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Full Length Research Paper

Bulk density, chemical composition and *in vitro* gas production parameters of Iranian barley grain cultivars grown at different selected climates

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Barley grain varieties adapted to grow under different climates of Iran (16 cultivars named Bahman, Makoeei, CB-79-10, Sahand, Reyhan03, Reyhan45, Fajer, Nosrat, Valfajer, Kavir, MB-82-12, AB-23-14, Nimrooz, Jenob, Dasht and Sahra) were provided (10 samples per each cultivars). Samples were assessed for bulk density (BD), chemical composition including organic matter (OM), crude protein (CP), ether extract (EE), acid detergent fiber (ADF), neutral detergent fiber (NDF), and soluble sugar. An in vitro gas production technique was also used to examine the effect of the cultivars evaluated on gas production parameters of the barley grains evaluated. Approximately, 200 mg of each sample was weighed into a 120-ml serum vial (n=4). Gas production was recorded at 2, 4, 6, 8, 12, 24, 36, 48, 72, and 96 h after the incubation. Data of 24 h gas production were also used to estimate the organic matter digestibility and metabolizable energy of the cultivars. Crude protein, soluble sugar, EE, ash, NDF and ADF concentrations of the barley samples investigated was on average of 108, 35, 30, 24, 238.4 and 72 g/kg, respectively; while the difference was significant (P< 0.001) among the cultivars. Dasht had the highest crude protein (CP) and the lowest NDF; while Makooei and Sahra showed the highest NDF and BD, respectively. The average of bulk density was 679 kg/m³ (from 615 to 720); there was a negative correlation between the bulk density and ADF (r = -0.77 and P< 0.001), while the correlation between the bulk density and metabolizable energy was positive (r = 0.69 and P< 0.05). Organic matter digestibility of the samples evaluated ranged from 75 to 81% (mean= 78%) and there was a significant differences (P< 0.01) among the cultivars.

Key words: Barley grain, digestibility, metabolizable energy, gas production.

INTRODUCTION

Barley is a principal feed grain in Iran, due to the limitations of climate and soil fertility. However, barley varieties differ in bulk density, chemical composition (Colkesen et al., 2005; Reynolds et al., 1992) and *in situ* degradability (Ghorbani and Hsdj-Hussaini, 2002). Approximately, 80 to 90% of barley starch is fermented in the rumen compared with 55 and 70% for sorghum and

*Corresponding author. E-mail: danesh@um.ac.ir. Tel: +98-511-8795618. corn, respectively (Nocek and Tamminga, 1991). Variety, location, cultivar, and environment, among other factors, interact to affect the rate and extent of ruminal cereal grain digestion (Van Barneveld, 1999). Differences in the chemical composition of barley cultivars have been reported as well as significant differences in the composition of the protein matrix and starchy endosperm of the grain (Palmer, 1993). Therefore, these differences might be expected to influence their degradability in the rumen (Colkesen et al., 2005).

In vitro gas production is a rapid method that detects small differences in nutritional characteristics between

Cultivar	Climate	BD	СР	NDF	ADF	Ash	SS	EE
Bahman	С	675 ^e	103.5 ⁿ	240.4 ^{bcde}	66.4 ^{eca}	20.9 ^e	26 [′]	28.7 ^{fegd}
Makooei	С	682 ^{cae}	110.3 ^{reg}	271.2ª	71.8 ^{becd}	25 ^{abc}	33.6 ^{dgfe}	29.8 ^{fegd}
CB-79-10	С	647 ^r	108.4 ^{rg}	232.9 ^{cde}	75.8 ^{abc}	25.5 ^{ab}	30.7 ^{ngr}	23.5 ^{nrg}
Sahand	С	672 ^e	88.7 ^J	251.4 ^{abc}	66.3 ^{ecd}	23.3 ^{bcde}	35.4 ^{ace}	21.2 ⁿ
Rayhan45	М	702 ^{abc}	115.7 ^{ac}	222.9 ^{de}	59.8 ^{ed}	24.7 ^{bcd}	27.1 ⁿⁱ	26 ^{hefg}
Rayhan03	М	677 ^{de}	111.3 ^{re}	242.5 ^{abcd}	76.1 ^{abc}	28.3 ^a	34.3 ^{dre}	26 ^{hefg}
Fajer	М	667 ^e	103 ⁿ	240.5 ^{bcde}	76.2 ^{abc}	22.5 ^{bcde}	54.3 ^a	41.8 ^a
Nosrat	М	697 ^{cbd}	95.9 [']	235.2 ^{cde}	70.4 ^{ecdb}	21.2 ^{de}	39 [°]	35.3 ^{DC}
Valfajer	М	705 ^{ab}	100.8 ⁿ	235.2 ^{cde}	58.4 ^e	21.5 ^{cde}	44.9 ⁰	36.9 ^{ab}
Kavir	М	641 ^r	115 ^c	246.5 ^{abcd}	86.6 ^a	23.9 ^{bcde}	29.3 ^{ngi}	35.9 ^{ab}
MB-82-12	М	706 ^{ab}	86.2 ^J	218.8 ^{de}	72.9 ^{bcd}	22.4 ^{cde}	44.4 ⁰	37.3 ^{ab}
AB-23-14	М	615 ⁹	120.2 ⁰	268.4 ^{ab}	81.9 ^{ab}	26 ^{ab}	36.4 ^{ca}	22.2 ^{ng}
Nimrooz	W	698 ^{cbd}	112.6 ^{de}	237.6 ^{abcd}	69.2 ^{bcde}	24.6 ^{bcd}	32.5 ^{dgre}	30.9 ^{becd}
Jenob	W	680 ^{de}	115.1 ^{ac}	226.5 ^{cde}	68.7 ^{bcde}	23.7 ^{bcde}	31.7 ^{gre}	28.7 ^{tega}
Dasht	W	715 ^{ab}	132.1 ^a	210.5 ^e	67.8 ^{cde}	23.5 ^{bcde}	20.8j	32.8 ^{bcd}
Sahra	W	720 ^a	107.3 ^g	234.9 ^{abcd}	60.7 ^{ed}	20.5 ^e	36.1 ^{dce}	29fecd
SEM	-	1.62	0.25	2.5	0.99	0.27	0.33	0.49
p-value	-	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01

Table 1. Bulk density (g/L) and chemical composition (g/kg DM) of barley grain cultivars grown at different Iran climates.

C: cold, M: moderate, W: warm, DM: dry matter, CP: crude protein, NDF: neutral detergent fiber, ADF: acid detergent fiber, SS: Soluble sugar, EE: ether extract, BD: bulk density. a,b,c,d: means within a column with differing superscripts are significantly different (P<0.05).

feedstuffs, allows for more frequent sampling compared with *in vitro* digestibility (DePeters et al., 2003). In a assessing the genetic improvement achieved through selection, the gas production method was used to provide information on DM and starch digestibility of different sorghum cultivars (Pedersen et al., 2000). The gas production method was also used to compare sorghum grain hybrids that differed in endosperm colour (Streeter et al., 1993) and to evaluate the effect of varieties, growing sites and grain species (Opatpatanakit et al., 1994). The present study was designed to determine bulk density, chemical composition, *in vitro* gas production kinetics and relationships between chemical composition and gas production kinetics of various Iranian cultivars of barley grain adapted to grow under different climates.

MATERIALS AND METHODS

Sampling and chemical composition

During summer 2009, sixteen cultivars of barley grain named Bahman, Makoeei, CB-79-10, Sahand, Reyhan03, Reyhan45, Fajer, Nosrat, Valfajer, Kavir, MB-82- 12, AB-23-14, Nimrooz, Jenob, Dasht and Sahra (10 samples per each cultivar) adapted to grow at different climates known as cold, moderate and hot (with the annual averages of daily maximum and daily minimum temperatures "16.3 and 3.1, 22.2 and 12.8, and 34.3 and 23°C, respectively, Table1) were provided (Fisher, 1968). Climate map of

Iran with barley grain cultivars selected for each climate has been shown in Figure 1. Due to the extensive areas of the cultivation, soil texture was different (Dewan and Famouri, 1961). Phosphorus (P) and potassium (K) fertilizer were applied to the each barley grain cultivar at a rate of 90 and 60 kg/ha during sowing, respectively.

Nitrogen (N) fertilizer was applied at a rate of 45 kg/ha during sowing and second applications of N fertilizers was applied at a rate of 45 kg/ha, 4 month later. Each cultivar was usually irrigated five times during the plant growth. After harvest, representative samples were analyzed for bulk density (Grain test weight scale, Seedburo Equipment Co., Chicago, IL). Samples were then ground to pass through a 1 mm sieve (Retsch Muhle mill, Retch EPP 15X20, Germany), and then were used for chemical analysis and in vitro gas production technique. Dry matter content of each sample was determined using a forced-air oven at 105°C for 24 h. Nitrogen content was determined using the Kjeldahl method (Kjeltec 2300 Autoanalyzer, Foss Tecator AB, Hoganas, Sweden) and CP was calculated as N × 6.25. Ash-free neutral detergent fiber (NDF) was determined, using thermo stable alpha amylase (Sigma A-3306), without sodium sulphite in the ND, according to Van Soest et al. (1991). Acid detergent fiber [(AOAC, 2000), ID 973.18] was determined and expressed exclusive of residual ash. Samples were also analyzed for ether extract [(AOAC, 2000), ID 920.39], and ash [(AOAC, 2000), ID 942.05] concentrations. Total sugar content was determined by an anthrone/sulphuric acid procedure (Southgate, 1976), using glucose as standard. The results for sugars are reported as glucose equivalents (in g/ 1000 g).

Gas production

Three fistulated adult Balochi male sheep (body weight: 49.5±2.5



Figure 1. Climate map of Iran with barley grain cultivars selected for each climate.

kg) were used as rumen liquor donor for gas production technique. Animals were fed a diet to meet their maintenance requirement (NRC, 1985). Sheep were fed a total mixed ration consisting of 0.8 kg DM alfalfa hay and 0.5 kg DM concentrate consisting of barley grain, sugar beet pulp, soybean meal, wheat barn and minerals (165 g CP/kg of DM). The ration was fed twice daily at 0800 and at 1500 h (Bilik and Lopuszanska-Rusek, 2010).

Rumen fluid was collected immediately before the morning feeding and strained through 4 layers of cheesecloth into a prewarmed CO₂-filled flask. All laboratory handling of rumen fluid was carried out under a continuous flow of CO₂. *In vitro* incubation of the samples was done using a manual pressure transducer technique. Approximately, 200 mg of each sample was weighed into a glass vial (n=4). Vials were pre-warmed at 39°C before the injection of 30 ml rumen fluid-buffer mixture into each bottle followed by incubation in a water bath at 39°C. Following inoculation, during gas pressure reading, vials were briefly and gently rolled to facilitate mixing and to maximize contact of the inoculums with the samples, which exhibited a slight tendency to adhere to the glass above or below the gas-liquid interface. Gas pressure measurement were made with a digital pressure gauge (model SEDPGB0015PG5 sensor unit, SenSym, Milpitas, Calif.) having a 0.01 lb/in² (or psi; 1 psi =0.06805 atmosphere) sensitivity. Measurements of the pressure and gas production were done at 2, 4, 6, 8, 12, 24, 36, 48, 72 and 96 h after the incubation. Total gas volumes were corrected for a blank incubation which contained only the buffered rumen fluid without any barley samples and weight of sample according to the equation proposed by Valentin et al. (1999):

$$GP (ml/200 \text{ mg DM}) = \frac{200 (V_t}{W} = \frac{V_0}{W} = \frac{V_b}{V_b}$$

where GP is corrected gas volume (ml), Vt is gas volume recorded in the vial containing the sample (ml) at time t (h), V₀ is volume in the vial with sample at 0.0 h of incubation, Vb is gas volume in the vial without sample, and W is weight of sample (mg). *In vitro* gas production was conducted in three run.

Data analysis

Cumulative gas production data were fitted to a model of Y = b (1-e^{ct}); where: Y = potential of gas production at time t; b = the asymptotic gas volume (ml); c = gas production constant rate (ml/h); t = incubation time (h). Metabolizable energy (ME) content and organic matter digestibility (OMD) were calculated using the equations of Menke and Steingass (1988) as:

ME (MJ/kg DM) = $0.157 \times GP + 0.0084 \times CP + 0.022 \times EE - 0.0081CA + 1.06$

OMD (%) = 0.9991 × GP + 0.0595 × CP + 0.0181 × CA + 9

where CA is ash in g/kg DM; EE is ether extract in g/kg and GP is the net gas production (ml/ 200 mg DM in 24 h).

Analysis of variance (ANOVA) was carried out to compare the bulk density, chemical composition and gas production kinetics data. The statistical significance of the differences between means was tested using the Duncan test at P<0.05. The Pearson correlations between chemical components and exponential fractional constant rate of gas productions were determined using the PROC CORR (SAS, 1999).

RESULTS AND DISCUSSION

Chemical composition of the barley grain cultivars is presented in Table 1. Bulk density was ranged from 615 to 720 g/L with a mean of 681 g/L; Sahra had the higher bulk density than those of the other cultivars (P< 0.001). The DM of barley cultivars ranged from 925 to 935 g/kg with a mean of 928.1 g/kg. Crude protein ranged from 88.7 to 132 g/kg with a mean of 107.9 g/kg. The CP content of Dasht was higher (P< 0.001) than those of the others. The NDF content of the samples ranged from 210.5 to 271.2 g/kg DM with a mean of 238.4 g/kg and Dasht had the lowest NDF level compared with those of the other cultivars. Campbell et al. (1995) reported that the CP values for six cultivars of barley grains grown at 12 different locations and indicated it ranged from 9.3 to18.2%. The CP level of the barley grain cultivars used in the present study was higher than those reported by Ghorbani and Hadj-Hussaini (2002) who showed that the CP level of 10 barley grain cultivars ranged from 90 to 117 g/kg DM. However, the range in CP content of barley grain cultivars observed in our study is still much smaller than those of 7.2 to 21.4, 9.3 to 18.2, and 12.2 to 15.9% reported by Reynolds et al. (1992), Campbell et al. (1995) and Colkesen et al. (2005), respectively. This might be due to the difference between cultivars and growing conditions. Bradshaw et al. (1992) showed that the environment and season variations might have impact on the chemical composition of barley grain cultivars. Present data of CP levels of the evaluated barley grain cultivars are similar to those reported by Ortega-Cerrilla et al. (1999b) and Yu et al. (2003).

The ADF concentration of the barley grain cultivars

used in the present study had less variance than those of reported by Ghorbani and Hadj-Hussaini (2002) and Woods et al. (2003), but were consistent with those reported by Colkesen et al. (2005). Ether extract and ash levels of the barley grain cultivars evaluated were in agreement with the findings of Shivus and Gullord (2002).

Gas production parameters and calculated amount of organic matter digestibility and metabolizable energy are presented in Table 2. The volume of gas produced ranged from 67.3 to 72 ml/ 0.2 g DM for the different cultivars with a mean ranging from 0.063 to 0.083 (ml/h). The OMD ranged from 75 to 81%, while Sahra had the highest of both OMD and metabolisable energy (P < 0.01) compared with those of the other cultivars. Among the cultivars. Sahand and Bahman had the highest and the lowest gas production constant rate. The amount of asymptotic gas volume and gas production constant rate of Sahand was significantly higher than those of the other cultivars (P< 0.01). Lanzas et al. (2007) showed that the fractional constant rate of gas production of the barley grain cultivars ranged from 0.20 to 0.29 h⁻¹. Surber and Bowman (1998) showed *in vitro* starch disappearance rates of 0.11 h^{-1} for barley (2 mm particle size). The discrepancies among studies may be attributed to methodological differences such as dissimilarities in the proportion of inoculums and buffer, type of buffer solution, processing effects and mathematical models used to evaluate the gas production parameters (Lanzas et al., 2007). Most of the kinetic data for grains are from the in situ studies. However, starch and dry matter digestion parameters derived from the in situ studies may not be directly compared with gas production technique. In situ methods assume two degradable pools: A soluble fraction that is considered to be degraded instantaneously and completely and an insoluble fraction that is degraded exponentially (Tamminga et al., 1990). In situ studies measure degradation rate for the slowly degradable pool, while gas production rates are derived from the entire degradable pool.

Correlation coefficients between chemical composition and OMD and ME of the evaluated barley grains are shown in Table 3. Acid detergent fiber was negatively correlated to OMD and ME (r = -0.619, P < 0.01 and r = -0.551, P < 0.02). Fife et al. (2008) observed lower correlation coefficients (r = -0.452, P< 0.001) between acid detergent fiber and *in vitro* digestibility. Doornbos and Newman (1989) showed a considerable variation in the chemical composition properties of the evaluated barley grain and noted that this was attributed to the variety rather than the grown location. Bulk density was positively correlated with ME and OMD (r = 0.695, P<0.01 and r = 0589, P=0.016), which agreed the results of Fife et al. (2008). Mathison et al. (1991) demonstrated that barley grain BD was not highly correlated with the

Cultivar	b (ml)	c (ml/h)	OMD (%)	ME (MJ/kg)
Bahman	71 <u>±2</u> ^{abcd}	0.06±0.01 ^e	77±0.1 ^{cd}	12.1±0.0
Makooei	70±2 ^{abcdf}	0.08±0.01 ^{ab}	78±1.5 ^{bC}	12.33±0.2 ^{bcde}
CB-79-10	70±2 ^{abcdf}	0.07±0.01	76±0.4 ^{cd}	11.9±0.1 ¹⁹
Sahand	73±2 ^a	0.08±0.01 ^a	80±0.3 ^{ab}	12.5±0.0 ^{abc}
Rayhan45	67±2g	0.08±0.01 ^{ab}	80±1.6 ^a	12.6±0.2 ^{ab}
Rayhan03	71±1 ^{abcde}	0.08±0.01 ^a	77±0.3 ^{cd}	12.1±0.1 ^{et}
Fajer	69±2 ^{cdefg}	0.08±0.01 ^a	77±0.3 ^{cd}	12.43±0.0 ^{abcd}
Nosrat	72±2 ^{ab}	0.06±0.01 ^{ed}	78±0.6 ^{cd}	12.4±0.1 ^{bcde}
Valfajer	71±2 ^{abcd}	0.08±0.01 ^{ab}	78±0.1 ^{bC}	12.5±0.0 ^{abc}
Kavir	68±2f ^g	0.07±0.01 ^{ced}	78±0.4 [°]	12.4±0.1
MB-82-12	71±2 ^{abcde}	0.08±0.01 ^{bced}	78±0.2 [°]	12.5±0.0 ^{abc}
AB-23-14	69±2 ^{erg}	0.08±0.01 ^a	75±0.6 ^d	11.7±0.1 ⁹
Nimrooz	69±2 ^{bcdegf}	0.07±0.01 ^{abc}	78±0.1 [°]	12.3±0.0 ^{bcde}
Jenob	69±2 ^{derg}	0.07±0.01 ^{abc}	78±0.6 [°]	12.2±0.1 ^{cde}
Dasht	69±2 ^{cdefg}	0.07±0.01 ^{abc}	77±0.2 ^{cd}	12.2±0.0 ^{bcde}
Sahra	72±2 ^{abc}	0.07±0.01 ^{abcd}	81±0.4 ^a	12.8±0.1 ^a
Р	<0.01	<0.01	<0.01	<0.01

Table 2. Gas production parameters, organic matter digestibility (OMD)* and metabolizable energy (ME)** content of different barley grain cultivars grown at different Iran climates.

*OMD = 0.9991 Gas + 0.0595 CP + 0.0181 CA + 9, **ME = 0.157 Gas + 0.0084 CP + 0.022 EE - 0.0081 CA + 1.06. b: the asymptotic gas volume. c: gas production constant rate.

Table 3.	Correlation coefficients ((r) between measures	of chemical com	position and OME	D and ME of barley	grain cultivars g	grown at
different	Iran climates.						

ltem	OMD ¹	СР	BD^1	ADF	NDF	Ash	FR	EE	SS	ME
OMD	1 ²	-0.24	0.59	-0.62	-0.21	-0.54	0.22	-0.05	0.01	0.89
CP	0.36	1	-0.15	0.17	0.22	0.38	0.14	-0.18	-0.61	-0.37
BD	0.02	0.59	1	-0.77	-0.31	-0.36	-0.10	0.34	-0.01	0.69
ADF	0.01	0.54	0.01	1	0.29	0.46	055	0.06	0.07	-0.55
NDF	0.45	.41	0.24	0.27	1	-0.04	-0.12	0.01	-0.15	-0.17
Ash	0.03	0.15	0.17	0.07	0.88	1	0.50	-0.09	0.13	-0.6
FR	0.42	0.62	0.70	0.84	0.66	0.05	1	-0.22	0.32	0.19
EE	0.84	0.49	0.20	0.83	0.98	0.72	0.40	1	0.52	0.44
SS	0.99	0.43	0.97	0.80	0.58	0.62	0.23	0.04	1	0.23
ME	0.01	0.01	0.01	0.03	0.51	0.01	0.94	0.09	0.35	1

¹OMD: Organic matter digestibility; CP: crude protein; BD: bulk density; ADF: acid detergent fiber; NDF: neutral detergent fiber; FR: fractional constant rate of gas production; EE: Ether extract; SS: soluble sugar; ME: Metabolizable energy. ² Coefficients above the diagonal element represent.

in situ degradability. They showed that barley grain with moderate BD (590 g/L) resulted in greater *in situ* degradability compared with those of heavier BD (640 g/L). It has been demonstrated that the environment temperature might impact the starch degradation considerably; the degradation was negatively correlated to the growth temperature (Anker-Nilssen et al., 2006). Moreover, other growth conditions might also influence

starch digestion characteristics (Tester et al., 1995; Anker-Nilssen et al., 2006).

Fractional constant rate of gas production of the samples evaluated in the present study was positively correlated with CP, ash, soluble sugar and OMD; and negatively correlated with BD, ADF, EE and NDF. There was no significant correlation between fractional constant rate of gas production and chemical composition except for ash (Table 3). Getachew et al. (2004) and Lanzas et al. (2007) found a poor correlation between the rate of gas production and chemical composition of the feed evaluated. The low correlation between chemical components and digestion parameters might be due to the narrow range of the chemical parameters (Lanzas et al., 2007). In addition, it has been demonstrated that the grain digestibility is primarily affected by the physical structure of the kernel rather than the chemical composition components.

Present results indicate that there is a significant difference in asymptotic gas volume, gas production constant rate, organic matter digestibility and metabolizable energy of different cultivars of Iranian barley grain. The result of this experiment also indicates that crude protein, ADF, and BD are positively correlated to in vitro organic matter digestibility of barley grain with ADF being the most consistent predictor. However, correlations between chemical constituents and gas production constant rates of barley cultivars were low. Results from these trials further emphasize the wide range in the variation of barley grain evaluated and underscore the need to improve upon the current method for predicting feed value of barley in livestock.

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