Herbage mass and chemical composition of the heterogeneous grasslands affected by harvesting time in Subtropical terrain Nepal

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ABSTRACT
A study was carried out to evaluate the productivity and chemical composition of heterogeneous grasslands at Agriculture and Forestry University (AFU) livestock farm. The four grassland ecotypes were chosen as upland north, upland south, lowland south and lowland north. The dominating herbage species and cover abundance by the botanical groups were studied on day before the harvesting. Later, the herbage dry matter productivity was estimated by quadrat cutting during May and June, 2017. Chemical analysis was done by using the proximate method for dry matter (DM), crude protein (CP), crude fiber (CF) and ether extract (EE) content. Research results showed that the AFU grassland dominated by perennial grasses and sedges followed by the forbs. The mean coverage of grasses and sedges was about 55%, whilst that of forbs was about 29% and the least was for legumes (about 4%). The cumulative herbage mass was about 1.53 t/ha on the DM basis, whilst the highest DM was found in the upland-south (1.74 t/ha) and the least was in the upland-north (1.334 t/ha). The proximate analysis further revealed that the site had no effect on CF content, whilst the CP was significant only at the second harvest for the lowland north (8.34%). Data revealed that the herbage composition might depend upon the soil moisture availability and geographical aspect. The dominance of perennial grasses at AFU grasslands revealed the yield stability, but needs the improvement through inoculation with leguminous forages for improved feed quality.

Keywords: Herbage cover, herbage mass, proximate composition, rangeland, species dominance, Vegetation ecotypes.


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INTRODUCTION

The grasslands or pasturelands are important components in agricultural production systems, by providing feed for livestock and by replenishing soil organic matter, preventing soil erosion and restoring soil fertility and so on (Fageria et al., 2010). The management for long-term sustainability of such lands often requires the contribution of short-term activities (Snyman, 1998), such as herbage species mix, moisture etc. The Pasture productivity and nutritive value are major factors that determine patterns of grazing distribution of livestock (Bailey et al., 1996), and herbage harvested for also the feeding of animals in the tie stalls. The aboveground herbage mass productivity and measures of nutritive value, vary between years and between the physiological stages within a given growing season at harvest (Givens et al., 1993; Beecher et al., 2015) and it may further vary upon the various grassland eco-types favored by different other biotic and abiotic factors (Snyman et al., 1993), and geographical aspects (Radcliffe, 1982; Bennie et al., 2006) and etc. The present study aimed at exploring the differences in herbage dry matter productivity and chemical composition at two harvesting regimen in four different grassland ecotypes in subtropical conditions of a close distant at Agriculture and Forestry University livestock farm.

MATERIALS AND METHODS

Site selection and layout

The current experimental set up (study area) was located inside the Agriculture and Forestry University, Rampur, Chitwan, Nepal. It lies at a latitude of 27°38′14.1″N and longitude 84°21′25.2″E (NMRC, 2017). Study area was located at an altitude of 257 m from the sea level. The experimental site had a humid and sub-tropical with cool winter climate (2 to 3°C) and hot summer days (43°C). The annual rainfall was 1500 mm, where monsoon period (about 75% of annual rainfall) started from mid-June to mid-September (NMRC, 2017). The study area had about 120 ha land occupied by native perennial species and about one-third of which was in lowland category. The selected sites had a mix mosaic of perennial vegetation. The four experimental sites; each having five blocks of 0.5 × 0.5 square meter served as the replicated sampling units for herbage mass productivity and forage samples for laboratory analysis.

Based on the water submergence (water visibility) and geographical aspect (north/south), the study grasslands were classified into broad four broad sites as upland north, upland south, lowland south and lowland respectively.

Herbage cover estimation

The herbage species dominance and botanical coverage were studied the day before the cutting or herbage sampling. Grasses, forbs, legumes, grass-like plants (sedges/cyperus) and bare patchiness were studied over the grassland to determine plan cover by following Gibbens and Beck (1987). Simple bird’s eye view method was used to measure the basal cover of each species and herbage species score was set from 0-100 from low to high abundance (basal cover). Identification of plant species was further completed. Five the most dominated plant species over the selected grasslands types were identified and presented in the present study. Later, the herbage cover of the botanical groups was recalculated.

Herbage sampling
Two cuttings were taken in the one-month interval during summer (May and June) at each experimental block of the grassland eco-types. At each site, five fixed plots (0.5×0.5 m²) were chosen randomly and fenced prior to herbage growth in April 2017. Sampling bag was labeled with a time of sampling and sites details from where it was taken. The vegetation covered by a quadrat frame 0.5 x0.5 m² was cut above 2 cm from the ground to mimic the general removal of vegetation by grazing cattle and buffaloes. Debris (dead matter) was avoided in the sample for subjecting to laboratory analysis. Fresh weight of herbage was weighted in the field by a digital balance (Ohaus Pro™). Plant identification and herbage cover estimation were conducted in the field during the cover estimates.

**Herbage mass estimation**

Herbage mass was estimated by cutting, drying and weighing the aboveground herbage. The green herbage mass was weighed on-site. Five quadrats-cut from each site were collected in well-labeled bags. Quadrat with the size of 0.5×0.5 m² was used for this purpose. Grass shoots were harvested above 2 cm from the ground assuming the available height of plant grazed by cattle and buffaloes.

**Laboratory analysis**

The laboratory activities were performed at Agriculture and Forestry University (AFU) animal nutrition laboratory. The harvested materials were dried to a constant temperature of 65°C in the oven at 48 hours and weighed to determine total dry matter productivity and The chemical composition of herbage samples was estimated by following the guidelines of the AOAC (1991).The crude protein content in samples was estimated by using the macro-Kjeldahl method, in which digestion, distillation, and titration were done. Later, the total ash content was determined for a 2 g dried milled (by Thomas Mill, 45 mm mesh size) of sample putting in a muffle furnace at 550 °C for eight hours. The Crude fiber was determined by washing a sample with acid and base in presence of heat and subtracting final weight with total ash content using the Gold Fish apparatus. A dried sample (2 g) was extracted with petroleum ether in Soxhlet Apparatus to remove the ether-soluble component present in it. Then, the extracted material was dried to a constant weight in an oven at 65°C for 48 hr. and the obtained weight was the fat content of the sample.

**Data analysis**

For data analysis, GenStat release version 11 (Payne et al., 2011) was used to analyze the data. The simple descriptive statistics were employed i.e. for herbage cover estimates. The following one way analysis of variance (ANOVA) model. The means were compared in terms herbage mass productivity and major chemical composition using the least significant difference (LSD) at 5% level of significance (Jan et al., 2009; Baral et al., 2016; Shrestha, 2019; Sharma et al., 2016; Kandel & Shrestha, 2019).

\[ Y_{ijk} = \mu + h_j + e_{ijk} \]  

Equation (1).

Where, \( Y_{ijk} \) = output of individual observation for parameter 

\( \mu \) = Over all mean for parameter Y 

\( s_i \) = Fixed effect of the \( i^{th} \) parameter 

\( e_{ijk} \) = Residual error
RESULTS AND DISCUSSION

**Dominated plant species in four sites**
In the southern upland sites, the mosaic of *Cynodon-Eleusine-Cyperus* was dominated, whilst in the northern uplands, the herbage mosaic of *Cynodon-Imperata-Cyperus* was dominated. The scale of dominance of top five species in both of the upland sites has been shown in detail in Table 1.

**Table 1. Dominated herbage species over an upland area**

<table>
<thead>
<tr>
<th>Plant species</th>
<th>Score</th>
<th>Plant species</th>
<th>Score</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Cynodon dactylon</em></td>
<td>10</td>
<td><em>Cynodon dactylon</em></td>
<td>9</td>
</tr>
<tr>
<td><em>Eleusine indica</em></td>
<td>9.5</td>
<td><em>Imperata cylindrica</em></td>
<td>8.5</td>
</tr>
<tr>
<td><em>Cyperus rotundus</em></td>
<td>6.5</td>
<td><em>Cyperus rotundus</em></td>
<td>6</td>
</tr>
<tr>
<td><em>Imperata cylindrica</em></td>
<td>6</td>
<td><em>Eleusine indica</em></td>
<td>5.5</td>
</tr>
<tr>
<td><em>Mimosa pudica</em></td>
<td>5</td>
<td><em>Xanthium strumarium</em></td>
<td>3</td>
</tr>
</tbody>
</table>

Note: A score of 0-10 was used based on USDA Forest Services Method for determination of species dominance.

Likewise in the southern lowland sites, the species combination of *Furena-Cyanodon-Digitaria* was dominated, whilst the *Persicaria-Rotala-Xanthium* mix was dominated (table 2).

**Table 2. Dominated herbage species in the lowlands.**

<table>
<thead>
<tr>
<th>Plant species</th>
<th>Score</th>
<th>Plant species</th>
<th>Score</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Furena umbellate</em></td>
<td>10</td>
<td><em>Persicaria barbata</em></td>
<td>10</td>
</tr>
<tr>
<td><em>Cyanodon dactylon</em></td>
<td>10</td>
<td><em>Rotala rotundifolia</em></td>
<td>9.5</td>
</tr>
<tr>
<td><em>Digitaria sanguinalis</em></td>
<td>9.5</td>
<td><em>Xanthium strumarium</em></td>
<td>8</td>
</tr>
<tr>
<td><em>Cyperus rotundus</em></td>
<td>9</td>
<td><em>Alternanthera sessilis</em></td>
<td>6</td>
</tr>
<tr>
<td><em>Commelia diffusa</em></td>
<td>5.5</td>
<td><em>Ageratum houstonianum</em></td>
<td>4.5</td>
</tr>
</tbody>
</table>

Note: A score of 0-10 was used (Scores based on USDA Forest Service Method).

*Cynodon dactylon*, *Eleusine indica* and grass-like plants (cyperus) were found in all sites. Other grass species found in those areas were *Amannia baccifera*, *Amannia baccifera*, *Smithia sensitive*, *Bidens Pilosa*, *Dysophylla auricularia*, *Bidens Pilosa*, *Smithia sensitive*, *Youngia japonica*, *Isachne globosa*, *Digitaria sanguinalis*, *Eragrostis variabilis*, *Blumea lacer*., *Alternanthera sessilis*, *Phyllanthus urinaria* etc. in upland and *Eleusine indica*, *Pteris vittata*, *Pteris vittata*, *Justicia diffusa*, *Lindernia crustaceaen*, *Lindernia crustaceaen*, *Xanthium strumarium* etc. in lowland. These indicated greater plant species diversity in grassland and an indication of grassland stability and higher primary productivity as reported by Daly et al., (1996) and Caldeira et al. (2001). Chaudhary and Devkota (2018) also reported grasses as predominant in rangelands whereas the proportion of legume was minimum in the subtropical grasslands as in the present study.
The occurrence of vegetative life form varied greatly among the grasslands ecotypes (Table 3). Upland north had the highest (47.5%) and lowland south had the lowest (37%) cover of grasses. Broadleaf herbage cover was highest in the upland north (33.4%) followed by lowland south (32.7%). Cover by legume was only with an average of 1.5 to 6% of basal cover. The cyperus or sedges were the highest in the upland south (19.3%) followed by lowland south (11.6%) and lowland north (9.4%). The herbage cover occupied by dead matter was only less than 4% across the experimental sites studied. The highest bare patchiness (open) was found in the upland south (10.7%) and lowest in Lowland south (7.8%) respectively. The detail of the herbage cover by the botanical groups has been presented in table 3.

Table 3. Percentage herbage cover in different rangeland types by botanical groups

<table>
<thead>
<tr>
<th>Range category</th>
<th>Grass</th>
<th>Forb</th>
<th>Legume</th>
<th>Grass-like</th>
<th>Dead matter</th>
<th>Bare patchiness</th>
</tr>
</thead>
<tbody>
<tr>
<td>Upland north</td>
<td>47.5</td>
<td>33.4</td>
<td>1.5</td>
<td>6.9</td>
<td>3.0</td>
<td>10.7</td>
</tr>
<tr>
<td>Lowland north</td>
<td>42.0</td>
<td>27.0</td>
<td>6.0</td>
<td>9.40</td>
<td>3.2</td>
<td>9.2</td>
</tr>
<tr>
<td>Upland south</td>
<td>43.8</td>
<td>24.4</td>
<td>2.5</td>
<td>19.30</td>
<td>2.8</td>
<td>8.4</td>
</tr>
<tr>
<td>Lowland south</td>
<td>37.0</td>
<td>32.7</td>
<td>4.5</td>
<td>11.6</td>
<td>3.2</td>
<td>7.8</td>
</tr>
<tr>
<td>Mean</td>
<td>42.6</td>
<td>29.4</td>
<td>3.62</td>
<td>11.8</td>
<td>3.1</td>
<td>9.0</td>
</tr>
<tr>
<td>SEM</td>
<td>15.9</td>
<td>16.1</td>
<td>16.0</td>
<td>9.3</td>
<td>4.5</td>
<td>3.4</td>
</tr>
</tbody>
</table>

Note: SEM= Standard error of mean.

Taking into account the herbage cover at all the study sites together, there were much more abundance of grasses than any forb or grass-like plants (cyperus) and legumes respectively. However, the occurrence of each botanical group varied greatly among the grassland types. The existence of variation in the frequency of botanical groups, particularly in the proportion of perennials may show a variation in stocking rates or nature of soil or a combination of the both. It has been repeatedly reported that grasslands are ecologically favored when perennials dominate the ecosystem.

**Herbage mass productivity**

The weight of herbage harvested and their respective dry weights were given in table 4. Site had significant contribution to the cumulative dry weight mass (P<0.05). The highest cumulative dry weight mass was observed at the upland south (1.738 t/ha) and the lowest in the upland north (1.338 t/ha), whereas it remained within the range of about 1.5 t/ha in other two sites. At first harvest, the harvesting time had no significant effect to herbage mass, whilst it was found significant at second harvest. Total fresh herbage yield in the first harvest was obtained 1768 kg/ha to 3212 kg/ha in first harvest and 2540 kg/ha to 4124 kg/ha in second harvest from this study (fresh mass data not presented here), which is concomitant to the reports of Pariyar (1998) and Pandey (2007) who estimated the total forage yield of tropical grassland of Nepal upto 4000kg/ha.

Table 4. Green and dry weight of herbage with their respective cumulative weight.

<table>
<thead>
<tr>
<th>Range type</th>
<th>Harvesting time</th>
<th>Accumulated dry weight, t/ha</th>
</tr>
</thead>
<tbody>
<tr>
<td>1st May</td>
<td>1st June</td>
<td></td>
</tr>
</tbody>
</table>

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Production and productivity of the grasslands, however, vary from 0.65 MT DM/ha/yr. to 3.60 MT DM/ha/yr. in different agro-ecological zones (LMP, 1993; Pariyar, 1998). Dry matter yield of grasses in the current study was within the range of 0.521 t to 0.721 t/ha in first harvest and 0.76 t/ha to 1.22 t/ha in the second harvest. The practical reasons associated to the lower herbage productivity in the first harvest might be due to the low rainfall as compared to the second harvest (Austin, 2002), that constrained the low soil moisture (Fay et al., 2003). It has been repeatedly stated in previous experiments that the perennial species productivity is associated with the seasonal rainfall (Cable, 1975; Fynn and O’Connor, 2000) and their richness (Sanderson et al., 2005). However, the study on effect soil factors on dry matter productivity were lacking in the present study.

**Herbage chemical composition**

The result of the chemical composition of grasses harvested from the study areas was presented in Table 5. Grass collected from grasslands was no difference (p<0.05) in levels of EE, CF and Ash content in all four sites. The protein content of different sites was slightly different. The protein content of herbage at first harvest was found no difference but protein content of herbage at second harvest was found significantly different in four sites. In the first harvest, lowland south was highest in protein content with 8.78% followed by upland north (7.76%) and upland south (7.7%). In the second harvest; lowland north was richest in protein content with 8.34% followed by upland south (7.96%) and lowland south (6.9%). Protein content was found highest in the first harvest. Mean crude fiber content of herbage was 29.22% in first harvest and 29.85% in the second harvest. Ether extract in all sites were not more than 4% of the dry matter. Average ash content of the herbage was 6.49% in first harvest and 6.82% in the second harvest.

Crude protein value ranged between 6.4 and 9.8% and showed an average of 8.9% in first harvest and 8.2% in the second harvest among the grasslands. This showed a low protein value of grassland. Daily protein requirements for ruminant could be expressed as 10.7% of dry matter intake or at least 13.5% to 14.5% crude protein is required for high producing dairy cows. Lower protein value was due to the poor richness of leguminous herbages. The protein content of herbage at first harvest was greater than that of the second harvest.
Table 5. Chemical composition of the herbage harvested at different grassland eco-types at AFU livestock farm

<table>
<thead>
<tr>
<th>Range category</th>
<th>First harvest (%)</th>
<th>Second harvest (%)</th>
<th>First harvest (%)</th>
<th>Second harvest (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CP</td>
<td>CF</td>
<td>EE</td>
<td>Ash</td>
</tr>
<tr>
<td>Upland north</td>
<td>7.76</td>
<td>29.42</td>
<td>3.04</td>
<td>6.76</td>
</tr>
<tr>
<td>Lowland north</td>
<td>7.50</td>
<td>29.18</td>
<td>3.0</td>
<td>6.28</td>
</tr>
<tr>
<td>Upland south</td>
<td>7.70</td>
<td>29.32</td>
<td>2.92</td>
<td>6.54</td>
</tr>
<tr>
<td>Lowland south</td>
<td>8.78</td>
<td>28.94</td>
<td>3.08</td>
<td>6.38</td>
</tr>
<tr>
<td>Mean</td>
<td>8.94</td>
<td>29.22</td>
<td>3.02</td>
<td>6.49</td>
</tr>
<tr>
<td>p-value</td>
<td>0.87</td>
<td>0.79</td>
<td>0.22</td>
<td>0.33</td>
</tr>
<tr>
<td>SEM (±)</td>
<td>1.25</td>
<td>0.35</td>
<td>0.05</td>
<td>0.19</td>
</tr>
<tr>
<td>CV (%)</td>
<td>28.03</td>
<td>2.68</td>
<td>3.9</td>
<td>6.43</td>
</tr>
</tbody>
</table>

Note: LSD= Least significant difference, SEM= Standard error of mean, CV= coefficient of variation.* indicated probability p<0.05.

It revealed that protein content of plant was decreased in subsequent harvesting in response to increasing the fiber content. But the CP content of herbage was low indicating that grazing only to supply required nutrition for the animals. The CP content in the present study was approximately in the range as reported by Chaudhary and Devkota (2018) which was 7.04-11.1% during post-monsoon and 8.4-11.4% during the start of winter. Tiwari (2005) estimated 21.9%, 12.13%, and 12% respectively for average dry matter, total ash and crude protein content of different grass species while studied 686 samples of different grass species collected from 28 districts of Nepal. Yield performance of animals raised by grazing depends on the nutrient content of the species present in grassland. Palatability of plants, one of the factor for species selection by animals, is positively correlated with the high soluble cell components. Therefore, grassland having plant species low in lignin and cellulose content is more preferable for grazing of animals to obtain better yield performance. In addition, the crude protein content has minor variation in upland and lowland grassland in the current study. The upland species may experience soil moisture deficit comparative to lowland as much of them were dominated by the grasses rather than legumes and in such cases, the low CP content could be expected (Seguin et al., 2002). The CP content being higher in the lowland north was unclear in comparison to lowland south but could be associated to the changes in soil nitrogen content. As expected, the soil mineral content variation between the grassland ecotypes might have acted on, which however limited the measurement in the present study. The change in CP content in the second harvest might be associated to the seasonal changes and developmental stage of herbage, however, the changes in herbage mass might be both affected by species diversity (Sanderson et al., 2004; Florine et al., 2006) and duration of growth, as well. The practical reason associated to high CP content at lowland north might be associated to the carryover effect of the continuous grazing (Pavlü et al., 2006) as a common phenomenon in the study sites and or rather high...
dominance of some leguminous and forbs species. However, to verify the carryover effect of the continuous stocking needs further verification by long term grazing experiments.

CONCLUSIONS
This study provides basic information on the variation of herbage m and proximate composition of range herbage available for livestock stall feeding and grazing as well in the Agriculture and Forestry livestock farm. Based on the findings of the study, it was concluded that studied grassland was rich in perennial grasses and sedges. The level of protein concentration of herbage was found below the animal requirements and that further suggests to incorporate legume pastures in the grasslands. The findings also revealed that the herbage productivity and composition differed with the grassland ecotypes and the research may further imply for the moisture availability and geographical aspect of the aspects.

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Author contributions
Prabin Pandey conducted the field experiment. Badrika Devkota-Barsila helped in data analysis. Shanker Raj Barsila designed the lab and field sampling protocols. Other co-authors involved in the manuscript revision and review.

Conflict of Interest
The authors declared no conflict of interest.

REFERENCES


