Experimental protocol: ‘Genetic variation in in vivo digestibility and voluntary intake by sheep in five Accessions of Napier

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Experiment Title:	Genetic variation in protein, fiber and in vitro digestibility effects on in vivo digestibility and voluntary intake by sheep in 5 Accessions of Napier grass
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Justification

In East Africa, a regular supply of milk provides nutritional security for many rural poor (Nicholson *et al*., 2003). Dairy also generates more regular household income and jobs than any other enterprise. In Kenya, resource poor smallholder dairy farmers produce more than 80% of the marketed milk (Peeler and Omore, 1997). In Central Kenya, 73% of agricultural households have dairy cattle (Staal et al., 2001) and in most districts in the area, households ranked dairy as the most important source of income (75% in Nairobi district). Livestock feeds to support dairy remain an issue and the low milk yields, high calf mortality and long calving intervals seen in many smallholder enterprises are predominantly due to insufficient good-quality feed.

Most dairy farmers practice a cut-and-carry zero grazing system. Currently, Napier grass (*Pennisetum purpureum*) is the most important forage crop in these systems in the

Central Kenya Highlands (Staal *et al.*, 1997; Orodho, 2006) and has been shown to constitute between 40 to 80% of the forage for the smallholder dairy farms. In Kenya alone, more than 0.3 million smallholder dairy producers (53%) rely on Napier grass as a major source of feed. The demand for Napier grass in Kenya is so high, that landless farmers plant along the highway verges and free land to cut and sell to animal owners. Demand for Napier has also been increasing rapidly over the last five years in Ethiopia with over 200,000 cuttings of best bet Napier accessions distributed from ILRI in 2003 and 1.4 million cuttings in 2004 (Hanson and Peters, 2003).

ILRI holds a germplasm collection of 60 accessions of Napier grass in trust under the auspices of FAO as a global public good as part of the forage genebank. Recognizing the importance of Napier for smallholder dairy systems, ILRI has been assessing the diversity it its collection since 1995.

In collaboration with national partners in the region, ten promising accessions of Napier were screened as part of a multi-site evaluation trial under the African Feed Resources Network in 1995 (Ndikumana and Kamidi, unpublished) to identify accessions and/or hybrids (*Pennisetum purpureum x Pennisetum typhoides*) of Napier grass that performed well in a variety of sub-Saharan African regions for use in the development of Napier based feeding packages for smallholder dairy farmers. Accessions for the trial were selected based on results of earlier work from ICRISAT in southern Africa. The five Napier accessions included in the study were accessions 15746, 16786, 16789, 16797 and 16798; and the four *Pennisetum* hybrids were accessions 16834, 16835, 16837, and 16838. These nine accessions were evaluated at ten sites in nine different sub-Saharan countries: Bouake, Cote d'Ivoire; Dschang, Cameroon; Holetta, Ethiopia; Kumasi, Ghana; Kakamega, Kenya; Kiajansoa, Madagascar; Makurdi, Nigeria; Morogoro and Tanga, Tanzania; and Kabanyolo, Uganda. Each accession was compared, along with a number of local varieties at each site, to find which ones performed best across the study sites (Ndikumana, 1995). Fifty three of the accessions were also planted in 1.5 x 2.7m plots in 3 replicates in a randomized block design at the ILRI Debre Zeit station for agro-morphological characterization.

Studies to assess the morphological and genetic variation in the collection and group similar accessions indicated a large variation, in yield and general morphology, although some accessions were so similar that they are probably clones (van de Wouw *et al*., 1999). Genetic diversity of the Napier grass collection was found to be fairly high and thus the collection probably represents a wide genetic base for this species (Lowe *et al*., 2003). Distinct groups of accessions were identified. Three dwarf accessions that might be useful for grazing systems, with an average height of 1.2 m, formed a distinct group. Although they had a lower yield, this was partly compensated by improved quality. A group of tall high yielding accessions, with one accession reaching a height of 3.4 meter 10 weeks after cutting, was distinguished. A large variation in hairiness was also observed. The accessions that are hybrids with Pearl Millet could not always be distinguished morphologically from true Napier grass, although some of them flowered earlier, had thinner stems and shorter leaves. However, the hybrids had a distinct genetic profile from the other accessions and could be easily recognized using DNA markers.

Results from the multi-locational trial and the agro-morphological characterization were used to identify five superior clones, four Napier and hybrid accession, for use in smallholder farming systems. All these promising accessions had a high percentage of leaves in the biomass harvested. The four Napier grass accession all belong to the same high yielding group of late flowering, robust plants. The hybrid accession had thinner stems and is flowers earlier than the other promising accessions.

More recent studies in 2002-4 using 56 accessions of Napier indicated considerable variation in nutritional quality traits (Solomon Teka, 2004). Diversity in nutritional traits can also be exploited to select superior accessions for use in farming systems. However, testing large numbers of accessions using standard laboratory chemistry is both time consuming and expensive.

The principal use of Napier grass is as forage for dairy animals and studies to assess the yield and nutritional values from a range of maturity types, management regimes and environments have been carried out (Zewdu *et al*., 2002a, 2002b, 2003). On average, Napier grown in Ethiopia yields around 40 t/ha fresh, with about 15 percent DM (ILRI, 2001). With such a low DM percentage Napier grass does have a lower feed efficiency rate; however it is a very palatable feed in the leafy stage (van de Wouw *et al.*, 1999) and is readily accepted by livestock. The ranking of accessions based on agronomic measurements of dry matter yield, protein concentration and in vitro estimates of digestibility do not necessarily correlate with the performance achieved by animals when fed these accessions. Animal feeding experiments need to be undertaken to verify the comparative performances of the different accessions when fed to animals. Sheep have frequently been used as a model in nutritional analysis providing the opportunity for using growing sheep to assess target quality traits, such as intake and digestibility, and then apply the findings to dairy cows.

Objective

- To assess five accessions of Napier grass for voluntary intake, in vivo digestibility and weight changes by sheep.

Null hypothesis

- Five accessions of Napier with superior agronomic/adaptive traits do not differ in terms of in vivo digestibility, voluntary intake and growth rates by sheep

Expected Output

- Variability in terms in vivo digestibility and intake assessed among 5 accessions with superior agronomic traits

Materials and Methods

Accessions

In this study the nutritive value of five accessions of Napier grass from the ILRI core germplasm will be determined in nutrition experiments with sheep. The five accessions include:

1. 14984
2. 16786
3. 16803
4. 16835
5. 16837

These accessions were selected on the basis of agronomic characteristics and broad adaptability from the regional trials (Ndikumana, 1995) and yield trials in Ethiopia (Van de Wouw *et al*., 1999). Each accession was grown in non-replicated plots of approximately 400 m^2 each under irrigation with application of fertiliser after each cut. The accessions were originally planted in 2002 and had been harvested twice a year for stem cuttings for propagation material until the start of the trial.

In September 2004 the five plots were cut at approximately 15 cm above ground level and divided into 8 subplots of 50 m^2. After 6-13 weeks, the 8 subplots were harvested again, cutting the grass (one sub-plot at a time) either after 6, 7, 8, 9, 10, 11, 12 or 13 weeks of re-growth. This cutting calendar was scheduled to have one subplot harvested every week in way that after the harvesting cycle would be completed every subplot could be harvested every 8 weeks. This cutting interval has proved to be adequate under good management conditions in Kenya and Ethiopia (Booman, 1997). The harvest schedule also provided information on growth curves for the 5 accessions

After harvesting the subplot at 13 weeks of re-growth, each sub-plot was divided into 7 sub-sub plots of approximately 7 $m^{2.}$ Each sub-sub plot was then harvested daily in a way that one sub-plot would be harvested totally in one week. Then the 7 seven sub-sub plots of the second subplot would be harvested in the second week, the third subplot in the third week, and so on until the eighth subplot would be harvested in the eighth week. After one full cycle of harvesting the whole plot the eight subplots the process would be repeated in a way that the material harvested daily will always be from 8-week old re-growth. The nutritional value of this material will be evaluated in vivo using sheep. After cutting at 15cm above the ground, the leaf and stem will be chopped by hand using a machete to pieces approximately 3cm long. The chopped material will be spread in thin layers and partially dried in the shade for 2 days before feeding as wilted green material.

Animals

Forty sheep divided in five groups (one group per accession) with approximately eight sheep per accession (exact number of animals in each group will vary depending on the amount of grass harvested) will be used in the study.
The groups will be constituted of young male lambs weighing 18-20 kg and will be randomly allotted to the 5 accessions. Each group will be fed during 12 weeks. The sheep will be purchased in local markets, quarantined for about 6 weeks, treated for internal and external parasites, vaccinated against common diseases and adapted to consuming wilted

green Napier grass before starting the experimental phase of each group. Initial weight will be used as criteria for allotting the animals to experimental groups in a way that the interval and mean of initial weight will be similar across groups. In each group, six sheep will be slaughtered at the beginning of the study to determine initial carcass characteristics.

Feeding of animals

During the study, the sheep will be individually fed. They will be given wilted green Napier grass *ad libitum* adjusting the level of offer weekly to allow for a refusal of approximately 15 % of the fodder offered. The sheep will receive no supplement other than a mineral mix. Refusals of grass will be collected and weighed daily and samples for chemical analysis taken weekly.

Monitoring of experiment

<u>Duration and weighing of animals</u>. Each group will be fed for 12 weeks. During this time the animals will be weighed for three consecutive days at the beginning, every 2 weeks afterwards and at the end of the feeding period.

<u>Slaughtering of animals</u>. All sheep will be slaughtered after 12 weeks of feeding. Carcass weight (hot and cold), dressing percentage, carcass gain, offals and mesenteric fat will be determined for each animal.

<u>Animal care</u>. Animal care and use will follow standards accepted internationally and will meet guidelines and procedures established by ILRI's Institute Animal Care and Use Committee (IACUC).

<u>Production per hectare</u>. Production of meat per hectare will be estimated as:
mean animal cold carcass weight x (animals fed per hectare) .

animals fed per hectare will be calculated as:
dry matter yield per hectare / (mean daily dry matter intake per animal x number of days)

<u>Estimates of chemical analysis</u>. All samples of grass offered will be analysed for DM, OM, CP, NDF, ADF and IVOMD

Response variables

In vivo digestibility, voluntary intake of dry matter and digestible organic matter, average daily gain, carcass weight, meat produced per ha.

In vivo digestibility, voluntary intake of dry matter and digestible organic matter, average daily gain and carcass weight will be determined directly in individual animals. Meat produced per hectare will be calculated as described above

Experimental design and data analysis

This is an exploratory non-replicated experiment in which individual animals (not plots) will be used as experimental units. The design corresponds to a Completely Randomised Design using initial weight as covariate. The model for analysis of digestibility, intake, growth rate and carcass weight will be;

$$Y_{ij} = u + B_1 (X_{ij}-x) + A_i + E_{ij}$$

where Y_{ij} is each response variable observed in jth animal in ith Accession, X_{ij} is initial weight of jth animal, x is average initial weight, A_i is the effect of ith accession and E_{ij} is random error.

Production of meat per ha will not be analyzed statistically due to lack of field replicates.

Calendar

Activity	Time
Lab analysis	May 2005 – May 2006
Lamb growth experiment	May - Aug 2005
Sample preparation sheep	Sep 2005
Preliminary report	Oct 2006
Final Report	Dec 2006

Budget (excludes production of Napier)

Salaries	$A
Services	$B
Supplies	$C
General expenses	$D
Lab analyses	$E

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