Experiment Protocol for Assessing Biomass Production of *Calliandra calothyrsus, Vernonia amygdalina* and *Morus alba* Forage Species Under Different Management Practices

Implemented by the Agroforestry Program, NaFORRI





Thematic Area: Technological options responding to demands and markets

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Abstract

This study aims at improving the forage production and utilization for increased milk productivity of Uganda's smallholder dairy systems. The study focuses on three candidate forage species, namely; *Calliandra calothyrsus, Vernonia amygdalina* and *Morus alba.* The choice of these species was based on findings of a baseline survey conducted among smallholder dairy farmers in Masaka and Mbale districts. The study will (i) assess the effect of varying cutting heights on the amount of biomass produced; (ii) evaluate the effect of spacing on biomass/foliage production and (iii) determine harvesting intervals for optimal foliage production. Complete Randomised Block Design will be used to establish an on-station Type 1 (Researcher designed, researcher managed) forage trial at the National Forestry Resources Research Institute (NaFORRI) research station at Kifu, Mukono District in central Uganda. The trial will have three blocks, each representing a different species. Each block will have three plots of different spacing i.e. 50cmx50cm (control), 75cmx75cm and 100cmx100cm. The plots of spacing of 75cmx75cm and 100cmx100cm will each have sub-plots which will be subjected to varying cutting heights at two levels of 25cm and 100cm, and varying cutting frequency. The spacing, 50cmx50cm (Control), will be evaluated at only one level of cutting height of 50cm. This will give 5 treatments for each block (50 X 50 X 50, 75 X 75 X 25, 100 X 100 X 25, 75 X 75 X 100 and 100 X 100 X 100). Each of these treatments will be replicated thrice yielding a total of 15 subplots in each block. The data to be collected from the trial will include Seedling survival (%), Plant height (m) and Biomass production (kg). Collected data will be entered in Microsoft Excel spreadsheet and later exported to Minitab statistical software and Genstat for analysis of variance (ANOVA) in biomass produced under different treatments (cutting height, cutting frequency and spacing). The AREPMEASURES procedure (Genstat) will be used to analyse changes in treatment effect over time to generate information not only about the effects of cutting frequency, cutting height and spacing, and interactions between them, but also how these may change over time. The information obtained from this study will be disseminated to small holder dairy farmers' networks, in workshops and published for wider outreach.

1 Title

Assessment of Biomass Production under Different Forage Management Practices

2 Location of Trial experiment

The experiment is located at the National Forestry Resources Research Institute (NaFORRI) research station at Kifu (0° 27'16.19" N and 32° 45'43.24"E) in Mukono District in central Uganda.

3 Investigators

The Principal Investigator (PI) of the study is Joel Buyinza, from NaFORRI and the table below shows the list of the research team.

Table 1: List of the other forage experiment research team members

No.	Name	Organization	Contact / Phone number*
1.	Kwaga Phiona	NaFORRI	kwagaphifi@yahoo.com
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11.	Opolot Vincent	NaFORRI	vopolot@yahoo.com
12.	Okullo Paul	NaFORRI	paul.okullo@gmail.com
13.	Bazanya Grace	MuZARDI	
14.	Kugonza Jane	ICRAF/ EADD-Project	kugonzajane@yahoo.com

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4 Background and justification

Smallholder dairy development can be a catalyst to agriculture-led economic development. In Uganda, however, most smallholder farmers realise low milk production due to poor nutrient composition of the fodder used, which mainly comprises natural pastures, browse and crop residues. Seasonal shortage of such feeds, especially during dry spells, further impedes the growth of the dairy sector in Uganda (DSIP,

2010).Though rich in fibre roughage, these feeds are deficient in proteins and other vital nutrients, which compromises the quality and quantity of milk produced (Gerrits, 1999). In addition, protein-deficient animal diets are not considered climate-smart since they result in high emission of methane as a by-product of digestion (Mugerwa *et al.*, 2013).

Planted grasses, forage legumes and improved fodder trees and shrubs on the other hand, can enhance forage availability and subsequently milk production. Forage legumes, shrubs and trees provide protein that is required for milk production and supplement animal diet, thus reducing methane emissions. The relatively high biomass yield renders planted grasses and forage legumes reliable sources of vital nutrients for dairy cattle. Forage trees and shrubs can also withstand extended periods of water stress as their deep root systems enable tapping of water and nutrients from deep into the soil profile (Kabirizi, 2004). In spite of these attributes, use of improved forage in Uganda's livestock systems is limited, and animal feed supplies remain largely dependent on wildly existing stands, which limits livestock productivity (DSIP, 2010)

4.1 Summary of Literature

According to Cheema *et al.,* (2011), provision of forage of adequate nutritional quality is fundamental in ensuring increased livestock production in the developing countries. Tree fodders are richer in protein and mineral contents than natural grasses and thus can be supplemented to low quality grasses (Aganga *et al.,* 2003). However, most small holder dairy farmers in Uganda graze exotics and crossbred cattle in the wet season and introduce stall feeding, with cut fodder, in the dry season (EADD, 2009). While a small number of households keeping dairy cattle make effort to plant improved pastures (Ekou, 2014), consequently, very few farms with improved dairy cattle produce enough fodder to meet the needs of their herds throughout the year (Balikowa, 2011). Saddul *et al.,* (2004) evaluated the forage potential of mulberry (*Morus alba*) under Malaysian conditions, and recommended fresh mulberry whole plant as a valuable supplemental source of nutrients to poor quality basal diets. A similar study by Shayo (1997) assessed the uses, yield and nutritive value of *Morus alba* trees for ruminants in the semi-arid

areas of central Tanzania. However, no study has been conducted to establish the appropriate management option for optimal forage production in Uganda.

4.2 Past research related to the project

In 2013, the agroforestry program at NaFORRI conducted a baseline survey to assess small holder dairy farmers' knowledge on forage production and utilization in the Lake Victoria crescent and Eastern Highlands Agro Ecological Zones (AEZs) of Uganda. Semi-structured questionnaires were administered to a total of 208 small holder dairy farmers in Masaka (100) and Mbale (108) districts. The research results reveal *Pennisetum purpureum*, *Calliandra calothyrsus, Musa paradisiacal,* and *Leucaena leucocephala* as the most prominent forage species used for feeding livestock. Also, forage production among small holder dairy farmers is significantly ($P < 0.05$) influenced by location, household size, land ownership, forage source and livestock feeding system. Although forage growing is not taken as a tradition among small holder dairy farmers, the main source of forage is on farm (67.2%). On the contrary, apart from chopping before feeding the animals, minimal processing is done to forage. These results formed a basis for establishment of an on-station forage management trial at Kifu in a bid to address the current forage production and utilization challenges among small holder dairy farmers in Uganda.

4.3 Links to other parts of the same project

This forage management trial directly links to five (6) other components of the forage project being implemented by the Agroforestry program at NaFORRI, namely;

1. Assessment of forage security situation of smallholder dairy farmers
2. Nutritive laboratory analysis of leaf samples (proximate analysis).
3. Confirmatory on-station experiments of candidate forage species using lactating cows.
4. Assessment of smallholder dairy farmers' networks.
5. Knowledge sharing through information packs, field visits and open days.
6. Forage seed multiplication and distribution.

7. On-farm scenarios on forage management, placement, attitude, preference and labour requirements

4.4 Hypotheses

H_{01}: Cutting height for Forage species does not influence the amount of biomass produced

H_{02}: The spacing of a particular forage species has no influence on biomass/foliage production

H_{03}: Forage harvesting intervals has no effect on foliage production

H_{04}: Use of cuttings of the three forage species has no influence on seedling survival

H_{05}: Cutting height has no influence on number of coppices on the forage species stump

Other hypotheses include;

Reducing cutting height to 25cm but increasing time between cutting regimes will give higher forage yields over a long time.

Increasing cutting height to 100cm but decreasing time between cutting regimes will give higher forage yields over a long time.

4.5 Potential Impacts

1. Enhanced milk production through promotion of appropriate forage management practises.
2. Reduced forage scarcity among dairy farmers, especially during the dry seasons.

5 Objectives

The objectives of the study are threefold i.e.

1) To assess the effect of varying cutting heights on the amount of biomass produced
2) To evaluate the effect of spacing on biomass/foliage production
3) To determine harvesting intervals for optimal foliage production

6 Methods

6.1 Trial type

The trial type is a Type 1 (Researcher designed, researcher managed)

6.2 Study units

The study units for the trial will be individual plants of *Calliandra calothyrsus, Vernonia amygdalina* and *Morus alba* forage species. The biomass of each forage species planted at different spacing will be assessed at varying cutting heights and intervals in 15 trial plots per species, for a period of 2 years. The study units will be compared to identify the appropriate scenario for optimal forage production in Uganda.

6.3 Location

Mukono district, Kifu (on-station), at a GPS location (0° 27'16.19" N and 32° 45'43.24"E) and elevation of 1129m above sea level.

6.4 Other study units

Other study units for this study will include (1) the forage leaves which will be used to carry out a nutritive laboratory analysis of leaf samples, (2) lactating cows for confirmatory on-station experiments on milk production when fed on each of the 3 forage species and (3) Small holder dairy farmers will be studied to assess smallholder dairy farmers' networks, knowledge sharing through information packs, field visits and open days. These units will be studied at different stages of the forage management trial.

6.5 Treatments_ The primary options being compared

The trial will involve three forage tree species; *Calliandra calothyrsus, Morus alba, Vernonia amygdalina.* The choice of these species was based on findings of a baseline survey conducted among smallholder dairy farmers in Masaka and Mbale districts.

The trial will have three blocks, each representing a different species. Each block will have three plots of different spacing i.e. 50cmx50cm (control), 75cmx75cm and 100cmx100cm. The plots of spacing of 75cmx75cm and 100cmx100cm will each have

sub-plots which will be subjected to varying cutting heights at two levels of 25cm and 100cm, and varying cutting frequency. The spacing, 50cmx50cm (Control), will be evaluated at only one level of cutting height of 50cm. See illustration below:

Block

Plots	Spacing (cm)	Cutting Height (cm)		
		25	50	100
1	50 X 50 (Control)		50 X 50 X 50	
2	75 X 75	75 X 75 X 25		75 X 75 X 100
3	100 X 100	100 X 100 X 25		100 X 100 X 100

This will give 5 treatments for each block (50 X 50 X 50, 75 X 75 X 25, 100 X 100 X 25, 75 X 75 X 100 and 100 X 100 X 100). Each of these treatments will be replicated thrice yielding a total of 15 subplots in each block. The cutting height and spacing at 30cm - 50cm is the known/conventional standard used (Saddul *et al.,* 2004; Shayo, 1997). Thus 50cm cutting height and 50cmx50cm spacing are used as control. The other spacing and cutting heights were chosen arbitrary to determine their effect on optimal biomass production

6.6 Layout

The trial will have three blocks, each representing a different species. Each block will have three plots of different spacing i.e. 50cmx50cm, 75cmx75cm and 100cmx100cm replicated thrice adding up to a total of 15 subplots in each block. Within the three plots, there will be three sub plots subjected to varying cutting heights of 50cm, 25cm and 100cm, as illustrated below.

C=*Calliandra calothyrsus* **M**=*Morus alba* **V**=*Vernonia amygdalina*

Spacing

1 = 75cmx75cm at 25cm (Area = 8.4375m^2)

2 = 75cmx75cm at 100cm

3 = 100cmx100cm at 25cm (Area = 15m^2)

4 = 100cmx100cm at 100cm

5 = 50cmx50cm at 50cm (Area = 3.75m^2)

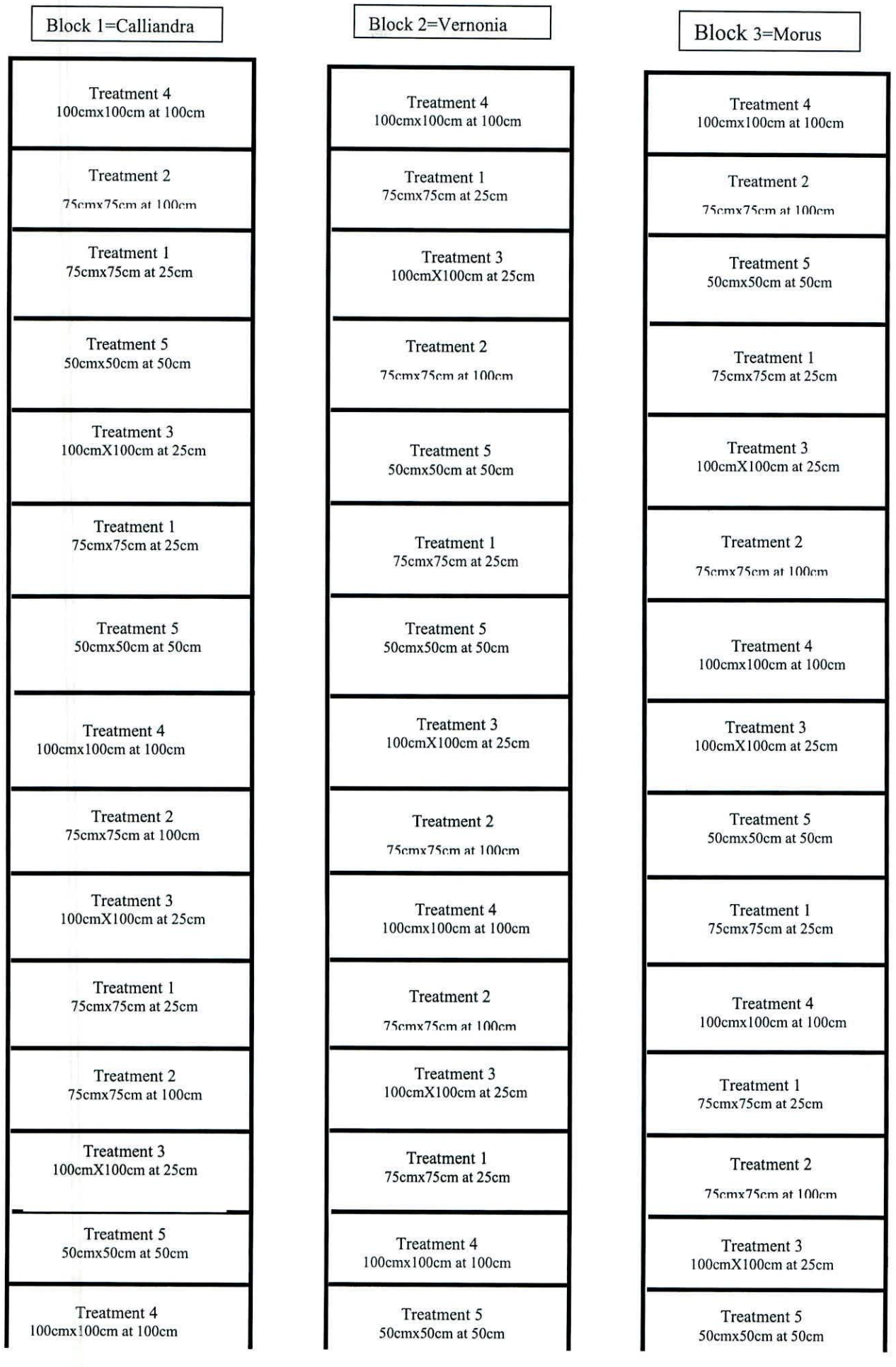
Total area = (8.4375x3) + (15x3)+3.75

=25.4+45+3.75

=74.0625m^2

Note:

1. Separation between blocks = 4m
2. Separation between plots = 0.5cm


Block 1=Calliandra
Treatment 4
100cmx100cm at 100cm
Treatment 2
75cmx75cm at 100cm
Treatment 1
75cmx75cm at 25cm
Treatment 5
50cmx50cm at 50cm
Treatment 3
100cmX100cm at 25cm
Treatment 1
75cmx75cm at 25cm
Treatment 5
50cmx50cm at 50cm
Treatment 4
100cmx100cm at 100cm
Treatment 2
75cmx75cm at 100cm
Treatment 3
100cmX100cm at 25cm
Treatment 1
75cmx75cm at 25cm
Treatment 2
75cmx75cm at 100cm
Treatment 3
100cmX100cm at 25cm
Treatment 5
50cmx50cm at 50cm
Treatment 4
100cmx100cm at 100cm
Block 2=Vernonia
Treatment 4
100cmx100cm at 100cm
Treatment 1
75cmx75cm at 25cm
Treatment 3
100cmX100cm at 25cm
Treatment 2
75cmx75cm at 100cm
Treatment 5
50cmx50cm at 50cm
Treatment 1
75cmx75cm at 25cm
Treatment 5
50cmx50cm at 50cm
Treatment 3
100cmX100cm at 25cm
Treatment 2
75cmx75cm at 100cm
Treatment 4
100cmx100cm at 100cm
Treatment 2
75cmx75cm at 100cm
Treatment 3
100cmX100cm at 25cm
Treatment 1
75cmx75cm at 25cm
Treatment 4
100cmx100cm at 100cm
Treatment 5
50cmx50cm at 50cm
Block 3=Morus
Treatment 4
100cmx100cm at 100cm
Treatment 2
75cmx75cm at 100cm
Treatment 5
50cmx50cm at 50cm
Treatment 1
75cmx75cm at 25cm
Treatment 3
100cmX100cm at 25cm
Treatment 2
75cmx75cm at 100cm
Treatment 4
100cmx100cm at 100cm
Treatment 3
100cmX100cm at 25cm
Treatment 5
50cmx50cm at 50cm
Treatment 1
75cmx75cm at 25cm
Treatment 4
100cmx100cm at 100cm
Treatment 1
75cmx75cm at 25cm
Treatment 2
75cmx75cm at 100cm
Treatment 3
100cmX100cm at 25cm
Treatment 5
50cmx50cm at 50cm

6.7 Inputs

a) Inputs for trial establishment

Seedlings/cuttings/wildlings	Strings
Hoes	Tape measure
Tractor	Pangas and slashers

b) Inputs for trial management

Weighing scale	Watering cans
Hose pipes	Hoes, pangas, slashers
Bow saws	Secateurs
Diameter tapes	Tape measures
Gunny bags/Sacks	Ribbons
Stationery (markers, note books, pens, pencils, clip boards)	Permanent markers

6.8 Management

The Principle investigator will be responsible for overseeing the implementation of this study. The research team comprises of technicians and scientists with forestry, agroforestry, biometrics, environment and livestock academic background, each with specific tasks (*See section 7.2 of this protocol*). The study activities have also been scheduled to ensure timely and effective execution of the study.

6.9 Experimental and Non - experimental variables

a) Experimental variables

- *Dependent variables*
    - Plant height
    - Biomass yield
    - Percentage seedling survival

- *Independent variables*
    - Coppicing height

- Frequency of cutting or harvesting intervals
- Spacing

b) Non- experimental variables

The key non- experimental variable in this study are the two wet and dry seasons experienced in the study area. Mukono normally experiences wet seasons (March to May and August to November) and dry seasons (May-July and December to April) with occasional overlaps of the two seasons. It has been assumed that these seasons may have an influence of forage biomass.

6.10 Data collection

The following data will be collected from the trial;

a) Seedling survival (% seedling survival)
b) Plant height(Average height)
c) Biomass production(subsample fresh weight, subsample dry weight, Total fresh weight and dry matter yield)

a) Seedling Survival (SS) assessment

The procedure for seedling survival assessment is as follows;

1. Count seedlings of each forage species at 2 (SM_2) and 5 (SM_5) months after transplanting or planting in the trial plots.
2. Calculate percentage seedling survival for each forage species during the establishment year as:

$$\text{Seedling Survival (\%)} = \frac{(SM_2 - SM_5)}{SM_5} 100$$

Where; SM_2 =Number of seedlings at 2 months after establishment

SM_5 = Number of seedlings at 5 months after establishment

b) Plant height

Height of all the individual plants in each replicate (plot) will be measured with a height rod metre stick every eight (8) weeks and the average plant height for each replicate/ plot recorded

c) Biomass production at varying cutting heights

1. Take the first foliage harvest (at the different cutting heights i.e 25cm, 50cm and 100cm) when plants are at the age indicated in Table 1. The variation in forage age at initial cutting is due to the differences in growth rates of the selected forage species. Subsequent harvest periods will be based on forage species, species phenology and the climatic changes such as dry and wet seasons (*See Table 1*).
2. At the first harvest (*see Table 1*), cut all the plants in each plot at the already pre determined cutting height (25cm, 50cm and 100cm).
3. Divide each plot into four (4) sub plots (Parcels), each representing one of the 4 growth stages or harvesting periods. The following harvesting periods (*Table 1*) will then be considered for the different forage species.

Table 1: Harvesting period/ growth stages across the different cutting heights

Forage species	**Date of establishment (D/MM/YYYY)**	**Age at initial cutting (Weeks)**	**Harvesting periods across the different forage cutting heights (Weeks)**			
Calliandra calothyrsus	11/04/2014	28	3	5	7	9
Morus alba	11/04/2014	33	3	6	9	12
Vernonia amygdalina	23/06/2014	24	4	8	12	16

4. Since each plot has 24 plants, each sub plot (parcel) will in this case have 6 plants for each growth stage or harvesting period across all the 3 replicates for each treatment.
5. At each of the subsequent harvesting period, cut all the plants (approximately 6 plants) in each sub plot/ parcel.

6. Sort materials divided into leafy (leaves and green parts of the shoot) and woody biomass (lignified material)
7. Fresh weigh the sorted material in the field, obtain the subsamples and oven dry at 80°C to constant weight for the determination of Dry Matter (DM).
8. Collect 2 other sub samples from the sorted material and use one for air drying and another for sun drying until constant weight has been obtained.

The proportions of the wet and dry weight of the leaf and stem samples will be recorded as percent dry matter (% DM) and will be used to calculate DM yield (kg) of leaf and stem per tree and consequently DM yield per hectare as follows;

DM yield/ tree = DM% x weight of fresh material (leaf and stem)/ tree (1)

The biomass of each of the components (leaf and stem) can also be computed independently as a product of the fresh weight of each component and the ration of the dry and fresh weight of the sub sample from that component (Brown, 1997). This can be represented as:

$$\text{Biomass} = \text{Fresh weight of forage component} \times \frac{\text{Dry weight of sub sample}}{\text{Fresh weight of sub sample}}$$

DM yield/ha =DM yield/tree x number of trees/ha (2)

DM yield/acre =DM yield/tree x number of trees/acre (3)

6.11 Data management

Data collection will be conducted by the institute technicians under Agro forestry programme. All data collected shall be entered into an Excel spread sheet and hard copies immediately filed. A copy of the data base will frequently be stored on an external back-up device for safety.

6.12 Data analysis, reporting and feedback

The collated data in MicroSoft Excel spreadsheet shall later be exported to Minitab statistical software for the exploratory data analysis (EDA) and for analysis of the treatment means. Using Genstat Statistical Software, Repeated Measures ANOVA Methods will be performed to assess the different treatment effects of cutting height, cutting frequency and spacing, over time, on the biomass produced.

Analysis of variance (ANOVA) will be used to analyse variation in biomass produced under different treatments (cutting height, cutting frequency and spacing). The AREPMEASURES procedure (Genstat) will be used to analyse changes in treatment effect over time. This carries out an ANOVA on the repeated measurements and checks the assumptions required (i.e. symmetry of the variance-covariance matrix). To adjust for partial failure of the assumptions it calculates a correction factor (Greenhouse-Geisser) for the degrees of freedom in the *plot.spacing.cutting hieght.cutting frequency.time* stratum. The correction increases the rigour of the F-tests in this stratum.

The analysis will also examine the data in terms of pairs of harvests. For example comparing each 3-weekly harvest with the 9-weekly harvests for *Morus alba* carried out in the same period. For biomass, the production from the 3-weekly cuts will be summed to compare with the biomass from the 9-weekly cut (because cumulative totals are of interest). For all the other variables, mean values from each pair of different weekly cuts will also be compared. This analysis will provide information not only about the effects of cutting frequency, cutting height and spacing, and interactions between them, but also how these may change over time.

The information obtained from this study will be disseminated to small holder dairy farmers' networks, in workshops and published for wider outreach.

6.13 Duration

Start date: April 2014 End date: April 2016 (24 months)

7 Implementation plan

7.1 Outputs

The trial experiment is expected to provide the following outputs;

- Effect of varying cutting heights on the amount of biomass produced
- Effect of spacing on biomass/foliage production
- Appropriate harvesting intervals for optimal foliage production

7.2 List of tasks

The key tasks to be undertaken during implementation of this study include the following;

1. Protocol development
2. Updating the protocol
3. Procuring planting materials
4. Preparation of trial sites
5. Planting of forage species in the trial plots
6. Beating up
7. Forage trial maintenance (e.g weeding)
8. Designing data collection tools
9. Cutting back and harvesting
10. Data collection and entry
11. Data analysis
12. Project supervision
13. Monitoring and evaluation
14. Reporting and reviewing
15. Feedback
16. Data management
17. Field liaison during on-farm experimentation
18. Technical backstopping on indigenous forage formulations

7.3 Timing

Table 2: A schedule of the forage trial tasks

Task	2014											2015												2016			
	F	M	A	M	J	J	A	S	O	N	D	J	F	M	A	M	J	J	A	S	O	N	D	J	F	M	A
Protocol development																											
Updating the protocol																											
Procuring planting materials																											
Preparation of trial sites																											
Planting of forage species in trial plots																											
Beating up																											
Forage trial maintenance																											
Designing data collection tools																											
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Monitoring and evaluation																											
Reporting and reviewing																											
Feedback																											
Data management																											
Field liaison during on-farm experimentation																											
Technical backstopping on indigenous forage formulations																											

7.4 List of partners

1. National Livestock Resources Research Institute (NaLIRRI)
2. National Agricultural Research Organization Secretariat (NAROSEC)
3. MuZARDI
4. World Agroforestry Centre (ICRAF)
5. East African Dairy Development project (EADD)

7.5 Roles of all partners and team members

Researcher name	Responsibility	Institution/ organisation
Jude Sekatuba	Project leader, overall assessment and reporting, design data collection tools, data collection and analysis	NaFORRI
Joel Buyinza	Updating protocol, Project supervision and data analysis	NaFORRI
Hillary Agaba	Project supervision, experimental design	NaFORRI
Gerald Ongodia	Data collection, analysis and reporting	NaFORRI
Kenneth Eryau	Data collection and management	NaFORRI
Phiona Kwaga	Design data collection tools, Data collection and management, trial maintainance	NaFORRI
Richard Akellem	Data collection and management	NaFORRI
Scovia Mudondo	Data collection and management	NaFORRI
Bazanya Grace	Dairy farmers' institutional collaboration	MuZARDI
Jolly Kabirizi	Technical backstopping on indigenous forage formulations	NaLIRRI
Jane Kugonza	Field liaison and on-farm experimentation	EADD/ ICRAF
Juma Ndhokero	Technical backstopping and data analysis	NAROSEC
Paul Okullo	Technical backstopping and experimental design	NaFORRI