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EFFECTS OF HYDROXYCINNAMIC ACIDS (FERULIC AND P-COUMARIC ACIDS) IN BARLEY CULTIVARS ON CELL WALL COMPONENTS DEGRADABILITY IN RUMEN

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Abstract. Barley grain contains hydroxycinnamic acids especially Ferulic (FA) and p-Coumaric acid (pCA) become cross-linked to cell wall polysaccharids as lignification commences that are the major inhibiting factors of biodegradability of plant cell walls in the rumen. Chemical characteristics, FA and pCA content of 11 Iranian barley cultivars determined. Using 3 fistulated ewes, the effects of FA and pCA content on ruminal degradation of dry matter (DM), neutral and acid detergent fiber (NDF and ADF) and lignin were studied. In barley cultivars, DM varied from 82.52 to 90.90 %; NDF varied from 9.64 to 27.34 % DM; ADF varied from 2.03 to 8.13 % DM and lignin varied from 0.87 to 3.03 % DM. The FA content ranged from 151.2 to 354.2 μg/g; and pCA content ranged from 114.5 to 444.4 μg/g of DM. Ruminal degradation parameters for DM, NDF, ADF and lignin were different between barley cultivars. The soluble fraction, slowly degradable, potential degradable, and undegradable fraction of DM were 2.92 to 56.33%; 42.64 to 91.45%; 65.68 to 98.97%, and 1.02 to 34.31%, respectively. The rate of ruminal degradation for DM varied among barley cultivars from 3.64 to 27.81% h^{-1}. The FA was related to rumen indigestible DM, NDF, ADF and lignin, while pCA correlated with ADF. Using multi-regression, FA and pCA were inhibiting factors of ruminal degradability for DM and cell wall components; and FA was the most effective factor to predict DM degradability, while both FA and pCA affected NDF and ADF ruminal degradability.

Key words: hydroxycinnamic acid, Ferulic acid, p-coumaric acid, barley, rumen, degradability

Introduction

Recently, increasing corn prices resulted in using more barely grain as main starch sources in dairy cattle rations. In barley *(Hordeum vulgare L.)*, the starch- and protein-laden endosperm is surrounded by a pericarp encased in a fibrous hull both of which are extremely resistant to damage by chewing and microbial degradation *(Beauchemin et al. 1994)*. Barley grain contains predominant phenolic compounds or low molecular weight hydroxycinnamic acids including FA and pCA *(Hernanz et al. 2001)*. The rate and extent of ruminal degradation of plant cell wall is negatively impacted by complex components such as lignin, cellulose, lignin-carbohydrate, and phenolic-carbohydrate, as well as FA and pCA is believed to be the major inhibiting factors to the ruminal biodegradability of plant cell walls *(Yu et al. 2005)*. However, livestock performance can be improved by increasing the digestibility of feeds.

The FA rapidly deposits in the cell walls at the early stage of lignification, subsequently pCA residue deposits continuously throughout the lignification *(Brett et al. 1999)*. The acylation of polysaccharides was done via feruloyl-CoA, coumaroyl-CoA, and the secretion of phenolic precursors, such as hydroxycinnamates amides and esters into the cell wall of dicotyledons, which were oxidatively linked to the cell wall polymers. The cell walls polysaccharids become cross-linked to monolignols via Hydroxycinnamic acids as lignification commences *(Santiago et al. 2006).* As bifunctional molecules with carboxylic and phenolic bonding sites, these Hydroxycinnamic acids can be involved in both ester and ether linkages. The presence of esterified phenolic compounds may protect the plant against pathogen infestation and generate a chemical barrier that improves disease resistance *(Santiago et al. 2006).* Furthermore, increases in dimeric and monomeric compound content following exposure to light were reported. These compounds influence the mechanical properties of the cell walls, such as rigidity during plant growth *(Miyamoto et al. 1994)*.

Barley grain contains 8% lignin *(NRC, 2001)*. There is no apparent lignin-degrading microorganisms or enzymes in the rumen therefore, its digestibility is relatively low and variable *(Van Soest, 1994)*. Lignin plays a negative role in ruminant nutrition, feed digestion and utilization through three ways *(Moore and Jung, 2001)* :1) lignin inhibits ruminal digestion as a physical barrier to restrict rumen microbes and enzymes acting; 2) lignin reduces plant energy availability by limiting animal fiber utilization, and 3) lignification restricts animal DM intake because it slows down plant DM digestibility and increases the rumen fill effect. The action of lignin seems to depend not only on their amount but also on other factors like cross linking and because of the chemical nature of this heterogeneous compound, it is nearly impossible to extract lignin in any pure form–especially once it polymerizes into ADL *(Raffrenato and Van Amburgh, 2010).* The relative abundance of lignin and the frequently of phenolic compounds cross-links with

polysaccharids appear to be the most important factors limiting energy utilization in barley grain and hull by rumen microorganism *(Casler, 2001)*. Variation of the content of hull, FA, pCA, NDF, ADF, ADL and characteristics of particle size reduction in various barley varieties may cause differences in the digestibility of barley grain. Therefore, greater knowledge about the relationship between the digestibility in the rumen and the specific chemical and physical profiles of barley grain will provide useful information for barley breeders and cattle producers. The objectives of this research were to identify interrelationships among FA and pCA and cell wall component of 11 barley cultivars and to determine their influence on DM, NDF, ADF and ADL ruminal degradation.

Material and Methods

Barley cultivars

Eleven barley cultivars were used as substrates in this experiment. These cultivars (Table 1) were grown at Karaj Research Station, Iran, in one field under the similar soil and environmental conditions.

Table 1. Variety and growing condition of eleven barley samples utilized in this study

	Variety [a]	Seed coat	Climate	Winter/spring variety
1	Bahman	Hulled	Cold mountains	Winter
2	Fajr30	Hulled	Mediterranean	Moderate
3	Kavir	Hulled	Mediterranean	Spring- autumn
4	Makooei	Hulled	Cold mountains	Winter
5	Nimrooz	Hulled	Hot coastal dry	Spring
6	Nosrat	Hulled	Mediterranean with spring rains	Moderate
7	Reyhan03	Hulled	Mediterranean	Spring- autumn
8	Sahra	Hulled	Caspian mild and wet	Spring-autumn
9	UH-12	Hulless	Mediterranean	Spring
10	Usef	Hulled	Mediterranean	Spring
11	Valfajr	Hulled	Mediterranean	Spring- autumn

[a] Eleven varieties of barley were grown at Karaj Research Station Farm (Karaj, Iran) using standard agronomic production practices for barley production.

Animals and diet

Three fistulated ewes (approximately 2 years old, Body weight = 35 ± 2 kg) those were equally fed a total mixed ration at maintenance level that included alfalfa hay

and barely grain with 75:25 ratios were used. Diets also contained vitamin-mineral premix, limestone, and salt. Water and mineral block were available over the experiment. The diets were offered in two equal meals at 0700 h and 1900 h. The animals were adapted to the basal rations for two weeks prior to ruminal incubation of the bags. All procedures used in this study were approved by the Animal Care and Use Committee of Proposing a National Ethical Framework for Animal Research in Iran *(Mobasher et al. 2008).*

Chemical Analyses

Feed samples were analyzed for dry matter (DM) by drying at 105°C. The neutral (NDF) and acid (ADF) detergent fibers were determined according to the procedure described by *Van Soest et al. (1991)*, and acid detergent lignin (ADL) was determined *(Feldsine et al., 2002)*. Two hydroxycinnamic acids (FA and PCA) in barley cultivars were determined using High Performance Liquid Chromatography (HPLC) and barley pretreatment for HPLC analysis was done using the method of *Hernanz et al. (2001)* with some modifications. For extraction, whole barley grain was cleaned, ground through a 1-mm mesh screen, hydrolyzed by adding 2 *M* NaOH solution (100 mL) per 1gr followed by incubation at ambient temperature for 16 h while samples wrapped with Aluminum foil. Then samples acidified with 6 *M* HCl to pH 2.6, and then extracted five times with equal volumes of ethyl acetate. The solutions were combined and evaporated to dryness with rotary evaporator at 45ºC. The residue was dissolved in 1 mL methanol HPLC grade and filtered through a 0.45 μm syringe filter (Millipore) and 20 μL samples were analyzed by HPLC using standard FA (46278) and pCA (C9008) that were purchased from Sigma. A Knaure smartline 1100 HPLC system and UV detector was employed. Separation was performed by isocratic elution with a mobile phase of water-acetic acid (98:2; v/v) (A) and methanol-butanol (92:8; v/v) (B), in a column C18 (250×4.6 mm, 5 mm). The gradient conditions were as follows 0 -10 min, 85% A and 15% B; 10 - 20 min, 50 % A and 50% B; 20 - 30 min, 85% A and 15 % B. Flow rate was 1 mL/min; and injection volume was 20 μL. The content of FA and pCA were calculated from chromatograms that were recorded at 245 nm.

Rumen incubation

Using the nylon bag technique, the barley samples were ground to pass a 2 mm screen. Then approximately 3 g of dry samples were weighed into 7×14 cm^2 and 40 ± 5 μm pore size nylon bags. Bags were incubated in the rumens of three ewes and were removed after 0, 1, 3, 6, 9, 12, 24, 36 and 48 h of incubation. Immediately after removing from the rumen, the bags were washed with cold tap water until clear and then were dried at 55°C for 48 h. The bags were weighed and residues were removed and then analyzed for DM, NDF, ADF and ADL. The disappearance of DM, NDF, ADF and ADL at each incubation time was calculated from the

proportion remaining in the bag after incubation in the rumen. The disappearance rate was fitted to the following equation given *(Orskov and McDonald, 1979)*:
Disappearance (%) = $a + b\times(1 - e^{-ct})$
where, a = soluble fraction (% of total), b = degradation fraction (% of total), t= time of rumen incubation (h), and c = rate of degradation (% h^{-1}). The effective degradability of DM, NDF, ADF and ADL was calculated by the equation of *Orskov and McDonald (1979):*
Effective degradability = $a + [(b \times c)/(c + k)]$
Where, k is the estimated rate of outflow from the rumen. Effective degradability of DM, NDF, ADF and ADL was estimated at ruminal outflow rates of 6% h^{-1}.

Statistical Analysis

Using a completely randomized design with eleven treatments with three replicates, the data were analyzed with the PROC GLM of SAS® (20). Duncan Multiple Range test were used for means comparison when significance was declared at $P<0.05$. In addition, PROC CORR procedure of SAS® (2002) was used to examine the correlations among the barley components such as FA, PCA, NDF, ADF and ADL and ruminal degradation parameters of DM, NDF, ADF and ADL. A multi-regression analysis was carried out using the PROC REG of SAS ® (2002) in order to develop prediction equations to show the effects of FA, pCA on ruminal degradation parameters of DM, NDF, ADF and ADL.

Results

Chemical compositions

The DM, FA, PCA, NDF, ADF and ADL content of the eleven barley cultivars were significantly different ($P<0.0001$; Table 2). Dry matter content varied from 82.52 to 90.90 % with a mean value of 88.35 %. Kavir and UH-12 had the highest and the lowest DM content. NDF varied from 9.64 to 27.34 % DM with mean of 22.55 % DM. ADF was much lower than NDF, ranging from 2.03 to 8.13 % DM, with an average of 6.26 % DM. In addition, ADL varied from 0.87 To 3.03 % DM with a mean of 2.18% DM. Bahman, Valfajr and Nimrooz had higher NDF, ADF and ADL. UH-12 had the lowest NDF. The FA content ranged from 151.2 to 354.2 µg/g with the highest in Bahman and lowest in UH-12, and PCA content ranged from 114.5 to 444.4 µg/g of DM with the highest in Valfajr and lowest in UH-12. In addition, the starch, CP, EE, ash and NFC content of were significantly different among the varieties (Table 2).

Table 2. Chemical compositions of different cultivars of barley grains cell wall [1, 2]

Cultivar	Unit	Bahman	Fajr30	Kavir	Makooei	Nosrat	Nimrooz	Reyhan03	Sahra	Valfajr	UH-12	Usef	SEM	P-value
DM	%	8910^{e}	90.39^{d}	90.90^{c}	88.08^{i}	88.08^{gh}	88.41^{h}	97.35^{a}	88.51^{g}	88.96^{f}	82.52^{j}	96.68^{b}	0.001	<0.0001
NDF	% DM	27.34^{a}	24.40^{c}	24.69^{b}	23.86^{f}	23.54^{g}	23.51^{gh}	24.26^{d}	23.41^{i}	23.46^{hi}	9.64^{j}	24.12^{e}	0.001	<0.0001
ADF	% DM	6.57^{e}	7.52^{b}	6.75^{d}	6.26^{h}	6.52^{f}	6.34^{g}	7.24^{c}	6.04^{i}	8.13^{a}	2.03^{k}	5.17^{j}	0.0004	<0.0001
ADL	% DM	2.02^{c}	2.01^{c}	2.00^{c}	3.01^{a}	2.50^{b}	3.03^{a}	3.00^{a}	1.5^{d}	2.03^{c}	0.87e	2.00c	0.004	<0.0001
FA	µg/g DM	354.2^{a}	222.5^{i}	277.1^{f}	277.6^{e}	329.4^{c}	265.4^{g}	277.5^{e}	257.3^{h}	333.4^{b}	151.2j	308.6^{d}	0.04	<0.0001
PCA	µg/g DM	192.2^{d}	237.7^{b}	178.4^{e}	219.4^{c}	162.3^{f}	118.2^{i}	222.5^{c}	131.5^{h}	444.4^{a}	114.5i	147.3^{g}	11.30	<0.0001
Starch	% DM	56.79^{l}	57.64^{j}	57.15^{K}	58.07^{h}	59.43^{c}	59.26^{d}	58.44^{f}	59.24^{de}	59.95^{b}	62.52^{a}	58.48^{g}	0.0001	<0.0001
CP	% DM	10.34^{f}	10.03^{f}	10.50^{e}	11.50^{a}	9.50^{h}	11.16^{c}	10.53^{e}	10.68^{d}	10.46^{e}	11.32^{b}	9.87^{g}	0.003	<0.0001
EE	% DM	2.86^{i}	4.17^{a}	3.58^{c}	2.99^{g}	3.51^{d}	3.08^{f}	2.62^{j}	2.91^{h}	3.65^{b}	2.75^{e}	3.11^{e}	0.0001	<0.0001
Ash	% DM	2.08^{gh}	2.23^{e}	2.38^{d}	2.49^{b}	2.11^{g}	2.44^{c}	2.82^{a}	2.01^{h}	2.10^{h}	1.97^{j}	2.16^{f}	0.0004	<0.0001
NFC	% DM	57.38^{j}	59.17^{i}	58.85^{f}	59.16^{i}	61.34^{b}	59.81^{g}	59.77^{h}	60.99^{c}	60.33^{d}	74.32^{a}	60.74^{c}	0.001	<0.0001

[a-k] means within a raw with differing superscripts are significantly different (P<0.05).

[1] DM, dry matter; NDF, neutral detergent fiber; ADF, acid detergent fiber; ADL, acid detergent lignin; FA, Ferulic acid; PCA, p-coumaric acid, CP, crud protein, EE, ether extract, NFC, non-fiber carbohydrate.

Rumen degradation kinetics

Ruminal degradation parameters were significantly different between barley cultivars for DM, NDF, ADF and ADL (Table 3). The soluble fraction ranged between 2.92 to 56.33 % of DM. Nimrooz had the highest (91.45%) slowly degradable fraction than others, and UH-12 had the lowest (42.64%) slowly degradable fraction (Table 2; $P<0.0001$). However, Bahman had higher (34.31%) and UH-12 had lower (1.02%) undegradable fraction for DM ($P<0.0001$). The potential degradable of DM in barley cultivars ranged between 65.68 to 98.97% ($P<0.0001$), that was highest for UH-12 and lowest for Bahman. The rate of degradation varied among barley cultivars and was lowest for Fajr30 cultivar (3.64% h^{-1}) and highest for UH-12 cultivar (27.81% h^{-1}; $P<0.0001$).

The soluble fraction for NDF differed ($P<0.0001$) from 0.16 in Valfajr to 10.61% in Nimrooz. This fraction for ADF ranged from 0.11 in Kavir to 3.0 % in Bahman, and in ADL differed ($P<0.0001$) from 0.03 in Fajr30 to 5.86 % in Bahman (Table 3). Also, the *b* fraction of NDF, ADF and ADL differed ($P<0.0001$) from 16.87 % in Usef to 42.80 % Bahman; 19.33 % in Valfajr to 10.07% in UH-12 and 4.36 % in Bahman to 23.85% in UH-12, respectively. The undegradable fraction for NDF, ADF and ADL ranged 54.39 % in Fajr30 to 82.06 % in Bahman; 79.78 % in UH-12 to 89.67 % in Valfajr, and 74.67 % in UH-12 to 89.77 % in Bahman, respectively. In contrary, the degradable fraction of NDF, ADF and ADL in barley cultivars were 17.93 % in Fajr30 to 45.60 % Bahman; 10.32 % in Valfajr to 20.22 % in UH-12, and 10.22 in Bahman to 25.33 % in UH-12, respectively. In addition, the lowest and greatest degradation rate of NDF were observed in Sahra (3.76 $\%h^{-1}$) and Fajr30 (35.83$\%h^{-1}$), respectively. Also, K_d for ADF ranged from 3.38 to 14.08$\%h^{-1}$ for UH-12 and Nimrooz, respectively; and for ADL ranged from 3.09 to 16.71$\%h^{-1}$ in Makooei and Bahman, respectively. In addition, the barley cultivars differed in effective degradability of DM, NDF, ADF and ADL (Table 3).

Table 3. Dry matter, neutral detergent fiber, acid detergent fiber and acid detergent lignin ruminal degradability parameters of barley cultivars

Item	Fajr-30	Nimrooz	Bahman	Sahar	Rey	Usef	Makooei	Kavir	Nosrat	Valfajr	UH-12	SEM	P-Value
Dry matter													
a	7.08^{e}	2.92^{g}	4.82^{f}	10.35^{d}	7.13^{e}	10.13^{d}	17.70^{b}	19.62^{b}	12.80^{c}	3.85^{f}	56.33^{a}	0.002	<0.0001
b	87.25^{ab}	91.45^{a}	60.86^{d}	87.6^{ab}	85.12^{ab}	60.72^{d}	72.20^{c}	73.38^{c}	81.85^{b}	89.01^{a}	42.64^{e}	0.006	<0.0001
a +b	94.34^{ab}	92.37^{ab}	65.68^{c}	97.95^{a}	92.25^{ab}	70.85^{c}	89.91^{b}	93.00^{ab}	94.65^{ab}	92.86^{ab}	98.97^{a}	0.006	<0.0001
c	5.66^{bc}	7.62^{bc}	34.31^{a}	2.04^{c}	7.74^{bc}	29.15^{a}	10.08^{b}	6.99^{bc}	5.34b^{c}	7.13^{bc}	1.02^{c}	0.006	<0.0001
K_d	3.64^{f}	5.13^{f}	9.74^{de}	11.46^{d}	21.39^{b}	8.12^{e}	12.13^{d}	15.15^{c}	11.14^{d}	12.06^{d}	27.81^{a}	0.002	<0.0001
EDDM[1]	39.63^{f}	43.18^{ef}	42.37^{ef}	67.80^{c}	73.60^{b}	45.03^{e}	65.97^{cd}	72.17^{b}	65.99^{cd}	63.27^{d}	84.99^{a}	0.004	<0.0001
Neutral detergent fiber													
a	5.90^{b}	0.16^{d}	1.05^{d}	0.67^{d}	0.90^{d}	0.34^{d}	5.71^{b}	0.32^{d}	1.49^{d}	10.61^{a}	2.89^{c}	0.001	<0.0001
b	39.70^{ab}	32.02^{cd}	16.87^{g}	25.70^{e}	34.05^{c}	42.80^{a}	39.70^{ab}	41.33^{ab}	30.29^{d}	21.37^{f}	38.13^{b}	0.003	<0.0001
a +b	45.60^{a}	32.18^{c}	17.93^{e}	26.37^{d}	34.95^{c}	43.14^{ab}	45.41^{a}	41.65^{b}	31.78^{c}	31.98^{c}	41.03^{b}	0.003	<0.0001
c	54.39^{e}	67.81^{c}	82.06^{a}	73.62^{b}	65.04^{c}	56.85^{de}	54.58^{e}	58.34^{d}	68.21^{c}	68.01^{c}	58.97^{d}	0.003	<0.0001
K_d	12.29^{a}	7.12^{d}	9.29^{bc}	3.76^{e}	7.97^{cd}	4.53^{e}	10.49^{b}	4.52^{e}	8.82^{c}	8.05^{cd}	5.12^{e}	0.001	<0.0001
EDNDF	32.55^{a}	17.55^{g}	11.30^{h}	10.39^{h}	20.32^{d}	18.73^{ef}	30.92^{b}	18.06f^{g}	19.44^{de}	22.66^{c}	20.39^{d}	0.001	<0.0001
Acid detergent fiber													
a	1.25^{cd}	2.39^{b}	1.48^{c}	1.48^{c}	0.21^{e}	0.78^{d}	3.00^{a}	0.11^{e}	0.20^{e}	0.25^{e}	0.88^{d}	0.0005	<0.0001
b	13.17^{bcd}	11.94^{bcd}	11.27^{bcd}	14.50^{bcd}	15.17^{abc}	15.73^{ab}	14.87^{abcd}	10.68^{cd}	15.51^{abc}	10.07^{d}	19.33^{a}	0.004	<0.0001
a +b	14.43^{bcde}	14.33^{bcde}	12.76^{cde}	15.98^{abc}	15.38^{bcd}	16.51^{abc}	17.88^{ab}	10.79^{de}	15.71^{abc}	10.32^{e}	20.22^{a}	0.004	0.003
c	85.57^{abcd}	85.67^{abcd}	87.24^{abc}	84.01^{cde}	84.61^{bcd}	83.48^{cde}	82.12^{de}	89.21^{ab}	84.28^{cde}	89.67^{a}	79.78^{e}	0.004	0.003
K_d	4.65^{cd}	14.08^{a}	9.25^{b}	4.95^{cd}	4.25^{cd}	3.87^{cd}	4.52^{cd}	7.49^{bc}	7.65^{bc}	9.19^{b}	3.38^{d}	0.004	<0.0001
EDADF	6.62^{ef}	10.69^{a}	8.22^{cd}	7.95^{d}	6.23^{fg}	6.93^{ef}	9.21^{b}	5.59^{g}	8.88^{bc}	6.32^{f}	7.19^{e}	0.0009	<0.0001
Acid detergent lignin													
a	0.03^{e}	1.55^{c}	5.86^{a}	4.76^{b}	0.50^{de}	4.75^{b}	0.40^{de}	0.87^{cde}	0.97^{cd}	0.68^{cde}	0.47^{c}	0.0008	<0.0001
b	12.34^{bc}	13.16^{b}	4.36^{e}	6.58^{de}	10.64^{c}	6.83^{d}	13.71^{b}	10.04^{c}	10.19^{c}	13.36^{b}	10.96^{a}	0.002	<0.0001
a +b	12.38^{cd}	14.71^{b}	10.22^{d}	11.34^{d}	11.14^{d}	11.59^{d}	14.11^{bc}	10.91^{d}	11.16^{d}	14.04^{bc}	25.33^{a}	0.002	0.0002
c	87.61^{ab}	85.29^{c}	89.77^{a}	88.65^{a}	88.85^{a}	88.40^{a}	85.88^{bc}	89.08^{a}	88.83^{a}	85.95^{bc}	74.67^{d}	0.002	0.0002
K_d	4.47^{cd}	4.09^{cd}	16.71a	12.49^{b}	7.36^{c}	12.74^{b}	3.09^{d}	5.23^{cd}	5.04^{cd}	4.52^{cd}	6.78^{c}	0.003	<0.0001
EDADL	5.24^{ef}	6.84^{c}	9.05^{b}	9.11^{b}	6.33^{cde}	9.22^{b}	5.03^{f}	5.54^{def}	5.62^{def}	6.39^{cd}	14.12^{a}	0.001	<0.0001

[a-h] means within a raw with differing superscripts are significantly different (P<0.05).

[1] DM, dry matter; NDF, neutral detergent fiber; ADF, acid detergent fiber; ADL, acid detergent lignin; FA, Ferulic acid; PCA, p-coumaric acid.

[2] *a*, Soluble fraction (%); *b*, slowly degraded fraction (%); *c*, undegradable fraction, *a +b*, degradation fraction (%); K_d, rate of degradation (% h^{-1}); EDDM, EDNDF, EDADF and EDADL, effective degradability of dry matter, neutral detergent fiber, acid detergent fiber, and acid detergent lignin, respectively (0.06 h^{-1}).

Discussion

Chemical compositions

The DM level of the barley grain cultivars used in the present study was lower than those reported by *Ghorbani and Hadj-Hussaini (2002)* who showed that the DM level of 10 barley grain cultivars ranged from 92 to 94 %. *Abdi et al. (2011)* reported that the DM values for 16 cultivars of barley grains and indicated it ranged from 92.5 to 93.5%. The NDF, ADF and ADL concentrations of the barley grain cultivars used in the present study had more variance than those of reported by *Du et al. (2009)*, that examined six Canadian barley varieties and reported NDF, ADF and ADL values varied from 17.6 to 21.9, 5.5 to 7.0 and 1.7 to 2.1 %DM. Also, the FA and pCA content ranged from 555 to 663 and 283 to 345 μg/g of DM, respectively *(Du et al. 2009)*. *Holtekjolen et al. (2006)* studied five varieties of hulled two-row barley grown in Norway in 2002 and observed that FA content varied from 512 to 723 μg/g of DM, and pCA content varied from 114 to 244 μg/g of DM. The pCA content in the present study was similar, but FA content was lower. This variation might be due to the difference between cultivars and growing conditions. The cultivars used this study were grown in the same field under the same soil and environmental conditions. Thus, variation between them is likely a result of the different cultivars type. *Hernanz et al. (2001)* indicated that the concentrations of FA and pCA in barley were influenced by the genotype. *Du et al. (2009)* showed that barley variety had a significant effect on the content of FA, pCA, NDF, ADF, ADL and hull contents in various barley cultivars, and concluded barley variety plays an important role in determining the quality of barley as a feed.

Rumen degradation kinetics

Ruminal degradation parameters were significantly different between barley cultivars for DM, NDF, ADF and ADL (Table 2) that were comparable to the results outlined by *Du et al. (2009)*. In contrary, the potential degradable fraction provides the major source of slowly fermenting starch for rumen microbes *(Ghorbani and Hadj-Hussaini, 2002)*. However, the quantitative importance of lignin in the cell wall, their variable structure, and a variety of cross-linkages between cell-wall components all have variable depressive effects on cell-wall carbohydrate degradation by microorganisms. *Bunzel et al. (2003)* suggested that FA, pCA, and other hydroxycinnamic acids, like Sinapic acid, may also play a similar role to FA in plant cell walls forming crosslinkages. The FA may also conjugate to cell wall nitrogenous compounds or proteins, and in this way FA regulates cell wall rigidity and decreases cell wall digestibility *(Van Soest 1994)*.

Also in present study, the disappearance kinetics of DM, NDF, ADF and ADL in the rumen differed among barley cultivars. Large differences in degradability among barley varieties can be attributed to broad vary in composition such as cell

wall components in barley or its hull. A good feed barley variety should have these traits: high in nutrients, good nutrient availability, slow rate of rumen starch fermentation and maintaining large particle size after mechanical processing *(Du and Yu, 2011)*. The DM soluble fraction had more variance than those of reported by *Khorasani et al. (2000)* that reported solubility of DM ranged from 35.2 to 59.4% in sixty Canadian barley cultivars. Also, *Lehmann et al. (1995)* reported solubility values of 25 to 40.7%. The difference in the proportion of the soluble fraction is related to a number of factors including bag pore size, particle size of the grain, and the ratio of the sample weight: bag surface area and the washing technique *(Ghorbani and Hadj-Hussaini, 2002).* Since the bag pore size was standardized across the trial, it can be assumed that the differences in the results may be attributed to variations in washing technique and an element of variation in grain particle sizes, resulting in different amounts of small particles being washed out rather than being truly soluble. *Ghorbani and Hadj-Hussaini (2002)* reported that DM slowly degradation fraction for 10 Iranian barley grain cultivars ranged from 42.2 to 49.0%, whereas, *Cleary et al. (2011)* reported the b values of DM varied from 46.6 to 63.1%, however in the present study had more variance than them (42.64 - 91.45%). Also, *Cleary et al. (2011)* reported DM undegradable fraction ranged from 5.3 to 27.6%, whereas, *Ghorbani and Hadj-Hussaini (2002)* showed that DM c fraction ranged from 13.5 to 36.0%. The degradable fraction is the portion of the grain which is slowly digested within the rumen when allowed sufficient time. It is an important source of slowly fermenting starch providing energy for the rumen microbes *(Cleary et al., 2011). Khorasani et al. (2000)* reported degradable values of 25 to 40.7%, whereas, *Du and Yu (2011)* reported *a* + b fraction ranged from 79.3 to 82.8%. In present study, UH-12 provided more nutrients for ruminants than others cultivars, because of its higher (98.97 %DM) potential digestible fraction and lower (1.02 %DM) undegradable fraction of DM. Also, UH-12 had lowest content of NDF, ADF, ADL, FA and pCA than the others (Table 2). UH-12 is a hull-less barley cultivar; and had the lowest fiber and phenolic components. The hull fraction of barley seed is usually high in fiber that is made up of cell wall polysaccharides such as cellulose and hemicellulose that are usually more resistant to degradation. Hull-less barley does have surrounding hull during its life cycle, but it is very loosely attached to the kernel and sheds readily, and therefore the kernel becomes naked during threshing. Also, it had highest rate of degradation in rumen and effective degradability of DM in comparison with other cultivars (27.81%). The rate of DM degradation within the rumen is influenced by a number of interactions between the rumen microorganisms and barley kernel tissue. The rate at which digestion occurs influences the rate of passage, site of digestion, form of substrates and the efficiency of feed utilization. The rate and extent of ruminal digestion is important as a high rate of degradation within the rumen causes the higher production of VFA for absorption, drop in pH which can result in ruminal acidosis, a reduction in microbial protein synthesis,

fiber digestion and feed intake *(Van Soest, 1994)*. Therefore, when hull-less cultivars such as UH-12, it is important to consider balancing the extent and rate of fermentation in the rumen. Fajr30 had lowest rate of DM degradation, therefore using Fajr30 in ration could decline occurrence of acidosis. *Cleary et al. (2011)* studied tow malting barley varieties and reported the K_d from 12.7 to 16.5 $\%h^{-1}$. Also, *Khorasani et al. (2000)* found that the K_d ranged from 20 to 62$\%h^{-1}$, whereas, *Ghorbani and Hadj-Hussaini (2002)* reported that the K_d varied from 25.6 to 31.5$\%h^{-1}$. UH-12 showed higher EDDM (84.99 %0.06h^{-1}), which indicated that UH-12 tended to be more extensively degraded in the rumen. *Ghorbani and Hadj-Hussaini (2002)* found the EDDM ranged from 75.4 to 79.5%0.08h^{-1}, and *Khorasani et al. (2000)* reported that it ranged from 73.8 to 89.0%0.09h^{-1}. In our study, EDDM had ranged from 39.63 to 84.99 % when we considered the passage rate 0.06%/h; Table 3).

There was a large variation between chemical compositions and DM, and NDF rumen degradability in Iranian barley cultivars. Chemical compositions were useful in some cases in making inferences about diet digestibility, but could not be used as the sole means of predicting nutritional quality. Digestibility of NDF is a major factors contributing to differences among barley cultivars that has higher fiber and lower starch content than most other grains. A range of variation for NDF digestibility exists. The NDF represents the total structural cell wall components (cellulose and hemicellulose as well as lignin except pectin), so rumen indigestion of NDF residue was lower than ADF and ADL, and averaged 64.35% (from 63 to 68% total tract undigested NDF for whole barley grain (*Feng et al., 1995)*). *Beauchemin et al. (2001)* found it was 53% for the whole barley grain. *Du and Yu (2011)* observed different effects of variety on the rumen undigested residues of barley NDF and ADF, except for ADL residues. Among the eleven Iranian cultivars, Bahman showed considerably higher NDF residue than others (82.6% of DM) that probably related to the highest NDF content in the Bahman (27.34% of DM, Table 2). In contrast, Fajr30 had the lowest NDF residue and the highest NDF potential degradable among cultivars, which might imply that most NDF in Fajr30 was degraded in rumen.

The ADF contains principally cellulose and lignin, which is less digestible than NDF. *Du and Yu (2011)* found that rumen undigested ADF for stream-rolled barley was 80% compared to 50 to 65% of undigested NDF. In this study, ADF residue averaged 85.05 and its potential degradable averaged 14.93%. Among the eleven cultivars, Valfajr had the highest ADF residue than others, and UH-12 had the lowest. Less ADF is always preferred in feed barley selection, whereas Valfajr had the highest original ADF.

Although ADL is thought of as low in digestibility, in the present study, roughly 2% of ADL was soluble in the rumen. Although lignin content in most plants and barley is relatively low, it is the most recalcitrant fiber component. *Du et al. (2009)* reported 10% of ADL was soluble in rumen. The ADL content of barley was quite

low (about 0.87 to 3.03% of DM). In practice, ADL digestibility of barley grain is seldom analyzed. Nevertheless, results showed that Bahman had highest ADL residue than others, and UH-12 had the lowest. Lignin is the typical complex phenolic polymer which impedes animal digestion of plant cell walls. In the animal alimentary tract, proanthocyanidins can inhibit protein digestion and utilization by forming an insoluble complex *(Slafer et al. 2001)*. There are also small quantities of simple phenolic acid residues such as FA and ρCA *(Slafer et al. 2001).*

The presence of excessive hydroxycinnamic acids (especially FA, pCA) in plant cell walls may reduce animal digestibility and productivity. Although phenolic acids (mainly FA and pCA) are present in comparatively low levels, they impose effective and important effects on the physical and chemical properties of barley. Free phenolic acids have oxidative properties and antibacterial functions which help to defend the kernel from micro-organism attack. When these phenolic acids form intricate cross-linkages with lignin and cell wall polysaccharides, they become the inhibitory factors for plant cell wall rumen degradation. Since most esterified pCA on lignin are not covalently attached to other cell wall polymers, they should not directly influence cell wall rumen degradability. Some cell wall models show how they can interfere with ferulate-lignin cross linking and in some cases reduce the proportion of lignin bound to cell wall. Ether linkage between FA and lignin has been used a measure of cross-linking between lignin and arabinoxylans and defined as the most important factor limiting energy utilization *(Casler, 2001)*. Ester-linked FA had generally a negative relationship except in Brown Mid Rib (BMR) corn hybrids for 24h and positive for 96h NDF digestibility *(Raffrenato and Van Amburgh, 2010).* The ferulate primarily form as esters of arabinoxylans and later they cross-link through ether linkages with lignin. So esters of FA should not necessarily limit NDF degradation. This has probably more to do with the degree of arabinoxylans substitution. *Raffrenato and Van Amburgh (2010)* found that forage groups demonstrated different relationships for digestibility from positive to negative in NDF residues, but the ADF residues were instead characterized by a consistent negative relationship among all forage groups and similar results were obtained for 96 h NDF digestibility. However, in this study, we obtained consistent negative relationship with potential degradable of DM, NDF, ADF, and ADL (Table 4). *Raffrenato and Van Amburgh (2010)* found that negative effect of etherified FA on NDF digestibility has been found in elongating internodes in maize but not in internodes that had stopped the elongation process and confirms the hypothesis that secondary cell wall development may mask the negative impact of etherified FA on NDF digestibility. Also, BMR corn shows higher content of etherified FA compared to conventional corn in NDF residues, demonstrating that etherified FA is not always a good indicator of cross-linking between lignin and arabinoxylans. However, this relationship changes when ADF residues were analyzed for ether linked FA, showing how the solubilization or branching of the lignin structure has in this case more importance than linkages.

Acid detergent solution in this case might dissolve those FAs that only etherified (instead of having and ester-ether linkage).

Table 4. Correlation between DM, NDF, ADF, ADL, FA and pCA of eleven varieties and ruminal degradability parameters

Item	NDF (g/kg)	ADF (g/kg)	ADL (g/kg)	FA	PCA
Chemical characteristics					
NDF(g/kg)					
ADF(g/kg)	0.830***				
ADL (g/kg)	0.704***	0.578***			
FA	0.679***	0.635***	0.441*		
pCA	0.292	0.629***	0.132	0.392*	
Degradation parameters of DM					
a	-0.843***	-0.860***	-0.613***	-0.715***	-0.350*
b	0.621***	0.792***	0.531**	0.288	0.362*
a +b	-0.276	-0.060	-0.090	-0.568***	-0.031
c	0.276	0.060	0.090	0.568***	0.031
K_d	-0.672***	-0.563***	-0.345*	-0.445**	-0.098
Degradation parameters of NDF					
a	0.037	0.314	-0.051	0.026	0.842***
b	-0.071	-0.320	0.065	-0.520**	-0.343
a +b	-0.056	-0.196	0.043	-0.505**	-0.015
c	0.056	0.196	-0.043	0.505**	0.015
K_d	0.343	0.464**	0.437*	0.163	0.391*
Degradation parameters of ADF					
a	0.188	-0.102	0.282	-0.140	-0.254
b	-0.475**	-0.615***	-0.227	-0.460**	-0.419*
a +b	-0.402*	-0.615***	-0.140	-0.477**	-0.470**
c	0.4022*	0.615***	0.140	0.477**	0.470**
K_d	0.200	0.324	0.322	0.367*	0.094
Degradation parameters of ADL					
a	-0.003	-0.220	-0.337	0.315	-0.347*
b	-0.710***	-0.539**	-0.256	-0.728***	0.004
a +b	-0.858***	-0.757***	-0.472**	-0.726***	-0.163
c	0.858***	0.757***	0.472**	0.726***	0.163
K_d	-0.029	-0.164	-0.339	0.288	-0.260

*P< 0.05, **P< 0.01, ***P< 0.001;

[1]*a*, Soluble fraction (%); *b*, slowly degraded fraction (%); *c*, undegradable fraction, *a +b*, degradation fraction (%); K_d, rate of degradation (% h^{-1}).

Correlation between chemical components and ruminal degradation parameters

Correlation between NDF with ADF, ADL, and FA and between ADF with ADL, FA and pCA was significantly high (Table 4). Also correlation between ADL with FA was significant, but between ADL with pCA was not statistically significant. However, correlation between FA and pCA was significant. The FA correlated to the content of NDF, ADF and ADL, but pCA only were significantly correlated to ADF. The correlation between FA and cell wall components such as NDF, ADF and ADL was relatively stronger than pCA**.** The high correlation could be explained by the different bonding models between FA and pCA in plant cell walls. The pCA is heavily esterified to lignin, and seldom linked to cell wall polysaccharides, while FA is esterified to polysaccharides, etherified to lignin, and forms cross-linkages between polysaccharides and lignin, and among polysaccharides *(Van Soest, 1994).* There is some evidence which suggests that phenolic acids may limit the digestibility of the plant cell wall in the ruminants. The FA and pCA are covalently linked to plant cell wall polysaccharides by ester bonds and to lignin by both ester and ether bonds *(Hernanz et al., 2001; Lam et al., 1992)* and directly or indirectly involved in affecting the digestibility of cell wall polysaccharides *(Grabber et al., 2004).* These phenolic acids are esterified to arabinoxylans within the plant cell wall, and digestibility of plant cell walls has been related to amounts of phenolic acids released by alkali treatment. Formation of ester bonds between phenolic acids and plant wall polysaccharides through *in vitro* syntheses, while not entirely representative of naturally occurring esters, reduced biodegradation of carbohydrates, further supports the contention that phenolic esters limit carbohydrate degradation by ruminal microorganisms.

Also, FA had positive correlation with rumen indigestible DM, NDF, ADF and ADL while pCA had just positive correlation with rumen indigestible ADF, and both had similar but negative effect on potential degradable fraction. The FA and pCA had effect on rapidly degradable fraction of DM, which for FA is relatively stronger than that pCA. FA and pCA are both esterified and etherified to plant cell wall components *(Du and Yu, 2011).* Also, FA negatively corrected with slowly degradable fractions of NDF, ADF and ADL, but pCA alone had significantly effect on slowly degradable of DM and ADF. The FA corrected with rate of degradability (K_d) fraction of DM and ADF, and pCA only corrected with rate of degradation fraction of NDF. Generally, results can be meaning that FA and pCA in barley grain reduce the degradability of barley grain in the rumen. The negative effects of barley fiber have been studied extensively. The NDF, ADF and ADL contents were significantly correlated to in situ rumen degradation kinetics of DM, except fraction of a+b and c were not significantly affected by NDF, ADF and ADL. These relations were negative with *a*, *a*+b and K_d fraction and positive with *b* and c fraction of DM. Cell wall fiber contents were a little correlated to *in situ*

rumen degradation kinetics of NDF, and showed no correlation effect with the ruminal degradability kinetics, except K_d. Ruminal degradability kinetics of ADF includes *b*, *a*+b and c significantly corrected with NDF and ADF, but had no correlation with ADL. The b, and *a+b* fractions of ADF negatively corrected to NDF and ADF; and the ADF c fraction positively corrected to NDF and ADF. Ruminal degradability kinetics of ADL includes b, a+b and c significantly corrected with NDF ADF and ADL.

The FA and pCA of barley grain reduce the ruminal degradability parameters of barley grain NDF, ADF and ADL. The rumen degradability of plant cell walls are improved by releasing FA and pCA from plant cell walls and by reducing FA cross-linking in the plant *(Jung and Phillips, 2008). Khorasani et al. (2002)* observed that FA content in barley grain positive effects on *in situ* residue of DM, NDF and ADF, but pCA positive effects only on residue of DM and NDF, which means that FA and pCA in barley grain had negative correlation on ruminal degradability of barley grain. *Jung and Phillips (2008)* also observed the negative correlation between the content of FA and the degradation of Lucerne cell walls. Our results showed that FA had more inhibitory effects than pCA. This probably results from the differences in bonding models. *Grabber et al. (2004)* reported that FA is extensively and covalently linked to cell wall components, forms ester/ether bridges between polysaccharides and lignin, and forms ester/ester bridges among polysaccharides, while pCA is esterified to lignin. Therefore, FA inhibits the degradability of plant cell wall polysaccharides while pCA is deemed to be an indicator of lignification and exerts a negative influence directly or indirectly through lignin. In addition, *Grabber et al. (2004)* suggested that lignin composition does not directly affect the degradability of cell walls by fungal enzymes or by rumen microorganisms. According to current information, barley cultivars with less FA and pCA content would be a good candidate for feed barley and the correlation analysis results implied that reduction of barley FA and pCA content could increase the degradability of barley grain in ruminants.

Prediction of ruminal degradability kinetics using FA and pCA

The multi-regression analysis to find the most important variable to predict of ruminal degradability kinetics using FA, pCA with a tested multi-regression model as follows:

Y (degradation kinetics) = $FA + pCA + FA^2 + pCA^2 + FA \times pCA + FA^2 \times pCA^2$

The best models deduced from the stepwise multi-regression analysis are presented in Table 5.

Table 5. The best models deduced from the stepwise multi-regression analysis

Predicted variable (y)	Prediction equations best model	R^2	Partial $R^2_{f,p}$	Partial $R^2_{p,f}$	p-value
DM (a)	y=67.09 – 0.19×FA	0.51	-	-	<0.0001
NDF (a)	y=2.66 – 0.00003×FA^2+0.00006×pCA^2	0.79	0.71	0.08	<0.0001
ADF (a+b)	y=2.66 – 0.02×FA– 0.01×pCA	0.36	0.22	0.09	0.002
ADL (b)	y=29.80 – 0.08×FA– 0.01×pCA	0.62	0.52	0.09	<0.0001

The linear and quadratic regression analysis further shows that the content of FA in barley grain accounted for 51% variation in DM rumen degradability. Variation in NDF rumen degradability was explained when a quadratic regression coefficient of FA^2 and pCA^2 were used in model (R^2 = 0.79; P<0.0001). Although the R^2 of partial regression for FA^2 was 0.79 alone and pCA^2 just explained 8% of variation in DM rumen degradability. In addition, the total variation in the rumen undegradable fraction FA and pCA against ADF degradability 0.36 % explained by model and content of FA and pCA in barley grain accounted for 22% (P=0.002) and 9% of the variation in the rumen degradation, respectively. Multi-regression linear and quadratic analysis also shows that both FA and pCA accounted for 62% (P<0.0001) variation in the rumen degradability of ADL.

Efekti hidroksikinamičnih kiselina (ferulnih i p-kumarnih kiselina) u sortama ječma na razgradivost komponenti ćelijskog zida u rumenu

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Rezime

Zrno ječma sadrži hidroksikinamične kiseline, posebno ferulnu (FA) i p-kumarnu (pCA), koje postaju ukrštene sa polisaharidima ćelijskog zida, kako počne lignifikacija, i koji su glavni inhibitorni faktori biorazgradljivosti zidova biljnih ćelija u rumenu. Određene su hemijske karakteristike, FA i pCA sadržaj 11 iranskih sorti ječma. Korišćenjem 3 fistulisane ovce, ispitivani su efekti sadržaja FA i pCA na vlaknastu degradaciju suve materije (SM), vlakna neutralnog i kiselog deterdženta (NDF i ADF) i lignina. U sortama ječma, DM je varirao od 82,52 do 90,90%; NDF je varirao od 9,64 do 27,34% SM; ADF je varirao od 2,03 do 8,13% SM, a od 0,87 do 3,03% SM. Sadržaj FA se kretao od 151,2 do 354,2 μg/g; i

sadržaj pcA JE varirao od 114,5 do 444,4 μg/g SM. Parametri degradacije u rumenu za SM, NDF, ADF i lignin su bili različiti zavisno od sorti ječma. Rastvorljiva frakcija, polako razgradiva, potencijalno razgradiva i nerazgradiva frakcija SM su bile 2,92 do 56,33%; 42,64 do 91,45%; 65,68 do 98,97% i 1,02 na 34,31%, respektivno. Stopa ruminalne degradacije za SM varirala je između sorti ječma od 3,64 do 27,81% h^{-1}. FA je bio povezan sa nerazgradivim u rumenu SM, NDF, ADF i ligninom, dok je pCA u korelaciji sa ADF-om. Koristeći multi-regresiju, FA i pCA su bili inhibirajući faktori razgradljivosti ruminalnih komponenti SM i komponenti ćelijskog zida; a FA je bio najefektivniji faktor za predviđanje razgradljivosti SM, dok su FA i pCA uticali na razgradivost NDF i ADF u rumenu.

Ključne reči: hidroksikinamična kiselina, ferulinska kiselina, p-kumarinska kiselina, ječam, rumen

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