HULL, FERULIC ACID, PARA-COUMARIC ACID CONTENT AND PARTICLE SIZE CHARACTERISTICS OF VARIOUS BARLEY VARIETIES IN RELATION TO NUTRIENT AVAILABILITY IN RUMINANTS

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ABSTRACT

The fibrous barley hull is the main reason for barley's low available energy relative to corn. Barley grain contains hydroxycinnamic acids (mainly ferulic acid (FA) and ρ-coumaric acid (PCA)) which are cross-linked to polysaccharides, therefore, limit cell wall degradability in the rumen. Paricle size of barley grain also affects the digestion of barley in the rumen. The objective of this study was to evaluate a set of barley varieties grown in Saskatchewan (Canada) and provided by Crop Development Center (CDC, Canada) and find a variety with low hull, FA, PCA and fiber content, while maintaining large particle size after mechanical processing, and having high nutrient availability.

Three studies were conducted to determine the content of barley hull, FA, PCA, neutral detergent fiber (NDF), acid detergent fiber (ADF) and acid detergent lignin (ADL) in various barley varities. Mean/median particle size of the barley grain after coarse dry-rolling was also determined. The relationships among these parameters and the digestibility of barley grain in ruminants were then assessed. Six barley varieties (AC Metcalfe, CDC Dolly, McLeod, CDC Helgason, CDC Trey and CDC Cowboy) from samples grown in three years (2003, 2004 and 2005) were evaluated in each study.

The first study determined the original content of barley hull, FA, PCA, NDF, ADF, ADL, and mean/median particle size of barley grain and evaluated the effects of barley variety. The results showed barley variety had a significant impact on the chemical and physical profiles of barley grain, with CDC Helgason and CDC Dolly showing relatively lower content of barley hull, FA, PCA, NDF, ADF, ADL, hemicellulose and cellulose, and moderate mean/median particle size, whereas McLeod and CDC Cowboy showed the opposite.

The second study involved two consecutive trials. Trial 1 was to assess differences in the in situ rumen degradability of dry matter (DM), FA, PCA, NDF, ADF and ADL at 12 and 24 h of rumen incubations. Results revealed that CDC Dolly consistently showed relatively lower rumen residues of DM, FA, PCA, NDF, ADF and ADL at 12

and 24 h, with McLeod being opposite. Barley variety displayed some effects on the digestibility of DM, FA, PCA, NDF, ADF and ADL at 12 and 24 h. Since CDC Dolly demonstrated relatively less content of hull, FA, PCA, NDF, ADF, ADL and mean/median particle size and higher rumen digestibility among the six barley varieties, while McLeod was the opposite, CDC Dolly and McLeod were selected for the third trial in order to compare differences in the rumen degradation kinetics of DM, FA and PCA. Trial 2 did not show significant differences in effective degradation of DM, FA, except for PCA. In general, CDC Dolly exhibited better degradability of DM, FA and PCA than McLeod.

The third study analyzed the correlation and regression between the original content of barley hull, FA, PCA, NDF, ADF, ADL and mean/median particle size in barley grain and rumen residual content of the corresponding parameters at 12 and 24 h of rumen incubation. Results showed that FA content in barley grain had a predominantly negative effect on DM degradability, while barley hull content affected the degradability of NDF and ADF.

In summary, the present studies show that hull and FA content in barley grain have negative effects on the degradability of barley grain in ruminants and also showed that CDC Dolly could be an ideal feed barley grain for ruminants due to its lower hull and FA content and higher rumen dry matter degradability.

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LIST OF ABBREVIATIONS

ADF	Acid detergent fiber
ADL	Acid detergent lignin
ANOVA	Analysis of variance
ANSI	American National Standards Institute
AOAC	Association of Official Analytical Chemists
CDC	Crop Development Center
Cell	Cellulose
CFIA	Canadian Food Inspection Agency
CRD	Completely randomized design
D	Slowly digestible fraction of material for in situ incubation (%)
DAD	Diode array detector
DDFM	Denominator degrees of freedom method
DM	Dry matter
EBC	European Brewery Convention
ED	Effective degradability
EDCP	Effective degradable crude protein (%)
EDDM	Effective degradable dry matter (%)
EDFA	Effective degradable ferulic acid (%)
EDPCA	Effective degradable ρ-coumaric acid (%)
FA	Ferulic acid
FAO	Food and Agriculture Organization of the United Nations
GM	Geometric mean diameter
h	Hour
Hemi	Hemicellulose
HPLC	High performance liquid chromatography
K_d	Rate of degradation of slowly digestible fraction (D fraction) (% h^{-1})
K_p	Rumen passage rate (% h^{-1})
LSD	Least significant difference
NDF	Neutral detergent fiber
NRC	National Research Council

P value	Probability of significance
PCA	ρ-Coumaric acid
PI	Processing index
R	Correlation coefficient
R(t)	Rumen residue at time t
R^2	Coefficient of determination
RCBD	Randomized complete block design
RSS	Residual sum of squares
RU	Rumen undegraded feed fraction
RUDM	Rumen undegraded dry matter
RUFA	Rumen undegraded ferulic acid
RUPCA	Rumen undegraded ρ-coumaric acid
S	Soluble fraction (%), estimated by washing without rumen incubation
SAS	Statistical Analytical System
SD	Standard deviation
SEM	Standard error of the means
SKCS	Single Kernel Characterization System
T_0	Lag time (h)
U	Undegradable fraction of material for in situ incubation (%)

1. INTRODUCTION

Barley, the second most important cereal grain in Canada is mainly used as feed for animals, but also fermented into alcohol for beer, processed into flour for food, and has lately been increasingly used for ethanol production (Statistics Canada, 2000-2008). The majority of the commercially available barley varieties are hulled malting types (Canadian Food Inspection Agency (CFIA) 2007). The hull composition and content are closely related to the value of barley as a feed grain as well as a fermenting and malting grain. Although barley contains more protein than corn, it has slightly lower available energy density (National Research Council (NRC) 2001). Barley's shortcoming in energy is mainly attributed to its greater content of hull, which represents approximately 13% of the weight of barley seed (Evers et al. 1999). It is extremely fibrous and indigestible for monogastric animals and partially degradable in ruminants. However, the adhesive barley hull is a desirable trait in the malting and brewing industry (Olkku et al. 2005). Ruminants that consume a large volume of hull-less barley may be predisposed to metabolic diseases because of the rapid fermentation nature of hull-less barley (Zinn et al. 1996). Therefore, it is impossible to eliminate hulled barley from the market. Due to the existence of large numbers of hulled barley varieties and the importance of barley to Canada's feed industry, it is economically significant to improve the nutritive quality of barley and barley hull as an energy feed for ruminants.

The rate and extent of plant cell wall degradation in the rumen is negatively impacted by complex plant cell wall components such as lignin, cellulose, lignin-carbohydrate, and phenolic-carbohydrate (Yu et al. 2005a). Phenolic constituents, especially hydroxycinnamic acids play an undesirable role in barley cell wall digestion in ruminants. Barley grain contains two major low molecular weight hydroxycinnamic acids: ferulic acid (FA, 4-hydroxy-3-methoxycinnamic acid) and ρ-coumaric acid

(PCA, 4-hydroxycinnamic acid) (Nordkvist et al. 1984; Hernanz et al. 2001). About 80% of FA and PCA are concentrated in barley grain's outer layer (barley bran) (Nordkvist et al. 1984; Hernanz et al. 2001; Sancho et al. 2001). FA is covalently cross-linked to polysaccharides by ester bonds and to lignin by both ester and ether bonds (Sun et al. 2002). FA also forms ester-ether cross-linking bridges between polysaccharides and lignin, and among the polysaccharides (Iiyama et al. 1990; Lam et al. 1992a). The ferulate dimers and trimers are involved in the bridging linkages as well (Hernanz et al. 2001; Rouau et al. 2003; Bunzel et al. 2005). Therefore, FA is believed to be one of the major inhibiting factors to the biodegradability of plant cell walls in the rumen (Yu et al. 2005a). PCA is mainly esterified to the cell wall polysaccharides and lignin, but does not form cross-linkages as FA does (Sun et al. 2002). PCA is believed to have no direct inhibitory effect on plant cell wall digestibility and is considered to be as an indicator of plant cell wall lignification (Jung and Allen 1995; Grabber et al. 2004).

Before feeding to cattle, barley grain is usually ground, rolled, cracked or crimped to breach the tough hull thereby improving the digestion and utilization of the grain (Owens et al. 1997). Barley particle size reduction obtained after mechanical processing is related to the grain's hardness, texture and composition (Camm 2008; Darlington et al. 2001; Psota et al. 2007). Therefore, different barley varieties could produce variable mean/median grain particle size after the same mechanical processing. Excessive processing should be avoided and fine particles should be minimized because high levels of small particles will not only lead to an unpalatable ration, but will also predispose cattle to digestive upsets (Owens et al. 1997). One of the desirable merits of barley as a ruminant feed is the hard-textured endosperm which can maintain larger particle size after dry-rolling, thus slowing down starch fermentation in the rumen (Bowman et al. 2001; Zhang et al. 2003).

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 and varied nutrient availability in ruminants. 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 project were: 1) to determine the content of barley hull, FA, PCA, NDF, ADF, ADL and mean/median particle size of barley grain (several varieties) obtained by coarse dry-rolling; 2) to estimate rumen digestibility of barley grain (several varieties) at 12 and 24 h of rumen incubation and to find a variety with low hull, FA, PCA and fiber content, maintaining large particle size after mechanical processing, and having high nutrient availability; and 3) to perform correlation and regression analyses among the available parameters to reveal the factors influencing the digestibility of barley grain in the rumen.

It is hypothesized that the barley variety with the lower content of barley hull, FA, PCA, NDF, ADF, ADL, larger particle size after coarse dry-rolling and less in situ rumen indigestible residues at 12 and 24 h of rumen incubation, would be a better feed barley for ruminants.

2. LITERATURE REVIEW

2.1 Basic Information on Barley and Cattle

2.1.1 Barley

Cultivated barley, *Hordeum vulgare* L., belongs to the grass family *Poaceae*, the tribe *Triticeae*, and the genus *Hordeum* (Shewry 1992) and is mostly harvested for its grain. Barley has good environmental adaptability; therefore, it is distributed throughout the world. Barley particularly prospers in the well-drained farmland with cool and semi-arid climates (Slafer et al. 2002) such as western Canada.

2.1.1.1 Barley Production in Canada

As one of the most widely grown cereal grains, barley ranks fourth in the world, behind wheat, corn and rice, while in Canada barley is second only to wheat (FAOSTAT 2005). According to FAO statistics (FAOSTAT 2005), Canada is among the top five barley producers and exporters in the world. Annual barley production in Canada is close to 12 million tonnes, with the majority of the production from western Canada (mainly Alberta and Saskatchewan) (Statistics Canada, 2003-2005). Roughly 43% is exported; 18% contributes to food and industrial usage; the remainder (39%) is used as animal feed (Statistics Canada, 2000-2008).

2.1.1.2 Barley Variety

Barley is an ancient cereal crop. Due to a long history of artificial selection, barley has evolved a great many varieties with diverse traits. With the development of modern barley breeding techniques, new barley varieties are continuously generated.

As per different categorizing standards, barley can be classified as malting and feed types, two-row and six-row types, hulled and hull-less types, rough, smooth, hooded awns and awn-less types, winter and spring types. In Canada, newly developed

barley varieties are designated pursuant to barley's different classes and agronomic features, and registered by the Canadian Food Inspection Agency (2003). So far, two hundred varieties of locally adapted barley varieties have been registered and cultivated in Canada (CFIA 2007). Among the varieties on the list, only three are winter types. Two-row and six-row types account for 85 and 115 varieties respectively, with 23 hull-less types. There are over 50 barley varieties produced in western Canada, including eight hull-less types, 13 malting types and some others suitable for the livestock industry (Manitoba Competitiveness Training and Trade Undated).

2.1.1.3 Malting Barley and Feed Barley

Historically, barley development has been directed toward the characteristics of growth, production and malting qualities because of the particular support from the malting and brewing industries (Slafer et al. 2002). Malting barley has a premium price over feed barley of $16 to 62 per tonne (Canadian Wheat Board 2007). Therefore, barley is grown primarily for malting purposes despite being Canada's primary feed grain. About 65% of the Canadian barley acreage is sown with malting varieties. In Saskatchewan, malting barley occupies more than 80% of the total barley acreage. Barley production in Canada often exceeds the demand for malting and brewing. Indeed, less than 20% of the total barley production (of top grades) is selected for the malting and brewing industry annually, with the majority going to the feed industry (Canadian Wheat Board 2007).

As far as the qualities and characteristics of malting and feed barley varieties are concerned, discrepancies do exist. Due to the specific malting and brewing requirement, brewers always look for malting barley with well-filled, high starch, low protein and soft grain (Slafer et al. 2002). Although the barley hull is inedible and is of low nutritive value for humans and animals, the presence of the hull is desirable. For malting barley, it is important that the hull remains intact with good resistence to hull peeling, thin and wrinkled traits (Edney, 1999; Roumeliotis et al. 2001). Traditionally, the importance of barley as feed grain is often overlooked and barley varieties not suitable for malting and brewing would be designated as feed barley. In the past, feed barley quality was

focused on agronomic and physical characteristics such as yield and test weight. A good feed barley variety requires higher nutrient content, better animal utilization and performance. Traits of interest include high energy density, elevated protein content and quality, large particle size after coarse dry rolling, slow rate of dry matter digestibility, low acid detergent fiber content, low phytate, low hull content and low content of hydroxycinamic acids (especially FA and PCA).

2.1.1.4 Two-row and Six-row Barley

Based on the arrangement of seeds in the ear, barley is divided into two-row and six-row type (Slafer et al. 2002). Two-row barley tends to have heavier test weight, plumper kernels, uniform seeds, less hull and fiber content, better feed conversion by animals and better resistance to diseases than six-row barley. Bowman et al. (2001) reported that six-row barley contained higher acid detergent fiber and lower starch content, and also had lower dry mater digestibility than two-row barley. Therefore, it is possible that two-row barley is more valuable for feeding animals. In addition, two-row barley dominates barley production in Canada (Canadian Wheat Board 2004-2006).

2.1.1.5 Hulled and Hull-less Barley

Just as the name implies, the main difference between hulled and hull-less barley is the presence or absence of the hull. In hulled barley, the hull is firmly attached to the kernel, while in its hull-less counterpart, the hull is loose and easily detached during threshing. Hull-less barley was developed primarily for swine and poultry (Bhatty 1999). The presence of the hull dilutes the nutrient content in regular barley. Despite the shortcomings of hulled barley as an animal feed, there are reasons for the prevalence of the hull. Foremost, it is the byproduct of the malting and brewing industry. Secondly, ruminants can partially digest barley hull. The adherent hull even helps to slow down barley starch fermentation and to prevent excessive accumulation of acid in the rumen (Zinn et al. 1996). Cattle performance experiments do not show an advantage of hull-less barley for ruminants (Zinn et al. 1996; Beauchemin et al. 1997;

Yang et al. 1997). Last but not least, hulled barley currently yields more and is more economical for animal producers than hull-less barley.

2.1.2 Specific Digestion Behavior in Ruminants

The physiological difference in digestion between ruminants and monogastric animals principally results from the different characteristics of their gastro-intestinal tract. Ruminants have four-compartment stomachs in which the rumen is the site for fiber digestion. Ruminants have evolved this special ability for fiber digestion through symbiosis with microorganisms. The rumen hosts billions of microorganisms, including bacteria, protozoa, fungi, archaea, and viruses (Dehority 2003). Rumen microflora contribute to the degradation and fermentation of protein, starch, fiber, and other recalcitrant substances through enzymatic activity. Undergoing fermentation in the rumen, plant cabohydrates are converted into organic acids and gases (Owens et al. 1998). In well-adapted and normally fed cattle, rumen microbial ecology is balanced. Rumen pH fluctuates from 5.7 to 7.3. The rumen is well buffered with saliva as a result of chewing activities (Beauchemin 2002). When cattle are fed heavily on soluble carbohydrate or rapidly fermenting starch (e.g. barley grain), organic acids produced in the rumen may exceed the buffering capability, resulting in low rumen pH which could induce animal digestive upsets and diseases (commonly acidosis, laminitis and liver abscesses) (Owens et al. 1998).

Cattle in western Canadian feedlot are fed ad libitum with a ration containing up to 90% barley grain (Personal communication, John J. McKinnon, University of Saskatchewan). However, barley is known as a “hot” grain, because its starch is rapidly degraded in the rumen. Therefore, in order to prevent animal digestive diseases for cattle fed on high levels of barley, special management should be practised, such as gradual increases of barley content in the feed, slowing down dry matter digestibility of barley grain, minimizing fine particles in feed, supplementing additives in feed and employing suitable feeding strategies (Owens et al. 1998; Anderson and Schroeder 1999; Ondarza 2006).

2.2 Physical and Chemical Profiles of Barley Grain

Barley belongs to the monocotyledonous grasses. Its seed is called grain or kernel, whereas the botanical term is caryopsis (Slafer et al. 2002). Barley grain has a complicated structure. The discrepancies in physical, chemical and digestive characteristics among various barley varieties can be better understood through examining the grain's structure and interactions among different tissues. Hulled barley grain is comprised of the hull, pericarp, testa, aleurone, endosperm and embryo (Figure 2.1) (Hough 1991). In a two-row barley kernel, the approximate proportions of these different parts are 9-14% hull, 2-3% pericarp and testa, 4-5% aleurone, 77-82% endosperm and 2-3% embryo on DM basis (Briggs et al. 2004; Priest and Stewart 2006). Barley bran is collectively made up of the hull, pericarp, testa and aleurone layer (Chakraverty et al. 2003).

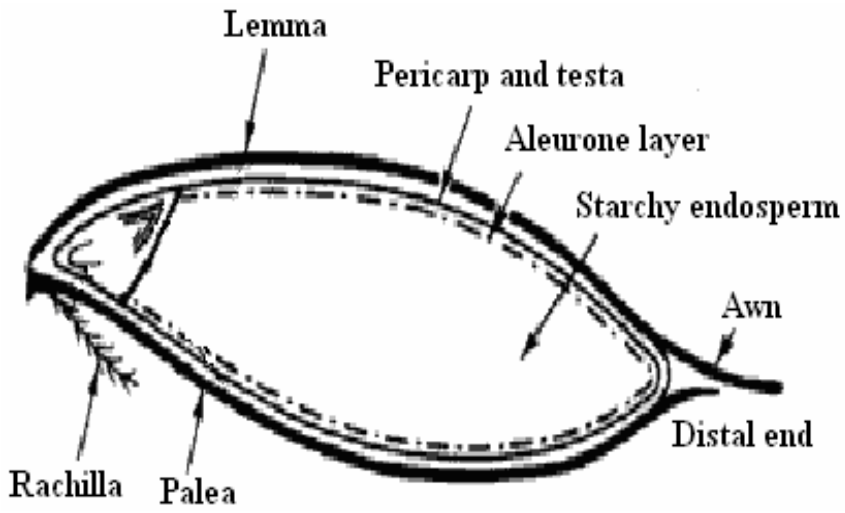


Figure 2.1. Diagram of longitudinal section of a barley grain.

Adapted from Hough (1991)

2.2.1 Barley Hull

2.2.1.1 The Composite Structure of the Hull

The seed of hulled barley is wrapped within a tough outer covering called the hull or husk. The hull is firmly cemented to the kernel, so it is not easily detached by threshing as occurs with hull-less barley. The hull is thin at the distal end and thicker at

the opposite end where the hull protects the embryo (Broderick and Vogel 1977). Anatomically, the hull consists of two scale-like bracts: lemma and palea (Dendy and Dobraszczyk 2001). The lemma is attached to the dorsal side of the grain, while the palea adheres to the ventral side (Hough 1991). In an awned barley type, the lemma is extended and reaches out from the distal end of the grain forming the barley awn (Olkku et al. 2005). During harvesting and threshing, the awn is mechanically broken off at a point 3-5 mm away from the tip of the grain. Otherwise, the awn may irritate an animal's lip, tongue and mouth, and thereby depress appetite. Good quality barley grain should appear bright with buff or tan hull colour (Broderick and Vogel 1977). Discolored or stained grain might indicate environmental, climatic or disease damage (Fox et al. 2001).

In a sound, ripe barley grain, the hull consists of dead cells forming four types of layers (Figure 2.2): the outer epidermis, fibres, spongy parenchyma and inner epidermis (Briggs 1998; Olkku et al. 2005). Hull is the only tissue in barley seed that is highly lignified. The external epidermis is greatly silicified (Briggs 1998), and becomes a physical barrier for microorganisms' initial attack (Agbagla-Dohnani et al. 2003b). The outermost two layers built up with thick-walled cells are compact, rigid and act as a protective barrier (Olkku et al. 2005). In contrast, the two inner layers consist of thin-walled cells (Olkku et al. 2005). Olkku et al. (2005) reported that a thin layer of hull with intact thin-walled cells indicated greater resistance of the hull to physical and chemical damages, compared with the thick hull in which some parenchyma cells are broken. The hull of barley is fused to the pericarp through a cementing layer. Cementing materials are secreted by the pericap epidermis cells during grain development (Olkku et al. 2005). The outer and inner epidermal layers contain waxy cuticles which reduce water and microorganism penetration. When pelleting ground barley, the hydrophobic cuticular layer of the hull may cause brittleness and crumbling of the pellets (Gallant et al. 1991). The specific structure and composition of the hull gives barley an abrasive surface. Barley hull thickness and skinning resistance property depends on its variety (Olkku et al. 2005).

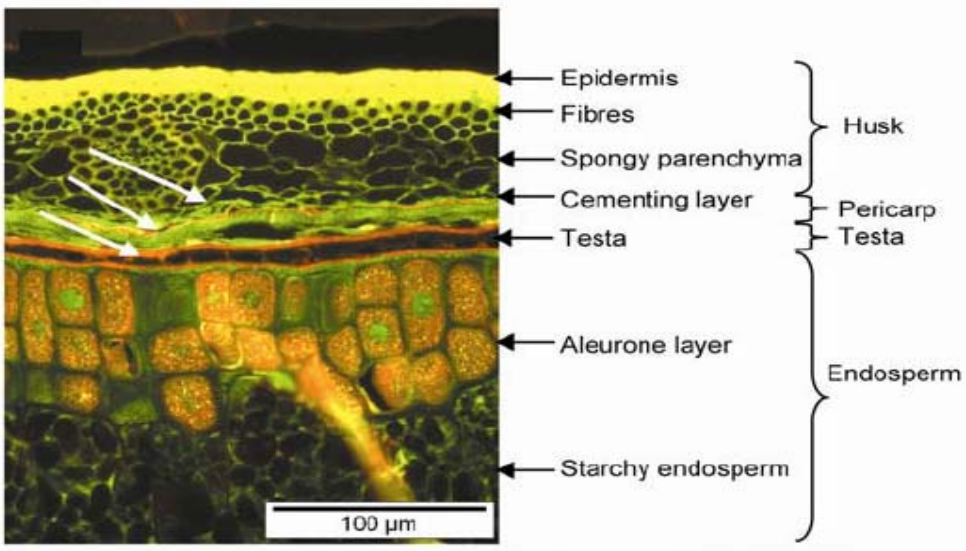


Figure 2.2. Micro-structure of barley hull and related tissues.
Source: Olkku et al. (2005)

2.2.1.2 The Chemical Constituents in the Hull

The hull is extremely fibrous and made predominantly of hemicellulose, cellulose, lignin and a small amount of ash and protein (Table 2.1) (Briggs 1998; Olkku et al. 2005). Grove et al. (2003) observed variation in content of neutral (NDF) and acid detergent fiber (ADF) and acid detergent lignin (ADL) in barley hulls of different varieties and under different agronomic management. Moore and Jung (2001) noted that many factors (e.g. environment, genotype and physiology) could affect fiber content in the plant. Since the hull contributes largely to the total content of ADF, NDF and ADL in barley grain, humans, monogastric animals and poultry have difficulty digesting the fibrous hull due to the lack of fibrolytic enzymes. Ruminants, however, can partially utilize the hull as an energy source. The poor quality of the hull actually dilutes the nutrient and energy content in barley grain, resulting in lower digestive energy in barley than in corn.

Table 2.1. Chemical composition of barley hull.

Composition	Average content	Content range
Cellulose (%)	27.7	26.5 – 28.8
Hemicellulose (%)	33.5	33.3 – 33.7
Pectin (%)	1.0	1.0
Lignin (%)	22.9	22.8 – 22.9
Ash (%)	5.1	5.05 – 5.16
Protein (%)	2.6	2.1 – 3.0
Others (%)	7.4	6.7 – 8.0

Adapted from Olkku et al. (2005)

Ash distribution is uneven throughout the kernel, being more concentrated in the hull (5.1% in the hull vs. 1.5% in the peeled kernel) (Kulp and Ponte 2000; Olkku et al. 2005). Of the ash, silica (SiO_2) contributes the greatest amount. The deposition of silica in the hull enhances its strength and rigidity and maintains the integrity of the hull, and thus improves the resistance to abiotic (e.g. arid soil) and biotic (e.g. diseases), which is beneficial for plant growth and kernel development (Liang et al. 2003). However, silica has a negative influence on plant cell wall digestibility in ruminants (Balasta et al. 1989; Agbagla-Dohnani et al. 2003b).

Barley hull contains significant amounts of phenolic compounds. Lignin is the typical complex phenolic polymer which impeds animal digestion of plant cell walls (Priest and Stewart 2006). Proanthocyanidins are complex phenols, having oxidative properties. In the animal alimentary tract, proanthocyanidins can inhibit protein digestion and utilization by forming an insoluble complex (Slafer et al. 2002). There are also small quantities of simple phenolic acid residues such as ferulic acid (FA) and ρ-coumaric acid (PCA) (Slafer et al. 2002; Priest and Stewart 2006). 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 hull, which will be discussed in detail later. Free phenolic acids have oxidative properties and antibacterial functions which help to defend the kernel from micro-organism attack.

However, when these phenolic acids form intricate cross-linkages with lignin and cell wall polysaccharides, they become the inhibitory factors for plant cell wall degradation in the rumen.

2.2.1.3 Factors Influencing Barley Hull Content

Barley grain has variable hull content ranging from 7 to 25% of the total seed weight (on DM basis) with the average being 13% (Evers et al. 1999). Many factors affect barley hull content, such as grain size, variety, growing environment, genetics and agronomic management.

Grain size: Physical or morphological aspects of barley grain can indicate the hull content. Plumper barley seeds should have lower hull content proportionately.

Variety: Collins et al. (1999) observed that barley hull content was associated with a region on chromosome 2H, which indicates the genetic basis for variety effect on barley hull content. 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 kernal becomes naked during threshing. Two-row types (10.4%) contain less hull content and thinner hull layer than six-row barley (12.5%) (Broderick and Vogel 1977; Evers et al. 1999). Variety effects on hull content are complicated because variety itself is related to the grain size, plumpness and test weight.

Growing environment: Geographic differences such as latitude create distinct climate and weather circumstances which sequentially affect barley growing conditions. In fact, any factor that influences barley grain composition also can change hull content directly or indirectly. Hull content gradually decreases as the growing sites change from low latitude (equator) to high latitude (the earth's poles) (Evers et al. 1999). Usually, long light patterns, low environmental temperature, less disease and predation stress increase plant metabolic pool and reserves, while reducing plant cell wall content and hull content (Van Soest 1994). Longer day-light may aid grain filling development and accordingly reduce hull content (Slafer et al. 2002). High temperature and drought during the grain filling period hinders starch accumulation and reduces barley grain weight and size (Savin and Nicolas 1996; Voltasa et al. 1999; Passarella 2002), which

consequently raises the hull ratio in the grain as a side effect. Fox et al. (2006) reported that both environment and genetic heritage had effects on barley hull content.

Agronomic management: Grove et al. (2003) reported that early-planted barley produced more hull, and contained more NDF and ADF in the hull than the later-planted counterpart. Erekul et al. (2007) found that application of excessive level of N-fertilizer lowered barley grain size, which will possibly affect barley hull content.

2.2.2 Dehulled Barley Kernel

2.2.2.1 Pericarp

Immediately below the hull is a thin waxy coat - the pericarp made up of compressed parenchyma cells without lignin and starch (Kulp and Ponte 2000; Olkku et al. 2005). Gaines et al. (1985) hypothesized that during growth, the pericarp epidermis secretes an unknown gluey material to form a cementing layer between the hull and outer layer of the pericarp. Therefore, in mature hulled barley cultivars, the hull is firmly fused to the pericarp, which results in difficulties in collecting and separating the hull on a large scale by mechanical or chemical methods (Briggs 1998). Wang et al. (1999) confirmed that the pericarp, as well as the hull was the most inhibiting structural barrier to ruminal digestion of barley. This is due to the waxy outer cuticle of the pericarp which is so compact that rumen microorganisms cannot attach to or penetrate it efficiently. Therefore, these authors suggested that the integrity of the pericarp, even for hull-less barley should be broken up to enhance digestion in the rumen. Furthermore, the cell wall of the pericarp mainly consists of arabinoxylans which are covalently linked to FA. Such cross-linkages may contribute to the recalcitrance of pericarp cell wall to rumen digestion and kernel hardness.

2.2.2.2 Testa

The testa is another seed-protecting thin layer cemented to the inner pericarp layer.The testa contains cellulose, lipids and polyphenoles (mainly proanthocyanidins and catechin). These constituents form a physical and chemical barrier to keep pathogens away from the endosperm and embryo. To some extent, proanthocyanidins

are considered to be an anti-nutrient factor because of their properties of chelating with iron and protein and their antibacterial functions.

2.2.2.3 Aleurone layer

The aleurone layer, three cells deep, is composed of live, thick-walled cells. It is the site for secreting hormones and many hydrolytic enzymes (Briggs 1998). The aleurone cell wall consists of mainly arabinoxylan and cellulose (McNeil et al. 1975). The thick, rigid and fibrous aleurone layer enveloping the starchy endosperm may help to delay starch fermentation in the rumen. When phenolic pigments are present in the aleurone layer, dehulled barley grain may show color such as a black, violet, purple or blue appearance. Bowman et al. (2001) reported that the phenolic pigments could influence feed barley quality, as barley with a light-colored aleurone had more digestible starch content, resulting in greater rumen digestibility of DM and starch.

2.2.2.4 Starchy Endosperm

Starchy endosperm, the bulkiest portion of the barley kernels is the energy reserve of the barley seed and the primary energy source for animals. The cell wall of this tissue is relatively thin, consisting of β-glucan (~71%) and arabinoxylan (~19%) (Noots et al. 2001). The endosperm is packed with starch (85-89%), protein (10%) and β-glucan. Starch exists in the form of large and small granules composed of amylopectin (75%, branched chain) and amylose (25%, straight chain). Waxy barley grain has less amylose and as a result, better animal digestibility (Svihus et al. 2005). β-glucan is positively related to barley grain hardness (Svihus et al. 2005) and this trait is assumed to benefit ruminants by slowing down starch degradation. Starch in the barley endosperm is loosely embedded in the protein matrix, while in the horny endosperm of corn, it is tightly encased and surrounded by protein. The starch and protein interaction creates difference in the rate and extent of starch digestion among cereals (McAllister et al. 1993; McAllister and Cheng 1996). The protein matrix can limit the accessibility of microbes and enzymes to the starch granules, so barley starch often ferments faster than corn (McAllister et al. 1993; McAllister and Cheng 1996).

2.2.2.5 Embryo

The embryo is the core of the grain and is responsible for the grain's reproduction. It is located in the dorsal innermost side (Hough 1991). This tissue is rich in protein (34%), nucleic acids, lipid (14-17%), ash and water, and also contains minimal amounts of cellulose, pectin, hemicellulose, and lignin (formed only with germination) (Briggs 1998).

2.3 Factors Affecting the Digestibility of Barley Grain in Ruminants

The rate and extent of barley grain degradation in the rumen is related mainly to three factors - animal feeding systems and eating behavior, the activities of rumen microorganisms, and the physical and chemical profiles of barley grain.

2.3.1 Animal Eating Behaviors and Feeding Methods

Feed passage rate from the rumen is positively related to feed intake. The more the animal eats, the faster rumen digesta passes through the gastro-intestinal tract and the lower the DM digestibility (Varga and Kolver 1997). Inclusion of effective fiber in barley-based diets contributes to more rumination, higher rumen pH, better starch fermentation and digestibility (Mertens 1997). Rumination and mastication promote barley digestion by fracturing the protective covering (hull, pericarp and testa), and continually secreting buffering saliva. Supplementary live microbial feed additives, rumen buffers, ionophores and antibiotics can change rumen fermentation stoichiometry, stabilize rumen pH, enhance fiber breakdown and increase the digestibility of barley grain (Varga and Kolver 1997). Researchers have done some tests to decrease the rumen starch fermentation rate by treating grain with glutaraldehyde and formaldehyde (Ortega-Cerrilla et al. 1999), and to increase the grain hull digestibility with multi-enzyme mixtures containing ferulic acid esterase (Yu et al. 2005a). Improved feeding strategies such as increased feeding frequency (Sutton et al. 1985; Varga and Kolver 1997), and night feeding (Schwartzkopf-Genswein et al. 2004) stabilize barley grain starch fermentation and increase DM digestibility.

The physical processing of barley such as grinding, rolling or cracking, breaches the resistant outer coat, and enlarges the grain surface for rumen bacterial attachment, thus increasing DM digestibility and improving animal performance as compared to feeding intact barley (Mathison 1996; Beauchemin et al. 2001). However, fine processing creates dust, which is not appetizing for cattle, and reduces feed intake (Beauchemin et al. 2001). In addition, over-processing increases the barley starch fermentation rate, induces rumen acidosis in animals fed a high-barley diet and impairs animal performance (Owens et al. 1997; Owens et al. 1998). Therefore, it is important to control the particle size of barley after mechanical processing in order to balance the extent and rate of fermentation in the rumen.

2.3.1.1 Importance of the Particle Size of Barley Grain after Coarse Dry-rolling

Barley bran is the natural defensive barrier for protecting the inner endosperm and embryo from damage by microorganism intrusion, chemical erosion and physical abrasion. In the rumen, the whole barley grain is almost completely indigestible (Wang et al. 1999). Experiments show that animals fed whole barley have significantly lower average daily gain and feed efficiency than those fed processed grain (Mathison 1996). Grain particle size reduction after processing positively affects DM digestibility (Mathison 1996).

Cattle retain feed particles via the reticulo-rumen orifice. Feed substances are broken down to a critical size (about 1.18 mm) in order to pass through the reticulo-rumen orifice to the hind gut (Poppi et al. 1985; Kononoff 2005). Particle size \reduction is accomplished through animal mastication, rumen digestion and feed processing. In Canada, cattle especially the finishing animals are fed high levels of barley. Physical processing (commonly coarse dry-rolling in western Canada) produces a moderate grain particle size to maximize animal performance. A compromise in particle size reduction is necessary to balance animal intake and the rate and extent of barley grain degradation in the rumen (Beauchemin et al. 1994). Reducing particle size of barley grain increases the surface area of the grain and exposes the starchy endosperm, thus allowing more hydration, greater bacterial and enzymatic digestion of

the starch. This leads to faster rumen emptying and greater animal intake. However, because barley grain contains a high content of fast fermenting starch, finely processed barley will be degraded too rapidly to synchronize with protein biodegradation of feed ingredients, and may coincide with increased digestive upsets and animal diseases (Owens et al. 1997). It is well known that matching the extent and rate of starch and protein digestion can optimize rumen fermentation, microbial production, and subsequently animal performance. The larger the grain particle size, the smaller the grain surface area, which means reduced microbial colonization and enzymatic attack, and thus a slower degradation of the grain in the rumen.

Particle size not only plays a role in the digestibility of the grain, but may also affect the digestion site of grain in the gastro-intestinal tract (Ewing et al. 1986; Koenig et al. 2003; Rémond et al. 2004), since feed particles have to be broken down to a certain critical size before leaving the rumen. Particle size reduction also improves handling and mixing properties. However, excessively small particle size (finely processed) increases the energy cost, dust problems, animal sorting, and does not improve the digestibility of barley grain in ruminants (NRC 2001) because of the faster passage rate and rumen acidosis. Indeed, the major concern for grain particle size in ruminant rations is the issue of animal health, because small particle size after mechanical processing is thought to enhance the rate of starch fermention in the rumen and cause a greater incidence of animal diseases (Mathison 1996; Owens et al. 1998; Beauchemin et al. 2001). Therefore, it is important to regulate the particle size of barley grain obtained after mechanical processing.

For steam rolling, Canadian scientists have proposed a processing index (PI) to regulate barley processing to maximize animal performance. The PI describes the change of volume percentage before and after processing, with critical standards of coarse (81%), medium (72.5%), medium-flat (64%) (the best for dairy cows) and flat (55.5%) (Yang et al. 2000). However, the PI method is not suitable for dry-rolled barley grain because of the greater amount of fines produced in the procesing.

In practice, coarse dry-rolling is still empirical and there is no definite critical particle size for ruminants and no consistent method employed in its analysis. The

common recommendation is to crack barley as coarsely as possible. Boyles et al. (2000) suggested that breaking the barley grain kernel into two to three pieces is acceptable. Mathison (1996) questioned the empirical method and suggested a re-evaluation of the relationship between particle size and animal performance.

The first challenge to estimate particle size after coarse dry-rolling is that currently there is no good particle size prediction model. The American National Standards Institute (ANSI) (2003) brought forward a more precise method to determine the particle size of ground grain, but ANSI specifically indicated that this prediction equation is not adequate to define the particle size of coarsely dry-rolled grain, because this processing does not produce regularly shaped particles. Therefore, scientists have developed and used several different equations to estimate the mean/median particle size of feed particles obtained after mechanical particle size reduction processing (Fisher et al. 1988; Pond et al. 1984).

2.3.1.2 Factors Affecting the Particle Size of Barley Grain after Coarse Dry-rolling

Coarse dry-rolling is a process to crack or crush barley grain by rollers, thereby reducing grain particle size and increasing grain surface area (Boyles et al. 2000). Different roller gaps, roller speeds and kernal sizes would lead to variable particle sizes. Unlike grinding, coarse dry-rolling seldom has an effect on the fibrous fractions in barley (Boyles et al. 2000).

The characteristics of barley particle size reduction are not only affected by mechanical settings, but also by barley variety, moisture content and kernel size of the grain, grain composition, endosperm and hull textures (Bowman et al. 2001; Campbell et al. 2001; Fang and Campbell 2003). One of the desirable traits of barley grain used as ruminant feed is the hard-textured endosperm which can maintain large particle size after dry-rolling (Bowman et al. 2001; Zhang et al. 2003). Bowman et al. (2001) observed that six-row barley (high in ADF, low in starch) produced a larger particle size than two-row barley after dry-rolling because starch content is negatively correlated to particle size reduction, whereas the reverse relationship exists between

ADF and particle size. Grain hardness is more determined by the interaction between the starch and protein matrix. Izydorczyk et al. (2005) reported that with increasing content of protein and β-glucan, barley grain becomes harder, which may be good for particle size maintenance. Barley hardness was related to the Hardness gene of hordoindolines proteins which negatively regulate barley grain's hardness, probably in the same way as in wheat, by loosening the linkages between starch and protein, thus produce a softer endosperm (Beecher et al. 2002; Svihus et al. 2005). Izydorczyk et al. (2003) discovered that β-glucan content has positive effect on grain's particle size after mechanical process, so barley containing higher β-glucan content required more milling energy and produced a higher proportion of large particles. The content of FA and PCA, and cross-linkages between cell wall components and FA, particularly in the pericarp, can enhance the grain's mechanical resistance (Antoine et al. 2003; Greffeuille et al. 2007), which may lead to larger particle size.

2.3.2 Fermentation Activities of Rumen Microorganisms

Feed digestion in the rumen is not performed by ruminant animals per se, but rather by the microorganisms. Any factor influencing the rumen environment (e.g. the strict anaerobic conditions, and narrow pH ranging from pH 6 to 7 for cellulose digesting bacteria), the ecological population of microorganisms (e.g. the biomass of fibrolytic bacteria vs. the amylolytic bacteria) or nutrient availability for micro-organisms (e.g. the match of carbohydrate and protein for energy and nitrogen supplement) will change the digestive capability of the microorganisms, and thus the digestibility of barley starch, protein and fiber (Varga and Kolver 1997). To initiate digestion of grain, microorganisms should adhere, colonize and penetrate the protective layers (hull, pericarp and testa) of the barley kernel. Therefore, microbes usually manage to digest barley grain by encroaching the inner vulnerable tissues such as the broken areas (Varga and Kolver 1997). Rumen bacteria also produce an array of enzymes to digest many substances captured in the rumen. The plant cell wall degrading enzymes (e.g. hemicellulase and cellulase) are unique in ruminants compared with many other domestic animals. Changing the rumen environment will

alter the critical niches for enzymatic activities, and thus lead to variable digestibility of feed constituents.

2.3.3 Physical and Chemical Effects on the Digestibility of Barley Grain

As has been described earlier, endosperm starch types, protein content and matrix and barley kernel particle size are all involved in the rate and extent of barley grain degradation. Van Soest (1994) indicated that the chemical constitution of a feed is the primary factor determining feed nutrient availability for animals. The endosperm is filled with starch, so it has the potential to be completely degraded in the rumen.

Barley bran is the main factor limiting the digestibility of the kernel. Nutritionally, the cell wall components of barley bran are difficult to digest, and even have anti-nutritional properties. For instance, cellulose and hemicellulose in the hull are slowly digestible and lignin is nearly indigestible and impedes the digestion of cellulose and hemicellulose. Silicatization, cutinization and lignification in the hull inhibit nutrient availability. Anti-nutrients such as high concentrations of tannins and phenolic compounds prevent animals from digesting and utilizing the nutrients. Structurally, the hydrophobic property, sealability, silicatization, cutinization and lignification of the hull prevent the attachment, colonization and penetration of barley grain by rumen microorganisms and their enzymes.

2.3.3.1 Fiber in Barley Grain

Barley hull is fibrous (94% fiber) in nature (Grove et al. 2003; Olkku et al. 2005). Although adequate fiber is required for ruminants, forages are the major and best fiber sources. The high fiber content in barley grain reduces its value as an energy source for ruminants, and is responsible for its relatively lower energy level compared to corn. Therefore, reducing the hull and fiber content in barley grain or changing the intrinsic chemical composition of the hull could hold promise for improvement in its DM digestibility and energy availability for ruminants.

Fiber is a complex entity with no nutritional, chemical, or physical uniformity and has many definitions (Van Soest et al. 1991). From the perspective of ruminant

nutrition, fiber is characterized chemically as a component of a feed with low solubility in certain solvents, is a nutritional entity with less animal digestibility than starch, and is often expressed in the form of NDF, ADF and ADL (Jung and Allen 1995).

2.3.3.1.1 Acid Detergent Fiber and Neutral Detergent Fiber

ADF and NDF are chemical analysis entities. Analysis methods were initially developed for forage-based samples. ADF is the standard method of Association of Official Analytical Chemists (AOAC) for measuring feed fiber which was established for estimating cellulose and lignin by removing hemicellulose with a weak acid detergent while leaving most of the cellulose and lignin undissolved in the feed residues (Van Soest et al. 1991; Moore et al. 2007). ADF is related to feed digestibility since it consists of cellulose and most of the indigestible lignin. High ADF indicates poorly utilizable fiber, reduced digestibility and lower digestible energy content.

NDF is used to evaluate the total plant cell wall content, and represents the insoluble substances in plant cell walls, mainly cellulose and hemicellulose, as well as lignin and some insoluble nitrogenous compounds (Moore et al. 2007). Depending on the source of feedstuff, other components such as pectin, galactans and β-glucans may also be detected in NDF residue. NDF is the best indicator of DM intake because it includes most of the bulky, slowly digesting fiber (hemicellulose and cellulose), which have physical fill effect in the rumen. NDF is widely used in feed quality determination and formulating animal rations, but it is not the AOAC standard method.

The difference between NDF and ADF is calculated as hemicellulose; and cellulose is calculated by subtracting ADL from ADF (Van Soest et al. 1991; Moore et al. 2007). Since hemicellulose and cellulose are slowly digestible cell wall components, feeding animals with feed high in them will lead to the accumulation of undigested feed residues in the alimentary tract, and consequently reducing animal intake (Jung and Allen 1995). Due to the existence of lignin in ADF and NDF, the digestibility of ADF and NDF varies with feed types, harvesting stage and the growing environment.

Jung and Allen (1995) indicated that feed fiber cannot be completely digested because its digestibility is controlled by both rumen digestion rate and rumen passage

rate. Fiber digestibility in the rumen fluctuates with diets. When a feed is completely composed of forage, NDF is a good indicator of animal DM intake (R=-0.76), while increasing concentrate level in the diet minimizes this relationship, because concentrate feed depresses fiber digestion in the rumen (Hoover 1986). The causes of this depression are: 1) rumen microbes prefer starch to fiber; 2) rapid fermentation of starch reduces rumen pH, which impairs activities of cellulolytic rumimal microbes and enzymes; 3) cellulolytic microbes fail in the competition for essential nutrients for their reproduction in the rumen (Hoover 1986; Russell and Wilson. 1996). Therefore, barley shows lower fiber digestibility than forage. Barley fiber (especially hull fiber) quality is not competitive with forage fiber. Barley hull contains 35-41% ADF, while barley silage contains 29% ADF. In addition, barley hull has small particle size, so it cannot provide ruminants with a good quality of physical effective NDF as forages do.

2.3.3.1.2 Lignin or Acid Detergent Lignin

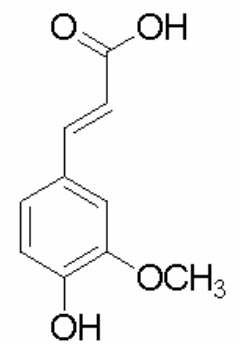
Lignin is the complex polymer of phenylpropanoid without definite form (Rowell et al. 2000). Barley grain and barley hull contain 2% and 8% lignin, respectively (NRC 2001; Grove et al. 2003). Although lignin content in most plants and barley is relatively low, it is the most recalcitrant fiber component. In animal nutrition, although lignin is not considered a nutrient, it is more related to fiber analysis and fiber digestion. In the past, lignin was considered to be indigestible and inert because no apparent lignin-degrading microorganisms or enzymes were found in the rumen (Van Soest 1994). Later evidences showed that lignin is actually digestible in the rumen, although the digestibility is relatively low and variable (Fahey and Jung 1983; Silanikove and Brosh 1989; Susmel and Stefanon 1993).

Lignin plays a negative role in ruminant nutrition, feed digestion and utilization through three ways (Moore and Jung 2001). First of all, lignin inhibits cellulose and hemicellulose digestion in the rumen by working as a physical barrier to restrict rumen microbes and enzymes acting on digestible polysaccharides. Second, lignin significantly reduces plant energy availability to animals by limiting animal fiber

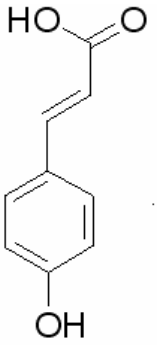
utilization. Third, lignification restricts animal DM intake because it slows down plant DM digestibility and increases the rumen fill effect.

2.3.3.2 Hydroxycinnamic Acids in Barley Grain

Hydroxycinnamic acids belong to phenolic acids, which contain an aromatic ring and other functional groups, and are omnipresent plant secondary metabolites. Hydroxycinnamic acids include ferulic acid, ρ-coumaric acid, caffeic acid, sinapic acid, and others. FA and PCA (Figure 2.3) are most abundant in cereal grain. They are important for physiological and biological functions in plants. However, the presence of excessive hydroxycinnamic acids (especially FA, PCA) in plant cell walls may reduce animal digestibility and productivity.



Ferulic acid



ρ-Coumaric acid

Figure 2.3. Chemical Structures of Ferulic acid and ρ-Coumaric acid

2.3.3.2.1 Functions of Ferulic Acid and ρ-Coumaric Acid in Plants

FA and PCA exist mainly in plant cell walls and are covalently linked to lignin by ester and ether bonds, and to polysaccharides mainly by ester bonds (Iiyama et al. 1990; Lam et al. 1992a; Kroon and Williamson 1999; Hernanz et al. 2001). FA forms ester-ether cross-linkages in plant cell walls, thereby providing cell wall rigidity and strength. This helps to protect plant cell walls from attack by pathogenic microorganism and microbiological degradation (Kroon and Williamson 1999). The cross-linkages of FA also limit the extensibility of plant cell walls; thereby controling seed germination, cell growth and maturation. Free FA and PCA have an astringent

taste and act as anti-nutritional agents (Kroon and Williamson 1999). More FA and PCA might be synthesized and released from plants during wound and disease infection. As antimicrobial agents, FA and PCA can inhibit microbial growth and impair their activities, with PCA having more effect than FA (Burritt et al. 1984; Wells et al. 2005). Furthermore, FA and PCA have antioxidant properties, which can protect plants from solar UV light and other radiation damage (Blokker et al. 2006). During the secondary plant cell wall development, the involvement of FA and its dimers provide nucleation sites for lignin synthesis and deposition in the plant cell wall (Iiyama et al. 1990; Ralph et al. 1995; Grabber et al. 2002).

2.3.3.2.2 Biosynthesis of FA, PCA and Formation of Bound FA

As noted by Iiyama and Lam (2001), FA and PCA are the intermediates of lignin biosynthesis (Figure 2.4). They are both derived from the plant phenylpropanoid pathway, which initiates from either the phenylalanine or tyrosine biosynthesis pathways, and involves many enzymes, with phenylalanine ammonia lyase (PAL) and tyrosine ammonia lyase (TAL) being the key enzymes for this biosynthesis. The secondary metabolites produced through this process include feruloyl-CoA and ρ-coumaroyl-CoA, which eventually lead to the formation of cell wall bound FA and PCA (Brett et al. 1999).

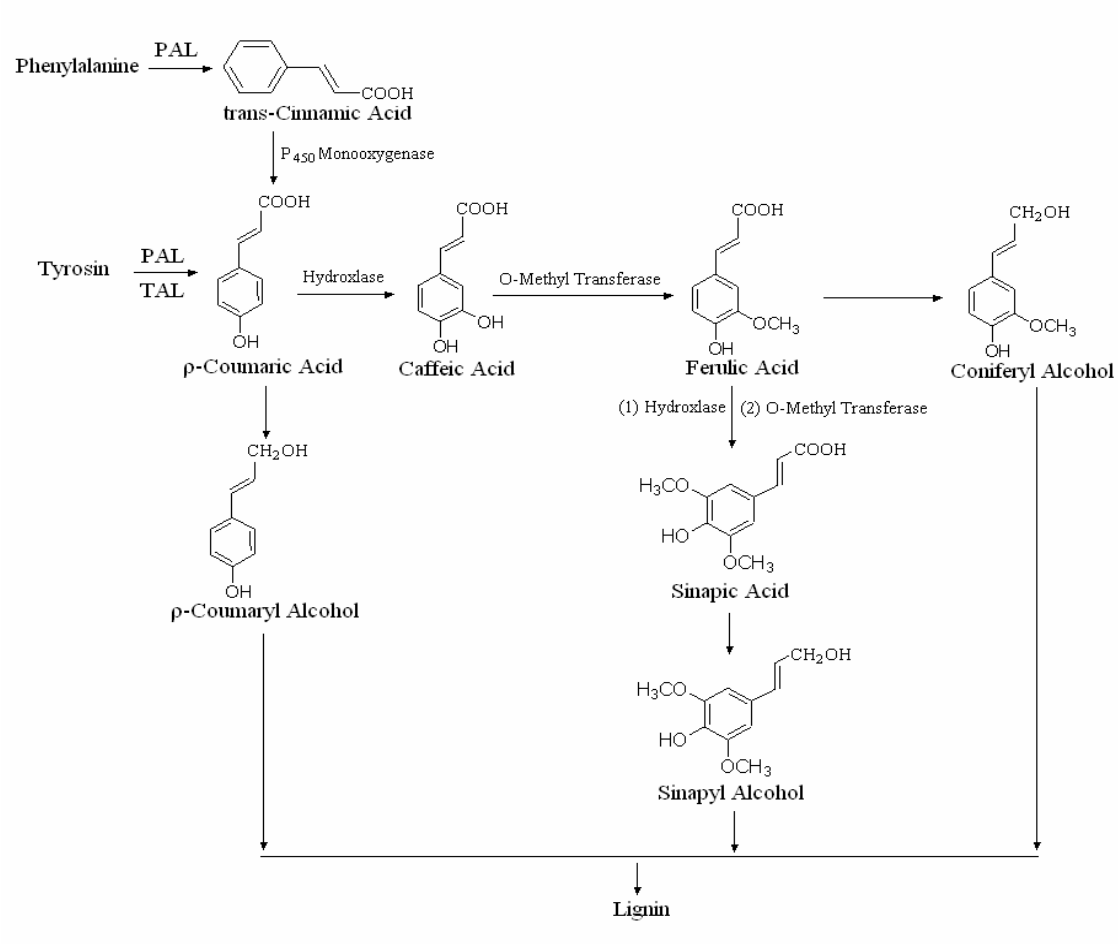


Figure 2.4. Pathway of hydroxycinnamic acids and lignin biosynthesis.

Adapted from Iiyama and Lam (2001) and Shahidi and Naczk (2003)

2.3.3.2.3 Ferulic Acid and ρ-Coumaric Acid in Barley

Barley grain contains two major low molecular weight hydroxycinnamic acids: FA and PCA (Nordkvist et al. 1984; Chemey et al. 1992). In barley grain, FA and PCA are mainly concentrated in the bran cell walls (about 80%), especially in the hull and aleurone layers with only about 5% in the endosperm and embryo (Nordkvist et al. 1984; Hernanz et al. 2001). In barley, FA and PCA are present in the form of free, soluble-conjugated and mainly insoluble but bound to plant cell wall components (Holtekjolen et al. 2006). Natural forms of FA and PCA exist only in trans forms, but they are prone to isomerization to cis forms (an artifact) when exposed to UV light during experimental extraction (Faulds and Williams 1999). FA also has many polymers and derivates such as monomer, dimers and trimers.

Like many other graminaceous plants such as wheat, oat and rice, barley FA and its derivatives are widely cross-linked to plant cell wall polysaccharides and lignin by either ester or ether bonds (Iiyama et al. 1990; Hernanz et al. 2001). Therefore, FA is assumed to be one of the most inhibiting factors in plant cell wall digestibility in ruminants (Jung and Allen 1995; Iiyama and Lam 2001; Grabber et al. 2004; Yu et al. 2005a). PCA is also covalently linked to polysaccharides (minor) and lignin (major), but PCA does not form the inhibitory cross-linkages as FA does. PCA is mainly considered to represent plant cell wall lignification rather than being a digestibility inhibitor (Jung and Allen 1995).

2.3.3.2.3.1 ρ-Coumaric Acid

PCA is believed to be both esterified and etherified to lignin, but to a lesser degree esterified to cell wall polysaccharides (Sun et al. 2002). PCA is not involved in forming ester-ether bridges between cell wall polysaccharides and lignin as is the case with FA (Ralph and Helm 1993; Sun et al. 2002). The majority of PCA is integrated into the plant cell walls during the secondary cell wall development. As lignification proceeds, large amounts of PCA are deposited and esterify to lignin in the secondary cell wall (Jung and Vogel. 1992; Ralph et al. 1994a). In addition, incorporation of syringyl units into lignin requires the involvement of PCA (Lu and Ralph 1999). As a consequence, PCA is considered to be an indicator of lignin deposition in plant cell walls. The inhibitory effect of PCA on plant cell wall digestibility is minor (Jung and Allen 1995; Grabber et al. 2004). It has been observed that the ratio of PCA/FA is negatively related to plant cell wall digestibility (Morrison et al. 1998; Vailhe et al. 2000). Grabber et al. (2004) proposed that PCA and FA are good indictors of total lignin content and lignin distribution in plant cell walls, respectively. Low ratio of PCA/FA means limited lignified plant tissues, whilst high ratio indicates an even distribution of lignification in plant tissues. However, many experiments seldom consider the inhibitory effects of FA in plants when calculating the ratio of PCA/FA. Inconsistent relationships between the ratio of PCA/FA and plant cell wall digestibility have been observed (Akin 1989; Buxton 1989; Grabber et al. 1992; Wilson and Hatfield 1997; Grabber et al. 2004).

Therefore, using the ratio of PCA/FA as a digestibility index or a plant breeding selection criterion needs further examination and validation.

2.3.3.2.3.2 Monomer of Ferulic Acid

The structure, content and anatomical distribution of FA in plant cell walls varies with plant growth stage and tissue. FA has two functional groups: -COOH and phenolic OH, which make it a bi-functional molecule. As a consequence, FA can bind to lignin via both ester and ether linkages and forms either inter or intra ester-ether bridges between lignin and polysaccharides, and among polysaccharides (mainly arabinoxylan in barley) (Figure 2.6) (Gubler et al. 1985; Sun et al. 2002). These ester-ether bridges modify plant cell wall extensibility and mechanical strength and reduce the digestibility of plant cell wall polysaccharides (Jung and Deetz 1993; Sun et al. 2002).

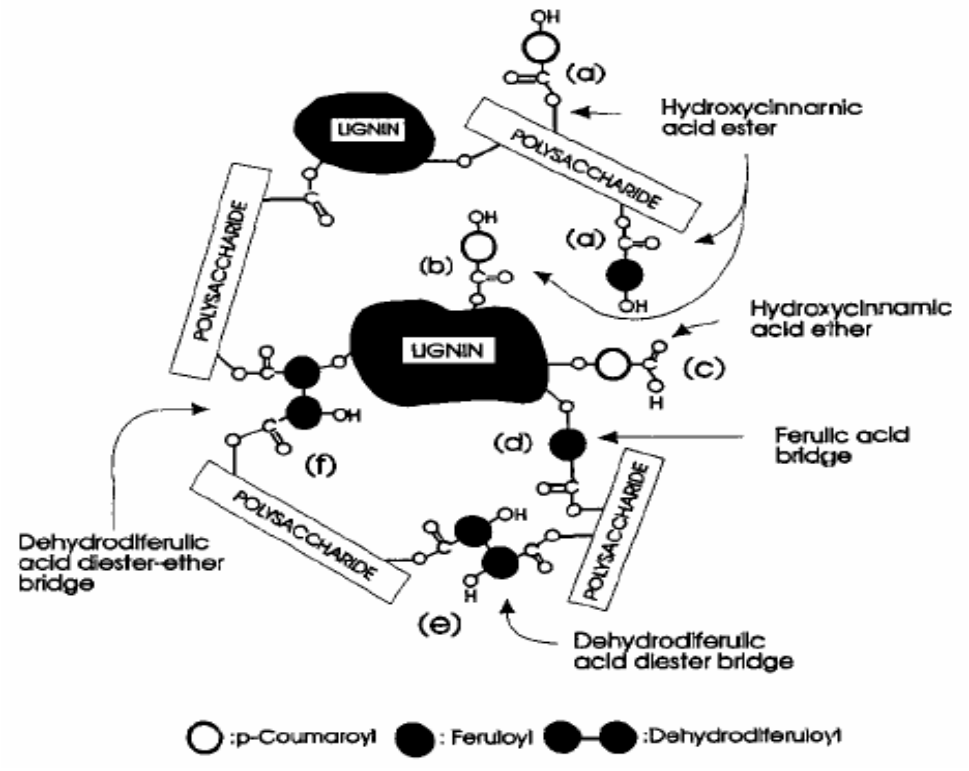


Figure 2.5. Cross-linkages between lignin and polysaccharides in plant cell walls. Source: Iiyama et al. (1994)

FA is deposited and esterified to polysaccharides and lignin in plants throughout the formation of the primary cell wall (Jung and Deetz 1993; Iiyama and Lam 2001). During the secondary wall development, the esterified FA-polysaccharides continue to ether-link to lignin, which leads to intricate ferulate ester-ether cross-linking bridges (Morrison et al. 1998; Iiyama and Lam 2001). Furthermore, esterified FA-polysaccharides are thought to direct and control lignification of plant secondary cell wall (Anderson and Schroeder 1999). Lignification of cell wall polysaccharides is promoted through active oxidative coupling, with the esterified FA-polysaccharides being the initiation and nucleation sites (Ralph et al. 1995). Therefore, FA is thought to enhance the inhibitory effects of lignin on plant digestibility in the animal (Jung and Allen 1995). In barley, the majority of the insoluble and bound FA exist in the cell walls of barley bran (Nordkvist et al. 1984; Hernanz et al. 2001), which consists of 23% cellulose, 32.7% hemicellulose and 21.4% lignin (Cruz et al. 2000). Therefore, in barley cell walls, FA is crosslinked to cellulose, hemicellulose by ester bonds and to lignin by ether bonds, which forms rigid ester-ether bridges. In contrast, corn bran is devoid of lignin, so ether linkage does not exist in corn (Saulnier et al. 1995) and corn cell walls are more digestible than barley.

The ether linkages of FA are inert and difficult to cleave in the rumen (McSweeney et al. 1994; Jung and Allen 1995). Although rumen microorganisms have the capacity to hydrolyze the ester-bonds of FA, such linkages reduce plant digestibility by affecting the rate of cell wall polysaccharide degradation (Moore and Jung 2001). The complex cross-linking bridges of FA place cell wall polysaccharides and lignin in a close physical proximity, which works as a steric obstacle and shields the esterified polysaccharides from enzymatic hydrolysis in the rumen (Moore and Jung 2001). When free FA is released in the rumen, it is harmful to rumen microorganisms (Chesson et al. 1982; Borneman et al. 1986; Lam et al. 1992b; Wells et al. 2005). For example, Akin et al. (1993) observed that rumen bacterial growth and function were impeded by FA released from ester-linked feruloyl arabinoxylans, when the concentration of FA was over 1 mM. Nevertheless, the amount of free FA and PCA released from concentrates in the rumen is low; and rumen microorganisms are capable

of degrading these acids (Chesson et al. 1999; Hernanz et al. 2001; Holtekjolen et al. 2006).

2.3.3.2.3.3 Dimers of Ferulic Acid

FA dimers are created based on the esterified FA-polysaccharides, either through oxidative coupling or through photodimerization (Myton and Fry 1994). Like FA monomers, the dimers also form cross-linkings among polysaccharides and between polysaccharides and lignin by ester and ether bonds (Figure 2.6) (Ralph et al. 1994b; Saulnier et al. 1999; Bunzel et al. 2001; Allerdings et al. 2005). As such, FA dimers also limit cell wall extensibility, control cell growth and expansion, stiffen plant cell walls, serve as the nucleation site of lignification, protect the plant against various biotic and abiotic damages, provide a better plant cell wall resistance to rumen microbial digestion, and thus reducing plant cell wall digestibility in ruminants (Ikegawa et al. 1996; Wakabayashi et al. 1997; Zarra et al. 1999; Mathew and Abraham 2004). Due to the increase of mechanical properties of the plant cell walls provided by dehydrodiferulic acid, the dimers are regarded to be the "molecular equivalent of spot welding a steel mesh frame" (Iiyama et al. 1994).

Although the content of individual FA dimers present in barley grain is much lower than FA monomer, with the total dimers summing up to roughly 200 μg/g, FA dimers are believed to be significant in enhancing the physical, mechanical and biochemical properties of the plant cell walls (Holtekjolen et al. 2006). Breaking up diferulic cross-linkages needs to interrupt more than one polysaccharide, which means that diferulic cross-linkages are relatively more indigestible than monomeric FA cross-linkages (Chesson et al. 1999).

2.3.3.2.3.4 Trimers of Ferulic Acid

Recently, several dehydrotriferulic acids have been isolated and identified from maize (Rouau et al. 2003; Bunzel et al. 2005). So far, these trimers are known to exist in very small quantities in grain and some isomers may be derived from experimental oxidative reactions as artifacts (Bunzel et al. 2005). Because of their specific

structures, some dehydrotriferulic acids are not involved in polysaccharide cross-linking while others may cross-link to two or three polysaccharides (Bunzel et al. 2005). Their role in the digestibility of plant cell walls is still under investigation.

2.3.3.2.3.5 Other Linkages of Ferulic Acid to Limit Plant Cell Wall Digestibility

Bunzel et al. (2003) isolated sinapate dehydrodimers and sinapate-ferulate heterodimers from cereals, and suggested that other hydroxycinnamic acids, like sinapic acid, may also play a similar role to FA in plant cell walls forming cross-linkages. This implies that sinapic acid may have effects on the digestibility of plant cell walls as well. 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 (Jung and Deetz 1993; Facchini et al. 2002; Oudgenoeg et al. 2002; Piber and Koehler 2005; Edreva et al. 2007).

2.3.4 How to Improve the Digestibility of Barley Plant Cell Walls (e.g. hull)?

Studies show that plant cell walls are structured with pores, the radius of which varies from 0.5 to 5.0 nm (Chesson et al. 1999). The cross-linking bridges of FA place the cell wall matrix components in much greater physical closeness and reduce the pore sizes. When the degradative enzymes are larger than >20kDa, the small pore sizes hinders enzymes from attacking, penetrating and digesting plant cell walls (Chesson et al. 1999). Low content of FA in plant cell walls would lead to less cross-linking of lignin and polysaccharides, and less compact arrangement of cell walls, thus improving rumen digestibility of plant cell walls. Therefore, barley varieties with low FA content could be bred or identified for better digestibility (Zupfer et al. 1998).

Agronomic management also should be taken into account, as injury, diseases, strong sunlight and high temperature result in higher level of FA and PCA content in plants (Miyamoto et al. 1994; Zupfer et al. 1998; Blokker et al. 2006).

Artificial chemical and biochemical treatments are expected to improve plant cell wall digestibility by removing the steric constraints and making plant cell walls more

accessible to rumen microbial and enzymatic attacks. For example, plant tissues treated with combined enzymes or a multienzyme cocktail of feruloyl esterases, ρ-coumaroyl esterases and cellulose can release hydroxycinnamic acids from the plant cell walls of barley, wheat and rye, thus improving their digestibility for animals (Faulds and Williamson 1991; Bartolomé et al. 1997; Sancho et al. 2001; Yu et al. 2005b). As is widely practiced, barley straw can be treated with ammonia and various hydroxides to improve its digestibility. One of the reasons is that the feruloyl ester bonds are alkaline sensitive and easily broken to release FA under mild alkali conditions (Sun et al. 2002).

2.4 Analyzing Methods for Barley Hull, Ferulic acid, ρ-Coumaric Acid, Particle Size and In Situ Rumen Digestion

2.4.1 Analysis of Barley Hull Content

Barley hull has been extensively analyzed for breeding purposes (Ibrahim 1971). Scientists have developed several methods for barley hull content analysis, including mechanical peeling, solution soaking and chemical eroding. In recent years there has been significant improvement in techniques to estimate barley hull content. So far, none of the measurement techniques has demonstrated sufficient accuracy or been accepted as an international standard for barley hull content analysis due to the difficulties caused by specific properties of barley and its hull (Briggs and Hough 1981). These include the fact that the barley kernel is not round shaped and bears a grooved furrow, and that the hull is very thin and fused tightly to the pericarp.

Milling or dehulling machines have been developed for separating hulls from cereal grain (Briggs and Hough 1981). But this approach for estimating barley hull content is not expected to completely remove the superficial hull without compromising the closely cemented pericarp, or even the endosperm. Mealy and steely barley grain may react differently to harsh mechanical abrasion. Furthermore, the hull on the ventral furrow side is very resistant to peeling.

Several soaking methods were reported to loosen and peel the hull by hand. Kleber and Franke (1959) tried to macerate barley grain in distilled water for 2 h to soften the hull and facilitate manual hull separation. Luff (1898) suggested steeping barley grain

in a 5% ammonia solution at 80^{o}C for 1 h before manual separation. Ibrahim (1971) found that this method gave relatively higher results by peeling off some pericarp and improved the method by soaking barley grain in a 5% ammonia solution at room temperature for 15 h and then removed the hull by hand. Briggs and Hough (1981) recommended that all these chemical soaking and manual hull-separating methods were laborious and inaccurate because they could not be employed for large scale barley hull analysis which led to unrepresentative sampling. In addition, a certain amount of soluble materials from barley could be leached by aqueous solution steeping.

Strong corrosive chemical reagents have also been employed to erode or bleach the hull. Veldhuiszen (1946, cited by Ibrahim 1971) first developed a rapid method to remove barley hull and analyze the content by digesting barley in a heated 50% sulfuric acid solution for seconds and then washed away the eroded hull with water rinse. Essery et al. (1956, cited by Whitmore 1961) modified this method by immersing barley in a 50% sulfuric acid solution at ambient temperature for 3 h. Whitmore (1961) considered 3 h to be too long and set up a new method for rapid determination of barley hull content. Barley grain was boiled for 3 min in a sodium hypochlorite solution with 10% available chlorine to get rid of the hull. Whitmore (1961) also compared the two methods (sulfuric acid and hypochlorite) and observed good correlation between them. Again, Briggs and Hough (1981) commented that these methods were not accurate because the corrosive chemicals would eat not only the barley hull, but also the inner pericarp if the reacting time was not well controlled. These methods require critical selection criteria for excluding broken, damaged and naked barley grain which may not represent the whole sample. However, compared to other methods, chemical eroding methods are relatively precise, economical, more rapid and less tedious. Also the hypochlorite method is more accurate and environment-friendly than sulfuric acid. The the European Brewery Convention (EBC) Analysis Committee (1998) adopted the sodium hypochlorite solution method as a routine method for measuring barley hull content by calculating the weight difference from the whole and the dislodged barley grain.

2.4.2 Quantification Methods for Ferulic Acid and ρ-Coumaric Acid

In barley grain, only trace amounts of FA and PCA are in free form, others are bound as soluble and insoluble forms. The bound FA and PCA can be hydrolyzed with mild alkaline solution (2N NaOH under room temperature) to break the ester-bonds between FA, PCA and cell wall polysaccharides, lignin, and therefore, to release free FA and PCA (Maillard and Berset 1995; Zupfer et al. 1998; Hernanz et al. 2001; Holtekjolen et al. 2006). Various analytical techniques are available to measure FA and PCA content in plant cell walls.

Early chromatographic methods used paper chromatography (PC) or thin-layer chromatography (TLC), which is fast, sensitive, versatile and inexpensive, but has obvious disadvantages of limited quantitative accuracy and is easily affected by impurities (Ellnain-Wojtaszek 1997; Sikorska et al. 2000; Mazol et al. 2004). UV spectrophotometeric assay is also a rapid, sensitive and reliable method to detect FA and PCA through UV absorption maxima, but the detection can be interfered with by solvents, pH, protein, phenolic acids, nucleic acids and amino acids. Capillary electrophoresis (CE), a versatile and highly efficient method, while still not generally accepted, could become an alternative or complementary to the HPLC technique (Peng et al. 2005; Kubán et al. 2006). Gas Chromatography (GC) is a popular chromatographic technique employed for identification and quantification of FA and PCA. However, GC analysis relies on the thermal-stability and volatility of samples; furthermore, derivatization of the sample is a challenge (Nordkvist et al. 1984; Ralph et al. 1994b; Dong et al. 2005; Stewart et al. 2005). HPLC is the most commonly applied method in separating and quantifying FA and PCA. Most HPLC methods for FA and PCA analysis are based on acidic mobile phase. The elution time for FA and PCA is quite long and considerable amounts of solvent are required (Guido et al. 1989; Kroon and Williamson 1996; Amariwiz and Weidner 2001; Pirjo et al. 2005). Olkowski et al. (2003) developed a rapid HPLC method based on basic mobile phase that significantly reduced the solvent amounts used in the mobile phase.

2.4.3 Particle Size Analysis of Barley Grain

Barley grain can be processed for the cattle industry by grinding, steam-rolling, temper-rolling and dry-rolling. Although steam-rolling and temper-rolling can reduce the amount of fines and enhance feed efficiency and animal performance (Mathison 1996, 2000; Beauchemin et al. 2001), they require the addition of extra heat, water, energy and money, and the cost may not be balanced by the limited improvement in feed efficiency and animal performance (Mathison 1996). Coarse dry-rolling is an extensive processing method with ease of handling, less effort and cost, and is widely applied in western Canadian cattle feedlots, but there is no standard method to control the particle size of barley grain after dry-rolling. In order to compare the differences of particle size distribution and intrinsic properties of various barley varieties, we need a suitable particle size analyzing method.

The American National Standards Institute (ANSI) (1998; 2003) proposed some particle size analyzing methods for chopped grasses and ground grain. According to the ANSI method, mechanically processed barley grain is sorted and separated into several fractions through a set of sieves with different screen pore sizes. Sub-samples from each sieving fraction are then collected and estimated with mathematical equations. The standard equation to assess finely ground barley particle size distribution is the geometric mean diameter calculation equation. However, ANSI (1998; 2003) suggests that geometric equation only applies to the analysis of particle size distribution for chopped forages, ground grain and some feed ingredients with spherical, cubic or other fixed shape particles. Coarsely dry-rolled barley grain does not satisfy these requirements because of indefinite shapes. Hence, some other prediction equations are required for particle size analysis for coarsely dry-rolled barley. In a situation where the particle size of rumen digesta, feces and esophageal exrusa needed to be determined, Pond et al. (1984) tested an exponential equation and recommended it instead of the geometric equation. Later on, Fisher et al. (1988) recommended another exponential equation. These equations have been adopted by different scientists to analyze the particle size distribution of some ruminant related feedstuffs, but these methods were always randomly selected and used for roughly estimating the particle size distribution

of barley grain. No one has tested and determined which equation is more accurate and suitable for coarsely dry-rolled barley grain.

2.5 Summary of Literature Review, Hypothesis and Research Objectives

Barley digestibility in the rumen is influenced by many factors such as the content of barley hull, hydroxycinnamic acids, fiber, and particle size after mechanical processing. So far, no research has been done on the relationship between the effects of hull, FA, PCA content, particle size after dry-rolling and rumen digestibility of various barley varieties from western Canada. The hypothesis of the research required in this thesis is that barley varieties with low content of barley hull, FA, PCA, fiber, and maintaining larger particle size after coarse dry-rolling would have beter feed value for ruminants. To address the hypothesis, physical and chemical analysis, and in situ rumen digestion experiments were carried out. The objectives were 1) to determine the variation in the content of hull, FA, PCA, NDF, ADF, ADL, and particle size obtained after coarse dry-rolling of various barley varieties from western Canada; 2) to inverstigate the relationship between the content of barley hull, FA, PCA, NDF, ADF, ADL, particle size obtained after coarse dry-rolling and nutritional utilization and availability of various barley varieties in ruminants using in situ rumen technique; 3) to find a relatively better barley variety as a ruminant feed grain with low content of hull, FA, PCA, fiber, and maintaining large particle size after coarse dry-rolling.

3. INVESTIGATION OF CHEMICAL AND PHYSICAL DIFFERENCES AMONG THE VARIOUS BARLEY VARIETIES GROWN IN WESTERN CANADA

3.1 Introduction

Barley is one of the principal feed grains for ruminants in western Canada. Approximately 10-14 million tonnes are produced annually in Canada (more than 90% of which are from western Canada), and over half of the national barley yield is used by the feedlot sector (Statistics Canada, 2000-2008). There are more than 50 barley varieties grown in western Canada, most of which are hulled two-row varieties (Brian G. Rossnagel, University of Saskatchewan). On average the barley hull represents roughly 13% of the grain by weight (Evers et al. 1999) which means that over one million tonnes of barley hull is produced annually. Moreover, barley hull is extremely fibrous (28% cellulose, 34% hemicellulose and 23% lignin) and low in valuable nutrients (Olkku et al. 2005). Nutritionally, the barley hull is indigestible for monogastric animals and only partially digestible for ruminants. The low digestibility of barley hull limits nutrient and energy availability to animals. Although barley contains higher protein than corn, it fails to compete in term of digestible energy, primarily due to the presence of the hull. However, the hull is an important component that may benefit ruminants by slowing down barley starch fermentation in the rumen and reducing metabolic diseases.

Due to the major usage of hulled barley by the malting and the cattle industries, it is economically important to develop a barley variety with less hull content and improved nutritional quality. In addition to lignin, researchers have found that hydroxycinnamic acid-carbohydrate complexes are another important inhibitory factor related to plant cell wall digestibility in ruminants. In barley and other cereal grains, FA and PCA are two major low molecular weight hydroxycinnamic acids (Nordkvist et al.

1984; Hernanz et al. 2001). FA and PCA are concentrated in the cell walls of the outer coverings of barley seeds, mainly in the bran (Nordkvist et al. 1984; Sancho et al. 2001). In gramineous plants such as barley, FA is covalently linked to cell wall polysaccharides (especially arabinoxylans) by ester bonds and to lignin mainly by ether bonds (Gubler et al. 1985; Sun et al. 2002). Through ester and ether linkages, FA is extensively involved in cross-linking between plant cell wall polysaccharides and lignin (Iiyama et al. 1990; Iiyama and Lam 2001; Sun et al. 2002). Furthermore, FA can dimerize and trimerize through oxidative coupling (Bunzel et al. 2001; Hernanz et al. 2001; Bunzel et al. 2005). Therefore, FA forms intra- and/or inter- molecular ester–ether bridges between lignin and cell wall polysaccharides. Although the role of FA in the digestibility of plant cell walls is not well elucidated, the proposed mechanism behind its negative effect on digestibility of cell wall polysaccharides is that FA cross-linkages increase steric interference to rumen microbial digestion and shield bound polysaccharides from enzymatic hydrolysis (Jung and Deetz 1993; Grabber et al. 1998).

PCA is mainly esterified and etherified to lignin in plant cell walls, and seldom linked to polysaccharides (Ralph and Helm 1993; Sun et al. 2002). Since PCA does not form ester-ether cross-linkages as FA, it is not considered to be directly involved in plant cell wall digestibility and primarily functions as an indicator of cell wall lignification (Jung and Allen 1995; Grabber et al. 2004). FA and PCA content in barley may be influenced by barley variety, growing environment, disease and agronomic management (Miyamoto et al. 1994; Zupfer et al. 1998; Blokker et al. 2006).

It is a common practice in the western Canada feedlot to coarsely process barley grain to breach the tough barley hull before feeding, thereby improving digestibility in the rumen. However, over-processing can lead to an unpalatable ration and reduce DM intake. It can also cause digestive problems such as acidosis, laminitis and liver abscesses due to the rapid rumen fermentation of starch. Larger particle size can reduce the surface area for microbial colonization and enzymatic attack, which in turn slows down the rate of barley starch degradation in the rumen without affecting the extent of digestion. Therefore, larger grain particle size is preferred in order to maximize barley

grain digestibility, animal intake and performance (Mathison 1996; Beauchemin et al. 2001). Barley particle size reduction obtained after dry-rolling is related to grain hardness, texture, and composition (Camm 2008; Darlington et al. 2001; Zhang et al. 2003; Psota et al. 2007). Mean/median particle size can be used to describe the differences in particle size among various barley varieties after mechanical processing. In order to properly predict mean/median particle size for coarse dry-rolled grain, a suitable model is required. Pond et al. (1984) and Fisher et al (1988) proposed different mathematic equations for analyzing mean/median particle size of substances with irregular shapes.

The main objectives of this study were 1) to determine the content of hull, FA, PCA, NDF, ADF and ADL in six barley varieties from three consecutive years; 2) to determine the mean/median particle size of barley grain obtained after coarse dry-rolling.

3.2 Materials and Methods

3.2.1 Barley Varieties and Samples

Barley varieties used in this project were obtained from the Crop Development Centre (CDC) and included AC Metcalfe, CDC Dolly, McLeod, CDC Helgason, CDC Trey and CDC Cowboy (Table 3.1). These were two-row malting or feed barley varieties, harvested from three different years (2003, 2004, 2005), and grown at the Kernen Crop Research Farm, University of Saskatchewan, Saskatoon, Saskatchewan, Canada.

Table 3.1. Barley samples used in the project

Barley variety	Barley type	Year		
AC Metcalfe	Two-row, Malting	2003	2004	2005
McLeod	Two-row, Feed	2003	2004	2005
CDC Dolly	Two-row, Feed	2003	2004	2005
CDC Helgason	Two-row, Feed	2003	2004	2005
CDC Trey	Two-row, Feed	2003	2004	2005
CDC Cowboy	Two-row, Feed (Forage)	2003	2004	2005

3.2.2 Determination of Barley Hull Content Using Modified European Brewery Convention (EBC) Method

The modified EBC method (Appendix 1) was used to analyze the hull content in the six barley varieties from three years. Barley grain (20 g) was boiled and digested for 3 min in a solution of 80 ml sodium hypochlorite (12%) (ClearTech Industries Inc., Saskatchewan, Canada) and 20 ml sodium hydroxide (3.125 N) (pellet, VWR International, Pennsylvania, USA). The dehulled samples were then dried and ground to determine barley hull content.

3.2.3 Determination of the Content of NDF, ADF and ADL

Eighteen barley samples (6 varieties × 3 years) were analyzed for NDF, ADF and ADL. Whole barley grain was ground through 1 mm pore-size mesh screens (Retsch ZM-100, Brinkmann Instruments Ltd., Ontario, Canada). Ground barley (0.5 g) was weighed and placed in F57 filter bags (Ankom Technology Corporation, Fairport, NY). All samples were treated with alpha-amylase (Anachemia Science, Anachemia Canada Inc. Winnipeg, MB) in 8 M urea (pellet, VWR International, Pennsylvania, USA) solution overnight (Van Soest et al. 1991). Alpha-amylase was used at 100 µl per 30 ml 8 M urea. After incubation, all bags for NDF analysis were rinsed ten times with warm tap water. NDF and ADF were determined in tandem using Ankom Fibre 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 (Van Soest et al. 1991). ADL was determined by oxidizing and removing the remaining carbohydrate residue in ADF with 72% H_2SO_4 (98%, VWR International, Pennsylvania, USA) (AOAC 1990).

3.2.4 Determination of Ferulic Acid and ρ-Coumaric Acid Content

3.2.4.1 Sample Preparation

Whole barley grain was ground through a 0.5 mm pore-size mesh screen followed by a 0.25 mm screen using a Retsch ZM–1 grinder (Brinkmann Instruments Canada Ltd, Ontario).

Alkaline hydrolysis of barley samples and extraction of FA and PCA were based on the method of Hernanz et al. (2001) with some modifications (Appendix 2). Ground barley grain (50 mg) was mixed with 0.75 ml 1% (w/v) α-amylase in a 0.05 M phosphate solution (pH 6.9) and incubated in a hot water bath (90 ^{o}C) for 1 h. The samples were then cooled at room temperature and centrifuged at 14,000 rpm for 20 min. The supernatant (S1) was collected and stored at -20 ^{o}C. The 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 were wrapped with aluminium tinfoil). After centrifugation (14,000 rpm, 10 min), the supernatant (S2) was collected and combined together with the initial supernatant (S1), acidified with 200 µl 6 M HCl to pH 2, and then extracted five times with equal volumes of ethyl acetate. The organic solutions were combined and evaporated until dry under N_2 in a heat block at 40 ^{o}C. The residue was dissolved in 1 mL of methanol/water (50:50, v/v) and filtered through a 0.45 µm syringe filter (Minipore, Bedford, USA) and 5 µl samples were analyzed by HPLC. All samples were prepared and analyzed in triplicate.

3.2.4.2 HPLC Condition

HPLC analysis of ferulic acid (FA, trans-4-Hydroxy-3-methoxycinnamic acid) (46278) and ρ-coumaric acid (PCA, trans-4-Hydroxycinnamic acid) (C9008) were

developed based on the method of Olkwoski et al. (2003) with some modifications. An Agilent 1100 series HPLC system was used, which consists of a system controller (HP Chem Station computer program), pump, auto-sample processor and photo diode array detector (DAD) (Interface 35900E). 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 auto-sampler. The 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 standard compounds and were quantified using the external standards. HPLC chromatogram is shown in Appendix 3. FA and PCA concentrations of sample extracts were extrapolated from the FA and PCA standard curves. The standards were prepared as stock solution 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.

3.2.5 Determination of Mean/Median Particle Size of Barley Grain Obtained after Dry-rolling

Barley samples were coarsely dry-rolled in a grain roller mill (Seven Grain Mill, Apollo Machine and Products Ltd., Saskatoon, Canada) in the College of Engineering (University of Saskatchewan, Canada) through a 1.55 mm gap. Particle size distribution of these cracked samples was determined by weight distribution. In brief, triplicate samples (100 g) were sifted through a stack of six test sieves plus one bottom pan arranged in descending sizes of square sieve apertures (Table 3.2), fitted in a Ro-Tap sieve shaker (Tyler Industrial Products, USA). 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 (ANSI 2003) sieving method.

Table 3.2. Aperture sizes for test sieves

Sieve No.	Aperture Size (mm)
6	3.36
8	2.36
12	1.70
16	1.19
20	0.84
30	0.58

After sieving, the fractions remaining on each screen were weighed, and particle size distribution was expressed in percentage cumulative weight oversize by adding up the weight on each sieve and those from all larger screens (ANSI 2003). The mean/median particle size values were estimated by fitting these data into Pond's equation (3.1) with 0 mm = 100% (Pond et al. 1984) (0 mm = 100% means that particles passing the 0.58 mm sieve are included in Pond's equation). The selection of this equation is explained in the Appendix 4. Data were computed using the NLIN procedure of the Statistical Analytical System (SAS) (SAS Institute, Inc. 2002).

Pond's Equation:

$$R = 100\, e^{-k(s-w)} \qquad (3.1)$$

Mean particle size = 1/k+w

Median particle size = 0.693/k+w.

Where:

s = sieve opening size (mm);

w = the smallest predictable particle size;

k = the decay constant of the exponential curve describes the proportionality constant between the percent of particles passed to the next sieve and the percent remained.

3.2.6 Statistical Analysis

Analysis of variance (ANOVA) was performed using the Proc Mixed procedure of SAS (2002). Experiments were carried out as a completely randomized design (CRD) with barley variety as a fixed effect and year as replication. Treatments were compared by the LSD test. Significance was declared at $P < 0.05$.

The model used for the analysis was:

$$Y_{ij} = \mu + t_i + e_{ij}$$

Where Y_{ij} is an observation of the dependent variable; 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.

3.3 Results and Discussion

3.3.1 Variety Effect on Barley Hull Content

The hull content for the six barley varieties (AC Metcalfe, CDC Dolly, McLeod, CDC Helgason, CDC Trey and CDC Cowboy) collected over three years (2003, 2004, 2005) varied from 9.4 to 10.7% with a mean value of 10.1% (Table 3.3). Significant effects of variety on hull content were detected ($P<0.05$), with McLeod and CDC Cowboy demonstrating the highest hull content, and CDC Dolly and CDC Helgason showing the lowest. The hull content in this experiment agreed with that reported by Evers et al. (1999) who indicated that barley hull content varies from 7 to 25% among two-row and six-row barley grain, while two-row barley commonly displays lower hull content with a mean content of 10.4%. Barley hull content is influenced by both environment as well as genetic factors. Evers et al. (1999) 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 growing season and tends to grow barley with low hull content. Fox et al. (2006) observed that hull content was associated with a genomic region on barley chromosome 2H. Such information provides the genetic basis for the variety effect on hull content.

Although CDC Dolly is not a new variety, it is still widely cultivated for feed barley in Canada, and is often used as a reference variety for barley breeders. CDC Dolly usually produces a heavier test weight than many other varieties (Government of

Alberta 2007). The results in this study indicate that CDC Dolly had a lower ($P<0.05$) hull content than McLeod and CDC Cowboy, but similar to CDC Helgason, CDC Trey and AC Metcalfe, while CDC Helgason had the lowest ($P<0.05$) hull content compared to the other barley varieties.

Table 3.3. Variation of variety effect on hull content in six barley varieties

Barley variety	Hull content (%DM)
McLeod	10.7 [a]
CDC Cowboy	10.4 [ab]
AC Metcalfe	10.2 [bc]
CDC Trey	10.1 [bc]
CDC Dolly	9.8 [cd]
CDC Helgason	9.4 [d]
SEM	0.11

[a, b, c, d] Different superscripts of in the same column are significantly different ($P < 0.05$).

CDC Cowboy is a barley variety oriented toward forage value and is quite low in grain yield production (Government of Alberta 2007). However, CDC Cowboy actually performed fairly well in kernel weight test, far better than CDC Helgason which was low in kernel weight test and high in yield test (Government of Alberta 2007). Therefore, it is reasonable to expect higher hull content in CDC Helgason than in CDC Cowboy. However, the present study has revealed the reverse results. Such inconsistencies between barley hull content and kernel weight, grain size or plumpness (Field Crop Development Centre and Lacombe Research Centre 2006; SeCan 2006; Government of Alberta 2007) implies that some other factors are involved in affecting barley hull content, such as genetic features, climate, agronomic management, disease resistance and soil adaptation (Evers et al. 1999; Fox et al. 2006). More research is needed to investigate factors determining barley hull content. In the present study, the

confounding factors such as weather, environment and agronomic management have been managed by applying the same agronomic practices in the same year for all the six varieties, so variety should have exerted its maximum effect on the difference of hull content in the barley samples.

3.3.2 Variety Effect on the Content of FA, PCA and PCA/FA in Barley Grain

Barley grain contains two major low molecular weight hydroxycinnamic acids: FA and PCA (Nordkvist et al. 1984; Chemey et al. 1992). They are covalently cross-linked to barley cell wall polysaccharides and lignin by ester and/or ether bonds (Iiyama et al. 1990; Lam et al. 1992a; Hernanz et al. 2001) and directly or indirectly involved in affecting the digestibility of cell wall polysaccharides (Jung and Allen 1995; Grabber et al. 2004). About 80% of FA and PCA is found in the bran cell walls and the rest in the cell walls of the endosperm (Hernanz et al. 2001). Rumen microorganisms are able to synthesize limited phenolic acid esterases to ultimately break down the ester bonds. The ether linkages however are difficult to cleave in the rumen anaerobic environment (McSweeney et al. 1994; Jung and Allen 1995). Therefore, only the esterified FA and PCA were analyzed in the present study. The 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 (Zupfer et al. 1998; Hernanz et al. 2001; Yu et al. 2002; Holtekjolen et al. 2006).

Table 3.4 shows the effects of barley variety on the content of FA and PCA, and ratio of PCA to FA (PCA/FA) in this study. Significant variety effects were detected ($P<0.05$). In all samples, barley grain contained a higher FA content than PCA, ranging from 555 to 663 μg/g DM for FA, and 283 to 345 μg/g DM for PCA. Accordingly, the ratio of PCA to FA varied from 0.49 to 0.56 (or FA/PCA 1.8 to 2.1). Hernanz et al. (2001) examined several European malting and feed barley varieties and found a range of 359 to 624 μg/g DM for FA content, 79 to 260 μg/g DM for PCA content, and 0.27 to 0.37 for ratio of PCA/FA. 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 DM, PCA content varied from 114 to 244 μg/g DM, and the ratio of

PCA/FA varied from 0.16 to 0.48. FA content in the present study was similar to their findings, but PCA content was slightly higher in our barley samples; consequently, the ratio of PCA/FA was also higher. This is likely a result of the different growing environments and varieties studied.

Table 3.4. Variation of variety effect on the content of FA, PCA and ratio of PCA/FA of whole barley grain in six barley varieties

Barley variety	FA (μg/g DM)	PCA (μg/g DM)	PCA/FA
McLeod	663 [a]	345 [a]	0.52 [ab]
CDC Cowboy	606 [b]	339 [a]	0.56 [a]
AC Metcalfe	594 [bc]	308 [b]	0.52 [ab]
CDC Helgason	581 [bcd]	283 [b]	0.49 [b]
CDC Trey	563 [cd]	301 [b]	0.53 [a]
CDC Dolly	555 [d]	306 [b]	0.55 [a]
SEM	13.4	10.1	0.015

[a, b, c, d] Different superscripts of in the same column are significantly different ($P < 0.05$).

In plant cell walls, FA is esterified to both polysaccharides and lignin, and mainly etherified to lignin (Jung and Deetz 1993; Morrison et al. 1998; Iiyama and Lam 2001; Sun et al. 2002). Through radical coupling reactions, FA forms cross-linkages between cell wall polysaccharides and lignin and between polysaccharides (Iiyama et al. 1994; Ralph et al. 1995). The ferulate cross-linkages strengthen plant cell walls to defend against pathogenic microorganisms and microbiological degradation (Kroon and Williamson 1999). It is of special interest in ruminants because ferulate linkages limit the digestibility of the plant cell wall in the rumen by forming a steric obstacle to degradation by rumen bacteria (Moore and Jung 2001). FA in barley grain is mostly concentrated in barley bran (Nordkvist et al. 1984; Hernanz et al. 2001). Comparison of FA content among the six varieties shows that barley variety significantly influenced

the FA content in barley grain ($P<0.05$). McLeod exhibited the highest ($P<0.05$) FA content, while CDC Dolly, CDC Trey and CDC Helgason were significantly lower ($P<0.05$) in FA content. Zupfer et al. (1998) also observed significant variety differences of FA content in barley grain. At the same time, they reported a strong genetic basis for the difference of FA content in barley, but did not observe a relationship between kernel weight and FA content.

PCA content among the six barley varieties was also significantly different ($P<0.05$). The ranking sequence showed that McLeod and CDC Cowboy had the highest PCA content ($P<0.05$), but statistical difference of PCA content among AC Metcalfe, CDC Dolly, CDC Trey, and CDC Helgason was not observed. PCA is mainly esterified and etherified to cell wall lignin and seldom linked to polysaccharides, so it is known as a good indicator of plant cell wall lignification (Jung and Allen 1995; Sun et al. 2002; Grabber et al. 2004). More PCA indicates more lignified plant cell walls (Jung and Allen 1995; Grabber et al. 2004). 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 ($P<0.05$) content of PCA which was in accordance with that of barley hull content.

The ratio of PCA/FA is proposed as an 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 (Grabber et al. 2004). In the present study, CDC Helgason had a lower ($P<0.05$) PCA/FA 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 was similar to CDC Dolly. Further study is required to assess the relationship among the FA and PCA content and hull content in barley grain.

3.3.3 NDF, ADF, ADL, Hemicellulose and Cellulose in Barley Grain

NDF, ADF, ADL, hemicellulose and cellulose of the six barley varieties are presented in Table 3.5. For all the five parameters, variety had a significant influence

(P<0.05). NDF values varied from 17.6 to 21.9 %DM with a mean of 19.5 %DM. ADF in barley grain was much lower than NDF, with a range from 5.5 to 7.0 %DM and 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 (NDF-ADF) and cellulose (ADF-ADL) were 13.5 %DM (from 12.2% to 14.9 %DM) and 4.1 %DM (from 3.8 to 4.9 %DM), respectively.

Table 3.5. Variation of variety effect on the content of ADF, NDF, ADL, hemicellulose and cellulose in six barley varieties

Variety	Original Samples				
	NDF (%DM)	ADF (%DM)	ADL (%DM)	Hemicellulose (%DM)	Cellulose (%DM)
McLeod	21.9 ^{a}	7.0 ^{a}	2.1 ^{a}	14.9 ^{a}	4.9 ^{a}
CDC Cowboy	20.5 ^{b}	6.4 ^{b}	2.1 ^{ab}	14.0 ^{ab}	4.4 ^{b}
AC Metcalfe	19.7 ^{bc}	5.7 ^{cd}	2.0 ^{bc}	13.9 ^{bc}	3.8 ^{c}
CDC Trey	19.0 ^{cd}	5.9 ^{c}	1.8 ^{cd}	13.1 ^{cd}	4.0 ^{c}
CDC Dolly	18.2 ^{de}	5.5 ^{cd}	1.8 ^{d}	12.6 ^{de}	3.7 ^{c}
CDC Helgason	17.6 ^{e}	5.5 ^{d}	1.7 ^{d}	12.2 ^{e}	3.8 ^{c}
SEM	0.41	0.13	0.05	0.30	0.12
Mean	19.5	6.0	1.9	13.5	4.1

$^{a, b, c, d}$ Different superscripts of in the same column are significantly different (P < 0.05); NDF: neutral detergent fiber, ADF: acid detergent fiber, ADL: acid detergent lignin, Hemicellulose = NDF - ADF, and Cellulose = ADF - ADL.

In general, McLeod and CDC Cowboy had higher (P<0.05) fiber content than the other barley varieties. CDC Helgason and CDC Dolly were relatively low in fiber content among the six examined barley varieties and did not differ from each other. In the present study, NDF and ADF content in the barley grain were slightly lower than the results from Fairbairn et al. (1999), while ADL was in accordance with NRC (2001). Studies have reported a wide variation in fiber content in barley, with NDF from 12 to

26 %DM, ADF from 4 to 8 %DM (Henry 1988 cited by Fairbairn et al. 1999) and ADL at approximately 1% (Fairbairn et al. 1999). NRC (2001) reported that NDF in barley grain was a 20.8 ± 8.6 %DM; ADF was 7.2 ± 2.8 %DM; and ADL was 1.9 ± 1.1 %DM. The calculated mean of hemicellulose was 13.6 %DM and the mean of cellulose was 5.3 %DM from NRC (2001), which are comparable to our results. All of this information implies that the fiber content varies in different barley varieties.

Ruminants are able to digest and utilize hemicellulose and cellulose as energy sources. Nonetheless, hemicellulose and cellulose contain less digestible energy than lipid, starch and protein. Therefore, barley grain containing more hemicellulose and cellulose will have lower energy density and digestibility. McLeod and CDC Cowboy contained more fiber content than the others and may not be good feed barley for ruminants. In contrast, CDC Helgason and CDC Dolly with less fiber content are better choices for feed barley as they would provide higher digestible energy at the same feeding level. Lignin, an important structural composition of plant cell walls, is attached to hemicellulose and cellulose and concentrated in the secondary plant cell walls (Rowell et al. 2000). Lignin is not carbohydrate and cannot be utilized as the energy source. Furthermore, no apparent lignin-degrading microorganisms or enzymes in the rumen can degrade lignin efficiently (Van Soest 1994). As a result, lignin is well-known as an inhibitor of plant cell wall digestibility. CDC Helgason and CDC Dolly with lower content of lignin may be a good choice for animal feed.

3.3.4 Mean/Median Particle Size of Coarsely Dry-rolled Barley Grain

As illustrated in Tables 3.6, barley variety exerted a significant effect on the mean/median particle size estimated from Pond's equation with 0 mm = 100% ($P<0.05$). The range of mean particle size estimated using Pond's equation was from 3.06 to 3.66 mm with an average value of 3.35 mm. Median particle size behaved similarly ranging from 2.71 to 3.04 mm and 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, while in the rank of median

particle size, it was CDC Cowboy, CDC Helgason, CDC Dolly, McLeod, AC Metcalfe, CDC Trey.

Table 3.6. Variation of variety effect on mean/median particle sizes of coarsely dry-rolled barley predicted by Pond's equation with 0 mm = 100% in six barley varieties

Barley Variety	Mean (mm)	Median (mm)
CDC Cowboy	3.66 [a]	3.04 [a]
CDC Helgason	3.39 [ab]	2.98 [a]
McLeod	3.35 [bc]	2.92 [a]
CDC Dolly	3.33 [bc]	2.94 [a]
AC Metcalfe	3.31 [bc]	2.84 [ab]
CDC Trey	3.06 [c]	2.71 [b]
SEM	0.073	0.047
Mean	3.35	2.91

[a, b, c, d] Different superscripts of in the same column are significantly different ($P < 0.05$).

Particle size reduction after mechanical processing is related to the grain's physical and chemical characteristics such as hardness. Camm (2008) reported that milling energy consumption had a positive relationship with barley endosperm hardness. Their results of the milling energy requirement and Single Kernel Characterization System (SKCS) hardness test from high to low were McLeod, CDC Dolly, CDC Helgason, and CDC Trey for the milling energy requirement, and McLeod, CDC Dolly, CDC Trey, and CDC Helgason for SKCS hardness test. In the present study, mean/median particle size of the same four barley samples demonstrated the similar trend of grain hardness with that of Camm (2008), with the exclusion of CDC Helgason. The similar trend potentially means that the particle size reduction of dry-rolled barley grain is related to grain hardness or milling energy consumption. 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, while no difference ($P>0.05$) was observed among CDC Helgason, McLeod, CDC Dolly and AC Metcalfe. The variety difference of barley grain is responsible for the intrinsic chemical composition (e.g. β-glucan, protein) and grain hardness (Andersson et al. 1999; Izydorczyk et al. 2003; Caldwell et al. 2004), which consequently influences barley particle size distribution after mechanical manipulation. Fairbairn (1999) observed a significant difference in particle size among 20 barley varieties even when grain was finely ground. Bowman et al. (2001) observed that NDF content could affect the particle size reduction of barley grain.

3.4 Conclusions and Implications

The results of chemical and physical analyses show that barley variety had a significant effect on the content of hull, FA, PCA, NDF, ADF and ADL in various barley varieties, and also on the mean/median grain particle size obtained after coarse dry-rolling. Therefore, barley variety plays an important role in determining the quality of barley as a feed.

Generally, the varieties of McLeod and CDC Cowboy consistently contained higher content of hull, FA, PCA, NDF, ADF and ADL 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. But when mean/median particle size obtained after coarse dry-rolling was compared, CDC Cowboy and CDC Helgason showed larger particle size, and therefore become more promising as feed barley. On the whole, CDC Dolly and CDC Helgason have lower content of hull, FA, PCA, NDF, ADF and ADL, and have moderate mean/median particle size after dry-rolling, so both are good candidates for feed barley according to the current study. However, the best way to predict barley quality is in situ rumen degradation experiment. Therefore, further experiments are needed to investigate the exact relationship between in situ digestibility and the physical, chemical measurements in these barley varieties.

4. IN SITU DEGRADATION CHARACTERISTICS OF HYDROXY-CINNAMIC ACIDS AND FIBER OF VARIOUS BALREY VARIETIES

4.1 Introduction

Barley grain is the principal energy source for cattle in western Canada. Barley seed contains mainly starch and protein which can be quickly fermented in the rumen. However, barley grain cell walls, especially in the bran consist of mainly fiber and other minor but important chemical components such as ferulic acid (FA) and ρ-coumaric acid (PCA), the presence of which could exert considerable effects on the rate and extent of grain degradation. It is well established that fiber is the major contributor to the relatively lower energy density in barley grain than in corn. Lignin is recognized as the most inhibiting constituent of plant cell wall with respect to digestion in animals. Among the physical fractions of barley grain, the hull is the most fibrous part, which contains roughly 85% fiber (Olkku et al. 2005). Therefore, barley hull is the most indigestible fraction.

Hydroxycinnamic acids (mainly FA and PCA) have been reported to adversely affect plant cell wall digestibility in ruminants. FA and PCA are the two principal hydroxycinnamic acids in barley grain, concentrated in the cell walls of the bran (Nordkvist et al. 1984; Chemey et al. 1992). FA and PCA are covalently linked to plant cell wall polysaccharides by ester bonds and to lignin by both ester and ether bonds (Iiyama et al. 1990; Lam et al. 1992a; Hernanz et al. 2001). However, FA is considered to have direct inhibitory influence on the digestibility of plant cell walls, while PCA is regarded primarily as an indicator of the degree of plant cell wall lignification (Jung and Allen 1995; Anderson and Schroeder 1999; Grabber et al. 2004).

Particle size is another important factor influencing barley digestion in the rumen. Whole barley grain fed to animals was shown to have significantly lower grain digestibility and animal performance compared to processed barley grain (Mathison

1996). In contrast to small particle size, large particle size reduces the surface area for rumen bacteria and enzymes to attack and digest, and accordingly slows down the fermentation rate of starch and digestion rate of barley grain in the rumen. It is also likely that particle size affects the extent of feed digestion by changing the digestion site and passage rate of feed in the digestive tract of cattle (Koenig et al. 2003; Rémond et al. 2004)

Feed degradability can be measured by using the in situ rumen nylon bag technique which reflects a more realistic rumen environment than in vitro and other methods. The first-order digestion kinetics equation (Ørskov and McDonald 1979) is the most widely applied model to describe the digestibility of individual feed components such as DM, protein, starch and fiber. With respect to in situ rumen degradability of DM, different varieties of barley grain demonstrated varying rate and extent of rumen degradation due to differences in the intrinsic physical structures and chemical components (Yu et al. 2003; Walker 2007). In practice, the in situ rumen digestibility of feed components varies due to many influencing factors such as feed processing, experimental techniques and animal status (Mustafa 1996; Vanzant et al. 1998). However, the in situ rumen nylon bag technique is still a widely used and useful method to compare and estimate feed quality.

The specific objectives of this study were 1) to investigate in situ rumen digestibility of DM, FA, PCA, NDF, ADF and ADL at 12 and 24 h rumen incubation among the six varieties; 2) to select the relatively better and worse barley varieties based on the initial comparison study of hull, FA, PCA, NDF, ADF, ADL, particle size and rumen digestibility to further analyze and compare the rumen digestion kinetics of DM, FA and PCA.

4.2 Materials and Methods

4.2.1 Barley Samples and Preparation

Barley samples used in this experiment were the same six barley varieties used in the Chapter 3: AC Metcalfe, CDC Dolly, McLeod, CDC Helgason, CDC Trey and CDC Cowboy from 2003, 2004 and 2005.

The particle size reduction procedure was the same as that previously described in the Chapter 3. 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., Saskatoon, Canada) in the College of Engineering, University of Saskathcewan.

4.2.2 In Situ Rumen Incubation

4.2.2.1 Animals and Diets

Three Holstein dry cows weighing approximately 670 kg were rumenly fistulated and housed individually in a box stall with bedded straw in the metabolism facilities at the University of Saskatchewan. The cows had ad libitum access to fresh water and were free to enter the exercise ground. The cows were fed twice daily at 0800 and 1600 h and received equal portions (7 kg 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 dairy concentrate and 6.8% fresh cow concentrate on dry matter base (Appendix 5). 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 Canadian Council on Animal Care (1993).

4.2.2.2 Rumen Incubation

Rumen degradation experiments were performed in two separate sets of in situ incubation trials following the nylon bag technique procedure described by Yu et al. (2003).

Trial 1: Rumen Degradability of DM, FA, PCA, NDF, ADF and ADL of Six Barley Varieties at 12 and 24 h Rumen Incubation

The first trial examed the rumen degradability of barley DM, NDF, ADF, ADL, FA and PCA of the six barley varieties at 12 and 24 h of rumen incubation. Coded nylon bags (Nitex 03-41/31 monofilament open mesh fabric, ScreenTec Corp., Mississauga, ON; 10 cm × 20 cm; pore size of 41 μm) were filled with 7 g of ground sample per bag. The ratio of sample weight to bag surface area was roughly 19 mg/cm^2. Three and four bags per sample were arranged for 12 and 24 h periods respectively to

ensure that there was sufficient sample residue for chemical analysis. Bags were randomized and individual bags were placed in a large mesh bag (28 nylon bags per mesh bag) with a heavy bottle for the ballast and placed in the ventral sac of the rumen at 2100 pm for 24 h and at 0900 am for 12 h according to the 'gradual addition/all out' schedule. The trial was carried out in three runs. Following removal from the rumen, all the bags were washed together under cold tap water without detergent for six rinses with gentle agitation until the effluent remained clear. Subsequently, bags were dried in a forced-air oven at 55 °C for 48 h, then left in the open air to air equilibrate for three days. The residues were weighed and pooled together according to treatment and incubation time. DM was determined based on pooled residues. Pooled samples were ground in a Retsch ZM–1 grinder (Brinkmann Instruments Canada Ltd, Ontario) using a 1-mm screen. Samples were mixed and separated into two portions: one for fiber analysis; the other was reground through a 0.25 mm screen size for determining FA and PCA content following the same procedure in the Chapter 3.

Trial 2: In Situ Rumen Degradation Kinetics of Two Barley Varieties

Based on the results from Trial 1, two barley varieties were selected for further analysis of rumen degradation kinetics in detail, which were CDC Dolly ("better") and McLeod ("poorer"), respectively. Trial 2 was performed for these two barley varieties to determine whether or not FA and PCA fit into the first-order digestion kinetics equation (Ørskov and McDonald 1979) and to compare the differences of the degradation characteristics for DM, FA and PCA. The two barley varieties were from three years (2003, 2004, 2005). Year was used as experimental replication. The incubation periods in Trial 2 were increased to eight incubations in the sequence of 72, 48, 24, 12, 8, 4, 2 and 0 h according to the 'gradual addition/all out' schedule (Appendix 6). To ensure enough residue left for further analysis, increasing bag numbers were arranged following the ascending incubation time which is listed in Appendix 6. Following incubation, all the bags (including 0 h bags) were washed and dried with the same procedure as Trial 1.

4.2.2.3 Rumen Degradation Kinetics

For Trial 1, the percentage of rumen indigestible residues of DM, FA, PCA, NDF, ADF and ADL at 12 and 24 h was calculated from the content of these components in original samples and in residues.

For Trial 2, the percentage of rumen indigestible residues of DM, FA and PCA at each incubation was calculated and fitted into the nonlinear model using the PROC NLIN procedure of SAS (2002) with iterative least squares regression (Gauss- Newton method) by following the first-order digestion kinetics equation (Ørskov and McDonald 1979) modified by Tamminga et al. (1990, 1994):

$$R(t) = U + (100 - S - U) \times e^{-K_d \times (t-T_0)} \quad (t > T_0)$$

As long as RSS meets the convergence criterion, the rumen degradation data is assumed to fit the Ørskov first-order kinetics model (Mustafa 1996). In the equation, R(t) means the amount of residue (%) of the original sample incubated after t hour. DM, FA and PCA were partitioned into three fractions based on the relative susceptibility to rumen degradation. Fraction S was defined as the instantly digestible or 'wash-out' fraction (S, %); fraction U corresponded to the undegradable fraction in the rumen (U, %); and fraction D was considered as the slowly degradable fraction (D, %), which was degraded exponentially in the rumen. T_0 (h) was lag time and K_d (% h^{-1}) was disappearance rate constant (K_d, % h^{-1}) were determined directly from this model, and fraction S was estimated from 0 h. The effective degradabilities (ED) for DM, FA and PCA were calculated as:

$$ED\ (\%) = S + D \times K_d/(K_p + K_d)$$

Where, K_p is the fractional passage rate and assumed to be 6% h^{-1} (Yu et al. 2003). The rumen undegraded proportions (RU) of DM, FA and PCA were determined as:

$$RU\ (\%) = U + D \times K_p/(K_p + K_d).$$

Where, S, U, D, K_p, K_d were defined as above.

4.2.3 Statistical Analysis

Analysis of variance (ANOVA) for rumen degradation parameters was performed using SAS (2002). Significance was declared at $P < 0.05$. Factorial treatment

arrangement (6×2: six varieties and two incubation times) and CRD experimental design were used to describe the effects of barley variety and rumen incubation time on the rumen degradation parameters (residue percentages of DM, FA, PCA, NDF, ADF and ADL), using the Proc Mixed in SAS (2002). Means were compared by LSD test. Rumen degradation parameters (S, D, U, K_d, T_0, ED, RU) for DM, FA and PCA were compared between two barley varieties seleted based on Trial 1 results with T test using PROC TTest procedure in SAS (2002).

4.3 Results and Discussion

4.3.1 Trial 1: Rumen Degradability of DM, FA, PCA, NDF, ADF and ADL of Six Barley Varieties at 12 and 24 h of Rumen Incubations

Tables 4.1 shows the effect of barley variety and/or rumen incubation time on the in situ rumen indigestible residues (%DM) of barley DM, FA, PCA, NDF, ADF and ADL as well as the interaction between the barley variety and rumen incubation time.

Effects of variety ($P<0.05$) were observed on the rumen indigestible residues of DM, FA, PCA, NDF and ADF, but not ADL residues ($P=0.14$). Rumen incubation time had a significant effect ($P<0.05$) on the rumen indigestible residues of barley DM, FA, ADF and ADL. The higher the indigestible residue percentage left in the rumen, the lower the digestibility of the grain. The interaction effect between barley variety and rumen incubation time on the rumen indigestible residues was not observed.

Table 4.1. Rumen degradability of DM, FA, PCA, NDF, ADF and ADL of six barley varieties at 12 and 24 h of rumen incubations

		In situ rumen indigestible residue at 12 and 24 h (%)					
		DM	NDF	ADF	ADL	FA	PCA
Variety	AC Metcalfe	46.3 ^{abc}	61.3 ^{a}	85.2 ^{d}	87.1	60.3 ^{cd}	71.7 ^{b}
	CDC Cowboy	45.4 ^{bc}	64.4 ^{a}	89.9 ^{ab}	91.3	64.1 ^{b}	74.2 ^{b}
	CDC Dolly	44.1 ^{c}	61.9 ^{a}	86.6 ^{cd}	87.0	59.9 ^{cd}	72.1 ^{b}
	CDC Helgason	49.4 ^{a}	57.8 ^{b}	88.8 ^{bc}	89.4	59.7 ^{d}	71.9 ^{b}
	CDC Trey	47.6 ^{abc}	57.7 ^{b}	88.4 ^{bc}	89.4	72.3 ^{a}	80.6 ^{a}
	McLeod	49.0 ^{ab}	62.8 ^{a}	92.3 ^{a}	89.7	63.6 ^{bc}	74.7 ^{b}
	SEM	1.31	1.17	1.03	1.28	1.30	1.54
Incub. Time	12 h	56.6 ^{a}	61.4	90.1 ^{a}	90.1 ^{a}	64.8 ^{a}	74.6
	24 h	37.4 ^{b}	60.4	87.0 ^{b}	87.8 ^{b}	61.8 ^{b}	73.8
	SEM	0.76	0.68	0.60	0.74	0.75	0.89
Statistical analysis		----------P value----------					
	Variety	< 0.05	< 0.05	< 0.05	0.14	< 0.05	< 0.05
	Time	< 0.05	0.35	< 0.05	< 0.05	< 0.05	0.55
	Variety×Time	0.88	0.71	0.74	0.97	0.61	0.33

$^{a, b, c, d}$ Different superscripts in the same column are significantly different ($P < 0.05$).

The average DM indigestible residue percentages at 12 and 24 h of rumen incubation were 56.6% and 37.4%, respectively, and were different ($P<0.05$) (Table 4.1). DM residues in the present study were higher than those of Yu et al. (2003) who observed that the indigestible 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 mm vs. 0.53 mm). CDC Dolly showed relatively low DM residues (44.1%) after rumen digestion, which indicated CDC Dolly has a high DM digestibility that is favorable for ruminants. In contrast, CDC Helgason was the poorest (49.4%) feed barley for ruminants.

The residue percentages of fiber in the forms of NDF, ADF and ADL were much higher than DM as the fiber is more recalcitrant to rumen digestion. NDF represents the total structural cell wall components including cellulose and hemicellulose as well as lignin, so rumen indigestible NDF residue was lower than ADF and ADL, and averaged 61.4% and 60.4% at 12 and 24 h, respectively. Feng et al. (1995) reported 63-68% total tract indigestible NDF for whole barley grain, while Beauchemin et al. (1999) found it was 53% for the whole barley grain indicating that a range of variation for NDF digestibility exists. The NDF residue difference between 12 and 24 h was not significant ($P>0.05$), which implies that most of NDF in barley grain was degraded within 12 h of rumen incubation. ADF contains principally cellulose and lignin, which is less digestible than NDF. Beauchemin et al. (2001) found that rumen indigestible ADF for stream-rolled barley was about 80% compared to 50-65% of indigestible NDF in steers. In the present study, ADF residue at 12 and 24 h averaged 90.1% and 87.0%, respectively. Rumen ADF residues were different among barley varieties and between the two rumen incubation time-points ($P<0.05$). Among the six varieties, McLeod showed higher ($P<0.05$) ADF residue than CDC Dolly and AC Metacalf. Therefore, AC Metcalfe and CDC Dolly were superior to McLeod in terms of feed barley quality. Although ADL is thought to be of low digestibility for animals, in the present study, roughly 10% of ADL was degraded in the rumen. Nelson (2001) also reported that 12.2% ADL was degradable when lambs were fed a coarsely dry-rolled barley based

diet. However, no statistical difference among barley varieties was detected for ADL rumen residues. The original content of ADL in barley was quite low (about 1-2%). This is perhaps because the original content of ADL in barley was quite low (1.7 – 2.1 %DM). In practice, ADL digestibility of barley grain is seldom analyzed.

To date, no one has studied the digestibility of esterified FA and PCA in barley using either in vitro, in situ or in vivo methods. Researchers are interested in FA and PCA in forages, because forages contain greater amounts of FA and PCA. In the present study, barley variety was also found to have significant effect on FA and PCA rumen residues ($P<0.05$) with highest FA and PCA rumen indigestible residues in CDC Trey. Although CDC Trey showed only moderate content of hull, FA, PCA and fiber, it exhibited higher ($P<0.05$) rumen indigestible residues of FA and PCA than others. The reason for this is not clear. With exception to CDC Trey, there was no difference in PCA residues among the remaining five barley varieties. FA residues at 12 and 24 h were different ($P<0.05$), decreasing from 64.8% to 61.8% on average. The change for PCA resiude was not obvious ($P = 0.55$), decreasing from 74.7% to 73.8%. This implies that FA in barley continued to be degraded in the rumen after 12 h of rumen incubation, while the degradation of PCA came to a plateau after 12 h rumen incubation. After the same rumen incubation periods (12 and 24 h), levels of FA residues were lower than PCA, indicating that FA was degraded to a relatively greater extent than PCA. Others have observed that esterified FA was degraded more quickly and to a greater extent than esterified PCA in forages (e.g. cocksfoot, orchardgrass) (Canale et al. 1990; Grabber and Jung 1991; Bourquin et al. 1994). However, PCA is more related to lignin, so its degradability probably will not affect much the digestion of plant cell wall polysaccharides.

When all the degradation parameters were compared, CDC Dolly showed relatively lower rumen residues of DM, FA, PCA, ADF and ADL compared to the other barley varieties, while McLeod seemed to be more resistant to rumen degradation with relatively higher in rumen residues of DM, PCA, ADF and ADF. In combination with the physical and chemical information reported in the previous chapter, we have found that CDC Dolly is more promising as a feed barley grain while McLeod is relatively

inferior. Therefore, these two barley varieties were selected for the next experiment for detailed comparison of in situ rumen digestion kinetics of DM, FA and PCA.

4.3.2 Trial 2: In Situ Rumen Degradation Kinetics of Ferulic Acid and ρ-Coumaric Acid in Two Barley Varieties

4.3.2.1 Degradation Characteristics of DM

The rumen DM degradation characteristics of CDC Dolly and McLeod are presented in Table 4.2. Figure 4.1 illustrates the degradation curve. General parameters of in situ DM degradation in barley were comparable to the results described by Yu et al. (2003) and Walker (2007). As expected, CDC Dolly provided more nutrients for ruminants than McLeod because of its higher ($P<0.05$) potential digestible fraction of DM (D, %DM) and lower ($P<0.05$) undegradable fraction of DM (U, %DM) ($P<0.05$) (82.8 vs. 79.3 %DM, 13.7 vs. 16.2 %DM) (Table 4.2). The main undegradable fraction of barley is the hull. CDC Dolly also contained less content of hull, FA, PCA and fiber than McLeod. So, it is reasonable that CDC Dolly showed higher D fraction and lower U fraction than McLeod. The slowly digestible fraction is nutritionally important for ruminants because it contributes to the growth of rumen microbes by providing starch as the energy source (Khorasani et al. 2000).

Table 4.2. In situ degradation kinetics of dry matter in CDC Dolly and McLeod

Parameter	Barley Variety		
	CDC Dolly	McLeod	P value
S (% DM)	3.5	4.6	0.06
D (% DM)	82.8	79.3	<0.05
U (% DM)	13.7	16.2	<0.05
Kd (% h^{-1})	10.8	8.1	0.18
T_0 (h)	0.95	0.52	0.26
EDDM (% DM)	56.2	49.8	0.11
RUDM (% DM)	43.8	50.2	0.11

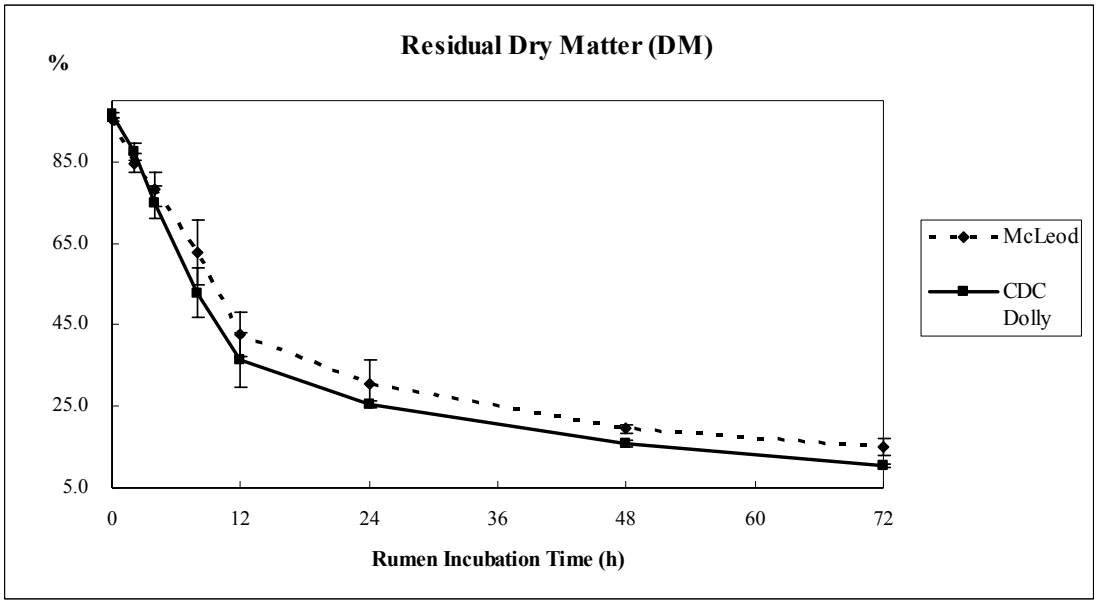


Figure 4.1. Dry matter residue of CDC Dolly and McLeod at various rumen incubations

CDC Dolly tended to exhibit a lower (P = 0.06) soluble portion of DM (S, %DM) (3.5 %DM) than McLeod (4.6 %DM) (Table 4.2), which could result from the difference of the intrinsic properties of barley grain (eg. the structural organization of protein, starch and the molecular structural chemical make-up) (McAllister et al. 1993; Walker 2007; Yu et al. 2008). Yu et al. (2003) observed large variation of soluble fractions between Valier and Harrington (1.5 vs. 11.6 %DM) which was owing to the difference of barley type (feed vs. malting). Rumen degradation rate (Kd, % h^{-1}) between CDC Dolly and McLeod did not differ (P = 0.18). Lag time (T_0, h) (0.95 h for CDC Dolly and 0.52 h for McLeod) was quite long compared to that of Yu et al. (2003) (0 - 0.2 h). This is likely due to a larger roller gap size (1.55 vs. 0.53 mm) used to process the grain in the present study. Lag time is a process of hydration and the initiation of rumen bacterial attachment (Mustafa 1996). Larger particle size could delay the process and lead to longer lag time. The effective degradation of DM (EDDM) between CDC Dolly and McLeod did not differ (P = 0.11), but CDC Dolly showed numerically higher EDDM (56.2 vs. 49.8 %DM), which indicated that CDC Dolly tended to be more extensively degraded in the rumen and of higher nutritional feed value than McLeod.

4.3.2.2 Rumen Degradation Characteristics of FA and PCA

The rumen degradation kinetics of FA and PCA were presented in Table 4.3 and Figure 4.2 for FA, and in Table 4.4 and Figure 4.3 for PCA. Canale et al. (1990) also employed the first-order digestion kinetics equation to describe the effect of alkali-treatment on in situ digestion of FA and PCA in orchardgrass and alfalfa. Bourquin et al. (1994) used the first-order exponential model to analyze in situ digestion profiles of DM, FA and PCA, and found that in situ degradation profiles of FA and PCA followed the patterns similar to that of DM.

Table 4.3 shows that there was no difference in the characteristics of FA degradation kinetics between CDC Dolly and McLeod except for U (%FA) and RUFA (μg/g DM) where CDC Dolly tended to have lower U and RUFA than McLeod ($P>0.05$). This indicates that FA in CDC Dolly tended to have a higher extent of rumen degration than McLoed.

Table 4.3. In situ rumen degradation kinetics of ferulic acid in CDC Dolly and McLeod

Parameter	Barley Variety		P value
	CDC Dolly	McLeod	
S (% FA)	22.2	18.4	0.26
D (% FA)	44.4	41.9	0.22
U (% FA)	33.4	39.7	0.08
Kd (% h^{-1})	4.4	4.4	0.99
T_0 (h)	0	0	
EDFA (% FA)	40.9	36.1	0.19
EDFA (μg/g DM)	226.8	237.8	0.51
RUFA (% FA)	59.1	63.9	0.19
RUFA (μg/g DM)	328.3	424.8	0.06

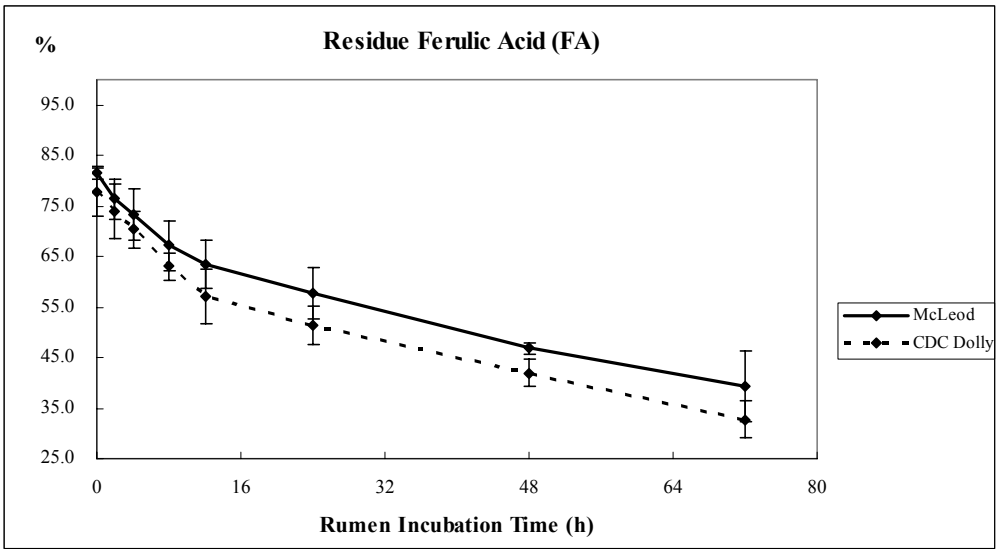


Figure 4.2. Ferulic acid disappearance of CDC Dolly and Mcleod at various rumen incubations

Table 4.4 shows in situ degradation kinetics of PCA in the two barley varieties. Significant differences were found in S (%PCA), U (%PCA), EDPCA (%PCA) and RUPCA (%PCA), which indicate that esterified PCA in CDC Dolly was more digestible than that in McLeod.

Table 4.4. In situ rumen degradation kinetics of para-coumaric acid in CDC Dolly and McLeod

Parameter	Barley Variety		
	CDC Dolly	McLeod	P value
S (% PCA)	17.1	10.9	<0.05
D (% PCA)	21.7	22.4	0.50
U (% PCA)	61.2	66.7	<0.05
Kd (% h^{-1})	9.7	9.2	0.85
T_0 (h)	0.81	0.97	0.72
EDPCA (%PCA)	30.3	24.4	<0.05
EDPCA (μg/g DM)	92.5	84.0	0.39
RUPCA (%PCA)	69.7	75.6	<0.05
RUPCA (μg/g DM)	213.0	261.2	0.06

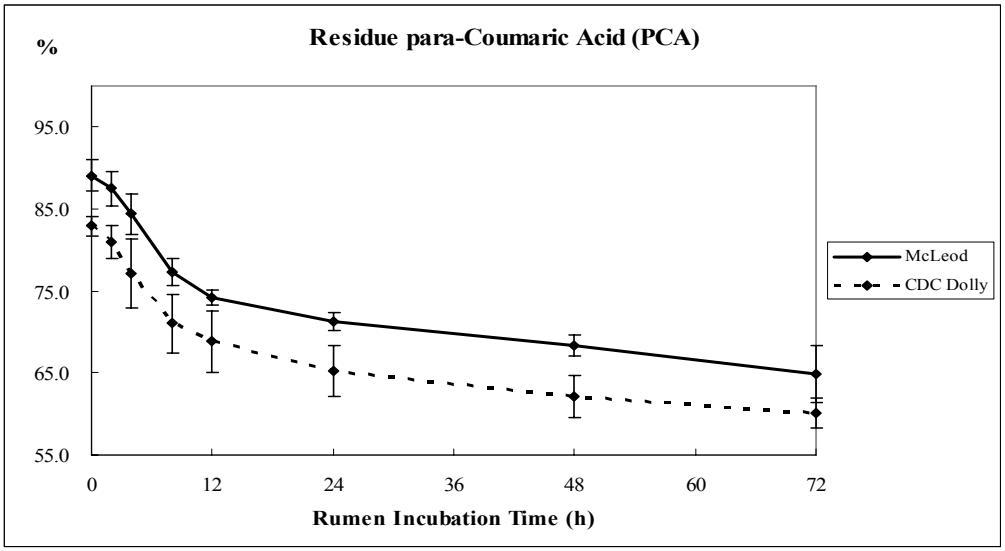


Figure 4.3. para-Coumaric acid disappearance of CDC Dolly and Mcleod at various rumen incubations

Comparing FA to PCA, FA had slower K_d (about 4.4% h^{-1}) than PCA (around 9.5% h^{-1}) which contrasted to the findings of Canale et al. (1990) for forages (7.8 % h^{-1} vs. 3.3 % h^{-1}). These authors also found that K_d for PCA increased with the maturity degree from prehead (3.3% h^{-1}) to head (7.5% h^{-1}) stage in forages. Bourquin et al. (1994) observed that animal diets and the degree of feed processing could affect K_d values of FA and PCA in orchardgrass. The discrete lag time for FA was 0 h, while for PCA it was around 0.9 h. One possible explanation is that due to the difference in chemical structure, the esterified FA is relatively more easily accessible and degradable for rumen microbes and their enzymes than the esterified PCA. Another potential reason is that FA is both esterified to lignin and polysaccharides and is rich in both barley hull and aleurone layer, while PCA is mostly esterified to lignin in barley hull (Maillard and Berset 1995). Furthermore, aleurone layer is more likely to shatter during rolling than barley hull, so more FA could be washed out as the aleurone powder goes, which would likely contribute to 0 h lag time for FA. Canale et al. (1990) observed a shorter lag time for FA than for PCA in orchardgrass hay. EDFA (%FA) was higher than EDPCA (%PCA), which means the esterified FA was more digestible than the

esterified PCA in the rumen (Canale et al. 1990; Grabber and Jung 1991; Bourquin et al. 1994; Agbagla-Dohnani et al. 2003a).

In general, most of the degradation kinetics parameters of DM, FA and PCA support CDC Dolly as a good feed grain.

4.4 Conclusions and Implications

Two consecutive in situ degradation experiments were carried out. The first trial was to determine and compare the differences in the rumen undegradable residues of DM, FA, PCA, NDF, ADF and ADL at 12 and 24 h of rumen incubation. The results show that barley variety had a significant effect ($P<0.05$) on rumen undegradable residue content of DM, FA, PCA, NDF and 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 content of rumen indigestible residues for most of parameters studied. In contrast, McLeod showed comparatively higher rumen residues and inferior digestibility. This information also implies that CDC Dolly could be a good candidate as a feed barley grain for ruminants.

Based on the first trial, two barley varieties (CDC Dolly and McLeod) were selected for further evaluation in the second experiment to study detailed degradation kinetics. This trial showed that the in situ rumen degradation parameters of FA and PCA fit the first-order digestion kinetics equation. This trial also showed that the esterified FA in barley grain was relatively more degradable than the esterified PCA. When degradation parameters for in situ degradation kinetics of DM, FA and PCA between CDC Dolly and McLeod were compared, no significant differences in EDDM and EDFA were seen, except for the significantly higher EDPCA of CDC Dolly than McLeod ($P<0.05$).

5. INVESTIGATION OF RELATIONSHIPS BETWEEN ORIGINAL CONTENT OF BARLEY HULL, HYDROXYCINNAMIC ACIDS, FIBER, PARTICLE SIZES IN VARIOUS BARLEY GRAIN AND IN SITU RUMEN DEGRADABILITIES

5.1 Introduction

Barley is the principle energy grain for ruminants in western Canada. It contains moderately higher protein than corn, which could save cattle feeders from supplementing with other protein feeds. However, barley grain comprises proportionally lower content of starch than corn (Walker 2007) and shows relatively less digestible energy available for animals due to the presence and dilution effect of barley hull (NRC 2001). The inferiority of barley in terms of energy density is mainly due to the existence of the fibrous barley hull (about 13% of the kernel) (Evers et al. 1999). As is widely known, fiber playes an important role in affecting feed digestibility and energy density.

Other chemical components such as hydroxycinnamic acids are also reported to impose adverse effects on the digestibility of plant cell walls for ruminants (Canale et al. 1990; Jung and Deetz 1993; Grabber et al. 2004; Yu et al. 2005a). Barley grain contains two major low molecular weight hydroxycinnamic acids: FA (ferulic acid) and PCA (ρ-coumaric acid) (Nordkvist et al. 1984; Hernanz et al. 2001), which are chiefly concentrated in the cell walls of barley bran (Nordkvist et al. 1984; Sancho et al. 2001). FA is esterified to both plant cell wall polysaccharides and lignin, and is also etherified to lignin (Gubler et al. 1985; Sun et al. 2002). The extensive cross-linkages of FA between polysaccharides and lignin, and among cell wall polysaccharides form steric obstacles to protect plant cell wall polysaccharides from hydrolysis and attack by rumen microorganisms, thus reducing the digestibility of the plant in the rumen (Jung and Deetz 1993; Iiyama and Lam 2001; Moore and Jung 2001; Sun et al. 2002). PCA is

chiefly esterified and etherified to cell wall lignin but seldom linked to polysaccharides, so PCA is not directly related to the digestibility of cell wall polysaccharides (Sun et al. 2002; Grabber et al. 2004).

It is common to coarsely dry-roll barley grain before feeding to cattle feedlots as dry-rolling helps to breach the rough barley hull, reduce the grain particle size, and improve its digestibility for ruminants (Mathison 1996). Particle size reduction is related to the intrinsic composition and structure of the grain. Processing barley has a positive effect on DM digestibility (Bradshaw et al. 1996). Nonetheless, excesively small particle size will not benefit the animal because rapid fermention of starch in the rumen will predispose cattle to greater incidences of disease and digestive problems. Therefore, maintenance of large particle size after the mechanical process is a preferred characteristic for a good feed barley grain.

The objectives of this study were to investigate the relationship between the hull, chemical composition, different mean/median particle size of various barley varieties, and the in situ rumen nutrient digestibility at 12 and 24 h of incubation.

5.2 Material and Methods

5.2.1 Barley Samples

Six barley varieties from three consecutive years (2003, 2004, 2005) were used and included AC Metcalfe, CDC Dolly, CDC Helgason, CDC Trey, CDC Cowboy and McLeod.

5.2.2 Chemical Analysis

Original content of barley hull, FA, PCA, ratio of PCA/FA, total of PCA+FA, NDF, ADF, ADL, hemicellulose, cellulose, mean/median particle size, and residue content of DM, FA, PCA, NDF, ADF and ADL at 12 and 24 h of rumen incubation were analyzed and reported in the previous chapters 3 and 4.

5.2.3 Statistical Analysis

SAS (2002) procedure "PROC CORR" was used to examine the correlations among the variables of the barley hull, FA, PCA, NDF, ADF, ADL, and mean/median particle size, and residue content of DM, FA, PCA, NDF, ADF and ADL at 12 and 24 h of rumen incubation.

In order to develop prediction equations to show the effects of hull, FA, PCA and mean/median particle size on rumen undegradable residues of DM, NDF, ADF and ADL at 12 and 24 h of rumen incubation, muti-regression analysis was carried out using the "PROC REG" procedure of SAS (2002) with a model as follows:

Y = Hull + FA + PCA + PCA/FA + Mean + Median

Where, Y = Rumen degradability of NDF, ADF, ADL at 12 and 24 h of rumen incubation.

The model selection used a stepwise option. All variables left in the final prediction model are significant at the 0.05 level.

5.3 Results and Discussion

5.3.1 Correlation Analysis between Original Content of Hull, FA, PCA, Fiber, Particle Sizes and Rumen Indigestible Residues at 12 and 24 h of Rumen Incubation

5.3.1.1 The Effects of Barley Hull

The correlation analysis results are presented in Table 5.1. As expected, barley hull content was highly correlated to the content of NDF, ADF, ADL, hemicellulose and cellulose ($P<0.001$). It is widely recognized that the hull is the major contributor of fiber in barley grain. NRC (2001) reported that barley seed contains 20.8% NDF, 7.2% ADF and 1.9% ADL, while the hull representing about 13% of the total kernel weight is comprised of 72-79% NDF, 35-41% ADF and 7.2-8.4% ADL (Grove et al. 2003), which means that more than 45% NDF, 65% ADF and 50% ADL are from the hull. As such, the hull is of little nutritional value for monogastric animals because they cannot synthesize cellulolytic enzymes to digest fiber. Although ruminants are well-known for their capacity to utilize plant fiber, the digestibility of barley hull is as low as 32%, with

only 14% disappearance for NDF (Grove et al. 2003). Therefore, a high percentage of barley hull will definitely lead to lower fiber digestion and lower digestible energy in barley. As seen in Table 5.1, in situ rumen indigestible residues of NDF and ADF were significantly correlated to barley hull content, which indicates that the higher hull content ($P<0.05$) results in lower NDF and ADF rumen digestibility.

However, the DM digestibility at both 12 and 24 h of rumen incubation was not significantly correlated with hull content ($P>0.05$) with low R value of 0.33 and 0.27, respectively. This is in contrast to the findings of Grove (2003). Some other factors may have exerted an effect on the DM digestibility rather than the barley hull. Although Grove (2003) observed that barley DM digestibility varied significantly with barley hull content, the author did not examine the effect of starch which was also significantly different among varieties. Barley hull had no significant ($P>0.05$) correlation with rumen residue content of FA, PCA and ADL. This indicates that not only the barley hull but also other factors, such as grain processing, content or structural matrix of starch and protein may play a role in determining rumen degradation of FA, PCA and ADL.

In general, the hull content was highly correlated to the content of FA, NDF, ADF, ADL, hemicellulose and cellulose in barley grain ($P<0.05$). Barley hull showed a significantly positive effect on rumen indigestible residue of NDF at 12 and 24 h, and ADF at 12 h ($P<0.05$). Therefore, among the hulled barley, the variety with less hull content would be preferred for ruminant feed.

Table 5.1. Correlation analysis for all parameters (original content of barley hull, FA, PCA, ration, total, NDF, ADF, ADL, hemicellulose, cellulose, mean/median particle size, and rumen in situ indigestible residues of DM, NDF, ADF, ADL, FA and PCA at 12 and 24 h of rumen incubation)

R value	Hull	FA	PCA	PCA/FA	PCA+FA	NDF	ADF	ADL	Hemi	Cell	Mean	Median
Original chemical and physical profiles in barley grain												
Hull	1.00	-	-	-	-	-	-	-	-	-	-	-
FA	0.57*	1.00	-	-	-	-	-	-	-	-	-	-
PCA	0.42	0.82***	1.00	-	-	-	-	-	-	-	-	-
PCA/FA	0.08	0.25	0.75***	1.00	-	-	-	-	-	-	-	-
PCA+FA	0.53*	0.96***	0.94***	0.49*	1.00	-	-	-	-	-	-	-
NDF	0.95***	0.76***	0.60**	0.15	0.72***	1.00	-	-	-	-	-	-
ADF	0.87***	0.71***	0.57*	0.15	0.68**	0.93***	1.00	-	-	-	-	-
ADL	0.84***	0.78***	0.64**	0.19	0.75***	0.91***	0.79***	1.00	-	-	-	-
Hemi	0.94***	0.75***	0.58*	0.15	0.71***	0.98***	0.85***	0.93***	1.00	-	-	-
Cell	0.78***	0.60**	0.47*	0.13	0.57*	0.82***	0.97***	0.61**	0.71***	1.00	-	-
Mean	0.31	0.70***	0.81***	0.57*	0.79***	0.45	0.35	0.49*	0.47*	0.25	1.00	-
Median	0.19	0.67**	0.79***	0.57*	0.76***	0.35	0.26	0.39	0.37	0.18	0.98***	1.00

* for P<0.05, ** for P<0.01, *** for P<0.001; Hemi = Hemicellulose, Cell = Cellulose, mean = mean particle size, median = median particle size

Table 5.1. (Cont'd)

R value	Hull	FA	PCA	PCA/FA	PCA+FA	NDF	ADF	ADL	Hemi	Cell	Mean	Median
Rumen in situ indigestible residue												
DM12	0.33	0.59**	0.42	0.04	0.54*	0.41	0.38	0.35	0.40	0.35	0.48*	0.50*
DM24	0.27	0.66**	0.57*	0.22	0.65**	0.38	0.26	0.40	0.41	0.17	0.60**	0.63**
NDF12	0.55*	0.34	0.18	-0.09	0.28	0.55*	0.48*	0.62**	0.55*	0.36	0.14	0.04
NDF24	0.63**	0.54**	0.67**	0.46	0.66**	0.67**	0.61**	0.75***	0.67**	0.49*	0.43	0.48*
ADF12	0.58*	0.33	0.12	-0.16	0.25	0.55*	0.64**	0.40	0.48*	0.66**	0.10	0.06
ADF24	0.46	0.49*	0.36	0.08	0.45	0.54*	0.67**	0.40	0.45	0.70***	0.33	0.30
ADL12	0.41	0.25	0.16	-0.03	0.22	0.42	0.44	0.49*	0.39	0.37	0.13	0.04
ADL24	0.36	0.11	-0.01	-0.09	0.06	0.32	0.39	0.31	0.28	0.37	0.09	0.07
FA12	0.35	-0.07	-0.11	-0.09	-0.09	0.22	0.22	0.18	0.21	0.22	-0.18	-0.21
FA24	0.33	0.13	0.16	0.10	0.15	0.27	0.31	0.32	0.24	0.28	0.01	-0.03
PCA12	0.28	-0.03	0.07	0.15	0.01	0.24	0.14	0.13	0.27	0.13	-0.10	-0.14
PCA24	0.26	0.04	0.12	0.14	0.08	0.28	0.31	0.31	0.25	0.28	-0.06	-0.11

* for P<0.05, ** for P<0.01, *** for P<0.001; Hemi = Hemicellulose, Cell = Cellulose, mean = mean particle size, median = median particle size

5.3.1.2 The Effects of FA and PCA, PCA/FA Ratio and total of FA+PCA

FA and PCA are both esterified and etherified to plant cell wall components. Rumen microorganisms can produce phenolic acid esterases to ultimately break down the ester bonds, while the ether linkages are difficult to cleave in the rumen anaerobic environment (McSweeney et al. 1994; Jung and Allen 1995). Therefore, the esterified FA and PCA of rumen degradability were investigated in the present study.

FA, PCA and FA+PCA were highly correlated to the content of NDF, ADF, ADL, hemicellulose and cellulose ($P<0.05$). This is due to their close relationship with cell wall components. FA and PCA are mainly (about 80%) contained in the cell walls of barley bran (hull, pericarp, testa and aleurone layer) (Nordkvist et al. 1984; Hernanz et al. 2001; Chakraverty et al. 2003). However, the relationship between barley hull content and PCA was not significant ($P>0.05$), but was significant for FA ($P<0.05$). This could be explained by the different bonding models between FA and PCA in plant cell walls. PCA is heavily esterified and etherified to lignin, and seldom linked to cell wall polysaccharides, while FA is esterified to both lignin and polysaccharides, etherified to lignin, and forms cross-linkages between polysaccharides and lignin, and among polysaccharides (Iiyama et al. 1990; Lam and Tiyama 2000; Sun et al. 2002). Therefore, PCA is more related to the total lignin content in barley seed instead of the hull content. As can be found in Table 5.1, the correlation between PCA and ADL ($P<0.01$) is relatively stronger than that between PCA and NDF, ADF, hemicellulose, cellulose ($P<0.05$).

Interestingly, FA and PCA were very strongly correlated to mean/median particle size obtained by coarse dry-rolling ($P<0.001$). Experiments on wheat showed that higher content of FA, PCA and cross-linkages between FA and cell wall polysaccharides improved wheat bran's mechanical properties of extensibility, elasticity and plasticity (Antoine et al. 2003; Greffeuille et al. 2007).

The present results show that FA had positive effects on rumen indigestible residues of DM at 12 and 24 h, NDF and ADF at 24 h ($P<0.01$), while PCA showed effects on rumen indigestible residues of DM and NDF at 24h ($P<0.05$), which means that FA and PCA in barley grain are associated with the digestibility of barley grain in

the rumen. Research has shown that rumen digestibility of plant cell walls are improved by releasing esterified FA and PCA from plant cell walls (Hartley 1983; Canale et al. 1990; Akin et al. 1991) and by reducing FA cross-linking in the plant (Jung and Phillips 2008). A computer model showed that in vitro rumen digestibility of hemicellulose was negatively correlated to the esterified FA and PCA, with FA having a greater negative influence than PCA (Jung et al. 1991). Jung et al. (1994) also observed a negative correlation between the content of esterified FA and the degradation of lucern cell walls. Casler and Jung (2006) observed a negative effect of esterified FA on 24h NDF in vitro digestibility in forages (Smooth bromegrass and Reed canarygrass). Rodrigues (2007) observed that FA and PCA content in hay showed negative effects on in situ digestibility of NDF and on the degradation rate of DM, but positive effects on effective digestibility of DM (EDDM). Their explanation was that FA and PCA content did not limit the extent of grass digestion, and the positive relationship between FA, PCA content and EDDM indicated that the deposition of FA and PCA and other cell wall nutrients were simultaneous. However, the nutrient deposition mechanisms in barley grain may be different from that in grasses, because the structural cell walls in barley grain are not evenly distributed as in grasses. In addition, FA imposed more of an impact on rumen digestion of DM, ADF and ADL at 12 and 24 h than PCA. Other authors have also observed more inhibitory effects of FA than PCA (Rodrigues et al. 2007). This probably results from the differences in bonding models.

The degradation of barley grain is a complex process, which is under the control of many factors. At 12 h of rumen incubation, more grain DM was retained than at 24 h; consequently, the effects of FA and PCA were more confounded by other factors (e.g. starch, protein). This explains why more influences were seen on 24 h residues rather than 12 h residues. In addition, FA and PCA seem to have almost no relationship ($P>0.05$) with rumen indigestible residues of ADL, FA and PCA at 12 and 24 h, which means that original FA and PCA content in barley grain did not affect the digestibility of ADL, FA and PCA in the rumen. Jung and Casler (1991) observed the different effects of lignin and esterified FA and PCA on the in vitro dry matter digestibility (IVDMD) of Smooth bromegrass leaf and stem and concluded that cross-linkages

among the cell wall components influenced cell wall digestibility to a greater degree than the concentration alone.

The ratio of PCA/FA did not exhibit noticeable effects on most items examined, with the exception to mean/median particle size. Based on the observations from grasses studies, Grabber et al. (2004) proposed that the ratio of PCA/FA indicates the degree of lignification of plant tissues. In our experiment, the total content of FA and PCA in barley grain was analyzed; nonetheless, the distribution of FA and PCA in the whole seed is actually not even, with barley bran dominating (about 80%) in overall content (Nordkvist et al. 1984; Hernanz et al. 2001). Even within the bran fractions, barley hull is far more heavily lignified than percicarp, testa and aleurone layer. Therefore, the ratio of PCA/FA probably is not a good indicator of lignification in barley grain as it is in forages. Accordingly, the ratio of PCA/FA did not show a high correlation with barley fiber content. As for FA+PCA, it showed the collective effects of FA and PCA.

Generally, FA and PCA were found to be highly correlated to barley fiber (NDF, ADF, ADL, hemicellulose and cellulose) ($P<0.05$). FA and PCA content in barley grain affected ($P<0.05$) mean/median barley particle size obtained after coarse dry-rolling. Both FA and PCA showed inhibitory effects on the digestibility of barley DM, NDF and/or ADF ($P<0.05$) in the rumen, with FA exhibiting more negative influences than PCA in general. Rumen digestibility of ADL, FA and PCA were not related to the original content of FA and PCA in barley grain. The ratio of PCA/FA was not related to most parameters. According to current information, barley varieties with less FA and PCA content would be a good candidate for feed barley.

5.3.1.3 The Effects of NDF, ADF, ADL, Hemicellulose and Cellulose

Fiber is notorious for its recalcitrant properties. It is not only resistant to rumen digestion, but also retards, and even inhibits the availability of other nutrients to animals. The negative effects of barley fiber have been studied extensively. Our results show that the fiber in the form of NDF, ADF, ADL, hemicellulose and cellulose were significantly positively related to rumen indigestible residue content of NDF and ADF

at either 12 or 24 h of rumen incubation ($P<0.05$). Numerical effects were found between barley fiber content and rumen indigestible residues of DM and ADL at 12 and 24 h. Profound differences would probably appear when the content of fiber is accumulated to a certain high degree, as is always seen in grasses and straws which contain more fiber (ADF, NDF, ADL) than barley grain. Rumen digestibilities of FA and PCA at 12 and 24 h were not significantly affected by the fiber. Fiber also showed some effects on particle size reduction after coarse dry-rolling. ADL and hemicellulose were positively correlated to mean particle size ($P<0.05$). Bowman et al. (2001) observed a positive relationship between ADF and particle size. Baumberger et al. (1998) stated that lignin could interact with starch and enhance the starch film's mechanical properties, while hemicellulose was reported to increase the extensibility of plant cell walls (Whitney et al. 1999). Barley bran cell walls are rich in fiber, which may, therefore, contribute to the particle size reduction during mechanical processing. However, barley hull and particle size were not significantly related. One possible explanation could be that fiber from the bran layers of pericarp, testa and aleurone play a more important role in determining the grain's mechanical resistance, as happens in wheat grain (Antoine et al. 2003).

In general, barley content of NDF, ADF, ADL, hemicellulose and cellulose were related to the rumen digestibility of NDF, ADF and ADL. Nutritionally, a barley variety with low fiber content would be an ideal feed grain with moderate content of fiber a benefit to maintain larger particle size during mechanical process.

5.3.1.4 The Effects of Mean/Median Grain Particle Size

A good feed barley variety should have high nutrient content, good nutrient availability, slow rate of rumen starch fermentation and maintain large particle size after mechanical processing. Although the degradation rate for processed barley grain with different particle size could not be directly compared, the in situ rumen digestion study at 12 and 24 h in the present study shows that larger mean/median barley particle size resulted in increased rumen indigestible residues of DM ($P<0.05$). This implies that larger particle size reduced DM digestibility. Researchers at the Dairy Research

and Technology Center in University of Alberta reported that barley particle sizes that were generated by grinding the barley through 2, 4 and 6 mm screens did not significantly affect effective dry matter digestibility (EDDM) and rumen degradation rate (K_d) of DM, but their results also showed a trend of decreasing EDDM with increasing grain particle size (Nikkhah et al. Undated). Further research is needed to disclose the relationship between particle size and barley digestibility in the rumen. No significant ($P>0.05$) effects were observed on the digestibility of NDF, ADF and ADL at 12 and 24 h. Boyles et al. (2000) stated that mechanical processing methods were employed to improve starch and protein digestibility of the grain; they further stated that the rolling method did not improve cellulose digestibility, a result also found in the present study. Mean/median particle size exerted negligible effects on FA and PCA digestion in the rumen.

In general, mean/median barley particle sizes obtained after coarse dry-rolling significantly reduced DM digestibility in the rumen at 12 and 24 h incubation, and showed no correlation effect with the digestibility of fiber, FA and PCA at 12 and 24 h rumen incubation.

5.3.2 Multi-regression Analysis between Hull, FA, PCA Content, Mean/median Particle Size and Rumen Indigestible Residues at 12 and 24 h of Rumen Incubations

The best models deduced from the stepwise multi-regression analysis are presented in Table 5.2. to illuminate the relationships between the in situ rumen indigestible residues of DM, NDF, ADF and ADL at 12 and 24 h and the original content of hull, FA, PCA, mean/median particle size.

Table 5.2. Multi-Regression analysis to find most important variables to predict rumen degradability using physiochemical characteristics: hull, FA, PCA, ratio, and mean/median particle size with tested multi-regression model as follows:
Model: Y (degradability) = Hull + FA+ PCA + PCA/FA ratio + Mean + Median

Predicted variables (Y)	Variable(s) selection [z] (Variables left in the model with P<0.05)	Prediction Equations Test model: Y= a + b1*x1 + b2*x2+ ….	Model R^2 value	RSD [y]	P value
DM12	Only FA left in the model	DM12 = 10.01 + 0.05 * FA	0.354	3.79	0.009
DM24	Only FA left in the model	DM24 = 10.89 + 0.04 * FA	0.439	2.84	0.003
NDF12	Only Hull left in the model	NDF12 = 27.17 + 3.38 * Hull	0.298	3.51	0.019
NDF24	Only Hull left in the model	NDF24 = 19.68 + 4.14 * Hull	0.392	3.49	0.005
ADF12	Only Hull left in the model	ADF12 = 54.71 + 3.50 * Hull	0.333	3.35	0.012
ADF24	Only FA left in the model	ADF24 = 64.12 + 0.04 * FA	0.236	3.90	0.041
ADL12	No variable met the 0.05 significant level for entry the model				
ADL24	No variable met the 0.05 significant level for entry the model				

[z] Model variable selection using multi-regression analysis with a stepwise option with variable selection: "slentry=0.05, slstay=0.05"
[y] RSD= Residue standard deviation. For other abbreviations, please see the abbreviation list in the text.

As described in the in situ rumen digestion experiments, FA showed a negative effect on DM digestibility in the rumen. The present regression analysis further shows that the original content of FA in barley grain accounted for 35.4% and 43.9% of the total variation in the rumen indigestible residues of DM at 12 and 24 h. However, this result contrasts to the observation of Rodrigues et al. (2007) on meadow hays. The discrepancy probably is originated from the different physical and chemical properties between grain and grasses. In barley grain, bran (possessing more than 80% of the total barley grain cell walls) is the protective layer for the inner vulnerable starchy endosperm and embryo. The presence of FA could enhance the resistance of the hull, pericarp, testa and aleurone layer to the digestion of rumen microorganisms and enzymes since FA is extensively cross-linked to cell wall polysaccharides and lignin (Jung and Deetz 1993; Grabber et al. 1998; Moore and Jung 2001).

Multi-regression analysis also shows barley hull was the most limiting factor for the in situ rumen digestion of NDF at 12 and 24 h with coefficients of determination (R^2) being 0.298 and 0.392, respectively ($P<0.05$). The hull is a large contributor to NDF content (about 45% of total NDF) in barley grain, so it is possible that higher hull content will lead to higher NDF residue in the rumen. The effects of FA and hull on DM and NDF residues increased when the incubation time proceeded from 12 to 24 h.

As for the in situ residues of ADF, hull content was the main source of variation at 12 h, but at 24 h, FA replaced hull and became the most restraining effect. The reason for this is unclear. Barley hull ADF represents 70% of total barley ADF content, so the hull is supposed to be a major effect on the ADF digestibility as is happened to NDF.

The ADL content in the hull occupies 50% of the total ADL in barley grain. Although barley hull was the dominating factor for determining rumen indigestible residues of ADL at 12 and 24 h, the influence of barley hull on ADL residues in the rumen was not significant ($P>0.05$). This is probably because the lignin content in the barley grain is very low averaging 1.9% and the digestibility of lignin is also small, even negligible (less than 10% of total lignin), which collectively leads to statistically insensitive results.

In general, barley hull and FA were the two most effective factors in determining the in situ rumen indigestible residues of barley DM, NDF and ADF, among the parameters of the original content of hull, FA, PCA and mean/median particle size. Accordingly, hull and FA content in barley grain should be considered when estimating rumen digestibility of DM, NDF and ADF in barley grain.

5.4 Conclusions and Implications

All the parameters from the physical and chemical analysis were collected and examined for correlation and regression models. These parameters included original content of barley hull, FA, PCA, NDF, ADF, ADL, hemicellulose, cellulose and mean/median particle size after coarse dry-rolling in 18 barley samples, rumen indigestible residues of DM, FA, PCA, NDF, ADF and ADL at 12 and 24 h of rumen incubation.

The correlation study showed that barley hull was highly correlated to NDF, ADF, ADL, hemicellulose and cellulose content ($R>0.78$, $P<0.001$) and significantly correlated to FA ($R=0.57$, $P<0.05$) but not to PCA ($R=0.42$, $P>0.05$). Barley hull was also significantly and positively related to rumen indigestible residues of NDF and ADF at either 12 or 24 h, but not to rumen residues of ADL, FA and PCA at 12 and 24 h. FA, PCA or FA+PCA were highly and positively correlated to barley NDF, ADF, ADL, hemicellulose, cellulose, mean/median particle size, and rumen indigestible residues of DM, NDF and ADF at either 12 or 24 h ($P<0.05$), with FA being more effective than PCA. The ratio of PCA/FA was not significantly correlated to most of the parameters. The original content of FA and PCA was not significantly correlated to the indigestible residues of ADL, FA and PCA at 12 and 24 h. NDF, ADF, ADL, hemicellulose and cellulose were highly interrelated ($P<0.05$) and were significantly and positively correlated to rumen indigestibe residues of NDF and ADF at 12 and 24 h, but not the residues of DM, ADL, FA, and PCA at 12 and 24 h of rumen incubation. Mean/median particle size of barley grain positively influenced the rumen indigestible residue content of DM, but not others. The correlation analysis results implied that reduction of barley hull, FA and PCA content could increase the digestibility of barley

grain in ruminants. Changing mean/median particle size of barley grain obtained after dry-rolling could contribute to the digestibility of DM in the rumen.

Multi-regression with model variable selection analysis revealed that FA was the factor most inhibiting to DM degradability of barley in the rumen, and was the most effective factor to predict DM digestibility, while hull was the most effective factor to determine NDF digestibility in the rumen. Both hull and FA affected ADF degradability in the rumen.

All the results indicate that breeding barley varieties with lower hull and FA content would result in higher digestibility, higher energy density and higher quality of feed barley.

6. GENERAL CONCLUSIONS

Barley is the principle energy grain for ruminants in western Canada. There are many hulled barley varieties grown in western Canada. Due to the extensive usage of barley grain in cattle diets, it is of economic importance to screen a good feed barley to maximize animal production. A variety with low content of hull, FA, PCA, fiber, and maintaining large particle size after mechanical processing would be the ideal feed barley for ruminants. The basic objective of this study was to compare the physical and chemical differences among the six barley varieties in relation to rumen nutrient availability and find a relatively ideal barley variety.

In the first study, chemical and physical profiles of barley samples were analyzed. This study showed that the variety had significant impacts ($P<0.05$) on the chemical and physical characteristics of barley, with CDC Helgason and CDC Dolly consistently showing relatively lower content of barley hull, FA, PCA and fiber than McLeod and CDC Cowboy, while mean/median particle size was not different among CDC Cowboy, CDC Helgason, McLeod and CDC Dolly. The results show that CDC Helgason and CDC Dolly would be promising as good feed barley varieties for ruminants.

The second study was to assess the differences in the in situ rumen digestibility of DM, NDF, ADF, ADL, FA and PCA at 12 and 24 h of rumen incubation. Barley variety again showed effects on rumen digestibility and the results revealed that CDC Dolly consistently showed relatively better rumen digestibility of DM, FA, PCA, NDF, ADF and ADL at 12 and 24 h, with McLeod the worst.The combinated results of the first and the second study showed that CDC Dolly was of superior quality and McLeod was of inferior quality as ruminant feed grain.

The third study was to compare the differences in the rumen degradation kinetics of DM, FA and PCA in CDC Dolly and McLeod. The rumen indigestible residue data were fitted into the first-order kinetics of degradation model. CDC Dolly showed lower

RUDM, RUFA, and RUPCA than McLeod. In general, CDC Dolly exhibited better digestibility of DM, FA and PCA than McLeod, which continues to indicate that CDC Dolly was of better quality as a feed barley grain for ruminants.

The last study examined the correlation and regression analysis between the data of the chemical and physical analysis and the in situ rumen digestibility at 12 and 24 h. Results show that the content of barley hull, FA, PCA, fiber and grain particle size all had negative effects on the rumen digestibility of barley grain, with FA having the most negative influence on barley grain's DM digestibility, and the hull being responsible for the inhibitory influence on the digestibility of NDF and ADF. FA affects barley grain's DM digestibility through its cross-linking with cell wall polysaccharides and lignin and works as a steric obstacle and shields the esterified polysaccharides from enzymatic hydrolysis in the rumen (Moore and Jung 2001).

The present studies show that CDC Dolly is the best candidate as a good feed barley grain for ruminants.

Future study could look at the quality of CDC Helgason which showed consistently low content of hull, fiber, FA, PCA and relatively large mean/median particle size. However, in the study on rumen in situ digestibility at 12 and 24 h, it did not perform well and failed to compete with CDC Dolly. If its low digestibility at 12 and 24 h resulted from the slow rate of digestion, CDC Helgason would also become a good candidate for feed barley.

Since FA and PCA showed negative effects on barley grain's digestibility, methods could be exploited to reduce their content in barley grain (mainly in the bran). This could be accomplished through barley breeding program or mechanical treatment of barley grain or feeding with enzymes and chemicals to improve nutrient availability.

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APPENDICES

Appendix 1. Comparison of Modified EBC Method and 50% H_2SO_4 Method (Whitmore 1961) in Determinantion of Barley Hull Content

A1.1 Modified EBC Method for Barley Hull Content Analysis

Four randomly selected barley samples were used for EBC method testing. They were AC Metcalfe (2003), RCSL 97 (2003), AC Metcalfe (2004) and CDC Trey (2004). Barley grain samples were screened according to EBC method (EBC 3.9 1998). Naked and broken seeds were removed by hand. 20 g of barley grain was boiled and digested for 3 min in the solution of 80 ml sodium hypochlorite (12%) and 20 ml sodium hydroxide (3.125 N). The dehulled samples were then dried and ground to determine barley hull content.

A1.2 50% H_2SO_4 Method (Whitmore 1961) for Barley Hull Content Analysis

The dehulling procedure of 50% H_2SO_4 (7664-93-9, 98%, VWR International, Pennsylvania, USA) (Whitmore 1961) was similar to the modified EBC method, except that 20 g samples were immersed in 100 ml 50% H_2SO_4 at room temperature for 3 h to remove the hull. The dehulled samples were also dried and ground to determine barley hull content.

A1.3 Comparison Between Modified EBC Method and 50% H_2SO_4 Method for Barley Hull Content Analysis

Barley grain contains a protective outer hull, which consists of lemma (attached to the dorsal side) and palea (attached to the ventral side) (Hough 1991; Dendy and Dobraszczyk 2001). The hull is thin compared to that in many other grains such as oat and rice, but it plays an important role by providing physical protection for the grain, supporting the growing acrospires, maintaining water balance, and even plays a role in photosynthesis when the hull is green (Olkku et al. 2005). In hull-less barley, the hull loosely sticks to the caryopsis and sheds easily during harvesting and threshing, while

in hulled barley, it is more tightly adhered to the pericarp, which is removable, but with difficulty (Evers et al. 1999). In barley, the hull is cemented to the pericarp and it is difficult to distinguish between the two by visual observation. This also makes mechanical and/or chemical dehulling difficult. So far, there is no accurate method for completely separating the hull from barley grain without compromising the pericarp or endosperm. Most of the existing methods such as the EBC method (EBC 1998) are suitable for comparison analysis. However, the high concentration of sodium hypochlorite solution (20% NaClO) required in the EBC procedure was not available for our experiment. Therefore, a relatively low concentration (12% NaClO) was used and the reacting time was adjusted from 80 sec to 3 min. The modified EBC method was compared with a method using 50% H_2SO_4 (Whitmore 1961).

Results from the modified EBC method and the 50% H_2SO_4 method are presented in Table A1.1. The hull content was slightly higher with the modified EBC method than with 50% H_2SO_4. However, both methods gave a similar trend in hull content and were highly correlated (R = 0.91). These results indicate that the modified EBC method is an acceptable method to compare differences in barley hull content among various barley varieties. The 50% H_2SO_4 method was difficult and dangerous to handle and quiet tedious, therefore, the modified EBC method was employed for analyzing barley hull content in the present study.

Table A1. Comparison of two methods for determination of barley hull content

Barley sample	Hull content (%DM)	
	Modified EBC method	50% H_2SO_4 method
AC Metcalfe, 2003	10.8 ± 0.1	9.2 ± 0.3
RCSL 97, 2003	10.3 ± 0.2	9.1 ± 0.2
CDC Trey, 2004	10.1 ± 0.1	8.2 ± 0.3
AC Metcalfe, 2004	9.1 ± 0.03	7.8 ± 0.1
Correlation coefficient (R)	0.91	

Appendix 2. Pretreatment before Alkaline Hydrolysis for Barley Grain

Barley grain contains a high content of starch which produces problems by swelling and gelation during alkaline hydrolysis and extraction and even during HPLC analysis. Researchers have introduced large quantities of barley grain (400-1600 mg) and washed the starch enriched grain powders with large volumes of ethanol and hexane (100 ml/g) (Nordkvist et al. 1984; Andreasen et al. 2000; Holtekjolen et al. 2006) to reduce the swelling and gelation problems. The washing step could eliminate some of the starch problems, but could still not work well in the present study. During saponification, the treated samples would swell to form a thick gelation which would complicate the hydrolysis and extraction. Therefore, we developed a new method to overcome the problem. We had tried several tests to optimize the reaction conditions, including incubation in hot water with heating, incubation in α-Amylase solution with cool water and hydrolyzing for different time (20 min, 30 min or 1 h), and incubation in α-Amylase solution in 90 ^{o}C for 20 min, 30 min or 1 h. After comparison, we found the best way to reduce swelling and gelating problem was to mix the ground barley grain (50 mg) with 0.75 ml 1% (w/v) α-Amylase dissolved in 20mM KH_2PO_4 buffer (pH 6.9), and incubated in 90 ^{o}C water for 1 h.

Appendix 3. HPLC chromatogram of PCA and FA in barley grain

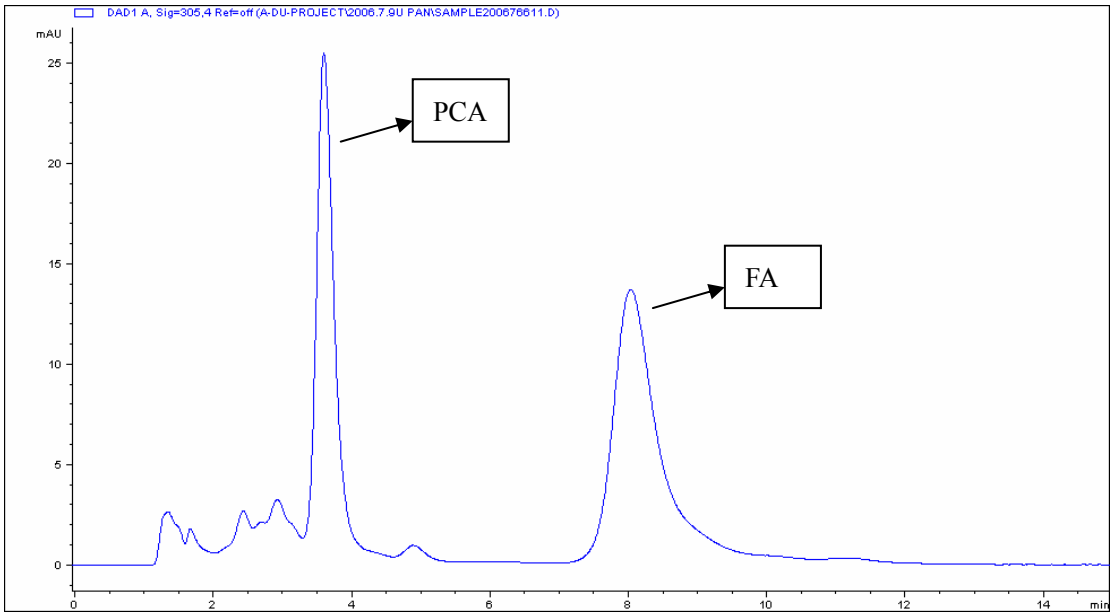

Figure A1. HPLC chromatogram at 305 nm of PCA and FA in barley grain extracted after alkaline hydrolysis

Appendix 4. Selection of the Best Model for Determining Mean/Median Particle Size of Coarsely Dry-rolled Barley Grain

A4.1 Mean/Median Particle Size Calculation with Three Equations

After sieving, the fractions remaining on each screen were weighed, and particle size distribution was expressed in percentage cumulative weight oversize by adding up the weight on each sieve and those from all larger screens (ANSI 2003). The mean/median particle size values were estimated by fitting these data into two exponential models: Fisher's equation (A1) (Fisher et al. 1988) and Pond's equation (A2) (Pond et al. 1984) with/without 0 mm = 100%. The particles passing the sieve 0.58 mm were included in Pond's equation with 0 mm = 100%, and discarded in Pond's equation without 0 mm = 100% in the calculation. Data were computed using the NLIN procedure of the Statistical Analytical System (SAS) (SAS Institute, Inc. 2002). 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 oversize.

Pond's Equation

$$R = 100\, e^{-k(s-w)} \qquad \text{(A1)}$$

mean particle size = 1/k+w

median particle size = 0.693/k+w.

Fisher's Equation

$$R = 100e^{-(s^{a}-b\times s)} \qquad \text{(A2)}$$

Where:

R = percentage cumulative weight oversize;

s = sieve opening size (mm);

w = the smallest predictable particle size;

k = the decay constant of the exponential curve describes the proportionality constant between the percent of particles passed to the next sieve and the percent remained.

a and b = mathematically estimated parameters.

Although the particle size of coarsely dry-rolled barley samples was not logarithmic-normally distributed, geometric mean diameter (GM) (A3) (ANSI 2003) was still calculated for the sake of equation comparison. The average particle size of materials retained on each sieve was fitted into the log-normal distribution curve (ANSI 2003) and the geometric mean diameter was calculated accordingly.

Equation for Predicting Geometric Mean Diameter (GM) of Log-normal Distribution

$$d_{gw} = \log^{-1}\left[\frac{\sum_{i=1}^{n} (W_i \log \overline{d_i})}{\sum_{i=1}^{n} W_i}\right] \qquad \text{(A3)}$$

where:

d_{gw} = geometric mean or median particle size of the whole materials, mm

d_i = nominal sieve opening of the ith screen, mm

W_i = the weight of particles retained on ith sieve, g

A4.2 Selection of the Best Model

Particle size distribution data (percentage cumulative weight oversize) was analyzed with three equations using the Gauss-Newton nonlinear iterative method. The statistical parameters used for equation comparison and selection of the best model were residue sum of squares (RSS) and coefficient of determination (R^2). Fisher et al (1988) only adopted RSS for their comparative study, while Pasikatan et al. (1999) used four indices to test eight distribution models for ground wheat. The best equation suitable for analyzing coarsely dry-rolled barley samples should give the smallest RSS value, with the largest R^2 value. In Table A2, the parameters of RSS and R^2, as well as the mean/median particle size predicted from Fisher's equation, Pond's equation (with and without 0 mm = 100%) and geometric mean diameter (GM) calculation equation were compared.

Table A2. Comparison of the parameters of RSS and R^2, as well as mean/median particle size predicted from Fisher's equation and Pond's equation (with/without 0 mm = 100%) for coarsely dry-rolled barley grain

Equations	Mean (mm)	Median (mm)	RSS	R^2
Fisher's	3.55 [a]	3.09 [a]	363.21 [a]	0.9917 [b]
Pond's (with 0 mm=100%)	3.35 [b]	2.91 [b]	68.66 [b]	0.9987 [a]
Pond's (without 0 mm=100%)	3.35 [b]	2.91 [b]	68.62 [b]	0.9984 [a]
Geometric Mean (GM)	2.75 [c]			
SEM	0.053	0.045	8.049	0.00049
P value		<0.05		

[a, b, c, d] Different superscripts of in the same column are significantly different ($P < 0.05$).

RSS from Pond's equation with and without 0 mm = 100% were 68.66 and 68.62, respectively, not significantly different ($P>0.05$). However, both values were significantly smaller ($P<0.05$) than RSS from Fisher's equation, indicating that Pond's equation was more suitable to model particle size data from coarsely dry-rolled barley grain than Fisher's equation. R^2 values ($P<0.001$) continued to support the point that Pond's equation (R^2: 0.9987, 0.9984) was better than Fisher's equation ($R^2 = 0.9917$). Within Pond's methods, no difference was found for RSS and R^2, but better potency was observed in Pond's equation with 0 mm = 100%, which included the observation of particles passing through the smallest sieve (0.58 mm). R^2 for Pond's equation with 0 mm = 100% was 0.9987. The estimation of mean/median particle sizes from Fisher's equation was larger than those from Pond's and GM calculation equation, with GM giving the smallest particle size.

Since the two parameters (RSS, R^2) denoted that Pond's equation was the best choice, in the following calculation and comparison, the Pond's equation with 0 mm = 100% was applied for computing mean/median particle size of coarsely dry-rolled barley samples expressed as percent cumulative weight oversize.

Appendix 5. Animal Diets during In Situ Experiments

Table A3. Standard dairy concentrate[1,2]

Integrant	%DM
Barley	56
Wheat	5
Oats	5
Dairy supplement pellets	33
Molasses	1

[1]Grain was dry rolled and mixed with supplement pellets.
[2]Proximate composition: 18.5% crude protein, 0.7% calcium, 0.8% phosphorus (DM basis).

Table A4. Fresh cow concentrate[1,2]

Integrant	%DM
Barley	51.05
Oats	5.0
Canola meal	11.6
Soybean meal	10.0
Wheat distillers dried grain	9.0
Corn gluten meal	3.0
Molasses	2.5
Golden flakes[3]	2.5
Canola oil	0.5
Mineral-vitamin mix[4]	3.0
Niacin-magnesium mix[5]	0.3
Cobalt-iodized salt	0.6
Sodium bicarbonate	0.6
Ground limestone	0.3
Dynamate[6]	0.05

[1]0.48 cm (3/16") pellets

[2]Proximate composition: 22% crude protein, 0.9% calcium, 0.85% phosphorus (DM basis).

[3]Dried fat supplement (Malaysian palm oil) distributed in Western Canada by Prairie Micro-Tech Inc., Regina, Saskatchewan.

[4]Formulated to provide 45 mg manganese, 63 mg zinc, 17 mg copper, 0.5mg selenium, 11000 I.U. vitamin A, 1800 I.U. Vitamin E per kg of dairy concentrate. The mix also contributes 0.14% magnesium, 0.48% calcium, 0.26% phosphorus, 0.23% sodium and 0.38% chloride to the total dairy concentrate. Prepared by Federated Co. Ltd., Saskatoon, Saskatchewan.

[5]Formulated to provide 1 g of niacin and 0.3 g of magnesium per kg of fresh cow concentrate.

[6]Contains 22% sulphur, 18% potassium, 11% magnesium (International Minerals and Chemical Corp., Mundelein, ILL).

Appendix 6. Nylon Bags and Incubation Time Arrangements for In Situ Experiments

Table A5. Nylon bags arrangement for 0 - 72 h of rumen incubation

Incubation period (h)	Bags/ period	Treat-ments	Total bags	% of total #bags	Assumed #bags /mesh bag /animal	Assumed #bags Incubated /animal	Real #bags incubated /animal
0 h[1]	2	6	-	-	-	-	-
2 h	2	6	12	0.08	30	2.5	3
4 h	2	6	12	0.08	30	2.5	3
8 h	2	6	12	0.08	30	2.5	3
12 h	3	6	18	0.13	30	3.7	4
24 h	4	6	24	0.17	30	5.0	5
48 h	5	6	30	0.21	30	6.4	6
72 h	6	6	36	0.25	30	7.5	8
Total	24		144	1		30	32

[1] Bags were not counted for rumen incubation bags at 0 h.

Table A6. Detailed ‘gradual in/all out’ rumen incubation schedule

Day	Incubation time	Incubation period (h)	# bags incubated in cow 1	# bags incubated in cow 2
day 1	21:00	72	8	8
day 2	21:00	48	6	6
day 3	21:00	24	5	5
day 4	9:00	12	4	4
	13:00	8	3	3
	17:00	4	3	3
	19:00	2	3	3
	21:00		32[1]	32[1]

[1] Total bags incubated in each animal in a consecutive incubation.