African Journal of Agricultural Research Vol. 7(10), pp. 1468-1474, 12 March, 2012
Available online at http://www.academicjournals.org/AJAR
DOI: 10.5897/AJAR11.391
ISSN 1991-637X © 2012 Academic Journals

Full Length Research Paper

Effect of climate on the *in vitro* first-order ruminal disappearance kinetics of dry matter in grain of semi-arid native barley cultivars

Einollah Abdi Ghezeljeh, Mohsen Danesh Mesgaran*, Hassan Nassiri Moghaddam and Alireza Vakili

Department of Animal Science, Faculty of Agriculture, Ferdowsi University of Mashhad, P.O. Box 91775-1163, Mashhad, Iran.

Accepted 8 February, 2012

An experiment was conducted to determine the *in vitro* first-order disappearance kinetics of dry matter in grain of six semi-arid native barley cultivars (*Hordeum vulgare*) adapted to grow at different climates known as cold (Bahman, Makoeei), moderate (Reyhan 03, Kavir) and hot (Dasht and Sahra). Samples (n=10) were assessed for bulk density (BD) and chemical composition. In addition, *in vitro* first-order kinetics of dry matter disappearance, medium pH and NH_3-N concentration were determined using fermentation culture. Data were fitted to an exponential equation model: $D_{(t)} = D_{(i)}.exp\ (-k.\ time) + I$; where $D_{(t)}$ was potentially a digestible residues at time *t*, $D_{(i)}$ was a potentially digestible fraction, *k* is a fractional rate constant of digestion (h^{-1}) and *I* was an indigestible fraction. Results showed there was significant effect of adaption of varieties to climate on bulk density and crude protein, ash, NDF (neutral detergent fiber), ADF (acid detergent fiber), soluble sugars and starch concentrations in grain of the barley cultivars evaluated ($P < 0.01$). Barley grain cultivars from cold climate had the highest concentration of NDF and starch, whilst hot climate cultivars showed the highest amount of BD and CP (crude fiber). The amount of ash, ADF and soluble sugar in moderate climate barley grain cultivars was higher than those of the other climates. *In vitro* first order disappearance of dry matter was significantly ($P < 0.01$) influenced by the climate. Digestible fraction, fractional rate constant of digestion and indigestible fraction of grain from cold (0.78, 0.15, 0.146), moderate (0.77, 0.16, 0.157) and hot (0.75, 0.19, 0.206) were not similar in pattern. Dasht and Bahman grains had the highest and the lowest fractional rate constant (0.23 and 0.12 h^{-1}, respectively). Barley grain cultivars from cold and hot climates had the largest digestible fraction and fractional rate constant of digestion, respectively. The relationship between NDF content (g/kg DM) and fractional rate constant of digestion (*X*) was negative: $NDF = -520.9X + 334.2$, $R^2 = 0.723$. It was concluded that ruminal dry matter disappearance kinetic differences do exist between barley grains cultivars obtained from different climates.

Key words: Barley, grain, climate, disappearance, starch.

INTRODUCTION

Starch digestion rate is important in regard to the risk of rumen acidosis, as a high rate of starch degradation in the rumen causes a severe drop in ruminal pH, which may reduce microbial protein synthesis, fiber digestion and feed intake, as well as, negatively impact the metabolic status of the animal (Hunt, 1996; Huntington, 1997; Nocek, 1997; Mills et al., 1999). Experiments on the metabolizable energy (ME) suggested a considerable variation between different barley cultivars (Anker-Nilssen et al., 2006). Moreover, growth conditions might also influence the grain starch digestion characteristics (Tester et al., 1995; Anker-Nilssen et al., 2006). These variations may be related to difference in starch granule

*Corresponding author. E-mail: danesh@um.ac.ir. Tel: +98-511-8795620. Fax: +98-511-8787430.

structure and associated components (Anker-Nilssen et al., 2006). In addition, there is a considerable variation in ruminal starch degradability among cereal species and also among cultivars within species (McAllister and Cheng, 1996; Mills et al., 1999; Offner et al., 2003; Svihus et al., 2005). Result of studies conducted by Kemalyan et al. (1991), Hartfield et al. (1993) and Khorasani et al. (2000) have confirmed that differences in the rate of degradation may occur between barley cultivars. Abdi et al. (2011) also showed that Iranian barley grain had different rumen fermentation characteristics. Barley grain cultivars are often developed and selected on the basis of agronomic and malting-quality characteristics only (Bowman et al., 2001). Therefore, barley breeding experts do not consider the nutritional characteristics for livestock. The objective of this study was to determine the *in vitro* first-order kinetics of dry matter disappearance, and mean pH and NH_3-N concentration of Iranian barley grain cultivars, adapted to semi-arid conditions in different climates including cool, moderate and hot, using fermentation culture.

MATERIALS AND METHODS

Sampling and chemical composition

During summer 2009, six cultivars of barley (*Hordeum vulgare*) named Bahman, Makoeei, Rayhan03, Kavir, Dasht and Sahra (10 samples per each cultivar) adapted to growth at different semi-arid climates known as cool, 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) were provided from the Seed and Plant Improvement Institute, Karaj, Iran. Phosphorus (P) and potassium (K) fertilizer were applied 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 for 4 months later. Barley was irrigated five times between seeding and harvest. Samples of each cultivar were pooled and a general sample was prepared. Barley grain 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 (RetschMuhle mill, Retsch EPP 15 × 20, Germany) and dried using a forced-air oven at 60°C for 48 h. Nitrogen content was determined using the Kjeldahl method (Kjeltec 2300 Autoanalyzer, Foss Tecator AB, Hoganas, Sweden) and CP (crude protein) 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 was determined and expressed exclusive of residual ash (AOAC, 2000), ID 973.18. 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 were reported as glucose equivalents (in g/1000 g). Starch was calculated as 0.9 × glucose content (Åman and Hesselman, 1984).

Substrates and medium preparation

The fermentation medium was prepared according to Arroquy et al. (2005) and reported by Rezaee et al. (2011). In a 1000-ml flask cellobiose 0.05 g, mineral I ;150 ml, mineral II; 150 ml, rumen fluid 400 ml, resazurin; 1 ml, distilled water; 300 ml, sodium carbonate; 4 g, and cysteine-HCL; 0.50 g were added, and boiled to eliminate oxygen from the media. Mineral I solution was composed of 3.0 g K_2HPO_4 in 1000-ml. Mineral II was made with KH_2PO_4 3.00g, NaCl 6 g, $(NH4)SO_4$ 6.0 g, $MgSO_4$, 7 H_2O 0.6 g, and $CaCl_2$. Rumen fluid was collected from three fistulated adult Balochi male sheep (body weight, 49.5 ± 2.5 kg). Sheeps were fed a total mixed ration including 0.8 kg DM alfalfa hay and 0.5 kg DM concentrate consisting of barley grain, sugar beet pulp, soybean meal and wheat bran (165 g CP/kg of DM). The ration was fed twice daily at 0800 and 1800 h. Rumen fluid was collected immediately before the morning feeding and strained through 4 layers of cheesecloth into a pre-warmed CO_2-filled flask, then centrifuged at 3000 RPM for 5 min. The supernatant was then centrifuged at 15000 RPM for 15 min. 45 ml of the medium was transferred into a 100-ml bottle containing the experimental sample and autoclaved at 120°C for 20 min. Each bottle was inoculated with 5 ml of cheesecloth strained rumen fluid and finely bubbled with CO_2, sealed and incubated. Prior to inoculation, the rumen fluid was incubated for 1 h in an incubation chamber at 39°C to allow large feed particles to rise to the top; taking care neither to include the large particles that rose to the top nor those that got sunk to the bottom. All bottles were crimped and placed in the incubator at 39°C, shaking them at regular intervals. Incubation was done for 2, 4, 8, 12, 16, 24, 36 and 48 h. At each sampling time, the fermentation bottles for that time were removed from the incubator, and the pH of each bottle was recorded directly (691 pH Meter, Metrohm, Series 01, Switzerland). The bottle content was filtered through nylon cloth (50 µm pore size). The remaining fraction after filtering was used for measuring dry matter (using air forced oven at 48°C for 48 h). The filtered fermentation fluid was analyzed for ammonia-N concentration using the steam distillation method (Kjeltec Auto 1030 Analyzer tecator, Hoganas, Sweden). This experiment was conducted in three runs, three bottles for each cultivar, at each incubation on each run.

Calculations and statistical analysis

In vitro first order parameters of dry matter disappearance of barley grain were determined using a first order exponential model. The model was:

$$D_{(t)} = D_{(i)}.exp\ (-k.\ time) + I$$

Where: $D_{(t)}$ = potentially digestible residues at any time
$D_{(i)}$ = potentially digestible fraction at any time
k = fractional rate constant of digestion (/*h*)
I = indigestible fraction at any time

The data for bulk density, chemical composition, medium pH, ammonia-N concentration and the quantity of dry matter disappearance were analyzed as a nested design, climate as the main treatment and within each climate cultivars of barley grain as the sub treatment (Snedecor and Cochran, 1980) by using the general linear model procedure of SAS, version 9.2 (SAS, 2003). The LSMEANS option was used to calculate and compare treatment means at P< 0.05. The statistical model was as shown:

$$Y_{ijk} = \mu + C_i + B_j(C_i) + \varepsilon_{ijk}$$

Where Y is the analyzed variable, μ is the overall mean, C_i is the effects of the climate (i= 1...3), $B_j(C_i)$ is the effect of the barley grain cultivar within the climate (j= 1…2) and ε is the residual.

RESULTS AND DISCUSSION

Bulk density and chemical composition of the barley grain

Table 1. Bulk density (g/l) and chemical composition (g/kg DM) in grain of the Iranian barley cultivars grown at different semi-arid climates including cold, moderate and hot.

Item	Cultivar						Climate			SEM	Significance	
	Makooei	Sahra	Reyhan 03	Bahman	Dasht	Kavir	Cold	Hot	Moderate		Cultivar	Climate
Bulk density	682^{b}	720^{a}	677^{b}	675^{b}	715^{a}	641^{c}	678.5^{b}	717^{a}	659^{c}	3.38	**	*
Crude protein	110.3^{c}	107.3^{cd}	111.3^{c}	103.6^{d}	132.1^{a}	116.5^{b}	107.0^{c}	119.7^{a}	113.7^{b}	0.56	**	**
Natural detergent fiber	270.1^{a}	243.3^{ab}	244.6^{ab}	243.8^{ab}	210.5^{b}	249.5^{a}	262.3^{a}	229.2^{b}	246.4^{ab}	4.1	**	*
Acid detergent fiber	71.8^{cb}	60.7^{c}	76.1^{b}	66.4^{cb}	67.8^{cb}	91.9^{a}	69.1^{b}	64.3^{b}	84.1^{a}	1.59	**	*
Ash	25.0^{b}	20.5^{d}	28.3^{a}	20.9^{cd}	23.5^{bc}	23.9^{b}	23.0^{b}	22.0^{b}	26.9^{a}	0.33	**	**
Soluble sugar	33.6^{ab}	36.1^{a}	34.3^{a}	26^{c}	20.8^{d}	29.3^{bc}	29.8^{ab}	28.4^{b}	31.8^{a}	0.53	**	**
Ether extract	27.8	29	26.3	28.7	32.8	35.9	28.3	30.9	31.1^{a}	1.11	NS	NS
Starch	586.2^{a}	551.7^{ab}	560.6^{ab}	553.8^{ab}	525.6^{b}	522.5^{b}	570.0^{a}	538.7^{b}	541.5^{b}	4.48	*	*

a, b, c, d Means within each row with differing superscripts are significantly different (P<0.05). SEM: Standard error of mean<0.05. *P< 0.05. ** P< 0.01. NS: Non-significant.

evaluated are presented in Table 1. The bulk density and crude protein content of the hot climate cultivars were higher (P < 0.001) than those of the other climates. As seen in Table 1, no significant difference in ether extract content was found in the comparisons made among the barley grain cultivars and climates (P > 0.05) evaluated under the present study condition. It has been previously reported that barley grains from cold climate have low fat content (Anker-Nilssen et al., 2006). Kiseleva et al. (2003) demonstrated that lipid content of starch is very sensitive to climate, with increasing temperature increasing fat content. It was proposed that the proportion of amylose–lipid complex (in cereal starches) increases with increasing growth temperature (Morrison, 1995). Tester (1997) showed that an increase of growth temperature leads to an increase of lipid content and a decrease of amylose content in barley starches. The soluble sugar content of barley grain cultivars in the moderate climate evaluated was higher (P < 0.05) in our experiment. The starch content of the cold climate barley grains was higher (P < 0.05) than those of the other cultivars. The starch content ranged from 522.5 to 586.2 g/kg with a mean of 552.3 g/kg; the highest (P < 0.05) concentration of starch was found in Makooei cultivar, whilst the lowest content of starch was found in a Kavir cultivar (Table 1). The mean of starch content of the barley grain cultivars used in the present study was lower than those reported by Holtekjolen et al. (2006) who showed that starch level of 39 barley grain cultivars ranged from 531.1 to 642 g/kg with a mean of 588 g/kg DM. Stevnebø et al. (2009) also reported that the starch content of 12 barley grain cultivars with variable amylose content ranged from 474 to 605 g/kg with a mean of 563 g/kg. The result of Herrera-Saldana et al. (1990) also indicated that the starch level of barley grain was 643 g/kg DM. The present study demonstrates that the total starch content decreased with increasing growth temperature. This can be explained by impaired starch synthesis as a result of higher growth temperature, leading to less starch per endosperm and smaller starch granules (Tester et al., 1995), or a reduction in numbers of initiated granules, rather than size (MacLeod and Duffus, 1988). The yield of a grain is determined by the rate of grain filling and the duration of the grain filling period (Wiegand and Cuellar, 1981). Grain yield is normally closely positively related to starch mass accumulated during grain development (Tester and Karkalas, 2001). The growth temperature is of course only one of several environmental factors that may affect the grain yield and the properties of starch, but it is thought to be the most important one (Tester and Karkalas, 2001).

The average fraction of dry matter disappearance in grain of barley cultivars grown at different semi-arid climates are presented in Table 2. During the initial incubation time (2, 4, 8 and 12 h), the dry matter disappearance rates of the hot climate barley grains were higher (P < 0.05) than those of the others, but when the incubation time was longer (24, 36 and 48 h) the dry matter disappearance of cold climate barley grains was increased (P < 0.05). This might be related to the higher starch content of the cold climate barley grains (Table 1).

The *In vitro* first-order parameters of dry matter disappearance in grain of the barley cultivars are

Table 2. The average fraction of dry matter decomposed in grain of Iranian barley cultivars grown at different semi-arid climates including cold, moderate and hot.

Incubation time (h)	Cultivar						Climate			SEM	Significance	
	Makooei	Sahra	Reyhan03	Bahman	Dasht	Kavir	Cold	Hot	Moderate		Cultivar	Climate
2	0.397^{ab}	0.434^{a}	0.390^{ab}	0.334^{c}	0.388^{b}	0.385^{b}	0.367^{b}	0.411^{a}	0.388^{ab}	0.006	*	*
4	0.490^{d}	0.512^{a}	0.482^{b}	0.438^{c}	0.501^{ad}	0.410^{e}	0.460^{b}	0.508^{a}	0.455^{c}	0.002	**	**
8	0.578^{bc}	0.597^{a}	0.572^{c}	0.513^{d}	0.608^{a}	0.562^{c}	0.541^{c}	0.601^{a}	0.567^{b}	0.003	**	**
12	0.692^{b}	0.740^{a}	0.730^{a}	0.648^{c}	0.701^{b}	0.709^{b}	0.670^{b}	0.722^{a}	0.713^{a}	0.002	**	**
16	0.747^{b}	0.802^{a}	0.771^{b}	0.778^{ab}	0.757^{b}	0.750^{b}	0.762	0.780	0.761	0.004	**	NS
24	0.794^{bc}	0.836^{a}	0.771^{c}	0.835^{a}	0.788^{bc}	0.817^{ab}	0.815^{a}	0.812^{a}	0.781^{b}	0.003	**	**
36	0.866^{a}	0.856^{a}	0.871^{a}	0.868^{a}	0.795^{b}	0.859^{a}	0.867^{a}	0.830^{b}	0.866^{a}	0.002	**	**
48	0.880^{b}	0.830^{a}	0.890^{ab}	0.888^{ab}	0.823^{c}	0.888^{ab}	0.884^{a}	0.860^{b}	0.889^{a}	0.001	**	**

a, b, c, d: means within a row with differing superscripts are significantly different (P < 0.05). *P < 0.05. ** P< 0.01. NS: Non-significant.

Table 3. The dry matter mean (± SEM) of first-order potentially digestible fraction (D), fractional rate constant of digestion (K) and indigestible fraction (I) in grain of the Iranian barley cultivars grown at different semi-arid climates including cold, moderate and hot.

Item	Cultivar						Climate			SEM	Significance	
	Makooei	Sahra	Reyhan 03	Bahman	Dasht	Kavir	Cold	Hot	Moderate		Cultivar	Climate
D	0.76±0.035^{b}	0.76±0.030^{b}	0.77±0.031^{ab}	0.80±0.029^{a}	0.74±0.025^{b}	0.78±0.032^{ab}	0.78±0.029^{a}	0.75±0.008^{b}	0.77±0.004^{ab}	2.33	*	*
I	0.17±0.021^{b}	0.16±0.015^{b}	0.16±0.016^{b}	0.13±0.021^{c}	0.22±0.012^{a}	0.15±0.021^{b}	0.15±0.021^{b}	0.19±0.031^{a}	0.16±0.006^{b}	0.27	**	**
K	0.17±0.020^{b}	0.18±0.016^{b}	0.17±0.016^{b}	0.12±0.011^{d}	0.23±0.018^{a}	0.14±0.014^{c}	0.146±0.023^{b}	0.206±0.022^{a}	0.157±0.015^{b}	0.002	**	**
R^2	0.94	0.97	0.97	0.99	0.98	0.99	0.98	0.95	0.97			

a, b, c, d: means within a row with differing superscripts are significantly different (P < 0.05). *P < 0.05. ** P < 0.01. NS: Non-significant.

presented in Table 3. Potentially digestible fractions of the cultivars ranged from 0.74 to 0.80 with a mean of 0.768. Bahman, one of the cold climate barley cultivars, had the highest and Dasht, one of the hot climate barley cultivars, had the lowest dry matter first order digestible fraction (P < 0.05). As seen from the data presented in Table 3, potentially digestible fractions in grain of the barley cultivars of cold climate were higher (P < 0.05) than those of the hot climate. The results of this experiment supports the finding of Anker-Nilssen et al. (2006) who reported that the growth temperature influenced the starch degradation considerably and barley cultivar dry matter degradation was negatively correlated to the growth temperature. The reason why and how the growth temperature affect starch degradation is complicated to explain, but several possibilities can be discussed. The shift in ratio between soluble and insoluble β-glucans could be an explanation for the decrease in starch degradation with increasing growth temperature. It is possible that the increase in soluble β-glucans actually leads to increased viscosity even in the small test tubes using *in vitro* method, thereby slowing down the starch degradation (Anker-Nilssen et al. 2006). Another explanation could be related to the protein, as the content increases with growth temperature (Table 1). The differences in degradation might also be caused by differences in the starch–protein matrix of the endosperm tissue. McAllister et al. (1993) suggested that the protein matrix surrounding starch granules is a major factor

Table 4. The mean of *in vitro* medium pH in grain of the Iranian barley cultivars grown at different semi-arid climates including cold, moderate and hot; measured though the incubation.

Incubation time	Cultivar						Climate			SEM	Significance	
(h)	Makooei	Sahra	Reyhan 03	Bahman	Dasht	Kavir	Cold	Hot	Moderate		Cultivar	Climate
4	6.64^{ab}	6.6^{ab}	6.60^{ab}	6.64^{ab}	6.66^{a}	6.58^{b}	6.64^{a}	6.63^{ab}	6.59^{b}	0.008	*	*
8	6.53^{ac}	6.43^{c}	6.47^{bc}	6.53^{a}	6.50^{ab}	6.50^{ab}	6.5	6.46	6.48	0.006	**	NS
12	6.38^{bc}	6.36^{c}	6.39^{b}	6.42^{a}	6.42^{a}	6.39^{b}	6.4	6.39	6.39	0.003	**	NS
16	6.33	6.32	6.35	6.35	6.29	6.29	6.34	6.31	6.36	0.009	NS	NS
24	6.27^{cb}	6.24^{c}	6.28^{b}	6.29^{b}	6.32^{a}	6.32^{cb}	6.28	6.28	6.27	0.003	**	NS
36	6.27^{d}	6.31^{c}	6.32^{bc}	6.32^{bc}	6.37^{a}	6.37^{a}	6.29^{b}	6.34^{a}	6.33^{a}	0.004	**	**
48	6.42^{a}	6.32^{b}	6.34^{b}	6.32^{b}	6.35^{b}	6.35^{b}	6.37	6.33	6.33	0.009	*	NS

a ,b, c, d: means within a row with differing superscripts are significantly different (P < 0.05). *P < 0.05. ** P < 0.01. NS: Non-significant.

responsible for differences in the ruminal starch digestion of cereal grains. The indigestible fractions of the barley grains evaluated in the present experiment are shown in Table 3. Bahman cultivar had the lowest and Dasht cultivar had the highest indigestible fraction as compared with those of the other cultivars (P < 0.05). The indigestible fraction of the cultivars obtained from the hot climate was higher (P < 0.05) than those of the other cultivars. The fractional rate constant of digestion in grain of barley cultivars evaluated in the present study ranged from 0.12 to 0.23 (/h) with a mean of 0.168 (/h). Bahman cultivar with the lowest and Dasht cultivar with the highest fractional rate constant of digestion were compared with those of the other cultivars (P< 0.05). Values for fractional rate constant of digestion for the barley cultivars (Table 3) were within the range of those found by the others using *in vitro* techniques (Sveibjornsson et al., 2007; Fox et al., 2003). Among different climates, barley grain cultivars obtained from the hot climate had the highest fractional rate constant of digestion as compared with those of the other cultivars (P < 0.05). This could be due to different amounts of NDF in these barley grain cultivars. The NDF fraction, directly or indirectly, has a clear impact on the ruminal digestion rate of barley grain starch (Stevnebo et al., 2009). In summary, it appears that the fractional rate constant of digestion of barley grain cultivars is positively correlated with the growth temperature.

Relationship between NDF and starch content (g/kg DM) in grain of the barley cultivars grown at different semi-arid climates including cold, moderate and hot with fractional rate constant of digestion (X) were conformed to NDF = -520.9X + 334.2, R^2= 0.723 and starch = 306.2X + 484.1, R^2= 0.116 Figure 1. Therefore, it is obvious that with an increase in the NDF content, fractional rate constant of digestion decrease. The results support findings of Stevnebø et al. (2009) who achieved similar results. Correlation between starch content and the fractional rate constant of digestion were poor (R^2= 0.12). Getachew et al. (2004), Lanzas et al. (2007) and Abdi et al. (2011) also found a poor correlation between the rate of gas production and the chemical composition of grain evaluated. The low correlation between starch content and digestion parameters might not be explained here (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.

The mean of *in vitro* medium pH in grain of barley cultivars at different incubation time are presented in Table 4. In all cultivars, the medium pH decreased from 4 to 24 h incubation. In an experiment of Rezaii et al. (2010), the pH of *in vitro* medium declined through the first 24 h and then stabilized. There was a significant difference (P < 0.05) *in vitro* medium pH of barley grain cultivars at each incubation time, excepting the 16 h incubation. The lowest pH appeared in Sahra (Table 4), one of the hot climate cultivars; and this could be due to its fractional rate constant of digestion.

The mean *in vitro* ammonia-N concentration in grain of barley cultivars at different incubation times is shown in Table 5. There was a significant difference (P < 0.05) among *in vitro* medium ammonia-N concentration in grain of barley cultivars sampled after 4, 8, 12, 24 and 48 incubations. The highest score of ammonia-N concentration of the medium incubated with Bahman cultivar was observed after 24, 36 and 48 h. In all cultivars, ammonia-N concentration curve was sigmoid, such that, after the initial increase in its concentration in the 4 and 8 h after incubation, the concentration gradually decreased with continued incubation time and at the end of the incubation time such that in 48 h after incubation, the highest concentration of ammonia-N was obtained.

When a comparison was made with the trend obtained from the *in vivo* research, the highest concentration of ammonia nitrogen in the initial time of incubation was obtained after 8 h of incubation, and then a decrease in incubation was observed. The concentration at the end of the incubation was inconsistent with the *in vivo* research results, due to the closed environments, secondary fermentation and degradation of microorganisms. Rezaii et al. (2011) showed that the incubation time had a significant effect (P < 0.01) on ammonia-N concentration.

Table 5. The mean of *in vitro* medium ammonia-N concentration (mg/dl) in grain of the Iranian barley cultivars grown at different semi-arid climates including cold, moderate and hot; measured trough the incubation.

Incubation time (h)	Cultivar						Climate			SEM	Significance	
	Makooei	Sahra	Reyhan 03	Bahman	Dasht	Kavir	Cold	Hot	Moderate		Cultivar	Climate
4	25.7^{a}	24.3^{b}	19.7^{c}	20.2^{c}	20.0^{c}	20.4^{c}	20.1^{c}	24.3^{a}	20.0^{a}	0.09	**	**
8	22.7^{a}	18.6^{b}	24.1^{a}	19.8^{b}	24.4^{a}	25.6^{a}	24.9^{a}	21.5^{b}	21.5^{b}	0.36	**	**
12	21.5^{bd}	22.8^{b}	17.1^{c}	23.5^{ab}	18.3^{cd}	21.0^{bc}	22.5^{a}	20.5^{ab}	19.1^{b}	0.54	*	*
16	18.7	19.3	20.9	20.3	17.1	20.3	19.3	18.4	20.6	0.50	NS	NS
24	22.1^{ab}	17.1^{c}	20.1^{ab}	21.2^{ab}	24.6^{a}	21.8^{b}	21.6	20.8	21.0	0.69	*	NS
36	22.3	21.5	17.8	21.2	26.1	23.6	21.8	23.8	20.7	0.76	NS	NS
48	27.6^{ab}	24.6^{b}	27.6^{ab}	24.8^{b}	30.8^{a}	28.3^{ab}	26.2	27.7	27.9	0.45	**	NS

a, b, c: means within a row with differing superscripts are significantly different ($P < 0.05$). *$P < 0.05$. ** $P < 0.01$. NS: Non-significant.

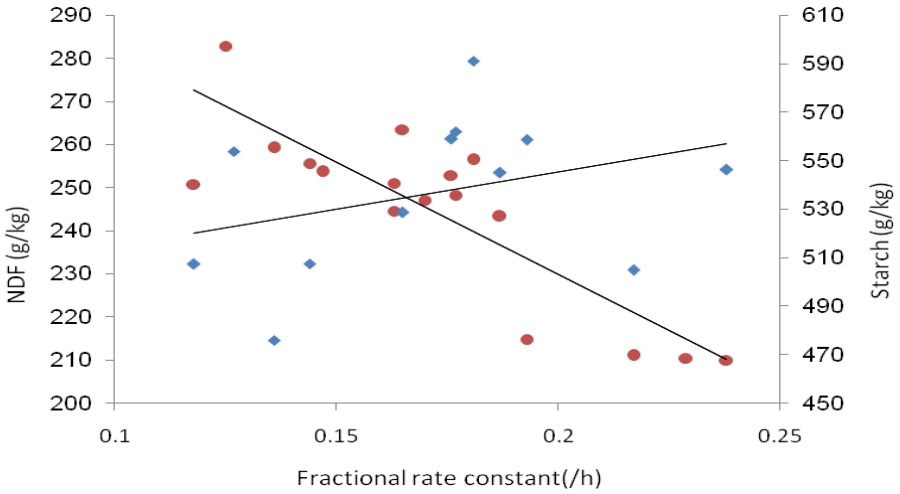

Figure 1. Relationship between NDF (●) and starch (■) content (g/kg DM) in grain of six Iranian barley cultivars grown at different semi-arid climates including cold, moderate and hot with fractional rate constant of digestion (X) were as *NDF* = -520.9*X* + 334.2, R^2= 0.723 and starch = 306.2*X* + 484.1, R^2= 0.116).

Ammonia-nitrogen concentration in the rumen fluid of cows (22 mg/dl) fed unprocessed grain has been reported (Tothi et al. 2003), and this is very consistent with the results of the present experiment. It was concluded that ruminal dry matter disappearance kinetic differences do exist between semi-arid barley grain cultivars obtained from different climates and that NDF content is a useful predictor of the grain digestibility. Furthermore, since ruminal dry matter disappearance kinetics and starch concentration were not correlated, rates of starch disappearance in barley grain could not be estimated from DM disappearance rates.

ACKNOWLEDGEMENTS

The authors wish to acknowledge the financial support received from the Ferdowsi University of Mashhad, Iran; Project Number 14789/1.

REFERENCES

Abdi GE, Danesh MM, Nassiri MH, Vakili A (2011). Bulk density, chemical composition and *in vitro* gas production parameters of Iranian barley grain cultivars grown at different selected climates. Afr. J. Agric. Res., 6: 1226-1232.

Åman P, Hesselman K (1984). Analysis of starch and other main constituents of cereal grains. Swedish J. Agric. Res., 14: 135-139.

Anker-Nilssen K, Færgestad EM, Sahlstrøm S, Uhlen AK (2006). Interaction between barley cultivars and growth temperature on starch degradation properties measured *in vitro* Anim. Feed Sci. Technol., 130: 3–22.

Arroquy JI, Cochran RC, Nagaraja TG, Titgemeyer EC, Johnson DE (2005). Effect of types of non-fiber carbohydrate on *in vitro* forage fiber digestion of low-quality grass hay. Anim. Feed Sci. Technol., 120: 93–106.

Bowman JGP, Blake TK, Surber LMM, Habernicht DK, Bockelman H (2001). Feed-quality variation in the barley core collection of the USDA National Small Grains Collection. Crop Sci., 41: 863–870.

Fox GG, Tylutki P, Tedeschi LO, Van Amburg ME, Chase LE, Pell AN, Overton TR, Russel JB (2003). The net carbohydrate and protein system for evaluating herd nutrition and nutrient excretion. Version 5.0. Animal Science Department Mimeo 213. Cornell University, Ithaca, NY, USA.

Getachew G, Robinson PH, DePeters EJ, Taylor SJ (2004). Relationships between chemical composition, dry matter degradation and *in vitro* gas production of several ruminant feeds. Anim. Feed Sci. Technol., 111: 57–71.

Hartfield PG, Peterson MK Clark CK, Glimp HA, Hemenway KJ, Ramsey WS (1993). Effects of barley variety and restricted versus *ad libitum* intake on rate, site and extent of digestion by wethers fed a high-energy diet. J. Anim. Sci., 71: 1390-1394.

Herrera-Saldanar E, huber J, Poore MH (1990). Dry matter, crude protein, and starch degradability of five cereal grains. J. Dairy Sci., 73: 238-2393.

Holtekjølen AK, Uhlen AK, Brathen E, Sahlstrőm S, Knutsen SH (2006). Contents of starch and non-starch polysaccharides in barley varieties of different origin. Food Chem., 94: 348–358.

Hunt CW (1996). Factors affecting the feeding quality of barley for ruminants. Anim. Feed Sci. Technol., 62: 37–48.

Huntington GB (1997). Starch utilization by ruminants: from basics to the bunk. J. Anim. Sci., 75: 852-867.

Kemalyan RE, Clark CK, Peterson MK, Newman CW (1991). *In vitro* dry matter disappearance rate and purine accumulation of proanthyocyanidine-free barley cultivars. Proc. West. Sect. Am. Soc. Anim. Sci., 40: 414-417.

Khorasani GR, Helm JH, Kennely JJ (2000). *In situ* rumen degradation characteristics of sixty grains as influenced by particle size. J. Dairy Sci. (Suppl. 1), 180. (Abstr).

Kiseleva VI, Tester RF, Wasserman LA, Krivandin AV, Popov AA , Yuryev VP (2003). Influence of growth temperature on the structure and thermodynamic parameters of barley starches. Carbohydrate Polymers. 51: 407–415.

Lanzas C, Fox DG, Pell AN (2007). Digestion kinetics of dried cereal grains. Anim. Feed Sci. Technol., 136:265–280.

MacLeod LC, Duffus CM (1988). Temperature effects on starch granules in developing barley grains. J. Cereal Sci., 8: 29–37.

McAllister TA Cheng KJ (1996). Microbial strategies in the ruminal digestion of cereal grains. Anim. Feed Sci. Technol., 62: 29–36.

McAllister TA, Philippe RC, Rode LM, Cheng KJ (1993). Effect of the protein matrix on the digestion of cereal grains by ruminal microorganisms. J. Anim., Sci., 71: 205–212.

Mills JAN, France J, Dijkstra J (1999). A review of starch digestion in the lactating dairy cow and proposals for a mechanistic model: 1 dietary starch characterization and ruminal starch digestion. J. Anim. Feed Sci. Technol., 8: 291–340.

Morrison WR (1995). Starch lipids and how they relate to starch granule structure and functionality. Cereal Foods World, 40: 437-446.

Nocek JE (1997). Bovine acidosis: implications on laminitis. J. Dairy Sci., 80:1005-1028.

Offner A, Bach A, Sauvant D (2003). Quantitative review of *in situ* starch degradation in the rumen. J. Anim. Feed Sci. Technol., 106: 81–93.

Rezaii F, Danesh Mesgaran M, Heravi Mousavi AR (2010). Effect of non-fiber carbohydrates on *in vitro* first order kinetics disappearance of cellulose. Iranian J. Veterin. Res., 11: 139-144.

Rezaii F, Danesh Mesgaran M, Heravi Mousavi AR (2011). Effect of the source of non-fiber carbohydrates on *in vitro* first order ruminal disappearance kinetics of dry matter and NDF of various feeds. Iranian J. Veterin. Res., 12: 222-229.

SAS (2003). Statistical Analysis System. User's Guide: Statistics, Version 9.2. SAS Institute, Cary, NC, USA.

Snedecor GW, Cochran WG (1980). Statistical methods, 7th ed., The Iowa State University Press, Ames, Iowa, USA.

Stevnebø A, Seppälä A, Harstad OM, Huhtanen P (2009). Ruminal starch digestion characteristics *in vitro* of barley cultivars with varying amylose content. Anim. Feed Sci. Technol., 148: 167-182.

Sveibjornsson J, Murphy M, Uden P (2007). *In vitro* evaluation of starch degradation from feeds with or without heat-treatments. Anim. Feed Sci. Technol., 132: 171–185.

Svihus B, Uhlen AK, Harstad OM (2005). Effect of starch granule structure, associated components and processing on nutritive value of cereal starch: A review. Anim. Feed Sci. Technol., 122: 303–320.

Tester RF (1997). Influence of growth conditions on barley starch properties. Inter. J. Biol. Macromol., 21: 37-45.

Tester RF, Karkalas J (2001). The effects of environmental conditions on the structural features and physicochemical properties of starches. Starch 53: 513–519.

Tester RF, Morrison WR, Ellis RH, Piggott JR, Batts GR, Wheeler TR, Morrison JIL Hadley P Ledward DA (1995). Effect of elevated growth temperature and carbon-dioxide levels on some physiochemical properties of wheat-starch. J. Cer. Sci., 22: 63–71.

Tothi R, Lund P, Weisbjerg MR, Hvelplund T (2003). Effect of expander processing on fractional rate of maize and barley starch degradation in the rumen of dairy cows estimated using rumen evacuation and in situ techniques. Anim. Feed Sci. Technol., 104: 71–94.

Wiegand CL, Cuellar JA (1981). Duration of grain filling and kernel weight of wheat as affected by temperature. Crop Sci., 21: 95–101.