UTILIZATION OF NEAR INFRARED REFLECTANCE SPECTROSCOPY FOR THE EVALUATION AND CHARACTERIZATION OF BARLEY IN WESTERN CANADA

By

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UTILIZATION OF NEAR INFRARED

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"One day, in retrospect, the years of struggle will strike you as the most beautiful."

-Sigmund Freud

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The first study evaluated near infrared reflectance spectroscopy (NIRS) for the determination of barley silage DM on as-is samples using either a commodity specific or broad based equation. A second study was conducted to evaluate a commercial NIRS prediction equation for barley grain, examining the nutrients of DM, CP and starch. Barley samples were selected as HIGH, MID or LOW for each nutrient group and the equation was tested using all samples or only the selected samples. Finally, a third study was conducted to evaluate NIRS as a selection tool for barley grain and the relationship between nutrient composition and digestion kinetics.

The results of the first study indicated that NIRS accurately predicts the DM of as-is barley silage ($R^2 = 0.98$, $p < 0.05$) using either a commodity specific or broad based equation. The second experiment indicates NIRS can accurately predict the DM and CP ($R^2 > 0.50$, $p < 0.05$), however did not accurately predict starch content of barley grain ($R^2 \leq 0.21$, $p < 0.05$). The third experiment indicates that NIRS holds promise as a selection tool for barley grain quality and a relationship exists being nutrient content and digestion kinetics. There was a significant relationship between the DM content of the sample and the rate of fermentation with LOW DM samples having a faster rate of fermentation than the MID and HIGH ($p < 0.05$). Gas production of LOW DM samples was greater between 8 and 23 hours of incubation compared to the HIGH and MID ($p < 0.05$). The MID CP had greater gas production (mL/g of substrate DM, $p \leq 0.05$) than the HIGH range, with LOW being intermediate. Correlations between the NIRS and lab determined chemical constituents and the gas production kinetics were examined. DM was negatively correlated ($p \leq 0.05$) with k and lag when measured with NIRS or in a lab, and CP was significantly ($p \leq 0.05$) negatively correlated with cumulative gas production (NIRS r = -0.31, lab r = -0.31), k (NIRS r = 0.48, lab r = 0.47), and lag (NIRS r = 0.30, lab r = 0.37).

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CHAPTER I

INTRODUCTION

Barley is a widely used feedstuff, both as a grain and as silage, for feedlot production in western Canada due to its availability and nutrient composition. Barley grain can vary greatly in quality and nutrient composition posing a challenge to feedlot operators in efficiently utilizing it in production. Two factors affecting barley grain quality are environmental conditions during growing and genetic variations of different cultivars (Anderson et al., 1984; Berdahl et al., 1976). The ability to assess the nutrient composition of barley grain and silage rapidly can greatly impact the utilization of it as a feedstuff on feedlot operations.

Near infrared reflectance spectroscopy (NIRS) has been developed as a method to analyze several commonly used feedstuffs in animal production. Stubbs et al. (2010) outlined the advantages of NIRS analysis to be a low cost analysis providing rapid results and is a non-destructive method compared to traditional laboratory analysis. In addition, Stubbs et al. (2010) describes NIRS technology as one which allows for a larger range of samples to be tested and multiple properties can be tested at one time. NIRS uses reference values of known samples that are similar to the test population to predict nutrient composition of new samples. However, the ability of NIRS to accurately predict

new sample composition is driven by how similar the samples being analyzed are to the population that was used to build the prediction equation (Aufrere et al., 1996).

The objectives of these experiments were to utilize NIRS technology to evaluate and characterize both barley grain and barley silage in feedlots in western Canada. First, NIRS technology was used to predict the DM content of barley silage using "as-is" equations where no additional preparation of the sample was required. Previous research indicates that NIRS can accurately predict the nutrient content of forages (Coelho et al., 1988, Coleman and Murray, 1993, Mathison et al., 1999, Brown et al., 1990); however most protocols would indicate samples must be dried and ground prior to NIRS analysis. A need for reduced sample processing prior to scanning was observed, and we felt confident that NIRS technology could be more widely used if as is calibrations were available for barley silage. Secondly, commercially available prediction equations for barley grain quality were examined. It was understood that differences in sample populations from the original equation population can decrease NIRS prediction accuracy; therefore a validation of commercially available technology for the barley population entering feedlots in western Canada was required.

In addition to nutrient composition of the grain, the fermentability of grain by ruminants is what drives its value as a feed (Lanzas et al., 2007). Given the variation in chemical composition of barley grain entering feedlots in western Canada it was expected that differences may also exist in fermentability. Therefore we examined the use of NIRS as a tool for selecting different populations of barley grain based on the nutrient composition, and the effect that NIRS grouping would have on in vitro fermentation measurements of IVDMD, rate of fermentation, maximum gas production and lag time.

We hypothesized that we could achieve high prediction accuracy of barley silage DM content when building an as is NIRS calibration, and that commercially available NIRS technology would provide accurate predictions for DM, CP, and starch of barley grain entering western Canadian feedlots. We also hypothesized that barley grain entering feedlots in western Canada differed in its fermentability and gas production measurements and that there would be a relationship between nutrient composition and these digestion kinetics. With the existence of this relationship the use of NIRS technology as a selection tool for barley grain entering feedlots in western Canadian feedlots could be expanded.

CHAPTER II

REVIEW OF LITERATURE

Barley Grain Use in Feedlot Production

Feedlots throughout Canada and the northern United States are able to utilize barley grain as one of the main ingredients for finishing beef cattle rations. This commodity can be utilized in these areas due to its availability as well as its high nutrient value. When compared to corn as a feedstuff for beef cattle, barley grain has proved to provide similar growth rates (Boss and Bowman, 1996; Boles et al., 2004) with no negative effects on hot carcass weight, fat thickness, LM area, percentage of internal fat, or USDA yield grade (Boles et al., 2004). Owens et al., (1997) demonstrated that when averaged across processing methods barley grain had similar results to corn in the animal performance variables ADG, DMI, and F:G. Additionally, animals on a barley ration proved to have lower feed costs associated with the ration, and therefore a lower cost per unit of gain compared with a corn based diet (Boss and Bowman, 1996). The feed cost per gain is dependent on feed prices; however, as barley is marketed for less than corn and produces a higher efficiency, it makes it an economically viable feedstuff for feedlot production (Boss and Bowman, 1996).

McLelland (1982) reported that approximately 90% of barley produced is utilized as animal feed. In contrast to this, much research and seeding has taken place related to malting barley varieties. The economic factors making malting varieties of greater value has increased production of these varieties so that almost 80% of the total acreage sown to barley in Alberta are varieties that would be eligible for malting (McLelland, 1982). This variety selection has increased the value of this commodity, making it higher than that of the feed quality barley varieties, despite the larger proportion of barley varieties being used for animal feed.

Digestible Energy

The Nutrient Requirements of Beef Cattle (NRC, 1996) describes digestible energy (DE) as the energy of the food minus the energy lost in the feces. The NRC (1996) also describes DE as an effective measure of diet digestibility and can be more easily measured than digestibility. Bhatty et al. (1974) described DE as being the key ingredient of feed grains and therefore, the major objective in selection of cultivars of barley should be related to the DE content. The DE measurement is a combination of the chemical components of a feedstuff; however, it does encounter the problem of overestimating or underestimating the value of feeds based on the measurement not considering the energy losses of digestion and metabolism (NRC, 1996). Nonetheless, based on its ease of measurement, it can still be considered a viable measure of the value of feedstuffs and is often used for research purposes. In 1972, the Canada Grains Council (as referenced by Anderson et al., 1984) described DE to be the single most important factor of the nutritional quality of feed grains in Canada. Grain as a source of DE is based on the components of grain: starch, protein, fat, NDF and ADF (Campbell et al., 1995).

As with many of the nutritive parameters of barley, the DE content can vary with cultivar or environment (Anderson et al., 1984; Berdahl et al., 1976). Several researchers have investigated the variability of DE content of barley and have measured significant ranges. Fairbain et al. (1999) evaluated 20 different barley samples and found, on average barley contained 2,934 kcal/kg DE, with a range of 2,686 kcal/kg to 3,133 kcal/kg DE. This range demonstrated a variation of 15.2% or 447 kcal/kg in the DE content of barley. Bhatty et al. (1975) also evaluated barley DE and found that there was a difference between the DE of hull-less cultivars and hulled cultivars. They reported that the DE of the hull-less cultivars was on average 3,918 kcal/kg and hulled varieties averaged 3,627 kcal/kg. From these data, we can conclude that there is significant variation in the cultivars of barley and their DE content, and with understanding the factors of different cultivars we could select barley to provide higher DE. Differences in rumen degradability exist between cultivars and types of barley and this could allow the potential for manipulation of barley to increase in digestibility (Lehman et al., 1995). Anderson et al. (1984) indicated in their research that to truly understand the variability of DE content, data must be collected and accounted for over a number of years before recommendations on nutritional parameters of cultivars can be made. It can be assumed from this observation that this would also allow for the accounting of environmental factors to be understood as well.

Starch

The starch content of barley is a significant factor in the energy content of the grain. Starch is the largest constituent of barley and accounts for 51%-64% of its make-

up (Holtekjolen et al., 2006). Kotarski et al. (1992) described how the structure of the grain kernel reflects their biological function, and that the starch content of barley comes primarily from the germ and endosperm, which is encased by the pericarp. Additionally, the starch contained in the endosperm is the most susceptible to digestion or processing. The starch content of barley is composed mainly of two polysaccharides (amylopectin and amylose) and the proportions of those are affected by species and variety of barley. The utilization of starch is dependent not only on the plant species but also the organism that is digesting the starch (Kotarski et al., 1992). The rate of fermentation of barley grain starch is higher in comparison to corn. Barley has a digestibility of 80% to 90% in the rumen, whereas corn and sorghum range from 55% to 70% (Nocek and Tamminga, 1991).

Huntington (1997) previously conducted an extensive review of literature and found that the rate of starch digestion and extent of such in the rumen is determined by different factors. These include dietary starch, diet composition, feed consumption, grain processing or alterations, and the adaptation of the rumen micro flora. However; there is no strong relationship observed between the actual starch intake and ruminal digestibility (Huntington, 1997). Several approaches have been adapted to control the starch digestion, in an attempt to mitigate the adverse effects seen with feeding high grain diets, including altering consumption, grain processing and feed additives (Huntington, 1997).

As the starch content of barley is a major energy constituent of the grain, various studies have been conducted trying to quantify the starch content of different cultivars of barley. Different studies have reported various ranges of starch content: 45%-56%, 56.80%-59.36%, and 48.3%-62.5% (Bhatty et al., 1974; Campbell et al., 1995, Khorasani

et al., 2000, respectively). From the results of this research, there is significant variability of starch content in barley. The starch content of barley can affect the animal performance (Boss and Bowman, 1996); as starch content increases; feed efficiency is improved (Engstrom et al., 1991).

Kong et al. (1995) looked at the variability of barley across regions of Canada to see if the differences in barley starch content varied by environment and year. From their research, Kong et al. were able to conclude that environment did have significant effects on starch content of barley, and that hull-less cultivars contained more starch than hulled barley. With the research conducted indicating starch effects on animal performance, and the understanding of the variability in starch content of barley, further research is needed to link these effects and understand the value of starch content in barley as a feedstuff in feedlot production.

Protein

The protein content of barley has been a driving factor in the malting industries and has therefore created pressure within the crop industry to select cultivars with specific low protein contents. Bole et al. (1980) explained how cultivars with protein contents <13.5% DM basis are those selected for malting. However, as stated with the highest proportion of barley being used in the animal feeding industry these criteria are not necessarily reflective of the needs for high feeding value in barley.

Research has been conducted to understand and evaluate the protein content of different cultivars of barley. Boila et al. (1995) saw no statistical differences in protein content of three barley cultivars, and exhibited the range of protein content to be 13.08%

to 13.33%. These results however contradict those found by Campbell et al. (1995) who did see statistical differences in six different barley cultivars. Campbell et al. (1995) showed a higher range of protein content of barley: 12.71% to 14.19%. The differences in these data can be attributed to different cultivars examined, as well as the increased number of samples evaluated by Campbell et al. (1995).

To further investigate the protein content of barley grain the specific amino acid composition can be reviewed. As the concentration of protein increases, the percentage of amino acid increases linearly (Boila et al., 1995). McBeath et al. (1960) investigated both the protein and amino acid composition of barley and the effects of feeding it to rats. Barley varieties differed in the amino acid proportions and protein content which can play a large role in animal production agriculture. One challenge in evaluating animal performance related to protein content and amino acid profiles of barley is that the ability to predict specific amino acid content based on protein content is variable with the R^2 values of amino acids predicted from protein content ranged from 0.29 to 0.85 (Boila et al., 1995). This variability in amino acid concentration is heightened by the fact that the protein content of barley is greatly affected by climate and environment (McBeath et al., 1960).

Bushel Weight

Barley is purchased by feedlots based on the bushel weight measurement of the grain. Bushel weight is commonly referred to as test weight or test volume, throughout the literature. This measure of density reflects the sum of the weights of each of the chemical components of the grain as well as the measure of space between kernels

(Campbell et al., 1995). The environment effects during a growing season can often result in barley that is of lower quality for malting, and lighter in bushel weight (Hanke and Jordan, 1963). Work has been done to indicate the nutritive parameters of barley and their link to the bushel weight of the crop. Campbell et al. (1995) indicated that test weight and protein plus starch content of barley had a correlation coefficient of 0.41 ($P < 0.05$) showing a positive relationship between those parameters. The starch and protein content of barley has a relationship to the bushel weight of barley as observed by Mathison et al. (1991). They had also separated barley into different bushel weight categories (43, 58 or 59, and 64 kg hL^{-1}) and found that the light weight barley had a starch content 9% less ($P < 0.05$) than that of the heavier two groups. A lower crude protein level was also seen for the light weight barley as compared to the heavier weight barley.

Hanke and Jordan (1963) segregated barley into three different bushel weight classes and fed it to lambs. Their research concluded that animals fed heavy weight barley ate more and gained significantly more weight than those fed light weight barley. Their findings demonstrate a linear relationship to bushel weight and live weight gains in animals. Mathison et al. (1991) however found that although the starch content of their light bushel weight was lower than that of the heavier barley, no statistical difference in ADG or DMI was observed. The differences could be seen in an increase in the DM:gain ratio (Mathison et al., 1991). These results agreed with those found by Grimson et al. (1987) who indicated DM:gain ratios of 5.80, 5.32 and 5.26 when feeding barley of bushel weights 48, 56 and 67 kg hL^{-1}. With results such as this, both research groups (Mathison et al., 1991; Grimson et al., 1987) concluded that a discounting mechanism in

the value of light weight grain would be applicable to compensate for the animal performance effects. Similar effects can be seen in other production sectors, with Hatfield et al. (1997) seeing the same test weight to performance relationships in lambs. The lambs fed heavier bushel weight barley had a higher gain:feed ratio (or a lower DM:gain ratio) (Hatfield et al., 1997). These results confirm the bushel weight effects throughout the animal production industry.

Digestibility and Digestion kinetics

Digestibility studies are important in understanding nutrition and feeding behavior; however these can be time consuming, expensive, and require considerable animal resources to perform (Mould et al., 2005). In vitro methods have been developed to provide rapid measures which require less substrate than traditional in situ procedures (Mould et al., 2005). Although in vitro techniques can add additional variability above the traditional methods, it does allow for the ability to control the environment the procedure is run in, which has led to greater understanding of rumen micro biome and function (Mould et al., 2005).

Digestibility between grain types as well as within types differs based on factors such as physical characteristics and the chemical composition, such as the protein matrix (Stevnebo et al., 2006). Saba et al. (1964) expressed that the in vitro dry matter digestibility of barley grain was 84.3%, and total digestion of nutrients to be 84.9%, giving it a higher digestibility coefficient than milo grain. Cleary et al. (2011) suggested that the largest portion of barley grain digestion is the starch digested in the rumen, producing VFA's and energy. Fife et al. (2008) reported a wide range in the digestibility

of barley grain with in vitro total digestibility ranging from 66.7 to 85.1 % and an average of 76.6% which is lower than that reported by Cleary et al. (2011). However the results of Fife et al. (2008) are similar to the reported 77.9 % digestibility coefficient of Fairbairn et al. (1999). Fife et al. (2008) and Fairbairn et al. (1999) were able to examine a large number of barley grain samples and therefore a greater range in in vitro digestibility would be expected based on the variation in the grain samples. Fife et al. (2008) concluded from their work that the chemical components of starch and NDF were related to in vitro digestibility of the grain in a laboratory setting, but that the variables measured in a laboratory did not predict the digestibility of the grain in the feedlot steers. Beauchemin et al. (2001) examined the effects of processing on digestion and found that DM digestibility in the total tract was not affected by increased processing; however, an increase in starch digestibility was due to processing, although the increase was of small magnitude. Increasing digestibility of a grain can be favorable as it increases ruminal fermentation and greater microbial protein synthesis (Feng et al., 1995); however, greater digestibility can increase the risk of acidosis (Beauchemin et al., 2001).

Digestion kinetics of microbial fermentation can be measured using an in vitro gas production technique which is based on the concept that gas is produced from the mixture of ruminal contents in relation to the amount of substrate fermented (Lopez et al., 2007). The objective of all in vitro systems is to mimic the environment of the gastro-intestinal tract and producing accurate gas production kinetics (Mould et al., 2005). Gas production techniques can be combined with degradability estimates and allow for measures of proportion of feed fermented as compared to that which is used for microbial growth (Rymer et al., 2005). In the use of gas production technology it is important to

utilize the appropriate inoculum for the samples being tested. Trei et al. (1970) found that when measuring gas production of processed grains the total gas production increased when samples were analyzed using inoculum from a grain fed steer compared to a hay fed steer. The inoculum with already high levels of amylase activity will show the effects of processing grains more clearly than roughage based diets (Trei et al., 1970). In addition, variations in gas production can be greatly affected by the ruminal fluid sample time, days, and animals; therefore creating additional sources of error in the technique that need to be managed (Trei et al., 1970).

Substantial research has been conducted regarding gas production techniques and cellulose digestion in roughages. Xiong et al. (1990) and Trei et al. (1970) both examined the use of gas production in grains and described the effects of processing on grain sources. Xiong et al. (1990) observed that the use of gas production techniques provides a quantitative approach to measuring starch availability, which is comparable to a measurement such as glucose release. These researches also concluded that gas production techniques can be a useful method in measuring grain processing effects on ruminal starch availability as well as provide an estimate of protein degradation (Xiong et al., 1990). Trei et al. (1970) found that correlations between gas production and VFA production and starch digestion were high; gas production can be used as a tool to predict these measurements. Trei et al. (1970) suggested that the gas production technique offers a rapid analysis of rate of digestion and can provide use in understanding the relative feeding value of processed grain.

Variation

The two factors that are known to cause the greatest effects on the nutritional constituents of barley are genetic and environmental variations (Anderson et al., 1984; Berdahl et al. 1976). Berdahl et al. (1976) described the greater of these two factors to be environmental conditions. The environmental conditions that impact this level of variance are soil moisture, soil temperature, solar radiation, air temperature, and precipitation (Khorasani, 2000).

Some variation of barley can be seen in the descriptors used in identification, such as vitreous, flinty, waxy, nonwaxy and opaque. These descriptors come from the variation seen in the endosperm layers of the grain (Huntington, 1997). With this increased variation in the content of barley we can see a reduction in the ability to accurately formulate rations and to predict animal performance (Fairbain et al., 1999). One important observation in understanding the variability of barley is that the variation within a variety is often greater for more nutritive measures than the variation among varieties (Fairbain et al., 1999). This conclusion indicates the even greater importance of understanding barley variability for animal agriculture.

From the literature presented, we can see that the value of feeding barley to beef cattle lays in the nutritive parameters of the grain. In maximizing the utilization of barley for feedlot production, we must understand both the nutrient components as well as the variability of those constituents. Further research must be conducted to understand the variability, seasonality and performance effects of feeding barley as well as combining these effects with the individual animal performance effects seen in beef cattle.

Near Infrared Reflectance Spectroscopy

NIRS has been developed as a method to predict the chemical composition and nutritional parameters of various commodities, it is most widely used in the agriculture sector in evaluating animal feeds (Foley et al., 1998). Accurate predictions of various animal feeds have been produced for nitrogen (protein), moisture, fiber, starch (Foley et al., 1998). All plant and animal tissue is made up of bonds between the atoms, and with NIRS these bonds are illuminated. This illumination causes bonds to stretch and bind; this will cause a bond specific wave motion at a specific frequency (Foley et al., 1998). NIRS is based on the concept that the C-H, O-H and N-H bonds absorb the radiation at specific frequencies and therefore the chemical makeup of tissues determines the amount of light absorbed and the wavelengths (Foley et al., 1998).

Stubbs et al. (2010) outlined the advantages of NIRS analysis to be a low cost analysis is provided with rapid results and a non-destructive method. In addition, Stubbs et al. (2010) describes NIRS technology as one which allows for a larger range of samples to be tested and multiple properties can be tested at one time. NIRS however is an empirical model which relies on calibration to a chemical analysis in order for the spectra to be "read" (Richardson et al, 2003). This means that a base or reference value of chemical analysis must be completed on a variety of samples prior to calibration development; at times up to hundreds must be analyzed to achieve acceptable accuracy in the predictions (Shenk et al., 1993). NIRS prediction accuracy is driven by how similar the samples being analyzed are to the population that was used to build the equation (Aufrere et al., 1996).

Foley et al. (1998) describes calibrations being built using a wide range of samples to ensure the range in the samples selected for analysis are similar to those in the equation, and that a regression equation is developed using the spectral absorbances and the subsequent lab analysis. Most often the calibrations are built using a dried and ground sample as the moisture content in samples can provide prediction inaccuracies (Abrams et al., 1988). Foley et al. (1998) explains that wet samples are inhomogeneous and the water interference can be a problem when developing calibrations for high moisture products. In addition, NIRS machines are often housed in laboratory conditions, making the sample preparation of drying and grinding a more feasible method (Gillon et al., 1999).

In an effort to reduce the costs associated with developing calibrations and to reduce the required reference laboratory analysis of samples, Shenk et al. (1993) examined the use of global NIRS equations. They explained that prior to this; local calibrations were built for each individual product to be analyzed using NIRS technology. Shenk et al. (1993) had the objective to create global equations from spectrally diverse samples that could offer accurate predictions for various products, allowing for a reduction in reference analysis. It was observed that you could eliminate laboratory work on spectrally similar samples, and therefore, eliminate duplicate laboratory analysis, so a focus could be put on new unanalyzed samples (Shenk et al., 1993). Shenk et al. (1993) concluded that this work had the potential to reduce chemical analysis costs in calibration development up to 89%. This concept of global calibrations has allowed the use of NIRS technology to expand and become a more commonplace analysis method. Global calibrations increase calibration robustness and maintain prediction accuracy, expanding NIRS use (Gillon et al., 1999).

NIRS prediction of forage quality

Understanding the nutrient quality of forages allows for the better management and utilization for cattle production (Brown et al., 1990). Forage analysis has predominately been completed as proximate analysis in a laboratory (Coleman and Murray, 1993). Research has indicated that NIRS technology can accurately predict nutrient quality, including the DM, CP, ADF, NDF, and animal digestibility values of hay, silages, and straw (Coelho et al., 1988, Coleman and Murray, 1993, Mathison et al., 1999, Brown et al., 1990). The ability of NIRS to predict nutrient composition in roughage sources has made it an asset to beef cattle operations.

Thiex and Richardson (2003) stated that one of the most widely used analysis of forages is moisture determination, and that accuracy in this measurement is most important for agriculture commodities due to the effect of moisture on weight, storage conditions, and its impact on nutrient dilution. NIRS calibrations were examined using the Karl Fischer moisture determination method and oven drying methods by Thiex and Richardson (2003) and the researchers suggested that Karl Fischer provided the most accurate measure of moisture whereas oven drying methods were biased by the removal of volatiles and other compounds. In using the Karl Fischer method for reference, NIRS equations for forages were developed and accurate results ($R^2 = 0.98$) were achieved in the prediction of forage moisture content (Thiex and Richardson, 2003).

In a study by Coelho et al. (1988) comparisons of various forms of forage analysis were conducted including microbial, enzymatic, chemical, and NIRS. These researchers examined several types of forage: alfalfa hay, mature ryegrass, Bermuda grasses, and

mixed hays. When utilizing the NIRS technology, the hays were ground and dried using the oven method prior to scanning in the research conducted by Ceolho et al. (1988). Ceolho et al. (1988) concluded that when using NIRS to predict nutrient composition they had higher R^2 values for the chemical constituents of forages than for the in vivo measurements, as would be expected from the higher variability in animal responses in a procedure. Coelho et al. (1988) suggested that proper calibration and large sample size numbers would determine NIRS's usage in forage analysis.

Mathison et al. (1999) suggested that understanding the nutrient composition of straw can be important in Western Canada as it is consumed both in cattle rations and when used as a bedding source. When examining the chemical composition of barley straw, Mathison et al. (1999) observed a range of values represented in their samples including CP content of 2.15% to 7.28%, NDF of 66.3% to 86.3%, and ADF of 37.3% to 54.4% as well as in various other measured parameters. The objective of these researchers was to determine if NIRS can accurately predict the nutrient and degradability characteristics of barley straw, as well as to see if a commodity specific (local) calibration had improved accuracy in results compared to a combined (global) equation (Mathison et al., 1999). Mathison et al. (1999) utilized ground and dried samples for NIRS scanning and found that NIRS could reasonably predict the nutrient composition of straw, with the exception of lignin content. They also found that the same level of accuracy was achieved for most parameters (NDF excluded) when either a local calibration or a global calibration was used. Mathison et al. (1999) were able to utilize NIRS technology for various nutrient parameters of barley straw and maintain a high level of prediction accuracy regardless of equation type used.

NIRS prediction of grain quality

NIRS technology has found many uses in the agriculture industry in predicting various feed ingredients and total mixed rations. Barley grain specifically, has been looked at by researchers due to its use as an energy source in animal diets. Zijlstra et al. (2011) examined NIRS's ability to predict the digestible energy (DE) content of barley for feeding pigs. These researchers developed their own equation using representative barley samples and tested it using independent samples. It was observed that NIRS technology could be used to accurately predict DE content of barley, despite their small sample size, and that to make a more robust calibration for DE content which could be used to segregate barley a larger sample size would be required (Zijlstra et al., 2011).

McCann et al., (2006) also examined the use of NIRS for predicting barley composition including DE concentration, in vitro ileal digestion of CP and total tract digestion in swine, and found that calibrations were able to be built with reasonable accuracy achieved. However, the R^2 values were reduced between the calibration and validation where samples outside of the calibration population were used. McCann et al. (2006) also concluded that a small sample size impacts the prediction accuracy and that more accurate results can be seen in smaller sample populations. McCann et al., (2006) acknowledged that the calibrations and validation procedures need to be expanded in order to firmly conclude that NIRS can accurately predict nutritional composition of barley grain.

In conclusion, NIRS technology provides advantages to the agriculture industry through its rapid analysis of various products. However, it is important to include a

validation of the technology when analyzing new products (Shenk et al., 1993). If unsuccessful results are seen, NIRS calibrations can be expanded or developed to include the constituents of desired interest. Use of NIRS technology can be inhibited by the need to dry and grind samples before analysis; however, research in conducting as-is analysis is an expanding area of interest. Literature suggests that NIRS can accurately predict chemical composition of barley grain and this can be used in production settings to improve animal production efficiencies.

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CHAPTER III

PREDICTION OF AS-IS BARLEY SILAGE DRY MATTER BY NEAR INFRARED REFLECTANCE SPECTROSCOPY

Abstract

The objective of this study was to evaluate the use of near infrared reflectance spectroscopy (NIRS) technology in determining the DM of barley silage. Two separate NIRS equations were built in an effort to improve DM prediction accuracy using the technology. First, a commodity specific equation with only barley silage samples included in the equation was developed. Second, a broad based equation was developed where both barley silage and barley straw samples were included in the equation. Fifteen samples of barley silage and straw were utilized and split into groups: water added (WTR) and fresh. Water was added to WTR samples to broaden the DM range. Samples were weighed, scanned (InfraXact, FOSS North America) and dried (55°C) in twelve, 4 hour intervals. DM was calculated and correlated to NIRS spectra at each interval. Silage samples were blocked by DM and randomized to validation (n = 128) or calibration (n = 639) sets. A commodity specific equation (SIL) was developed from the silage calibration set [SE of calibration (SEC) = 3.77, R^2 = 0.98]. A broad based equation (SIL-STR) was derived (SEC = 2.93, R^2 = 0.98) using a calibration set (n = 1,406) consisting

of the silage calibration set (n = 639) and straw samples (n = 767). The R^2 and SE of prediction (SEP) for the validation of SIL and SIL-STR, using the independent validation set, were 0.98 and 3.78, 0.98 and 3.96, respectively. Barley silage DM content can be accurately predicted using NIRS with broad based or commodity specific calibrations.

Introduction

Silage is a major commodity used throughout feedlots in Western Canada and the United States. Feedlots are including silage in rations at significant rates; therefore, understanding the composition of this feedstuff is important to balance the nutritional needs of feedlot cattle. Accurate nutritional information of forages is important for producers in allowing them to make production predictions and inferences (Coelho et al., 1986). Thiex and Richardson (2003) stated that no analysis is more widely used in the agriculture sector than that of moisture. Understanding the moisture contained in a commodity is important for producers when they determine storage conditions, the cost of the commodity, and ultimately converting nutrients to a DM basis (Thiex and Richardson, 2003). In order to achieve optimum intake of animals we must be able to accurate quantify the moisture contained within the forages (Thiex and Richardson, 2003). Traditional laboratory DM analysis utilizes a “loss on drying” where moisture is evaporated from a sample. This analysis can be susceptible to several errors including weighing errors, humidity fluctuations, drying time, and temperature uniformity. In addition, this analysis requires specific sample preparation and a long drying time which can make it less than ideal for rapid analysis (Thiex and Richardson, 2003).

NIRS technology is a non-destructive and rapid analysis method that has been demonstrated to provide accurate DM and quality trait predictions for various commodities (de Boever et al., 1995). NIRS application with forages has been used primarily in determining DM, protein, and ADF in dried hay (Abrams et al., 1987). One limitation of previous NIRS analysis has been that samples must be dried and ground prior to scanning which adds analysis time and cost (Foley et al., 1998). Drying and grinding steps result in additional processing for feedlots and additional sources of error in scanning results. It was identified that using NIRS technology to characterize silage on an "as-is" basis would be a beneficial advancement throughout the feedlot industry.

NIRS technology has often been used in single commodity applications, where individual commodity equations must be built for each new application. Shenk et al. (1993) stated that the practice of developing new calibrations for each group of samples is costly and could be avoided by the combination of different samples into one calibration. Therefore, to increase the usefulness of NIRS as an analytical tool, and to decrease laboratory costs, selected calibrations have been built to include several commodities. These "broad based" calibrations would be more robust and could lead to more advanced and diverse uses of the technology if prediction accuracy can be maintained. The study examined the use of an as-is calibration for barley silage DM, where both broad based and commodity specific equations were developed to test prediction accuracy using both equation development methods.

Materials and Methods

Sample Collection. Samples used for this project were 15 barley silage samples taken from four different feedlots, and 15 barley straw samples originating from three feedlots in western Canada. Each sample was split and duplicates put into two groups: water added (WTR) and natural. Samples in the WTR group had water added to them prior to scanning and subsequent oven drying to increase the moisture content and broaden the DM range based on publication: Silage Fermentation and Preservation (Schroeder, 2004). Samples were refrigerated for 24 h prior to the start of oven drying to allow those in the WTR group to absorb the moisture. Samples were then removed from the refrigerator, removed from their plastic bags and any unabsorbed moisture was left in the bag as not to affect the initial sample weight. Samples and trays were weighed and scanned prior to the start of drying. All NIRS spectra were collected using commercially available technology (InfraXact, FOSS North America, Eden Prairie, MN). Samples were put into the drying oven set at 55 °C for 48 h. Samples were removed from the oven every 4 h and left to cool at room temperature for one h. Following cooling, all samples were individually weighed and scanned. Time in the oven was calculated as actual time in oven and did not account for any cooling time.

DM was calculated for each 4 h interval as:

$$\text{DM \%} = (\text{Sample Weight at interval} / \text{Initial Sample Weight}) * 100$$

Calibration Development. DM data and spectra were correlated using WINISI software (FOSS North America, Eden Prairie, MN) utilizing wavelengths 1100-1848 nm. Barley silage spectra and calculated DM values were blocked by the calculated DM value and randomized into either the validation set or calibration set. The calibration sets were

utilized in building equations whereas the validation set was used to validate the created equations. A commodity specific equation (SIL) was developed using only the barley silage spectra and calculated DM results. A broad based equation (SIL-STR) was developed using the spectra and calculated DM values of the barley silage samples from the calibration set combined with the spectra and calculated DM values of the barley straw samples. Equations were developed using WINISI software using optimal math conversions and treatments (Aufrere et al., 1996 and Hervera et al., 2012).

Statistical Analysis. Linear regression analysis was conducted to establish the relationship between the NIRS predicted values and the oven determined DM values. The spectrum from the independent barley silage validation set was passed through each developed equation to obtain predicted DM values. For each equation, those predicted DM values were correlated to the determined DM values using PROC REG of SAS (SAS Inc., Cary, NC).

Results and Discussion

Sample Characteristics All moisture was determined to be lost from drying in the oven at 55 °C for 48 h for the barley silage samples (Figure 1.1). A similar range was achieved in both calibration sets as well as the validation sets (Table 1.1). A very diverse range of DM values, 22% to 100% DM, was achieved by the addition of water to the samples. The proper separation of samples into the validation and calibration sets allows for testing of the calibration across a broad range of DM values ensuring the same level of prediction accuracy at various DM values. As Duncan et al. (1987) indicated it is important to include all possible extremes of the samples in a calibration model. Brown et

al. (1990) echoed these observations when examining NIRS usage for extension purposes and suggested a wide range of sample variation becomes important when predicting composition of the unknown samples. Including straw samples in the SIL-STR equation increased the number of samples in the calibration to 1406 observations compared to the 639 of the commodity specific SIL equation.

Equation statistics are displayed in Table 2. A high coefficient of determination value ($R^2 = 0.98$) for both equations was observed in equation development. This R^2 value is the same as equations built by Abrams et al. (1987) for silage DM using various silage types including alfalfa, orchard-grass, timothy, and bromegrass mixtures. These results can be compared with those of Abrams et al. (1955) as both experiments used samples in the as-is form with drying performed prior to scanning. It is important to note that our samples were not ground prior to scanning which increases potential errors in the spectrum collection. Abrams et al. (1955) indicated that finer grinding can be an important factor in removing air gaps from the scanning results. However, our results indicate that further grinding of silage samples is not required to achieve accurate predictions for DM as the samples used in this study were not ground prior to scanning and yielded high prediction accuracy for DM determination ($R^2 = 0.98$). From the results of this and Abrams et al. (1955) it appears that building an as-is calibration for barley silage is a viable option with NIRS technology. It is important to note that the standard error of calibration (SEC) and standard error of cross validation (SECV) were both decreased in the SIL-STR equations as compared to the SIL equation as would be expected from having an increased number of observations available. Our SEC was higher than that reported by Abrams et al. (1955) who reported 1.80. This could be

expected given our high range of DM values compared to the 20-57.5% they investigated.

Prediction of Validation Set. Figures 1.2 and 1.3 display the regression analysis results from the predictions using the SIL and SIL-STR equations, respectively. A high coefficient of determination ($R^2 = 0.98$, $P < 0.05$) was achieved utilizing the independent validation set and both equations. These figures display that the validation set tested across a broad range of DM values (25% to 100%) which would make it suitable for a wide range application in testing DM of barley silage as-is.

The results of this study demonstrate that by including several commodities into one NIRS equation the robustness of a calibration increases and prediction accuracy is not compromised. Foley et al. (1998) have suggested that high moisture feeds can be difficult to predict using NIRS as the water interferes with other compounds however; one solution to this is a robust calibration with various components such as has been created with these calibrations. These results are comparable with Brown et al. (1990), who also concluded that broad based equations provide the advantage of reducing the need to build individual commodity calibrations. As Shenk et al. (1993) indicated, robust multi-commodity calibrations can allow for a reduction in laboratory costs over time, and can lead to a wider spread application of NIRS technology in forage DM analysis.

Conclusion

The use of barley straw and barley silage in a broad-based NIRS DM calibration equation successfully predicts the DM content of barley silage across a broad range of DM values. Calibrations for barley silage DM can be developed on as-is samples, with

the elimination of drying and grinding of samples before scanning. The ability to accurately predict the DM of barley silage allows for precise inclusion levels of silage in feedlot cattle rations, NIRS can provide these results more rapidly than conventional oven drying. The ability to perform as-is analysis of barley silage could lead to an increase in the adoption of NIRS technology on site for producers. Further research should be conducted to validate the broadness of the calibration and its ability to predict various silage types and expanded as new crops are to be analyzed.

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Table 1.1. Simple statistics for sample DM included in calibration and validation sets

		Calibration Set	
Statistic	Validation Set	SIL	SIL-STR
N	128	639	1406
Min.	22.55	22.87	22.87
Max.	100.00	100.00	100.00
SD	26.28	26.00	21.26
Mean	76.39	76.68	86.83

Table 1.2. Equation statistics for commodity specific or broad based barley silage calibrations

Equation	n[a]	SEC[b]	R^2	SECV[c]
SIL	634	3.77	0.98	3.89
SIL-STR	1384	2.93	0.98	2.96

[a]Samples were removed from SIL (5) and SIL-STR (16) equations after being determined spectral outliers

[b]SEC = Standard error of calibration

[c]SECV = Standard error of cross validation using 5 groups for cross validation

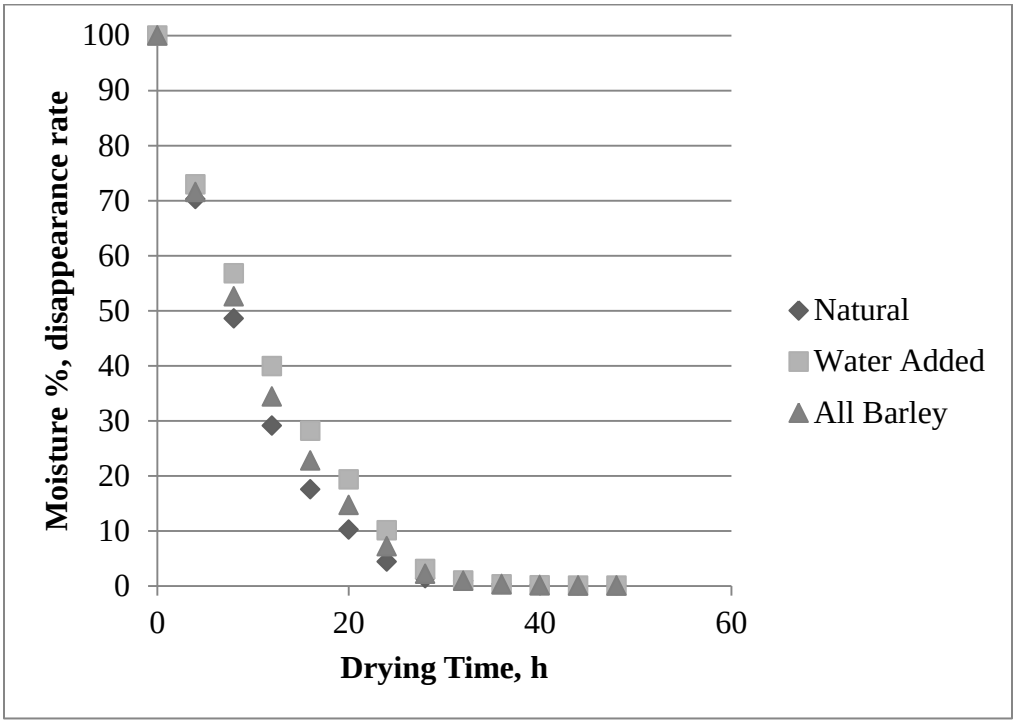

Figure 1.1. Average moisture disappearance rate of all barley silage samples over 48 h.

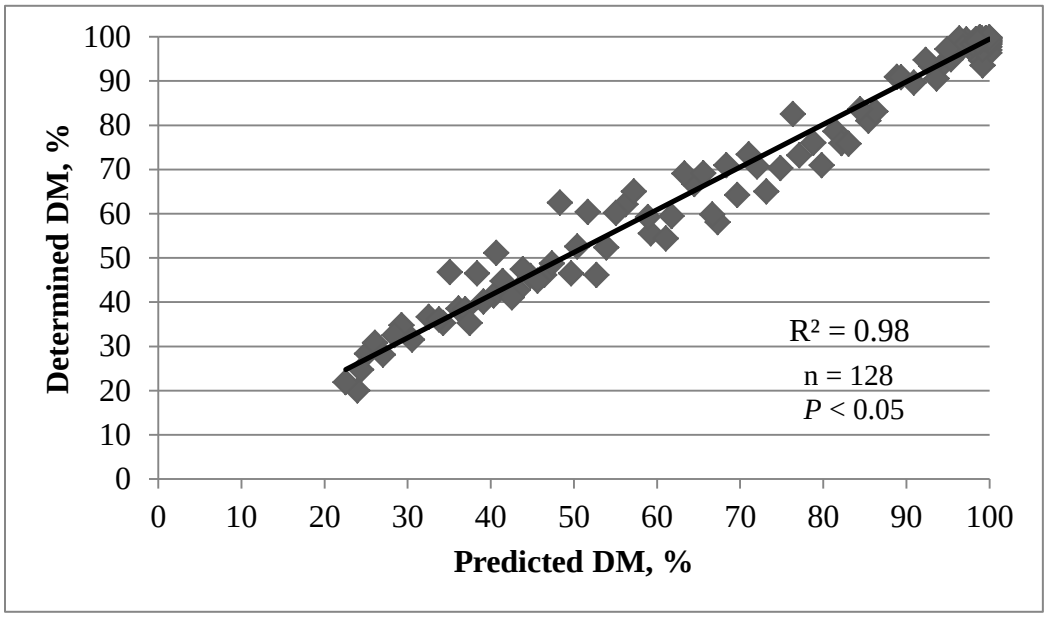

Figure 1.2. Commodity specific NIRS equation (SIL) predictions of independent validation set (n = 128).

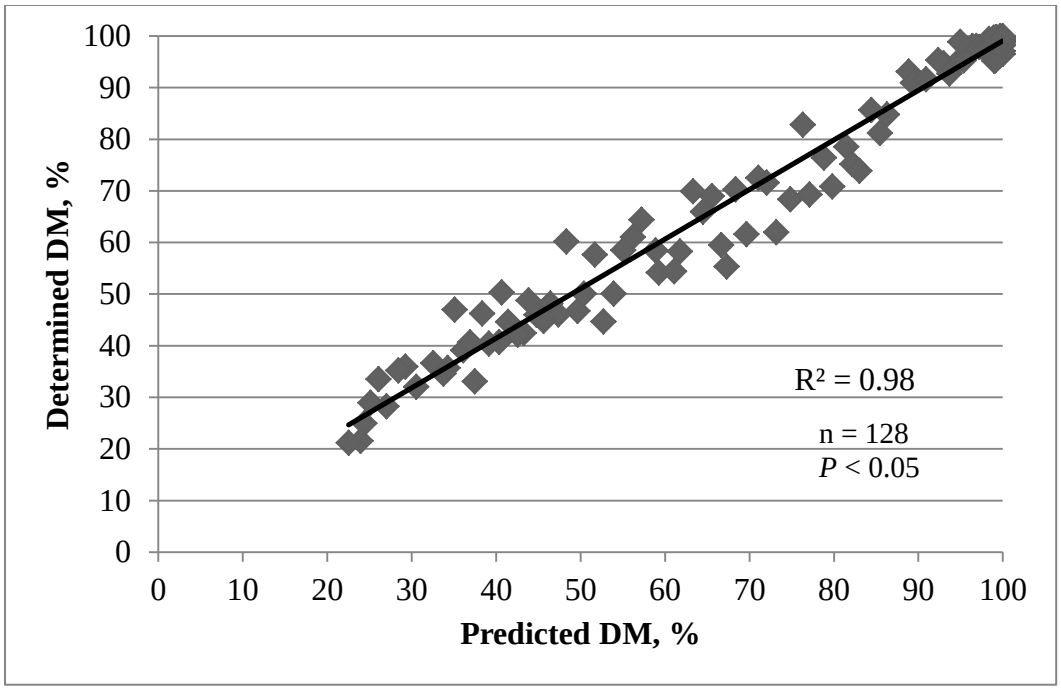

Figure 1.3. Broad based NIRS equation (SIL-STR) predictions of independent validation set (n = 128).

CHAPTER IV

EFFECT OF NUTRIENT COMPOSITION VARIABILITY OF BARLEY GRAIN ENTERING FEEDLOTS IN WESTERN CANADA ON NEAR INFRARED REFLECTANCE SPECTROSCOPY PREDICTIONS USING COMMERCIALLY AVAILABLE TECHNOLOGY

Abstract

Near infrared reflectance spectroscopy (NIRS) has been used to accurately predict the nutrient composition of animal feed commodities. Feedlots are challenged with large variation in nutrient composition of commodities entering their operations. The objective of this study was to examine the variation in nutrient composition and calibration prediction accuracy of NIRS technology. Barley samples (n = 111) were selected from six feedlots in western Canada between April and August, 2012, representing a range in nutrient compositions as predicted by NIRSS using commercially available NIRS prediction equations (InfraXact, FOSS North America, Eden Prairie, MN). Samples were selected for high, middle, or low nutrient composition of starch, fat, CP, and DM. Laboratory analysis was completed and correlated to the predicted NIRS values for starch, CP, and DM. PROC REG of SAS 9.3 (SAS Institute, Cary, N.C.) was used to determine correlations between laboratory assayed and NIRS values. When comparing NIRS and laboratory predictions of DM and CP for all samples, there was a strong

correlation (R^2 = 0.74 and 0.57, respectively, $P < 0.05$) whereas for starch predictions, there was a poor correlation (R^2 = 0.12, $P < 0.05$) when comparing NIRS and starch analysis. Regression analysis was conducted to evaluate NIRS predictions across the range (high, mid or low) of each constituent (starch, CP, and DM). Similar or improved R^2 values for all parameters were observed, [DM = 0.91 (n = 27), CP = 0.63 (n = 24), and starch = 0.21 (n = 27) ($P < 0.05$)] when the predictions were tested across the range. NIRS technology can adequately predict across a variable range of barley samples in western Canada for DM and CP. However, prediction accuracy decreases when greater variation exists in the population to be tested. Accurate predictions can be obtained for DM and CP content of barley arriving to feedlots in western Canada using commercially available NIRS prediction equations; however starch content is not accurately predicted with this current application.

Introduction

Barley grain is a readily available source of dietary energy and is utilized in feeding ruminant livestock in many parts of the world (Dehghan-banadaky et al., 2007). Feedlots utilize different grain sources based on factors such as availability, cost of the grain, and processing (Owens et al., 1997). Western Canadian feedlots are able to utilize barley grain in feeding beef cattle due to its energy content and ready availability in that area. Owens et al. (1997) demonstrated feeding barley grain resulted in the same animal performance in ADG, DMI and feed efficiency as other cereal grains, including corn, oats, and wheat, when averaged across processing methods. The energy content of barley grain is due to its chemical components of starch, fat, fiber, and protein (Campbell et al., 1995). However, significant variation occurs in these chemical components in barley

grain, especially throughout Canada, due to different cultivars and different environmental conditions during the growth phase of the plant production (Campbell et al., 1995).

Previous researchers indicated that the ability to accurately predict animal performance and to formulate specific diets for cattle is greatly affected by variation in energy content of barley (Fairbairn et al., 1999). Kong et al. (1995) examined an extensive selection of barley cultivars in Canada and found that in the 75 different cultivars the starch content ranged from 51% to 62%, the CP content ranged from 12% to 16%, the NDF content ranged from 8% to 18%, and the ADF ranged from 2% to 7%. Campbell et al. (1995) found even greater variation in the chemical composition of eight cultivars of barley where the starch content ranged from 48% to 65%, protein content from 9% to 18%, NDF content from 12% to 20%, and ADF ranged from 5% to 9%. The increase in variation of select cultivars makes chemical analysis of barley grain of greater importance when feeding beef cattle.

NIRS technology has been used to accurately analyze several agriculture commodities, including forages, cereal grains, and composite feeds (Brown et al., 1990; Ziljstra et al., 2011, Aufrere et al., 1996). In previous research by Ziljstra et al. (2011) they were able to accurately predict the DE value of barley grain for feeding swine. NIRS technology provides accurate, rapid, and non-destructive analysis of feedstuffs and therefore has many advantages over traditional laboratory assays, including the ability to determine several components simultaneously (Stubbs et al., 2010).

NIRS is a predictive technology that requires access to large amounts of laboratory results as the reference method for prediction equations. (Shenk et al., 1993). Historically NIRS equations were developed for each individual commodity; however, Shenk et al. (1993) suggested that NIRS product libraries can be developed using laboratory results from various similar commodities to produce equations that will yield satisfactory results for new samples. Combining spectrally similar products into a library and producing global calibration equations, allows for a reduction in laboratory costs of up 89% (Shenk et al., 1993). Shenk et al. (1993) however did suggest that constant validation of global calibrations is required and that the addition of new samples into the product library might be required to continue to achieve high levels of accuracy in predictions. The advancement in NIRS global predictions has allowed researchers to move away from individual NIRS equations in favor of a global equation approach. Commercially available NIRS technology has been developed based on the concept of global library equations which has allowed for the wider application of NIRS technology. However, as Shenk et al. (1993) cautioned, a validation process of NIRS library equations is required to ensure accurate results when new unknown samples need to be analyzed. The objectives of this study were 1) to examine the variation in chemical composition (DM, CP and starch content) of barley grain entering feedlots in Western Canada and 2) to evaluate the effect of variation in chemical composition on NIRS prediction accuracy of barley grain using commercially available technology.

Materials and Methods

Barley Samples Whole barley samples entering 6 feedlots in Western Canada were sampled prior to unloading at the facility between September 2011 and February 2012.

Whole samples were scanned using commercially available NIRS technology (InfraXact, FOSS North America, Eden Prairie, MN) and the distributions of the NIRS results for DM, CP, fat and starch were plotted. Based on the distributions of the subpopulation tested, study population samples were then selected from April to August 2012 from the same 6 feedlots as the top 10% (high), middle 10% (mid), and bottom 10% (low) for each parameter and at random (RANDOM) from all samples entering the 6 feedlots during this time. Selection criteria of study population (n = 111) is described in Table 2.1.

Laboratory Analysis Following selection, whole barley samples (n = 111) were ground through a 2 mm screen using a Wiley grinding mill. DM analysis was conducted using a forced air oven at 55 °C for 48 hours. CP and starch were determined using AOAC methods 992.23 and 996.11, respectively, with the total starch assay kit obtained from Megazyme Int. Ireland Ltd. (Wicklow, Ireland). Fat analysis was not completed on the samples; however the fat selected samples were still included in the total study population with the RANDOM samples to represent all samples entering feedlots in western Canada. The chemical composition of the total study population is summarized in Table 2.2. Chemical composition of those samples meeting the selection criteria of DM, CP, and starch is demonstrated in Table 2.3. In addition to these, 33 samples were selected for their fat content or RANDOM from the samples arriving at the feedlot.

Statistical Analysis Laboratory determined and NIRS predicted values for DM, CP, and starch was analyzed using PROC REG of SAS 9.3 (SAS Institute, Cary, NC). Regression analysis was performed in two ways to characterize the relationship between NIRS predictions and chemical composition: 1) all samples in the study population were included in the analysis for each nutrient (DM, CP, and starch, 2) only samples selected

as HIGH, MEDIUM or LOW in the sample population for each nutrient were included in the statistical analysis.

Results and Discussion

The samples selected for the experiment was reflective of the range of whole barley samples received at various feedlot locations in Western Canada for ration formulation. This was achieved by collecting data on barley entering feedlots for 6 months prior to sample selection. In addition, the DM and CP values observed were similar to those reported for barley of different types in the NRC (1996). Figure 2.1 shows the spectrum collected for the study population (n = 111), and confirms that samples were spectrally different from each other and, therefore, a greater amount of variability was achieved in the selection of the study population. Although spectrum differences were not specifically selected for in this experiment, these differences were expected given the selection based on nutrient composition.

Reasonable accuracy was observed in the regression analysis of the NIRS predicted DM and lab determined DM for all study samples ($R^2 = 0.74$, $p < 0.05$, Figure 2.2). When testing across the range and removing the variability of the other nutrient parameters, using only the selected DM samples, improved accuracy was observed in regression analysis ($R^2 = 0.91$, $p < 0.05$, Figure 2.3). As demonstrated in Figures 2.2 and 2.3 a wide range of DM values in the samples was examined, with samples ranging from 81 % to 95 % DM. These results would follow the same conclusion as Garnsworthy et al. (2000) who examined NIRS's prediction of wheat nutrient parameters and concluded NIRS predictions of chemical constituents are accurate. However, our observed

prediction accuracy was not as high as Garnsworth et al. (2000), who reported an R^2 value of 0.94 for DM of wheat. The difference in R^2 could be attributed in part to our samples being scanned as whole kernels whereas, Garnsworthy et al. (2000) ground samples prior to NIRS scanning.

Regression analysis of the CP content predicted by NIRS and the lab determined CP values yielded moderate prediction accuracy when all samples were included in the analysis ($R^2 = 0.57$, $p < 0.05$, Figure 2.4), and improved accuracy when only those samples selected for CP content were included in the model ($R^2 = 0.63$, $p < 0.05$, Figure 2.5). Foley et al. (1998) explained that the protein determination in agricultural plants and products is the most common application for NIRS technology in this industry; however, our results would indicate that only moderate accuracy is achieved in the CP prediction of whole barley grain and may not make NIRS a suitable technology in CP determination. Results from this study indicate a much lower prediction accuracy for CP content than de Boever et al. (1995) found in using NIRS to predict compound feeds of cattle. De Bouver et al. (1995) included a small number of samples in their study and therefore, their improved accuracy ($R^2 = 0.96$) could be related to a smaller sample size examined.

Finally, NIRS prediction and lab determined starch content were analyzed for all samples. There was a low level of accuracy demonstrated ($R^2 = 0.12$, $p < 0.05$, Figure 2.6). A wide range of starch content was observed across the samples from 33 % to 67 % for all samples. When only those samples selected for their starch content were analyzed, accuracy was improved ($R^2 = 0.21$, $p < 0.05$, Figure 2.7) but not to a reasonable or acceptable level. Garnsworthy et al. (2000) also observed that starch prediction accuracy was the lowest of the nutritive parameters examined in wheat however, the researchers

experienced a much higher level of accuracy (R^2 = 0.78) in starch predictions than the current study observed. Garnsworth et al. (2000) tested over a reduced range of starch contents compared to the current study population and may have been able to achieve a higher coefficient of determination due to a reduction in sample variability. Nonetheless, these results and the results of Garnsworthy et al. (2000) would indicate that even though prediction accuracy can vary, starch content in cereal grains is a difficult chemical constituent to predict using NIRS technology.

Conclusion

Commercially available NIRS technology demonstrates moderate prediction accuracy for the DM and CP content of whole barley grain entering feedlots in Western Canada. Improvements to the technology would be required to improve prediction accuracy of starch content. Previous research using NIRS technology has been done with dried and ground samples (Foley et al., 1998). Whole barley samples did not achieve the same level of accuracy as is seen in other agriculture products when they are dried and ground samples. Improvements in this technology and the base prediction equation need to be expanded to include more samples in the equation and have a more robust equation. In contrast, providing an individual barley grain starch equation with more barley sample analysis completed may allow for prediction accuracy to be increased.

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Table 2.1. NIRS selection criteria for barley grain study population sampling from April to August 2012

Parameter	High	Mid	Low
DM, %	> 89.36	87.01 - 85.33	< 83.62
Fat, %	> 3.12	2.19 - 1.60	< 1.08
Starch, %	> 61.50	58.85 - 58.40	< 55.74
CP, %	> 11.31	9.67 - 9.34	< 7.70

Table 2.2. Chemical composition (DM basis) of barley samples entering feedlots in western Canada (n=111)

Parameter	Avg	Min	Max	SD
DM, %	90.58	81.14	95.16	2.62
CP, %	11.38	7.59	16.48	1.39
Starch, %	52.48	32.71	66.94	6.42

Table 2.3. Chemical composition of selected samples for DM, CP, and starch groups (n = 78)

	High			Mid			Low		
Parameter	n	%	SD	n	%	SD	n	%	SD
DM, %	10	92.82	0.70	10	90.27	0.13	7	85.47	0.24
CP, %	9	12.50	0.53	5	10.50	0.23	10	9.20	0.32
Starch, %	7	57.50	0.32	10	53.19	0.42	10	48.60	0.42

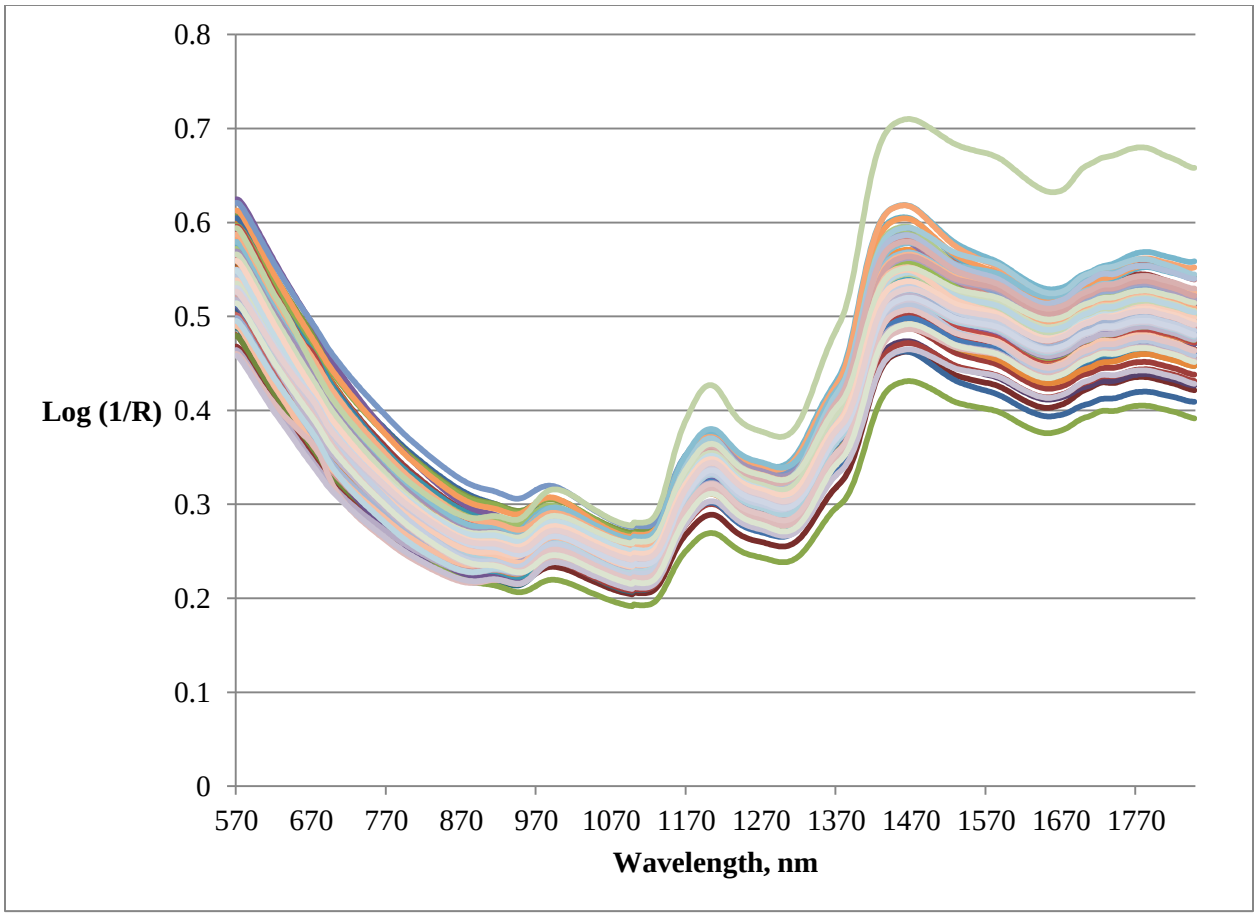

Figure 2.1. Spectrum of whole barley samples selected at 6 feedlots in Western Canada (n = 111)

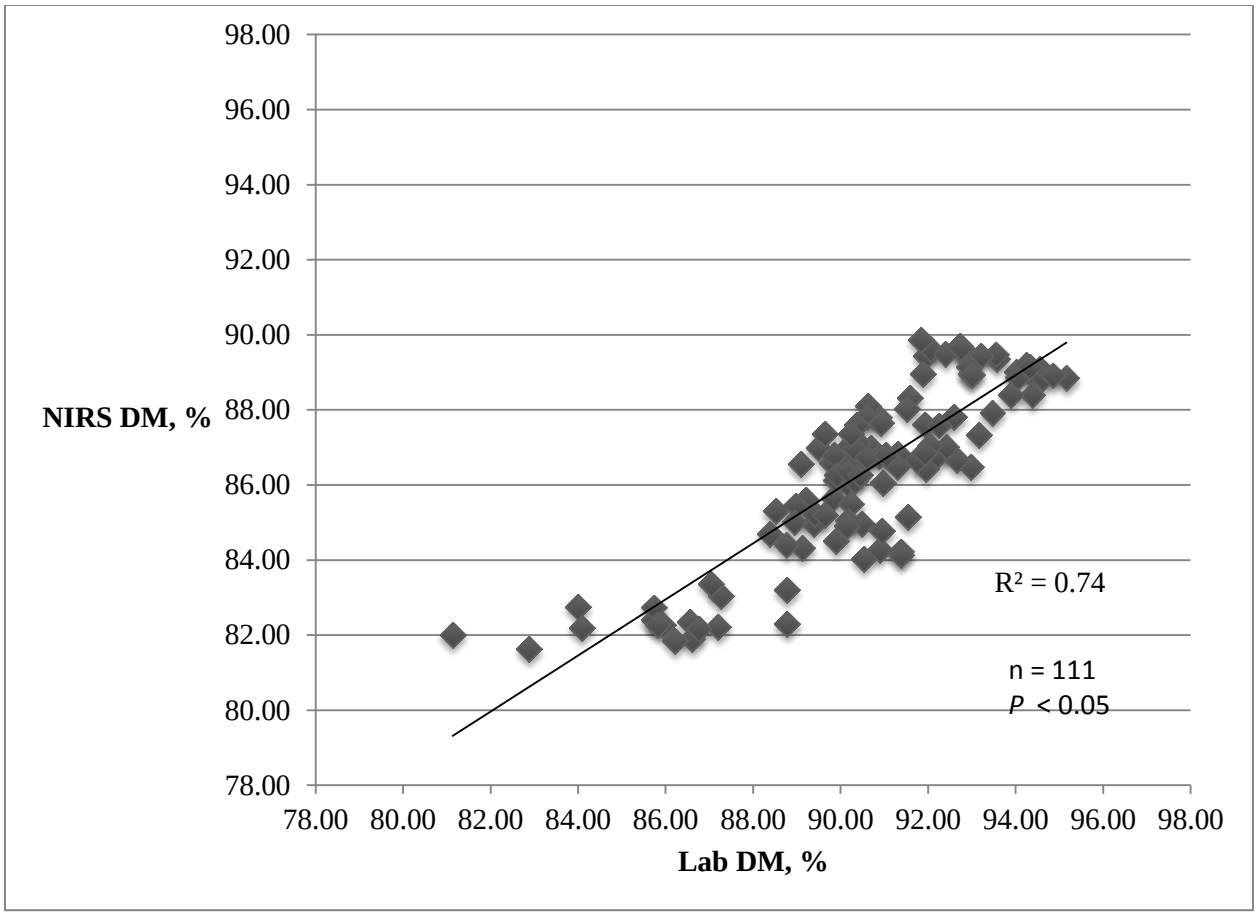

Figure 2.2. Validation of NIRS DM predictions for barley grain.

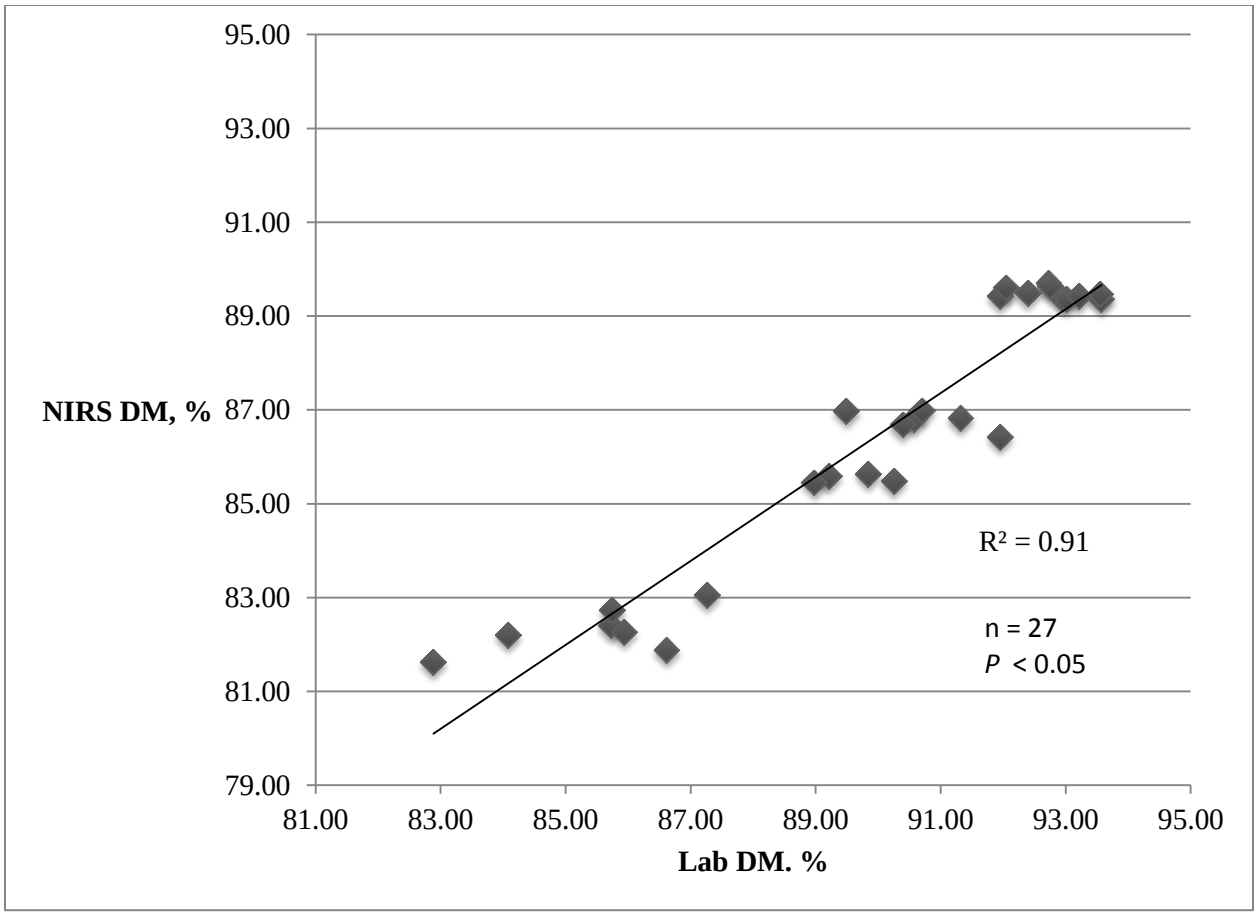

Figure 2.3. Validation of NIRS DM predictions for selected samples of barley grain. LOW samples were selected using NIRS predictions of DM < 83.62%, MID samples were selected for DM 85.33 - 87.01 % and HIGH samples were selected for DM > 89.36 %.

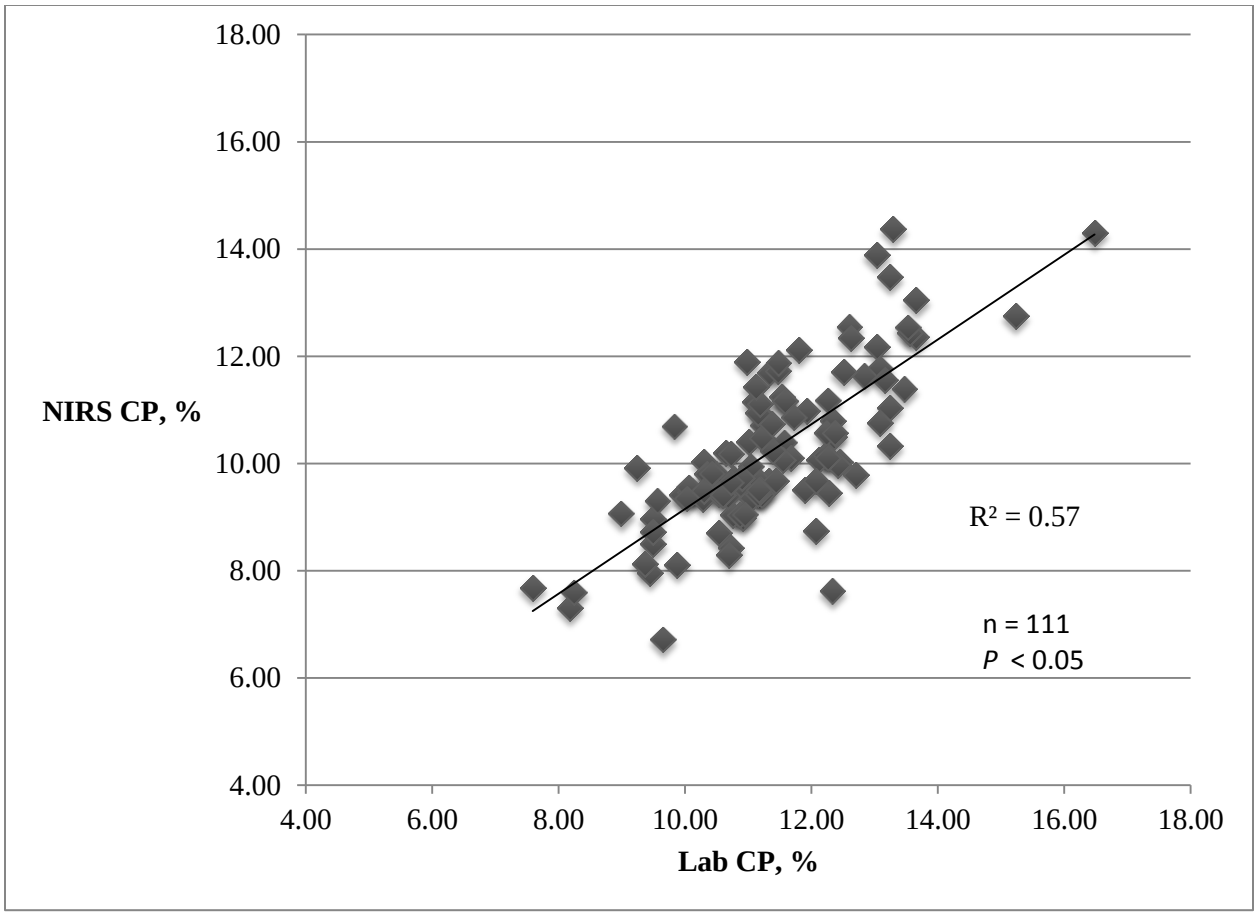

Figure 2.4. Validation of NIRS CP predictions for barley grain.

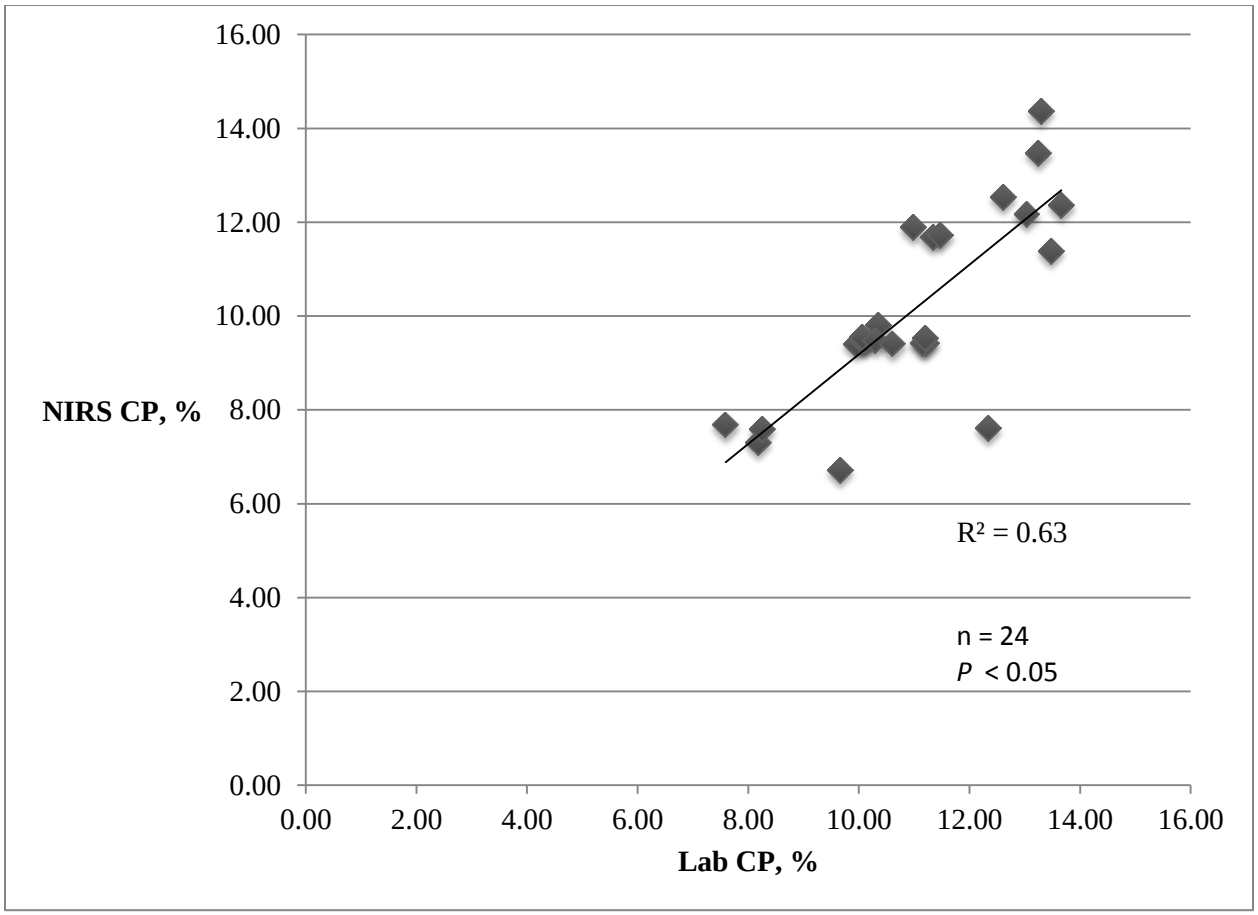

Figure 2.5. Validation of NIRS CP predictions for selected samples of barley grain. LOW samples were selected using NIRS predictions of CP < 7.70 %, MID samples were selected for CP 9.34 – 9.67 % and HIGH samples were selected for CP > 11.31 %.

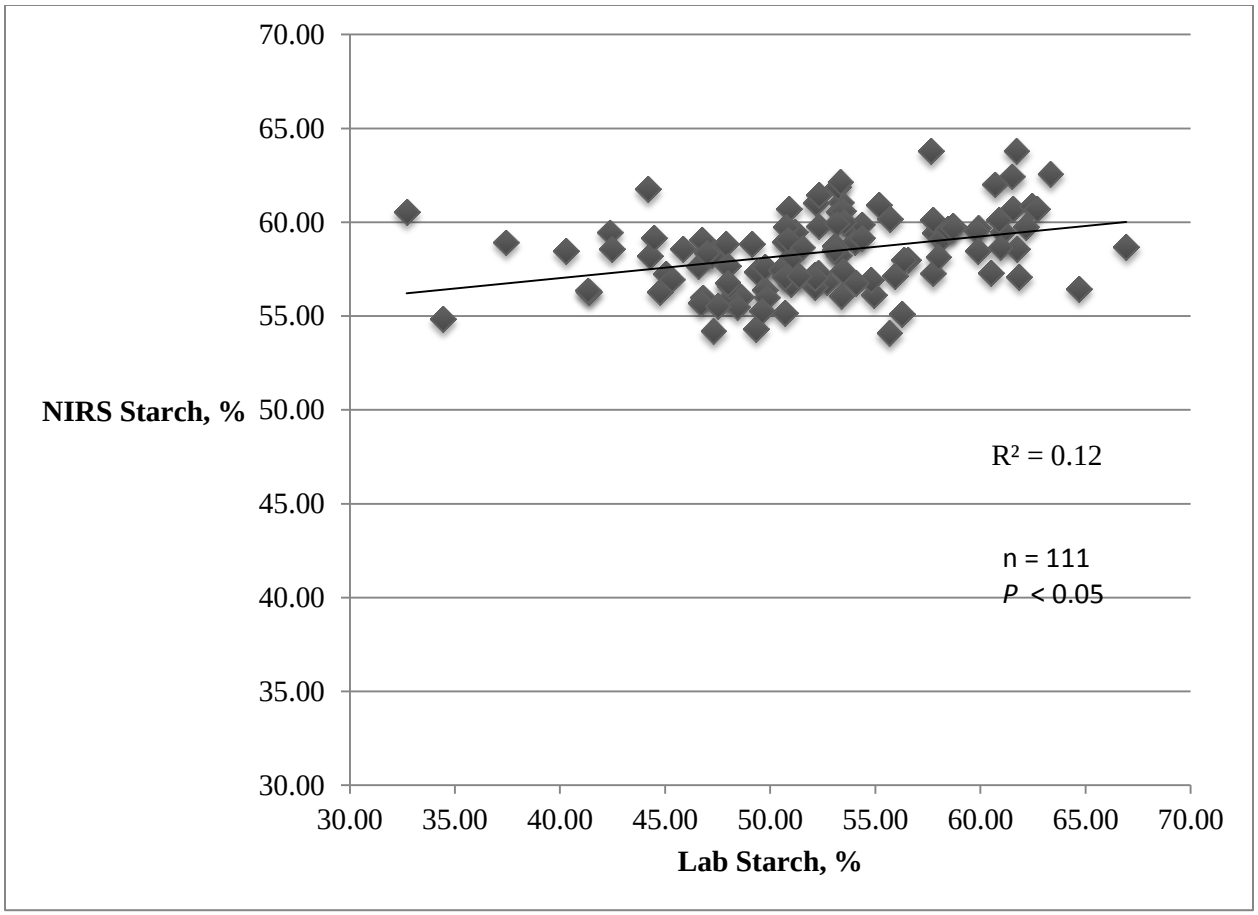

Figure 2.6. Validation of NIRS starch predictions for barley grain.

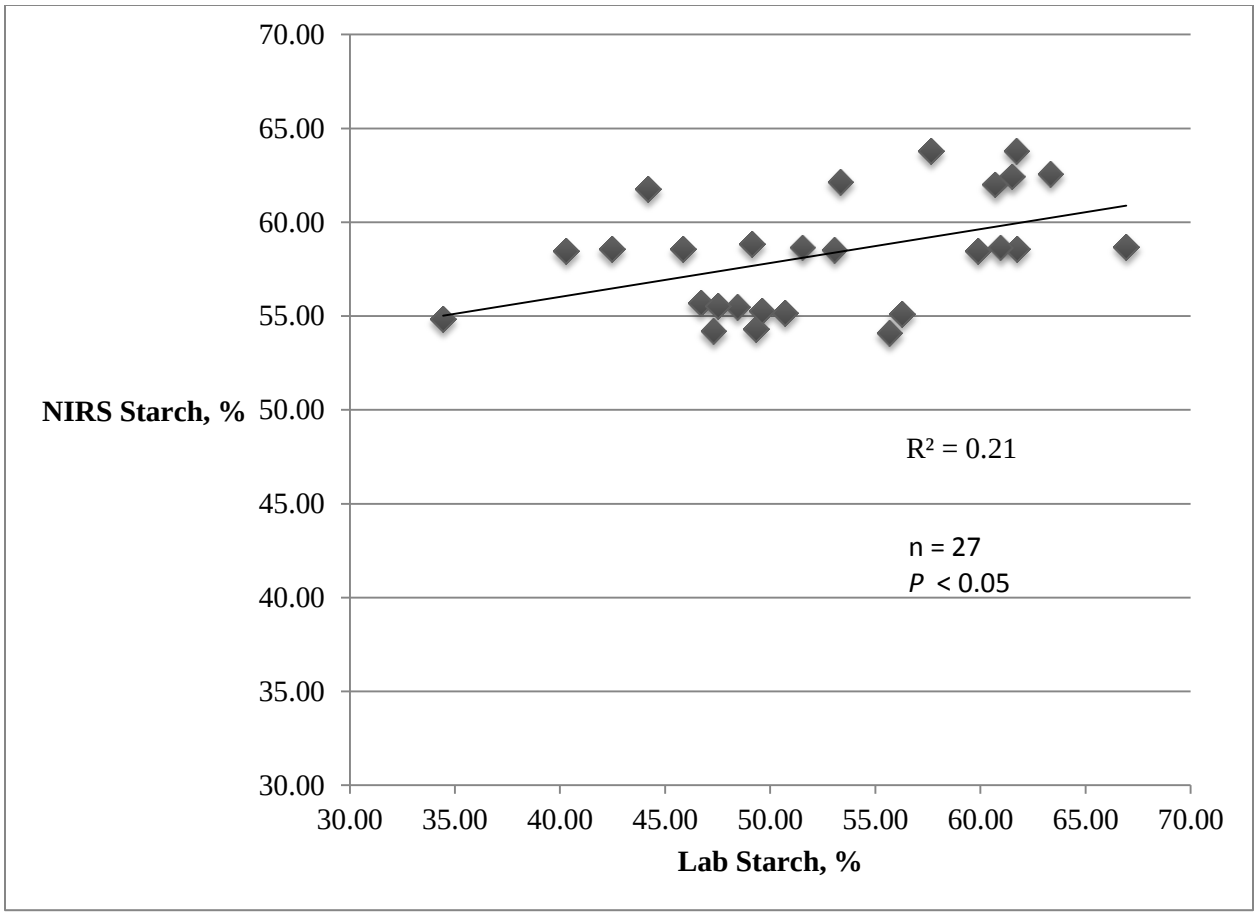

Figure 2.7. Validation of NIRS starch predictions for selected samples of barley grain. LOW samples were selected using NIRS predictions of starch < 55.74 %, MID samples were selected for starch 58.40 – 58.85 % and HIGH samples were selected for DM > 61.50 %.

CHAPTER V

EFFECT OF NEAR INFRARED REFLECTANCE SPECTROSCOPY SELECTION GROUP ON IN VITRO FERMENTATION AND GAS PRODUCTION KINETICS OF BARLEY GRAIN IN WESTERN CANADA

Abstract

The objective of this study was to evaluate the use of NIRS technology as a selection tool for barley grain and the subsequent in vitro fermentation measurements of those NIRS determined groups. Barley samples were scanned with NIRS technology and placed into groups based on being in the top 10% (high), middle 10% (mid), or bottom 10% (low) for each parameter of DM, CP, and starch. Ruminal fluid was collected from one cannulated, non-lactating, Holstein cow adapted to a high concentrate diet to evaluate IVDMD and gas production kinetics. Low DM samples had a faster rate of fermentation (k) ($p \leq 0.05$) compared to both the mid and high DM ranges. Cumulative gas production was also found to be greater for low range DM samples between hours 8 and 23 of incubation compared to both the high and mid-range samples ($p \leq 0.05$). The mid-range selected CP samples had greater gas production (mL/g of substrate DM, $p \leq 0.05$) than the high range, with low range selected samples being intermediate. Correlations between both the NIRS

and lab determined chemical constituents and the gas production kinetics were examined and found that DM was negatively correlated with k when DM was measured with NIRS or in a lab (r = -0.34 and r = -0.45, respectively, $p \leq 0.05$). DM measured by NIRS (r = -0.26) or in a lab (r = -0.27) was negatively correlated with lag ($p \leq 0.05$), and CP was significantly correlated with gas production (NIRS r = -0.31, lab r = -0.31, $p \leq 0.05$), k (NIRS r = 0.48, lab r = 0.47, $p \leq 0.05$), and lag (NIRS r = 0.30, lab r = 0.37, $p \leq 0.05$). Starch determined in the lab was significantly correlated with gas production (r = 0.19, $p \leq 0.05$). Differences in in vitro digestion kinetics do exist between different groups of barley entering feedlots in western Canada based on their nutrient composition of DM and CP. NIRS technology may be used as a selection tool for nutrient composition for barley grain.

Introduction

Barley is used in Western Canadian feedlot diets in large proportions due to its high energy content and availability in the area. Barley entering feedlots can be very diverse, based on the physical and chemical characteristics of the grain. The two factors that are known to have the greatest effect on the nutritional constituents of barley are the genetic variation and environmental variation during growing (Anderson et al., 1984; Berdahl et al., 1976). Lehman et al. (1995) confirmed that differences in rumen degradability of barley are affected by the type and cultivar of barley grain. Boss and Bowman (1996) described that the differences in barley varieties relates to a difference in animal performance, carcass quality grade and intake of digestible starch. Cleary et al. (2011) examined the effects of barley variety, seeding rate, location and nitrogen fertilization rates, and concluded that the effects did not have a consistent response on

barley grain digestion. Digestion kinetics can be measured using an in vitro gas production technique which is based on the concept that gas is produced from the mixture of ruminal contents in relation to the amount of substrate fermented (Lopez et al., 2007). The objective of all in vitro systems is to mimic the environment of the gastro-intestinal tract and producing accurate gas production kinetics (Mould et al., 2005). Gas production techniques can be combined with degradability estimates and allow for measures of proportion of feed fermented as compared to that which is used for microbial growth (Rymer et al., 2005). Trei et al. (1970) found that correlations between gas production, VFA production and starch digestion were high; indicating that gas production can be used as a tool to predict these measurements. Trei et al. (1970) suggested that the gas production technique offers a rapid analysis of rate of digestion and can provide use in understanding the relative feeding value of processed grain. Understanding that variation in barley composition can lead to differences in digestion and animal performance, a rapid measure of barley composition would aid in prediction of animal performance in feedlot production.

Near infrared reflectance spectroscopy (NIRS) has been developed as a method to predict the chemical composition and nutritional parameters of various commodities; it is most widely used in the agriculture sector in evaluating animal feeds (Foley et al., 1998). Accurate predictions of feeds have been produced for nitrogen (protein), moisture, fiber, starch, and more, of various animal feeds (Foley et al., 1998). Stubbs et al. (2010) outlined the advantages of NIRS analysis to be a low cost analysis is provided with rapid results and a non-destructive method. In addition, Stubbs et al. (2010) describes NIRS

technology as one which allows for a larger range of samples to be tested and multiple properties can be tested at one time.

The variation of barley grain entering feedlots in Western Canada may have an effect on animal performance. The use of NIRS technology on site at feedlot locations allows for rapid analysis of grains and a quick determination of feeding value. The objective of this study was to determine the use of NIRS technology as a selection tool for barley grain based on its predicted chemical composition, and the gas production kinetics and in vitro dry matter disappearance (IVDMD) of the selected barley grain.

Materials and Methods

Whole barley samples entering 6 feedlots in Western Canada were sampled prior to unloading at the facility between September 2011 and February 2012. Whole samples were scanned using commercially available NIRS technology (InfraXact, FOSS North America, Eden Prairie, MN) and the distributions of the NIRS results for DM, CP, fat and starch were plotted. Based on the distributions of the sub population tested, study population samples were then selected from April to August 2012 from the same 6 feedlots as the top 10% (high), middle 10% (mid), and bottom 10% (low) for each parameter. Originally 111 samples were selected based on their nutrient composition or at random. From this population, samples were separated into their groups and 78 samples were utilized for the gas production kinetics based on their DM, CP or starch characteristics as predicted by NIRS. Group characteristics are displayed in Table 3.1. NIRS prediction accuracy of each parameter Ash, DM, CP, and starch were described in Chapter IV.

Laboratory Analysis Ruminal fluid used for the digestion kinetics and in vitro fermentation was collected from one ruminally cannulated, non-lactating, Holstein cow. The animal was housed at the Willard Sparks Beef Research Center (WSBRC) in Stillwater, OK and fed a high concentrate diet containing > 50% cracked corn, prairie hay, and corn gluten feed as basal ingredients. Corn was used as the readily available grain source for the WSBRC and was expected to be an acceptable substitute to a barley based diet for rumen microbe populations. Feed was offered once daily with free choice access to water. Adaptation to the diet was done for 21 days prior to initial ruminal fluid collection. Ruminal fluid was collected between 4 and 6 h post feeding, and strained through 4 layers of cheese cloth and transported to the laboratory in a sealed thermos immediately following collection. Ruminal fluid was used immediately for laboratory procedures.

IVDMD IVDMD was conducted using an adapted procedure of Galyean (2010), where 0.5 ± 0.05 g of substrate was utilized and samples were completed in triplicate. Samples were weighed into a 50-mL centrifuge tube. McDougall's buffer and ruminal fluid were mixed at a ratio of 3:1, with a total of 36 mL being added to the tube with the sample, and four blanks were included in each run. Tubes were purged with CO_2 and capped with rubber stoppers and placed into a 39 °C waterbath. Contents of tubes were gently agitated every 6-8 h during the procedure for 48 h. Following the 48h incubation with ruminal fluid samples were taken from the 39 °C waterbath and placed into an ice bath for approximately 5 minutes. Stoppers were removed and 3 mL of HCl was added to each tube and gently swirled. Following the addition of HCl 2 mL of 5% pepsin was added and again the tube was gently swirled. Tubes were placed back into the 39 °C waterbath

with rubber stoppers inserted for an additional 24 h. Gentle agitation of tubes occurred every 6-8 h following the 24 h incubation.

Following the 24 h pepsin digestion, samples were removed from the water bath and filtered through No. 4 filter paper. Filter paper and residue of each sample was then dried in a forced air oven for 48 h at 55 °C. IVDMD was then calculated as follows and converted to a percentage through multiplying by 100:

$$\text{IVDMD} = \frac{\text{sample weight (DM basis)} - (\text{undigested residue weight} - \text{avg. blank weight})}{\text{Sample weight (DM basis)}}$$

In vitro kinetics of gas production Eighteen gas pressure monitor modules (Ankom Technology Corp.) were used in duplicate for each sample, with two additional blank modules for each run. Each 250 mL module received 0.7 ± 0.01 g of sample and 50 mL of a 3:1 mixed McDougall's buffer and ruminal fluid solution. Each flask was flushed with CO_2 and the monitor cap fastened. Flasks were inserted into a 39 °C shaking water bath set at 45 rpm (Thermo Fisher Scientific Inc.) for 24 h. Each monitor cap sends gas pressure data to a base coordinator unit wirelessly, and was set to send data every 30 min during the 24 h period. To eliminate gas pressure buildup the equipment was set to release pressure at 20.7 kPa and the computer monitored the gas pressure released providing a computer calculated cumulative gas pressure at each time interval. Gas pressure was measured in psi and then converted to milliliters of gas produced per gram of DM incubated using the following equation: (Ankom Technology Corp.):

$$G = (V_h/P_a) \times P_t$$

where G is gas volume, V_h is headspace volume, P_a is atmospheric pressure, and P_t is pressure measured by the transducer.

Statistical Analysis The duplicate gas production measurements and triplicate IVDMD measurements were averaged within run. The laboratory analysis of DM, CP, starch, and IVDMD were analyzed using the MIXED procedure of SAS (SAS Institute Inc., Cary, NC). A nonlinear model was used to fit the data from the Ankom Gas Pressure Monitor, where the nonlinear model was the modified Gompertz equation (Schofield et al., 1994) which included the parameters of maximum gas production (M), rate of gas production, (k) and lag time (l). The parameters M, k, and l were analyzed as repeated measures using PROC GLIMMIX of SAS (SAS Institute Inc.) as a 3 x 5 factorial where nutrient range and NIRS selection group were included in the model and run was included as a random effect. Gas production data was analyzed hourly for 24 h. For all statistical analysis, significant effects were observed at $\alpha \leq 0.05$, and tendencies declared at p – values between 0.05 and 0.10.

Results and Discussion

Laboratory analysis of the NIRS selected groups and the sample within each range reflected that differences were not achieve between all groups for a selected nutrient (Table 3.1), however DM and CP were different between the three ranges: high, medium, and low ($p \leq 0.05$). Differences were seen between the starch high and low ranges with the medium being an intermediate ($p \leq 0.05$). As described in previous chapters, the accuracy of the DM and CP predictions by NIRS were moderately good ($R^2 > 0.57$, $p \leq 0.05$), while the starch predictions were less accurate ($R^2 = 0.12$, $p \leq 0.05$)

which may account for the unachieved differences between the starch groups when using NIRS as the selection tool.

The average IVDMD across all barley samples originally selected (n = 111) was 82.95% (Table 3.2), and was comparable to the results presented by Hatfield et al. (1997) who presented the IVDMD of heavier bulk density barley to be 82.4%. Our overall average maximum gas production for all barley samples was 286.62 mL/g of substrate DM, with an average rate and lag of 20.10 % /h and 0.56 h, respectively. Given the relatively small amount of research completed on cereal grain gas production, as well as the dissimilarities in the methods, processing, and statistical analysis these results are difficult to compare directly to previous research. One observation found both in our data and in that presented by Lanzas et al. (2007) is that, although lag can be measured in vivo due to factors such as microbial attachment, the in vitro measurement of lag may be more difficult to measure due to the processing of a ruminal fluid buffer solution, methodology of incubation and the specifics of the inoculum used.

In vitro fermentation measurements of only the selected DM samples are presented in Table 3.3. A significant increase ($p \leq 0.05$) in the rate of fermentation was seen in the low DM samples as compared to the middle and high groups. These results would be similar to those seen by Getachew et al. (2005) who found that when steam was added to cereal grains, the fermentation was higher after 8 h than a whole grain. The additional moisture in the grain allows for a more rapid fermentation and a higher rate expressed in the gas production measurements. A tendency for lag time to be longer for the low DM samples was observed compared to the high samples, with no difference detected between the middle and either of the other ranges. The increased lag time of a

low DM sample would be similar to the prolonged lag time that Wang et al. (2003) observed in tempered barley samples compared to non-tempered samples. No significant differences were seen in the IVDMD % or maximum gas production (mL/g of substrate DM) between the high, medium, and low DM groups. These in vitro fermentation results would support the work of Wang et al. (2003) who explained that animal performance effects are observed when grain moisture is increased above 10%, which could be explained by the increased rate of fermentation we saw between the low group having a moisture content of >10% as compared to the other two ranges of high and medium both being <10%. Mathison et al. (1997) also concluded that performance of animals did not differ if tempering was done on grains that originally contained > 13% moisture. The higher rate of fermentation relating to moisture content has also been demonstrated in the use of steam flaking in corn, where a faster rate of fermentation is seen in steam flaked corn than dry rolled corn ($p = 0.01$) (Leibovich et al., 2009). Our results would support the conclusion that having >10% moisture in a barley grain impacts the rate of fermentation which may be why animal performance effects are seen at this level of moisture (Wang et al. 2003, Mathison et al., 1997), and that moisture content of grain is a driver in the rate of fermentation of grains in vitro.

In vitro fermentation measurements of CP selected samples are presented in Table 3.4. No difference ($p \geq 0.05$) was detected across the ranges for the IVDMD % or the lag period. There was a significant difference ($p \leq 0.05$) in the maximum gas production between the high and middle groups with the middle group having the most gas produced (311.57 mL/g of substrate DM) and the high group having the least gas produced (280.90 mL/g of substrate DM), and the low range was intermediate (299.20 mL/g of substrate

DM). There were no differences observed for rate of fermentation between the ranges ($p > 0.05$). The concept of protein content and fermentation rate is not well understood in barley grain however; the protein matrix which encapsulates starch granules could be a factor in understanding rate of fermentation (Kotarski et al., 1992). Kotarski et al. (1992) suggests that the physical structure of the protein and carbohydrates in the kernel is what limits digestion rates and is more important in influencing digestibility than the chemical composition of the nutrients in the grain or the physical processing of the grain. The samples used in this experiment were all processed the same however; no information is directly known regarding the structural form of protein or carbohydrates and therefore these results are difficult to interpret.

No differences ($p \leq 0.05$) were observed for the starch selected samples for the in vitro fermentation measurements of IVDMD %, maximum gas production or rate of fermentation (Table 3.5). A tendency was observed for the high starch group to have a longer lag time than the mid group with the low selected samples having an intermediate lag time. Our results did not demonstrate a difference in the rate of fermentation between the ranges of selected samples as Khorasani et al. (2000) was able to observe in different cultivars. Given the observed differences in rate of fermentation by Khorasani et al. (2000) of different cultivars, they explained that this characteristic of rate of fermentation is important to examine as samples with a slower rate of fermentation would lessen the incidence of digestive disorders.

In examining the correlations between both the NIRS predicted and lab determined chemical constituents and in vitro fermentation measurements similar results were observed (Table 3.6 and Table 3.7). Significant correlations exist between the rate

of fermentation and DM content of barley grain either predicted by NIRS (r = -0.34, $p \leq 0.05$) or determined in a laboratory (r = -0.45, $p \leq 0.05$), with a higher correlation between the laboratory value. A significant ($p \leq 0.05$) negative correlation was also observed for the DM content of barley grain and the lag period both for the NIRS predicted (r = -0.26) and lab determined (r = -0.27). CP content was negatively correlated to the total gas production for both the NIRS prediction and lab determined (r = -0.31 for both, $p \leq 0.05$). Positive correlations were observed for the NIRS predicted and lab determined CP for k (r = 0.48 and 0.47, respectively, $p \leq 0.05$) and lag (r = 0.30 and 0.37, respectively, $p \leq 0.05$). A significant correlation ($p \leq 0.05$) was determined between the gas production and the lab determined starch content (r = 0.19), with no significant correlations ($p \geq 0.05$) observed in the NIRS predicted starch content and in vitro fermentation measurements. Previous research by Lanzas et al. (2007) also found no significant correlation between starch content of barley grain (r = 0.31, $p \geq 0.05$). In addition, Getachew et al. (2005) examined correlations between chemical components and gas production, with specific focus on methane production and found a similar correlation for 24 h methane production and CP (r = -0.45) as we found for total gas production. As Lanzas et al. (2007) discussed the low correlations of chemical composition and rate of fermentation may be due to the physical structure of the kernel rather than the chemical make-up which makes predicting rates based on chemical composition difficult.

Analysis of cumulative gas production over time was performed, by nutrient group and by range (Figures 3.1, 3.2, and 3.3). Cumulative gas production of the DM selected samples showed significant ($p \leq 0.05$) differences at hour 6 where the low DM

selected samples had significant higher cumulative gas production ($p \leq 0.05$) than the mid or high range samples. There were no differences in cumulative gas production observed between the mid and high range samples throughout the 24 h period. The higher cumulative gas production of the low range samples was observed from h 6 to h 23. At h 23 there was no difference ($p \geq 0.05$) observed between any of the three ranges of DM selected samples for cumulative gas production.

Cumulative gas production of the CP selected samples show significant differences at 8 h where the low CP selected samples had significantly lower cumulative gas production ($p \leq 0.05$) than the mid or high range samples. There were no differences in cumulative gas production observed between the mid and high range samples at this 8 h time. The lower cumulative gas production of the low range samples was observed from 8 h to 14 h. For the 14 h there was a significant difference between all three groups ($p \leq 0.05$) with the low selected samples having the lowest cumulative gas production, the high selected samples having an intermediate cumulative gas production and the mid selected samples having the highest cumulative gas production. From 15 h to 24 h there was no difference observed between the high range and low range samples for cumulative gas production, however the mid-range showed significantly higher gas production ($p \leq 0.05$) than both of these ranges.

Finally, there were no differences observed between the starch ranges for cumulative gas production (Figure 3). Due to the difficulty of predicting starch content of barley grains by NIRS these results may have been expected. Although the starch content of the grain was determined to be different for the low group as compared to both the mid

and high group, this is not directly reflected as a difference in cumulative gas production over time.

Conclusion

Understanding nutrient composition of grains is important for animal production and NIRS technology has allowed for rapid analysis to be performed. When NIRS predictions of nutrient composition are accurate this technology can be utilized as a tool for producers to select different populations of barley grain. In vitro digestion kinetics can further demonstrate the feeding value of grains. Fermentation rates, cumulative gas production, and hourly gas production can differ due to DM and CP nutrient concentrations in barley grain, and understanding these measurements and relationships can allow for greater precision in animal feeding with potential for reduction of digestive issues and increased animal performance.

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Table 3.1. Chemical composition of selected samples for DM, CP, and starch groups (n = 78)

	High			Mid			Low		
Parameter	n	%	SD	n	%	SD	n	%	SD
DM, %	10	92.82[a]	0.70	10	90.27[b]	0.13	7	85.47[c]	0.24
CP, %	9	12.57[a]	0.53	5	10.50[b]	0.23	10	9.20[c]	0.32
Starch, %	7	57.50[a]	0.32	10	53.19[ab]	0.42	10	48.60[b]	0.42

[abc]means within a row with different superscripts differ at $p < 0.05$

Table 3.2. Average in vitro fermentation measurements of barley entering feedlots in Western Canada (n = 111)

Item	Mean	SD
IVDMD, %	82.95	4.40
Gas production, mL/g of substrate DM	286.62	20.20
k, mL /h	20.10	3.13
k, %/h	7.06	1.32
Lag, h	0.56	0.53

Table 3.3. Effects of NIRS selection range on in vitro fermentation measurements for DM selected samples

Item	High	Mid	Low
n	10	10	7
IVDMD, %	83.94	82.20	83.21
Gas production, mL/g of substrate DM	273.41	279.62	280.04
k, %/h	6.06[a]	6.35[a]	7.65[b]
Lag, h	0.43[x]	0.34[y]	0.68[xy]

[ab] means within a row with different superscripts differ at $p < 0.05$.

[xyx] means within a row with different superscripts tended to differ at $0.5 \leq p \leq 0.1$.

[1]Parameters were estimated by fitting a modified Gompertz function, with k = fractional rate of fermentation, and Lag = duration of the lag phase.

Table 3.4. Effects of NIRS selection range on in vitro fermentation measurements for CP selected samples

Item	High	Mid	Low
n	9	5	10
IVDMD, %	81.78	84.53	83.11
Gas production, mL/g of substrate DM	280.90[a]	311.57[b]	299.20[ab]
k, %/h	7.76	6.63	6.34
Lag, h	1.02	0.73	0.78

[ab] means within a row with different superscripts differ at $p < 0.05$.

[xyx] means within a row with different superscripts tended to differ at $0.5 \leq p \leq 0.1$.

[1]Parameters were estimated by fitting a modified Gompertz function, with k = fractional rate of fermentation, and Lag = duration of the lag phase.

Table 3.5. Effects of NIRS selection range on in vitro fermentation measurements for starch selected samples

Item	High	Mid	Low
n	7	10	10
IVDMD, %	84.77	82.11	80.76
Gas production, mL/g of substrate DM	280.24	281.29	277.41
k, %/h	8.67	8.13	8.36
Lag, h	0.78[x]	0.52[y]	0.73[xz]

[ab] means within a row with different superscripts differ at $p < 0.05$.

[xyx] means within a row with different superscripts tended to differ at $0.5 \leq p \leq 0.1$

[1]Parameters were estimated by fitting a modified Gompertz function, with k = fractional rate of fermentation, and Lag = duration of the lag phase.

Table 3.6. Correlations of NIRS predicted chemical constituents and in vitro fermentation measurements

Item	DM	CP	Starch
IVDMD, %	0.09	< 0.001	0.11
Gas production, mL/g of substrate DM	0.03	-0.31*	0.12
k, % h	-0.33*	0.52*	0.06
Lag, h	-0.26*	0.30*	0.03

*$p \leq 0.05$

Table 3.7. Correlations of lab determined chemical constituents and in vitro fermentation measurements

Item	DM	CP	Starch
IVDMD, %	0.05	< 0.001	0.10
Gas production, mL/g of substrate DM	0.06	-0.31*	0.19*
k, % h	-0.40*	0.51*	-0.08
Lag, h	-0.27*	0.37*	0.08

*$p \leq 0.05$

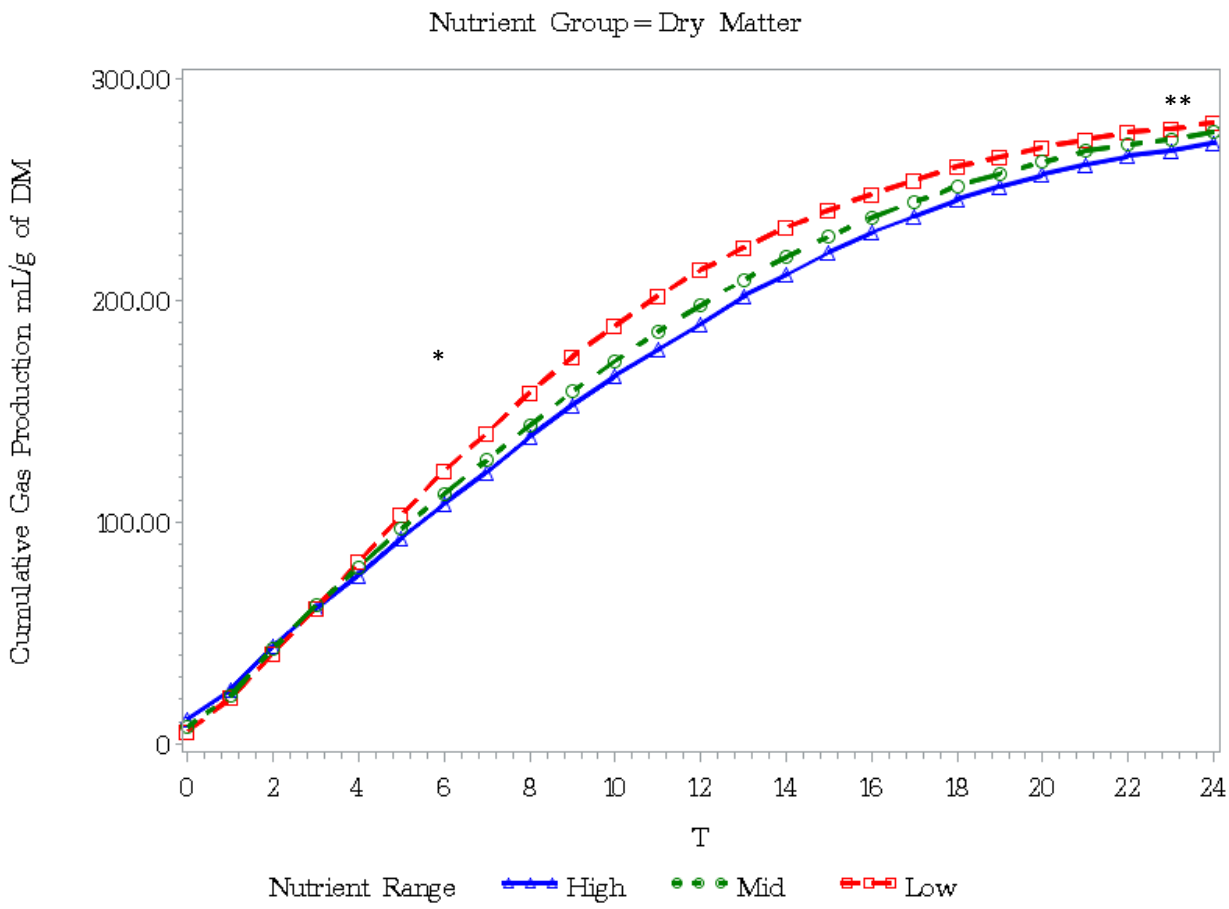

Figure 3.1. Gas production of DM selected samples over a 24 h period. Samples included were those selected as high DM (n = 10), middle DM (n = 10), and low DM (n = 7). * indicates that at hour 6 cumulative gas production significantly differed ($p \leq 0.05$) between the low DM selected samples and the other two ranges, with the low selected samples having a higher gas production than both the middle and high groups. ** indicates that at 23 h to 24 h the three ranges do not significantly differ ($p \geq 0.05$) in cumulative gas production.

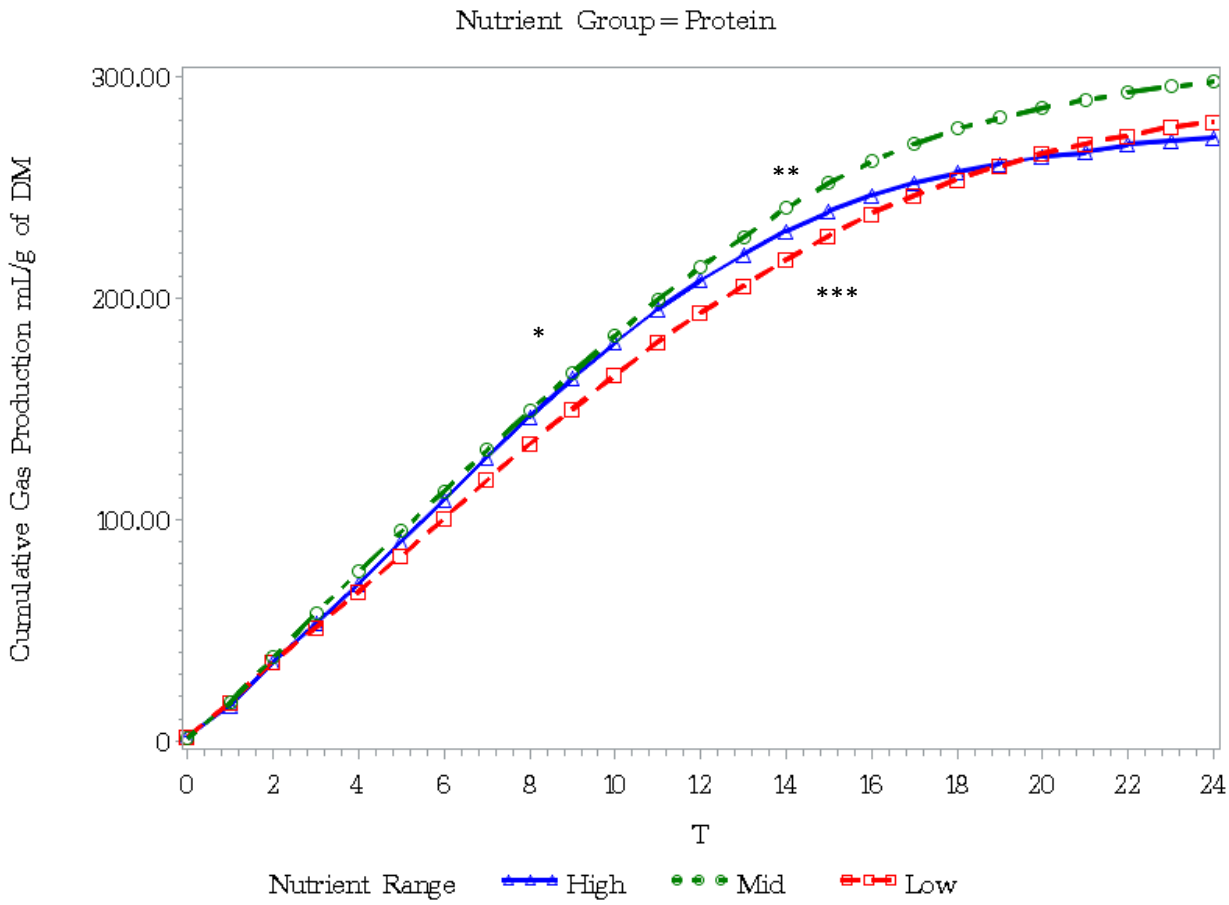

Figure 3.2. Gas production of CP selected samples over a 24 h period. Samples included were those selected as high DM (n = 9), middle DM (n = 5), and low DM (n = 10). * indicates that at hour 8 cumulative gas production significantly differed ($p \leq 0.05$) between both the high and mid ranges and the low range with no difference being observed between the high and mid selected samples. At h 8 the low selected samples having a lower gas production than both the middle and high groups. ** indicates that at hour 14 all three ranges differed ($p \leq 0.05$) for gas production. *** indicates that at hour 15 until 24 h the mid group differs significantly from the low and high groups with no significant difference ($p \geq 0.05$) in cumulative gas production between the low and high ranges.

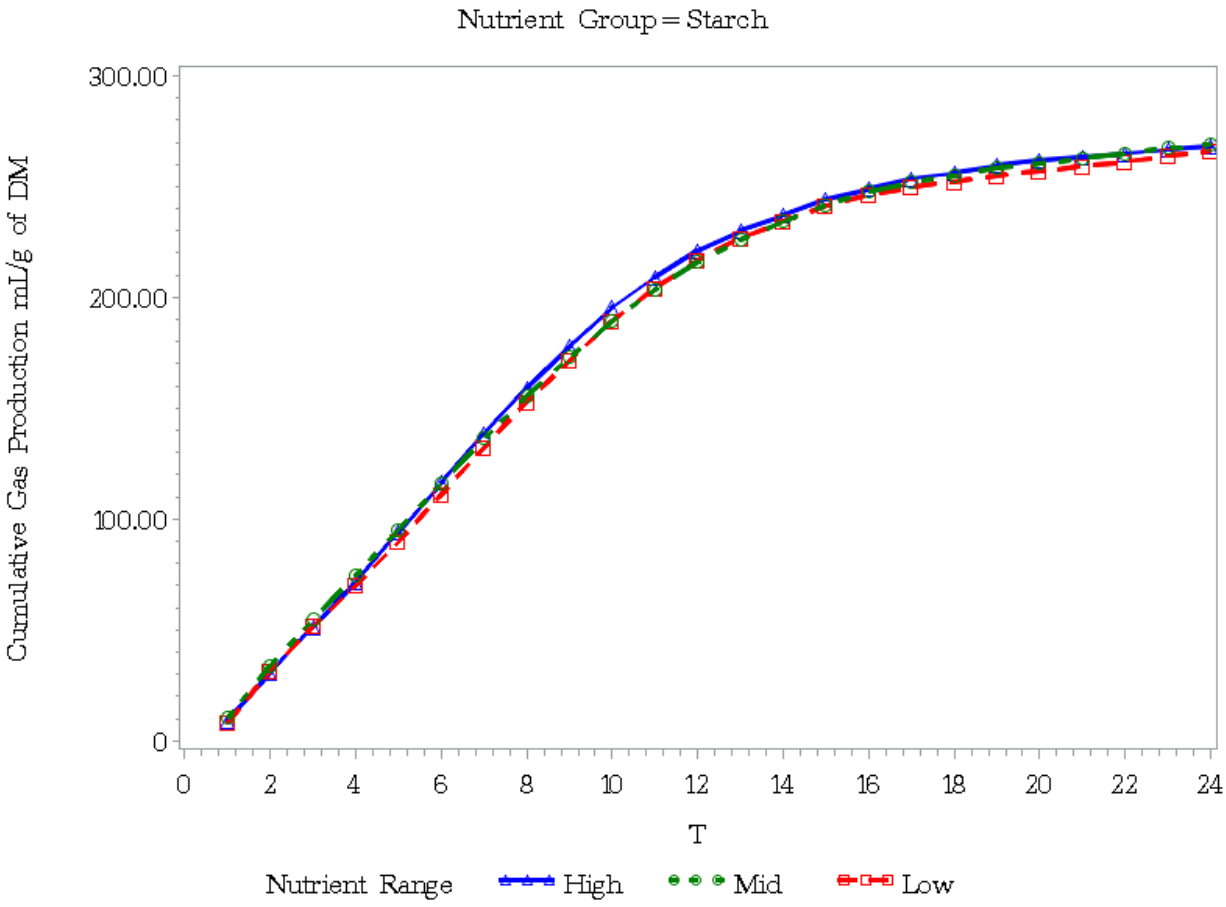

Figure 3.3. Gas production of starch selected samples over a 24 h period. Samples included were those selected as high DM (n = 7), middle DM (n = 10), and low DM (n = 10).

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