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Biomass Yield, Chemical Composition, *In-Vitro* Organic Matter Digestibility and Gas Production of Morphological Fractions of Mulberry (*Morus alba*) Plant Harvested at three Cutting Stages

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Abstract: Bio-mass yield (BMY), chemical composition, in-vitro organic matter digestibility(IVOMD) and gas production (GP), metabolizable energy (ME), methane and short chain fatty acid (SCFA) productions of Mulberry (*Morus alba.)* plant fractions (leaf, stem edible branch and whole plant) harvested at three cutting stages were evaluated. The plants were harvested at 6,8 and 10 months after planting which was denoted as stage 1,2 and 3 respectively. Among morphological fractions the highest ($p<0.05$) dry matter yield was for stem while the lowest ($p<0.05$) was for edible branch. The DM yield increased ($p<0.001$) with increasing stage of maturity for all fractions. The CP content decreased ($p<0.001$) with increasing stage of maturity for leaf and edible branch. The neutral detergent fiber (NDF) content for stage 1 and 2 was lower than stage 3 for leaf, edible branch and whole plant. The acid detergent fiber (ADF) for stage 1 in leaf was lower than that of stage 2 and 3. The IVOMD of leaf and whole plant was similar for stage 1 and 2 which was significantly higher than the third cutting stage. For edible branch and stem IVOMD decreased ($p<0.001$) with increasing stage of maturity. The highest ($p<0.05$) gas production was from leaf while the lowest was for stem. Gas production from immediately soluble fraction (a) showed lower and negative value among plant fraction except whole plant. Gas production from insoluble but degradable fraction and the potential gas production (a+b) was highest ($p<0.05$) for edible branch while the lowest ($p<0.05$) was for stem. The IVOMD, ME and SCFA decreased ($p<0.05$) with increasing stage of maturity. Methane production from leaf decreased with increasing stage of maturity at 24 hours of incubation. The highest amount of methane production was from leaf and whole plant at first and second stage of cutting. High CP and IVOMD and low fiber content for Mulberry leaf indicate that it could serve as protein supplement and the other fractions of mulberry is also use as alternative feed resources for low quality forages. First cutting stage is recommended as the right stage to obtain optimum protein content and digestibility. On the other hand to obtain optimum bio-mass yield, the third cutting stage is a good stage for harvesting. Further feeding trial is recommended by authors to verify the quality of the leaf on local Arsi-Bale sheep breed.

Key words: Crude Protein, Dry Matter · Fiber Content · *In vito* digistability · Leaf · Metabolizable Energy · Methane Gas Production

INTRODUCTION

Animal feeds in terms of quantity and quality is essential for body weight gain, milk production, efficient reproduction and high profits. A major factor in increasing livestock productivity is through feeding balanced nutrients and the required amount. Improved animal health and parasite control, breeding and management will also be important, but a major emphasis must be placed on providing better and quality nutrients [1]. Crude protein (CP), dry matter digestibility (DMD), metabolizable energy (ME) and fiber content are often used as indicators of forage quality.

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Maturity stage at harvest is the most important factor determining forage quality of a given species [2]. Moreover maturity stage had great effects on chemical composition. Protein content and soluble sugars in leaves decrease with the maturity of leaves [3], while fat, ash, neutral detergent fiber (NDF) and acid detergent lignin (ADL) constituents' increase with maturity [4]. Leaves are higher in quality than stems and the proportion of leaves in forage declines as the plant matures. The predominance of the stem with the advancing maturity results in an increase in crude fiber, lower levels of fermentable carbohydrates and protein and poor digestibility [5]. Forage consumption by animals is also influenced by maturity at harvest; as plants mature and become more fibrous, intake potential decreases and also the rate at which fiber is digested slows. Dry matter (DM) intake and *in-vitro* dry matter digestibility (IVDMD) of leaf is also decreased with increasing of maturity [5]. This is because fiber is more difficult to digest than the non-fiber components of forage.

Forage yield and quality are influenced by various factors such as environmental and soil conditions, species, stage of growth, leaf to stem ratio, diseases and pests and plant chemical composition [6]. The production of leaf and total dry matter per hectare of mulberry depends on the variety, location, plant density, harvesting season and cutting frequencies [3 -5]. Climate (moisture and solar radiation) and soil fertility are determining factors on productivity. In this regard, the result of Azim *et al.* [7] reported earlier indicated that the CP value of mulberry leaf is higher in spring than winter season. Another study showed that the nutritional value of mulberry leaves is higher at mid-stage in spring and at early stage in autumn [8]. There is also evidence that, increasing intervals between harvests significantly increased the fresh and DM yields of plant fractions of mulberry [5]. The same source further noted that delayed harvesting resulted in higher yields and greater plant persistence, but associated with marked reduction in the leaf: stem ratio and CP content and a corresponding increase in lignification's. Rumen degradable protein (RDP) is also found to decrease with the increasing maturity [4]. However, yields (dry matter content) are higher in late development stages compared with early growth stages [9]. Understanding the growth stage of forage plants in general is important for making good management and harvesting time decisions. There is little information available which assess the effects of stage of maturity on biomass yield, chemical composition, in vitro digestibility and gas production of Mulberry (*Morus a.*) in Ethiopia. Therefore, this study was designed to evaluate effect of stage of maturity on biomass yield, chemical composition, digestibility, *in-vitro* gas production, short chain fatty acids, methane productions and metabolizable energy content of mulberry plant harvested at three cutting stages.

MATERIALS AND METHODS

Description of the study area: The study was conducted in Hawassa Agricultural Research center situated in southern regional state which is 275km from Addis Ababa Ethiopia. Hawassa Agricultural Research Center is found in the geographic extent that ranges from 07° 03' 19.1'' to 07° 04' 00.2'' north latitude and from 38° 31' 08'' to 38 ° 31' 01.8'' east longitude. It has an undulating topography with altitude ranging from 1695 to 1713 m.a.s.l. It receives mean annual rainfall of about 948 mm. It has a bimodal rainfall pattern extending from March to September. Its mean annual maximum and minimum temperatures are 27.3°C and 12.6°C, respectively. It is endowed with 200-240 consecutive length of growing period (LGP) and a tepid to cool sub humid (SH) agro-ecology [10]**.** The soil type of the farm is Vitric Andosols with 80-152 cm depth and its slope ranges from 0-2%. The soil is slightly acidic to neutral with top soil (0-30 cm) pH values ranging between 6.4 and 6.9 [11].

Planting Materials and Experimental Designs: Stalks were collected from cultivated Mulberry (*Morus a*.) plant from research center. Experimental fields were hand dug three times and plots (2.1m x 1.4m) were prepared and arranged in a randomized complete block design (RCBD) against slope gradient of the land. Plots were arranged in three blocks each with 3plots in a row. Space between plots and rows was 70cm and planting were established in 3 rows on each plots. Four stalks with 50cm length were planted 70cm apart from each other in a rows. Planting of stalks was carried in August 2015. Irrigation was done every three days during rain fall shortage period and also weeding was done to maintain uniform establishment and production of plant until the end of data collection.

Data Collection and Sampling Procedure: After 6, 8 and 10months the 1st, 2nd and 3rd cuttings were collected respectively. For each cutting three plots, one plot from each block was randomly selected. The whole plants in the plots were harvested and fractionated into the leaf, edible branch and stem which were weighed separately to determine fresh and dry biomass yield. Sub-sample of each fraction of the plant was taken from each cutting stages separately and brought to laboratory by using

plastic bags. Dry matter content of the sample was determined after oven drying at 105ºC overnight. Samples for chemical analysis were dried at 65ºC for 48hr and ground to pass through 1mm sieve size for chemical analysis, *in-vitro* digestibility and *in-vitro* gas production.

Chemical Analysis:- Dry matter (DM), ash, ether extract (EE), crude protein (CP), neutral detergent fiber (NDF), acid detergent fiber (ADF) and acid detergent lignin (ADL) were analyzed according to standard procedures [12, 13].

In vitro* Digestibility Experiment:** for determination of ***in vitro organic matter digestibility rumen fluid was collected from two rams by inserting suction rumen tubes into the rumen before morning feeding and added into a pre-warmed thermos flask [14]. The fluid was strained through double layers of cheese cloth and added into a Jar. McDougall's buffer solution was prepared and approximately 0.5 gram of milled forage sample was weighed and sealed into fiber bags. The buffer solution with rumen fluid and 26 bags (24bags containing feed sample and 2bags without feed samples) were incubated in each jar for 48h at 39°C. Fermentation was stopped after 48h and acidified pepsin was added. Then bags were incubated in a Jar 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 (IVOMD) was determined as the quantity of organic matter (OM) lost during fermentation and subsequent pepsin digestion.

***In vitro* Gas Production:** for determination of *in-vitro* gas production was analyzed according to the procedure of Menke and Steingas [15]**.** Ruminal fluid was collected from two rams by inserting suction rumen tubes into the rumen before morning feeding and added into a pre-warmed thermos flask [14]. The fluid was strained through double layers of cheese cloth before added into glass syringe. The syringes were pre-warmed at 39°C before addition of 30ml of buffer mixture and rumen liquor into each syringe. McDougall's buffer solution was prepared and about 200 mg of dry sample was incubated with rumen fluid in a calibrated glass syringe of 100 ml in duplicate. The syringes were shaken gently 30 min after the start of incubation and every hour for the first 10h of incubation. Syringes with buffered rumen fluid without feed sample were also included in duplicate as a control. All the syringes were incubated in a water bath maintained at 39°C. Gas production was recorded after 3, 6, 12, 24, 48, 72 and 96h of incubation. The gas production characteristics were estimated by fitting the mean gas volumes to the exponential equation

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

where:

- G = The gas volume of gas produced (ml/200mg OM) at time t,
- a = The gas production from the immediately soluble fraction (ml),
- b = The gas production from the insoluble but degradable fraction (ml),
- a + b = is the potential gas production (ml) and
- c = the rate constant of gas production (fraction/h) [16].

Organic matter digestibility (OMD) was calculated from the equation:- ***OMD (%) = 14.88 + 0.889G24 + 0.45CP*** [15] where:- OMD = organic matter digestibility at 24 hours; CP=Crude protein content of feed samples and G24 = G24= Gas production value (ml/200mg) at 24hrs of. Metabolizable energy (ME) was calculated from equation: ***ME (KJ/gDM) = 2.2 + 0.136G24 + 0.057CP*** [15] where:- G24=Gas production value (ml/200mg) at 24hrs of incubation; CP=Crude protein content of feed samples. Short-chain fatty acids (SCFA) were estimated as: ***SCFA = 0.0239G24 - 0.0601***.[15]; where:- G24 = Gas production value (ml/200mg) at 24hrs of incubation.

Measurement of Methane Production: Methane (CH_4) production after 24 and 72 hours of incubation was measured by connecting lower end of syringe with another small (20ml) syringe with a needle containing 4.0ml of NaOH (10M) which was then introduced latter into incubated contents, thereby avoiding gas escape [17]. Mixing of contents with NaOH allowed absorption of CO_2 and volume remaining in syringe is considered to be CH_4. Net methane and gas productions were calculated by differences of methane and total gas in test syringe and corresponding blank. Methane concentration was calculated as net methane production/net gas production [4].

Statistical Analysis: Statistical analyses were performed using general linear model procedure of Statistical Analysis System (SAS)[18]. The model used for analysis was:

$$Yijk = \mu + Ai + Bj + A*B(ij) + e(ijk)$$

*w*here *y* is the parameter studied μ is overall mean, *Ai* is fixed cutting stages (i=1,2,3), *Bj* is fixed plant parts (j=1,2,3) and *A*B(ij)* is interaction between cutting stages and plant parts and *e(ijk)* is error term. Mean were compared using least significance difference (LSD) and significance was declared at $p < 0.05$.

RESULTS AND DISCUSSION

Effect of Cutting Stages on Forage Biomass Yield: Fresh and dry matter yield of plant parts is presented in Table 1. There was an increase ($P<0.05$) in biomass yield with an increase in harvesting stage. The highest fresh yield was from stem (59.9tone/ha) followed by leaf (47.9tone/ha) and edible branch (34.1tone/ha). Leaf production at the 3rd cutting stage in the current study was higher than the results reported earlier (10tonDM/ha/year) [19] and [20] and close to results reported earlier (15 to 22 tons/ha/year) earlier [8] and it was lower than the result reported earlier (20ton of DM/ha) for mulberry leaf harvested at the end of rainy season and beginning of mid dry season [21]. A dry matter yield of 15.5ton/ha/year and 45.2ton/ha/year was also reported for edible leaves [19] and young stems [20].

Chemical Composition and *in-vitro* Organic Matter Digestibility (*IVOMD*): Effect of cutting stage and plant parts on chemical composition and *in-vitro* organic matter digestibility of cultivated Mulberry plant is presented in Table 2. The highest ($p<0.05$) ash content was recorded for leaf (18.2%) during third cutting stage and lowest was for stem (4.47%). Ash from leaf in the current result is higher than the result reported earlier for leaf and pods of *Acacia* and *prosopis* [22]. Crude fat (EE) did not shown significant ($p>0.05$) difference among plant parts and within each cutting stages. However, the highest EE was recorded from leaf at first cutting stage.

The highest CP was recorded from leaf (26.5%) at first cutting and lowest from stem (4.16%) at third cutting stages. The CP content of mulberry leaf from first cutting stage in this study was higher than results reported [23, 8, 46 and 24] earlier. However, it is comparable with the result (26.06%) reported for different species of mulberry harvested at 60 days of cutting interval [25] and lower than the 28.1-35.8% CP reported for Mulberry leaf harvested at 3-7week[5]. Crude protein content of edible branch at third cutting stage presented in this study was in line with result reported earlier [24]. Crude protein content recorded for stem in this study was lower than the 8.3% reported for mulberry harvested at 7th week of age [5].

Crude protein contents (20.28-26.5%) of mulberry leaf in three cutting stages in the current study is within 12-30% reported for legume tree leaf [26] showings that mulberry leaf could serve as an important protein sources. The CP content in the leaf is above the recommended minimum requirements for lactation (12%) and growth

Table 1: Effects of cutting stages on fresh and DM yield of Mulberry

	Morphological fractions					
Yield	Cutting stage	Leaf	Edible branch	Stem	SEM	p-value
Fresh yield (g/plant)	1	413^{cB}	207^{cC}	835^{cA}	54.0	<.0001
	2	821^{bB}	506^{bC}	1217^{bA}	87.6	0.0002
	3	1174^{aB}	836^{aC}	1467^{aA}	85.3	0.0003
	SEM	77.13	43.1	100.4		
	p-vale	<.0001	<.0001	0.0007		
DM yield (g/plant)	1	128.1^{cB}	71.7^{cC}	318.1^{cA}	20.22	<.0001
	2	284.2^{bB}	145.6^{bC}	553.8^{bA}	38.22	<.0001
	3	343.9^{aC}	349.3^{aB}	784.6^{aA}	31.79	<.0001
	SEM	23.49	16.77	45.3		
	p-value	<.0001	<.0001	<.0001		
Fresh yield (tone/ha)	1	16.9^{cB}	8.4^{cC}	34.1^{cA}	2.2	<.0001
	2	33.5^{bB}	20.6^{bC}	49.7^{bA}	3.59	0.0002
	3	47.9^{aB}	34.1^{aC}	59.9^{aA}	3.48	0.0003
	SEM	3.1	1.77	4.1		
	p-value	<.0001	<.0001	0..0008		
DM yield (tone/ha)	1	5.2^{cB}	2.93^{cC}	12.9^{cA}	0.84	<.0001
	2	11.6^{bB}	5.9^{bC}	22.6^{bA}	1.57	<.0001
	3	14.03^{aB}	14.3^{aB}	32.0^{aA}	1.29	<.0001
	SEM	0.96	0.69	1.85		
	p-value	<.0001	<.0001	<.0001		

Different letters ''^{ABC}'' in a row and ''^{abc}'' in a column shows significant ($p<0.05$) difference for each plant parts and cutting stages respectively SEM = Standard error of mean, P = probability

Table 2: Chemical composition and organic matter digestibility of cultivated Mulberry (*Morus a.*) plant parts in different cutting stage

		Chemical composition							
Plant part	Cutting stages	DM	Ash	EE	CP	NDF	ADF	ADL	IVOMD
Leaf	1	93.4b	15.3c	3.11	26.5 a	25.6b	12.7b	4.95	86.9a
	2	94.7a	16.7b	1.05	23.3b	27.6b	14.8a	6.60	84.7a
	3	93.7b	18.2a	1.13	20.3c	35.7a	15.1a	7.58	75.9b
	SEM	0.10	0.35	1.21	0.81	2.11	0.27	0.62	1.45
	P-value	0.0017	0.0082	0.2968	0.0104	0.0332	0.0059	0.0526	0.0092
Edible branch	1	93.6c	7.46b	0.42	12.1a	53.0b	38.1	6.36	70.3a
	2	94.6a	8.37a	0.56	10.3b	54.8ab	38.5	6.56	55.2b
	3	94.4b	6.47c	0.88	8.16c	56.2a	39.3	7.29	48.9c
	SEM	0.029	0.114	0.573	0.479	0.714	0.464	0.757	6.533
	P-value	0.0001	0.0011	0.7330	0.0088	0.0471	0.1770	0.5113	0.0066
Stem	1	94.6	5.43a	2.16	5.39	67.3	50.8	7.53	54.4a
	2	95.6	4.71b	0.59	4.81	67.4	48.8	9.23	41.6b
	3	96.2	4.47c	0.59	4.16	67.7	47.7	8.29	30.6c
	SEM	1.152	0.062	0.435	0.328	0.501	0.699	0.418	4.616
	P-value	0.4523	0.0012	0.0561	0.0736	0.7143	0.0471	0.0603	0.0323
Whole plant	1	95.9	10.8b	1.47	23.1	41.7b	27.8	5.04	82.4a
	2	96.3	11.1b	0.86	15.4	40.9b	25.7	6.17	75.9a
	3	95.7	12.7a	1.36	13.1	44.8a	29.3	6.20	66.9b
	SEM	0.674	0.175	0.509	3.798	0.766	2.861	0.899	2.903
	P-value	0.7083	0.0034	0.5209	0.1483	0.0312	0.5278	0.4421	0.0291
P-value	Plant part	0.0004	<.0001	0.0010	<.0001	<.0001	<.0001	0.0003	<.0001
	Cutting stages	0.0054	<.0001	0.0424	0.0005	<.000	0.5181	0.004	<.0001
	Interaction	0.4872	<.0001	0.2837	0.1215	0.0019	0.1344	0.3150	0.3421

Means with different superscript within column are significantly different at ($p<0.05$); DM = Dry matter; OM = Organic matter; EE = Ether extract; CP = Crude protein; NDF = Neutral detergent fiber; ADF = Acid detergent fiber; ADL = Acid detergent lignin; IVOMD = *In-vitro* organic matter digestibility and p = probability

(11.3%) in ruminants [9], which can justify its inclusion in diet of lactating and growing ruminants. Moreover, the CP content 13.1 – 23.0% of whole mulberry plant from three cutting stage in this study is higher than minimum level of CP required for optimum microbial growth (7-14%) for ruminant and required for optimum rumen function (7- 8%) [27] and it is also above 8% CP required to satisfy maintenance requirement of ruminant animals [26]. Differences in CP content of plants could be an attribute of harvesting season, age of the leaves at harvesting, frequency of harvesting, climate and soil [28].

The value of NDF, ADF and ADL were shown significant ($p<0.05$) difference among morphological fraction within each cutting stages. Highest ($p<0.05$) value of NDF, ADF and ADL was recorded from stem under third cutting stage (Table 2). The NDF value of mulberry leaf harvested at the first cutting stage in this experiment is lower (67.3%) than previous result reported for mulberry [28, 8 and 24]. Average ADF content of mulberry leaf harvested at third cutting stage was lower than the result reported for mulberry [24, 28] Feed with more than 65% NDF and 55% ADF content were classified as low quality feeds and fodder trees with ADF values less than 31% are rated as having superior quality [29]. The ADL content of mulberry leaves was lower at the third cutting as compared with earlier reports [30 and 24] but higher than the value reported earlier [31]. The ADL content of mulberry leaf, edible branch, stem and whole plant in this study is below the maximum level of 10% which was indicated to limit DM intake [28]. Mulberry bark and stem are also reported to be edible [21, 32]. Cattle consume whole biomass of Mulberry if it is finely chopped [33]

In-vitro organic matter digestibility (IVOMD) was significantly ($p<0.05$) different among morphological fractions within each cutting stages and among plant fractions in a cutting stages. The highest IVOMD was recorded for leaf under first cutting and lowest for stem under third cutting. Result of IVOMD% of leaf from first cutting in this study is higher than 77.9% and 82.1% reported for leaf of mulberry [24 and 30] respectively. The IVOMD from leaf, edible branch and whole plant from three cutting stages is higher than earlier report for selected semi-arid browse forages except Prosopis Africana [34]. The value of IVOMD for leaf in the three cutting stage and the whole plant is higher than earlier reports for *Desmodium intortum* and *Sesbania sesban* leaf and whole plant [41].

Table 3: Pearson correlation coefficient (r) matrix among cell wall component (NDF, ADF and ADL), crude protein and *in-vitro* dry matter digestibility IVOMD

	NDF	ADF	ADL	CP	IVOMD
NDF	1.00				
ADF	0.73*	1.00			
ADL	0.88*	0.97*	1.00		
CP	-0.94*	-0.92*	-0.99*	1.00	
IVOMD	-0.99*	-0.74*	-0.88*	0.94*	1.00

CP = Crude protein, NDF = Neutral detergent fiber, ADF = Acid detergent fiber, ADL = Acid detergent lignin, IVOMD = *In vitro* organic matter digestibility
*Significant difference ($p<0.05$)

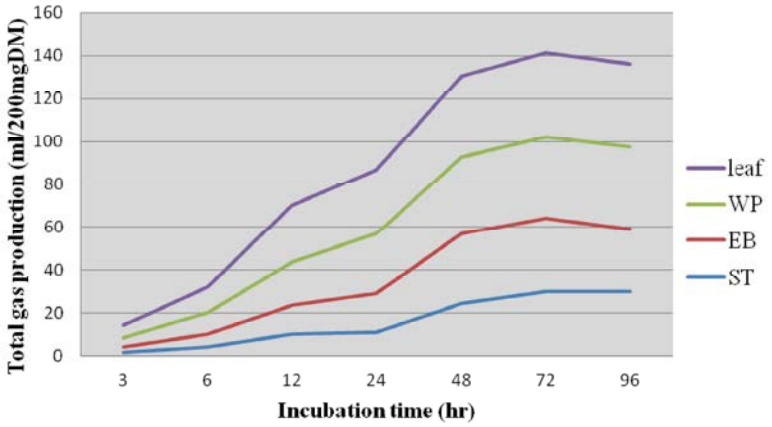

Fig. 1: *In-vitro* gas production of morphological fraction of mulberry plant

Fiber components such as, NDF, ADF and ADL were negatively correlated ($p<0.05$) with both CP content and IVOMD. On the other hand, there was positive correlation between protein content and IVOMD. Neutral detergent fiber (NDF) negatively affects IVOMD followed by ADL and ADF (Table 3). Stem had significantly reduced CP content and IVOMD in berseem clover [35]. Vegetative leaves declines as structural constituent increased with advancing maturity [36] which indicates that an increase in stem fraction in forages reduces IVOMD and CP and increases fiber content. Therefore, maximum IVOMD was observed at 1st stage of cutting and it declined with advancing stages of cutting.

***In-Vitro* Gas Production and Characteristics:** *In-vitro* gas production and characteristics of morphological fractions of cultivated mulberry plant is presented in Fig 1 and Table 4 respectively.

Throughout incubation hours, the highest gas production was for leaf while the lowest was for stem Figure 1. The gas production from leaf at 24 and 48hr of incubation in this study was higher than the result reported for browse species and for stinging nettle [31, 37] and gas production at 24, 48 and 72hr of incubation period are in line with earlier results (23-61ml, 33-70ml) reported for different vegetable wastes[38]. However, it is lower than the result reported earlier for different Oak species [47]. The gas production from leaf in this study was faster during the first 24hr of incubation and it declined with increasing incubation time.

The gas production from edible branch was faster after 24hr of incubation time than leaf and it declined with increasing of incubation time. Gas production from the immediately soluble fraction "a" in this study was lower and shown negative value. This agrees with earlier reports for stinging nettle [31], for grasses, woody species and forbs [40], for twigs and whole forage of *Desmodium intortum* [41], for different morphological fraction of maize stover [42] and for *A. tortolis* and *P.juliflora* [22].

The negative values could be due to differences in lag phase of fermentation of insoluble feed components that led to a deviation from exponential curve of fermentation [43].

Gas production from insoluble but degradable fraction "b', potential gas production (a+b) the rate constant "c" of gas production and lag time "L" are significantly ($p<0.05$) different between morphological fraction. The highest "b" fraction was recorded for whole plant at first cutting stage and the lowest from edible branch from cutting stage three. The highest "b" fraction

Table 4: Gas production characteristics (constants) of the morphological fraction of Mulberry plant

		Morphological fractions				
Variables	Cuttings	Leaf	Edible branch	Stem	Whole plant	p-value
a	1	-7.35^{bC}	-5.75^{bB}	-2.15^{aA}	-5.1^{bB}	0.0001
	2	-10.35^{cC}	-5.7^{bB}	-2.25^{aA}	-6.25^{cB}	<.0001
	3	-4.35^{aC}	-2.65^{aB}	-2.6^{bB}	-1.6^{aA}	0.0005
	p-value	<.0001	<.0001	0.0049	<.0001	
b	1	50.25^{aB}	46.75^{aC}	39.75^{bD}	51.25^{aA}	<.0001
	2	48.25^{bA}	36.05^{bC}	34.1^{cD}	42.2^{bB}	<.0001
	3	41.15^{cB}	32.05^{cD}	46.1^{aA}	37.3^{cC}	<.0001
	p-value	<.0001	<.0001	<.0001	<.0001	
a + b	1	43.15^{aB}	40.5^{aC}	37.35^{bD}	46.15^{aA}	0.0002
	2	37.65^{bA}	30.1^{bD}	31.6^{cC}	35.7^{bB}	<.0001
	3	37.3^{cB}	29.15^{cD}	43.25^{aA}	35.4^{bC}	<.0001
	p-value	<.0001	<.0001	<.0001	<.0001	
c	1	0.0887^{aA}	0.0416^{bC}	0.0255^{bD}	0.0552^{bB}	<.0001
	2	0.0865^{bA}	0.0557^{aC}	0.0304^{aD}	0.0606^{aB}	<.0001
	3	0.0461^{cA}	0.0372^{cC}	0.0133^{cD}	0.0445^{cB}	<.0001
	p-value	<.0001	<.0001	<.0001	<.0001	
L	1	1.45^{cD}	3.15^{aA}	2.15^{bB}	1.7^{bBC}	0.0045
	2	2.65^{a}	3.1^{a}	2.25^{b}	2.6^{a}	0.0863
	3	2.15^{bB}	2.2^{bB}	4.4^{aA}	1.5^{bC}	0.0008
	p-value	0.0009	0.0025	0.0002	0.0220	

*Means within a row with different superscripts ^{ABCD} and in column ^{abc} differ significantly ($p<0.05$), a = Gas production from the immediately soluble fraction (ml), b = gas production from the insoluble but degradable fraction(ml), a + b = potential gas production (ml), c = the rate constant of gas production (fraction/hr), L = lag time, SEM = standard error of mean, 1 = cutting stage one, 2 = cutting stage two and 3 = cutting stage three

from whole plant could be due to low CP content as compared with leaf. This is in agreement with earlier report for husk a fraction with the lowest CP content [42]. However, the total gas production in this study from whole plant at 72hr of incubation was the highest compared with leaf, edible branch and stem. The "b" fractions in the current study for whole plant is higher compared with earlier report for *M.olifera* and *M.stenoptala* leaf [44] but lower than that of *A. tortolis* and *P.juliflora* [22].

Potential gas production (a+b) was higher from whole plant at first cutting and lower from edible branch from third cutting stage. The potential gas production (a+b) from whole plant in this study was lower than the value reported earlier for *M.olifera* and *M. stenoptala* leaf [44] and *A. tortolis* and *P.juliflora* [22]. The rate constant of gas production (c) was higher for leaf at first cutting stage and lower for stem at third cutting stages. The lag time (L) was higher for stem at third cutting stage and while the lowest for leaf at first cutting stages. The lag time(L) for morphological fractions in this study was lower than the result reported earlier for *A. tortolis* and *P.juliflora* except *prosopis* leaves and pods treated with rumen liquor [22]. The different results observed in gas production parameters among morphological fractions indicate that there is a difference in rate and extent of fermentation characteristics.

Organic Matter Digestibility, Metabolizable Energy and Short Chain Fatty Acids: Effect of cutting stages and plant parts on organic matter digestibility (OMD), metabolizable energy (ME) and short chain fatty acids (SCFA) is presented in Table 5. The IVOMD, ME and SCFA decreased ($p<0.05$) with increasing stage of maturity. Organic matter digestibility of leaf recorded from first cutting in this study is higher than the result reported for leaf, stem and whole plant of stinging nettle [31] and for *Acacia* and prosopis leaf treated with rumen liquor, effective microbes, commercial yeast and wood ash and lower than acacia leaf treated with calcium hydroxide and polyethylene glycol (PEG) [22]. Akinfemi [38] has also reported lower result for different vegetable wastes except potato. The OMD of stem in this study harvested from first cutting stage is higher than the result reported for alternative feed resources, but it is lower than hay, Artichoke leaves and stems [18]. Metabolizable energy value of mulberry leaf in this study is higher than the result reported for *Acacia* and *prosopis* leaf treated by rumen liquor, effective microbes, commercial yeast and wood ash and lower than *Acacia* leaf treated by calcium hydroxide and polyethylene glycol [22].

Higher result for ME was reported from leaf and edible branch of Mulberry, rain tree seed and unconventional feed than in the current study [24, 45]. Metabolizable energy result obtained from leaf in the

Table 5: Effect of stage of maturity and plant parts of mulberry on%organic matter digestibility, metabolizable energy and short chain fatty acids during 24hr incubation period.

	Parameters			
Plant parts	Cutting stages	OMD (%)	ME (MJ/kg DM)	SCFA (µmol/g DM)
Leaf	1	72.62a	8.61a	0.80a
	2	65.9b	7.61b	0.66b
	3	59.59c	6.62c	0.51c
Edible branch	1	64.92a	5.85a	0.46a
	2	52.21b	5.24b	0.37b
	3	45.48c	4.70c	0.30c
Stem	1	51.21a	4.29a	0.26a
	2	47.62b	4.09b	0.23b
	3	44.5c	3.49c	0.12c
Whole plant	1	70.12a	8.07a	0.74a
	2	60.06b	6.88b	0.59b
	3	53.89c	6.08c	0.49c
	Cutting stages	<.0001	<.0001	<.0001
P-value	Plant parts	<.0001	<.0001	<.0001
	Interaction	0.0008	0.0013	0.0253

*Means within and among fractions with different superscripts differ significantly ($p<0.05$). DM = Dry matter, ME = Metabolizable energy, OMD = Organic matter digestibility, SCFA = Short chain fatty acids,

Table 6: Methane gas production and concentration at 24h and 72h of incubation

			Cutting stages				
Incubation periods (hr)	Parameters	Plant parts	1	2	3	SEM	P-value
24	Gas production (ml/0.2gDM)						
		Leaf	12.5aA	10.5bA	7.75cA	0.61	0.0102
		Edible branch	6.5aB	3.5bB	2.5bB	0.71	0.0225
		Stem	4.0C	3.5B	3.0B	1.22	0.7401
		Whole plant	10.5A	10.5A	8.5A	0.71	0.1028
		SEM	0.94	0.71	0.88		
		P-value	0.0018	0.0007	0.0063		
	Gas concentration (ml/total gas volume)						
		Leaf	0.35	0.35AB	0.32B	0.01	0.1671
		Edible branch	0.30a	0.25bC	0.22bC	0.01	0.0076
		Stem	0.17	0.17B	0.15A	0.01	0.1424
		Whole plant	0.33	0.39A	0.37B	0.03	0.2585
		SEM	0.05	0.03	0.05		
		P-value	0.2077	0.0168	0.0054		
72	Gas production (ml/0.2gDM)						
		Leaf	18.5A	18.75A	16.25A	1.04	0.1643
		Edible branch	11.5B	9.75C	9.25B	0.87	0.1539
		Stem	6.75C	4.0D	1.75C	1.44	0.0891
		Whole plant	14.0aB	12.5bB	9.0cB	1.15	0.0409
		SEM	1.44	0.9	0.77		
		P-value	0.0037	0.0004	0.0002		
	Gas Concentration (ml/total gas volume)						
	Leaf	0.49	0.47A	0.49A	0.04	0.1128	
	Edible branch	0.37	0.34B	0.31B	0.47	0.4308	
		Stem	0.29	0.22C	0.12C	0.04	0.0577
		Whole plant	0.34	0.36B	0.29B	0.05	0.1304
		SEM	0.05	0.03	0.04		
		P-value	0.1186	0.0023	0.0021		

Means with different superscript letter "ABCD" in a column and "abc" in the rows are significantly ($p<0.05$) different, SEM = Standard error of mean, P = probability, 1 = cutting stage one, 2 = cutting stage two and 3 = cutting stage three

Table 7: Correlation among protein, fiber fractions, digestibility, gas production and energy content of mulberry leaf

	CP	NDF	ADF	ADL	IVOMD	TGP	CH4	ME
CP	1.00							
NDF	-0.97**	1.00						
ADF	-0.95**	0.99**	1.00					
ADL	-0.76**	0.71**	0.64**	1.00				
IVOMD	0.94**	-0.92**	-0.90**	-0.84**	1.00			
TGP	0.98**	-0.94**	-0.92**	-0.86**	0.97**	1.00		
CH4	0.96**	-0.92**	-0.89**	-0.89**	0.97**	0.99**	1.00	
ME	0.73	-0.73	-0.69	-0.93	0.84	0.83	0.86	1.00

** Significant difference ($p<0.05$), CP = Crude protein, NDF = Neutral detergent fiber, ADF = Acid detergent fiber, ADL = Acid detergent lignin, IVOMD = *In vitro* organic matter digestibility, TGP = Total gas production, CH4 = Methane, ME = Metabolizable energy.

current study is higher than result reported for stinging nettle [31] and for rice straw and *Leucaena* [18]. Short chain fatty acids (SCFA) of leaf from first cutting presented in this study is higher than result reported on leaf flower of *S. nettle* and *Acacia* leaf treated by rumen liquor, effective microbe and commercial yeast [31, 22]. However, it was lower than acacia leaf treated with calcium hydroxide, PEG and wood ash [22]. Lower result of SCFA was reported for onion and higher for potato [38].

Methane Production and Concentration: Total methane (CH_4) gas production and concentration values are presented in Table 6. The highest ($p<0.05$) CH_4 production and concentration was recorded from the leaf and the lowest from the stem under the first and second cutting stages for both incubation time and from third cutting stages in 72h of incubation.

The highest ($P<0.05$) gas production and concentration was recorded from whole plant at third cutting stages. Gas production ranged from 2.5-12.5ml/200mgDM in24hr of incubation and from 1.75-18.75ml/200mgDM in 72hr of incubation time. Methane gas production from stem in 24hr of incubation time was higher than gas production in 72hr of incubation time. Methane gas production at 72hr of incubation from leaf at the first cutting is in line with the result earlier reported for Acacia leaf (14.5-18.8ml/200mgDM) and pods (17.6-20.5ml/200mgDM) and it is lower than the result reported for *prosopis* leaf and pods except leaf treated by wood ash[40]. Methane production and concentration at 24 and 72hr of incubation earlier reported [31] is lower than the result obtained from all fractions of mulberry plant in the current study.

Correlation of Crude Protein, Fiber Content, IVOMD, ME, Total Gas and Methane Production: Correlation among crude protein (CP), fiber content (NDF, ADF and ADL), *in-vitro* organic matter digestibility (IVOMD), metabolizable energy (ME), total gas (TG) and methane (CH_4) production is presented in Table 7. Fiber fractions (NDF, ADF and ADL) were negatively correlated ($p<0.05$) with CP, IVOMD, TGP, CH4 and ME. The CP content showed positive correlation ($P<0.05$) with IVOMD, TGP, CH_4 and ME. The above findings of total gas production agree with result reported for Stinging nettle [31]. Gas production from insoluble but degradable fraction "b" was highest for edible branch which had low CP content than leaf.

CONCLUSION

Leaf is the major plant part which contains more CP and had highest digestibility than edible branches and stem. However, edible branches and stem would be also utilized as alternative feed resources in order to fill the gap during feed shortage period for ruminants. Harvesting at the first cutting stage is recommended to obtain optimum protein content and digestibility. But, to maintain high bio-mass yield; cutting at the third stage is recommended to collect more yield than first cutting stage. In general the different fractions or whole plant of mulberry are of high nutrient content and good digestibility that are comparable to other leguminous forages and can be used as supplements to low quality roughages.

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