Cassava leaves and azolla as crude protein supplement feed to east african short horned Zebu Heifers

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The study was undertaken to explore the nutritive potential of cassava leaves and Azolla as supplementary source of crude protein to cattle fed on either mixed range grass hay or Bracharia Mulato II. Two experiments were carried out, the first was the determination of the biomass yield of Azolla, its relative growth rate and doubling time while the second was a feeding trial to determine the performance of small East African Shorthorned Zebu heifers of similar age and weight. The heifers (four each) were randomly assigned to six treatments in the feeding trial that ran for 16 weeks. The doubling time for Azolla was 5 days while the relative growth rate was 0.15 g/g/day. The chemical analysis indicated that Azolla is rich in crude protein (18.8%) and minerals (3.29% calcium, 2.66% potassium and 0.15% phosphorus and other minerals in trace levels). All the supplemented groups showed more weekly weight gains ranging from 0.75 to 1.11 kg per day more than the control. Overall, the treatment with Azolla or cassava showed slightly higher but insignificant increases in weight gains. Azolla and cassava leaves can therefore be considered as potential feed for livestock.

Key words: Arid and range lands, cattle, doubling time, relative growth rate.

INTRODUCTION

Improving protein supply for ruminants is a strategy for increasing productivity in ruminants that have a high protein requirement. This strategy is important when feeding young animals following weaning, those that are pregnant as well as those in lactation. The development of new feed types from the available feed resources is one approach that may help farmers overcome the low nutritional content and seasonal availability of feed for beef cattle production. The new feeds to be introduced need to be highly adaptable to the environment and have appreciably high productivity. Some of the tropical feeds that are high in crude protein and with high growth rates...
include Cassava leaf meal, Azolla and Brachiaria muta (Antoniewicz et al., 1995, García et al., 1995; Parashuramulu et al., 2013). In a study that was carried out by Oni et al. (2010), it was found that West African Dwarf goats that had been fed with on Panicum maximum and supplemented dried cassava leaves led to an improvement in nitrogen retention, apparent digestibility, weight gain as well as dry matter intake. In an experiment by Marjuki et al. (2008), the inclusion of cassava leaves on a dry matter (DM) basis was up to 30%. 

In the tropical areas of Africa, cassava forms part of the staple foods grown as its production is efficient and requires minimal inputs and management. The crop has proved suitable with potential to assist in curbing food scarcity in the continent of Africa (Hahn et al., 1992). Cassava roots are low in protein at about 2% crude protein (CP) and because of this, most of the times it is denigrated however it has a caloric value at 16 MJ ME/Kg DM (Oerke et al., 1994). Cassava leaves are high in CP, ranging from 16.7 to 39.9% which compares favourably with forages generally regarded as good protein sources such as lucerne with a crude protein content ranging from 16.9 to 30% (Antoniewicz et al., 1995; García et al., 1995). In Africa, scientists have recognized cassava as a crop with high potential as animal feed, just as it has been used in many countries in Europe. As a tradition in many tropical countries, cassava constitutes about 20 to 40% of the feed used by livestock (Oppong-Apane, 2013). This knowledge on the use of cassava for feeding livestock needs to be enhanced and made available to more people.

Another potential livestock feed is Azolla, commonly known as floating fern that is found in most parts of the world, both the temperate as well as tropical ecosystems (Arora and Singh, 2003). It is particularly common on farm dams and other still water bodies. Azolla has an association that is symbiotic with Anabaena azollae, the nitrogen fixing algae, which enables it to be highly productive. It has been claimed that Azolla is one of the fastest-growing plants on Earth (Miranda et al., 2018) with the potential to produce 347 tonnes/ha of fresh material, or an average daily yield of 818 to 955 kg/ha. In a study carried out in India on the effect of Azolla on cattle growth, there was a significant increase the daily live weight gain for the animals supplemented with Azolla as compared to the control (Chatterjee et al., 2013). This study was carried out to determine biomass yield and nutrient contents of Cassava leaves and Azolla as well as their effect on growth performance of cattle.

MATERIALS AND METHODS

The experiment was conducted at the KALRO Kiboko research centre in Southeastern Kenya. The centre is approximately 1,000 m above sea level and lies between latitudes 2°10'S and 2°25'S and longitudes 37°40'E and 37°55'E (Hatch et al., 1984). The average annual rainfall is 548 mm and average minimum and maximum temperature is 16.6 and 29.4°C respectively (Jimmy et al., 2017). The annual mean temperature is 23°C and the average evapotranspiration of the site is 2000 mm. Samples of Azolla were collected then dried on a paper towel by blotting and then 10 g each were placed in a bucket and grown for 10 days, this was repeated twice. The floating mat of Azolla was harvested from each bucket after the 10 days of growth. The harvested material was then dried by blotting with a paper towel before weighing with an electronic balance to determine the biomass. The time taken by the Azolla to double (doubling time, Dt) was determined as reported by Badayos (1989), while the relative growth rate (g/g/d) were determined as by Hechler and Dawson (1995):

\[ Dt = \frac{0.693 t}{\ln (w_f-wo)} \]

Where: \( t = \) growth duration (days); \( w_f = \) final biomass (g), and \( wo = \) initial biomass (g). The feeding trial was a completely randomized design which had 24 Small East African Zebu yearlings aged about 18 months and having a mean weight of 110 ± 18.4 kg. The animals were stall-fed for 16 weeks. Two weeks were used for adapting the yearlings to the feeds and assessing the yearlings’ intake of the feeds while data were collected for fourteen weeks. The 24 heifers were randomly assigned to the feeds while the feeds were also randomized among the six treatments. The yearlings were fed such that they received dry matter equivalent to 3% of their body weight. The performance of the heifers was monitored through their body weight gain with weekly weighing done in the morning before feeding. Supplementary feeding with Azolla or cassava leaves was done in the morning (8:00 a.m.). Supplementary materials were fed first to improve on their intake because it was observed that the yearlings preferred grass and not the test materials. One half of the amount of feeding materials (which varied between 2.55 and 4.41 kg depending on treatment) was given at 10.00 a.m. while the other half was given at mid-afternoon (2.00 p.m.) to minimize wastage. Weighed amount of feed was offered to the yearlings and intake determined by collecting and weighing the remains before the next feeding. Water was given ad libitum. A mineral block (Afa Bora® stock lick) was provided in each enclosure such that the animals had ad libitum access. Ticks and flies were controlled using a Pour-on acaricide (Ectopop 020 SA) while disease treatment was done whenever symptoms were observed. All the yearlings were dewormed with Albendazole 10% suspension before the start of the experiment. The treatment feeds were:

- Treatment 1 were fed with mixture of range grasses (control)
- Treatment 2 were fed Brachiaria muta II
- Treatment 3 were fed Range grass plus Cassava leaf meal
- Treatment 4 were fed Range grass plus Azolla
- Treatment 5 were fed Brachiaria muta II plus Cassava leaf meal
- Treatment 6 were fed Brachiaria muta II plus Azolla

Samples of the experimental feeds were collected, dried and then ground through a 1.00 mm sieve harmer mill. The samples which were ground were dried at 105°C overnight in the oven to find out its DM. The Kjeldahl procedure (AOAC, 2005), was used to determine the percent crude protein (CP). The neutral detergent fibre (NDF) and the acid detergent fibre (ADF) was evaluated using the Van Soest method (Van Soest et al., 1991). Burning of samples for 8 h at 550°C in a muffle furnace was used to determine the ash content. The mineral contents were determined using Near-infrared spectroscopy (NIRS). The growth performance data of the heifers were analyzed statistically by one-way Analysis of Variance (ANOVA) (Steel and Torie, 1980). Duncan’s New Multiple Range Test was used to separate means at 5% significance level. The statistical model used was \( Y_{ij} = \mu + \alpha_i + e_{ij} \), where \( Y_{ij} \) is the weight
Table 1. Relative growth rate and doubling time of azolla.

<table>
<thead>
<tr>
<th>Initial weight (Lwt) of Azolla</th>
<th>Duration in days</th>
<th>Final weight (Fwt) of Azolla</th>
<th>Fwt/Lwt</th>
<th>In(Fwt/Lwt)</th>
<th>Doubling time (days)</th>
<th>RGR</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>10</td>
<td>40.0</td>
<td>4.00</td>
<td>1.39</td>
<td>5.00</td>
<td>0.139</td>
</tr>
<tr>
<td>10</td>
<td>10</td>
<td>33.8</td>
<td>3.38</td>
<td>1.22</td>
<td>5.69</td>
<td>0.122</td>
</tr>
<tr>
<td>10</td>
<td>10</td>
<td>40.2</td>
<td>4.02</td>
<td>1.39</td>
<td>4.98</td>
<td>0.139</td>
</tr>
<tr>
<td>10</td>
<td>10</td>
<td>57.4</td>
<td>5.74</td>
<td>1.75</td>
<td>3.97</td>
<td>0.175</td>
</tr>
<tr>
<td>10</td>
<td>10</td>
<td>40.6</td>
<td>4.06</td>
<td>1.40</td>
<td>4.95</td>
<td>0.140</td>
</tr>
<tr>
<td>10</td>
<td>10</td>
<td>42.6</td>
<td>4.26</td>
<td>1.45</td>
<td>4.78</td>
<td>0.145</td>
</tr>
<tr>
<td>10</td>
<td>10</td>
<td>54.0</td>
<td>5.40</td>
<td>1.69</td>
<td>4.11</td>
<td>0.169</td>
</tr>
<tr>
<td>Average</td>
<td></td>
<td>44.1</td>
<td></td>
<td></td>
<td>4.78</td>
<td>0.147</td>
</tr>
</tbody>
</table>

Table 2. Nutritive content of the experimental feeds (the mineral contents were determined using NIRS).

<table>
<thead>
<tr>
<th>Chemical composition</th>
<th>DM</th>
<th>%CP</th>
<th>%NDF</th>
<th>%ADF</th>
<th>%P</th>
<th>%Ca</th>
<th>%K</th>
<th>%Mg</th>
<th>%TDN</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bracharia muta II</td>
<td>93.6</td>
<td>14.1</td>
<td>59.1</td>
<td>31.7</td>
<td>0.510</td>
<td>0.930</td>
<td>2.21</td>
<td>0.320</td>
<td>60.1</td>
</tr>
<tr>
<td>Azolla</td>
<td>93.2</td>
<td>18.8</td>
<td>52.1</td>
<td>44.2</td>
<td>0.150</td>
<td>3.29</td>
<td>2.66</td>
<td>0.450</td>
<td>43.6</td>
</tr>
<tr>
<td>Mixed Range Grass</td>
<td>93.7</td>
<td>5.54</td>
<td>58.3</td>
<td>44.7</td>
<td>0.460</td>
<td>0.81</td>
<td>2.18</td>
<td>0.280</td>
<td>50.1</td>
</tr>
<tr>
<td>Cassava leaves</td>
<td>93.5</td>
<td>29.0</td>
<td>40.0</td>
<td>29.2</td>
<td>0.610</td>
<td>2.17</td>
<td>2.84</td>
<td>0.470</td>
<td>68.0</td>
</tr>
</tbody>
</table>

gain over time (dependent variable), μ is the overall weight gain mean, α, is effect of treatment i, and ε is a random error.

RESULTS AND DISCUSSION

The doubling time for Azolla was about 5 days while the relative growth rate was 0.15 (Table 1). The findings were different from those of Peters et al. (1980) in which the relative growth rates were found to be 0.35 to 0.39. In another study, Talley and Rains (1980), found the relative growth rate of Azolla filiculoides, to be between 0.245 and 0.277 g/g day. In these studies, the doubling of biomass of Azolla took about 2 days. The prevailing conditions of the environment and the growth media and the species of Azolla could be the cause of differences in the growth rates. In another study, Peters (1990) found that Azolla grows well in water that is of shallow depth and of little disturbance. Nutrients’ availability to Azolla is mainly dependent on water and not the soils (Kushari and Watanabe, 1992).

The nutritive value of the feed materials used in the feeding experiment are shown in Table 2. The range grasses and Azolla had more ADF than Bracharia and Cassava leaves. Cassava leaves had higher levels of phosphorous than the supplement Azolla. Similarly, there were higher levels of potassium in cassava as compared to Azolla. Azolla however had more calcium than cassava leaves. Cassava leaves also had more total digestible nutrients (TDN) as compared to Azolla. Between the two grasses, Bracharia had more TDN than the range grass hay. Bracharia also had more phosphates than Azolla. Azolla had more calcium, potassium and magnesium as compared to Bracharia.

The weekly increase in the weights of the heifers are shown in Figure 1. The performance of the heifers across the six treatments was statistically insignificant (P > 0.05). The mean effects of the treatments on the heifers showed that supplementing with Azolla had the highest positive effect followed by cassava and Bracharia supplemented with Azolla. Those fed with Bracharia also had a higher weight gain than those fed on range grasses alone (Figures 1 and 2).

The values for the chemical composition of cassava foliage were within the range of values reported by Alli Ballogun (1995) and Oni et al. (2011). This confirms its potential as a source of supplemental protein to grass characterized by low nutrient levels in assisting the rumen microbes. Therefore, in the rumen, the amount of ammonia generated would be high and promote an efficient digestion process (Orskov, 1995). Norton (2003) observed that optimal rumen microbial activity requires levels of ammonia that can only be provided by feeds whose crude protein content is higher than 8%.

The experimental diets (Table 3), except the range grasses, had a higher crude protein content than 8% which suggested that nutritionally they could be of better quality. It has been shown that intake of protein has an important effect on the performance of ruminants due to elevated levels of available nitrogen that is fermentable and needed by the bacteria in the rumen.

For the rumen to function normally in ruminants, enough insoluble fibre material, which is linked to digestion of cellulose and rumination, is required. Tropical feed intake
Performance of the zebu heifers fed on brachiaria and mixed range grass basal diets supplemented with azolla and cassava leaves.

Improved digestibility of feeds as well as intake and better animal performance (Klopfenstein et al., 2001) is enhanced by moderate fibre levels which in turn facilitates the microbial
organisms to colonize the ingesta in the rumen. The NDF, ADF and ADL and their concentrations in the diets affects the intakes of cellulose, hemicellulose and diet digestibility (Harper and McNeill, 2015). The fermentation rate and amount of feed consumed is reduced by too much NDF although very low levels of fibre can result in very rapid fermentation in the rumen which result in acidosis. It was observed that the groups of heifers supplemented either with Azolla or cassava leaves tended to finish their allocated feeds. The observed high total DM intake among some of the treatments corroborates earlier findings that increasing level of cassava leaf supplementation improves intake of both dry matter and nitrogen in goats and cattle fed a grass diet and supplemented with ammoniated rice straw and rice straw with para grass, respectively (Do et al., 2002; Sath et al., 2013). The low DM intake observed in heifers fed solely on range grass hay could be due to the low level of crude protein in the range grass compared to the other supplemented treatments as low protein content diets have been determined to cause reduced intake by animals (Iflut, 1988).

According to Pillai et al. (2002), there was an increase in milk yield by between 15 and 20% when commercial feeds were mixed with 1.50 to 2 kg of Azolla. About 15 to 20% of Azolla can replace similar quantity of commercial feeds such as oil cake, without any change in milk yield (Pillai et al., 2002). Also, it was realized that feeding Azolla leads to an improvement in the quality of milk as well as longevity and the health of animals. The nutritive value of Azolla compares favourably with other high valued protein sources. As indicated in Table 2, above, it has crude protein of up to 26.3%. This makes Azolla a good candidate for feeding animals. As reported by Sculthorpe (1967), large quantities of Azolla have been harvested in various parts of Africa and Asia and used to feed cattle and pigs.

### Table 3. Percentage composition of the six treatment diets fed to the heifers.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Type of feeds used</th>
<th>Composition</th>
<th>% Dry matter intake</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Range grasses</td>
<td>100%</td>
<td>2.9</td>
</tr>
<tr>
<td>2</td>
<td><em>Brachiaria mulato II</em></td>
<td>100%</td>
<td>3.3</td>
</tr>
<tr>
<td>3</td>
<td>Range grass plus Cassava leaf meal</td>
<td>74:26</td>
<td>3.25</td>
</tr>
<tr>
<td>4</td>
<td>Range grass plus <em>Azolla</em></td>
<td>64:36</td>
<td>3.2</td>
</tr>
<tr>
<td>5</td>
<td><em>Brachiaria mulato II</em> plus Cassava leaf meal</td>
<td>94:6</td>
<td>3.5</td>
</tr>
<tr>
<td>6</td>
<td><em>Brachiaria mulato II</em> plus Azolla</td>
<td>91:9</td>
<td>3.4</td>
</tr>
</tbody>
</table>

The supplementation of range grasses with Azolla and cassava leaves improved feed intake, digestibility, and weight gain in the heifers. This opens an opportunity for smallholder farmers rearing cattle as an alternative supplement for their animals. Azolla which has a potential to doubles its weight every 3 or so days should be further explored as an alternative source of protein especially in areas where there are surface water bodies such as dams and rice paddies. *Brachiaria mulato II* should also be promoted as a good substitute to range grasses especially due to its high CP value.

**CONFLICT OF INTERESTS**

The authors have not declared any conflict of interests.

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