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Feeding value potential of mulberry (*Morus alba*) leaf meal to replace concentrate mix

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The experiment was carried out to estimate the feeding value of mulberry (*Morus alba*) leaf meal by evaluating its chemical composition *in vitro* organic matter digestibility and *in sacco* dry matter (DM) degradability. This study aimed to assess the potential of mulberry foliage in supplementing the feed of ruminant animals during the dry season, when other feed resources are scarce and their quality generally fall short of animal requirements. Dry matter (DM), crude protein (CP), ash, ether extract (EE), crude fiber (CF), neutral detergent fiber (NDF), acid detergent fiber (ADF) and acid detergent lignin (ADL) contents of the different diets were determined. *In vitro* organic matter digestibility was determined by two-stage method. *In sacco* rumen degradability was measured using three rumen fistulated Boran-Holstein Friesian cross steers at 0, 6, 12, 24, 48, 72, and 96 h. The composition of mulberry leaf meal compares favorably with that of the concentrate mixture in most of the nutrients. The ash content of mulberry leaf meal was more than double that of the concentrate mixture. Similarly, the calcium value of mulberry leaf meal was more than threefold to that of calcium content of concentrate mixture. Mulberry leaf meal alone had the greatest values for slowly degradable fraction (b) than the diets with less proportion of mulberry, whereas mulberry leaf meal alone (T5) and 75 g concentrate mix + 259.7 g mulberry leaf (T4) had significantly (p>0.05) less soluble fraction (*a*), and effective degradability (*ED*) values as compared to the diets with less proportion of mulberry. In a nutshell, all treatment diets recorded more than 66% DM degradability at 24 h, which implied that they were all greatly degradable in the rumen.

Key words: Chemical composition, degradability, digestibility, *Morus alba.*

INTRODUCTION

In areas like Ethiopia where livestock are closely integrated with crop production, crop residues are considered as valuable sources of ruminant feeds. However, crop residues are fibrous having low digestible organic matter and low crude protein content, and generally are low in nutritive value. This low nutritive values restrict its utilization by rumen microorganisms and consequently by the host animal. An adequate

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supply of nitrogen (N) to rumen microbes is crucial to obtain maximum rate of plant cell digestion as well as a high microbial protein synthesis (Adugna, 2008; Gül et al., 2010; Gizaw et al., 2010).

There are several alternatives for improving the performance of ruminants fed with low nutritional quality fibrous diets. Supplementation with concentrate could improve the nutritional value of low quality roughage such as straws which could be an alternative. However, the concentrate diets are often expensive and not accessible to smallholder farmers. In many tropical countries and regions, there has been a focus on indentifying and using locally available shrubs and tree leaves as cheaper alternative supplements for ruminants because of their high nutritive value and positive effects on rumen function (Yao et al., 2000).

Additionally, several works in different places have reported the potential of the mulberry (*Morus alba*) forage for sustainable ruminant production (Yulistiani et al., 2008; Martín et al., 2007; Kandylis et al., 2009; Soca et al., 2010; Salinas et al., 2011; González and Martín, 2016). Hence, mulberry might be potentially used as supplement to low quality roughage diets due to its high protein content (Sanchez, 2002) and degradability (Saddul et al., 2005). Beside its high protein content, the degradability of its organic matter is also high (Saddul et al., 2005) thus, can supply fermentable energy in the rumen. Moreover, this can create favorable condition in the rumen for plant cell wall degrading microorganisms (Yulistiani et al., 2008).

Therefore, this research was carried out to compare the chemical composition, *in vitro* digestibility as well as *in sacco* degradability of sole mulberry leaf meal, concentrate mix and their mixtures.

MATERIALS AND METHODS

Sample preparation and experimental design

Leaves from mulberry that were harvested from nearby farmers and nursery areas were dried under a shade for 4 to 5 days until the leaves were easily crushed when pressed in the hand and packed in a sack for later use. The concentrate mixture which was purchased from animal feed shop comprised of wheat bran and noug seedcake at the ratio of 2:1 on dry matter (DM) bases, in that order.

Five treatment diets were designed in such a way that concentrate mix was progressively replaced by mulberry leaf meal from 0 to 100% at iso-nitrogenous level. The diets include, 300 g concentrate mix alone (T1), 225 g concentrate mix + 86.55 g mulberry leaf (T2), 150 g concentrate mix + 173.1 g mulberry leaf (T3), 75 g concentrate mix + 259.7 g mulberry leaf (T4) and 346.2 g mulberry leaf alone (T5).

Chemical analysis

Chemical composition of the feed samples was determined at Holeta Agricultural Research Center, animal nutrition laboratory, Ethiopia. Samples of partially dried feeds were dried overnight at 105°C in a forced draft oven for determination of total dry matter content. Samples were analyzed for DM, ash, and nitrogen (N) using the procedure of AOAC (1990) and crude protein (CP) was calculated as N x 6.25.

Neutral detergent fiber (NDF), acid detergent fiber (ADF) and acid detergent lignin (ADL) were analyzed using the procedures of Van Soest et al., (1991).

In vitro organic matter digestibility

In vitro organic matter digestibility was determined by the two-stage method of Tilley and Terry (1963). Samples were incubated for 48 h with rumen fluid and buffer followed by additional 48 h digestion with pepsin and HCl. The residue was ashed in a muffle furnace at 550°C for 5 h.

Rumen fluid was obtained from rumen fistulated Boran x Holstein Friesian steers kept at maintenance dietary condition with diets containing hay supplemented daily with 4 kg of concentrate mixture, which involved 74, 25 and 1% wheat bran, noug seed cake and salt, respectively.

In sacco dry matter degradability

Rumen degradability of the samples was determined by incubating about 3 gm of sample in a nylon bag (41μm pore size and 6.5x14 cm dimension) in three rumen fistulated Boran x Holstein Friesian steers kept at Holeta Agricultural Research Station. The steers were offered grass hay supplemented daily with 4 kg of concentrate mixture, comprised of 74, 25 and 1% wheat bran, noug seed cake and salt, respectively and kept in individual pens. The feed samples were incubated for 0, 6, 12, 24, 48, 72 and 96 h. Duplicate nylon bag containing samples were incubated in three rumen fistulated animals by placing the samples at different hours and taking them out at the same time (sequential addition).

At the end of the incubation period, sample containing bags, including zero hour bags were hand washed in a running tap water. The washed bags were then dried in an oven at 105°C for 24 h to determine dry matter. The dried bags were taken out of the oven and allowed to cool down in desiccators and weighed immediately. The ruminal *in sacco* DM degradability at each incubation time was determined as follows;

$$\text{DMD (g/kg DM)} = (\text{DM in feed sample} - \text{DM in residue})/\text{DM in feed sample}$$

DMD data was fitted to the exponential equation $p = a+b\,(1-e^{-ct})$ as described by Ørskov and McDonald (1979), where p is the amount of nutrient degraded (%) at time t, a is the intercept of the degradation curve at time zero and represent as degradability of soluble fraction (%), b is the rumen-insoluble, but slowly degradable fraction (%), e is base for natural logarithm, c is the rate constant for degradation of b fraction (%/h), t is the incubation time (h) and the lag phase (L) represented as;

$$L = (1/c)\ \log_e [b/(a+b - A)]$$ (Ørskov and Ryle, 1990)

Potential degradation (PD) was estimated as (A + B), while effective degradability (ED) of DM was calculated using the formula:

$$ED = A + [B{*}c/(c + k)]$$

Where A, B and C are described above and k is rumen outflow rate which is assumed to be 0.03/ h for roughage feeds (Ørskov and McDonald, 1979). The calculation of the equation was carried out using the Neway program (Chen, 1996).

Table 1. Chemical composition of the feed ingredients.

Chemical composition	Treatments				
	T1	T2	T3	T4	T5
DM (%)	90.2	89.5	89.2	89.5	91.8
OM (%DM)	92.34	89.70	86.06	85.59	84.30
Ash (%DM)	7.66	10.30	13.94	14.41	15.70
CP (%DM)	22.03	22.13	20.01	18.51	18.49
CF (%DM)	13.27	12.84	13.56	13.78	14.17
NDF (%DM)	35.18	34.19	35.23	36.78	37.98
ADF (%DM)	20.17	19.47	21.66	19.91	22.32
ADL (%DM)	3.39	3.42	3.36	3.83	4.19
EE (%DM)	7.16	6.33	5.91	5.34	4.15
Ca (%DM)	0.6	0.76	1.08	1.45	2.11
P (%DM)	1.02	0.81	0.94	0.84	0.77

MLM= Mulberry leaf meal; CM= Concentrate mix (2:1 of wheat bran to noug seed cake); DM=Dry matter; OM=Organic matter; CP=Crude protein; CF= Crude fiber; NDF=Neutral detergent fiber; ADF = Acid detergent fiber; ADL = Acid detergent lignin; EE= ether extract; Ca= calcium; P= phosphorus.

Statistical analysis

Data from the experiments on *in vitro* organic matter digestibility and *in sacco* dry matter degradability were subjected to the analysis of variance (ANOVA) in a randomized complete block design, using the general linear model procedure of SAS (2008). Individual differences between means were tested using Tukey HSD test. In all comparisons, the level of significance was set at $\alpha = 0.05$.

RESULTS

Chemical composition of the feeds used in the experiment

The diets were formulated to meet the Crude protein (CP) requirements of yearling Tigray highland lambs. The composition of mulberry leaf meal compares favorably with that of the concentrate mixture in most of the nutrients which were better in its ash content than the concentrate mixture (Table 1).

The dry matter content seems similar across the different treatments, whereas the organic matter content declined as the proportion of concentrate mixture decreases. Ash content of mulberry leaf meal in the current study was more than double to that of the concentrate mixture. Similarly, the calcium value of mulberry leaf meal was more than threefold to that of concentrate mixture.

In vitro organic matter digestibility and *in sacco* dry matter degradability

There was less difference in *in vitro* organic matter digestibility (76.42 to 77.78%) across the different treatments in the current study. All tested diets resulted more than 76% *in vitro* organic matter digestibility. The *in sacco* dry matter degradability characteristics of sole mulberry foliage and concentrate mix or their mixtures are presented in Table 2.

The greatest and least soluble fraction (A) was recorded in the diet with higher proportion of concentrate mix and in sole mulberry leaf meal, respectively. The amount of washable materials (A) significantly increased as the proportion of concentrate mix increases.

The washable materials were significantly higher in T1 and T2, than the remaining treatments. Unlike the washable materials, the reverse is true in the case of potential degradation for non-water soluble materials (B). The insoluble but fermentable fractions increased as the proportion of mulberry leaf meal advanced. The slowly degradable fraction (B) value obtained in mulberry leaf meal was superior (53.2%) as compared to values recorded for the sole concentrate mix.

Potential degradability of T4 and T5 in the current study was significantly less than those of T1, T2 and T3. There was less difference in the rate of degradation across the different treatments in this study. Slight increase in degradation rate constant was observed until T3, which declined steadily at higher mulberry leaf meal level.

The trend of degradation of different levels of mulberry leaf meal in combinations with concentrate mixture showed a time dependent increase (Figure 1). This trend (T1 and T2) increased consistently up to 24 h but continues steadily up to 48 h. However, the progress seems constant beyond 48 h. Nevertheless, the trend of degradation for T3, T4 and T5 was slow up to 6 h; subsequent, T3 increased rapidly to 12 h compared to T4 and T5 which continues to increase exactly the same as T1 and T2. From 6 to 24 h, T4 and T5 showed similar increment. After 24 h T1, T2, T3 and T4 started to

Table 2. *In sacco* dry matter degradability characteristics of sole mulberry foliage and concentrate mix or their mixtures (%DM).

Parameter	Treatments					SEM	SL
	T1	T2	T3	T4	T5		
A	26.2[a]	26.1[a]	24.1[b]	22.7[c]	22.2[c]	0.25	***
B	51.1[b]	51.1[b]	52.5[ab]	52.6[ab]	53.2[a]	0.52	*
PD	77.0[a]	77.2[a]	76.6[a]	75.3[b]	75.3[b]	0.30	***
ED	63.6[a]	63.5[a]	62.8[a]	60.5[b]	58.9[c]	0.36	***
C/hr	0.087	0.089	0.090	0.086	0.084	0.00	NS
L (hr)	4.27[a]	4.43[a]	5.03[b]	4.90[b]	4.87[b]	0.08	***
In vitro DOM	77.46	77.47	76.98	77.78	76.42	1.34	0.861

[a-c] Means with different superscripts in a column within a category differ; *** = P<0.001; * = P<0.05; NS = not significant; SEM = standard error of the mean; SL= significant level A = rapidly soluble fraction; B = insoluble but fermentable fraction; PD = potential degradability; C = the rate of degradation of B; L = lag time; ED = effective degradability; DOM = Digestibility of organic matter; h = hour.

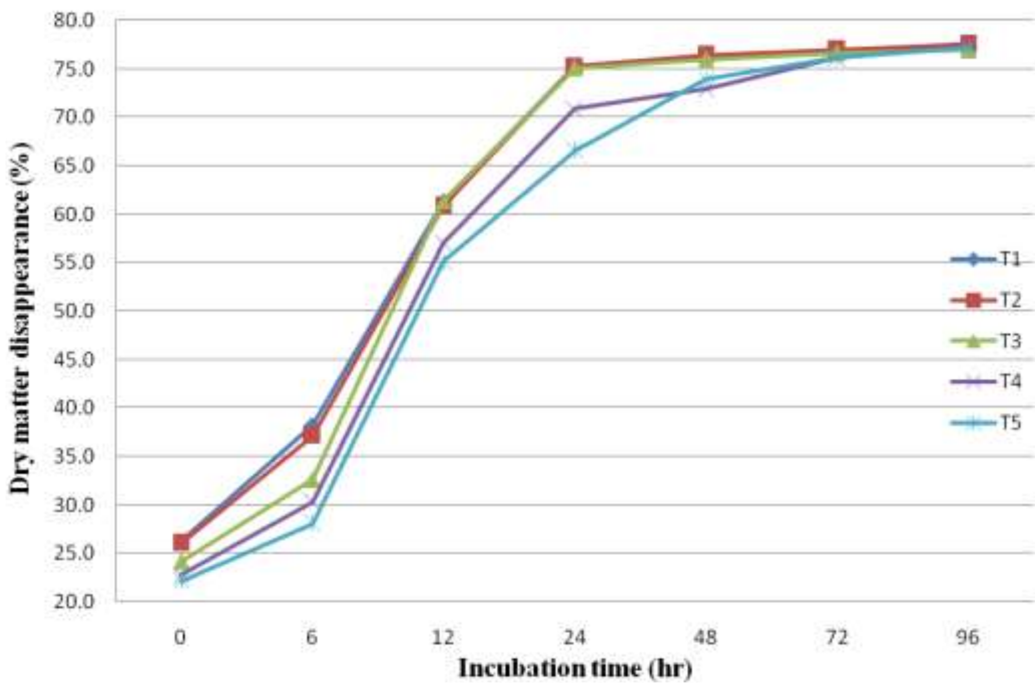

Figure 1. *In sacco* dry matter disappearances of the different treatment feeds.

decrease their degradation rate, while T5 (sole mulberry leaf meal) keeps increasing up to 48 h which continued increasing slowly until 72 h. As shown in Figure 1, the extent of degradation for all treatment diets beyond 72 h seems similar. After 48 h incubation the total dry matter degradability values showed non-significant effect across the different treatment diets.

Additionally, T1, T2 and T3 showed significantly higher degradability values than that of T4 and T5 in 12 to 48 h incubation. In a nutshell, all treatment diets recorded more than 66% DM degradability at 24 h, which implied that they were all potentially degradable in rumen.

DISCUSSION

Chemical composition

Since the feeds used in the current experiment were dried under sun, their dry matter was expectedly high (Table 1). The organic matter content declined as the proportion of concentrate mixture decreases. This might be due to higher ash content of mulberry leaf meal. The NDF, ADF and ADL compositions of the treatment diets were not deviated largely, justifying the comparable potential of mulberry foliage to that of concentrate mix.

The CP content of mulberry leaf meal obtained in this study was within the range of values (18 to 25%) obtained by Ba et al. (2005), Kandylis et al. (2009) and Vu et al. (2011).

The NDF content of sole mulberry leaf meal was to some extent higher than 31.1 and 26.4% reported by Vu et al. (2011) and Habib et al. (2016) respectively, whereas, its ADF content was within the range values (17.4 to 24.7 %) detected by Kabi and Bareeba (2008) as well as Habib et al. (2016). The ADL content of mulberry leaf meal in the present study is in accordance with the results that Kandylis et al. (2009) and Atiso et al. (2012) have reported 4.1and 4.65%, respectively. In three clones of mulberry leaves, Schmidek et al. (2000) noted comparable NDF (30.2 to 39.3%) and ADF (17.2 to 21.7%) results with the present finding.

Feeds with high ADF content could lower the availability of nutrients since there is a negative relationship between ADF and digestibility of feeds (McDonald et al., 2002). Moreover, Kabi and Bareeba (2008) also demonstrated that NDF, ADF and ADL contents increased with increasing maturity. However, the values for fiber fractions of mulberry leaf meal obtained in this experiment were low. This idea further corroborated the comparative supplementary feeding value of mulberry for ruminants. According to Lonsdale (1989) feeds that have <12, 12 to 20 and >20% CP are classified as low, medium and high protein sources, respectively. Hence, mulberry leaf meal in the current study could be categorized among the medium upper limit protein source feed that can serve as a protein supplement for low quality crop residues, particularly during the dry season. Similarly, Benavides (2000) also noted that mulberry leaves have a high potential as protein-rich forage supplement which is used in feed for ruminants, monogastrics and rabbits.

The ash content (15.4%) of mulberry reported by Habib et al. (2016) was comparable with the current finding. Similarly, Singh and Makkar (2002) stated that since mulberry leaves are rich in mineral matters like calcium, nitrogen, sulphur and other minerals they have the potential to be used as a supplementary feed for improving livestock productivity which conforms to the current study.

According to McDowell (1997), the recommended Ca to P ratio for ruminants is 2:1 to 4:1. Hence, the ratio obtained in the current study was within this range and the good mineral contents of mulberry foliage in this study further provoke its use as good alternative source of feed for ruminants. Ether extract obtained from the current study was higher than the value reported by Hurtado et al. (2012) (2.1%) whereas, it was comparable with 4.21 and 3.69% as reported by Wang et al. (2012) and Doran et al. (2007), respectively. This suggests that, mulberry is not an energy deficient plant. Therefore, the leaf from this plant can be considered as a cheap and affordable supplement for ruminant animals particularly in herds of small scale rural farmers.

***In vitro* organic matter digestibility**

The comparable digestibility of mulberry with the commercial concentrate mixture ensures the potential of the leaf, supplementing the less quality crop residues in ruminant production. The results that *in vitro* organic matter digestibility have been obtained, which are relatively higher than the results for *in vitro* digestibility of browse species during wet (68%) and dry season (72%) as reported by Solomon et al. (2010).

Moreover, Shayo (1997) reported that *in vitro* digestibility of mulberry leaves was 82.1% which is to some extent larger than the current study. Similarly, Bakshi and Wadhwa (2007) also noted that *in vitro* digestibility of mulberry leaves was higher than other forage species. The better digestibility of mulberry could be attributed to high CP level and increased concentration of ammonia nitrogen in rumen (Hristov et al., 2004). Higher ammonia nitrogen in rumen improves microbial activity and growth of fibrolytic bacteria resulting in more DM digestibility (Griswold et al., 2003). The narrow range of *in vitro* digestibility observed in the current study among the different treatment feeds might be a reflection of similarities in their chemical composition and potential of the mulberry being comparable with concentrate mix.

The threshold level of NDF in tropical plants beyond which feed intake of ruminants is affected is 60% (Meissner et al., 1991). Tree forages with a low NDF concentration (20 to 35%) are usually of great digestibility (Bakshi and Wadhwa, 2007). The NDF result of this study is in accordance with the results reported by Schmidek et al. (2000) and Cheema et al. (2011) for multipurpose trees and shrub species. Therefore, the NDF (37.98%) value obtained in the present experiment could be regarded as medium. This attribute can induce even greater fermentation rate, thus, improving its digestibility (Van Soest, 1994).

***In sacco* degradability**

In sole mulberry leaf meal, T5 had the greatest value for slowly degradable fraction (*b*) than the diets with less proportion of mulberry. Additionally, T5 and T4 had significantly ($p<0.05$) less soluble fraction (*a*) and effective degradability (ED) values compared to the diets with less proportion of mulberry (Table 1). The higher washable materials in T1 than T5 might indicate relatively higher content of dusty and small particles which could easily pass or wash from the bag, rather than a greater solubility. Similarly, Promkot and Wanapat (2003) reported that the result of the rumen bag technique depends on the way the feed is prepared and the pore size of the material from which the bag is made. Unlike the washable materials, the opposite is true in the case of the potential degradation for non-water soluble materials (B).

According to Belachew et al. (2013), multipurpose trees could be assigned to great (> 450 g/kgDM), medium (400 to 450 g/kgDM), and low (< 400 g/kgDM) quality groups based on their ED values. All the experimental diets in the current study including the mulberry foliage belonged to great quality group (Table 2). This further clarifies the comparable nutritional potential of mulberry foliage with that of concentrate mix and justifies the nutritive potential of mulberry which could supplement the less quality cereal crop residues in ruminant feed.

6 h post incubation; the greatest DM disappearance was recorded for T1 and T2, which have higher proportion of concentrate mix in the diet. Even though DM disappearances of mulberry leaf meal were lower compared to concentrate mix, 6 h post incubation; these results were comparable with other multipurpose trees. Belachew et al. (2013) noted that 6 h post incubation, the greatest DM disappearances was recorded for the leaves of multipurpose trees like *Ekebergia capensis* (32.12%) and *Melaleuca lanceolata* (32.60%) and the least DM disappearances was determined for *Ficus sycomorus* (15.82%) and *Rehmannia glutinosa* (16.87%) leaves. In the present study, dry matter disappearance increased with rumen incubation time for all experimental feeds. This is in accordance with the findings of Belachew et al. (2013), Lebopa et al. (2011), Vranic et al. (2009), Lanyasunya et al. (2006) and Tesema et al. (2003) which confirmed that *in sacco* degradation of dry matter increase as incubation time advances.

The obtained results for potentially degradable and rate of degradation are higher than the results reported by Suchitra and Wanapat (2008). However, the washable (39.2), potential degradability (84.7), and effective degradability (64.2) values were higher than the values obtained in the current study. The rate of degradations obtained in the current study (0.084 to 0.090) were higher than most of the multipurpose trees reported by Belachew et al. (2013) and to some extent is in accordance with that of Suchitra and Wanapat (2008). They have reported that the rate of degradation for Cassava hay (0.086) was highest compared to the other treatments, resulting in low gut fill which could lead to higher intake and animal production.

Furthermore, the lag time was at its minimum for T1 (4.27 h) and then increased which showed maximum value for T3 (5.03 h). In short, the amount of dry matter found degraded in all tested feeds at 12 and 24 h which are more than 50 and 66%, respectively, and implied that they were all greatly degradable in the rumen.

Generally, the results of the current study indicated that mulberry foliage appears to be rich in protein and minerals and low in fiber contents. Mulberry leaf meal by virtue of having high CP, good Ca to P ratio, promising digestibility and digestion rate as well as good extent of digestion is compatible to concentrate mix.

Conclusion

Mulberry leaf meal could potentially be used as a supplement to poor quality roughage diets, particularly during the dry season which could substitute the costly and not easily accessible protein concentrates by poor farmers in Ethiopia.

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CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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