Vegetation types and rangeland species nutritional values and forage quality indicators at various phonological stages

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Information on different rangeland plants’ nutritive values at various growth stages is important in rangelands management. This information helps rangeland managers to choose proper grazing times to achieve higher animal performance without detrimental effects on the rangeland vegetations. Effects of various plant parts’ growth stages and vegetation types on reserve carbohydrates and forage quality indicators were investigated during 2009 and 2010. Plant samples were collected from natural rangelands in Iran with completely randomized block (CRB) design. The species included, two grass species (Secale montanum and Festuco ovina), two forbs (Lotus corniculatus and Sanguisorba minor) and two shrubs (Kochia prosterata and Salsola rigida). Aerial plant parts’ samples were oven-dried at 80°C for 24 h, then analyzed for soluble carbohydrates, crude protein (CP), acid detergent fiber (ADF), dry matter digestible (DMD) and metabolizable energy (ME). Results showed that plants at the seedling stage had more reserve carbohydrates and from the three vegetation types (grass, forbs, and shrub), forbs contained more soluble carbohydrates as compared to the other two (grasses and shrubs). Differences in soluble carbohydrate contents of different species at various growth stages in 2 years were statistically significant. The forage quality indicators (CP, ADF, DMD and ME) in different species, in various vegetation types, in the 2 years were statistically significant, except for the CP.

Key words: Phonological stage, forage quality, protein, fiber.

INTRODUCTION

Study of the chemical compounds in the rangeland plants used for livestock feed, and information on the effects of the environmental conditions on changing these compounds are very important in rangelands management. Also, information on the forage feed value is essential knowledge for rangelands management because the forage feed value varies in different conditions (Biondini et al., 2006; Graza and Fulbright, 2008; Low and Andrews, 2007; Dongmei et al., 2005). On the other hand, the nutritional needs of the animals are different in various environmental conditions and at different physiological stages of plants (Mcdowell, 2005; Norton and waterfall, 2003; Shinde et al., 2000; Underwood, 2001).

Researchers believe that several factors affect the forage feed value. Sulc et al. (2009), Ayan et al. (2010) and White (2003) reported that the most important factor for change in the forage feed value is the vegetation covers’ growth stage, and the forage plants have different feed values at various phonological stages. Oddy et al. (1993) and Larbi et al. (2011) stated that the movement of the plant nutrients from the leaves and stems to roots and seeds is important for changes in forage feed value. Different rangeland plant species have been studied by

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several researchers and all of these investigators have reported that the differences in forage feed values in various plant species resulted in differences in plant metabolisms (Coyne and Cook, 1991; Davidson and Milthorpe, 1995; Graber, 1991; Deregibus et al., 2002; Hyder and Sneva, 2003). Different factors that affect forage feed values such as CP, ADF, NDF (neutral detergent fiber), ME, have been studied by several investigators (Menke and Trilca, 1985, 1993; Moore and Biddingscomb, 1994; Orodo et al., 2000). Information on the compounds that provide food reserves in plants is very important for rangeland managers. The knowledge of how these compounds are made in plants and in which plant parts are concentrated more can be a great help in identifying the appropriate grazing time, number of grazing livestock and the length of the grazing period. The lack of information and awareness may cause irreparable and irreversible damage to the rangeland plants. Physiological changes in different plants at various phenological stages are different because various species in terms of growth rate, germination, type of the leaves, stems, roots, height, are different from each other. This is essential in a time when the rangeland management is based on the carbohydrate reserve and plant energy providing capability. So, knowledge of carbohydrate production, transport, storage and use in plants can help the rangeland managers to take proper care of the pasture plant species (Mikic et al., 2010; Richards and Caldwell, 2005). The most important information for the balance in stoking rate and rangelands capacity is probably the data on the forage quality and to determine the capacity of a pasture. It is required to determine the forage nutritive value because animal performance in the grazing season has direct relationship with forage feed value. This information helps the rangeland manager to balance between the available forage and the animal’s nutrition needs, and using these factors enables him to obtain maximum animal performance. The forage quality and the forage feed value in plants are affected by several factors, including vegetation stages, grazing intensity and plant species. Among these, vegetation stage is probably the most important factor influencing the compounds found in plants.

MATERIALS AND METHODS

In this study, 6 plant species that were harvested from the rangelands were investigated. The species included 2 grasses (Secale montanum and Festuco ovina), 2 forbs (Lotus corniculatus and Sanguisorba minor) and 2 shrubs (Kochia prosterata and Salsola rigida). Each plot species was replicated 5 times and each replicated plot contained 5 plants. So, for each species, 25 plants were selected (each 5 plants were one replication). Plant species were harvested from the natural rangeland habitats. The samples were dried in the shade at room temperature. As the respiration and photosynthesis in clipped plants continue after the clipping for few minutes and this affected the soluble carbohydrates in order to measure their soluble carbohydrates contents, the plant materials should have either been dried immediately or put in a cool place. Therefore, the mobile freezer was used, and the frozen plant samples were used for chemical analysis. Then, plant materials were put in the oven and dried at 80°C for 24 h, except the plant samples that were used for the forage quality analysis which were dried in the room temperature. Then, the plant materials were ground. The plant sampling dates in the 2 years were different, because the plants started their growth a few days late in the second year. The following measurements were performed on the samples.

Measurement of the chemical compounds

For the measurement of the soluble carbohydrates, the phenol-HSO₄ (sulphuric acid) method was used. In this method, 0.5 g of dried plant sample was taken and 15 ml ethanol 80% was added to it, heated to warm temperature by a heater, then centrifuged at 3000 rpm for 10 min. Then, the centrifuge was turned off and the clear solution in the flask was separated. This was repeated for 2 replications, then the aliquots taken from these 2 replications were mixed and put in an oven at 70 to 80°C. After 1 h, its volume was raised to 100 ml by adding distilled water to it. Then, 4.7 ml Ba(OH)₂ (barium hydroxide) was added to it. After 3 min, 5 ml ZnSO₄ (Zink sulfide) was added to it and thoroughly mixed. Then, 35 ml of it was centrifuged at 3000 rpm for 10 min, then, 2 ml of this aliquot was used for spectrophotometry at 485 nm (nano-meter). In this study, 2 ml H₂O and H₂SO₄ were used for control. Data obtained with this method were on ppm (mg/l) units and the following formula was used to convert the data to carbohydrate in the plant dry matter:

\[ C(\%) = (V/106.DM) \times 100 \]

Where, C is the soluble carbohydrates, V is the volume of the soluble carbohydrates that was obtained by spectrophotometry in PPM (parts per million) and DM is grams (g) dry matter that was used for soluble carbohydrates measurement by this method.

Measurement of the CP (crude protein)

Measurement of the CP in these plant species was done by evaluation of the N content of the plants, assuming that all the proteins in the plants contained 16% nitrogen (16% N) and all the nitrogen was used for protein synthesis. Then, the following formula (Bidlock and Devald 1999) was used to calculate the CP:

\[ CP(\%) = 100/16 \times \%N = 6.25 \times \%N \]

Bidlock and Devald (1999) stated that this formula includes the non-protein nitrogen too, so the amount of the calculated protein by this formula is more than the actual protein. Therefore, measurement of the CP content of the plants by this formula is over estimated. This method is known as Kejeldahl 2.

Measurement of the ADF (acid detergent fiber)

To measure the ADF content of the plants, the Fibertec was used. For this purpose, 1 g of the ground sample was put into glass tubes in the Fibertec, then 100 ml ADS (acid detergent solution) was added and boiled for 1 h. For preparation of the ADS, 20 g BrNH₂(CH₂)₃ (three methyl bromide) was mixed with 10 ml H₂SO₄ (sulfuric acid). After 1 h, all materials in the solution disappeared, except the cellulose, lignin and the minerals. Then, the samples were washed with distilled water and acetone in the cold extraction device and the samples were placed in the oven at 120°C for 2 h,
Table 1. Analysis of variance of forage quality indicators.

<table>
<thead>
<tr>
<th>Year</th>
<th>Sources of Variance</th>
<th>df</th>
<th>Mean squares of the indicators</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>CP (%)</td>
</tr>
<tr>
<td>2009</td>
<td>Sp</td>
<td>5</td>
<td>267.7**</td>
</tr>
<tr>
<td></td>
<td>PS(Sp)</td>
<td>12</td>
<td>164.9**</td>
</tr>
<tr>
<td></td>
<td>Error</td>
<td>72</td>
<td>0.531</td>
</tr>
<tr>
<td></td>
<td>CV (%)</td>
<td>-</td>
<td>5.91</td>
</tr>
<tr>
<td>2010</td>
<td>Sp</td>
<td>3</td>
<td>150.6**</td>
</tr>
<tr>
<td></td>
<td>PS(Sp)</td>
<td>8</td>
<td>155.2**</td>
</tr>
<tr>
<td></td>
<td>Error</td>
<td>48</td>
<td>0.134</td>
</tr>
<tr>
<td></td>
<td>CV (%)</td>
<td>-</td>
<td>2.86</td>
</tr>
</tbody>
</table>

**Significant at the 0.01 probability level; Sp, species; PS, phonological stages; CV, coefficient of variation.

![Figure 1](image.png)

Figure 1. Soluble carbohydrates (g/kg) in 6 rangelands species in 2009.

afterwards, the sample weights were measured with a digital scale, and put in the electric furnace at 500°C for 3 h. In the electric furnace, all of the sample’s cellulose and lignin were burnt and only the minerals remained. These samples were taken out of the electric furnace and their weights were measured with a digital scale. By comparing the weights of the samples before and after the electric furnace, the ADF was obtained using the following formula:

$$ADF(\%) = \frac{\text{Samples' weight before the electric furnace} - \text{Samples' weight after the electric furnace}}{\text{Initial sample weight (1 g)}} \times 100$$

This method of the ADF measurement is according to the AOAC formula (Association of Official Analytical Chemists).

Measurement of DMD (dry matter digestible)

To measure the DMD, the following formula was used (Fonnesbeck and Davidson, 1985).

$$DMD(\%) = \frac{88}{9N} - \frac{0.779 \times ADF}{2}$$

Where, DMD is digestible dry mater and ADF is acid detergent fiber. So, DMD has direct relationship with plant nitrogen (N) content and inverse relationship with the ADF content of the plants.

Measurement of ME (metabolisable energy)

After the DMD was found, the following formula was used to calculate the ME based on MJ unit.

$$ME = 0.17 \times DMD\%-2$$

RESULTS AND DISCUSSION

The results of this study showed that the soluble carbohydrates values were different in the 6 studied species and their mean values were significantly different at the 1% probability level. Also, the mean square of the forage quality indicators in different species was significant at the 1% probability level (Table 1). The 6 studied species had different means of soluble carbohydrates (Figures 1 and 2). Sanguisorba minor in
the first year (2009) and *Lotus corniculatus* in second year (2010) had the highest soluble carbohydrates, while *Salsola rigida* had the lowest soluble carbohydrates. Duncan’s test indicated that the 6 species were in 6 different groups in 2009 and in 4 different groups in 2010 (since the studied grasses were annual grass species, the data on the grasses were taken only in 2009).

### Soluble carbohydrate reserves in phonological stages

The results of the analysis of variance (ANOVA) indicated that the phonological stages were significant at the 1% probability level and the mean values of the soluble carbohydrates in the 6 species at the 3 phonological stages were different. In both 2009 and 2010, the soluble carbohydrate reserves at the third phonological stage (seedling) were maximum, at the beginning of the vegetative stage were minimum, and at the flowering stage were between the first stage of the vegetation and the seedling stage (Figures 3 and 4).

### Soluble carbohydrate reserves in vegetation cover types

The average of the soluble carbohydrate reserves in the three types of the vegetation cover, grass, forb and shrub were significant at the 1% probability level, and the mean values of the soluble carbohydrates in these three vegetation cover types were different. The means showed that forbs have the highest carbohydrate reserves and grasses and shrubs had the same level. The means of the carbohydrate reserves in 2009 were 58.47 in forbs, 39.56 in grasses, 37.71 in shrubs and in 2010 were 76.9 in forbs and 43.4 g/kg in shrubs. The grasses in this study were annual and did not have any
re-growth in the next year (2010) (Figures 5 and 6).

**Results of the forage quality indicators**

The results of the analysis of variance (ANOVA) showed that the mean values of the four important indicators of the forage quality including CP, ADF, ME and DMD were significant at the 1% probability level, and the species were different in this regard. These results were as follows:

**Crude protein (CP) (%)**

As shown in Figures 7 and 8, at the two years, 2009 and
2010, *S. minor* had the highest CP and *K. prostrata* had the lowest CP, while, the two species *K. prostrata* and *S. rigida* had the same level of CP.

2009 and 2010 showed the highest value of ME for *Secale montanum* and *L. corniculatus*, while *F. ovina* had the lowest ME in both years (Figures 9 and 10).

### Metabolizable energy (ME)

The mean values of the metabolizable energy (ME) in

### Percent of digestible dry matter (DMD%)

The results of the analysis of variance (ANOVA) indicated
that the mean values of DMD for different species were different in both years (2009 and 2010). The highest mean of DMD was for \textit{L. corniculatus} and the lowest was for \textit{F. ovina} (Figures 11 and 12).

\textbf{Forage quality indicators in different vegetation cover types}

The results of the analysis of variance (ANOVA) showed that the three vegetation cover types including: grass, shrub, and forb showed that their mean values in both years (2009 and 2010) were different at the 1% probability level (Figures 13 and 14). So, forage quality indicators were different in the three vegetation cover types.

\textbf{Cp (%)}

Forbs had the highest average of CP and shrubs had the lowest.

\textbf{ADF (%)}

Grasses had the highest mean of ADF and forbs had the lowest.

\textbf{ME (%)}

Forbs had the highest rate of ME, and the grasses and...
shrubs were in the next rank.

**DMD (%)**

Forbs had the highest rate of DMD, and the grasses and shrubs had the lowest.

**Conclusions**

The entry and the exit of the animals to the pastures and animals' performance during the livestock grazing season are under the direct influence of soluble carbohydrate reserves in rangelands species. Study of the vegetation cover types showed that forbs, grasses and shrubs have different carbohydrate reserve contents. Therefore, management of the rangelands that contain these three types of vegetation covers should be done with careful attention. The forage quality indicators, including DMD, ME, ADF and CP in various species were different. It seems, in different plant species, the main constituents of the plants' structure such as type of roots and leaves, leaves arrangement, stems length and growth rates determine the quality of the plants.

Changes in the chemical compounds of these 6 rangelands species showed that phonological stage is the most important effective factor on forage quality. Therefore, according to these results, in order to improve the rangelands conditions and selecting suitable grazing system and grazing time, the following two factors are essential. The place of the food reserves in the rangeland's species as well as the nutritive value of the plant species during the growth period in order to provide
the nutritional needs of the animals and ensure the regrowth of the rangelands' plant species.

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REFERENCES


Figure 14. The average percentage of the indicators of the forage qualities (CP, ADF, ME, and DMD) in the two vegetation cover types (forb and shrub) in 2010.