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Physicochemical Characteristics, Hydroxycinnamic Acids (Ferulic Acid, *p*-Coumaric Acid) and Their Ratio, and in Situ Biodegradability: Comparison of Genotypic Differences among Six Barley Varieties

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Barley contains hydroxycinnamic acids, mainly ferulic acid (FA; 3-methoxy-4-hydroxycinnamic acid) and *p*-coumaric acid (PCA; 4-hydroxycinnamic acid). Ferulic acid is produced via the phenylpropanoid biosynthetic pathway and covalently cross-linked to polysaccharides by ester bonds and to components of lignin mainly by ether bonds. Various studies have consistently indicated that FA is among the factors most inhibitory to the biodegradability of cell wall polysaccharides. *p*-Coumaric acid is also covalently linked to polysaccharides (minor) and lignin (major), but does not form the inhibitory cross-linkages as FA does and is considered to represent cell wall lignification. The objectives in this study were to (1) determine genotypic differences in physicochemical characteristics in terms of (a) two major low molecular weight hydroxycinnamic acid profiles (FA, PCA, PCA-to-FA ratio, which are associated with digestion and lignification), (b) particle size distributions (mean, median), (c) hull content, and (d) digestion-resistant fiber fractions and (2) determine genotypic differences in in situ solubilization kinetics of FA and PCA. The barley varieties grown during three consecutive years (2003, 2004, and 2005) included AC Metcalfe, CDC Dolly, McLeod, CDC Helgason, CDC Trey, and CDC Cowboy. These barleys were grown at the Kernen Crop Research Farm (KCRF, University of Saskatchewan) and managed using standard agronomic production practices. Results showed that there were significant differences in hull content ($P < 0.05$) among the barley varieties, with Mcleod having the highest (11% DM) and CDC Dolly and CDC Helgason the lowest hull content (9% DM). Ferulic acid ranged from 555 to 663 μg/g of DM ($P < 0.05$). *p*-Coumaric acid ranged ($P < 0.05$) from 283 to 345 μg/g of DM. PCA-to-FA ratios ranged ($P < 0.05$) from 0.49 to 0.56. Mean particle size ranged ($P < 0.05$) from 3.06 to 3.66 mm, and median particle size ranged ($P < 0.05$) from 2.71 to 3.04 mm. In situ DM degradability ranged from 44 to 49%. In situ solubilized FA fractions ranged ($P < 0.05$) from 60 to 72% and of PCA ranged ($P < 0.05$) from 71 to 81%. In conclusion, CDC Dolly was best and McLeod barley was poorest as feed barley in terms of hull and FA contents. There were significant genotypic differences in FA, PCA and their ratio, hull content, particle size distribution, and in situ solubilization of FA and PCA among the barley varieties.

KEYWORDS: Ferulic acid; *p*-coumaric acid; barley varieties; nutrient availability; physicochemical characteristics

INTRODUCTION

Hydroxycinnamic acid−carbohydrate complexes are an important inhibitory factor related to cell wall digestibility in ruminants. In barley, ferulic acid (FA; 3-methoxy-4-hydroxycinnamic acid) and *p*-coumaric acid (PCA; 4-hydroxycinnamic acid) are two major (or richest) low molecular weight hydroxycinnamic acids (*1*). FA and PCA are concentrated in the cell walls of the outer coverings of barley grain, mainly in the bran (*1*, *2*). Ferulic acid is produced via the phenylpropanoid biosynthetic pathway and is covalently linked to cell wall polysaccharides (especially arabinoxylans) by ester bonds and to lignin mainly by ether bonds (*3*−*5*). Through ester and ether linkages, FA is extensively involved in cross-linking plant cell wall polysaccharides and lignin (*4*, *6*). Furthermore, FA can dimerize and trimerize through oxidative coupling (*7*). Therefore, FA forms intra- and/or intermolecular ester−ether bridges between lignin and cell wall polysaccharides. Although the role of FA in the digestibility of cell walls is not well elucidated, the proposed mechanism behind its

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negative effect on digestibility of cell wall polysaccharides is that FA cross-linkages increase steric interference of rumen microbial digestion and shield bound polysaccharides from enzymatic hydrolysis (*8*, *9*). Various studies have consistently indicated that FA is among the factors most inhibitory to the biodegradability of plant cell wall polysaccharides in the rumen (*8*).

PCA is the second most concentrated hydroxycinnamic acid in barley. It is mainly esterified to lignin in plant cell walls and seldom linked to polysaccharides (*4*, *10*). Because PCA does not form ester−ether cross-linkages as FA does, it is not considered to be directly involved in the plant cell wall digestibility and is more indicative of cell wall lignification (*11*, *12*).

Barley hull is a main reason for lower digestible energy (DE) of barley versus corn. Lower barley DE is mainly attributed to its excessive hull, which represents approximately 13% of the weight of barley grain (*13*). It is extremely fibrous and indigestible for monogastric animals and only partially degradable in ruminants.

It is a common practice in western Canada feedlots to coarsely process barley grain to breach the tough barley hull before feeding, thereby improving rumen digestibility. However, overprocessing can lead to an unpalatable ration and reduced DM intake and can cause digestive problems. Larger particle size can reduce the surface area for microbial colonization and enzymatic attack. An optimal grain particle size is preferred and required to maximize barley grain digestibility, feed intake, and performance (*14*, *15*). Barley particle size reduction obtained after dry-rolling is related to grain hardness, texture, and composition (*16*, *17*). Mean/median particle size can be used to describe differences in particle size among various barley varieties after processing. Pond et al. (*18*) and Fisher et al. (*19*) proposed different mathematic models (or equations) for analyzing mean/median particle size of substances with irregular shapes.

The objectives of this study were to determine (1) the effect of barley variety on the physicochemical characteristics or profiles in terms of FA, PCA, and PCA-to-FA ratio, genotypic variation in hull content, fiber fraction content (NDF, ADF, ADL, hemicelluloses, and cellulose) from different barley varieties from three consecutive production years; (2) the effect of barley variety on the variation in mean/median particle size of barley grain obtained after coarse dry-rolling; and (3) the genotypic variation of in situ solublization kinetics of the two major hydroxycinnamic acids (FA and PCA).

MATERIALS AND METHODS

Barley Varieties and Growing Conditions during 2003 through 2005. Six, two-row hulled barley varieties (AC Metcalfe, CDC Dolly, McLeod, CDC Helgason, CDC Trey, CDC Cowboy) were grown at the Kernen Crop Research Farm (KCRF), University of Saskatchewan, Saskatoon, SK, Canada, during three consecutive years (2003, 2004, and 2005). All barley plots were managed using standard agronomic production practices. The information on barley varieties and growing conditions, highest mean temperatures and rainfall during the three consecutive years, are presented in **Table 1**.

Table 1. Variety and Growing Conditions of Six Barley Samples Utilized in This Study

			growing year		
barley variety[a]	barley type	barley spike type	2003	2004	2005
1AC Metcalfe	malting	2-row	2003	2004	2005
2CDC Dolly	feed	2-row	2003	2004	2005
3McLeod	feed	2-row	2003	2004	2005
4CDC Helgason	feed	2-row	2003	2004	2005
5CDC Trey	feed	2-row	2003	2004	2005
6CDC Cowboy	feed (forage)	2-row	2003	2004	2005
			climate weather condition		
highest mean temperature (°C)			20.9	17.3	17.5
rainfall (mm)			190	305	455

[a] Six varieties of barley were grown at the Kernen Crop Research Farm (University of Saskatchewan, Saskatoon, Canada) using standard agronomic production practices for barley production.

Determination of Total Barley Hull Content Using the Modified European Brewery Convention (EBC) Method. A preliminary experiment was conducted to compare the modified EBC method (*20*) and the Whitemore method (*21*). The method described here was used for this study. Briefly, 20 g of barley grain was boiled and digested for 3 min in a solution of 80 mL of sodium hypochlorite (12%) (Clear Tech Industries Inc.) and 20 mL of sodium hydroxide (3.125 N) (pellet, VWR International). Samples were then dried to determine barley hull content.

Determination of FA and PCA Contents and PCA/FA Ratio. *Pretreatment for Hydroxycinnamic Acids Analysis*. Whole barley grain was ground through a 0.5 mm mesh screen followed by grinding through a 0.25 mm screen using a Retsch ZM-1 grinder (Brinkmann Instruments Canada Ltd.). Ground barley grain (50 mg) was mixed with 0.75 mL of 1% (w/v) α-amylase in a 0.05 M phosphate solution (pH 6.9) and incubated in a hot water bath (90 °C) for 1 h. Samples were then cooled at room temperature and centrifuged at 14000 rpm for 20 min. Supernatant (S1) was collected and stored at −20 °C. Precipitated pellets were hydrolyzed by adding 2 M NaOH solution (0.55 mL) followed by incubation at ambient temperature for 16 h in the dark (samples wrapped with foil). After centrifugation (14000 rpm, 10 min), supernatant (S2) was collected and combined with the initial supernatant (S1), acidified with 200 μL of 6 M HCl to pH 2, and then extracted five times with equal volumes of ethyl acetate. The solutions were combined and evaporated to dryness under N_2 in a heat block at 40 °C. The residue was dissolved in 1 mL of methanol/water (50:50, v/v) and filtered through a 0.45 μm syringe filter (Millipore), and 5 μL samples were analyzed by HPLC. All samples were prepared and analyzed in triplicate.

HPLC Analysis. Standard FA (46278) and PCA (C9008) were purchased from Sigma. An Agilent 1100 series HPLC system, which consists of a system controller (HP Chem Station computer program), pump, autosample processor, and photodiode array detector (DAD) (Interface 35900E), was used. Separation was performed by isocratic elution with a mobile phase of 5.5% methanol, pH 8.0, and 20 mM K_2HPO_4−KH_2PO_4 in a reverse phase PRP-1 column (Hamlton, 150 × 4.6 mm, 5 μm, pH 1−13) at room temperature. The isocratic elution flow rate was 1 mL/min, and samples (5 μL) were introduced into the column using an autosampler. Detection was monitored at 305 nm. FA and PCA in samples were identified by comparison of retention time and DAD-UV spectra with that of standards and were quantified using external standards. FA and PCA concentrations of sample extracts were extrapolated from the FA and PCA standard curves ranging from 1.0 to 100 μg/mL. Standards were prepared as stock solutions at 2 mg/mL in methanol. Calibration curves were calculated on the basis of the linear correlation between concentration of standards and the area of the FA and PCA peaks. All samples were analyzed in triplicate.

Determination of Mean/Median Particle Size of Barley Grain after Coarse Dry-Rolling. Barley samples (with moisture content of ca. 10%) were coarsely dry-rolled in a grain roller mill (Sven Grain Mill, Apollo Machine and Products Ltd.) at the College of Engineering (University of Saskatchewan) using a 1.55 mm gap (feedlot practice). Particle size distribution of these cracked samples was determined as weight distribution. In brief, triplicate samples (100 g) were sifted through a stack of six test sieves plus one bottom pan arranged in descending sieve aperture sizes (sieves 6, 8, 12, 16, 20, and 30 with aperture sizes of 3.36, 2.36, 1.70, 1.19, 0.84, and 0.58 mm, respectively), fitted in a Ro-Tap sieve shaker (Tyler Industrial Products). The duration of sieving (rotation and tapping) was determined by sieving initially for 1 min and increasing to 5 min until sifting had reached equilibrium according to the American National Standards Institute sieving method (*22*).

After sieving, fractions remaining on each screen were weighed, and particle size distribution was expressed in percent cumulative weight over size by adding together the weight on each sieve and those from all larger screens (*22*). Mean/median particle size values were estimated by fitting these data in an exponential model (*19*): Pond's equation with 0 mm = 100%. Particles passing through the 0.58 mm sieve were included in Pond's equation with 0 mm = 100%. Data were computed using the NLIN procedure of the Statistical Analytical System (SAS). Mean particle size was calculated as the weighted average of sample particle sizes, and median particle size was determined to be equivalent to the value at 50% of the percentage cumulative weight over size.

$$\text{Pond's equation}: R = 100 \times e^{-k(s-w)}$$

where mean particle size = $1/k + w$; median particle size = $0.693/k + w$; R = percentage cumulative weight oversize; s = sieve opening size (mm); w = smallest predictable particle size; k = decay constant of the exponential curve describing the proportionality constant between the percent of particles passed to the next sieve and the percent remaining.

In Situ Rumen Incubation. *Animals and Diet*. Three dry Holstein cows weighing an average of 670 kg were ruminally fistulated and housed individually in 9 × 6 m box stalls with bedded straw at the metabolism facilities at the University of Saskatchewan. Cows had ad libitum access to fresh water and were free to enter the exercise ground. Cows were fed twice daily at 8:00 a.m. and 4:00 p.m. and received equal portions (7 kg of DM at each feeding time) of total mixed ration, consisting of 56.8% barley silage, 10.2% alfalfa hay, 4.5% dehydrated alfalfa pellets, 21.6% standard (multiparous) dairy concentrate, and 6.8% fresh (primiparous) cow concentrate according to the National Research Council Dairy Requirement. The diet was introduced over a 10-day adaptation period. The animals used in the experiment were cared for according to the guidelines provided by the Canadian Council on Animal Care (*23*).

Rumen Incubation at 12 and 24 h. All barley samples were coarsely dry-rolled through a 1.55 mm roller gap in a grain roller mill (Sven Grain Mill, Apollo Machine and Products Ltd.) at the College of Engineering, University of Saskatchewan. Ruminal degradability of DM, NDF, ADF, and ADL and ruminal solubility of FA and PCA at 12 and 24 h of incubation were determined using the nylon bag technique procedure described by Yu et al. (*24*) with three runs. After incubation, the bags were removed from the rumen and rinsed under a cold stream of tap water to remove excess rumen contents. Bags were washed with cool water without detergent and subsequently dried at 55 °C for 48 h. Dry samples were stored in a refrigerated room (4 °C) until analysis. Residues were pooled according to treatments and incubation times and ground through a 0.25 mm screen size for FA and PCA content determination following the same procedure as described before and ground through a 1 mm screen (Retsch ZM-1, Brinkmann Instruments (Canada) Ltd.) for NDF, ADF, and ADL chemical analysis.

Chemical Analysis of Fiber. NDF, ADF, and ADL were analyzed for 18 barley samples (6 varieties × 3 years). Whole barley grain was ground through 1 mm mesh screens at 10000 rpm (Retsch ZM-100, Brinkmann Instruments Ltd.). Half a gram of ground barley sample was accurately weighed and filled into an F57 filter bag (Ankom Technology Corp.). All samples were treated with α-amylase (Anachemia Science, Anachemia Canada Inc.) in 8 M urea (pellet, VWR International) solution overnight. α-Amylase was used at 100 μL per 30 mL of 8 M urea. After incubation, all bags for NDF analysis were rinsed 10 times with warm tap water. NDF and ADF were determined in tandem using an Ankom Fiber Analyzer (Ankom Technology) by boiling samples in neutral detergent solution (Ankom Technology) for 75 min and acid detergent solution (Ankom Technology) for 60 min, respectively (*25*). ADL was determined by oxidizing and removing the remaining carbohydrate residue in ADF with 72% H_2SO_4 (98%, VWR International) (AOAC, 1990). Hemicellulose and cellulose were calculated.

Statistical Analysis. Statistical analyses were performed using the Proc Mixed procedures in SAS. Experiments were carried out as a completely randomized design (CRD) with barley variety as a fixed effect and year as replication and as a random effect. Variance component estimation was done by REML. The DDFM Satterthwaite option was considered for approximating the degrees of freedom for means. Treatments were compared by LSD. Significance was declared at $P < 0.05$.

Table 2. Genotypic Differences and Variation of Hull Content in Six Barley Varieties Produced during Three Consecutive Years, Using a Modified EBC Method[a]

no.	barley variety	hull content (% DM)
1	McLeod	10.7 a
2	CDC Cowboy	10.4 ab
3	AC Metcalfe	10.2 bc
4	CDC Trey	10.1 bc
5	CDC Dolly	9.8 cd
6	CDC Helgason	9.4 d
	SEM	0.11

[a] Different letters in the same column indicate significant differences ($P < 0.05$). SEM, standard error of means.

For physiochemical profile data, the model used for the analysis was $Y_{ij} = \mu + t_i + e_{ij}$, where Y_{ij} is an observation of the dependent variables, μ is the overall mean, t_i is the fixed effect of the ith barley variety ($i = 1-6$), and e_{ij} is the error term specific to the barley variety corresponding to the ith treatment.

For rumen solublibility of FA, PCA, and fiber (NDF, ADF, and ADL) at 12 and 24 h of incubation, a factorial treatment arrangement and CRD experimental design were used to describe differences between barley varieties and rumen incubation time on rumen degradation parameters (residue percentages of DM, FA, PCA, NDF, ADF, and ADL) using the Proc Mixed in SAS. Barley variety and rumen incubation time were considered to be fixed effects. Means were compared by Fisher's protected LSD.

RESULTS AND DISCUSSION

Variation in Hull Content among Barley Varieties. Hull content varied ($P < 0.05$) from 9 to 11% with a mean value of 10% (**Table 2**). Significant variety differences for hull content were detected ($P < 0.05$), with McLeod and CDC Cowboy demonstrating the highest hull content and CDC Dolly and CDC Helgason the lowest. Hull content in this experiment agreed with that reported by Evers et al. (*13*), who indicated that barley hull content varies from 7 to 25% among two-row and six-row barley grains, whereas two-row barley commonly displays lower hull content with a mean of 10%. Barley hull content is influenced by environment and genetic factors. Evers et al. (*13*) stated that barley growing in higher latitudes produces less hull. Canada is located in the very northern latitudes, where relatively low temperature prevails during the barley growing season and tends to produce low hull content barley. Olkku et al. (*26*) found that barley hull thickness and skinning resistance property depends on its variety. Fox et al. (*27*) observed that barley hull content was associated with a genomic region on barley chromosome 2H. Such evidence provides the genetic basis for the variety difference for barley hull content.

Although CDC Dolly is not a new variety, it is a widely cultivated feed barley in Canada and is often used as a reference barley variety by barley breeders. CDC Dolly usually produces heavier test weight grain sample than many other varieties (*28*). The results of the current study also indicated that CDC Dolly had a lower ($P < 0.05$) hull content than McLeod and CDC Cowboy, but similar to that of CDC Helgason, CDC Trey, and AC Metcalfe, whereas CDC Helgason had the lowest ($P < 0.05$) hull content.

Genotypic Variation in Hydroxycinnamic Acids (FA, PCA) and Their Ratio. Rumen microorganisms are able to synthesize limited phenolic acid esterases to ultimately break down ester bonds (*8*, *11*). Ether linkages, however, are difficult to cleave in the anaerobic rumen environment (*11*). Therefore, esterified FA and PCA were analyzed in the present study. Esterified FA and PCA are alkaline sensitive and can be released by mild alkaline hydrolysis (2 N NaOH) at room temperature and are usually analyzed by HPLC.

Table 3 shows differences between varieties for FA and PCA contents and ratio of PCA to FA (PCA/FA) for the six varieties studied. Significant variety differences were detected ($P < 0.05$). In all samples, barley grain contained higher FA than PCA, ranging from 555 to 663 μg/g of DM for FA and from 283 to 345 μg/g of DM for PCA. Accordingly, the ratio of PCA to FA varied from 0.49 to 0.56 (or FA/PCA from 1.8 to 2.1). Hernanz et al. (*29*) examined several European malting and feed barley varieties and found ranges of 359−624 μg/g of DM for FA content, 79−260 μg/g of DM for PCA content, and 0.27−0.37 for PCA/FA ratio. Holtekjolen et al. (*30*) 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, PCA content varied from 114 to 244 μg/g of DM, and the PCA/FA ratio varied from 0.16 to 0.48. FA content in the present study was similar, but PCA content was slightly higher; consequently, the PCA/FA ratio was also higher. This is likely a result of the different growing environments and varieties studied.

Through radical coupling reactions, FA forms cross-linkages between cell wall polysaccharides and lignin and between polysaccharides (*5*). Ferulic acid cross-linkages increase stiffness and rigidity of plant cell walls to defend against pathogenic microorganisms and microbiological degradation (*31*). This is of special interest in ruminants because FA linkages limit the rumen digestibility of the plant cell walls by forming a steric obstacle to degradation by rumen bacteria. FA in barley grain is mostly concentrated in the bran (*1*, *29*). Comparison of FA content among the six varieties shows that barley variety significantly influenced the FA content in barley grain ($P < 0.05$). McLeod was highest ($P < 0.05$) in FA content, whereas CDC Dolly, CDC Trey, and CDC Helgason were significantly lower ($P < 0.05$).

Table 3. Genotypic Differences and Variation in the Hydroxycinnamic Acids—Ferulic Acid (FA), *p*-Coumaric Acid (PCA), and Ratio of PCA/FA Content in Six Barley Varieties Produced during Three Consecutive Years[a]

		hydroxycinnamic acids		
no.	barley variety	FA (μg/g of DM)	PCA (μg/g of DM)	ratio PCA/FA
1	McLeod	663 a	345 a	0.52 ab
2	CDC Cowboy	606 b	339 a	0.56 a
3	AC Metcalfe	594 bc	308 b	0.52 ab
4	CDC Helgason	581 bcd	283 b	0.49 b
5	CDC Trey	563 cd	301 b	0.53 a
6	CDC Dolly	555 d	306 b	0.55 a
	SEM	13.4	10.1	0.015

[a] Different letters in the same column indicate significant differences ($P < 0.05$). SEM, standard error of means.

The PCA content among the six barley varieties was also significantly different ($P < 0.05$). Ranking showed McLeod and CDC Cowboy had the highest PCA with no statistical difference for PCA content among AC Metcalfe, CDC Dolly, CDC Trey, and CDC Helgason. PCA is mainly esterified to cell wall lignin and seldom linked to polysaccharides, so it is as a good indicator of plant cell wall lignification (*4*, *11*, *12*). More PCA indicates more lignified plant cell walls (*11*, *12*). Barley bran, especially the hull, is the most lignified tissue in barley grain. It is possible that PCA content may relate to barley hull content or the degree of lignification in the hull. Current results show McLeod and CDC Cowboy had the highest ($P < 0.05$) PCA content in accordance with high hull content in these varieties.

The PCA/FA ratio is proposed as another indicator for plant tissue lignification, with limited lignified plant tissues having a low ratio and a high ratio indicating an even distribution of lignification in plant tissues (*12*). In the present study, CDC Helgason had a lower ($P < 0.05$) PCA/FA ratio than CDC Cowboy, CDC Dolly, and CDC Trey. Correspondingly, in the comparison of barley hull content, CDC Helgason also showed signifcantly lower hull content than CDC Cowboy and CDC Trey, but similar to that of CDC Dolly. Further study is required to assess the relationship among the FA and PCA contents and barley hull content.

Genotypic Variation in Fiber (NDF, ADF, ADL, Hemicellulose, and Cellulose). The NDF, ADF, ADL, hemicellulose, and cellulose content of the six barley varieties is presented in **Table 4**. For all five parameters, varieties showed significant differences ($P < 0.05$). NDF varied from 17.6 to 21.9% DM with mean of 19.5% DM. ADF was much lower than NDF, ranging from 5.5 to 7.0% DM with a mean of 6.0% DM. ADL varied from 1.7 to 2.1% DM with an average of 1.9% DM. By difference, the contents of hemicellulose and cellulose were 13.5% DM (from 12.2 to 14.9% DM) and 4.1% DM (from 3.8 to 4.9% DM), respectively.

McLeod and CDC Cowboy had higher ($P < 0.05$) NDF, ADF, ADL content than the other barley varieties. CDC Helgason and CDC Dolly were relatively low in fiber and did not differ from each other. NDF and ADF contents were slightly lower than the results of Fairbairn et al. (*32*), whereas ADL was in accordance with the NRC value. Studies have reported a wide variation in barley fiber content, with NDF from 12 to 26% DM, ADF from 4 to 8% DM, and ADL around 1% (*32*). The calculated mean from NRC of hemicellulose was 13.6% DM and the mean of cellulose was 5.3% DM, which are comparable to results here. All of this implies that fiber content varies between barley varieties.

Ruminants are able to digest and utilize hemicellulose and cellulose as energy sources. Nonetheless, hemicellulose and

Table 4. Genotypic Differences and Variation in the Digestion-Limiting Fiber Content (ADF, NDF, ADL, Hemicellulose, and Cellulose) in Six Barley Varieties Produced during Three Consecutive Years[a]

		fiber content in the original samples				
no.	barley variety	NDF (% DM)	ADF (% DM)	ADL (% DM)	hemicellulose (% DM)	cellulose (% DM)
1	McLeod	21.9 a	7.0 a	2.1 a	14.9 a	4.9 a
2	CDC Cowboy	20.5 b	6.4 b	2.1 ab	14.0 ab	4.4 b
3	AC Metcalfe	19.7 bc	5.7 cd	2.0 bc	13.9 bc	3.8 c
4	CDC Trey	19.0 cd	5.9 c	1.8 cd	13.1 cd	4.0 c
5	CDC Dolly	18.2 de	5.5 cd	1.8 d	12.6 de	3.7 c
6	CDC Helgason	17.6 e	5.5 d	1.7 d	12.2 e	3.8 c
	SEM	0.41	0.13	0.05	0.3	0.12
	mean	19.5	6.0	1.9	13.5	4.1

[a] Different letters in the same column indicate significant differences ($P < 0.05$). SEM, standard error of means; NDF, neutral detergent fiber; ADF, acid detergent fiber; ADL, acid detergent lignin; hemicellulose, calculated as NDF − ADF; cellulose, calculated as ADF − ADL.

cellulose contain less DE than lipid, starch, and protein. Therefore, barley grain containing more hemicellulose and cellulose will have lower energy density and digestibility. McLeod and CDC Cowboy had more fiber than the other varieties evaluated. In contrast, CDC Helgason and CDC Dolly with less fiber would be better choices for feed and might provide animals with higher DE at the same feeding level. Lignin, an important structural component of plant cell walls, is attached to hemicellulose and cellulose and concentrated in the secondary cell walls (*33*). As opposed to hemicellulose and cellulose, lignin is not a polysaccharide, but a phenolic complex (*33*); thus, lignin cannot be utilized as an energy source. Furthermore, no apparent lignin-degrading microorganisms or enzymes in the rumen can degrade lignin efficiently. As a result, lignin is a well-known inhibitor of plant cell wall digestibility. Therefore, the less lignin barley has, the better the feed quality. CDC Helgason and CDC Dolly with lower lignin should be superior feed.

Mean/Median Particle Size of Coarsely Dry-Rolled Barley Grains: Magnitude of Difference and Genotypic Variation. As illustrated in **Table 5**, there were significant differences ($P < 0.05$) in the mean/median particle size (estimated from Pond's equation with 0 mm = 100%) among barley varieties [A preliminary experiment was conducted, and the Pond's equation was the best choice after three model comparisons: Fisher's equation, Pond's equation, and the Meometric Mean equation (*19*, *22*, *34*).] The range of mean particle size estimated using Pond's equation was 3.06−3.66 mm, with an average value of 3.35 mm. Median particle size ranged from 2.71 to 3.04 mm, with an average of 2.91 mm. Numerically, the predicted sequence for mean particle size from large to small was CDC Cowboy, CDC Helgason, McLeod, CDC Dolly, AC Metcalfe, CDC Trey, whereas the rank of median particle size was CDC Cowboy, CDC Helgason, CDC Dolly, McLeod, AC Metcalfe, and CDC Trey.

Particle size reduction after mechanical processing is related to grain physical and chemical characteristics such as hardness. Camm and Rossnagel (*35*) reported that milling energy consumption was positively related to barley endosperm hardness. Their results of milling energy requirement and single kernel characterization system (SKCS) hardness tests from high to low were McLeod, CDC Dolly, CDC Helgason, and CDC Trey for milling energy; and McLeod, CDC Dolly, CDC Trey, and CDC Helgason for SKCS hardness. In the present study, mean/median particle size of the same four barley samples demonstrated a trend of grain hardness similar to that of Camm and Rossnagel (*35*), with the exclusion of CDC Helgason. The similar trend potentially means that particle size reduction of dry-rolled barley grain is related to inherent grain hardness. However, in the present study, the significant difference in Pond's mean/median particle size was detected only between CDC Cowboy, CDC Helgason, and CDC Trey, whereas no difference ($P > 0.05$) was observed among CDC Helgason, McLeod, CDC Dolly, and AC Metcalfe. The genetic makeup of barley grain is responsible for intrinsic chemical composition (e.g., β-glucan, protein matrix conformation) (*36*) and grain hardness, which consequently influences barley particle size distribution after mechanical manipulation. Fairbairn (*32*) observed that even when grain was finely ground, a significant difference in particle size among 20 barley varieties was detected.

Table 5. Genotypic Differences and Variation in Mean and Median Particle Sizes of Six Coarsely Dry-Rolled Barley Varieties Produced during Three Consecutive Years and Predicted by Pond's Equation with 0 mm = 100%[a]

		particle size distribution	
no.	barley variety	mean (mm)	median (mm)
1	CDC Cowboy	3.66 a	3.04 a
2	CDC Helgason	3.39 ab	2.98 a
3	McLeod	3.35 bc	2.92 a
4	CDC Dolly	3.33 bc	2.94 a
5	AC Metcalfe	3.31 bc	2.84 ab
6	CDC Trey	3.06 c	2.71 b
	SEM	0.073	0.047
	mean	3.35	2.91

[a] Different letters in the same column indicate significant differences ($P < 0.05$). SEM , standard error of means.

Genotypic Variation in in Situ Rumen Solubilization of Hydroxcinnmaic Acids (FA, PCA) and Fibers of Six Barley Varieties at 12 and 24 h Incubation. Table 6 shows the differences between barley varieties and rumen incubation time on in situ rumen undigested residues (% DM) of DM, FA, PCA, and fibers (NDF, ADF, ADL) and also shows the interaction between the barley variety and rumen incubation time.

Different effects ($P < 0.05$) of variety were observed on the rumen undigested residues of barley DM, NDF, ADF, FA, and PCA except for ADL residues ($P = 0.1393$). There was a significant effect ($P < 0.05$) of rumen incubation time on rumen undigested residues of barley DM, ADF, ADL, and FA. No interaction on rumen undigested residues from barley variety and rumen incubation time was observed.

Average DM residue percentages at 12 and 24 h of rumen incubation were 56.6 and 37.4%, respectively, and were significantly different ($P < 0.05$). DM residues in the present study were higher than those of Yu et al. (*24*), who observed that the undigested residues of coarsely dry-rolled barley (Harrington and Valier) were approxmately 21% at 24 h. This discrepancy could have resulted from the different grain particle sizes, as the roller gap used in current experiment was larger (1.55 vs 0.53 mm). CDC Dolly showed relatively low DM residues (44.1%) after rumen digestion, indicating CDC Dolly has high DM digestibility, which is favorable for ruminants. In contrast, CDC Helgason was poorest (49.4%). Although CDC Helgason demonstrated lower hull and fiber than CDC Dolly, it showed higher levels of rumen undigested DM residues. This could result if CDC Helgason had a property of slow rate of DM digestion in the rumen. If so, CDC Helgason could be a good feed barley as well, but more experiments are needed to test this assumption.

Residue percentages of fiber in the form of NDF, ADF, and ADL were much higher than DM as fiber is more recalcitrant to rumen digestion. Neutral detergent fiber represents the total structural cell wall components including cellulose and hemicellulose as well as lignin, so rumen indigestion of NDF residue was lower than ADF and ADL and averaged 61.4 and 60.4% at 12 and 24 h, respectively. Feng et al. (*37*) reported 63−68% total tract undigested NDF for whole barley grain, whereas Beauchemin et al. (*15*) found it was 53% for the whole barley grain, indicating a range of variation for NDF digestibility exists. The difference of rumen undigested NDF between 12 and 24 h was not significant, which might imply that most NDF in barley grain was degraded at 12 h of rumen incubation. Acid detergent fiber contains principally cellulose and lignin, which is less digestible than NDF. Beauchemin et al. (*15*) found that rumen undigested ADF for stream-rolled barley was about 80% compared to 50−65% of undigested NDF. In the present study, ADF residue left at 12 and 24 h averaged 90.1 and 87.0%, respectively. It is found to be statistically different among barley varieties and between the two rumen incubation time points ($P < 0.05$). Among the six varieties, McLeod showed considerably higher ADF residue than CDC Dolly and AC Metcalfe. Less ADF is always preferred in feed barley selection. Therefore, AC Metcalfe

Table 6. Effect of Barley Variety on in Situ Rumen Solubility of Ferulic Acid (FA), *p*-Courmaric Acid (PCA), NDF, ADF, and ADL) at 12 and 24 h of Rumen Incubations[a]

	in situ rumen undigested residue (% of total) at 12 and 24 h					
		hydroxycinnamic acids		fibers		
	DM (%)	FA (%)	PCA (%)	NDF (%)	ADF (%)	ADL (%)
variety						
Metcalfe	46.3 abc	60.3 cd	71.7 b	61.3 a	85.2 d	87.1
CDC Cowboy	45.4 bc	64.1 b	74.2 b	64.4 a	89.9 ab	91.3
CDC Dolly	44.1 c	59.9 d	72.1 b	61.9 a	86.6 cd	87.0
CDC Helgason	49.4 a	59.7 d	71.9 b	57.8 b	88.8 bc	89.4
CDC Trey	47.6 abc	72.3 a	80.6 a	57.7 b	88.4 bc	89.4
McLeod	49.0 ab	63.6 bc	74.7 b	62.8 a	92.3 a	89.7
SEM	1.31	1.30	1.54	1.17	1.03	1.28
time						
12 h	56.6 a	64.8 a	74.6	61.4	90.1 a	90.1 a
24 h	37.4 b	61.8 b	73.8	60.4	87.0 b	87.8 b
SEM	0.76	0.75	0.89	0.68	0.60	0.74
statistical analysis						
variety (*P* value)	0.0356	<0.0001	0.0006	<0.0001	<0.0001	0.1393
time (*P* value)	<0.0001	0.0065	0.5538	0.3538	0.0004	0.0292
variety × time (*P* value)	0.8842	0.6073	0.3328	0.7101	0.7387	0.9653

[a] Different letters in the same column indicate significant differences ($P < 0.05$).

and CDC Dolly were superior to McLeod in terms of feed barley quality. Although ADL is thought of as low in digestibility, in the present study, roughly 10% of ADL was soluble in the rumen. Nelson (*38*) also reported 12.2% degradable ADL when lambs were fed a coarsely dry-rolled barley-based diet. However, no statistical difference among barley varieties was detected for ADL residues. The original content of ADL in barley was quite low (about 1−2%). This could contribute to analytical error and low sensitivity of the statistical analysis. In practice, ADL digestibility of barley grain is seldom analyzed.

To our knowledge, there are few studies of the solubility of esterified FA and PCA in barley using either in vitro or in situ methods. Researchers are usually interested in FA and PCA in forages, because forages contain more FA and PCA than cereal grains. In the present study, there was a significant effect of barley variety on rumen undigested FA and PCA ($P < 0.05$). Although CDC Trey showed only moderate hull content, fiber, FA, and PCA, it had significantly higher rumen undigested FA and PCA than others. The reason for this is not clear. With the exception of CDC Trey, there was no difference in rumen undigested PCA among the remaining five varieties. Rumen undigested FA at 12 and 24 h was significantly different ($P < 0.05$), decreasing from 64.8 to 61.8% on average. The difference for rumen undigested PCA was not obvious ($P = 0.5538$), from 74.6 to 73.8%. This implies that FA in barley could continue to be solublized in the rumen after 12 h of rumen incubation, whereas the solubilization of PCA plateaued after 12 h of incubation. After the same incubation periods (12 and 24 h), FA showed less rumen undigested fraction than PCA, indicating that FA was solublized to a greater extent than PCA. Others have observed that esterified FA was more solubilized and more quickly than esterified PCA in forages (e.g., cocksfoot, orchardgrass).

When all degradation parameters were compared, CDC Dolly had relatively lower residues of DM, FA, PCA, NDF, ADF, and ADL compared to the other barley varieties, whereas McLeod seemed to be more resistant to rumen degradation. In combination with the original physical and chemical information, CDC Dolly is more promising as a feed barley grain, whereas McLeod is relatively inferior. Future studies will be carried out to investigate the detailed solublization kinetics of FA and PCA in barley and their relationship to nutrient availability in ruminants.

Physicochemical analyses show that there were significant differences between barley varieties for hull content, FA, PCA, fiber fraction (NDF, ADF, ADL), and mean and median grain particle sizes. Therefore, barley variety plays an important role in determining the quality of barley as a feed. Generally, varieties McLeod and CDC Cowboy consistently had higher hull content, FA, PCA, and fiber compared to CDC Dolly and CDC Helgason. Therefore, from a nutritional point of view, CDC Dolly and CDC Helgason are more valuable than McLeod and CDC Cowboy. However, when mean/median particle sizes obtained after coarse dry-rolling were compared, CDC Cowboy and CDC Helgason had larger particle size and, therefore, become more promising as feed barley. On the whole, CDC Dolly and CDC Helgason have lower hull content, FA, PCA, fibers, and moderate mean/median particle size after dry-rolling, so both are good candidates for feed barley.

In situ degradation results show that there were significant differences between barley varieties for rumen undegradable residue content of DM, FA, PCA, and fibers (NDF, ADF) either at 12 or 24 h of rumen incubation, but only a numerical effect on ADL. Among the six barley varieties, CDC Dolly demonstrated relatively lower rumen residues. In contrast, McLeod showed comparatively higher residues and inferior digestibility. This information also implies that CDC Dolly would be a good candidate as a feed barley for ruminants.

In conclusion, there were significant genotypic differences and variations in barley hull content, particle size distribution, FA, PCA, and PCA/FA ratio, as well as in situ rumen degradability among the barley varieties evaluated. Further study is needed on the quantitative relationship between the physicochemical characteristics, hydroxycinnamic acids in barley, and nutrient availability in ruminants.

ABBREVIATIONS USED

ADF, acid detergent fiber; ADL, acid detergent lignin; DE, digestible energy; DM, dry matter; EBC, European Brewery Convention; FA, ferulic acid (3-methoxy-4-hydroxycinnamic acid);

HPLC, high-performance liquid chromatography; NDF, neutral detergent fiber; PCA, *p*-coumaric acid (4-hydroxycinnamic acid); REML, restricted maximum likelihood; RSS, residue sum of squares; SEM, standard error of mean.

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