Herbage Intake and Digestibility Considering Chlorophyll Content of Tropical Grass-legume Mixed Forages under Melia Azedarach Tree Shade

Shanker Raj Barsila*
Agriculture and Forestry University, Rampur, Chitwan, Nepal
Corresponding author: srbarsila@afu.edu.np.

Abstract

Purpose: The impact of native multipurpose trees on herbage mass productivity was researched at low profile in Nepal using physiological markers to the herbage intake and digestibility studies.
Methods: Under this context, the experiment was done with glycine (*Neonantia weightii*) and green leaf desmodium (*Desmodium intortum*) mixed with the Napier (*Pennisetum purpureum*) and blue panic (*Panicum antidotale*) planted under three levels of shade of Melia trees by using the Split Plot Design (SPD). Each plot was replicated three times, with one open. Standard agronomic practices were followed in the growth of the forages, whereas three shade levels (heavy, medium, and low) were maintained by pruning the lowest branches of Melia tree and also by measuring light intensity using a Lux meter. Additionally, 100 g of fresh leaf tissues were subjected to a chlorophyll analysis. After regrowth, herbage mass was collected from a 1 m^2 quadrant cutting over the ground at 60 and 75 days in August and September, respectively. Lab analysis was done on the sampled forages for proximate fractions, and a chlorophyll assay was performed on 100 g of fresh leaf tissues collected. Four goats were used in the herbage digestibility experiment using the metabolic crates during the second year of observation. Afterwards, the link between the digestibility attributes, herbage consumption, and relative dried weight yield of the herbage mass was noted.
Results: Research results had shown that the forage combination only affected $p < 0.05$ the total chlorophyll content, while the shade level $p < 0.05$ and forage mixture had significant effects $p < 0.05$ on the relative dried weight yield. Later, it was found that the dry matter yield, dry matter intake, and digestibility of dry matter (DM), crude protein (CP) and crude fiber (CF), respectively, have a significant positive correlation $p < 0.05$ with the total chlorophyll concentration.
Conclusion: The study's findings proved the Melia tree's broad effect on under-storey forages and provided the possibility of the establishment of an alternate model for forage development in Nepal's southern plains to have utilization by cultivating forages under tree shade, provided optimum shade is maintained by looping lower branches of Melia tree

Keywords: Chlorophyll content, Herbage mixture, Herbage intake, Herbage digestibility,Silvopasture,

1 Introduction

As a direct result of tree density and light penetration, one of the elements influencing herbage production understory in permanent tree-planted systems is the quantity of photosynthetically active radiation (PAR) available (dos Santos Neto et al., 2023). Under shade, the quantity of light is limited which slows down forage crops/pasture growth (Smith & Whiteman, 1983). This causes various morphological and physiological changes under understorey forages (Guenni et al., 2018). The ability of the majority of C4 grasses to sustain themselves in shading is attributed to a decreased rate of photosynthesis. This variation may be accounted for by changes in morphological characteristics, such as an increase in leaf area (Ludlow et al., 1974), increased above-ground competition (Casper & Jackson, 1997; Ong & Leakey, 1999), and ecosystem-dependent tree and forage species (Wilson, 1996). Shade-tolerant plants raised in shadow have different photosynthetic response characteristics than shade-intolerant plants acclimated to greater irradiance (Bond et al., 1999; Dodd et al., 2005; Baig et al., 2005). Many tropical and temperate forage/pasture species have shown benefits from the integration of trees and fodder; however, little is known about the pigment composition (chlorophyll content), forage productivity, and its relationship to proximal crude protein, crude fiber, and digestibility along with dry matter content. Although protein and energy are frequently the first two limiting factors impacting production responses from tropical pasture-based production systems, it is largely unknown how these two aspects relate to mixed forages/pasture produced under, for example, Melia tree shade. The amount of energy released for the animal to use is largely determined by how digestible its diet is. Consequently, pasture and forage species digestibility is crucial (Hughes et al., 2014). In

light of this, it is imperative to estimate the digestibility and total chlorophyll content of tropical pasture/forage species before grazing/ harvesting to determine the amount of CP in the herbage that is needed to meet domestic herbivores' nutrient requirements. It has been demonstrated that crude protein content is a reliable indicator of pasture digestibility (Njidda & Ikhimioya, 2010). There are currently about 200 species of fodder trees recognized in Nepal; however, little is known about the relationships that result in the production of under-storey forages under their shade, such as under Melia shade (Barsila & Devkota, 2008), shade of *Bauhinia purpurea* (NARC, 2002), some legume fodder trees (NARC, 1998), and apple orchards (Shrestha and Bastola, 2000).
Understanding the physiology of understory herbage is necessary to know more about the silvipastoral system's integration of trees and fodder (Devkota, 2000). This knowledge could also help to increase the system's potential adoption among farmers. As far as we are currently aware, no research has been done on the significance of chlorophyll concentration in predicting the dry matter productivity and digestibility of understory herbage. The quantities of nitrogen and chlorophyll in green leaves are strongly positively correlated (Boussadia et al., 2011; Mendoza-Tafolla et al., 2019). Forage productivity during the dry season is positively impacted by native trees that survive in grasslands. Agroforestry techniques that boost grassland productivity could be a useful countermeasure to the damaging impacts of widespread livestock farming-related deforestation. Thus, the objective of the current research was to generate current-time nutritional data through the proximate monitoring of chlorophyll. Information regarding the determination of ideal shade level and the forage mixture's tolerance understory when planted as an intercrop is scarce. The research also focused on the effects of intercropping two tall-growing grasses with legumes as forage on production, pigment (total chlorophyll) concentration, and intake digestibility of the feed in both medium shade and full sunlight to determine most scientific facets for understory cultivation of potential forage species.

2 Materials and methods

2.1 Experimental site

The experiment was conducted at the silvopastoral unit of the Agriculture and Forestry University (AFU) Livestock Farm under the 14 years old *Melia azedarach* shade planted by maintaining 1200 stems per ha; 5 m apart with an average of 10 m height and the average tree girth measured before the experiment was 12 inches. The site had a sandy loam characteristic of soil, having OM content of 3.59%, 2.1% organic C and 0.1% soil N respectively measured for 15 cm depth.

2.2 Experimental setup

The experiment was laid in a plot split-plot design having the main plot as shade level maintained by pruning the lowermost canopies up to 3 m (heavy shade), 4 (medium shade) and 5 m (low shade) height, respectively with sun plot (open) as a control). The subplots were the mixtures of two grasses i.e. napier *(Pennisetum purpureum)* and blue panic (*Panicum antidotale*); and glycine (*Neonantia weightii*) and green leaf desmodium (*Desmodium intortum*), so altogether the four grass/legume mixtures) with a size of 3.5 × 4 m was used as treatments.

2.3 Shade management

Following the standard agronomic practices, the forages in mixtures were planted in the experimental plots. The main plots were pruned to maintain the shade level and further defined the shade level by Lux meter (Model No 44147) which was validated as 72% irradiance to open as low, 64% open as medium, and 58% irradiance to open as the heavy shade. Later the canopy closure ratio was taken as a reference measured one year before for the main plots as 0.64, 0.54 and 0.43 for heavy, medium and low shade.

2.4 Herbage sampling

A quadrat of 1m × 1m was used to sample the herbage by manual cutting above the ground referenced to fresh herbage weight and later the samples were subjected to lab analysis.

2.5 Proximate analysis

The proximate analysis of the harvested herbage samples was done by following the procedures of AOAC (Horwitz et al., 1970). The harvested herbages were oven-dried and estimated to herbage productivity on a hectare basis. Later the samples per plot were subjected to grinding with a mill at 45 mm mesh size. Further, the ground herbage samples were used to estimate the proximate fractions (dry matter, crude protein, crude fibre, total ash, ether extract etc.).

2.6 Chlorophyll analysis of grasses

For the assay of total chlorophyll, the fresh leaf samples were prepared following the guidelines of Witham et al. (1971) with the use of the colorimetric method. Further, the total chlorophyll indices were calculated by the following formula (Bansal et al., 1999):

$$\text{Total Chlorophyll (mg/g)} = \frac{[20.2 \cdot (D_{645}) - 8.02 \cdot (D_{663}) \times V]}{1000} \tag{1}$$

where, D is the optical density (D) at a particular wavelength which was measured in mm i.e., 663, 645 mm V-final volume of 80% acetone extract, and W=fresh weight of tissue (g)

2.7 Digestibility trial

Only blue panic was used for its digestibility study considering the limited facility and logistic support available at the AFU. Accordingly, blue panic grown in shade and full sun (open) were fed to six-month-old Khari bucks for four days. Feed offered and refused was recorded daily for each animal. The feed intake herein has been considered as feed offered minus feed refused. The apparent dry matter digestibility of the feed offered to the experimental bucks was calculated as:

$$\text{Digestibility} = \frac{\text{Feed Intake} - \text{Faecal Output}}{\text{Feed Intake}} \times 100 \tag{1}$$

The digestibility of different nutrients, such as dry matter (DM), crude protein (CP) and crude fiber (CF) were also calculated from the four-day average intake of blue panic.

2.8 Statistical analysis

The data was analyzed by the two-way analysis of the variance (ANOVA) model using R statistics (version 4.3.2) whereas Least Significance Difference (LSD) was used to compare means at α=0.05.

3 Results

3.1 Dry matter productivity of forages under shade

The details of the effect of different levels of shade on the dried weight yield of forage grasses with different legume mixtures have been shown in Table 1. Both the shade level and forage mixture had a significant effect (p¡0.05) on the dried weight yield. The same trend persisted in the calculated dried weight yield as well. Napier had a low dried weight yield as compared to blue panic in all the shade levels. The highest dried weight yield was in the open followed by the low shade, while it was the lowest in the case of heavy shade, respectively (Table 1).

3.2 Chlorophyll content of plants

On July 20, of the first year, the effect of shade on the amount of chlorophyll in non-leguminous forages was found statistically similar ($p > 0.05$) (Table 2). On the other hand, the chlorophyll concentration was lowest in open conditions (0.20 mg/gm of green leaf) and highest in low shadow (0.40 mg/g of green leaf). Conversely, the species difference in the chlorophyll concentration of non-leguminous forages was significant ($p < 0.05$). Accordingly, in low shade (0.64 mg/g of green leaf), Napier had the highest chlorophyll content, while in open settings (0.21 mg/g of green leaf), it was the lowest one (Table 2). The chlorophyll content of non-leguminous forages at harvest on August 25 of the second year of sampling, was not significantly affected by shade ($p > 0.05$) (Table 2). Nonetheless, overall grew in comparison to the prior year. The leaf tissue in an open state had the highest chlorophyll content (1.03 mg/g), whereas in the lowest shade, the value of chlorophyll level was lowest (0.74 mg/g). Chlorophyll concentration varied significantly between species ($p < 0.01$); blue panic had the largest quantity (0.95 mg/g green leaf tissue) in medium shade with the lowest amount (1.18 mg) in heavy shade (Table 2). The interactive effect of shade on the forages in the mixture was non-significant ($p > 0.05$) for the chlorophyll content of the non-leguminous component (Table 2)

Table 1: Dried weight and relative dried weight yield of common tropical forages in a mixture under three levels of Melia tree shade in Chitwan, Nepal.

Shade level	Forage mixture	Dried weight yield (t/ha)			% Relative dried weight (RDW)		
		Year 1	Year 2	[1]cumulative	Year 1	Year 2	[2]cumulative
Heavy							
	a	2.52	1.85	4.37	45.32	20.66	32.99
	b	1.08	1.30	2.38	19.42	14.55	16.99
	c	9.74	15.91	25.65	175.18	177.39	176.29
	d	8.91	16.81	25.65	175.18	177.39	176.29
	Mean	5.56	8.97	14.53	100	100	100
Medium							
	a	3.43	3.03	6.46	49.48	25.61	37.55
	b	1.83	2.51	4.34	26.37	21.26	23.82
	c	11.27	19.92	31.19	162.62	168.56	165.59
	d	11.19	21.84	33.02	161.53	184.78	173.16
	Mean	6.93	11.82	18.75	100	100	100
Low							
	a	4.11	5.09	9.20	32.13	33.34	32.74
	b	3.78	4.35	8.13	29.54	28.5	29.02
	c	22.77	26.10	48.87	178.01	170.91	174.46
	d	20.49	25.54	46.03	160.22	167.26	163.74
	Mean	12.79	15.27	28.06	100	100	100
Open							
	a	25.19	28.29	53.48	76.18	54.15	65.17
	b	27.15	29.83	56.98	82.09	88.73	85.41
	c	41.42	37.90	79.32	125.24	122.72	118.98
	d	38.50	38.46	76.96	116.4	114.4	115.40
	Mean	33.07	33.62	66.69	100	100	100

a=Napier+Desmodium, b= Napier+Glycine, c= Blue panic+Desmodium and d= Blue panic+ Glycine. Statistical analysis: Both Shade levels had a significant effect $p < 0.05$*, Forage mixture had a significant effect, and interactive effects were nonsignificant (not presented in the table).*
[1]*Cumulative dried weight yield (DM) of two consecutive years of harvests*
[2]*Cumulative of relative dried weight yield of two consecutive years of harvests*

3.3 Herbage intake and digestibility

The voluntary intake of blue panic mixed forages under stall-fed condition by the bucks for four days has been presented in Table 3. Accordingly, the daily fresh herbage intake of blue panic was found higher (17.17%) in goats grown under medium shade. Accordingly, dry matter intake was found 7.27% higher per day. In addition, the daily faeces voided by the goat were 3.27% less for shade-grown herbage. The digestibility of fresh green herbage and dry matter was found higher if it were grown under shade. The digestibility of fresh green matter was 2.25%-unit higher than that of open (Table 3). The dry matter digestibility (DMD) of herbage grown under medium shade was 4.6% unit higher than that of herbage grown under full sun. Likewise, the crude protein and crude fibre digestibility of shade-grown herbage was 4.38% and 3.58% higher than that of open respectively (Table 3).

3.4 Relationships of total chlorophyll, dry matter and the proximate fractions

The correlation matrix (Table 4) showed that the total chlorophyll was a significant contributor to the relative dry weight yield of the mixed herbages. Likewise, the major feed fractions. The CP and CF content were also significantly correlated with the total chlorophyll content. The increasing DM content was also observed to significantly correlate with the crude fibre content, with a declined CP content.

Table 2: Average chlorophyll content of grasses grown in a mixture with different legumes at different shade levels of Melia azedarach in Chitwan, Nepal

Shade level	Forage mixture	Year 1	Year 2
Heavy			
	a	0.41	0.88
	b	0.38	0.56
	c	0.223	0.95
	d	0.23	0.95
	Mean	0.31	0.84
Medium			
	a	0.36	0.75
	b	0.37	0.93
	c	0.29	1.18
	d	0.29	1.09
	Mean	0.3275	0.9875
Low			
	a	0.35	0.47
	b	0.64	0.47
	c	0.34	1.01
	d	0.25	1.02
	Mean	0.395	0.7425
Open			
	a	0.21	0.95
	b	0.21	0.95
	c	0.18	1.11
	d	0.18	1.11
	Mean	0.195	1.03
Analysis of variance			
Shade Level		NS	NS
Forage Mixture		p<0.05	P<0.01
Interaction		NS	NS
SEM		0.07	0.18
LSD		0.2	0.56

NS= no significant difference at $p < 0.05$, SEM= standard error of mean,
LSD= least significant difference, a= mixture of Napier+Desmodium, b=Napier+Glycine,c= Blue panic+Desmodium, d=Blue panic+Glycine

Table 3: Digestibility of mixed forage species grown under shade in Chitwan, Nepal

	Intake (kg/day)	% Digestibility		
		DM	CP	CF
Open	1.65	75.86	47.47	76.88
Medium shade	1.77**	80.46***	51.85***	80.46***
% Difference	+6.78	+5.72	+8.45	+4.45
SEM	0.005	0.005	0.008	0.008
T-test	p<0.01	p<0.001	p<0.001	p<0.001

DM= dry matter (dried weight), CP= crude protein, CF= crude fibre

Table 4: Correlation matrix of the dried weight yield and nutritive components with total chlorophyll content in Chitwan, Nepal

Total chlorophyll		RDW	DM	CP	CF
Total chlorophyll	1	0.59*	0.41	0.04*	0.01*
RDM		1	0.51	0.02*	0.37
DM			1	-0.69**	0.77***
CP				1	-0.57

$RDW = Relativedriedweightyield(\%), DM = driedweightyield, CP = \%crudeproteinandCF = \%crudefibrecontent$

4 Discussion

4.1 Herbage productivity under Melia tree shade

The analysis of the relative dried weight of herbage mass revealed that the blue panic combined with either green leaf desmodium or glycine produced a larger dry matter yield which is in agreement with the findings of Barsila & Devkota (2008) under Melia shade and also with other species mixture at varied levels of shade (Angadi et al., 2022). Numerous studies have confirmed the relatively low biomass under shade (Barsila & Devkota, 2008; Lima et al., 2022). The results of the present study could be dealt with other additional physiological data, but other research on pasture species' tolerance to shade has shown that shade affects light interception differently in forages because of variations in vegetative cover development (Evans & Poorter, 2001). For collecting solar irradiance, leaves have a well-documented response to changes in light intensity in their morphology and physiology (Tsubo and Walker, 2004; Franco & Dillenburg, 2007). According to Abraham et al. (2014), this response varies depending on the forage species' tolerance to shade and the amount of shade it is exposed to. This could account for the observed differences in biomass allocation between the legumes combined with Blue Panic and Napier in the current study, or the anticipated advantage of Blue Panic's soil moisture adaptation over that of Napier (Abraham et al., 2014).

4.2 Chlorophyll content, DM digestibility and major chemical constituents under Melia tree shade

Grass and legume intercrops in terms of potential species are chosen primarily for their best combined effect to produce DM and also for their natural adaptation to tolerate shade. The efficiency with which a crop converts solar energy into dry matter (DM) determines crop growth and yield in the absence of resource constraints (Allard et al., 1991; Sinclair & Muchow, 1999). Although forages in shade have a unique morpho-physiological response to decreased sunlight, little is known about their ability to adjust to shading levels brought on by intercropping systems, particularly with Melia tree shade. The shadow level in the present study had no discernible effect on the total amount of chlorophyll. This might indicate that the chosen species can withstand some shade (see reviews by Wong, 1991; Eriksen & Whitney, 1982). Nevertheless, Blue panic exhibited a higher chlorophyll content than Napier. Findings of majority of the studies have demonstrated that the total chlorophyll content in under-storey species increased linearly with increasing shade levels (da Cruz et al., 2021; Umesh et al., 2023). Despite this, the results of this study support the findings of Angadi et al., (2022), as the authors reported that shade lowers the amount of chlorophyll. Except for the time of the harvest, other factors that may affect the pigment concentration include the year that under-storey pastures developed, the persistence of plantation trees and their standing per hectare, and competition between pasture species. The latter is less well-known in the context of open plots' higher chlorophyll content than that of shaded plots. Given the current study's environment, which included a significant rise in the total chlorophyll content, a more thorough understanding of the local pasture elements would be possible with the use of data analysis across the year effect (Table 2).

The quantity and digestibility of forages grown in shade by domestic animals are restricted in different ways. A functional component of plant tissue, digestibility (on a DM basis) not only indicates the nutritional content but also has a major impact on how much feed ruminants take (Lin et al., 2001; Stuth et al., 2003). It is generally accepted that ageing is the cause of the decline in digestibility over time (Fick et al., 1994; Borreani et al., 2003). This is rendered feasible by the forage fraction's quick accumulation of lignin under open settings, which is adversely related to the ruminants' ability to digest it (Jung, 1989). Goats seem to choose young leaves over stems, and stems typically contain more lignin, therefore their preference for herbage may also be related to the digestibility findings (Akin, 1989). The CP content normally increases (de Oliveira et al., 2020) and the CF decreases when forage plants become more mature (Barsila & Devkota, 2008).

The chemical makeup of leaf tissues, particularly the contents of the cell wall, is similarly impacted by decreased light. According to Blair et al. (1983), plants cultivated forages in shade have higher levels of crude protein (CP) and nitrogen than plants grown in full light. Shade also affects the amount and digestibility of acid detergent fibre (ADF) and neutral detergent fibre (NDF) (Kaligis & Mamonto, 1991; Norton et al., 1991). The slower rate of lignification of the stem and leaves, however, may have contributed to the increased feed intake and digestibility of the shade as found in this study, even though the digestibility feeding trial was carried out later in the growth cycle. There have been reports of a significant increase in the dry matter intake of cattle grazing on shade-grown fodder (de Oliveira et al., 2020). Increased digestion of DM from both C_3 and C_4 grass species cultivated in shade was also noted by Kephart and Buxton (1993). According to Akin (1989) and Gebrehiwot et al. (1996), leaves are more digestible than stems because they contain more protein and less fibre. A 60%–70% of the fibre in the grass and 40%–50% of the fibre in legumes may be digested by ruminants (Buxton et al., 1995). The digestibility (of crude fibre or DM) not only indicates the nutritional content but also has a major impact on how much feed ruminants take (Lin et al., 2001; Stuth et al., 2003).

5 Conclusion

The findings of this study demonstrated that, as a physiological approach for herbages to tolerate tree shade, the shading impact decreased DM productivity and chlorophyll content, which may be used as a tool to boost net photosynthesis under shade. It was evident that the total dry matter, crude protein (CP) and crude fibre (CF) digestibility content is equally associated with the total chlorophyll content. However, a decrease in CP was observed when DM increased because of an increase in CF content in the forages over time under tree shade. Nevertheless, a larger-scale study with a sizable number of ruminants is required to evaluate the under-storey forage intake and digestibility more precisely.

Conflict of Interest:

Author declares that there is no conflict of Interest.

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