Comparative studies of 3 Foxtail Millet (Setaria italica) cultivars at different phonological stages in Karaj region, Iran

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Abstract
The determination of forage quality is one of the most important factors essential to appropriate field management. The most important factor, effective on forage quality, is the growth stage; by studying on phonological stages of the forage, suitable grazing time can be determined. Since different cultivars of forage crops have different feed qualities in each of their growth stages, for the purpose of investigation of genetic diversity of forage yields and their quantitative characters, we studied three foxtail millet cultivars, namely KFM1, KFM6 and KFM9 in three phonological stages including vegetative growth stage, flowering stage and seeding stage by use of a factorial experimental design with a complete block basic design with 3 replications in Karaj, Iran during 2008-9. Except for Acid Detergent Fiber (ADF) and crude fibre percentage, the difference between the other feed quality indices as well as wet and dry yields, among the different cultivars and the three growth phases of the cultivars, were significant (P<0.01). Further, the interaction effect between different cultivars and also the phonological growth stages was statistically significant only in connection with wet and dry yield. Proportional to the development of growth stages, approximately in all cultivars, the crude protein percentage and digestive dry material percentage are decreased and the fibre content increased. In general, according to the measured indices, KFM1 had the highest feed quality and, taking into account its appropriate feed quality, was selected as the outstanding cultivar. The vegetative growth stage (1st phonological stage), from the point of view of feed quality indices, was higher than the other two stages (except for water soluble carbohydrates); but, considering relatively small forage energy difference between stages 1st and 2nd phonological stages, and also higher production and readiness of the fields (ranges) for grazing, the 2nd phonological stage (flowering stage) was selected as the best time for grazing (by use of grazing systems).

Key words: Foxtail millet, forage yield, forage digestibility percent, crude protein percent, growth stage.

Introduction
The food shortage and increasing trend of global population, especially in the developing countries, has caused serious concerns in connection with future food production. The role of forage crops in animal feed and, consequently, supply of human’s food requirements, are an outstanding concern. In line with development of milk and meat products plan in the country, the extension of forage production and access to the new resources of feeds are among priorities of the Office of Forage Crops of Division of Forage Crop Research Centres. The importance of appropriate and adequate feed of the ruminants, necessities that the feed quality of each of feed items and their ingredients to be determined by use of correct and standard systems.

Foxtail millet has been among feed grasses which attracted more attention and focus of the researchers in the recent years. Foxtail millet is mainly used as a feed crop. In addition, foxtail millet is a qualitative forage crop and has appropriate palatability.5,11

Foxtail millet has rapid growth rate in tropical climates and is among the plant with high water efficiency 8. This crop is produced in poor and low efficiency soils of Europe and tropical and sub-tropical regions of Asia. As foxtail millet has high growth rate, it could be used as second crop in crop rotation program 13. Meanwhile, the production of this crop is common in areas with low precipitation up to 2000 m altitude 6.

Foxtail millet is produced in Northern Iran (Mazandaran Province) and its seeds are used for preparation of a kind of local food and birds feed 4 and its advantage is the lack of inhibitors (such as prussic acid) in its composition. The high growth rate of foxtail millet in poor soils in comparison with other forage crops and its high crude protein content (16% w/w) is among its other advantages 12,16.

John’s investigations 7 showed that the best time for harvesting of foxtail millet is the flowering stage up to booting stage and it contains a crude protein (CP) content of 10 to 14% and 57 to 60% of total digestible nutrient if it is harvested at the beginning of flowering stage. Neville et al. 14 concluded that the delay in foxtail millet harvesting results in rapid decrease of CP percentage; hence ADF and NDF are increased. Weichenthal et al. 19 showed that foxtail millet has 12.1% crude protein, 62% NDF and its ADF content is 36% and has digestible dry material in the laboratory equal to 70% (w/w).

Nleya and Jeranyama 15 have concluded through an experiment that foxtail millet has 8 to 13% crude protein, 32% ADF, 61% NDF and 53 to 73% digestible dry material. Mehrani et al. 10 have studied 10 cultivars of foxtail millet in Karaj, Iran and concluded that they showed significant difference with each other and the treatments 1, 3 and 9 with 5.5, 5 and 5 ton.ha-1 yields, respectively, have the highest dry feed production rate 10.

The most important aims of this work are: 1. Evaluation of genetic variation for DM yield, 2. Study of quality traits during growth and
3. Identify and introduction of the best varieties of foxtail millet.

Materials and Methods
For the purpose of study and comparison of genetic diversity and the qualitative characters of three foxtail millet cultivars (KFM1, KFM6, KFM9) in three phonological stages: vegetative growth, flowering and seeding stages, a factorial experimental design using a complete block design with 3 replications were assessed.

For the purpose of implementation of the experiment, a land with 800 m² in a farm with 400 hectare area located in Karaj, Iran with longitude and latitude of 51° 6’ E and 35° 59’ N, respectively and 1321 m altitude, which had been prepared for the experiment. The field was tilled, disk applied and levelled by leveller and 200 to 250 kg ha⁻¹ ammonium phosphate and 100 to 150 kg ha⁻¹ urea fertilizer was applied. The seeds of each cultivar were sown in each plot with 60 cm raw distance. The seeds of each cultivar were sown in each plot with 60 cm raw distance. The flowering and seeding stages, a factorial experimental design using a complete block design with 3 replications were assessed. The plots were irrigated with 7 to 10 days intervals using corrugation irrigation method. The hoeing of the furrows and weeding took place after germination. Since the harvesting took place in three stages, the border lines and 0.5 m of both ends of plots were removed first and the harvesting took place in 12 m border lines and 0.5 m of both ends of plots were removed first and the harvesting took place in three stages. The field was tilled, disk applied and levelled by leveller and 200 to 250 kg ha⁻¹ ammonium phosphate and 100 to 150 kg ha⁻¹ urea fertilizer was applied. The seeds of each cultivar were sown in each plot with 60 cm raw distance. The sowing took place on 26th May, as per the plan. The plots were irrigated with 7 to 10 days intervals using corrugation irrigation method. The hoeing of the furrows and weeding took place after germination. Since the harvesting took place in three stages, the border lines and 0.5 m of both ends of plots were removed first and the harvesting took place in 12 m border lines and 0.5 m of both ends of plots. The harvested plants were weighed immediately after harvesting and the wet yield was calculated. A 1 kg sample was prepared from each treatment and was dried at 80 °C for one week. The dried samples were weighed first and the dry yield was calculated; then it was milled well and dispatched to the laboratory for determination of nutrient value. Near Infrared (NIR) spectrometer, belonging to Research Laboratory of State Forests and Ranges Organization, was used for determination of protein percentage, crude fibre, ash percentage, Acid Detergent Fibre (ADF) and dry material percentage.

After calibration of the NIR instrument, the measurement of qualitative characters was carried out following the methods of Jafari et al. The gathered data were analyzed by using MSTATC and SAS9 software.

Results
The statistical parameters including maximum, minimum, mean, standard error and coefficient of variations (CV) of means of the three cultivars of foxtail millet in its three growth stages have been presented in Table 1. The results of analysis of variance (ANOVA) of the characters and the significance level of the mean squares of the cultivars in the growth stages and the interaction effects have been included in Table 2. Table 3 contains the results of means comparison between the cultivars and the growth stages. The interaction effect of the cultivars and growth stages in different characters are shown in figures 1-8.

Table 1. Summary of statistical data of each of studied characters of 3 foxtail millet cultivars in growth stages.

<table>
<thead>
<tr>
<th>Statistical factor</th>
<th>Dry matter yield</th>
<th>Wet matter yield</th>
<th>Protein percentage</th>
<th>Digestibility</th>
<th>ADF</th>
<th>WSC</th>
<th>Crude fiber</th>
<th>Ash percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean</td>
<td>7.3</td>
<td>26.6</td>
<td>9.7</td>
<td>63.0</td>
<td>31.4</td>
<td>5.5</td>
<td>40.3</td>
<td>8.7</td>
</tr>
<tr>
<td>Minimum</td>
<td>4.8</td>
<td>19.3</td>
<td>5.3</td>
<td>56.2</td>
<td>26.2</td>
<td>2.2</td>
<td>37.1</td>
<td>6.7</td>
</tr>
<tr>
<td>Maximum</td>
<td>11.8</td>
<td>37.0</td>
<td>13.3</td>
<td>69.7</td>
<td>35.3</td>
<td>8.8</td>
<td>44.7</td>
<td>10.5</td>
</tr>
<tr>
<td>Standard E</td>
<td>0.4</td>
<td>0.9</td>
<td>0.4</td>
<td>0.5</td>
<td>0.4</td>
<td>0.3</td>
<td>0.2</td>
<td></td>
</tr>
<tr>
<td>SD</td>
<td>2.0</td>
<td>4.8</td>
<td>2.0</td>
<td>2.8</td>
<td>2.1</td>
<td>2.1</td>
<td>1.8</td>
<td>1.0</td>
</tr>
<tr>
<td>CV</td>
<td>28.0</td>
<td>18.7</td>
<td>21.0</td>
<td>4.5</td>
<td>6.8</td>
<td>38.1</td>
<td>4.4</td>
<td>11.7</td>
</tr>
</tbody>
</table>

Table 2. ANOVA of 3 foxtail millet cultivars in three phonological stages.

<table>
<thead>
<tr>
<th>Variation source</th>
<th>DF</th>
<th>Dry matter yield</th>
<th>Wet matter yield</th>
<th>Protein percentage</th>
<th>Digestibility</th>
<th>ADF</th>
<th>WSC</th>
<th>Crude fiber</th>
<th>Ash percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>R</td>
<td>2</td>
<td>1.9</td>
<td>5.2**</td>
<td>2.4</td>
<td>7.2**</td>
<td>1.9**</td>
<td>0.1**</td>
<td>0.1**</td>
<td>0.4**</td>
</tr>
<tr>
<td>Cultivar(A)</td>
<td>2</td>
<td>3.2**</td>
<td>37.5**</td>
<td>18.1</td>
<td>21.6**</td>
<td>2.1**</td>
<td>0.7**</td>
<td>6.7**</td>
<td></td>
</tr>
<tr>
<td>Phonological stage(B)</td>
<td>2</td>
<td>11.6**</td>
<td>102.7**</td>
<td>13.2**</td>
<td>23.8**</td>
<td>10.2**</td>
<td>17.1**</td>
<td>5.3**</td>
<td>3.1**</td>
</tr>
<tr>
<td>A*B</td>
<td>2</td>
<td>15.5**</td>
<td>53.4**</td>
<td>1.3**</td>
<td>6.9**</td>
<td>7.3**</td>
<td>1.3**</td>
<td>0.3**</td>
<td>0.2**</td>
</tr>
<tr>
<td>Error</td>
<td>16</td>
<td>0.7</td>
<td>5.9</td>
<td>0.8</td>
<td>4.6</td>
<td>3.8</td>
<td>1.9</td>
<td>4.2</td>
<td>0.3</td>
</tr>
</tbody>
</table>

Table 3. Mean comparison of yield and qualitative characters of 3 foxtail millet cultivars in three phonological stages.

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Dry yield</th>
<th>Wet yield</th>
<th>Protein percentage</th>
<th>Digestibility</th>
<th>ADF</th>
<th>WSC</th>
<th>Crude fiber</th>
<th>Ash percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>KFM1</td>
<td>6.58 b</td>
<td>26.55 a</td>
<td>11.26 a</td>
<td>64.69 a</td>
<td>31.09 a</td>
<td>4.39 b</td>
<td>39.96 a</td>
<td>9.23 a</td>
</tr>
<tr>
<td>KFM6</td>
<td>7.74 a</td>
<td>27.04 a</td>
<td>9.94 b</td>
<td>62.73 ab</td>
<td>31.96 a</td>
<td>4.88 b</td>
<td>40.28 a</td>
<td>9.06 a</td>
</tr>
<tr>
<td>KFM9</td>
<td>7.46 a</td>
<td>23.28 b</td>
<td>7.75 c</td>
<td>61.63 b</td>
<td>31.16 a</td>
<td>7.30 a</td>
<td>40.54 a</td>
<td>7.65 b</td>
</tr>
<tr>
<td>Vegetative growth</td>
<td>6.01 b</td>
<td>24.47 b</td>
<td>10.52 a</td>
<td>64.20 a</td>
<td>31.02 a</td>
<td>3.95 b</td>
<td>40.13 a</td>
<td>9.29</td>
</tr>
<tr>
<td>Flowering</td>
<td>8.23 a</td>
<td>29.43 a</td>
<td>10.17 a</td>
<td>63.69 a</td>
<td>30.59 a</td>
<td>6.09 a</td>
<td>39.56 a</td>
<td>8.51 b</td>
</tr>
<tr>
<td>Seeding</td>
<td>7.55 a</td>
<td>22.97 b</td>
<td>8.26 b</td>
<td>61.16 b</td>
<td>32.62 a</td>
<td>6.53 a</td>
<td>41.09 a</td>
<td>8.14 b</td>
</tr>
</tbody>
</table>

The means of the treatments with same connotations were not significantly different as per Duncan’s multi-range test at P<0.05.

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ton.ha$^{-1}$, respectively, belongs to flowering stage. The interaction effect at P<0.01 was significant for dry and wet yield; where KFM9 had the highest dry yield in seeding stage with 10.8 ton.ha$^{-1}$ yield and the lowest in vegetative growth stage with 5.3 ton.ha$^{-1}$ (Fig. 1). KFM6 cultivar had the highest wet yield in its flowering stage with 34.7 t/ha yield.

**Crude protein percentage and digestibility**: The quality and nutritional value of the plant have direct relationship with their crude protein content and digestibility and reverse relationship with ADF and crude fibre$^{1}$. The statistical results showed that there is significant difference between the cultivars from the points of view of protein percentage and digestibility percentage (P<0.01). KFM1 with 11.26 and 64.69% had the highest protein and digestibility, respectively. There was significant difference between different growth stages at P<0.01; where the vegetative growth stage showed 10.52 and 64.20% protein and digestibility among other stages respectively. The results of studies carried
out in USA correspond with our obtained results at the early flowering stage. Despite non-significance of the interactive effect, the highest protein percent and digestibility belongs to KFM1 at vegetative growth stage (Figures 3 and 4).

**ADF and crude fibre percentage:** The results of ANOVA of ADF and crude fibre percentage showed that there is no significant difference between the cultivars and the difference between the growth stages is only significant for ADF percentage at P<0.05. Nevertheless, KFM1 had the lowest ADF and crude fibre content (highest digestibility). The mean comparison between different growth stages showed that the flowering stage had the lowest ADF and crude fibre percentage. The interactive effect between two characters was not significant although KFM1 had the lowest crude fibre and ADF except for hemicellulose (Figures 5 and 6).

**Ash and water soluble carbohydrate percentage:** There were significant difference between the cultivars and also the growth stages when the two above characters are concerned (P<0.01). KFM9 with 7.30% and KFM1 with 4.39 had the highest and lowest water soluble carbohydrate content, respectively. Also, the seeding stage with 6.53% water soluble carbohydrate had the highest level. Despite non-significance of the interaction effect, KFM9 had higher carbohydrate content in seeding stage (Figure 7). KFM1 and also the vegetative growth stage showed the highest ash content.

**Discussion**

In general, the results of the research showed the highest wet and dry yield in seeding stage and this is because of timely establishment of the plant and its better utilization of the environmental conditions.

As we pointed out earlier, the majority of qualitative parameters of the plant are decreased with progress of growth and phonological stages of the plant. Stodart et al. pointed out that the nutrition quality of the forages show considerable differences depending on different places and times. The majority of forage plants have the highest nutrition value and quality at the beginning of their vegetative stage, where, due to decrease in nutrition value of the plant at their maturity stage, the plants have no appropriate quality. As the plant reaches its maturity stage, the structural carbohydrate content is increased, hence the protein concentration and digestibility of the plant and the value of metabolic energy is decreased. In our study, we found that the crude protein content and digestibility of the plant decreased as the plant reached its maturity stage.

The water soluble carbohydrates (WSC) had its lowest level at flowering stage and the highest value at seeding stage; because the need to conservator and strength textures increase while the plants grow and these textures have been mainly made of cellulose, hemicellulose and lignin. Therefore, at the end of growth period, the water soluble carbohydrates are transformed into structural carbohydrates. The minimum value of carbohydrates at the beginning of the first growth stage is due to lower ratio of the stem textures to the leaf.

Since the young cells form the majority of young plant structure, has thin cell wall and a little ADF and fibre content; but simultaneous with aging, the cell wall becomes thicker and harder and the fibre and ADF contents are increased.

As we found in this study, the ADF and fibre content increased proportional to increase in growth stage and the highest ADF content (the lowest digestibility) belongs to seeding stage.

The date of introduction and exit of livestock to the field (or range) depends on both soil and plant conditions. As mentioned earlier, the forage quality is in its highest level at the beginning of the season (spring). But, due to high precipitation at this stage and wet land, the soil will be compacted and this leads to rapid soil erosion and, on the other hand, in case of heavy grazing, the plant will have little time for recovery. On the other hand, the early harvesting of the plant may decrease the yield and stem stability and these two factors should be considered in any decisions making process. Harvesting at the early growth stage will have the highest loss and is the worst time for harvesting, because the total inorganic carbon (TAC) content is in its lowest level.

We should combine these factors with another important fact that the plant yield and its resistance to the grazing in the second growth stage (flowering) is higher than the first stage and this is an important fact for animal husbandry. Therefore, we can conclude that the more appropriate time for grazing is the second phonological stage (flowering), which seems to be better than all other stages, provided, however, that the appropriate grazing system to be implemented to allow the field (range) to reproduce and recover and the number of the livestock should be in harmony with the real capacity and capability of the range (field).

**Conclusions**

The results in this study establish a general framework for the domestication history of foxtail millet. The results obtained confirmed that there were significant differences among phonological growth stages for forage yield and also important quality traits such as; dry matter digestibility and crude protein. Present research proved that forage quality decrease while forage yield increase from vegetative stage to flowering stage. The best stage for harvesting is flowering due to high forage yield and plant resistance. The varieties of KFM1 and KFM6 are recommended for future plant breeding research since they can give high yield and good quality of forage.

**Acknowledgements**

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