

Chemical Composition, *In vitro* Organic Matter Digestibility and Kinetics of Rumen Dry Matter Degradability of Morphological Fractions of Stinging Nettle (*Urtica simensis*)

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Abstract: Chemical composition, *in vitro* organic matter digestibility (IVOMD) and DM degradability (DMD) of morphological fractions (leaf, flower, stem and whole forage) of stinging nettle (*Urtica simensis*) forage was evaluated through chemical analyses, *in vitro* and *in sacco* studies. Flower had highest ($p < 0.05$) DM (18.6%) and IVOMD (85.5%) but stem showed the lowest (15.2%DM and 75.5%IVOMD) percentages. Leaf contained highest CP (29.5%) and ash (23.9%) content. Stem had highest NDFom (52.2%), ADFom (36.4%) and ADL (8.2%) content of all morphological fractions. All morphological fractions had sufficient levels of calcium (from 0.44 to 1.43%DM) and phosphorus (from 0.08 to 0.46%DM). The *in sacco* DMD after 96h of incubation was highest for flower followed by leaf, whole forage and stem in decreasing order. Flower had highest ($p < 0.05$) washing loss (A), potentially degradability (PD) and effective degradability(ED) fractions but stem had the lowest ($p < 0.05$). Stem had lowest ($p < 0.05$) insoluble but slowly degradable fraction (B) of all the other morphological fractions. ADFom and NDFom were negatively correlated ($p < 0.05$) with CP content ($r^2 = -0.95$ and -0.89 respectively), IVOMD ($r^2 = -0.72$ and -0.82 respectively) and DMD ($r^2 = -0.69$ and -0.82 respectively). CP was positively correlated ($p < 0.05$) with IVOMD ($r^2 = 0.5$) and DMD ($r^2 = 0.47$). The CP, fiber contents, DMD and IVOMD values indicated that leaf and flower have potentials to be used as feed supplements for ruminant. However further animal experiment is needed to validate this conclusion.

Key words: Morphological Fraction • Stinging Nettle • Chemical Composition • Dm Degradability

INTRODUCTION

Livestock feed resources in Ethiopia are mainly natural pasture, crop residues and agro-industrial by-products. In Ethiopia, livestock have low productivity because they are fed almost entirely on natural pasture and crop residues. Natural pasture that is estimated to contribute to 80–90% of livestock feeds and whose quality is seasonally variable, is the main source of feed in arid and semi-arid pastoral areas, while crop residues contribute up to 50% of the feed supply in mixed-farming system [1]. Overgrazing and seasonal feed shortage are evident in the country. As the grazing area is heavily populated and as there has been virtually no effort to limit the livestock population, the natural pasture are over stocked and over grazed. Thus animals are not even able to meet their maintenance energy requirement and as a rule lose a substantial amount of weight [2, 3].

The chemical composition and digestibility of forages are influenced by plant species, environmental factors, plant morphological fractions and stage of maturity at harvest. Stage of maturity at harvest is the major factor affecting morphology and forage quality. As plants advance in maturity the leaf-stem ratio decreases thereby increases cell wall concentration with in stems and usually also in leaves. As a result the proportion of cell soluble components decrease. This indirectly decreases the digestibility of total herbage [4].

In smallholder livestock production system, under cut and carry forages feeding system practices, leaves are commonly used as feed for small ruminants. However leaf loss is quite common during harvesting, transporting and storage of forages. Hence studying the nutritive value of each morphological fraction of forages is more appropriate in addition to studying the entire whole plant [5].

Earlier it was reported that stages of maturity at harvest had effect on biomass production, chemical composition and *in vitro* organic matter digestibility of cultivated stinging nettle forage [6]. In the same report the chemical composition (CP and fiber fractions) and IVOMD of stinging nettle were influenced as advancing stages of maturity.

The chemical composition and digestibility of different varieties of stinging nettle have been reported by several authors [6-10]. However adequate studies on the nutrient concentration and nutritive value of different morphological fractions of stinging nettle (*Urtica simensis*) are not available. This study was thus designed to determine the chemical composition, *in sacco* DM degradability and *in vitro* organic matter digestibility of morphological fractions of stinging nettle (*Urtica simensis*).

MATERIALS AND METHODS

Study Site and Sample Collection: The study area is located in the southern Ethiopia (7°06'-9°38'N and 38°25'-35°53'E) with an elevation range of 1851-2759 masl. The annual rainfall range of the area is between 1000 and 1200 mm. The whole stinging nettle plants were randomly collected from naturally grown plants. Forage samples were taken approximately when three fourth of the plant stand was flowered. Fresh sample was separated into leaves, flower, stem and whole forage representing fractions, were separately weighed fresh on the field. Samples were oven-dried in a forced air to constant weight at 65°C for 48h. A portion of each dried sample was ground through 1.0 mm sieve using a Wiley mill (Thomas-Wiley, Laboratory Mill Model 4, Arthur H. Thomas Company, Philadelphia, PA, USA) for chemical analysis and *in vitro* organic matter digestibility (IVOMD) while the remaining portion was ground through 2.0 mm sieve for *in sacco* DM degradability.

Chemical Analysis: Dry matter (DM), ash and ether extract (EE) were determined using AOAC [11]. Nitrogen (N) content was determined by the Kjeldahl method and crude protein (CP) was calculated as $N \times 6.25$. Neutral detergent fiber (NDFom) was determined by procedures described by Van Soest *et al.* [12] and Sulfite and α -amylase were not used as reagents. Acid detergent fiber (ADFom) and acid detergent lignin (ADL) were analyzed using the procedures described by Van Soest and Robertson [13]. Both NDFom and ADFom were reported exclusive of residual ash. Phosphorous and calcium were analyzed using atomic absorption spectrophotometer.

In sacco DM Degradability: The *in sacco* DM degradability of the sample was evaluated using three fistulated steers with rumen cannula. Samples from each of the four morphological fractions were ground at 2 mm sieve size. Three replicate samples, weighing about 2.5g, were packed and sealed with nylon bags. The bags were put into the rumen of fistulated steers and were removed at an interval of 3, 6, 12, 24, 48, 72 and 96 hrs. All bags were inserted at the same time, just before the morning feeding. The zero hour was obtained by soaking the bags in a water bath maintained at 39°C for 1 hour. After the incubation period, the bags were withdrawn then were hand washed under running tap water until the water coming out of the bags was clear. The washed bags and contents were then dried for 48 hours at 65°C in a draught oven to determine DM disappearance. Washing loss was determined by similarly washing duplicate bags containing samples that were not incubated in the rumen. Duplicate bags were dried in the same way to determine the DM content of the samples.

The degradability constants were determined using the exponential equation:

$P = a + b(1 - e^{-ct})$ as described by Ørskov and McDonald [14], using the Neway Excel program [15], where P = % DM degradability at time t. The lag time was estimated by fitting the model $p = A$ for $t = t_0$, $p = a + b(1 - e^{-ct})$ for $t > t_0$ [16]. The degradation characteristics of the feeds was defined as A = washing loss (readily soluble fraction); B = (a+b)-A, representing the insoluble but fermentable fraction; c = the rate of degradation of B and the lag phase (L) = $(1/c) \log_{10}[b/(a+b-A)]$ [17]. Potential degradation (PD) of DM was calculated according to Dhanoa [18] using the formula $ED = A + [Bc/(c+k)]$ at rumen outflow rates (k) of $0.05h^{-1}$.

In vitro Organic Matter Digestibility: *In vitro* ruminal organic matter digestibility was determined according to the procedure of Tilley and Terry [19]. Ruminal fluid was collected using rumen tubes from local breed (Arsi-Bale) rams early in the morning. Rumen digesta was collected into a pre-warmed thermos flask. McDougall's buffer (based on the composition of sheep saliva) was prepared. Approximately 0.5g of milled forage samples were weighed into 50 ml centrifuge tubes. McDougall's buffer (based on the composition of sheep saliva) and ruminal fluid were added and tubes were incubated in water bath for 48h at 39°C. Four blank tubes containing ruminal fluid and buffer without feed sample were included. Centrifuge tubes were agitated manually three times per day. Fermentation was stopped after 48h and acidified pepsin was added.

Then tubes were incubated in water bath for another 48h at 39°C. The contents were then filtered and the residue dried and weighed. After drying, residue was ashed. *In vitro* ruminal organic matter digestibility was determined as the quantity of OM lost during fermentation and subsequent pepsin digestion.

Statistical Analysis: Statistical analyses were performed using the general linear model (GLM) procedure of the Statistical Analysis System [20]. Significance between individual means was identified using Fishers Least Significant Difference (LSD) and significance was declared at $P < 0.05$. Pearson's correlation coefficient was used to determine the relationship among chemical composition, IVOMD and DM degradability.

RESULTS

Chemical Composition and *in vitro* Organic Matter

Digestibility: Chemical composition and *in vitro* organic matter digestibility (IVOMD) of morphological fractions of stinging nettle forage is presented in Table 1. The DM and EE content were significantly highest ($p < 0.05$) in flower than in the other morphological fractions and the least was for stem. Leaf showed highest ($p < 0.05$) crude protein (CP) content followed by whole plant and flower and the least was for stem. Ash content was highest ($p < 0.05$) for leaf and least for stem. Stem showed highest ($p < 0.05$) cell wall fractions (NDFom, ADFom and ADL) and leaf had lowest. Highest fiber and lowest CP content was in stem and the vice versa in leaf. Highest Ca and P contents were observed in flower which was significantly higher ($P < 0.05$) than stem, leaf and whole plant. The highest IVOMD was recorded in flower followed by leaf and whole forage and the least was for stem.

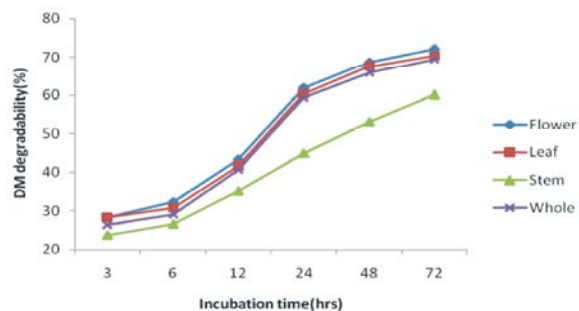


Fig. 1: *In sacco* DM degradability of morphological parts of stinging nettle forage

***In Sacco* DM Degradability:** The *in sacco* DM degradability (DMD) of morphological fractions of stinging nettle forage is presented in Table 2 and Figure 1. The overall *in sacco* DMD tended to be higher in flower and lower in stem than in the other morphological fractions. Flower showed highest ($P < 0.05$) DMD at all time of incubation. However the DMD after 3, 6 and 48h of incubation were similar for flower and leaf parts. On the other hand stem part showed lowest ($P < 0.05$) DMD than all other morphological fractions at all incubation time.

The *in sacco* degradability parameters of different morphological fractions of stinging nettle forage is presented in Table 2. The highest ($P < 0.05$) washing loss (A) was for flower followed by leaf part, whole forage and stem. The insoluble but slowly degradable fraction (B) was significantly ($P < 0.05$) lower for stem than the other morphological fractions. However it was similar among flower, whole forage and leaf despite observed numerical differences. The potentially degradable (PD) fraction was highest ($P < 0.05$) for flower. Leaf and whole forage showed higher ($P < 0.05$) potentially degradable (PD) value over stem which exhibited the least among all morphological fractions. The degradation rate (c) was lower ($P < 0.05$) for

Table 1: Chemical composition and *in vitro* organic matter digestibility of morphological fractions of stinging nettle forage

	Leaf	Flower	Stem	Whole	SEM	P value
DM (%)	17.6 ^b	18.6 ^a	15.2 ^d	16.7 ^c	0.22	<0.0001
CP (%DM)	29.5 ^a	22.7 ^c	12.7 ^d	25.5 ^b	0.82	<0.0001
EE (%DM)	2.1 ^b	2.5 ^a	1.3 ^c	2.2 ^b	0.07	<0.0001
ADFom (%DM)	13.3 ^d	18.3 ^c	36.4 ^a	21.5 ^b	0.20	<0.0001
NDFom (%DM)	31.8 ^d	33.0 ^c	44.8 ^a	39.5 ^b	0.63	<0.0001
ADL (%DM)	3.6 ^d	7.6 ^b	8.2 ^a	4.3 ^c	0.22	<0.0001
Ash (%DM)	23.9 ^a	12.8 ^d	16.2 ^c	21.6 ^b	0.39	<0.0001
IVOMD (%)	80.7 ^b	85.5 ^a	75.5 ^d	78.5 ^c	0.62	<0.0001
Ca (mg/100gDM)	805.7 ^b	1432.0 ^a	444.7 ^d	738.0 ^c	17.09	<0.0001
P (mg/100gDM)	136.6 ^b	462.7 ^a	83.6 ^c	126.3 ^b	13.50	<0.0001

*Means within a row with different superscripts differ significantly ($p < 0.05$); DM= Dry matter, CP= Crude protein, EE= Ether extract, ADFom= Acid detergent fiber, NDFom= Neutral detergent fiber, ADL= Acid detergent lignin, IVOMD= *In vitro* organic matter digestibility

Table 2: *In sacco* DM degradability and degradability characteristics of the morphological fractions of stinging nettle forage

Parameters	Plant parts				P value	MSE
	Flower	Leaf	Stem	Whole		
Incubation time (hrs)						
24	61.8 ^a	60.4 ^b	44.9 ^c	59.3 ^b	<0.0001	0.51
48	68.7 ^a	67.5 ^a	53.1 ^c	65.8 ^b	<0.0001	0.63
72	72.1 ^a	70.4 ^b	60.1 ^c	69.4 ^b	<0.0001	0.53
96	77.6 ^a	75.9 ^b	64.5 ^c	75.5 ^b	<0.0001	0.52
Degradability parameters						
A (%)	23.9 ^a	23.7 ^b	20.3 ^d	22.3 ^c	<0.0001	0.46
B (%)	51.9 ^a	50.6 ^a	45.8 ^b	50.6 ^a	<0.0001	0.52
PD (%)	75.8 ^a	74.3 ^b	66.1 ^c	73.2 ^b	<0.0001	0.54
c (h)	0.049 ^a	0.048 ^a	0.029 ^b	0.048 ^a	<0.0001	0.001
L (h)	1.7 ^b	1.9 ^{ab}	0.1 ^c	2.1 ^a	<0.0001	0.11
ED (%)	47.8 ^a	46.8 ^{ab}	37.3 ^c	45.1 ^b	<0.0001	0.71
RSD	2.7 ^b	2.9 ^{ab}	1.6 ^c	3.1 ^a	<0.0001	0.12

*Means within a row with different superscripts differ significantly ($p < 0.05$).

A= Washing loss, B= insoluble but slowly degradable, PD= potential degradability, c =degradation rate, L= lag time, ED= effective degradability, RSD = residual standard deviation).

Table 3: Pearson correlation coefficient (r) matrix of chemical composition, IVOMD and DM degradability of of stinging nettle forage

	ADFom	NDFom	ADL	CP	IVOMD	PD	ED	DMD
ADFom	1.00							
NDFom	0.98*	1.00						
ADL	0.70*	0.57*	1.00					
CP	-0.95*	-0.89*	-0.86*	1.00				
IVOMD	-0.72*	-0.82*	-0.03	0.50*	1.00			
PD	-0.83*	-0.86*	-0.49*	0.84*	0.86*	1.00		
ED	-0.84*	-0.76*	-0.52*	0.85*	0.84*	0.87*	1.00	
DMD	-0.69*	-0.82*	0.01	0.47	0.97*	0.81*	0.68*	1.00

*Significant difference ($p < 0.05$)

ADFom= Acid detergent fiber, NDFom= Neutral detergent fiber, ADL= Acid detergent lignin, IVOMD= *In vitro* organic matter digestibility, PD= Potential degradability, ED= Effective degradability, DMD= Dry matter degradability

stem. However there was no significant difference ($P < 0.05$) among flower, leaf and whole forage which exhibited higher rate of degradation (c) over stem. The highest ($P < 0.05$) lag time (L) was recorded in whole forage and leaf followed by flower. Leaf showed intermediate lag time (L) between whole plant and flower. However stem showed the least ($P < 0.05$) lag time (L) among all morphological fractions. The effective degradability (ED) was higher ($P < 0.05$) for flower and leaf followed by whole forage, whereas there was no significant difference between leaf and whole forage as the latter was intermediate between the former and flower. The least ($P < 0.05$) effective degradability (ED) was observed in stem.

Correlation among Chemical Composition, IVOMD and DMD: Relationship among chemical composition, IVOMD and rumen DM degradability (DMD) parameters

(ED and PD) is presented in Table 3. Fiber fractions (ADFom and NDFom) were negatively correlated ($p < 0.05$) with CP content, IVOMD and DMD parameters (PD and ED). Similarly lignin content (ADL) showed the same trend as fiber fractions. On the other hand CP showed positive correlation ($p < 0.05$) with IVOMD and DMD parameters.

DISCUSSION

Chemical composition of forages could be affected by several factors including stages of maturity, environmental conditions and morphological fractions. In the current study different morphological fractions of stinging nettle showed differences in chemical composition. The protein content of all fractions was different, with highest in leaf and lowest in stem. The CP content in leaf, flower and whole forage was above 20

percent, which could be used as protein supplement for poor quality roughages fed to small ruminants [21]. The protein content of leaf obtained in the current study is comparable with reports of previous studies [22, 23]. It is higher than the results of Getachew *et al.* [24] and Rafajlovska *et al.* [25] who found the protein content of leaf ranged between 25-26 and 18-26 percent respectively. It is also lower than the results (33%) of Rutto *et al.* [26] and Adhikari *et al.* [27]. The protein content of stem found in the current study is in agreement with the results of Rafajlovska *et al.* [25].

Leaf showed highest CP and lowest fiber fractions (NDFom, ADFom and ADL). This result is in agreement with previous studies in leguminous trees and cereal crops. Debela and Tolera [28] reported highest CP and lowest fiber (NDFom, ADFom and ADL) contents in leaves than pods and whole forage part in *Moringa oleifera* and *Moringa stenopetala*. Debela *et al.* [5] also found that leaves of *Sesbania sesban* and *Desmodium intortum* had highest CP and lowest fiber (NDFom, ADFom and ADL) content compared to twigs, pods and whole forage. Leiber *et al.* [29] also reported that highest CP and lowest NDFom contents were in leaf of buck wheat compared to flower, stem, grain and whole plant fractions.

Tolera *et al.* [30] also reported highest CP and lower fiber (NDFom, ADFom and ADL) content in vegetative parts (leaf blade and leaf) compared to stem of maize forage. Similarly, Nurfeta *et al.* [31] reported that leaf lamina and midrib had higher CP content compared with pseudostem of *enset* (*Ensete ventricosum*). Tolera *et al.* [32] described leaf portion of cowpea and silver-leaf desmodium forage legumes had higher CP and lower NDFom and ADFom contents compared to stem.

The IVOMD of whole stinging nettle forage obtained in the current study is comparable with previous findings [6, 33]. The highest IVOMD in flower and leaf would be related to lower fiber and lignin content of these fractions. On the other hand lowest IVOMD in stem might be associated with high lignin content of this fraction [5]. Yeheyis *et al.* [34] reported that stem fraction had significant effect on decline of CP content and IVOMD in berseem clover. This indicates that an increase in stem fraction in forages usually leads to low value of IVOMD and CP and high fiber. The quantity and quality of the leaf fraction may be more important than the total amount of forage. Under some grazing conditions, where large quality differences available between leaves and stems, animals prefer to eat the leaf than the stem [4].

The calcium (Ca) and phosphorous (P) contents obtained in this study are comparable with the results of Getachew *et al.* [24] but higher than the results reported

by Rutto *et al.* [26], Pradhan *et al.* [22] and Civelek and Balkaya [23]. These variations might be attributed with the differences in stinging nettle varieties studied, as the latter are temperate varieties. In the present study all fractions had adequate content of Ca and P which meet the recommended requirements for different classes of livestock [35]. However the ratio of Ca to P obtained in the current study were 3.0:1.0 (in leaf), 5.3:1.0 (in stem) and 5.8:1.0 (in the flower and whole forage). All morphological fractions were out of the normal recommended ratio of 2:1. Under such condition if stinging nettle is fed to animals as single ration, supplementation of P might be needed to adjust to recommended ratio and for proper utilization in the animal system [36].

The disappearance of feed is a composite of the different digesting substrates plus passage rate. Soluble matter ferments rapidly, leaving more slowly available insoluble matter to dominate the later phases of digestion [37]. The observed differences in the DM degradation (DMD) characteristics of morphological fraction of stinging nettle could be due to the differences in chemical composition. The difference in DMD among morphological fractions observed in the current study is in agreement with the previous studies on other legume trees and forages [5, 28]. The higher DMD of flower and leaf fractions reported in our work could be attributed to the high soluble cell fraction available in these fractions [37]. The lower DM degradability value in stem fraction might be related to the higher ADFom, NDFom and ADL contents of the fraction [5, 28].

The higher washing loss (A) of leaf and flower over stem shows the presence of more soluble components in the former fractions than the latter. Moreover the value of (A), which represents the readily soluble materials, could affect cell-wall degradation rates through its effect on the rumen microbial population and enzyme systems, because it is a source of nutrients for the rumen microorganisms [38]. Higher values observed in insoluble but slowly degradable (B), potentially degradable (PD) and effective degradability (ED) for leaf and flower could be related to the lower fiber and higher CP content compared to the stem. In addition the high PD of leaf and flower fractions reported in the current study could be associated with the presence of higher quantity of soluble nutrients (A) in these fractions [30, 39].

Rate of digestion refers to the quantity of feed that can be digested per unit of time and it is essential function of the diet. The composition of the diet and its quality determine the speed of digestion. Generally soluble components are fermented very rapidly while less soluble substrates are attacked more slowly. Structural carbohydrates like cellulose are fermented more slowly

than the other insoluble storage carbohydrates such as starch [37]. Higher rate of degradation (c) obtained in flower and leaf in the current study could be related to the availability of soluble cell components in these morphological fractions. Contrarily lower rate of degradation (c) in stem fraction, which is the determining factor for effective degradation, could be associated with high fiber and lignin content of this fraction. On the other hand the high protein content, low fiber and lignin composition and high proportion of readily digestible materials could maintain high degradation rates in flower and leaf fractions [31, 32, 40].

Forage materials with high protein content are usually able to supply fermentable protein which can be used to enhance digestibility while NDFom and ADFom are related to poor digestibility. CP and IVOMD were positively correlated while NDFom, ADFom and ADL and were negatively correlated with DMD. This is in accordance with previous reports on other legume forages [5, 41]. The negative correlation of cell wall components with DMD could be due to the fact that lignin causes depression in digestibility by physical encrustation and chemical bonding with structural carbohydrates which inhibit the activity of rumen microbial enzymes thereby lowering down the fermentative rate which in turn causes a decline in digestibility [36].

CONCLUSION

Forage quality could be affected by several factors including proportion of morphological fractions. Producing suitable quality forage for a given situation requires understanding the factors that govern forage quality and then implement management accordingly. Analysing forages through chemical analysis and DM digestibility and degradability could be used to determine the quality of the forage.

Morphological fractions varied in chemical composition, IVOMD and *in sacco* DM degradability. The differences in fiber component and CP content among different morphological fractions influenced digestibility. Hence fiber components (NDFom and ADFom) negatively influenced IVOMD and *in sacco* DMD, whereas CP influenced in the reverse way.

The values obtained from the current study attested that leaf and flower are of high nutritive value and have potentials to be used as alternate feed supplements for ruminants. However in order to optimize their use as animal feed supplements, further animal feeding studies are needed, so that optimal level of inclusion in the diet and feeding conditions will be properly defined.

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REFERENCES

1. Mengistu, A., 2005. Feed resources base of Ethiopia: Status and opportunities for integrated development. pp: 377-386. Proceedings of the 12th Annual Conference of the Ethiopian Society of Animal Production (ESAP). Addis Ababa, Ethiopia, August 12-14, 2004, ESAP (Ethiopian Society of Animal Production).
2. Negesse, T., H.P.S. Makkar and K. Becker, 2009. Nutritive value of some non-conventional feed resources of Ethiopia determined by chemical analyses and an *in vitro* gas method. *Animal Feed Science and Technology*, 154: 204-217.
3. Tolera, A., 2012. Feed resources availability and quality vs. animal productivity. In: A. Tolera, A. Yami and D. Alemu (eds.). *Livestock Feed Resources in Ethiopia: Challenges, Opportunities and Need for Transformation*, pp: 37-46.
4. De Santis, G., A. Iannucci and E. Chiaravalle, 2007. Changes during growth in cell wall components of berseem clover under different cutting treatments in a Mediterranean region. *Italian Journal of Agronomy*, 3: 321-331.
5. Debela, E., A. Tolera, L.O. Eik and R. Salte, 2011. Nutritive value of morphological fractions of *Sesbania sesban* and *Desmodium intortum*. *Tropical and Subtropical Agroecosystem*, 14: 793-805.
6. Andualem, D., T. Negesse and A. Tolera, 2015. Biomass yield, chemical composition and *in vitro* organic matter digestibility of stinging nettle (*Urtica simensis*) from four locations at three stages of maturity. *Livestock Research for Rural Development*. <http://www.lrrd.org/lrrd27/8/and u27159.html>.
7. Sultan, J.I., I. Rahim, M. Yaqoob, M.I. Mustafa, H. Nawaz and P. Akhtar, 2009. Nutritional evaluation of herbs as fodder source for ruminants. *Pakistan Journal of Botany*, 41: 2765-2776.
8. Purcell, P.J., T.M. Boland, M. O'Brien and P. O'Kiely, 2012. *In vitro* rumen methane output of forb species sampled in spring and summer. *Agriculture Food Science*, 21: 83-90.

9. Wencelová, M., Z. Váradýová, K. Mihaliková, D. Jalè and S. Kišidayová, 2014. Effects of selected medicinal plants on rumen fermentation in a high-concentrate diet *in vitro*. The Journal of Animal and Plant Sciences, 24: 1388-1395.
10. Kulivand, M. and F. Kafilzadeh, 2015. Correlation between chemical composition, kinetics of fermentation and methane production of eight pasture grasses. Maringá, 37: 9-14. Doi:10.4025/actascianimsci.v37i1.24336.
11. AOAC, 2005. Official methods of analysis of the official analytical chemists, 18th Edn. (W. Horwitz, Eds.), Association of Official Analytical Chemists, Washington DC.
12. Van Soest, P.J., J.B. Robertson and B.A. Lewis, 1991. Methods for dietary fiber, neutral detergent fiber and non-starch carbohydrates in relation to animal nutrition. Journal of Dairy Science, 74: 3583-3597.
13. Van Soest, P.J. and J.B. Robertson, 1985. Analysis of forages and fibrous foods. A Laboratory Manual for Animal Science 613, Cornell University, USA.
14. Ørskov, E.R. and I. McDonald, 1979. The estimation of protein degradability in the rumen from incubation measurements weighted according to rate of passage. Journal of Agricultural Science, 92: 499-503.
15. Chen, S.B., 1995. Neway Excel: An Excel Application Program for Processing Feed Degradability data, Rowett Research Institute, Bucksburn, Aberdeen, UK.
16. McDonald, I., 1981. A revised model for the estimation of protein degradability in the rumen. Journal of Agricultural Science, 96: 251-257.
17. Ørskov, E.R. and M. Ryle, 1990. Energy Nutrition in Ruminants. Elsevier, Oxford, pp: 149.
18. Dhanoa, M.S., 1988. On the analysis of Dacron bag data for low degradability feeds, Grass Forage Science, 43: 441-444.
19. Tilley, J.M.A. and R.A. Terry, 1963. A two stage technique for *in vitro* digestion of forage crops. Journal of British Grassland Society, 18: 104.
20. SAS, 2005. SAS Release 9.1. Institute Inc., Cary, NC, USA.
21. National Research Council, 2007. Nutrient requirements of small ruminants: Sheep, Goats, Cervids and New World Camelids. National Academy of Sciences, Washington, DC, USA.
22. Pradhan, S., S. Manivannan and J.P. Tamang, 2015. Proximate, mineral composition and antioxidant property of some wild leafy vegetables. Journal of Science and Industrial Research, 74: 155-157.
23. Civelek, C. and A. Balkaya, 2013. The nutrient content of some wild plant species used as vegetable in Bafera plain located in the black sea region of Turkey. The European Journal of Plant Science and Biotechnology, 7: 62-65.
24. Getachew, E.A., G.H. Desse and G.D. Addis, 2013. Nutritional profile of Samma (*Urtica simensis* steudel) leaves grown in Ethiopia. International Journal of Science Innovative. and Discovery, 3: 153-160.
25. Rafajlovska, V., Z. Kavrakovski, J. Simonovska and M. Srbinoska, 2013. Determination of protein and mineral contents in stinging nettle. Quality of Life, 4: 26-30. DOI: 10.7251/QOL1301026R.
26. Rutto, L.K., Y. Xu, E. Ramirez and M. Brandt, 2013. Mineral properties and dietary values raw and processed Stinging nettle (*Urtica dioica* L.). International Journal of Food Science. 2013, doi: 10.1155/2013/857120.
27. Adhikari, B.M., A. Bajracharya and A.K. Shrestha, 2015. Comparison of nutritional properties of Stinging nettle (*Urtica dioica*) flour with wheat and barley flours. Food Science and Nutrition, doi: 10.1002/fsn3.259.
28. Debela, E. and A. Tolera, 2013. Nutritive value of botanical fractions of *Moringa oleifera* and *Moringa stenopetala* grown in the mid-Rift Valley of southern Ethiopia. Agroforestry System, 87: 1147-1157.
29. Leiber, F., C. Kunz and M. Kreuzer, 2012. Influence of different morphological parts of buckwheat (*Fagopyrum esculentum*) and its major secondary metabolite rutin on rumen fermentation *in vitro*. Czech Journal of Animal Science, 57: 10-18.
30. Tolera, A. and F. Sundstøl, 1999. Morphological fractions of maize Stover harvested at different stages of grain maturity and their nutritive value of different fractions of the stover. Animal Feed Science and Technology, 81: 1-16.
31. Nurfeta, A., L.O. Eik, A. Tolera and F. Sundstøl, 2008. Chemical composition and *in sacco* dry matter degradability of different morphological fractions of 10 enset varieties, Animal Feed Science and Technology, 146: 55-73.
32. Tolera, A. and F. Sundstøl, 2001. Prediction of feed intake, digestibility and growth rate of sheep fed basal diet of maize Stover supplemented with *Desmodium intortum* hay from dry matter degradability of the diets. Livestock Production Science, 68: 13-23.

33. Baloyi, J.J., N.T. Ngongoni and H. Hamudikuwanda, 2008. Chemical composition and ruminal degradability of cowpea and silver leaf desmodium forage legumes harvested at different stages of maturity. *Tropical and Subtropical Agroeco system*. From <<http://www.redalyc.org/articulo.oa?id=93980107>>
34. Yeheyis, L., A. Tsegahun, A. Sebsibe, T. Mekonen, N. Ziku and A. Amame, 2008. Identified indigenous feed resources and their nutritive value in Menz-Gera district of Amhara region, Proceedings of the 3rd annual regional conference on completed livestock research activities, 1 - 4 September, 2008. pp: 1-10.
35. De Santis, G., A. Iannucci, D. Dantone and E. Chiaravalle, 2004. Changes during growth in the nutritive value of components of berseem clover (*Trifolium alexandrinum* L.) under different cutting treatments in a Mediterranean region. *Grass Forage Science*, 59: 378-388.
36. McDowell, L.R., 1997. Minerals for Grazing Ruminants in Tropical Regions. Third edition, University of Florida, Gainesville, Florida, USA., pp: 81.
37. Datt, C., M. Datta and N.P. Si Ngh, 2008. Assessment of fodder quality of leaves of multipurpose trees in subtropical humid climate of India. *Journal of Forest Research*, 19: 209-214.
38. Van Soest, P.J., 1994. *Nutritional Ecology of the Ruminant* (2nd Ed). Comstock Publishing Association, Cornell University Press, Ithaca.
39. Abebe, A., A. Tolera, Ø. Holanda, T. Ådnøya and L.O. Eikad, 2012. Seasonal variation in nutritive value of some browses and grass species in Borana rangeland, southern Ethiopia. *Tropical and Subtropical Agroecosystem*, 15: 261-271.
40. Gusha, J., N.T. Ngongoni and T.E. Halimani, 2013. Nutritional composition and effective degradability of four forage trees grown for protein supplementation. *Online Journal of Animal Feed Research*, 3: 170-175.
41. Kaitho, R.J., I.V. Nsahlai, B.A. Williams, N.N. Umunna, S. Tamminga and J. Van Bruchem, 1998. Relationships between rumen degradability, gas production and chemical composition of browses. *Agroforestry System*, 39: 129-144.