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Evaluation by Chemical and *in vitro* Gas Production Techniques of Foxtail Millet Grown in Northern Iran

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Abstract: An experiment was conducted at the Agricultural Research Station of Eraghi Mahalleh, Gorgan, in Northern Iran. The aim of the experiment was to measure the nutritional value of foxtail millet forage grown in Northern Iran with different sowing dates and at different densities. Foxtail millet (*Setaria italica* L.) was cultivated under different conditions of sowing date and plant density, using a factorial arrangement in a randomized completely design with 6 replications. The sowing dates as as first factor were July 1, 16 or 31, the plant densities as a second factor were 30, 45 or 60 plants m^{-2}. The results showed that the sowing date and plant density had a significant effect on the content of NDF and ADF ($p<0.05$). When the various treatments were compared, the forage produced from millet grown with a sowing date of July 16 and a density of 30 plants m^{-2} contained the greatest amount of protein (119.3 g kg^{-1}). The highest ash content (100 g kg^{-1}) was obtained from plants grown with a sowing date of July 1 and a density of 45 plants m^{-2}. The sowing date, the density and the interaction of these two factors did not have a significant effect on Ca and or P content of millet forage ($p<0.05$). The DMD of millet forage from different treatments varied between 418 and 589 g kg^{-1}. Similarly, the Digestible Energy (DE) varied between 7.5 and 10.5 and the Metabolizable Energy (ME) between 6.0 and 8.7 MJ kg^{-1}. The highest rate of rumen gas production was related to an incubation time of 12 h (46.8-58.8 mL/200 g DM). A sowing date of July 1 and a density of 60 plants m^{-2} are recommended to produce foxtail millet with optimal characteristics for animal nutrition in the climatic conditions of the Gorgan-Golestan province in Northern Iran. This is because these conditions led to the greatest production of dry matter and an optimal chemical composition (especially, with regard to the protein content).

Key words: *Setaria italica*, sowing date, plant density, feed evaluation, foxtail millet, Gorgan

INTRODUCTION

In Iran, feed prices have been continuously increasing but animal products such as milk and meat are relatively cheap. For this reason, there is now a significant move to perform nutritional evaluation of non-traditional feed sources for ruminant livestock in an attempt to reduce the cost of the diet. Forage is the major constituent of the diet of ruminant animals and it provides energy, proteins and minerals. The principal use of foxtail millet, *Setaria italica* L., including its forage and silage is to feed cows and sheep (Boval *et al.*, 2004). Foxtail millet, an important small millet crop, is widely cultivated in semi-arid regions of the subtropics. This crop germinates comparatively well in drought-prone areas and in very poor soils, where other crops fail to grow. The stage of growth, sowing date and plant density are the most important factors that influence the composition and nutritive value of forages (Howell, 1990; Fulkerson *et al.*, 2006). The factors that determine the appropriate sowing date for any plant are rainfall, temperature, light and day length (Berzenyi, 1990). Plant density and sowing date can affect on nutritive value as well as yield of plant (Dhingra *et al.*, 1986).

Although, foxtail millet is the plant used to meet the nutritional requirements of ruminants and monogastric animals in some part of Iran, there are no previous reports of the nutritive value of foxtail millet forage under different conditions of cultivation. Accurate prediction of forage quality under different conditions of cultivation would allow targeting of harvest or grazing to meet the desired levels of nutritional composition for specific requirements of the animal (Valente *et al.*, 2000; Cortes *et al.*, 2005).

The nutritional value of a ruminant feed is determined by the concentrations of its chemical components, as well as their rate and extent of digestion. Determination of the digestibility of feeds *in vivo* is laborious, expensive, requires large quantities of feed and is largely unsuitable

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for single feedstuffs, thereby making it impractical for routine evaluation of feed. The *in vivo* method is also subject to errors associated with the use of digesta flow, microbial markers and inherent variation between animals. Evaluation *in vitro* involves less expensive and more rapid alternatives (Alfredo *et al*., 2005).

The use of *in vitro* Gas Production (GP) techniques to estimate the digestibility of feed is based on the empirical relationships between digestibility and GP. The potential of GP to predict digestibility has been investigated by various researchers (Menke *et al*., 1979; Khazaal *et al*., 1995).

The main aim of this study was to determine the nutritional value of foxtail millet forage in terms of its chemical composition, *in vitro* GP and calculations of the Metabolizable Energy (ME) and Organic Matter Digestibility (OMD) in millet produced under different cultivation conditions.

MATERIALS AND METHODS

Location, cultivation and harvesting condition: The research was conducted in 2007 at the Agricultural Research Station of Eraghi Mahalleh, Gorgan, Iran, which lies between 36°54′ Northern latitude and 54°25′ Eastern longitude. Foxtail millet was cultivated at intervals of 15 days (July 1, 16 and 31), at three plant densities (30, 45 and 60 plant m^{-2}) for each sowing date. Foxtail millet forage was harvested with a hand sickle, when the seeds were at late maturity (the milky-doughy phase) in any sowing date.

Forage preparation: Whole plants were cut into pieces with a small chopper. Fresh forage samples were dried at 60°C in a forced air oven. The samples were ground to pass through a 1 mm sieve in a Wiley mill and were used for chemical analysis, assay of *in vitro* Gas Production (GP) and calculation of *in vitro* Dry Matter (DM) digestibility.

Chemical composition: Dry Matter (DM) was measured by drying the samples at 105°C overnight and the amount of ash was determined by igniting the dry samples in a muffle furnace at 525°C for 8 h. The content of Nitrogen (N) was measured by the Kjeldhal method (AOAC, 1992) and the amount of Crude Protein (CP) was calculated as N×6.25. The Ether Extract (EE) was measured by the method recommended by AOAC (1992). The content of Neutral Detergent Fiber (NDF) and Acid Detergent Fiber (ADF) was determined by the method of Van Soest *et al*. (1991).

***In vitro* digestibility:** The experimental protocol was approved by the Gorgan University of Agricultural Sciences and Natural Resources and local laws and regulations governing the use of experimental animals were adhered to. A modification of Tiilley and Terry (1963) two-stage technique was used to determine the DM and OMD *in vitro* of the experimental samples.

***In vitro* gas production:** Rumen fluid was obtained from three fistulated steers of the Taleshi breed, which were fed twice daily with a diet containing foxtail millet hay (60%) and concentrate (40%). The concentrate consisted of wheat (74%), sunflower meal (24%), calcium carbonate (0.99%), salt (1%) and a vitamin and mineral mixture (0.01%). The forage samples (0.200 g dry weight) were incubated in triplicate in rumen fluid in calibrated 100 mL glass syringes following the procedure of Menke and Steingass (1988).

The syringes were pre-warmed at 39°C before the injection of 30 mL rumen fluid-buffer mixture into each syringe, followed by incubation in a water bath at 39°C. The syringes were gently shaken 30 min after the start of incubation and then every hour for the first 10 h of incubation. Readings of GP were recorded before incubation (0) and 3, 6, 12, 24, 48, 72 and 96 h after incubation. Total gas values were corrected for blank incubation. cumulative gas production data were fitted to the model of Orskov and McDonald (1979):

$$y = a + b\,(1\text{-}e^{ct})$$

Where,

- a = The gas production from the immediately soluble fraction (mL)
- b = The gas production from the insoluble fraction (mL)
- c = The gas production rate constant for the insoluble fraction
- t = The incubation time (h)
- y = The gas produced at time (t)

The ME (MJ kg^{-1} DM) content of the experimental forage and silage was calculated using the equations of Menke *et al*. (1979) as follows:

$$\text{ME (MJ kg}^{-1}\text{ DM)} = 2.20 + 0.136\ \text{GP} + 0.057\ \text{CP} + 0.0029\ \text{CP}^2$$

where, GP is the net gas production in 24 h (mL/200 mg).

The DE (MJ kg^{-1} DM) of the experimental samples was calculated using the equation of Heany and Pigden (1963).

Statistical analysis: The experimental design was a factorial randomized completely block design, 3 (sowing date) × 3 (plant density) with 6 replications. Data on chemical composition, GP parameters, DM and OM digestibility *in vitro*, DE and ME were subjected to one-way analysis of variance using the general linear model function of SAS, (1988). Multiple comparisons were performed using Duncan's multiple-range test (Snedecor and Cochran, 1980).

RESULTS

Chemical composition: The chemical composition of the experimental samples of foxtail millet is presented in Table 1. The NDF (g kg^{-1}) ranged from 484-525, the ADF (g kg^{-1}) from 404-438, the CP from 104-125, the EE (g kg^{-1}) from 24-19, ash (g kg^{-1}) from 63-100, Ca (g kg^{-1}) from 1.7-2.1 and P (g kg^{-1}) from 1.1-1.5. As expected, the cell wall content (NDF) was the main chemical constituent of the foxtail millet forage. The sowing date and plant density had significant effects on the percent of NDF and ADF of foxtail millet forage ($p<0.05$).

Digestibility and energy: Table 2 shows the digestibility and energy content of experimental plant. In Table 3 it can be observed that the Dry Matter Digestibility (DMD) and Organic Matter Digestibility (OMD) of forage made from foxtail millet varied from 418-589 and 361-591 g kg^{-1}, respectively.

The Digestible Energy (DE) of the forage ranged from 8.1-10.9 and the ME from 6.5-8.7 MJ kg^{-1}. The effect of the interaction between sowing date and plant density on the DMD and DE was not significant ($p<0.05$).

***In vitro* gas production:** *In vitro* production of rumen gas from the forage of foxtail millet is presented in Table 3.

There were differences ($p<0.05$) in GP (mL/200 g DM) among treatments and with duration of incubation and the time by feed interaction. Potential GP differed among different samples of the forage made from foxtail millet. There was a wide range in the rate of GP among treatments ($p<0.05$).

Table 1: Chemical composition of foxtail millet forage under different conditions of sowing date and plant density

	Component (g kg^{-1})							
Treatments[1]	DM	Ash	CP	EE	NDF	ADF	Ca	P
Sowing date (July 1)								
Plant density (30/m^2)	423^{a}	78^{bc}	111^{b}	23^{a}	499^{bc}	416^{bc}	1.7^{a}	1.1^{b}
Plant density (45/m^2)	322^{a}	100^{a}	105^{ab}	20^{a}	525^{a}	438^{a}	2.1^{a}	1.4^{a}
Plant density (60/m^2)	34.8^{a}	95^{ab}	110^{a}	24^{a}	498^{bc}	416^{bc}	1.9^{ab}	1.3^{ab}
Sowing date (July 16)								
Plant density (30/m^2)	384^{a}	77b^{c}	125^{ab}	24^{a}	484^{c}	404^{c}	1.7^{b}	1.1^{b}
Plant density (45/m^2)	379^{a}	81^{abc}	116^{b}	23^{a}	502^{abc}	419^{abc}	2.0^{ab}	1.3^{ab}
Plant density (60/m^2)	339^{a}	94^{ab}	117^{b}	20^{a}	513^{ab}	428^{ab}	1.9^{ab}	1.4^{ab}
Sowing date (July 31)								
Plant density (30/m^2)	366^{a}	72^{c}	111^{b}	19^{a}	513^{ab}	428^{ab}	2.0^{ab}	0.15^{a}
Plant density (45/m^2)	331^{a}	63^{c}	110^{a}	23^{a}	513^{ab}	427^{ab}	1.9^{ab}	0.13^{ab}
Plant density (60/m^2)	368^{a}	68^{c}	104^{b}	22^{a}	506^{ab}	422^{ab}	1.7^{ab}	0.13^{ab}
SE	42.7	6.2	4.8	1.6	7.6	6.2	0.1	0.01

Table 2: Digestibility and energy content of foxtail millet forage under different treatments

	Variables				
Treatments[1]	DMD (g kg^{-1})	OMD (g kg^{-1})	DOMD (g kg^{-1})	DE (MJ kg^{-1})	ME (MJ kg^{-1})
Sowing date (July 1)					
Plant density (30/m^2)	487^{ab}	444^{ab}	397^{ab}	8.9^{ab}	7.1^{ab}
Plant density (45/m^2)	418^{b}	361^{b}	324^{b}	7.5^{b}	6.0^{b}
Plant density (60/m^2)	485^{ab}	441^{ab}	396^{ab}	8.8^{ab}	7.1^{ab}
Sowing date (July 16)					
Plant density (30/m^2)	488^{ab}	452^{ab}	406^{ab}	8.9^{ab}	7.1^{ab}
Plant density (45/m^2)	448^{ab}	531^{ab}	375^{ab}	8.1^{ab}	6.5^{ab}
Plant density (60/m^2)	541^{ab}	452^{ab}	478^{ab}	9.9^{ab}	8.0^{ab}
Sowing date (July 31)					
Plant density (30/m^2)	482^{ab}	452^{ab}	400^{ab}	8.8^{ab}	7.0^{ab}
Plant density (45/m^2)	507^{ab}	491^{ab}	450^{ab}	9.3^{ab}	7.4^{ab}
Plant density (60/m^2)	589^{a}	591^{ab}	543^{a}	10.9^{a}	8.7^{a}
SE	46.8	53.3	48.9	0.92	0.74

Means within the same column with different superscripts are significantly different ($p<0.05$)

Table 3: Rumen gas production *in vitro* (mL/200 mg DM) from foxtail millet forage under different treatments and incubation times

	Incubation time (h)								
Treatments[1]	2	4	6	8	12	24	48	72	96
Sowing date (July 1)									
Plant density (30/m^2)	34.2^{ab}	37.0^{bc}	41.2^{abcd}	45.3^{ab}	56.7^{ab}	47.7^{b}	41.7^{cd}	47.3^{c}	49.9
Plant density (45/m^2)	34.0^{abc}	36.8^{bc}	40.7^{bcd}	44.7^{abc}	58.2^{ab}	48.4^{b}	41.3^{d}	48.3^{abc}	49.8
Plant density (60/m^2)	33.7^{bc}	36.3^{c}	40.5^{bcd}	44.7^{abc}	56.3^{ab}	47.8^{b}	41.9^{bcd}	46.1^{c}	47.9
Sowing date (July 16)									
Plant density (30/m^2)	35.0^{a}	38.0^{ab}	43.2^{a}	47.7^{a}	58.8^{a}	47.3^{b}	42.1^{bcd}	47.2^{c}	49.2
Plant density (45/m^2)	34.8^{a}	36.8^{bc}	40.7^{bcd}	44.6^{abc}	54.7^{ab}	47.6^{b}	43.0^{bcd}	47.8^{c}	50.8
Plant density (60/m^2)	33.0^{c}	36.1^{c}	40.0^{cd}	43.5^{bc}	48.4^{c}	48.3^{b}	44.6^{bc}	50.2^{ab}	53.0
Sowing date (July 31)									
Plant density (30/m^2)	33.3^{bc}	36.8^{bc}	39.7^{d}	42.1^{c}	48.6^{c}	47.2^{b}	45.0^{b}	50.8^{a}	53.6
Plant density (45/m^2)	33.5^{bc}	37.7^{ab}	42.3^{abc}	45.9^{ab}	52.7^{bc}	50.4^{a}	48.6^{a}	50.2^{ab}	52.6
Plant density (60/m^2)	34.0^{abc}	3.4^{a}	42.8^{ab}	45.9^{ab}	52.9^{bc}	51.5^{a}	51.2^{a}	50.3^{ab}	52.9
SE	0.31	0.37	0.71	0.89	1.67	0.65	0.99	0.83	0.86

Means within the same column with different superscripts are significantly different ($p<0.05$)

DISCUSSION

The main aim of the research was to determine the nutritional value of foxtail millet forage in terms of the chemical composition, digestibility, energy content and *in vitro* production of rumen gas.

Forages are composed of structural and non-structural constituents. The sowing date and plant density had significant effects on the percentage of NDF and ADF in the experimental samples, which are structural components of foxtail millet. This is because, the most important factor that influences the composition and nutritional value of forage is the length of the vegetative period. When, the plant becomes mature, its need for structural tissues will be increased, consequently, the rate of synthesis of structural carbohydrates, such as NDF and ADF, will be increased (Coors *et al*., 1997; Jensen *et al*., 2005). The vegetative period of the experimental plants with sowing dates of July 1, 16 and 31 was 76, 69 and 64 days, respectively. A shortening of the vegetative period causes a decrease in the DM of a single plant and the total mass and consequently, the fiber content of the plant is reduced (Coors *et al*., 1997; Arzadun *et al*., 2006).

The effect of plant density on the NDF and ADF was significant and the highest values of NDF and ADF occurred in plants with a sowing date of July 1 and a plant density of 30 plants m^{-2}. This result is reasonable; because a decrease in the plant density results in reduced competition between plants for light, water and nutrients. Individual plants are therefore able to mature and as a result the rate of DMD decreases, consequently promoting production of plant fiber (Van Soest *et al*., 1991; Gorge, 1999; Mohanty *et al*., 2000; Beakou *et al*., 2008).

A delay in the sowing date and increased plant density caused an increase in the percentage of CP. This result is in agreement with the results of similar experiments on other gramine plants such as wheat. It may be explained by the fact that a delay in planting caused a decrease in the vegetative period (76, 68 and 64 days, respectively) and a decrease in the leaf area of each plant. This will have caused a decrease in the fiber content and an increased level of protein in the plants (Aaron *et al*., 2005).

The ash content in forage is representative of the amount of minerals in the plant tissues. With a sowing date of July 31 and a plant density of 30 plants m^{-2} the ash content was at its highest level. This result is in agreement with a previous study that reported that an increase in the plant density causes a decrease in fiber content and organic matter content. This, in turn, causes an increase in ash content (Grattan *et al*., 2004). It should also be mentioned that an increase in the leaf:stem ratio of a plant (leafiness) causes an increase in plant protein. In this experiment the effect of plant density on the percentage of protein was not significant, probably because of an insufficient number of replicates and low plant density (Hattab and Harb, 1990). The surface of a leaf will be increased as a result of increasing plant density. As a consequence, the number of photosynthetic organs per unit of leaf surface and the growth rate, will increase and as a result, the DM mass and protein content of the plant will increase (Hattab and Harb, 1990).

The different treatments had no effect on the digestibility and energy content of foxtail millet. Different factors can influence the digestibility and energy content of forage, of which the stage of growth is the most important. The lack of effect of the treatments on the digestibility and energy content of the experimental plants may have been due to inappropriate plant density and/or plant distance in the treatments (Heany and Pigden, 1963; McDowell and Valle, 2000; Givens *et al*., 2008).

In all treatments, the highest level of gas production occurred after to 12 h incubation. This means that fermentation of forage is maximal at this stage of incubation in ruminants. In general, this parameter is mostly related to the ration and its constituents, for example, for more readily digestible carbohydrates it is 12-16 h and for less digestible carbohydrates it is 24-96 h (Nikpour *et al*., 1987; Blummel *et al*., 1997; Adesogan *et al*., 1998; Kinan and Krishnamoorthy, 2007; Vanic *et al*., 2008).

CONCLUSION

The results of the study indicate that a delay in the sowing date and reducing the period of growth will cause a reduction in the fiber content of foxtail millet and an increase in its nutritional value. In general, increasing plant density causes competition among plants for light, water and nutrients. Plant density causes a decrease in plant DM weight, consequently the fiber content of the plant will increase. The sowing date did not affect the protein content, unlike plant density. The aim of livestock producers is generally to gain more energy from the forage mass. In contrast, in agronomy the aim is to gain more dry matter per hectare. In this experiment, the effect of sowing date on protein production was not significant, so we can conclude that a sowing date of July 1 and a plant density of 60/surface unit can be recommended from the point of view of economics.

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