *Trifolium montanum*, *Festuca ovina* and *Alopecurus textilis* were 2.31, 1.93 and 1.75 Mcal/kg DM, respectively. The mean level of predicted CP, ME and OMD for plants, especially in grasses (*Festuca ovina* and *Alopecurus textilis*) throughout the year, second and third stages of sampling (mid and late summer) and comparing the nutrient requirements of Moghanian sheep (native breed in Ardabil province) with these data show that only at early stage of sampling (late spring and early summer), one can supply sufficient nutrient for domestics whereas the ewes are pregnant at mid and end of summer and these nutrients are insufficient for them therefore, we have recommended the use of supplementary feeding in diets of grazing ewes for ideal productivity performance.

**Key words:** Quality traits, Gas production, *Trifolium montanum*, *Festuca ovina*, *Alopecurus textilis*.

## Introduction

In many traditional stock framings of ruminants such as Sabalan rangelands in north west of Iran, fresh forage represents the main component of diets for ruminants during the spring and summer seasons. Knowledge of nutritive value of forages is more important for planning the forage utilization in grazing seasons and developing an optimal feeding regime for ruminants. Furthermore, the optimization of feeding strategy is essential to control the forage wastage and minimize the environmental effects. The chemical composition and digestibility of fresh forage can be estimated on the basis of herbage species, mix samples of forages, maturity stage, climatic conditions and fertilizer application (Adesogan, 2002). In order to facilitate the production efficiency, small and large herds are assembled during the grazing seasons in Sabalan rangelands. Sabalan rangelands are located in North West of Iran in Ardabil province with temperate weather in spring and summer and cold weather in fall and winter. The Sabalan Mountain contains approximately 471 plant species in 200,000 Km<sup>2</sup>. The quality of forages affected by the maturity stage has been intensively studied throughout the history of animal science because of its importance to the biological and economical performance of ruminant based production systems. There are some In vitro techniques available to evaluate the nutritive value of feeds at relatively low cost. The use of In vitro gas method to estimate the digestion of feed is based on the measured relationships between the In vivo digestibility of feeds and In vitro gas production in combination with the feed's chemical composition (Menke and Steingass, 1988). The In vitro gas production technique developed by Menke *et al.*, (1979) is a very useful tool for the rapid screening of feeds to assess their potential as energy sources for ruminant

animals (Blummel and Becker, 1997) assuming that the volume of produced gas reflects the end result of the substrate fermentation to Short Chain Fatty Acids (SCFA), microbial biomass and the neutralization of SCFA. This technique has been used by Blummel and Qrskov (1993) to determine gas production at several incubation times. The obtained values could describe the pattern of feed fermentation using the model proposed by Larbi *et al.*, (1996). The gas produced from incubating 200 mg of food dry matter during 24 hours can be used with the concentration of Crude Protein (CP) and ash to estimate the Metabolizable Energy (ME) and Organic Matter Digestibility (OMD) (Menke *et al.*, 1979). In vitro gas production technique has been found to predict the nutritive value of forages reliably (Makkar *et al.*, 1997). Blummel and Ørskov (1993) reported high positive correlation between In vitro gas production and degradability values of dry matter at various incubation periods ( $r=0.95$  to  $0.97$ ). The objective of this study was to estimate the In vitro gas production of three indigenous and dominant plants namely *Festuca ovina*, *Alopecurus textileis* (as grasses) and *Trifolium montanum* (as legume) with ascertaining their nutritive value, determining the effect of grazing stages and growing period on chemical composition, gas production, OMD and ME and calculating the correlation coefficients among chemical composition, sampling stage and gas production rate (at 24 hour incubation) of these plants.

## Materials and Methods

### Study area

This study was conducted in west of Ardabil province around Sabalan Mountain at an elevation of 1800 to 3800 m above sea level. The extent of study area was 200000 Km<sup>2</sup> with 471 plant species. Mean annual rainfall is 500 mm. The biomass

production of study area is ranged from 250 in Shrublands to 750 kg/ha in Forbs and Grasslands. This area is consisted of 60-70 percents of grasses, 15-25 percents of forbs and the rest consisted of other plants such as shrubs. The predominant vegetative cover was *Festuca ovina*. Studied plants in this assay are dominant plant types in Sabalan rangelands.

### Sampling and Experimental Analyses

Samples were collected from forb (*Trifolium montanum*) and grasses (*Festuca ovina* and *Alopecurus textilis*) of Sabalan rangeland. They were clipped manually with special scissors at three grazing stages in 25<sup>th</sup> May (flowering stage), 13<sup>th</sup> July (seeding stage) and 27<sup>th</sup> August (ripening stage) in 2006 from study area and finally, 45 main samples were collected. All samples were ground through a 1 mm screen for In vitro gas production technique, incubation and chemical analysis. Dry Matter (DM), Crude Protein (CP), Ether Extract (EE) and ash content of samples were determined (AOAC, 1990). Neutral Detergent Fiber (NDF) and Acid Detergent Fiber (ADF) were examined using the method proposed by Van Soest *et al.*, (1994). Rumen fluid inoculum was collected from the animal before the morning feeding under vacuum pressure via the rumen fistula into a 2 liter glass flask and into two pre-warmed 1 liter thermos flasks which were then transported to the laboratory. The medium preparation was described by Makkar *et al.*, (1997). Mixed rumen fluid inoculums were obtained from two fistulated taleshian native steers (weighed 240±10 kg). The animals were fed twice a day. Water and a mineral lick were available *in libitum*. The feed sample of approximately 0.5 g on a fresh weight basis was transferred into a 50 ml serum bottle (Sommart *et al.*, 2000). The bottles were pre-warmed in a hot air oven at 39°C for about 1 h prior to the

injection of 40 ml of rumen fluid medium (using a 60 ml syringe) to each bottle. The bottles were closed with rubber stoppers, sealed and incubated in a hot air oven at 39°C. The rate of gas production was measured by reading and recording the gas volume after incubation using a 20 ml glass syringe connected to the incubation bottle with a 23 gauge, 1.5 inch needle. Readings of gas production were recorded from 2 to 96 h after incubation periods. Amounts of cumulative gas volume at 2, 4, 6, 12, 24, 48, 72 and 96 h after incubations were fitted using the equation  $Y = a + b [(1 - \text{Exp}(-ct))]$  (Ørskov and McDonald, 1979) where a is the intercept which ideally reflects the fermentation of soluble fraction, b is the fermentation of insoluble fraction, c is the rate of gas production, (a+b) is potential extent of gas production and Y is gas production at time 't'. In vitro digestibility of organic matter was measured at 24 h after incubation as  $\text{OMD} = 14.88 + 0.889 \text{ GP} + 0.45 \text{ CP} + 0.0651 \text{ XA}$ . The ME will be calculated as  $\text{ME, MJ DM} = 2.20 + 0.136 \text{ GP} + 0.057 \text{ CP} + 0.0029 \text{ CP}^2$  where GP is gas volume at 24 h and CP is the percentage of crude protein in feedstuffs.

### Statistical Analyses

All data obtained from the trials were subjected to the analysis of variance procedure of SAS, 2001. Means were separated by Duncan Multiple Range Test (DMRT). The level of significance was determined at  $P < 0.05$ . In vitro gas production data were fitted to the asymptote exponential model using Naway-Excel computer program (Macaulay Institute, Aberdeen, UK). Analysis of variance was carried out on chemical composition, In vitro gas production, organic matter digestibility and ME contents using the GLM procedure.

## Results

### Chemical Composition of Selected Plants

The chemical compositions of selected plants are presented in (Table 1). The crude protein content of the plants is ranged from 8.01 in *Alopecurus textilis* to 18.65% in *Trifolium montanum*.

The CP content of *Trifolium montanum* was considerably higher than two other species. There is not enough information available in terms of chemical composition for *Trifolium montanum* and *Alopecurus textilis* but there are some accessible data for *Festuca ovina*. There was considerable variation between forage samples for *Trifolium montanum* as compared to forage samples for two other species in terms of some chemical composition. Cell wall contents (NDF and ADF) which represent the most important fraction of dry matter for all forages are ranged from 41.83 to 73.03 and 26.97 to 42.18, respectively. Ash content of forages is ranged from 5.97 to 8.95%. *Trifolium montanum* had the highest ash content while *Alopecurus textilis* had the lowest. Significant differences in some chemical compositions were observed in the forages among three stages of maturity (grazing stage) in this experiment (Tables 2, 3 & 4).

Tables 2, 3 and 4 show that differences in chemical composition among three grazing stages in *Trifolium montanum* (as a Forb, Legume) were lower than two other species (as Gramineae). The CP, ADF and NDF contents in *Trifolium montanum* did not show substantial variation but in grasses, there was significance variation among the grazing stages ( $P < 0.05$ ).

### Gas Production Characteristics and Prediction of ME and OMD

Chemical analysis is essential for understanding the nutritional potential of forages but it is not sufficient. Data of gas production during the fermentation period, ME and OMD are given in (Table 5). Gas

production from the fermentation of forages was measured at 2, 4, 6, 8, 12, 24, 48, 72 and 96 hours. A comparison of gas production characteristics and estimated parameters of treatments indicated some significant differences among them except a and b intercepts ( $P < 0.05$ ). The values of a intercept for all samples were negative at the incubation times in this study.

The results indicate that cumulative gas volumes at all incubations were significantly different ( $P < 0.05$ ) between forages. Curves of cumulative gas production for each treatment are presented in (Fig. 1). Study of correlation between chemical composition and gas test parameters at 24 hours showed some significance differences almost in all cases.

## Discussion

### Chemical Composition of Selected Plants

There are many factors that affect CP content such as stage of growth (Promkot and Wanapat, 2004), maturity, species or variety (Von Keyserlingk *et al.*, 1996), soil types (Baloyi *et al.*, 1997) and high leaf-stem ratio of *Trifolium montanum*. These results are in agreement with the suggestion of Jerry *et al.*, (1989). And also, there are many factors that may affect fibrous (NDF and ADF) content such as growth stage (Promkot and Wanapat, 2004), maturity and species or variety (Agbagla-Dohnani *et al.*, 2001; Von Keyserlingk *et al.*, 1996), drying methods, growth environment (Mupangwa *et al.*, 1997) and soil types (Thu and Preston, 1999). The difference of ash content was probably due to the species. Turhan *et al.* (1997) pointed out that the ash content average of *Festuca ovina* was 16.22%. The variation of present study is in agreement with the studies carried out by Givens *et al.*, (1990) indicating that chemical composition, DM digestibility and energy value of forages varied significantly according to the herbage variety, growth

stage, year of harvest and vertical belts. Daalkhaijav (2000) showed that they tend to become more mature and their nutritive values generally decrease as the plant age is increased. Differences among grasses and legume in terms of chemical composition at three stages of maturity are due to high leaf-stem ratio of *Trifolium montanum* and a low decrease in leaf chemical compositions. Regardless of maturity stage, leaves were always more digestible than stems confirming Lambert *et al.*, (1989). It is well accepted that forage degradation in the rumen is mainly affected by the cell wall content and its lignification as lignin is an indigestible fraction and acts as a barrier that limits the access of microbial enzymes to the structural polysaccharides of the cell wall. Ammar (2002) reported that NDF, ADF and lignin were significant and negatively correlated with In vitro digestibility.

#### **Gas Production Characteristics and Prediction of ME and OMD**

These data suggested that a lag phase may occur in the early state of incubation due to the delay in microbial colonization of substrate. Several authors (Blummel and Ørskov, 1993; Khazaal *et al.*, 1995) have also reported negative values with various substrates while using mathematical models to fit gas production kinetics. This is because of a deviation from the exponential cause of fermentation or delays in the onset of fermentation due to the microbial colonization. It is well known that the value for absolute  $a$  ( $|a|$ ) that is described ideally reflects the fermentation of the soluble fraction. The soluble fraction could be easily attached by microorganisms in the rumen and increase the gas production (Table 5). In this study, the absolute  $a$  was the highest for *Trifolium montanum*. The gas volume at asymptote ( $b$ ) described the fermentation of the insoluble fraction that in *Trifolium*

*montanum*, is lower than two others. This result may reflect the proportion of their fiber components and protein contents. Menke *et al.*, (1979) suggested that gas volume at 24 hours after incubation has a relationship with metabolizable energy in feedstuffs. Sommart *et al.*, (2000) suggested that gas volume is a good parameter to predict the digestibility, fermentation end-product and microbial protein synthesis of the substrate by rumen microbes in the In vitro system. Additionally, In vitro OMD was shown to have high correlation with gas volume (Sommart *et al.*, 2000; Nitipot and Sommart, 2003). These data suggested that a lag phase may occur in the early state of incubation due to the delay in microbial colonization of the substrate. Several authors (Blummel and Ørskov, 1993; Khazaal *et al.*, 1995) have also reported negative values with various substrates while using the mathematical models to fit gas production kinetics. This is due to a deviation from the exponential cause of fermentation or delays in the onset of fermentation because of microbial colonization. It is well known that the value of absolute  $a$  ( $|a|$ ) described ideally reflects the fermentation of soluble fraction. The soluble fraction could be easily attached by microorganisms in the rumen and increase gas production (Table 5). In this study, the absolute  $a$  was the highest for *Trifolium montanum*. The gas volume at asymptote ( $b$ ) described the fermentation of the insoluble fraction that is lower than two others in *Trifolium montanum*. This result might reflect the proportion of their fiber components and protein contents. Menke *et al.*, (1979) suggested that gas volume at 24 h after incubation has a relationship with metabolizable energy in feedstuffs. Sommart *et al.*, (2000) suggested that gas volume is a good parameter to predict the digestibility, fermentation end-product and

microbial protein synthesis of the substrate. Additionally, In vitro organic matter digestibility was shown to have high

by rumen microbes in the In vitro system. correlation with gas volume (Sommart *et al.*, 2000; Nitipot and Sommart, 2003).

Table 1. Chemical Composition of Plants (DM basis)

parameters	<i>A. textilis</i>		<i>T. montanum</i>		<i>F. ovina</i>		Sig.
	Mean	SD	Mean	SD	Mean	SD	
DM	95.86	0.24	95.48	0.49	95.92	0.22	ns
CP	b 8.01	3.47	a 18.65	2.8	b 9.37	2.59	*
NDF	a 73.03	7.43	b 41.83	7.9	a 68.63	5.59	*
ADF	a 42.18	8.03	b 26.97	5.1	a 36.87	5.2	*
ASH	b 5.97	1.55	a 8.95	1.4	b 6.77	1.7	*
EE	0.77	0.4	1.04	0.26	1.09	0.48	ns

Means in rows with no common letters (a–d) differ significantly ( $p < 0.05$ ).

Table 2. Effect of sampling stage on chemical composition of *Festuca ovina*

Parameters	Stage 1		Stage 2		Stage 3		Sig.
	X	SD	X	SD	X	SD	
DM	96.2a	0.22	95.82b	0.25	95.76b	0.14	*
CP	11.6a	2.27	8.75b	1.77	7.66b	1.4	*
NDF	61.88b	2.5	71.98a	5.32	72.02a	3.2	*
ADF	31.14b	2	37.31a	4.12	42.14a	3.08	*
ASH	8.27a	1.17	6.27b	3.22	5.75b	1.55	*
EE	1.47a	0.26	0.98 ab	0.53	0.79b	0.29	*

Means in rows with no common letters (a–d) differ significantly ( $p < 0.05$ ).

Table 3. Effect of sampling stage on chemical composition of *Alopecurus textilis*

Parameters	Stage 1		Stage 2		Stage 3		Sig.
	X	SD	X	SD	X	SD	
DM	96.02a	0.8	95.97a	0.57	95.52b	0.57	*
CP	10.11a	1.7	8.41ab	5.2	4.64b	0.32	*
NDF	65.37b	5.1	75.40a	5.8	78.65a	1.33	*
ADF	35.5b	7.18	46.9ab	5.65	47.5a	3.53	*
ASH	6.7	1.3	5.45	2.16	5.4	1.55	ns
EE	0.89	0.43	0.79	0.66	0.57	0.31	ns

Means in rows with no common letters (a–d) differ significantly ( $p < 0.05$ ).

Table 4. Effect of sampling stage on chemical composition of *Trifolium montanum*

Parameters	Stage 1		Stage 2		Stage 3		Sig.
	X	SD	X	SD	X	SD	
DM	95.77	0.33	95.1	0.45	95.23	0.77	ns
CP	20.03	2.77	17.09	2.61	16.52	3.81	ns
NDF	35.8b	5.77	48.43a	6.1	48.67a	7.07	*
ADF	23.3	3.74	31.61	3.95	30.33	5.37	ns
ASH	9.87	0.8	7.7	1.41	8.13	2.11	ns
EE	1.1	0.21	1	0.19	0.97	0.5	ns

Means in rows with no common letters (a–d) differ significantly ( $p < 0.05$ ).

Table 5. In vitro gas production at incubation time (ml/g DM), predicted Metabolizable Energy (ME) (MJ/kg DM) and organic matter of digestibility content of forages

Incubation time	<i>A. textilis</i>		<i>T. montanum</i>		<i>F. ovina</i>		Sig.
	Mean	SD	Mean	SD	Mean	SD	
2	3.84 b	0.69	5.99 a	0.43	4.62 b	0.53	*
4	5.74 b	2.2	10.31 a	0.84	6.42 b	1.75	*
6	7.85 b	3.66	17.56 a	3.14	8.44 b	2.75	*
8	10.09 b	5.3	25.87 a	6.0	11.22 b	3.84	*
12	16.29 b	8.0	34.90 a	6.7	17.25 b	5.39	*
24	35.46 b	9.57	50.96 a	7.05	40.04 b	5.63	*
48	49.24 b	7.73	58.15 a	4.21	56.76 a	2.45	*
72	57.53 b	7.32	61.43 ab	7.35	64.48 a	3.98	*
96	67.91 b	10.92	66.05 ab	11.38	74.99 a	10.09	*
Parameters							
C	0.042 b	0.014	0.069 a	0.014	0.03 b	0.01	*
la <sup>1</sup>	2.26	0.78	4.09	1.48	2.7	0.62	ns
A	2.26	-	4.09	-	2.7-	-	ns
B	78.2 ab	9.47	67.3 b	1.7	84.62 a	7.27	*
la+ b <sup>1</sup>	80.5 ab	9.8	71.4 b	3.15	86.77 a	7.25	*
a+ b	75.97 ab	-	63.23 b	-	81.37 a	-	*
ME	1.75 b	0.31	2.31 a	0.13	1.93 b	0.2	*

Means in rows with no common letters (a-d) differ significantly ( $p < 0.05$ ).

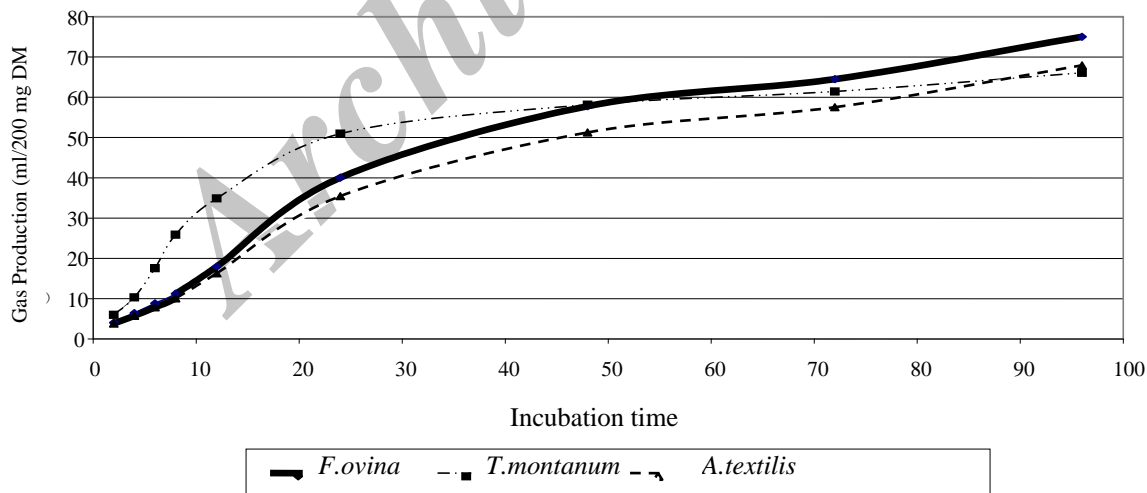


Fig. 1. Cumulative gas production of plants at different times of incubation time

## References

- Adesogan, A. T., 2002. What are feeds worth? A critical evaluation of selected nutritive value methods. Proceeding 13<sup>th</sup> annual Florida ruminant nutrition symposium. 33-47.
- Agbagla-Dohnani A., Noziere P., Clement G. and Doreau M., 2001. In sacco degradability chemical and morphological composition of 15 varieties of European rice straw, *Anim. Feed Sci. Technol.* **94**: 15-27.
- Ammar, H., 2002. Composition quimica, digestibilidad cinetica de fermentacion ruminal in vitro de arbustos. Ph. D. tesi, Universidad de Leon, Spain.
- AOAC, 1990. Official Methods of Analysis, 15th ed. Association of Official Analytical Chemists, Arlington, VA, USA.
- Baloyi J. J., Ngongoni N. T., Topps J. H. and Ndlovu P., 1997. Chemical composition and degradability of *Brachystegia spiciformis* (musasa) leaves and stems harvested over 4 months from three site in Zimbabwe, *Anim. Feed Sci. Technol.* **69**: 179-186.
- Blummel, M. and K. Becker, 1997. The degradability characteristics of fifty-four roughages and roughage neutral detergent fibers as described by in vitro gas production and their relationship to voluntary feed intake. *Br. J. Nutr.* **77**: 757-768.
- Blummel M. and Ørskov E. R., 1993. Comparison of in vitro gas production and nylon bag degradability of roughages in predicting feed intake in cattle, *Anim. Feed Sci. Technol.* **40**: 109-119.
- Daalkhajjav, Daltanzul, Ts., 2000. Chemical content and rumen degradability of desert steppe pasture. Research institute of animal husbandry, Zaisan, Ulaanbaator 10153. PP: 1-7.
- Givens, D. I., Jeannie, M., Everington and Adamson, A. H., 1990. The nutritive value of spring grown herbage produced on farms throughout England and Wales over 4 years. II. The prediction of apparent digestibility In vivo from various laboratory measurement. *Anim. Feed Sci. Technol.* **27**: 173-181.
- Jerry, L., Holechek. Pieper, Dcarlton, H., 1989. Comparative nutritive value of range forage. Range management principle and practice, 274-276.
- Khazaal K., Dentinho M. T., Ribeiro J. M. and Ørskov E. R., 1995. Prediction of apparent digestibility and voluntary feed intake of hays fed to sheep: Comparison between using fiber component, in vitro digestibility or characteristics of gas production or nylon bag degradation, *Anim. Sci. Jour.* **61**: 521-538.
- Lambert M. G., Jung G. A., Harpster H. W., Budding P. J., Wewala G. S., 1989. Forage shrubs in North Island hill country. III. Forage digestibility. *New Zeal. Jour. Agric. Res.* **32**: 491-497.
- Larbi, A., J. W. Smith, I. O. Adekunle and I. O. Kurdi, 1996. Studies on multipurpose fodder trees and shrubs in West Africa: Variation in determinants of forage quality in *Albizia* and *Paraserianthes* species. *Agroforest. Syst.*, **33**: 1-11.
- Makkar, H. P. S. and K. Becker, 1997. Nutrients and antiquality factors in different morphological parts of the *Moringa oleifera* tree. *Jour. Agric. Sci.*, **128**: 311-322.
- Menke K. H and Steingass, H., 1988. Estimation of the energetic feed value obtained from chemical analysis and in vitro gas production using rumen fluid, *Animal Res dev.* **28**: 7-55.
- Menke K. H., Raab L., Salewski A., Steingrass H., Fritz D. and Schneider W., 1979. The estimation of the digestibility and metabolizable energy content of ruminant feedstuffs from the



- gas production when they are incubated with rumen liquor. *Jour. Agri. Sci.* **93**: 217-222.
- Mupangwa J. F., Ngongoni N. T., Topps J. H. and Ndlovu P., 1997. Chemical composition and dry matter of forage legumes *Cassia rotundifolia* cv. Wynn, *Lablab purpureus* cv. Highworth and *Macroptilium atropurpureum* cv. Siratro at 8 weeks of growth (pre-anthesis), *Anim. Feed Sci. Technol.* **69**: 167-178.
- Nitipot P. and Sommart K., 2003. Evaluation of ruminant nutritive value of cassava starch industry by products, energy feed sources and roughages using in vitro gas production technique. Proceeding of Annual Agricultural Seminar for year 2003, 27-28 January, KKU. 179-190.
- Promkot C. and Wanapat M., 2004. Ruminant degradation and intestinal digestion of crude protein of tropical resources using nylon bag and three-step in vitro procedure in dairy Cattle. Proceedings of the Agricultural Seminar, Animal Science/Animal Husbandry. Held at Sofitel Raja Orchid Hotel 27-28 January.
- Ørskov E. R. and McDonald I., 1979. The estimation of protein degradability in the rumen from incubation measurements weighted according to rate of passage. *Jour. Agri. Sci. (Camb)*. **92**: 499-504.
- SAS, 2001. Institute Inc., SAS/STAT User's Guide, Version 6, Fourth Edition, Volume 1, Cary, N. C., SAS Institute INC.
- Sommart, K., Parker D. S., Rowlinson P. and Wanapat M., 2000. Fermentation characteristics and microbial protein synthesis in an in vitro system using cassava, rice straw and dried ruzi grass as substrates, *Asian-Aust. Jour. Anim. Sci.* **13**: 1084-1093.
- Thu N. V. and Preston T. R., 1999. Rumen environment and feed degradability in swamp buffaloes fed different supplements, *Livestock Research for Rural Development*, **11**: 1-7.
- Turhan, S., S. Sagsoz, I. Akgun and M. Tosun, 1997. Determination of some cytological and agricultural characters in wild sheep's Fescue (*Festuca ovina*). Turkey 2<sup>nd</sup> Field Crops Congress, 450-454.
- Von Keyserlingk M. A. G., Swift M. L., Puchala. R. and Shelford V., 1996. Degradability characteristics of dry matter and crude protein of forages in Ruminants, *Anim. Feed Sci. Technol.*, **57**: 291-311.
- Van Soest, P. J., 1994. Nutritional Ecology of the Ruminant, second ed. Cornell University Press, Ithaca, NY. USA.