

THE USE OF NEAR INFRARED REFLECTANCE
SPECTROSCOPY FOR THE CHARACTERIZATION OF
WHEAT AND BARLEY GRAIN ENTERING
FEEDLOTS IN WESTERN CANADA

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"Learn from yesterday, live for today, hope for tomorrow. The important thing is to not stop questioning."

-Albert Einstein

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Abstract: The first experiment evaluated the use of near infrared reflectance spectroscopy (NIRS) for the nutrient prediction of wheat grain and the factors affecting in vitro dry matter digestibility (IVDMD) and in vitro kinetics of gas production of wheat grain. Wheat samples ($n = 75$) were selected from three feedlots in Alberta from September 2011 to April 2012 to represent a range in DM, CP, starch, and fat. The prediction models for DM, CP, and starch were tested and the effect of each nutrient on in vitro fermentation parameters were evaluated. A second experiment was conducted evaluating the effects of a barley spectra index on in vitro fermentation parameters and feedlot performance of yearling cattle. Results of the first experiment demonstrate that NIRS can accurately predict ($R^2 = 0.90$) the CP content but not DM or starch ($R^2 = 0.17$ and 0.02 , respectively) across a broad range of composition. High DM samples had greater IVDMD ($P < 0.05$) than low and medium DM samples. Rate of gas production of high starch samples was lower than low starch samples and higher for high CP samples than medium and low CP samples. Results of experiment two indicate that segregating barley by spectra index may improve cattle performance by minimizing variability in substrate supplied to the rumen. Cattle fed LOW, MED, or HIGH spectra index barley had greater DMI ($P = 0.02$), tended to have greater HCW and live- and carcass adjusted ADG ($P = 0.08, 0.09, 0.07$, respectively) than cattle fed the unsegregated CON. Likelihood of Yield Grade 1 carcasses was greatest ($P = 0.05$) in steers fed MED treatment barley. As treatment group increased there was: a linear decrease in DM ($P = 0.02$); linear increase in CP ($P < 0.01$); a tendency for a linear decrease in starch ($P = 0.07$); linear decrease in the color variables brightness and red:green scale ($P = 0.02$ and 0.04 , respectively); and linear increases in 1,000-kernel weight and kernel diameter ($P < 0.05$).

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CHAPTER I

INTRODUCTION

Barley grain is the principle feed grain used in the cattle feeding industry in the northwest regions of the United States and much of Canada due to climate and soil restrictions that impede the production of corn (Campbell et al., 1995; Boss and Bowman, 1996). Wheat grain is often used in place of, or in addition to barley based rations for cattle production when availability or favorable pricing make feeding wheat more cost effective (Riley, 1984). The variation in barley and wheat grain has been demonstrated (Bhatty et al., 1974; Zijlstra et al., 1999). This variation is largely due to environmental and genetic factors associated with different cultivars (Anderson et al., 1984; Berdahl et al., 1976).

The wide range in variation in nutrient composition of these grains invariably leads to differences in digestibility and utilization, and energy supplied to the animal. Increasing competition for grain commodities from human, fuel, and other livestock markets will continue to drive the cost of production up. Producers will be forced to feed cattle more precisely, with less wastage, more efficient utilization of nutrients, and using fewer resources, in order to maintain the sustainability of their operations.

The most practical and commonly used method of quantifying grain quality on-site has been bushel weight (Grimson et al., 1987). Previous research, however, has demonstrated little correlation between bushel weight and nutrient value (Campbell et al., 1995), digestible energy (Bhatty et al., 1974), or animal performance (Grimson et al., 1987). An alternative technology producers can use to manage the variation in commodities entering the feedlot is Near Infrared Reflectance Spectroscopy (NIRS). The ability of NIRS to predict nutrient composition has been demonstrated (Williams, 1975; Hunt et al., 1977; Norris et al., 1976; Sinnaeve et al., 1995; De Boever et al., 1993) for various types of feed commodities including concentrates, grasses, and silages.

The objectives of this project were to evaluate the use of NIRS technology for the characterization of wheat and barley grain entering feedlots in western Canada. First, samples were selected from multiple feedlots in western Alberta to represent a range in nutrient composition basis the overlying population. These samples were utilized to evaluate the existing calibration model for wheat grain and also explore the factors affecting *in vitro* digestibility and kinetics of gas production of wheat grain. In a subsequent experiment, an index was developed to quantify spectral differences of barley samples. The effects of this index were then tested related to IVDMD, kinetics of gas production, and feedlot performance of yearling cattle.

We hypothesized that the current, commercially available NIRS technology would be able to accurately predict the nutrient composition of wheat grain and that estimation of these parameters would help to understand the factors affecting digestibility and fermentation *in vivo*. Furthermore, we hypothesized that that a spectra index would be more indicative of animal performance than any individual nutrient.

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CHAPTER II

REVIEW OF LITERATURE

Wheat grain

Wheat grain is often used in finishing diets for cattle due to availability and favorable pricing compared to corn or barley (Riley, 1984), particularly in western Canada. Owens et al. (1997) summarized data from 39 feed trials with 1,440 head of cattle fed a variety of concentrate grain sources and utilizing a variety of processing methods. The authors observed that ADG, DMI, and feed efficiency of cattle fed wheat grain were not different than that of cattle fed corn or barley when averaged across all processing methods. Furthermore, they noted that the observed and body weight (BW)-adjusted metabolizable energy (ME) of wheat was shown to be not different than corn or barley and better than dry rolled milo. Erjaei et al. (2012) observed that, when used in growing and finishing diets fed to cattle, starch is vital as the main energy constituent of wheat grain and that carbohydrate utilization is key to improving cattle efficiency and performance. A large variation in CP and starch of Canadian wheat varieties was reported by (Zijlstra et al. (1999)) resulting in variation in chemical composition and DE. Nutrient variation is a reflection of genetic and environmental effects and the economic impact of

this variation compounds the need for improved chemical composition determination when used in animal production Zijlstra et al. (1999).

Wheat grain has also been widely used in Canada as a grain source for ethanol production. About one third of the 1.5 billion liters of fuel ethanol produced in Canada each year is produced from the fermentation of wheat grain (Canada Renewable Fuels Association, 2007). Wheat grain has roughly half the oil content and substantially more protein than corn grain (National Research Council, 1996). Roughly 70% of the weight of wheat grain is starch (Gibb et al., 2008), most of which is fermented by yeast to produce ethanol during the fermentation process. This results in approximately a threefold increase in the concentration of non-starch components. Although starch is typically considered the biggest driver of digestible energy content of feed grains, Klopfenstein et al. (2008) indicated that the total energy of wet corn distillers grains can be as high as corn grain. Larson et al. (1993) had reported previously that utilization of these grains can decrease as inclusion levels increase. Nonetheless, wheat distillers grains can be an inexpensive energy and protein source in feedlot diets. Gibb et al. (2008) reported that when wheat distillers grains are included at only 5% in barley based finishing diets, they can exceed protein requirements of finishing cattle. Furthermore, dried distillers grains with solubles from wheat have similar energy content as barley in backgrounding diets, and inclusion of up to 20% wheat DDGS in barley based finishing rations has minimal effect on animal performance (Gibb et al., 2008).

Barley grain

Use in feedlot production

In the northern and western regions of the United States and much of Canada, barley is the principle feed grain used for growing and finishing beef cattle as well as lactating dairy cattle, due to climate and soil limitations that impede the production of corn (Campbell et al., 1995; Boss and Bowman, 1996). Though corn and milo are the preferred concentrates for cattle finishing in the southern and south western United States, Boss and Bowman (1996) reported similar growth rates and improvements in carcass quality grades of cattle fed corn and barley. Furthermore, Ovenell-Roy et al. (1998) found that carcass weight and loin muscle area of cattle fed barley were not different than those of cattle fed corn. Finally, Maltin et al. (1998) suggested that beef from cattle fed barley may be redder than those fed corn due to an increase in heme pigments, although these observations were not supported by Nelson et al. (2000) who found no differences in retail color score of beef from cattle fed barley or corn based finishing diets. Owens et al. (1997) reported that, when averaged across all processing methods, ADG of cattle fed barley was not different than that of cattle fed wheat, corn, milo, or oats and that DMI and F:G conversion was not different for cattle fed corn, wheat, or oats. Furthermore, the observed ME of barley and wheat were not different, a trend that continued when figures were adjusted for BW of cattle fed the respective grains. With barley prices typically lower than corn, feed cost of gain can be lower for barley based rations than corn based rations.

Variability in composition

Variation in composition of barley grain has been investigated by several researchers. (Bhatty et al. (1974)) reported ranges in crude protein of western grown barley grain to be from 12% to 17%; fiber content ranged from 4%-6%; and starch

content ranged from 44% to 56%. Furthermore, the amylose content of barley starch ranged from 23% to 33% and the range in B-glucan content was from 1.2% to 2.7%. This variation in composition was then attributed to a range of 3,184 to 3,558 kcal/kg digestible energy, determined in a mouse feeding model. Fairbairn et al. (1999) evaluated barley samples from five different varieties grown in Alberta, Saskatchewan, and Manitoba that were selected to be those used most heavily in the Canadian swine industry. In a feeding trial using pigs, the authors found that 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. Bhatti et al. (1979) reported an 8.2% difference in DE of hull-less cultivars and hulled cultivars (3,918 kcal/kg versus 3,918 kcal/kg). Though these experiments were conducted with pigs, similar variation in energy content can be expected in ruminant diets.

Digestibility

Lehman et al. (1995) investigated differences in *in situ* rumen degradability of different cultivars of barley after Givens et al. (1993) reported differences in barley composition of two- and six-row varieties and Bhatti et al. (1974) reported differences in chemical composition of various cultivars. Lehman et al. (1995) found that two row barley was more degradable at all time points than six row barley, hullless varieties were more degradable than hulled varieties at all time points except the initial wash, and rough-awned varieties were more degradable than smooth-awned varieties. Furthermore, there was little difference in the degradability of feed and malting types (Lehman et al., 1995). Givens et al. (1993) suggested that slower rates of ruminal degradation may be superior to more highly fermentable varieties in cattle production as increased incidence

of metabolic issues such as bloat, ruminal acidosis, and grain overload would be expected to increase with rate of degradation. Furthermore, Owens et al. (1986) reported that starch digested in the small intestine provided 42% more energy to the host animal than starch degraded in the rumen. Ultimately, Lehman et al. (1995) concluded that, since there were differences in rumen degradability across various varieties of barley, genetic selection and plant breeding could be conducted to improve animal performance in the feedlot.

Approximately 90% of the barley grown in Alberta is used in livestock feeds (McLelland, 1982). Nutrient composition of barley entering feedlots can be very diverse and mostly due to genetic variation (Anderson et al., 1984), cultivar (Fairbairn et al., 1999) or environmental and growing conditions (Berdahl et al., 1976; Fairbairn et al., 1999). The variation in barley composition can make accurate diet formulation difficult (Wiseman et al., 1982). Several studies have investigated the differences in chemical composition of different cultivars of barley (Bhatty et al., 1974; Bhatty et al., 1979), however data describing differences in digestibility or animal performance due to barley variety or cultivar are inconsistent. Lehman et al. (1995) reported differences in rumen degradability of barley due to cultivar of barley grain. Further, Boss and Bowman (1996) reported differences in animal performance, carcass quality, and intake of digestible starch due to barley variety. Cleary et al. (2011) however, was not able to detect differences in digestibility of barley due to differences in variety, seeding rate, location grown, or nitrogen fertilizer application rate.

In vitro fermentation

In the rumen, starch reactivity to amyloglucosidase is dependent on the disruption of or solubilization of the starch granule during processing. The protein matrix encapsulating wheat starch is primarily gluten, which is highly soluble in rumen fluid. This is in contrast to the protein matrix encapsulating corn starch, which is composed primarily of the promalin zein which is very insoluble in rumen fluid and, consequently, serves as a barrier to ruminal enzymatic degradation of the starch (Zinn, 1992). Furthermore, Zinn (1992) noted improvements in rumen microbial efficiency and greater ruminal nitrogen degradation (93% versus 55%).

The analytical gas production technique (López et al., 2007) can be used to evaluate rate of digestion by measuring cumulative gas production at different incubation time points. In this technique, substrates of interest are inoculated with a microbial inoculum and fermentation measurements are measured *in vitro*. The objective of this technique is to mimic the environment of a specific section of the gastrointestinal tract and microbial inoculum should therefore contain similar species and concentrations of microbes (Mould et al., 2005). The method operates under the principle that gas produced in the system is the result of microbial and enzymatic digestion of the sample (López et al., 2007). Mathematical equations have been developed to relate the quantity of gas produced to the rate of digestion with the assumption that total gas production is directly proportional to the rate of fermentation of the sample (France et al., 2000). Blank modules are typically included in the analysis to account for gas produced by the microbial digestion of solubilized feed matter that was present in the rumen of the donor animal(s) prior to ruminal fluid collection.

When a feed sample is mixed with microbial inoculum it is degraded and the degraded fraction is either utilized to support microbial growth or fermented to produce fermentation acids and by product gases. Gas production data can be interpreted in combination with in vitro fermentation measurements to determine the fraction of sample that was allocated to microbial biomass and that which was degraded (Rymer et al., 2005). Correlations have also been demonstrated between gas production and total volatile fatty acids, in vitro starch digestion, and in vitro dry matter disappearance (Trei et al., 1970) indicating that analytical gas production techniques can be used as a tool to estimate these parameters and concluded that gas production by rumen microorganisms may be useful as a guide to 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

Feed cost of gain accounts for 65-80% of the total cost of feedlot cattle production. Characterizing the inherent variability in feedstuffs using wet chemistry takes a considerable amount of time and is expensive. In large scale cattle feeding operations where large quantities of feed ingredients are procured, processed, and fed on a daily basis, conducting proximate analysis or wet chemistry in a commercial laboratory can be time and cost prohibitive. With the use of Near Infrared Reflectance Spectroscopy (NIRS), real-time nutrient compositions can be measured on site.

The composition of plant and animal tissue is ultimately reflected in the types of bonds between atoms and groups of atoms (Foley et al., 1998). The primary constituents of plant and animal tissue are carbon, nitrogen, oxygen and hydrogen. Near Infrared light (750-2500 nm) is absorbed primarily by C-H, N-H, and O-H bonds (Osborne et al., 1993). When a sample of light is irradiated, the bonds between atoms and functional groups vibrate at characteristic frequencies. Incident light with frequencies matching that of the bonds is absorbed while incident light with differing frequencies is transmitted or reflected (Foley et al., 1998). The chemical composition of a biological sample determines the number of each of these types of bonds, therefore the spectrum generated from the reflectance of near infrared light on a sample contains information regarding the composition (Burns and Ciurczak, 2007). The relationship between absorbed energy and the concentration of a specific type of bond or functional group in a sample is complicated by overlapping spectral bands from differing functional groups present in the sample. Since there is currently no mathematical relationship quantifying the interference of heterogenous components within a sample, NIRS is a secondary analytical technique and must be calibrated to samples of known chemical composition determined using standard primary methods (Givens and Deaville, 1999) .

The first practical applications of NIRS were in the grain industry. Williams (1975) and later Hunt et al (1977) investigated the application of the technology for testing composition of wheat grain in Canada and the United States, respectively. The findings of these investigators led to the adoption of NIRS as the official protein testing method for the marketing of wheat grain in both countries. Norris et al. (1976) were the first to investigate the application of the technology in forages for the feeding of sheep.

They showed that NIRS could be used to accurately predict ($R^2 \geq 0.90$) CP, ADF, NDF, L, and IVDMD; DMD, DMI, and DEI they were able to predict with a lesser degree of accuracy ($R^2 = 0.78, 0.64, 0.72$ respectively). Early NIRS research, though valuable, was limited by computing power available at the time. With the advent of more powerful, personal computers in the 1980's, rapid developments in the use and application of NIRS to grain and forage testing occurred (Givens and Deaville, 1999).

Traditional methods of analysis for animal feeds are typically expressed in terms of energy and protein and are derived using wet chemistry analyses and *in vitro* and *in vivo* metabolism methods. These methods are time consuming, expensive, and not practical in commercial environments. Research indicates that NIRS will become an invaluable tool for feed analysis, especially as emphasis transitions from energy and protein content to total nutrient supply (Givens and Deaville, 1999). Furthermore, NIRS may be used to predict the concentrations of and total VFAs from microbial degradation in the rumen (Sinnaeve et al., 1995). Additional uses of NIRS include the identification of unknown or poorly classified feedstuffs (De Boever et al 1993), mineral analysis (Clark et al., 1987; Smith et al., 1991; De Boever et al., 1994), specific amino acid analysis in addition to total nitrogen content (Williams et al., 1984). There are also potential applications of NIRS for the prediction of physical characteristics that may have secondary effects on nutritional value or animal utilization. The ability to predict kernel hardness of whole wheat has been investigated (Williams, 1997) and Edney et al. (1995) attempted to use NIRS to predict kernel plumpness of whole barley.

Most of the original work utilizing NIRS for analysis of feeds involved the scanning of oven dried and milled samples. This has a number of obvious drawbacks that

take away from the appeal for an onsite, real time analyzer. The use of NIRS for the prediction of nutrient composition, intake and digestibility of fresh forages was investigated by multiple researchers including Abrams et al. (1988), Sinnaeve et al. (1995), and Gordon et al. (1998) and it was ultimately concluded that NIRS predictions of undried and unmilled samples were also acceptable.

NIRS is desirable for nutrient prediction of feedstuffs largely due to its rapidity, no reagents are needed, no sample preparation is required, and multiple analyses are able to be performed simultaneously (Givens and Deaville, 1999). NIRS has been enthusiastically adopted by many sectors of the agriculture industries (Foley et al., 1998); and those interested in evaluating and improving the nutritive value of feedstuffs for livestock are some of the biggest users of the technology (Shenk and Westerhaus, 1994). Some NIRS methods are considered official analyses (AOAC 1990).

Use of NIRS in beef cattle production

NIRS has been shown to be able to successfully predict the voluntary intake of cattle. Park et al. (1997) showed that NIRS could predict voluntary intake of grass silage of 192 beef cattle to within ± 5.05 g DM/ kg LW. These results were supported by those of Steen et al (1998), who found that NIRS could more effectively predict intake than other laboratory methods. Many researchers have also attempted to predict in vivo digestibility of forages (Lindgren, 1983; Robert et al., 1986). Barber et al. (1990) showed that NIRS could predict organic matter digestibility more accurately than a wide range of commonly used laboratory methods. Others have successfully developed calibrations for

the prediction of digestibility and metabolizable energy of other forages including cereal straws and grasses (Givens et al., 1992).

There has been considerable effort devoted to the development of calibrations for chemical composition of concentrates. One of the earliest applications of NIRS was the estimation of the protein content of cereal grains (Givens and Deaville, 1999). In an attempt to improve rolling precision of barley, Edney et al. (1995) investigated the ability of NIRS to predict kernel plumpness. Williams (1997) explored calibrations for kernel hardness of wheat grain. Others have investigated the use of NIRS for mineral prediction. Smith et al. (1991) successfully predicted the magnesium content of perennial ryegrass and Clark and Lamb (1991) were able to develop calibrations for the prediction of calcium, phosphorous potassium and magnesium.

Limitations of NIRS

Williams (1975) noted that while infrared reflectance spectroscopy would likely be a major breakthrough in the routine analysis of cereal grains, its application depends as heavily upon mathematical statistics—mainly multiple linear regression analysis—as it does upon the phenomenon of infrared radiation. (Givens and Deaville (1999)) in their review also note that one of the chief disadvantages of NIRS technology is the complexity in choice of data treatment in addition to the technology's dependence on laborious and time consuming calibration procedures, and the expense of the instruments themselves. Perhaps one of the biggest drawbacks of NIRS is the fact that it is a secondary method of analysis. NIRS predictions often include error from several sources—the standard error of prediction (SEP), error associated with the primary

(laboratory) methods against which the instruments are calibrated, as well as any error associated with actual sample preparation and scanning. Lanari *et al.* (1991) had samples of dried beet pulp, lucerne, and hay analyzed by wet chemistry at twenty feed analysis laboratories and reported coefficients of variation of 17.8% for ether extract, 27.3% for lignin, and 7.4% for NDF. Additionally, Beever *et al.* (1996) reported coefficients of variation of 12.7% and 16% for CP and starch respectively, of corn silage samples analyzed at two independent laboratories.

The use of NIRS to segregate barley based on several chemical characteristics upon arrival to the feedlot has been investigated (Hussey, 2012). Here the authors found improvements in carcass adjusted-ADG and feed intake of cattle fed barley that had a lower starch:NDF ratio than cattle fed barley determined to be higher in starch:NDF as predicted by NIRS. In a separate trial, Hussey (2012) investigated the affect of segregating barley based on NIRS predicted DE content and reported a linear increase in rate of prime carcasses and a linear decrease in feed intake as the DE content of the barley increased. Furthermore, Hussey (2012) reported differences in metabolic mortality due to NIRS predicted DE treatment. These results indicate that NIRS may be able to be used to not only predict composition, but predict animal performance.

Current methods of quantifying grain quality

Canadian grown barley and wheat can provide 70-80% of the energy requirements of beef cattle (Bhatty et al., 1974) and in Canada, DE is considered the single biggest indicator of nutritional quality of feed grains (Christison and Bell, 1975).

The Canada Grains Council (1972) suggested that bulk weight is the most practical measure of energy content of feed grains.

Commercial feedlots and feedmills typically purchase barley based on the quality criteria of volume weight and moisture content (Grimson et al., 1987). It has generally been assumed that the feeding value of barley weighing more than 58 kg hL⁻¹ is considerably greater than lighter barley (Mathison et al., 1991). Volume weight and bushel weight are measurements of the sum of each of the chemical constituents as well as a measure of the space between kernels (Campbell et al., 1995). Using samples from six cultivars of barley and eight cultivars of wheat collected in duplicate from twelve locations over three years, Campbell et al. (1995) analyzed the correlations between test weight and CP, starch, fat, NDF, and ADF respectively. The chemical constituent of barley for which the highest correlation coefficient was observed was NDF ($R^2 = -0.66$). The relationship between CP content and test weight of barley was not significant ($P > 0.05$) and the correlation coefficients for the relationships between starch and fat were also relatively poor ($R^2 = 0.43$ and 0.29 , respectively). Similar results were presented for wheat grain. Correlation coefficients for test weight and protein, starch and fat were 0.14, 0.15, and 0.10, respectively; and the chemical constituent for which the strongest relationship to test weight was NDF ($R^2 = -0.44$). Furthermore, the correlation coefficients for test weight and DM for barley and wheat were 0.06 and 0.03, respectively (Campbell et al., 1995).

Utilizing samples of seventeen cultivars of hard and soft Canadian wheat and twenty-nine cultivars of two- and six row barley in a feeding experiment utilizing mice, Bhatti et al. (1974) found that the correlation coefficients for the relationship between

bulk weight of hard and soft wheat and digestible energy were -0.71 and -0.17 respectively; when sample sets for hard and soft wheat were combined the correlation coefficient was -0.59. The relationships were even worse for bulk weight and DE for 2-row and 6-row barley ($R^2 = -0.01$ and -0.04 , respectively). Plumpness was highly correlated with DE of soft wheat ($R^2 = -0.87$) but only moderately correlated with DE of hard wheat ($R^2 = -0.54$). 1000 kernel weight of soft wheat was a slightly better indicator of DE ($R^2 = -0.69$) than bulk weight, but it was a slightly worse indicator of DE of hard wheat ($R^2 = -0.55$). 1000 kernel weight was a better indicator of 2-row and 6-row barley DE than bulk weight ($R^2 = 0.13$ and 0.18 , respectively) but still a poor indicator overall ($R^2 = 0.28$). Plumpness of 2-row and 6-row barley was a better indicator of DE ($R^2 = 0.04$ and 0.16 , respectively) but still poor overall ($R^2 = 0.28$).

The work of Campbell et al. (1995) and Bhatta et al. (1974) indicate that bushel weight, plumpness, and 1000 kernel weight are poor indicators of overall grain quality. Additional research should be done to determine an accurate method of predicting grain quality so that feedlot producers can effectively manage grains coming into the feedlot.

Grimson et al. (1987) evaluated the relationships between volume weight and processing method on feedlot performance of yearling beef steers. Barley was selected as being light, medium, or heavy VW (47.8, 55.6, 66.6 kg hL⁻¹, respectively). It was found that cattle consuming light VW barley tended ($P = 0.086$) to have greater DMI than medium or heavy VW barley from d 0-27, though they also had significantly higher ($P = 0.017$) DM:G than cattle consuming heavy VW barley from d 27-55. DMI and ADG of cattle consuming heavy, medium, or light VW barley was not different ($P < 0.05$) during any of the examined time periods.

Chemical composition of barley is affected by volume weight (Mathison et al., 1991), but volume weight is only an indirect measurement of starch and fiber (Engstrom et al., 1992). Mathison et al. (1991) reported barley of 43, 59, and 64 kg hL⁻¹ volume weights differed in gross energy, CP, Calcium, Phosphorous, ADF, NDF, starch, and ash. High volume weight barley was higher in GE than medium weight barley and CP and starch than light weight barley and lower in ADF, NDF, and ash than light weight barley. Digestibility of these barleys and an additional 66 kg hL⁻¹ variety were analyzed. Apparent digestibility of fiber was lowest for cattle consuming the higher volume weight (lower fiber) barley. DM and energy digestibility of heavy and light volume weight barley were lower than medium weight barley. Starch digestibility was not different for cattle consuming high, medium, or light volume weight barley. However, organic matter digestibility of low volume weight barley was 2% less than steers fed the heavier three barleys. Although the light barley contained more fiber and less starch than the heavier barleys, no differences in ADG, DMI, or DM:G conversion of cattle fed 43, 69, 64 or 66 kg hL⁻¹ barley were observed (Mathison et al., 1991). These observations are supported by those of Grimson et al. (1987) who observed no differences in ADG of 192 steers fed light (47.8 kg hL⁻¹) versus heavy (66.6 kg hL⁻¹) barley. The exception is that (Grimson et al. (1987)) observed a 10% increase in DM:G ratio in cattle fed light versus heavy barley, although this difference was ascribed to the increased ADF content of lower volume weight barley.

Furthermore, Engstrom et al. (1992) reported differences in volume weight of commercial lots of barley that did not always correspond to differences in starch, CP, ADF, or NDF. Engstrom et al. (1992) found that B- glucans ultimately had no detectible

effect on digestion or utilization of barley in feedlot cattle, and ultimately suggested that ADF may be a better indicator of feed efficiency in feedlot cattle. Christison and Bell (1975) stated that criteria used for assessing milling and malting characteristics failed to effectively evaluate feed grains since they are not based on nutritive value. They reported that the single most important selection criterion for feed grains is available (i.e. digestible or metabolizable) energy, and of secondary importance is crude protein.

Physical characteristics of grain

Peterson et al. (2001) indicated that visual wheat grain color may be correlated to other grain traits such as protein content, hardness, vitreousness, and kernel shape and size. Lukow et al. (2012) analyzed samples of Canadian grown hard white spring wheat and reported that the combined effects of geographic location and weather conditions (agro-climatic zone) contributed 8.6% to and 21.8% of the variation in the grain quality parameters of grade and yield, respectively. Furthermore, the color variables L^* , a^* (red:blue), and b^* were also affected by agro-climatic zone and year. The agro-climatic zone accounted for 13.0% to 17.9% of the variation in color measurements, while year accounted for 71.3% to 78.6% of the variation. Furthermore, kernel brightness was highly correlated with kernel yellowness ($R^2 = 0.79$), similar to results for domestic wheat reported by Peterson et al. (2001). Lukow et al. (2012) reported that kernel color L^* and b^* values were negatively correlated to grain hardness index values ($R^2 = -0.49$ and -0.28 , respectively), again similar to results of Peterson et al. (2001) ($R^2 = -0.21$ and -0.26 , respectively).

Lukow et al. (2012) reported correlations of kernel color variable a^* with season maximum temperature ($R^2 = 0.53$) and mean growing temperature ($R^2 = 0.49$). Growing season maximum mean temperature had the greatest effect (32%) on kernel redness. Kernel yellowness was also moderately correlated to growing season mean maximum ($R^2 = 0.42$) and extreme maximum temperature ($R^2 = 0.42$), and mean maximum temperature accounted for 21% of the variation in yellowness.

Lukow et al. (2012) reported significant differences in kernel dimensions and weight across agro-climactic zones. Kernel hardness was affected by growing season mean minimum temperature and longitude as well as protein content and kernel diameter.

Protein content has been shown to be inversely related to yield in several studies (Stewart and Dwyer, 1990; DePauw et al., 2007; Oury and Godin, 2007). Lukow et al. (2012) and Smith and Gooding (1996) also reported correlations of protein content to growing season temperature. Kernel color variables L^* and b^* values have been shown to be negatively correlated to protein content (Matus-Cádiz et al., 2008; Lukow et al., 2012). (Chen et al., 1972) also reported positive correlations between kernel redness and test weight.

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CHAPTER III

VALIDATION OF NEAR INFRARED REFLECTANCE SPECTROSCOPY TECHNOLOGY FOR THE PREDICTION OF NUTRIENTS, IN VITRO DRY MATTER DIGESTIBILITY AND GAS PRODUCTION KINETICS OF WHEAT GRAIN

Abstract

Near infrared reflectance spectroscopy (NIRS) has been used to accurately predict the nutrient composition of feedstuffs. Considerable variation is observed in nutrient profiles of wheat grain used as an energy source in beef cattle diets. The objective of this study was to investigate the use of NIRS as a selection tool for wheat grain entering feedlots in western Canada by evaluating current prediction models with wet chemistry and in vitro fermentation measurements. Wheat samples (n = 75) were selected from three feedlots in western Canada from September, 2011 to April, 2012, representing a range in nutrient compositions as predicted by NIRS (InfraXact, FOSS North America, Eden Prairie, MN). Samples were selected for HIGH, MEDIUM, or LOW nutrient composition of CP, starch and DM as predicted by NIRS. Selected samples were then laboratory analyzed. DM was determined by placing samples in a forced air oven at 55°C for 48 h and measuring moisture loss. Starch and CP were determined using AOAC methods 992.23 and 996.11, respectively. Kinetics of gas production were

measured with gas pressure monitor modules (Ankom Technology Corp.) and IVDMD was determined using a procedure adapted from (Galyean, 2009). Lab values were correlated to the NIRS predictions for CP, starch and DM using PROC CORR of SAS 9.3 (SAS Institute, Cary, N.C.). NIRS predictions and laboratory values for CP were correlated for all samples ($R^2 = 0.86$, $P < 0.01$) whereas there were poor correlations for lab values and NIRS predictions for starch ($R^2 = 0.29$, $P = 0.29$) and DM ($R^2 = 0.42$, $P < 0.01$). Regression analysis was conducted to evaluate NIRS predictions across the ranges (HIGH, MED or LOW) of each constituent, CP, starch and DM. Improved R^2 values for each parameter were observed [CP = 0.95, $P < 0.01$; starch = 0.29, $P = 0.09$; DM = 0.45, $P = 0.09$] when only samples selected as HIGH, MED, or LOW for that constituent (n = 15 ea.) were included in the model. Cumulative gas production (M) was greater for Mid DM samples than Low or High DM samples but rate (k) and lag (l) of high DM samples were lower ($P < 0.05$) than Mid or Low DM samples. K and L of Low CP samples were lower than Mid or High CP samples ($P < 0.05$). There tended ($P = 0.10$) to be a linear effect on rate of gas production as starch content increased. These results indicate that NIRS technology is able to accurately predict CP of wheat samples in western Canada for a broad range of CP content. Furthermore, differences in amount of and rate at which gas is produced in vitro across ranges of DM and starch content indicate a greater need for accurate prediction of these nutrients by NIRS.

Introduction

Wheat grain is often used in place of, or in addition to, barley based cattle finishing diets in western Canada due to availability and favorable pricing (Riley, 1984). Owens et al. (1997) summarized data from 39 feed trials with 1,440 head of cattle fed a

variety of concentrate grain sources and utilizing a variety of processing methods. The authors observed that ADG, DMI, and feed efficiency of cattle fed wheat grain were not different than that of cattle fed corn or barley when averaged across all grain processing methods. Furthermore, they noted that the observed BW-adjusted ME of wheat in the review was shown not to be different than corn or barley and better than dry-rolled milo. Erjaei et al. (2012) observed that, when used in growing and finishing diets fed to cattle, starch is vital as the main energy constituent of wheat grain and that carbohydrate utilization is essential to improving cattle efficiency and performance. A large variation in CP and starch of Canadian wheat varieties was reported by Zijlstra et al. (1999) resulting in variation in chemical composition and DE. Bhatta et al. (1974) observed differences in DE content of seventeen cultivars of Canadian grown wheat due to cultivar and origin; furthermore, the authors observed moderate to strong correlations (correlation coefficients > 0.5) of DE with physical characteristics including 1,000-kernel weight, plumpness, and chemical characteristics including fiber, ash, and gross energy content. Nutrient variation is a reflection of these factors as well as genetic and environmental effects and the economic impact of this variation compounds the need for improved chemical composition determination when used in animal production (Zijlstra et al., 1999).

In the rumen, starch reactivity to amyloglucosidase is dependent on the disruption of or solubilization of the starch granule during processing. The protein matrix encapsulating wheat starch is primarily gluten, which is highly soluble in ruminal fluid. This is in contrast to the protein matrix encapsulating corn starch, which is composed primarily of the prolamins zein which is very insoluble in ruminal fluid and, consequently,

serves as a barrier to ruminal enzymatic degradation of the starch (Zinn, 1992). Furthermore, Zinn (1992) noted improvements in rumen microbial efficiency and greater ruminal nitrogen degradation (93% versus 55%) of wheat versus corn.

The analytical gas production technique (López et al., 2007) can be used to evaluate rate of digestion by measuring cumulative gas production at different incubation time points. In this technique, substrates of interest are inoculated with a microbial inoculum and fermentation measurements are measured *in vitro*. The objective of this technique is to mimic the environment of a specific section of the gastrointestinal tract and microbial inoculum should therefore contain similar species and concentrations of microbes (Mould et al., 2005). For this reason, microbial inoculum used in this experiment was ruminal fluid collected from an individual animal in combination with an artificial buffer (McDougall, 1948) similar to that of bovine saliva. The method operates under the principle that gas produced in the system is the result of microbial and enzymatic digestion of the sample (López et al., 2007). Mathematical equations have been developed to relate the quantity of gas produced to the rate of digestion with the assumption that total gas production is directly proportional to the rate of fermentation of the sample (France et al., 2000). Blank modules are typically included in the analysis to account for gas produced by the microbial digestion of solubilized feed matter that was present in the rumen of the donor animal(s) prior to ruminal fluid collection.

When a feed sample is mixed with microbial inoculum it is degraded and the degraded fraction is either utilized to support microbial growth or fermented to produce fermentation acids and by-product gases. Gas production data can be interpreted in combination with *in vitro* fermentation measurements to determine the fraction of sample

that was allocated to microbial biomass and that which was degraded (Rymer et al., 2005). Correlations have also been demonstrated between gas production and total volatile fatty acids, in vitro starch digestion, and in vitro dry matter disappearance (Trei et al., 1970) indicating that analytical gas production techniques can be used as a tool to estimate these parameters and Trei et al. (1970) concluded that gas production by ruminal microorganisms may be useful as a guide to determining the relative feeding value of processed grain.

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, among other common parameters common to animal feeds (Foley et al., 1998). Stubbs et al. (2009) outlined the advantages of NIRS analysis as a non-destructive, low cost analysis that provides rapid results. In addition, Stubbs et al. (2009) described NIRS technology as one which allows for a larger range of samples to be tested and multiple properties to be tested simultaneously.

The objective of this experiment was to evaluate the use of commercially available NIRS technology for the nutrient prediction of wheat grain entering feedlots in western Canada, and the effect of nutrient range on in vitro dry matter digestibility, as well as lag, rate, and total gas production.

Materials and Methods

Sample Selection Whole wheat samples entering nine feedlots in Western Canada were sampled prior to unloading at the facility from September 2011 to April 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 apparent distributions of the overlying population, selection criteria for the study population were determined. Study population samples were selected from September 2011 to April 2012 from three of the nine feedlots and were selected as being in the top 10% (high), middle 10% (mid), and bottom 10% (low) for each of the four identified parameters (ex. 15 total samples selected for CP: 5 high, 5 Mid, and 5 low). Additional samples (n = 15) were selected at random (RANDOM) to be included in the study population. Criteria of study population (n = 75) is described in Table 3.1.

Laboratory Analysis All procedures involving live animals were approved by the Oklahoma State University Care and Use Committee.

Following collection and scanning at the feedlot, samples were sent to Oklahoma State University where laboratory analyses were performed. All samples were ground through a 2 mm screen using a Wiley grinding mill (Thomas Scientific, Swedensboro, NJ). Analysis of DM was conducted using a forced air oven at 55 °C for 48 h. Ash was determined by incinerating samples in a muffle oven at 500 °C for 8 hours. Crude protein analysis was conducted using a LECO nitrogen analyzer (LECO Corporation, St. Joseph, Michigan) according to procedure 992.23 of the Association of Official Analytical Chemists (AOAC). Starch analysis was conducted using sequential enzymatic digestion steps starting with thermostable α - amylase to solubilize starch into maltodextrins, followed by amyloglucosidase to degrade the maltodextrins to D - glucose, which is oxidized to D - gluconate in the last step, releasing 1 mol of hydrogen

peroxide (Megazyme Int. Ireland Ltd., Wicklow, Ireland), the concentration of which was then measured quantitatively using a calorimeter (SpectraMax M3 Plate Reader, Molecular Devices, LLC, Sunnyvale, California) according to AOAC method 996.11. Each starch run included quadruplicate blanks, duplicate corn starch controls, and a duplicate internal control. Both NDF and ADF were determined using a procedure adapted from Van Soest et al. (1991) utilizing the Ankom system (Ankom[®], Tech. Co., Fairport, NY, USA). All analyses were performed in duplicate. For DM, and CP, coefficients of variation of 0.5 and 2% were used to determine sample acceptability and the need for subsequent re-runs (Galyean and May, 1989). For ADF and NDF, a CV of 5% was used and for ash a CV of 2% was used. A CV of 5% was used for replicate samples in starch analysis.

Ruminal fluid used for the analysis of in vitro fermentation and gas production kinetics 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 wheat based diets for rumen microbe populations. Feed was offered once daily and water was offered *ad libitum*. The animal was adapted to the diet over a period of 21 days prior to the first ruminal fluid collection. Ruminal fluid was collected between 4 and 6 h post feeding, was strained through 4 layers of cheese cloth into a 2 L pre-warmed thermos, and transported to the Ruminant Nutrition Laboratory (Stillwater, Ok). Within approximately 30 minutes of sampling, ruminal fluid was used for the culture of inoculum.

IVDMD IVDMD was conducted using an adapted procedure of (Galyean, 2009), 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 CO₂ and capped with rubber stoppers and placed into a 39 °C water bath. Contents of tubes were gently agitated every 6-8 h for 48 h. Following the 48h incubation with ruminal fluid, samples were taken from the 39 °C water bath 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. After the addition of HCl, 2 mL of 5% pepsin was added and again the tube was gently swirled. Rubber stoppers were reinserted and tubes were placed back into the 39 °C water bath for 24 h. Tubes were gently agitated 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 Watman's No. 4 filter paper. Filter paper and residue of each sample was dried in a forced air oven for 48 h at 55 °C. IVDMD was calculated as follows and expressed as a percentage:

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

Sample weight (DM basis)

In vitro kinetics of gas production Eighteen gas pressure monitor modules (Ankom Technology Corp.) were used in combination with 250 mL serum bottles in duplicate for each sample. Two blanks were used in each run and were treated similarly to treatment

serum vials but with no substrate added, which provided an estimate of gas production from the microbial inoculum itself. Each 250 mL module received 0.7 ± 0.01 g of sample and McDougalls buffer (37.5 mL; (McDougall, 1948) and 12.5 mL of ruminal fluid (50 mL of 3:1 buffer to ruminal fluid ratio). Each flask was flushed with CO₂ after the addition of McDougall's buffer: ruminal fluid mixture and the monitor cap then fastened. A 24 hour incubation was chosen because preliminary observations indicated that maximum gas production occurred prior to 24 h and this was supported by observations of Getachew et al. (2005) analyzing corn samples. Flasks were inserted into a 39 °C shaking water bath set at 45 rpm (Thermo Fisher Scientific Inc.) for 24 h. Gas pressure data collected by each gas pressure monitor module was sent wirelessly to a base coordinator unit every 30 minutes for 24 h. To eliminate gas pressure buildup the pressure monitor modules released gas from within the flask when the pressure inside the flask reached 20.7 kPa. Gas volumes released from the flasks in this manner were included in cumulative gas production readings at each time point. Gas pressure was measured in psi and then converted to mL 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. Gas production for each sample was corrected for gas production introduced into the system by the ruminal fluid (based on blank serum vials).

Statistical Analysis

Prediction model validation Laboratory values and NIRS predictions for corresponding parameters for all samples were determined using PROC CORR of SAS (SAS Institute Inc., Cary, NC) to determine the relationship between NIRS predictions and laboratory determined values. The initial analysis included all (n = 75) samples. Subsequent analyses were conducted where only the samples selected as high, medium, or low for DM, CP, or starch were included in the model. Fat analysis was not able to be conducted on these samples, so regression analysis was not performed for only the fat-selected samples.

In Vitro Analyses 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 PROC GLIMMIX 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 as main effects and run was included as a random effect. In the subsequent analysis, laboratory-determined values for DM, CP, starch, NDF and ADF were included in the model as main effects with run included as a random effect. In both analyses, gas production data were analyzed hourly for 24 h. For all statistical analyses, significant effects were observed at $P \leq 0.05$, and tendencies declared at P – values between 0.05 and 0.10.

Results and Discussion

Selection criteria for the study population for high, medium, and low DM, CP, and starch samples are presented in Table 3.1. Wet chemistry results and corresponding NIRS predictions for DM, ash, CP and starch of the study population ($n = 75$) are presented in Table 3.2, and for only the samples selected for each DM, CP, and starch can be found in Table 3.3. These results were used to validate existing prediction models for respective parameters.

Regressing the NIRS predictions for DM against the lab determined DM values demonstrated poor accuracy (Figure 3.1, $R^2 = 0.42$, $P < 0.01$) of the prediction model. When only the samples selected for analysis due to being either high, medium, or low in DM content ($n = 15$) were included in the regression model, the prediction accuracy increased (Figure 3.2, $R^2 = 0.45$, $P = 0.09$). The inaccuracy of the NIRS prediction model for DM is demonstrated further by the average DM content of each of the five samples selected for being high, medium, or low for these parameters—the attempted difference was not achieved for high and medium samples (Table 3.4). Although wet chemistry DM values of the high and medium NIRS predicted DM samples were greater than the wet chemistry values of the NIRS predicted low DM samples, using NIRS prediction for sample selection did not achieve the attempted range.

When DM-selected samples were analyzed by NIRS predicted range of DM it was observed that high DM samples had significantly more ($P < 0.05$) cumulative gas production than middle and low DM samples at all-time measurements (Figure 3.8). Interestingly, NIRS predicted high DM samples also had greater IVDMD than mid DM samples (Table 3.6), similar to previous observations that cumulative gas production is highly correlated with IVDMD (Trei et al., 1970; France et al., 2000). Furthermore, these

results are supported by those of Wang et al. (2003) who observed increases in animal performance of cattle when moisture was greater than 10%. IVDMD of NIRS predicted low DM samples was not different than that of NIRS predicted high or mid DM samples. Furthermore, cumulative gas production of NIRS predicted low DM samples was not significantly different ($P > 0.05$) than middle DM samples for hours 0 through 11. There was a tendency ($P = 0.08$) for cumulative gas production of NIRS predicted low DM samples to be greater than NIRS predicted mid DM samples at hour 12. Cumulative gas production of NIRS predicted low DM samples was greater than NIRS predicted mid DM samples ($P < 0.05$) for hours 13 through 24. There were no differences in lag (h) or rate of gas production (k; mL/h) in NIRS predicted high, medium or low DM samples. This is explained by the poor prediction accuracy of the DM calibration used.

Overall, the range of DM as predicted by NIRS was a poor indicator of total gas production or gas production kinetics, but wet chemistry lab determined DM values were strong indicators of kinetics of gas production parameters (Table 3.6). Gas production was significantly higher ($P = 0.01$) for high DM samples than medium or low DM samples when wet chemistry DM values were used. Ironically the rate and lag of gas production of high DM samples was significantly lower than medium or low DM samples ($P < 0.01$). Differences in lag or rate of gas production across ranges in DM have been reported for other grains (Wang et al., 2003), supporting the results seen when wet chemistry DM values were used.

Although significant differences were observed between cumulative gas production and range of DM as determined by wet chemistry (Table 3.6), cumulative gas production of individual samples was poorly correlated with NIRS predictions for DM

(Table 2.11; $R^2 = 0.13$, $P > 0.05$) and lab determined DM values (Table 3.11; $R^2 = 0.02$, $P > 0.05$). Lab determined and NIRS predictions for DM were negatively correlated with rate of gas production ($R^2 = -0.25$, -0.45 respectively, $P < 0.05$).

Crude protein was the only wheat prediction model for which the prediction accuracy was deemed acceptable (Figure 3.4, $R^2 = 0.86$, $P < 0.01$). Similar to observations for DM, when only samples selected for CP were included in the regression model, the prediction accuracy increased (Figure 3.5, $R^2 = 0.95$, $P < 0.01$). Figure 3.9 shows cumulative gas production of NIRS predicted CP samples analyzed by range. NIRS predicted high CP samples had greater cumulative gas production ($P < 0.05$) than NIRS predicted low CP samples at all-time measurements except hours 2 through 4 at which time points NIRS predicted high CP samples had a tendency ($P > 0.07$) to be greater than NIRS predicted low CP samples. These observations contradict those of Lanzas et al. (2007) who reported a negative correlation between gas production and CP content of wheat grain although it should be noted that the magnitude of the Spearman correlation coefficient was relatively weak (-0.08). Interestingly, Lanzas et al. (2007) reported a stronger positive association between cumulative gas production and neutral detergent insoluble crude protein.

Cumulative gas production of NIRS predicted High CP samples did not differ from mid CP samples from 0 to 7 hours but was significantly greater for NIRS predicted high CP samples than NIRS predicted low CP samples from hours 8 through 24. NIRS predicted Low CP samples had significantly less cumulative gas production ($P < 0.05$) than NIRS predicted mid CP samples for hours 0 through 2 and 6 through 11. NIRS predicted Low CP samples tended ($0.05 < P > 0.1$) to have less cumulative gas

production from hours 3 through 5 and 12. No differences were observed in cumulative gas production of NIRS predicted low CP and mid CP samples from hours 13 through 24. No differences in cumulative gas production were observed across wet chemistry CP ranges but lab determined low CP samples had significantly smaller k and L values ($P < 0.05$). Furthermore, CP content had a significant linear effect on rate and lag of gas production ($P < 0.05$), indicating that CP content affects rate of in vitro degradation of wheat grain. The effect of CP content on digestibility of wheat is not well understood, but Kotarski (1992) suggested that the protein matrix encapsulating the starch granules may be a factor in understanding rate of fermentation in barley. This is not supported by the results of the current experiment however, as the protein matrix encapsulating starch is understood to serve as a barrier to microbial digestion (Zinn, 1992), in which case the low CP sample should have had greater rates of fermentation.

The highest correlation coefficient observed for NIRS predicted parameters and in vitro fermentation measurements was for rate of gas production (k; mL/h) and CP (Table 3.11, $R^2 = 0.35$, $P < 0.05$). Not surprisingly, the highest correlation coefficient between lab determined values and in vitro fermentation measurements was also observed for CP and k (Table 3.11, $R^2 = 0.46$, $P < 0.05$). This is explained by the prediction accuracy of the CP calibration model and the chemical composition of the NIRS predicted high, medium, and low CP samples (Table 3.4). Total gas production, rate and lag of gas production and IVDMD were poorly correlated with NIRS predictions and lab determined values for CP. Our results for IVDMD are not supported by those of Barton et al. (1976) who reported a strong relationship ($R^2 = 0.90$, $P < 0.01$) between CP and IVDMD of tropical grasses.

Lastly, the prediction model for starch was deemed unacceptable (Figure 3.6; $R^2 = 0.12$, $P = 0.29$) and the relationship was also not significant. When only the samples selected for starch content were included in the model the coefficient of determination was improved (Figure 3.7, $R^2 = 0.29$, $P = 0.09$), however the NIRS predictions were still considered inaccurate. Additionally, the average starch content as determined by wet chemistry of the five samples selected for being high starch as predicted by NIRS was 58.38%, and the average wet chemistry starch composition of the samples selected for medium starch as predicted by NIRS was 58.77% (Table 3.4). While samples selected for being high and medium in starch content were higher than that of samples selected by NIRS to be low starch, wet chemistry results of these samples indicate that the true range in starch composition of the population may not have been represented by the study population.

It is important to note that NIRS scans were generated from whole kernel wheat rather than ground and/or dried samples. A limitation of previous applications of NIRS is that samples must be dried and ground prior to scanning, adding time and cost to the analysis (Foley et al., 1998). While we can speculate that scanning ground samples may have resulted in more accurate predictions, the technology would have lost appeal to feedlot operators as a tool for *real time* analysis had grinding been required prior to scanning. As such, no sample preparation (i.e. grinding or drying) was done prior to scanning in this experiment.

Figure 3.10 shows cumulative gas production of samples selected for starch analyzed by range. No differences were observed in cumulative gas production between NIRS predicted high, medium, or low starch samples at any time period. In addition to

total gas production, rate and lag of gas production as well as IVDMD were poorly correlated with NIRS predictions and lab determined values for starch (Table 3.8). However, there were also no significant effects of starch content as determined by wet chemistry on IVDMD, cumulative gas production, or lag time but it tended ($P = 0.10$) to have a negative linear effect on rate of gas production. These results are supported by the observations of Lanzas et al. (2007) who reported poor correlations between total volume of gas produced and starch content ($R^2 = -0.08$, $P > 0.10$). 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. Regardless, it has been reported by multiple authors that digestibility is affected more by the physical structure of the kernel of the grain rather than its chemical composition (Kotarski, 1992; Lanzas et al., 2007)

The prediction model for ash was also determined to be unsatisfactory (Figure 3.3) as the coefficient of determination was $R^2 = 0.09$ and this relationship was found to be insignificant ($P = 0.46$). Prediction models for NDF and ADF for wheat were not available using the technology evaluated in this experiment but ultimately should be developed; as such, wet chemistry analyses for these parameters were conducted on all samples. Correlations of in vitro parameters with wet chemistry derived NDF and ADF values are presented in Tables 3.9 and 3.10 respectively. NDF was not correlated with IVDMD (Table 3.11, $R^2 = 0.06$, $P = 0.62$), maximum gas production (Table 3.11, $R^2 = 0.03$, $P = 0.79$), rate (Table, 3.11, $R^2 = -0.16$, $P = 0.17$), or lag of gas production (Table 3.11, $R^2 = -0.07$, $P = 0.53$). Although rate of gas production was not significantly correlated to individual NDF values, range of NDF content tended to have a negative

linear effect on rate of gas production (Table 3.9, $P = 0.10$). ADF was loosely correlated with IVDMD (Table 3.10, $R^2 = -0.23$, $P = 0.05$) but was not correlated with maximum gas production ($R^2 = -0.05$, $P = 0.68$), rate ($R^2 = -0.003$, $P = 0.98$), or lag of gas production ($R^2 = 0.04$, $P = 0.76$). Results of our study, at least for IVDMD, contradict those of Barton et al. (1976), who reported a significant negative correlation between IVDMD and NDF of tropical and temperate grasses ($R^2 = -0.62$, $P < 0.01$) and a significant positive correlation between ADF of temperate grasses ($R^2 = 0.62$, $P < 0.05$), respectively. Relationships between NDF and ADF with IVDMD (Barton et al., 1976) as well as the relationship between total gas production and rate of fermentation (France et al., 2000) indicate that NDF and ADF may effect kinetics of gas production, though our results do not support that.

Conclusion

Significant variation exists in the composition of wheat grain entering feedlots in western Canada and the United States, regardless of one feedlots proximity to another. These observations reinforce the need for accurate nutrient determination of wheat grain. The prediction models for wheat grain utilized in this study were overall poor. The only parameter in our analysis that NIRS predicted in an acceptable range was CP. In order for NIRS technology to add value to production systems as a real-time, on-site tool for nutrient prediction, current prediction models for starch, DM, and ash must be improved in samples scanned whole. Additionally, the prediction model for fat should be evaluated for its prediction accuracy. Furthermore, NDF and ADF prediction models should be developed and validated. The study population used here would be acceptable for NDF and ADF calibration development. Since these samples were selected to represent a

range in DM, CP, starch, and fat, it follows that they represent a broad range in NDF and ADF content as well. The NDF content of the study population ranged from 11.7% to 47.7%. The average NDF content was 19.3%, substantially higher than book values (NRC, 1996). The ADF content of the study population ranged from 3.29% to 5.84%, averaging 4.26% which is similar to reference values (NRC, 1996). An additional population with similar range in composition will need to be selected and analyzed in order to validate the prediction models.

Understanding the variation in nutrient composition of wheat grain becomes increasingly important as fluctuations in commodity prices and high demand for barley create situations where wheat is competitively priced for feedlot rations in Canada and the pacific northwest. In vitro fermentation and gas production measurements can provide valuable information regarding the rate and extent of digestibility in vivo and be useful in determining overall feed value. Much of the appeal of NIRS to feedlot operators lies in its rapidity. In order for it to be marketable to producers, an acceptable level of accuracy must be achieved. Adding to the challenge of achieving desirable predictions are the typical inter- and intra- lab variation in wet chemistry analyses. For many chemical parameters, multiple wet chemistry methods are available for determination. In order to achieve maximum prediction accuracy, the error associated with both the prediction model itself, and the reference methods against which it is calibrated, must be minimized. More precisely formulating rations as well as improving animal health and performance through a better understanding of the relationships of cumulative gas production, rates of fermentation, and total digestibility, will ultimately enable feedlots to be more productive and more profitable.

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Tables and Figures

Table 3.1. Near infrared reflectance spectroscopy selection criteria for barley grain study population (dry matter basis).

Item	High	Mid	Low
Dry Matter, %	> 87.3	86.4 – 87.0	< 85.6
Crude Protein, %	> 15.3	12.7 - 14.7	< 10.8
Starch, %	> 70.5	66.2 - 70.1	< 63.6

Wheat grain samples (n = 75) were selected from whole wheat entering 3 feedlots in western Canada which represented the top 10% (High), middle 10% (Mid), and bottom 10% (Low) for each parameter listed relative to a subpopulation tested at each site between September 2011 and February 2012.

Table 3.2. Average difference between laboratory determined wet chemistry values and near infrared spectroscopy predictions for all wheat samples (n = 75).

Item	LAB	NIRS	R²	P - value
Dry Matter, %	91.3 ± 1.29	87.8 ± 1.29	0.42	< 0.01
Ash, %	1.71 ± 0.17	2.0 ± 0.24	0.09	0.46
Crude Protein, %	14.6 ± 2.13	13.8 ± 2.07	0.86	< 0.01
Starch, %	61.8 ± 6.94	67.8 ± 3.35	0.12	0.29

Wheat grain samples (n = 75) were selected from whole wheat entering 3 feedlots in western Canada. Samples were scanned on-site using commercially available NIRS technology (Foss North America). A subsample was sent to Oklahoma State University for wet chemistry analysis.

All values are presented as the mean ± the standard deviation.

Table 3.3. Average difference between laboratory determined wet chemistry values and near infrared spectroscopy predictions for study population wheat samples (n = 15 ea.)

Item	LAB	NIRS	R²	P - value
Dry Matter, %	90.6 ± 1.01	87.4 ± 1.81	0.45	0.09
Crude Protein, %	14.2 ± 2.75	13.5 ± 2.81	0.95	< 0.01
Starch, %	60.6 ± 6.38	67.7 ± 4.31	0.29	0.09

Wheat grain samples (n = 75) were selected from whole wheat entering 3 feedlots in western Canada. Samples were scanned on-site using commercially available NIRS technology (Foss North America). A subsample was sent to Oklahoma State University for wet chemistry analysis.

All values are presented as the mean ± the standard deviation.

Table 3.4. Chemical composition of selected wheat samples for DM, CP, and starch groups (n = 45) as determined by wet chemistry.

Item	High			Mid			Low		
	n	%	SD	n	%	SD	n	%	SD
DM, %	5	90.8	0.68	5	91.2	0.72	5	89.6	0.97
CP, %	5	17.5	0.43	5	14.0	0.58	5	11.1	0.67
Starch, %	5	58.4	4.08	5	58.8	7.00	5	64.5	6.88

Table 3.5. Average in vitro fermentation measurements of wheat samples (n = 75) entering feedlots in Western Canada .

Item	Mean	SD
IVDMD, %	90.3	5.80
Gas production, mL/g of substrate DM	287	17.20
k, mL / h	26.2	3.69
Lag, h	2.20	0.83

Table 3.6. Effects of DM as determined by NIRS and wet chemistry on in vitro fermentation measurements for wheat samples.

Item	NIRS				P- values				Lab				P- values			
	High	Mid	Low	SEM	Range	Lin	Quad	High	Mid	Low	SEM	Range	Lin	Quad		
N	5	5	5					25	25	25						
IVDMD, %	93.4 ^a	86.5 ^b	90.0 ^{ab}	2.01	0.09	0.24	0.06	90.0	90.2	91.0	2.40	0.76	0.50	0.78		
Gas production, mL/g of substrate DM	293	265	251	11.2	0.16	0.35	0.09	291 ^a	280 ^b	289 ^a	4.26	0.01	0.64	< 0.01		
k, mL / h	30.1	26.9	24.2	1.41	0.22	0.11	0.52	23.8 ^b	27.3 ^a	27.9 ^a	0.77	< 0.01	< 0.01	0.05		
Lag, h	2.30	2.59	2.48	0.355	0.84	0.74	0.64	1.84 ^b	2.33 ^a	2.38 ^a	0.210	< 0.01	< 0.01	0.16		

^{ab} means within a row with different superscripts differ at $P < 0.05$.

^lParameters were estimated by fitting a modified Gompertz function, with k = fractional rate of fermentation, and Lag = duration of the lag phase.

Table 3.7. Effects of CP as determined by NIRS and wet chemistry on in vitro fermentation measurements for wheat samples.

Item	NIRS				P- values			Lab				P- values		
	High	Mid	Low	SEM	Range	Lin	Quad	High	Mid	Low	SEM	Range	Lin	Quad
N	5	5	5					25	25	25				
IVDMD, %	91.8	91.7	93.4	4.12	0.71	0.51	0.65	89.9	90.2	91.1	2.40	0.74	0.45	0.84
Gas production, mL/g of substrate DM	286 ^{ab}	273 ^b	315 ^a	9.30	0.02	0.05	0.03	288	282	289	4.6	0.17	0.78	0.07
k, mL / h	26.5 ^a	23.2 ^b	21.9 ^b	0.812	< 0.01	< 0.01	0.37	27.8 ^A	27.9 ^A	23.4 ^B	0.81	< 0.01	< 0.01	< 0.01
Lag, h	2.66	2.16	1.67	0.523	0.16	0.06	0.99	2.31 ^A	2.34 ^A	1.90 ^B	2.33	0.03	0.03	0.16

^{ab} means within a row with different superscripts differ at $P < 0.05$.

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

Table 3.8. Effects of starch as determined by NIRS and wet chemistry on in vitro fermentation measurements for wheat samples.

Item	NIRS				P- values			Lab				P- values		
	High	Mid	Low	SEM	Range	Lin	Quad	High	Mid	Low	SEM	Range	Lin	Quad
N	5	5	5					25	25	25				
IVDMD, %	92.1	90.9	89.6	3.27	0.39	0.18	0.95	90.4	91.5	89.3	2.30	0.33	0.47	0.19
Gas production, mL/g of substrate DM	271	271	266	7.4	0.89	0.99	0.64	289	288	282	4.40	0.18	0.11	0.36
k, mL / h	27.6 ^a	26.1 ^{ab}	24.0 ^b	1.80	0.49	0.25	0.90	25.6	26.0	27.3	0.81	0.21	0.10	0.55
Lag, h	2.65	2.70	2.06	0.217	0.12	0.06	0.38	2.20	2.24	2.13	2.35	0.84	0.69	0.67

^{ab} means within a row with different superscripts differ at $P < 0.05$.

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

Table 3.9. Effect of NDF as determined by wet chemistry on in vitro fermentation measurements.

Item	High	Mid	Low	SEM	Range	Lin	Quad
n	25	25	25				
IVDMD, %	90.8	90.8	89.7	2.33	0.69	0.50	0.64
Gas production, mL/g of substrate DM	286	289	284	4.4	0.29	0.46	0.16
k, mL / h	25.3 ^b	25.8 ^b	27.9 ^a	0.79	0.02	0.01	0.30
Lag, h	2.22	2.18	2.17	0.210	0.96	0.79	0.94

^{ab} means within a row with different superscripts differ at $P < 0.05$.

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

Table 3.10. Effect of ADF as determined by wet chemistry on in vitro fermentation measurements.

Item	High	Mid	Low	SEM	Range	Linear	Quad
n	25	25	25				
IVDMD, %	90.0	91.2	90.4	2.38	0.72	0.80	0.43
Gas production, mL/g of substrate DM	288	286	286	4.5	0.87	0.70	0.71
k, mL / h	26.1	26.4	26.4	0.86	0.95	0.76	0.89
Lag, h	2.20	2.24	2.13	0.216	0.86	0.74	0.68

^{ab} means within a row with different superscripts differ at $P < 0.05$.

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

Table 3.11. Correlations of chemical constituents and in vitro fermentation measurements as determined by NIRS and wet chemistry.

Item	NIRS			Lab				
	DM	CP	Starch	DM	CP	Starch	NDF	ADF
IVDMD, %	0.05	-0.14	0.14	-0.12	-0.10	0.23*	0.06	-0.23**
Gas production, mL/g of substrate DM	0.13	-0.12	0.01	0.02	-0.10	0.12	0.03	-0.05
k, mL / h	-0.25*	0.35*	0.26*	-0.45*	0.46*	-0.14	-0.16	-0.003
Lag, h	0.03	0.180	0.26*	-0.25*	0.24*	0.03	-0.07	0.04

* $P \leq 0.05$

** $P < 0.10$

Pearson correlation coefficients presented for the correlation between NIRS predictions and corresponding wet chemistry values for each of the parameters DM, CP, and starch.

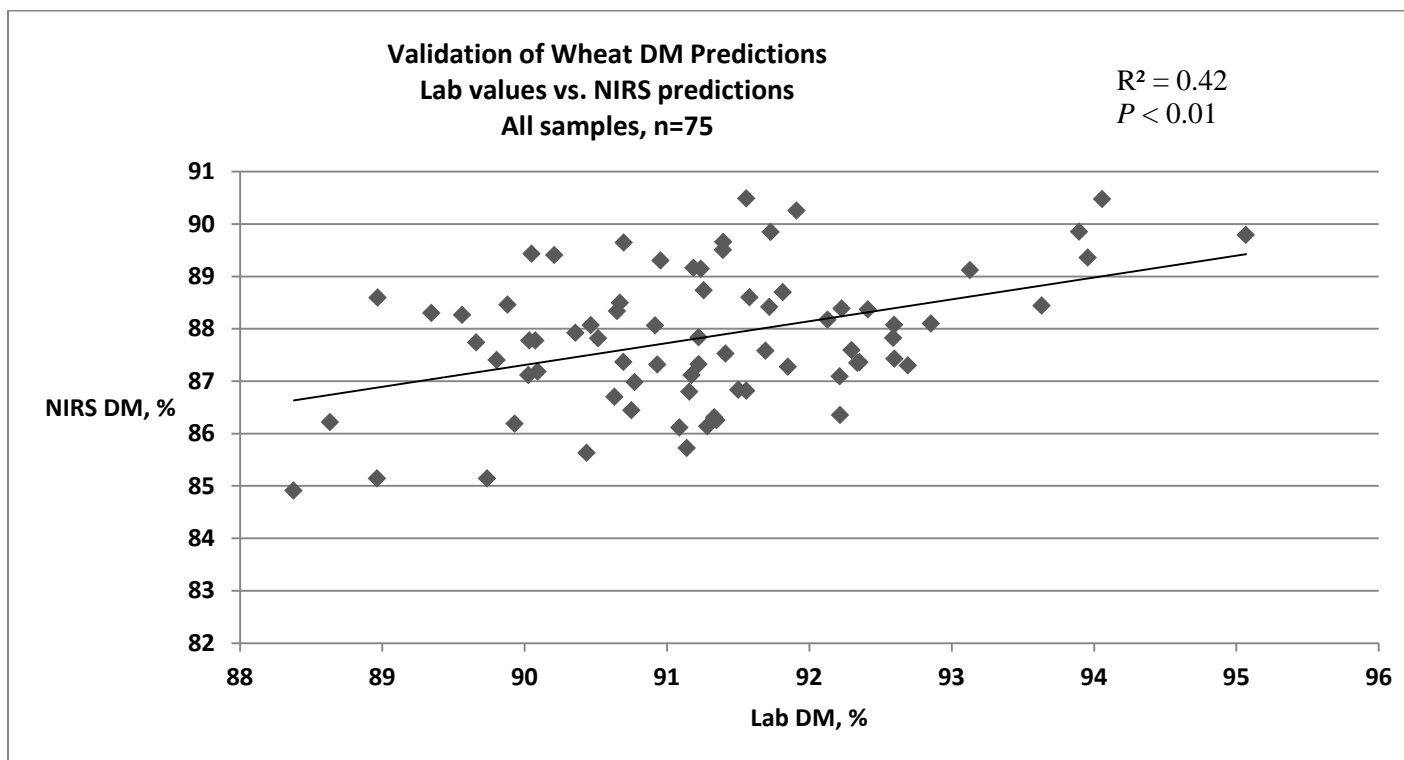


Figure 3.1. Validation of NIRS predictions of DM for all wheat grain samples.

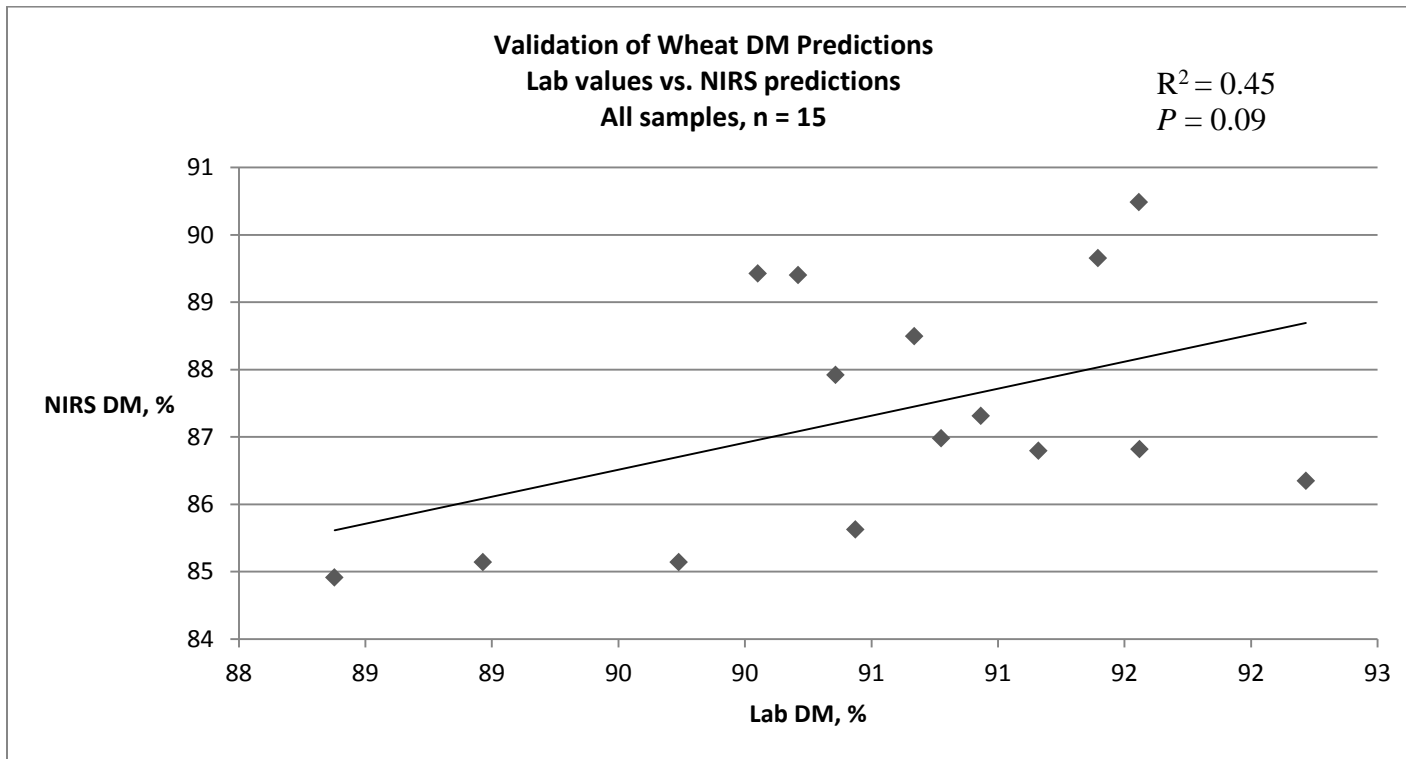


Figure 3.2. Validation of NIRS predictions of DM for wheat grain samples selected for DM.

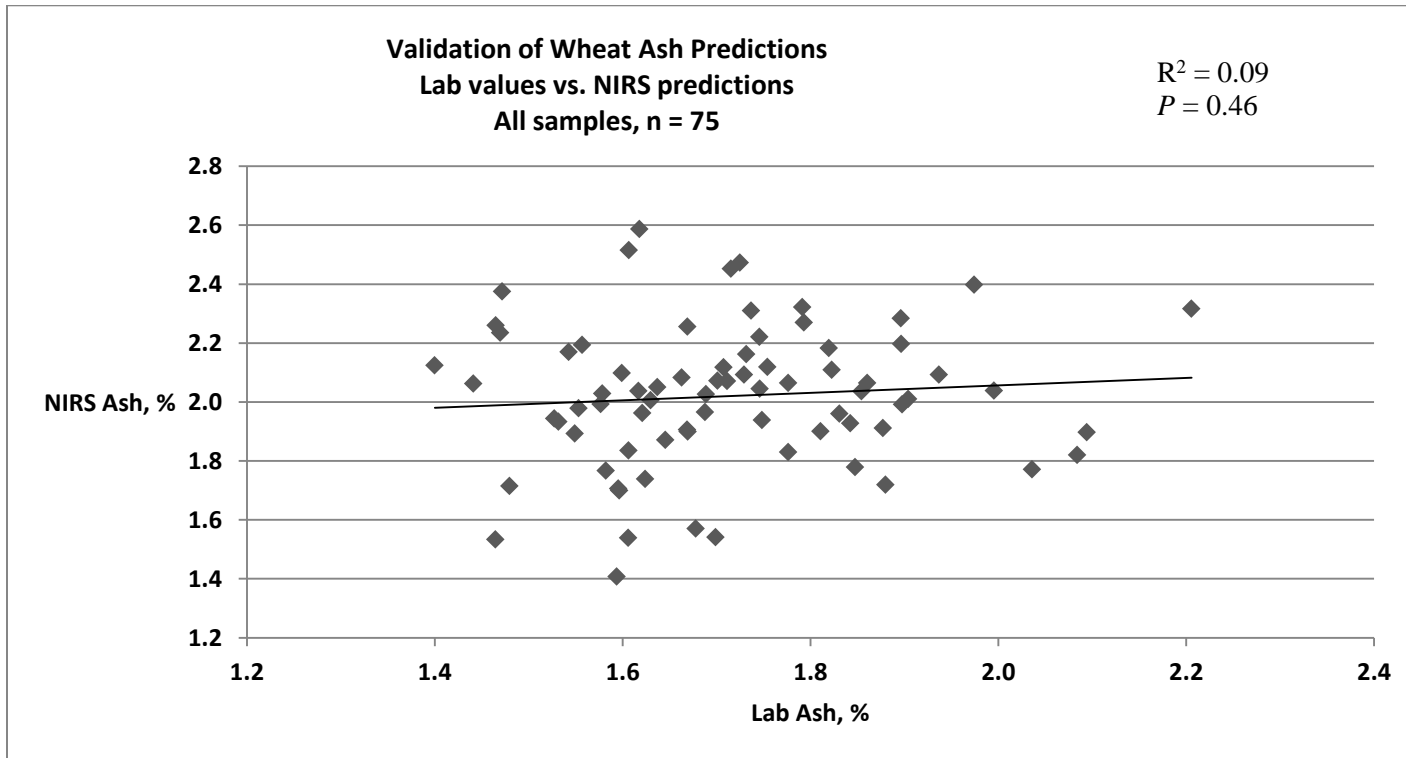


Figure 3.3. Validation of NIRS predictions of ash for all wheat grain samples.

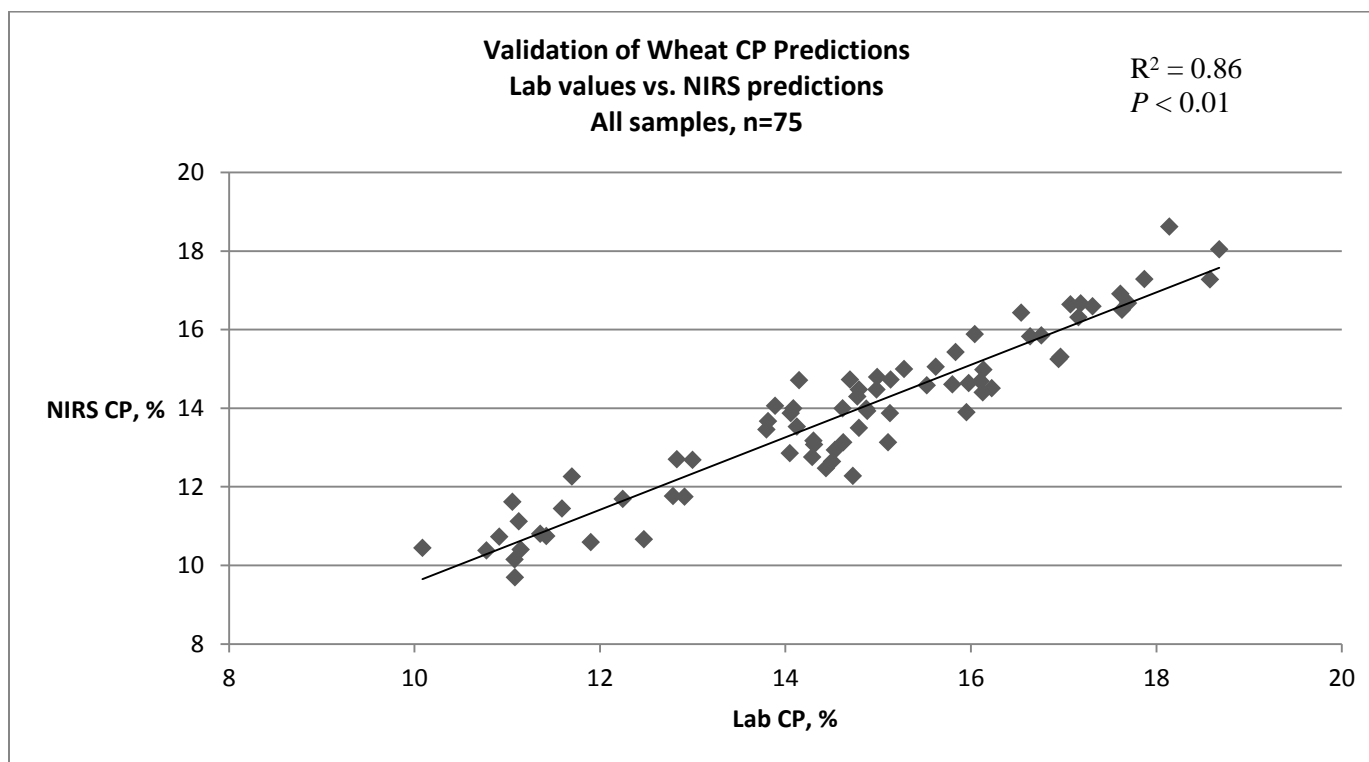


Figure 3.4. Validation of NIRS predictions of CP for all wheat grain samples.

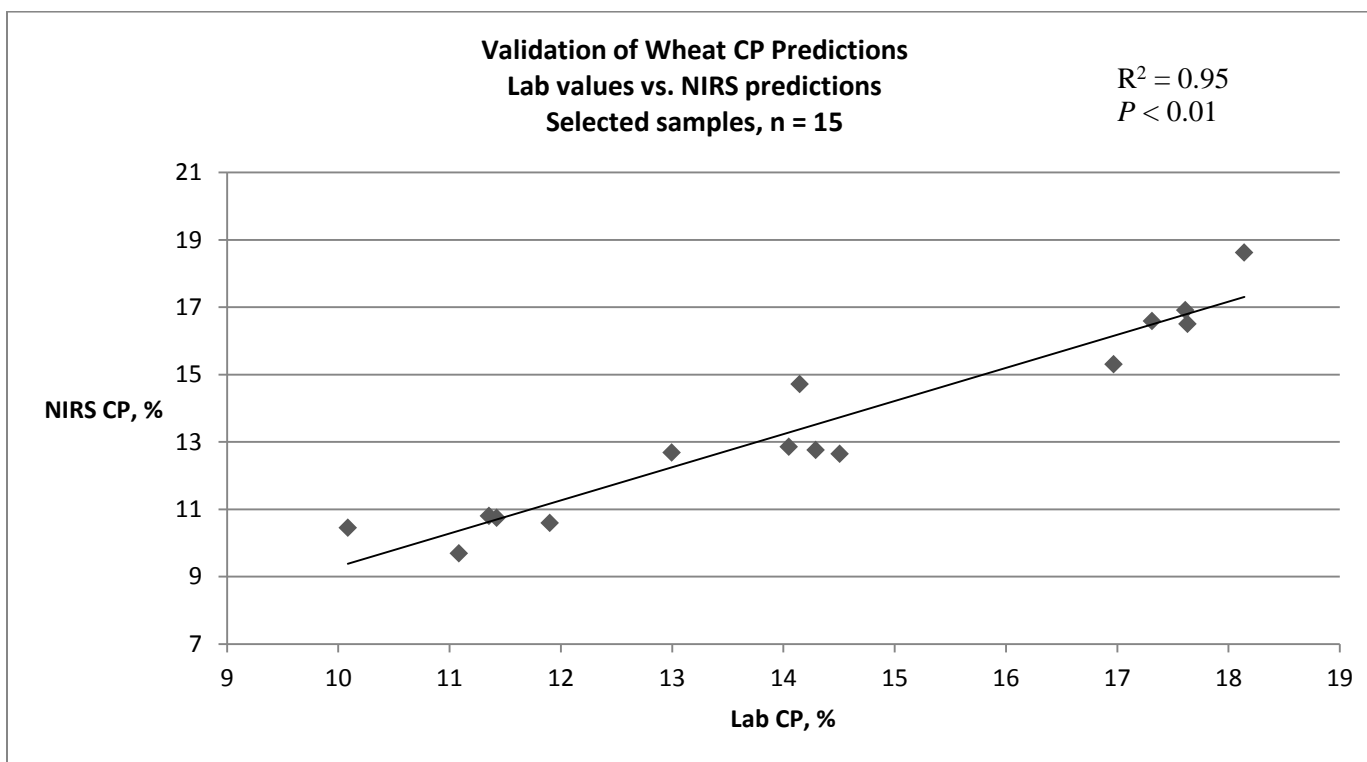


Figure 3.5. Validation of NIRS predictions of CP for wheat grain samples selected for CP.

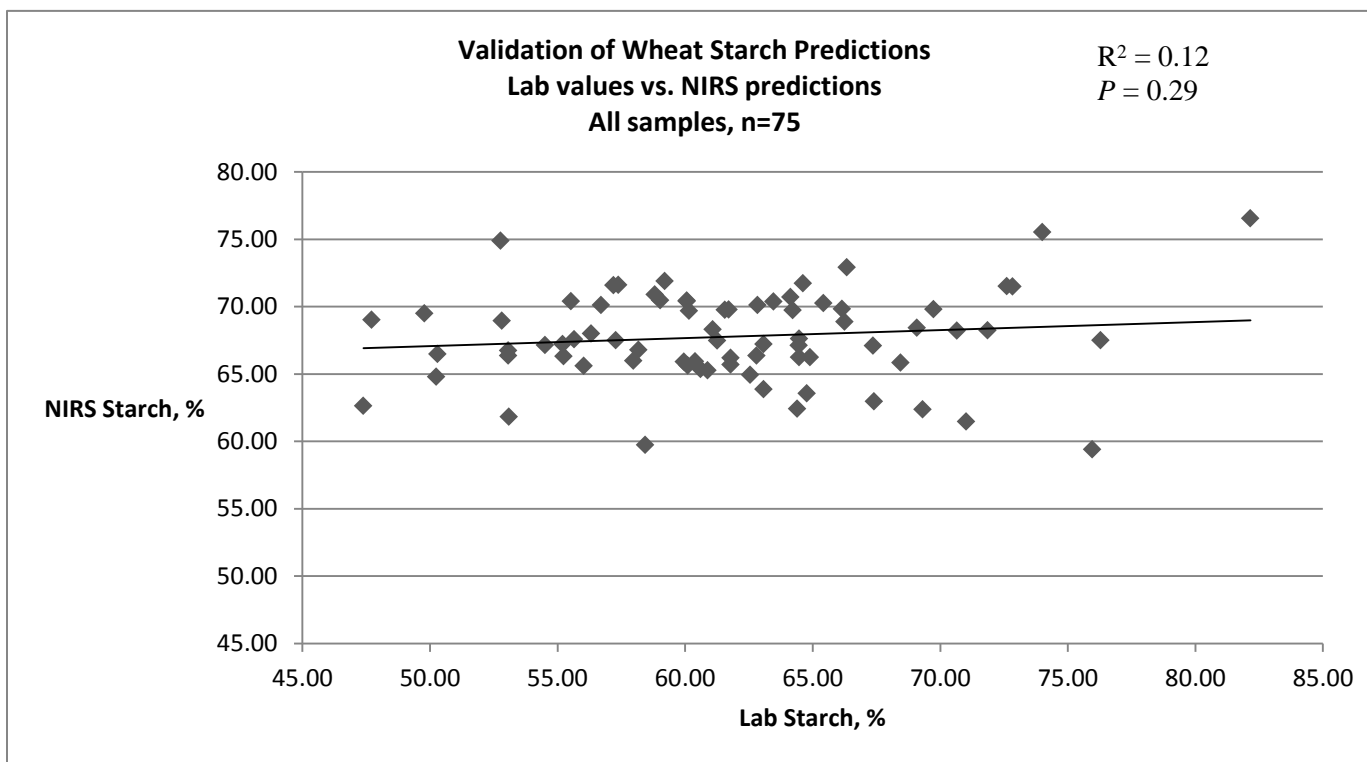


Figure 3.6. Validation of NIRS predictions of starch for all wheat grain samples.

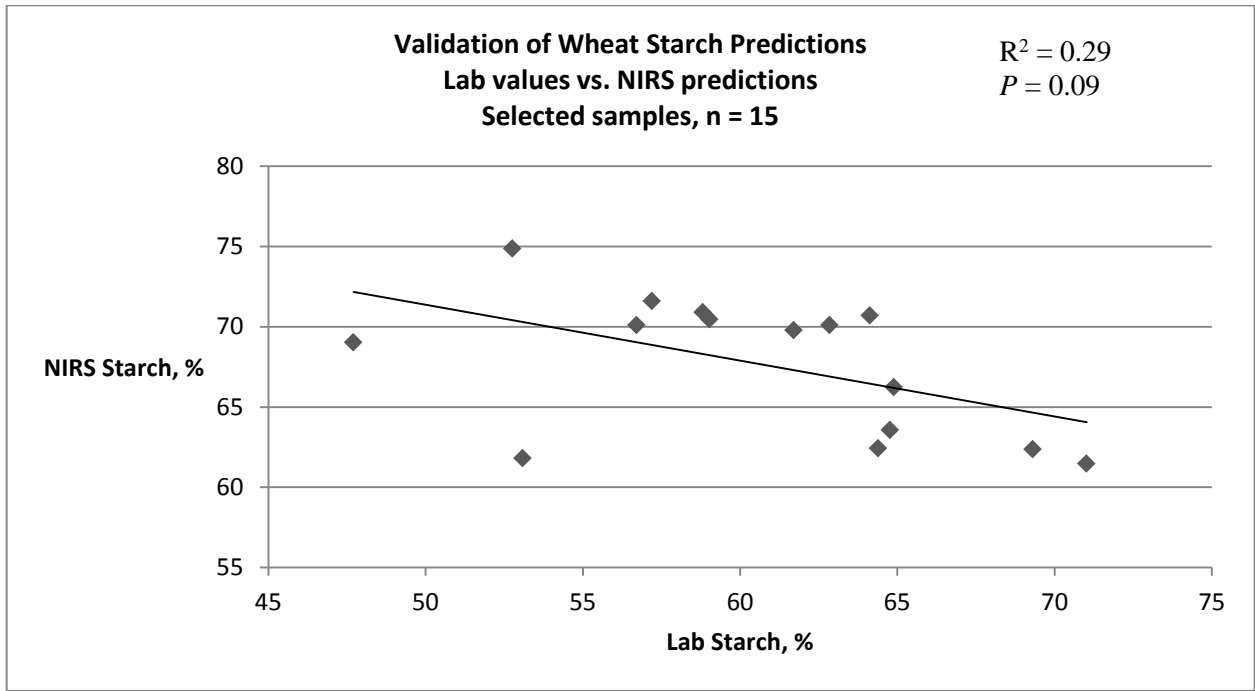


Figure 3.7. Validation of NIRS predictions of starch for wheat grain samples selected for starch.

Nutrient Group = Moisture

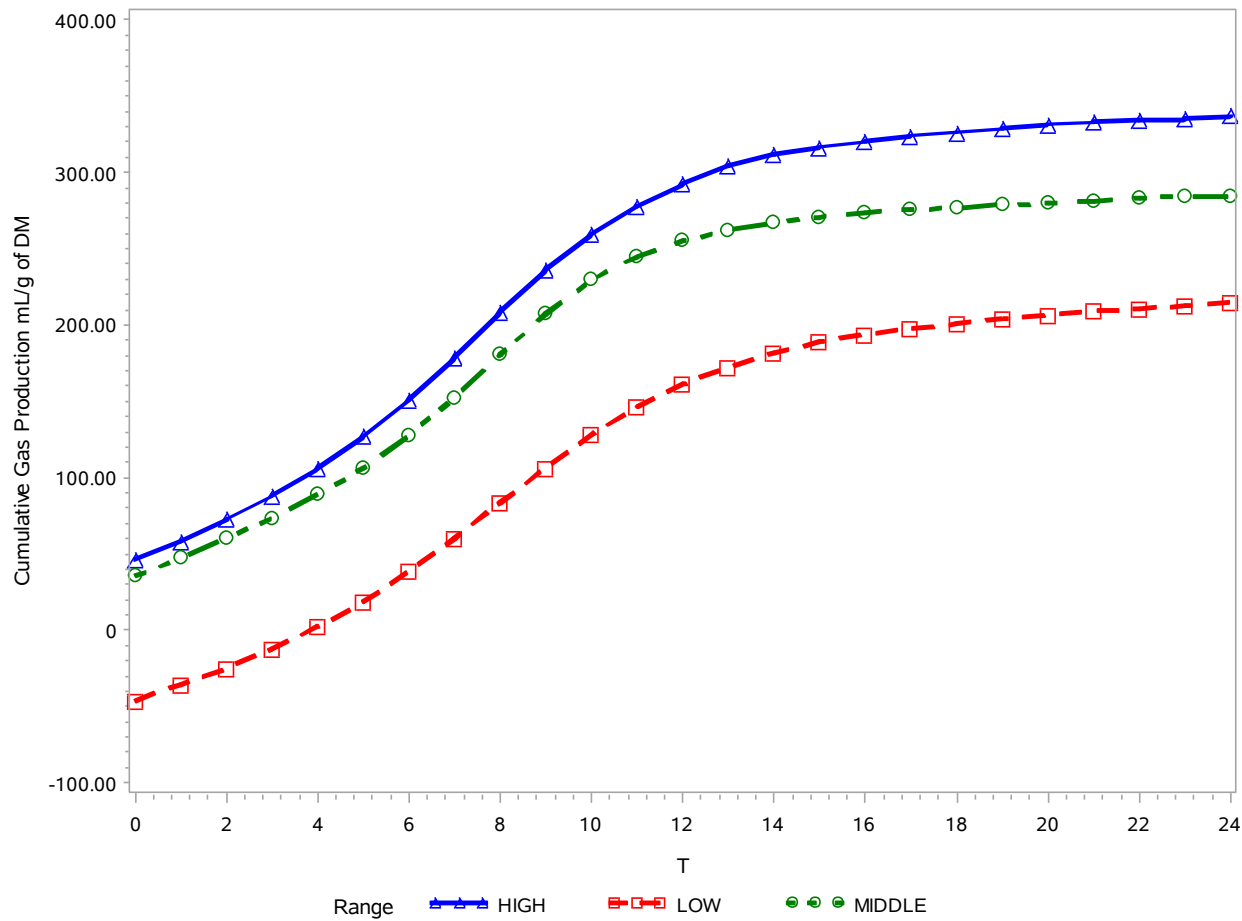


Figure 3.8. Gas production of DM selected samples over a 24 h period. Samples included were those selected as high DM (n = 5), middle DM (n = 5), and low DM (n = 5).

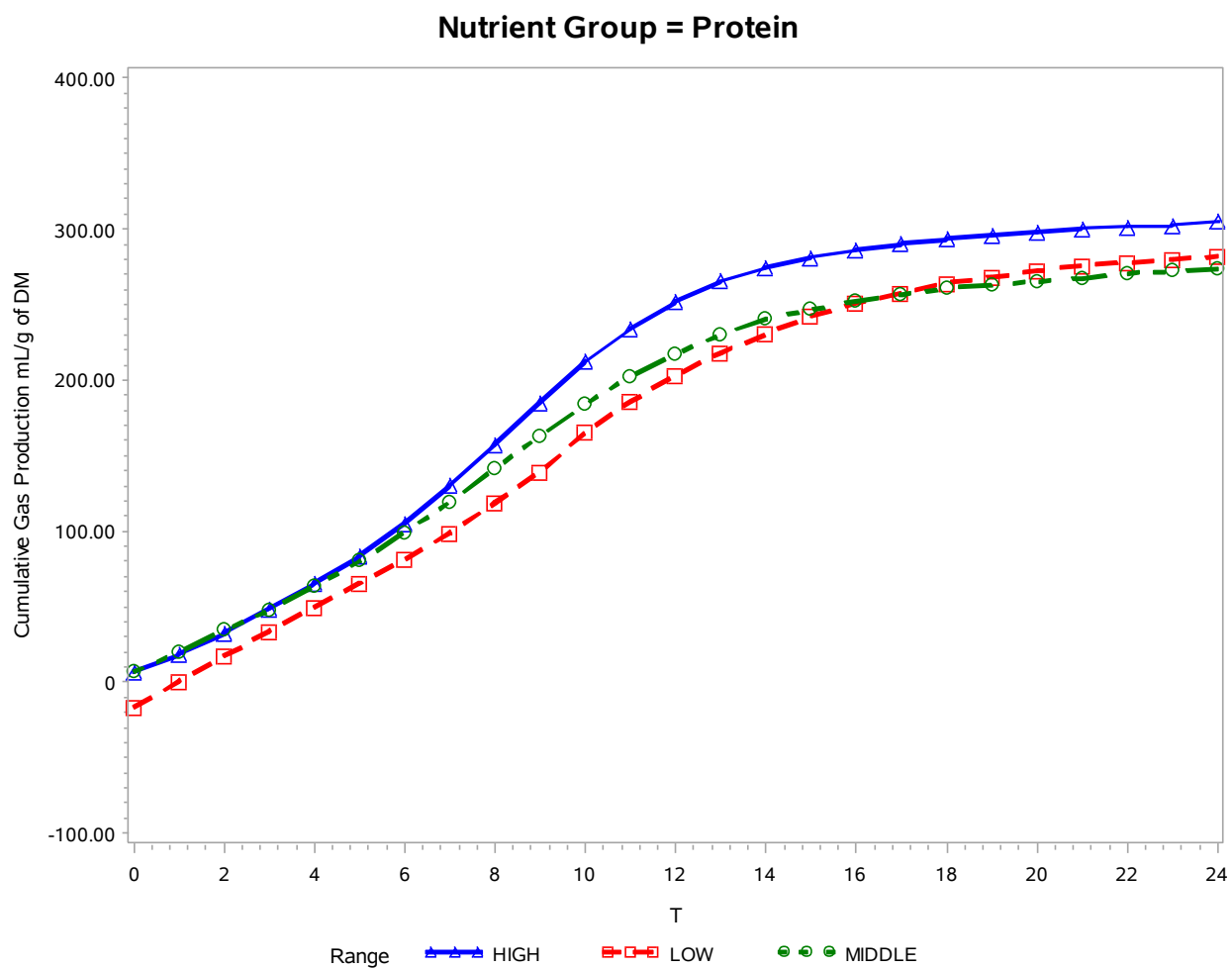


Figure 3.9. Gas production of CP selected samples over a 24 h period. Samples included were those selected as high CP (n = 5), middle CP (n = 5), and low CP (n = 5).

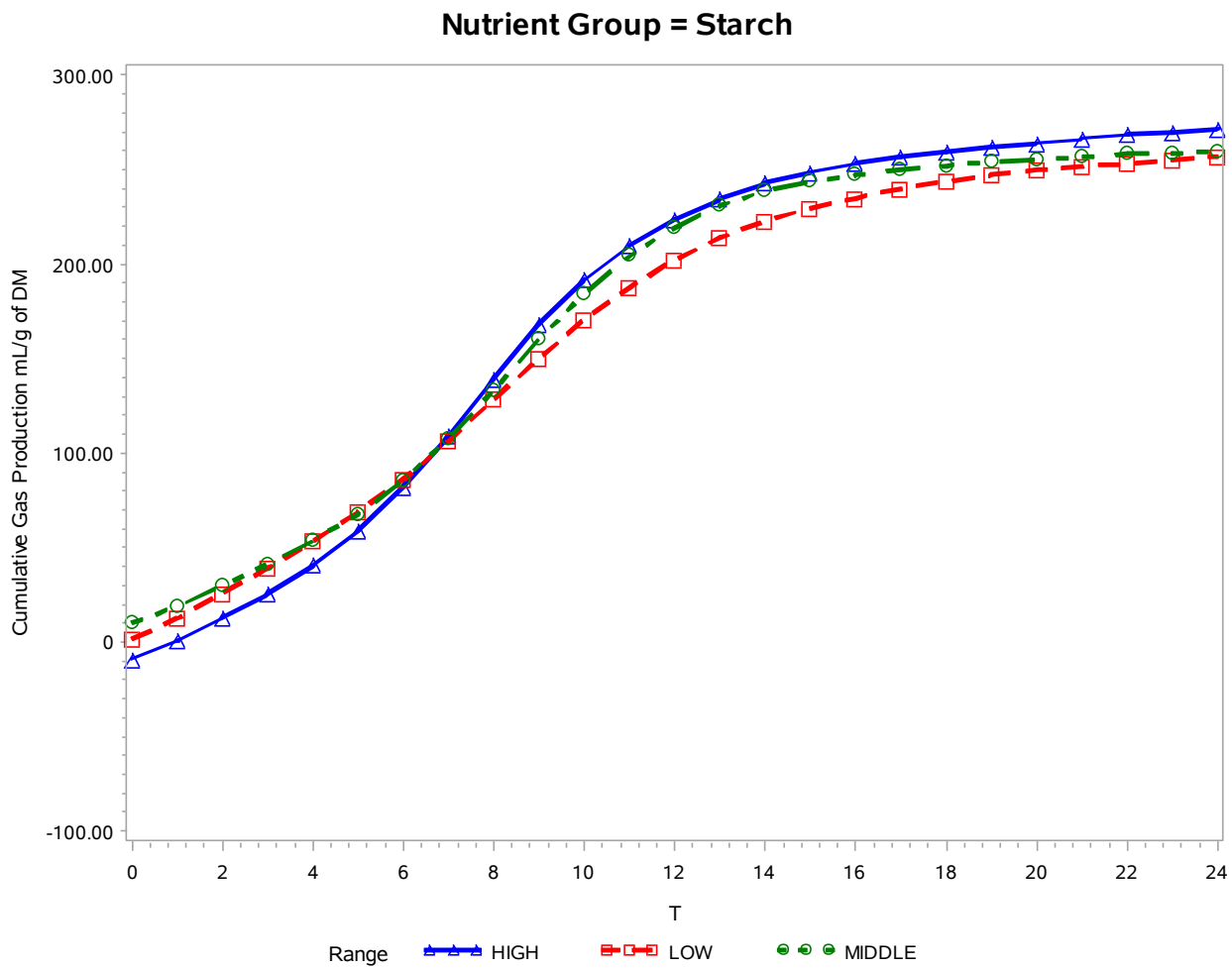


Figure 3.10. Gas production of starch selected samples over a 24 h period. Samples included were those selected as high starch (n = 5), medium starch (n = 5) and low starch (n = 5).

CHAPTER IV

EFFECT OF NIRS SEGREGATION OF BARLEY ON IN VITRO FERMENTATION, GAS PRODUCTION KINETICS, FEEDLOT PERFORMANCE AND CARCASS CHARACTERISTICS OF YEARLING CATTLE

Abstract

This study was conducted to evaluate the effect of near infrared reflectance spectroscopy (NIRS) segregation of barley grain on in vitro fermentation, gas production kinetics, feedlot performance and carcass characteristics of yearling cattle. Approximately 13,000 barley samples collected from six feedlots in western Canada from October 2011 to October 2013 were scanned utilizing commercially available NIRS technology (InfraXact, Foss North America, Eden Prairie, Mn.) and the spectra were characterized utilizing a proprietary Index calibration (Feedlot Health Management Services, Ltd.) for total barley composition. The following experimental groups for barley were established based on the distribution of Index values from the overlying population: LOW (barley with index values in the 15th percentile or lower), MED (index values between the 42.5 and 57.5th percentiles) and HIGH (index values in the 85th percentile or higher). Barley samples falling into each of these categories (n = 9, 15, 15) were selected for analysis. A control sample set (n = 15) was selected from across the distribution with a similar

frequency. A non-linear model was used to fit the data from the gas pressure monitor modules (modified Gompertz equation) for the parameters maximum gas production (M), rate (k), and lag time (l). M, k, and l were analyzed as repeated measures using PROC GLIMMIX (SAS Institute Inc., Cary, NC). IVDMD and gas production kinetics analyses were conducted according to published procedures (Galyean, 2009; May et al., 2010). IVDMD tended to be different across treatment groups ($P = 0.10$) and while M was not different ($P = 0.61$), k and L of gas production across treatment groups were different ($P = 0.03$ and 0.07 , respectively). A feeding trial was then conducted utilizing 480 head of crossbred yearling steers (BW = 1127 ± 60 lbs) and heifers (BW = 1095 ± 57 lbs), blocked by weight (6 replicates) within gender and randomly assigned to one of four experimental groups: LOW, MED, HIGH, CTRL. Barley inclusion in experimental diets was determined using the same frequencies as those utilized in the in vitro portion of the experiment with the exception of LOW index barley being redefined as that in the 33rd percentile or lower and CTRL barley was not segregated. Final performance data were analyzed using PROC GLIMMIX (SAS Institute Inc., Cary, NC) with experimental group and gender included as main effects and replicate and shipment day included as random effects. Cattle consuming segregated barley had greater DMI ($P = 0.02$), and tended to have higher HCW and live- and carcass adjusted ADG ($P = 0.08, 0.09, 0.07$, respectively) than cattle fed the unsegregated CON. Likelihood of Yield Grade 1 carcasses was greatest ($P = 0.05$) in steers fed LOW treatment barley. Samples of LOW, MED and HIGH treatment barley fed in the trial were collected and analyzed for chemical and physical characteristics. As treatment group increased there was: a linear decrease in DM ($P = 0.02$); linear increase in CP ($P < 0.01$); a tendency for a linear

decrease in starch ($P = 0.07$); linear decrease in the color variables brightness and red:green scale ($P = 0.02$ and 0.04 , respectively); and linear increases in 1,000-kernel weight and kernel diameter ($P < 0.01$ and 0.04 , respectively). These results indicate that NIRS segregation of barley can improve animal performance, possibly by minimizing variation in substrate supplied to microorganisms in the rumen or improving rate and extent of digestion. Additional research needs to be done to more fully understand the performance effects of barley segregation by NIRS.

Introduction

Feed cost of gain accounts for 65-80% of the total cost of feedlot cattle production. Characterizing the inherent variability of feedstuffs and understanding effects on animal performance will undoubtedly improve the ability to more precisely finish cattle and ultimately be more profitable.

In the northern and western regions of the United States and much of Canada, barley is the principle feed grain used for growing and finishing beef cattle as well as lactating dairy cattle, due to climate and soil limitations that impede the production of corn (Campbell et al., 1995; Boss and Bowman, 1996). Though corn and milo are the preferred concentrates for cattle finishing in the southern and south western United States, Boss and Bowman (1996) reported similar growth rates of cattle fed corn and barley and improvements in carcass quality grades of cattle fed barley. Furthermore, Ovenell-Roy et al. (1998) found that carcass weight and loin muscle area of cattle fed barley were not different than those of cattle fed corn. Finally, Maltin et al. (1998) suggested that beef from cattle fed barley may be redder than those fed corn due to an

increase in heme pigments, although these observations were not supported by Nelson et al. (2000) who found no differences in retail color score of beef from cattle fed barley or corn based finishing diets. Owens et al. (1997) found that, when averaged across all processing methods, ADG of cattle fed barley was not different than that of cattle fed wheat, corn, milo, or oats and that DMI and F:G conversion was not different for cattle fed barley, corn, wheat, or oats. Furthermore, the observed ME of barley and wheat were similar, a trend that continued when figures were adjusted for BW of cattle fed the respective grains. Furthermore, with barley prices often competitively priced relative to corn and wheat, feed cost of gain can be lower for barley based rations.

The analytical gas production technique (López et al., 2007) can be used to evaluate rate of digestion by measuring cumulative gas production at different incubation time points. In this technique, substrates of interest are inoculated with a microbial inoculum and fermentation measurements are measured in vitro. The objective of this technique is to mimic the environment of a specific section of the gastrointestinal tract and microbial inoculum should therefore contain similar species and concentrations of microbes (Mould et al., 2005). The method operates under the principle that gas produced in the system is the result of microbial and enzymatic digestion of the sample (López et al., 2007). Mathematical equations have been developed to relate the quantity of gas produced to the rate of digestion with the assumption that total gas production is directly proportional to the rate of fermentation of the sample (France et al., 2000). Blank modules are typically included in the analysis to account for gas produced by the microbial digestion of solubilized feed matter that was present in the rumen of the donor animal(s) prior to ruminal fluid collection.

When a feed sample is mixed with microbial inoculum it is degraded and the degraded fraction is either utilized to support microbial growth or fermented to produce fermentation acids and by product gases. Gas production data can be interpreted in combination with in vitro fermentation measurements to determine the fraction of sample that was allocated to microbial biomass and that which was degraded (Rymer et al., 2005). Correlations have also been demonstrated between gas production and total volatile fatty acids, in vitro starch digestion, in vitro dry matter disappearance (Trei et al., 1970) indicating that analytical gas production techniques can be used as a tool to estimate these parameters and concluded that gas production by rumen microorganisms may be useful as a guide to the relative feeding value of processed grain.

Traditional methods of analysis for animal feeds are typically expressed in terms of energy and protein and are derived using wet chemistry analyses and in vitro and in vivo metabolism methods. These methods are time consuming, expensive, and not practical in commercial environments. NIRS is desirable for nutrient prediction of feedstuffs largely due to its rapidity, no reagents are needed, no sample preparation is required, and multiple analyses are able to be performed simultaneously (Givens and Deaville, 1999). It has also been indicated that NIRS may have the potential to identify and characterize physical characteristics of grains that may have secondary effects on nutritional value or animal utilization. The ability to predict kernel hardness of whole wheat has been investigated (Williams, 1997) and Edney et al. (1995) attempted to use NIRS to predict kernel plumpness of whole barley.

Barley can provide 70-80% of the energy requirements of beef cattle (Bhatty et al., 1974) and in Canada, DE is considered the single biggest indicator of nutritional

quality of feed grains (Christison and Bell, 1975). The Canada Grains Council (1972) suggested that bulk weight is the most practical measure of energy content of feed grains. Commercial feedlots and feedmills typically purchase barley based on the quality criteria of volume weight and moisture content (Grimson et al., 1987), due to the general assumption that feeding value of heavier barley is greater than that of lighter barley (Mathison et al., 1991).

Relationships between volume weight and chemical constituents of barley grain have been found to be inconsistent but overall poor. Mathison et al. (1991) reported differences in gross energy, CP, Calcium, Phosphorous, ADF, NDF, starch, and ash of barley of different bushel weights. Engstrom et al. (1992) reported differences in volume weight of commercial lots of barley that did not always correspond to differences in starch, CP, ADF, or NDF. Campbell et al. (1995), however, reported weak correlations of test weight to starch and fat ($R^2 = 0.43$ and 0.29 , respectively), and no relationship between test weight and CP ($P > 0.05$). The only chemical constituent analyzed that was even moderately correlated to test weight was NDF ($R^2 = -0.66$, $P < 0.05$). Furthermore, Bhatta et al. (1974) reported poor relationships between bulk weight and DE for 2-row and 6-row barley ($R^2 = -0.01$ and -0.04 , respectively) when fed to mice. 1,000-kernel weight was a better indicator of 2-row and 6-row barley DE than bulk weight ($R^2 = 0.13$ and 0.18 , respectively) but still a poor indicator overall ($R^2 = 0.28$). Plumpness of 2-row and 6-row barley was a better indicator of DE ($R^2 = 0.04$ and 0.16 , respectively) but still poor overall ($R^2 = 0.28$).

Mathison et al. (1991) reported that apparent digestibility of fiber was lowest for higher volume weight barley but DM and energy digestibility of heavy and light barley

were lower than medium weight barley. In this study, starch digestibility was not different for cattle consuming high, medium, or light volume weight barley. However, organic matter digestibility of low volume weight barley was 2% less than steers fed the heavier three barleys. Although the light barley contained more fiber and less starch than the heavier barleys, no differences in ADG, DMI, or DM:G conversion of cattle fed 43, 69, 64 or 66 kg hL⁻¹ barley were observed (Mathison et al., 1991). Although Grimson et al. (1987) reported a 10% increase in DM:G, they reported no differences in ADG of steers fed light versus heavy barley.

The work of Campbell et al. (1995) and Bhatta et al. (1974) indicate that bushel weight, plumpness, and 1,000-kernel weight are poor indicators of overall grain quality. Christison and Bell (1975) stated that current criteria used for assessing milling and malting characteristics fail to effectively evaluate feed grains since they are not based on nutritive value. There is a need for a rapid and accurate method to predict barley grain feed value so that feedlot producers can effectively manage the variation in barley coming into their feedlots.

Materials and Methods

Barley Spectra Index

A Barley Spectra Index (BSI, Feedlot Health Management Services, Ltd., Okotoks, Ab.) was developed using the results of 13,000 NIRS scans of barley from six feedlots in Alberta that were collected from October, 2011 to October, 2013. Samples of incoming loads of barley at the study site were scanned using commercially available NIRS technology (InfraXact, FOSS North America, Eden Prairie, Mn.) and the load was

categorized as HIGH, MED, LOW, or intermediate (INT) spectra relative to the baseline population (WinISI, FOSS North America, Eden Prairie, Mn.). For experiment 1, samples with a BSI in the 85th percentile or higher were classified as HIGH, samples with a BSI between the 42.5th and 57.5th percentile were classified as MED, samples with a BSI in 15th percentile or lower were classified as LOW and samples with a BSI between LOW and MED or between MED and HIGH samples were classified INT. The same distributions were used for Experiment 2 with the exception of LOW barley, which was reclassified as barley falling in the 33rd percentile.

Experiment 1

Barley Samples

Whole barley samples entering 9 feedlots in western Canada and the US 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 samples tested, study population samples were then selected from April to August 2012 from 6 of the 9 feedlots. Samples were selected for being in the top 10% (high), middle 10% (mid), and bottom 10% (low) for either DM, CP, starch, or fat, and at random (RANDOM). Selection criteria of study population (n = 111) is described in Table 4.1.

Laboratory Analysis

Following collection and scanning at the feedlot, samples were sent to Oklahoma State University where laboratory analyses were performed. All samples were ground

through a 2 mm screen using a Wiley grinding mill (Thomas Scientific, Swedensboro, NJ). DM analysis was conducted using a forced air oven at 55 °C for 48 h.

All procedures involving live animals were approved by the Oklahoma State University Care and Use Committee.

Ruminal fluid used for the in vitro fermentation and gas production kinetics 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 wheat based diets for rumen microbe populations. Feed was offered once daily and water was offered *ad libitum*. The animal was adapted to the diet over a period of 21 days prior to the first ruminal fluid collection. Ruminal fluid was collected between 4 and 6 h post feeding, was strained through 4 layers of cheese cloth into a 2 L pre-warmed thermos, and transported to the Ruminant Nutrition Laboratory (Stillwater, Ok). Within approximately 30 minutes of sampling, ruminal fluid was used for the culture of innoculum.

IVDMD

Analysis of IVDMD was conducted using an adapted procedure of (Galyean, 2009), 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

CO₂ and capped with rubber stoppers and placed into a 39 °C waterbath. Contents of tubes were gently agitated every 6-8 h 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. After the addition of HCl, 2 mL of 5% pepsin was added and again the tube was gently swirled. Rubber stoppers were reinserted and tubes were placed back into the 39 °C waterbath for 24 h. Tubes were gently agitated 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 Watman's No. 4 filter paper. Filter paper and residue of each sample was dried in a forced air oven for 48 h at 55 °C. IVDMD was calculated as follows and expressed as a percentage:

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

In vitro kinetics of gas production

Eighteen gas pressure monitor modules (Ankom Technology Corp.) were used in combination with 250 mL serum bottles in duplicate for each sample. Two blanks were used in each run and were treated similarly to treatment serum vials but with no substrate added, which provided an estimate of gas production from the microbial inoculum itself. Each 250 mL module received 0.7 ± 0.01 g of sample and McDougalls buffer (37.5 mL; (McDougall, 1948) and 12.5 mL of ruminal fluid (50 mL of 3:1 buffer to ruminal fluid ratio). Each flask was flushed with CO₂ after the addition of McDougall's buffer:

ruminal fluid mixture and the monitor cap then fastened. A 24 hour incubation was chosen because preliminary observations indicated that maximum gas production occurred prior to 24 h and this was supported by observations of (Getachew et al. (2005)) analyzing corn samples. Flasks were inserted into a 39 °C shaking water bath set at 45 rpm (Thermo Fisher Scientific Inc.) for 24 h. Gas pressure data collected by each gas pressure monitor module was sent wirelessly to a base coordinator unit every 30 minutes for 24 h. To eliminate gas pressure buildup the pressure monitor modules released gas from within the flask when the pressure inside the flask reached 20.7 kPa. Gas volumes released from the flasks in this manner were included in cumulative gas production readings at each time point. Gas pressure was measured in psi and then converted to mL 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. Gas production for each sample was corrected for gas production introduced into the system by the ruminal fluid (based on blank serum vials).

Experiment 2

Study Facility

This study was conducted at a commercial feedlot (Chinook Feeders, Nanton, Alberta, Canada) using 48 small-sized research pens. Each small pen measured 9.52 m by 37.93 m and had a capacity of 20 animals. The basic design of the feedlot was

representative of the standard design used in commercial feedlots in Alberta. Open-air, dirt-floor pens were arranged side by side with central feed alleys, continuous pour concrete bunks and 20% porosity wood-fence windbreaks. There was a central hospital and weighing facility that were each equipped with a hydraulic chute, an individual animal scale, a chute-side computer for recording individual animal data (*i*FHMS, Feedlot Health Management Services Ltd. (FHMS), Okotoks, Alberta), and separation alleys to facilitate the return of animals to designated pens.

Study Animals

A total of 480 cross-bred yearling beef steers (1127 ± 60 lb.) and 480 crossbred yearling beef heifers (1095 ± 57 lb.) were selected from a candidate population at a routine handling event. At this time, all animals received an infectious bovine rhinotracheitis (IBR) virus and bovine parainfluenza-3 (PI₃) virus combination vaccine (Bovi-Shield[®] IBR-PI₃, Zoetis Canada, Kirkland, Québec), topical permethrin for parasite control (Boss[™] Pour-On Insecticide; Engage Animal Health Corporation, Guelph, Ontario), and a trenbolone acetate/estradiol growth promoting implant (steers received Revalor[®]-S (Merck Animal Health) and heifers received Revalor[®]-200 (Merck Animal Health)).

Experimental Design

Steers and heifers were randomly assigned to one of four experimental diet groups in a randomized complete block design: LOW, MED, HIGH, and CON. All animals received a barley-based finishing diet. A proprietary Barley Spectra Index (BSI) was used to segregate barley (as described in the Barley Allocation section below) for the LOW,

MED and HIGH experimental groups, while barley was not segregated (as per normal feedlot practice) for the CON group. All animals received their respective study diets from allocation until shipment for slaughter.

Heifers and steers were allocated to the study independently (6 d apart). Within a gender, animals were stratified based on the average of individual weights captured on two consecutive days. Allocation occurred (Day 0) such that each replicate was made up of animals with similar body weight (BW) allotted from heaviest to lightest, and that each pen within a replicate had the same average BW. By stratifying cattle this way, two marketing groups for each gender were established: Heavy (average initial BW of steers and heifers was 1177 lb. and 1143 lb., respectively) and Light (average initial BW of steer and heifers was 1078 lb. and 1048 lb., respectively), each made up of three replicates (12 pens). Animals were followed from allocation to harvest, with cattle from each experimental group within a marketing set sent to the same packing plant on the same day. One day prior to shipment for slaughter, final individual live weight was recorded for each animal.

Barley sampling and allocation

Individual hoppers or independent compartments on a truck were treated as independent loads and were thus sampled and scanned independently. Barley loads were sampled with a grain probe (KC Supply Inc, Kansas City, Mo) once for every 3,000 lb. of barley in the load. Samples were taken at equally spaced intervals within the load. All samples from each load were combined and subsequently poured through a grain separator (Humboldt Manufacturing, Schiller Park, IL) and re-composited three times

before scanning to ensure thorough mixing and homogeneity of the sample. Samples were then analyzed using commercially available NIRS technology (InfraXact, FOSS North America, Eden Prairie, MN) using a proprietary calibration for whole barley. Once samples were scanned they were retained for later chemical and physical analyses (Tables 4.9 and 4.10, respectively). Barley was segregated by classification and kept in independent grain storage bins until rolling. All barley was dry rolled using a portable, PTO-powered grain roller to achieve a target barley processing index (BPI) of 80%. All rolled barley was kept in individual outdoor bays. Barley of each type was rolled approximately every seven days as needed.

Feeding program

Water and standard mixed complete feedlot diets, formulated to meet or exceed the National Research Council nutrient requirements for beef cattle, were offered *ad libitum* throughout the feeding period. Feedlot diets were blended in truck-mounted mixer boxes equipped with electronic load cells. Diets were delivered to the pens once daily and daily feed allowances to each pen were recorded.

Candidate animals for this study were conditioned to a high concentrate diet over a period of 28 days prior to and shortly after allocation and then received the trial diets until harvest. Heifers remained on the step 4 ration through Day 3 and were stepped up to the finishing diet on Day 4. Study-specific barley was included in heifer diets beginning on Day 1. Steers were held on step 4 diet through Day 4 and were stepped up to the finishing diet on Day 5. Study-specific barley was included in rations for steers beginning on Day 0. The high concentrate diet consisted of approximately 90.58% barley, 7.5% barley silage, and 1.92% granular supplement on a 100% DM basis (Table

4.4) and was the same for all treatment groups. LOW, MED, and HIGH treatment barley was included in the respective treatment diets. Barley included in control diets was not segregated on arrival.

Diets fed to the study animals from allocation until harvest contained monensin to control coccidiosis and bloat (Rumensin[®], Elanco Animal Health, Division of Eli Lilly Canada Inc., Guelph, Ontario) at a level of 25 mg/kg diet DM and an in feed antimicrobial (Tylan[®]; Elanco Animal Health) to control liver abscesses, included at a level of 11 mg/kg DM. Heifers received melengestrol acetate to improve feed utilization and to suppress estrus (MGA[®] 100 Premix, Zoetis Canada). Steers received a beta-agonist (Optaflexx[®] 100 Premix, Elanco Animal Health) for 21 days prior to slaughter.

Feedbunk Sampling

Feed bunk samples were collected weekly from three randomly selected pens from each experimental group within a gender throughout the course of the study. For each pen, equal sized feed samples were collected from the beginning, middle, and end of the bunk and were composited to create one sample. Weekly samples were dried for dry matter determination and a monthly composite of each diet within a gender was analyzed for chemical make-up. The samples were frozen and stored at FHMS for potential future assay. A summary of the dry matter and chemical analysis of the mixed complete diets is presented in Table 4.5.

Animal Health

Experienced animal health personnel that were blinded to the experimental status of each pen observed the study animals once daily for evidence of disease. Animals deemed

to be “sick” by the animal health personnel were individually sorted from pen mates, moved to the hospital facility, diagnosed, and treated as per the computerized treatment protocols provided by FHMS veterinarians. The treatment events, including the treatment date, the presumptive diagnosis, drug(s) administered, and dose(s) used were recorded using *iFHMS*. Animals were returned to their designated research pen immediately following treatment except for one animal that required euthanasia.

Marketing

The target carcass weight for animals in the study was between 870 and 885 lb. for steers and 810 and 840 lb. for heifers depending on the average initial BW of the replicate within a marketing set. The Heavy heifer and steer marketing sets were killed on the same day and were 87 and 81 days on feed (DOF) respectively. The Light heifer and steer marketing sets were killed on the same day and were 102 and 96 DOF respectively. All animals were processed at Cargill Meat Solutions, High River, Alberta. Individual carcass records, linked by the Canadian Cattle Identification Agency tag, were obtained for all animals on trial.

Data Collection and Management

Over the course of the trial, all individual animal data were collected using *iFHMS*. At enrollment, initial weight and hip height were measured for each animal to assess the homogeneity of the animals in each experimental group. Daily feed data were captured electronically using the data collection systems in each feed truck and these data were electronically uploaded and stored in the feedlot administrative software system. At slaughter, the quality grade (QG), yield grade (YG), and weight of each carcass were

collected using the data capture system in place at the packing plant. All study data were entered or electronically imported into a spreadsheet program (Microsoft® Office Excel 2010, Microsoft Corporation, Redmond, Washington), collated, and verified. Ancillary production variables were calculated for each pen to describe the feedlot production system. Outcome variables describing feedlot performance (both live weight basis and carcass weight basis), carcass characteristics, and animal health were calculated. The carcass characteristic variables included the proportion of QG (Prime, Canada AAA, Canada AA, Canada A, B4 and D1) and YG (Canada 1, Canada 2 and Canada 3) observed in each group.

Physical and chemical characteristics of barley grain

Physical characteristics of barley were measured for loads as they were segregated for use in the trial. The BPI and bushel weight were determined using a Cox funnel and 0.5 L measure (Labtronics Manufacturing, Winnipeg, Manitoba). Plumpness was determined using a three-layer sieve (Labtronics Manufacturing). Analysis for the color variables brightness (L), red/blue scale (a) and yellow/green (b) (Minolta Colorimeter, Konica Minolta Sensing Americas, Inc, Ramsey, NJ) as well as the physical attributes of hardness, 1,000-kernel weight, and kernel diameter were measured using the Single Kernel Characterization System (SKCS 4100, Perten Instruments, Inc., Springfield, Il.) according to AACC method 55-31 at the Kansas State University Wheat Quality Lab.

Nutrient determination of barley and TMR samples was conducted at Servitech Laboratories (Hastings, Ne.)

Statistical Analysis

In Vitro Fermentation Analyses The duplicate gas production measurements and triplicate IVDMD measurements were averaged within run and analyzed using PROC GLIMMIX 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 for were analyzed as repeated measures using PROC GLIMMIX of SAS (SAS Institute Inc.) where sample was the experimental unit, treatment group was the main effect in the model, and run was included as a random effect. Gas production data was analyzed hourly for 24 h. For all statistical analyses, significant effects were observed at $P \leq 0.05$, and tendencies declared at P – values between 0.05 and 0.10.

Performance Data The baseline, ancillary production, feedlot performance, and carcass characteristic data for steers and heifers were analyzed using PROC GLIMMIX of SAS (SAS Institute, Inc.) for experimental group effects and adjusted for intra-replicate clustering of observations (SAS Institute Inc., 2009). Pen was the experimental group, experimental group and shipment day were included as main effects, and replicate was included in the model as a random effect. Linear, quadratic, and “CTRL vs. Experimental Group” contrasts were conducted. Baseline variables were tested as covariates of the feedlot performance variables, and included in the final models for the performance variables when significant ($P < 0.050$) covariate effects were detected.

Barley grain characteristics Physical and chemical characteristics of barley loads fed in the trial were analyzed using PROC GLIMMIX (SAS Institute, Inc) with treatment group included in the model.

Results and Discussion

Results from the analyses of IVDMD, maximum gas production, rate of gas production, and lag of gas production for the total population are presented in Table 4.2. Results of these analyses for barley samples in each treatment group are presented in Table 4.3. IVDMD tended ($P = 0.10$) to be effected by treatment group. IVDMD of CON barley was lower than MED barley and not different than LOW or HIGH. There tended ($P = 0.06$) to be a quadratic response in IVDMD across LOW, MED, and HIGH treatment groups, but IVDMD of LOW, MED and HIGH treatment groups was not different ($P = 0.76$) than CON barley. Cumulative gas production was not different for barley in any treatment group nor were the linear, quadratic, or “NIRS v. CON” contrasts. Treatment group had a significant effect on rate of gas production ($P = 0.03$), with LOW treatment barley having the slowest rate of gas production and CON, MED, and HIGH treatment barley were not different. There was also a linear ($P = 0.01$) response in rate of gas production across LOW, MED, and HIGH treatment groups, but rate of gas production was not different for CON samples compared to the LOW, MED, and HIGH treatment barley. It is important to note that rate of gas production of CON barley samples was higher than the LOW and similar to the MED and HIGH barley. This is not surprising given that the CON samples were selected to represent each of the other three treatment groups. Duration of the Lag phase of gas production tended to be effected by treatment. LOW treatment barley samples had the shortest Lag time while HIGH

samples had the longest Lag time ($P < 0.05$). Lag time of CON and MED samples were not different than one another or LOW or HIGH treatment samples. Again, a significant linear increase ($P = 0.01$) is seen in Lag time of LOW, MED, and HIGH samples and Lag time of CON samples was roughly in the middle. This is not surprising given the average nature of the CON population.

Barley samples were collected from LOW, MED, and HIGH treatment barley loads ($n = 26, 18, \text{ and } 27$, respectively) as they were allocated to the trial bins. Wet chemistry results of these samples are presented in Table 4.9. Treatment group had a significant effect ($P = 0.05$) on DM, with LOW samples having greater DM content than HIGH samples and MED samples being not different than LOW or HIGH. This was a significant linear decrease ($P = 0.02$) in DM across LOW, MED, and HIGH treatments. Treatment had a significant effect on CP as well ($P = < 0.01$), with LOW barley samples being lowest in CP, HIGH samples being highest, and MED samples being not different than LOW or HIGH. There was a linear increase in CP ($P = < 0.01$) across LOW, MED, and HIGH TRT groups. There were no differences due to TRT or linear or quadratic responses ($P > 0.05$) in starch, ADF, fat, DE, ME, NEM, NEG, or TDN. There tended ($P = 0.07$) to be a linear decrease in starch across treatment groups.

Analysis of the physical parameters of bushel weight, plumpness, color, 1,000-kernel weight, kernel hardness, and kernel diameter were conducted and results are presented in Table 4.10. There were significant effects of treatment group on 1,000-kernel weight and kernel diameter ($P < 0.01$ and 0.05 , respectively), and significant linear increases were seen across treatment groups ($P < 0.01$ and 0.04 , respectively).

Interestingly, bushel weight and plumpness, as determined using equipment and

procedures common to feedlot production in Alberta, were not different ($P = 0.46$ and 0.41 , respectively) across treatment groups.

Barley color is described using three variables (Table 4.10). Brightness (L) was significantly different across treatments ($P = 0.01$) with LOW and MED treatment barley being brighter than HIGH treatment barley and a significant linear response was observed ($P = 0.02$). Treatment group also had a significant effect on the red: green scale ($P = 0.05$) with HIGH barley expressing less red and more green pigment than LOW and MED samples. As with brightness, a significant ($P = 0.04$) negative linear response was observed in red:green scale across treatment groups. Hardness was not affected ($P = 0.84$) by treatment group and no linear or quadratic responses were observed ($P > 0.56$).

Interim weights of steers and heifers were taken at 45 and 50 DOF, respectively, and interim data are presented in Table 4.6. No interactions were observed between the main effects of treatment and gender, so data were analyzed with gender nested within rep to produce 12 replicates of each treatment group. Significant differences ($P < 0.01$) were observed due to treatment group for the performance variables DMI, ADG, and F:G. Cattle fed segregated barley had greater DMI and ADG and lower F:G than cattle fed the unsegregated control barley ($P < 0.01$). For each variable, response of cattle fed LOW, MED and HIGH were not different ($P > 0.05$) from one another and no linear or quadratic effects were observed for DMI, ADG, or F:G across of cattle fed LOW, MED, or HIGH treatment barley.

Final performance data for steers and heifers are presented in Table 4.7. Steers fed HIGH treatment barley had the greatest DMI and steers in the CON group had the

lowest DMI; DMI of steers fed LOW and MED treatment barley were not different. No linear or quadratic responses were observed in intakes across LOW, MED, or HIGH treatments, but all cattle fed segregated barley ate significantly more ($P = 0.02$) than cattle fed the unsegregated control. There were no differences in initial weight ($P > 0.05$) of steers across treatment groups, There were differences in HCW ($P < 0.05$) across treatment groups that were reflective of differences in ADG, particularly on a carcass-adjusted basis. Cattle fed CON treatment barley had the lowest ADG on a live and carcass adjusted basis, not surprisingly they also had the lightest carcasses. Steers fed LOW treatment barley had the heaviest carcass weights but also the greatest ADG on live and carcass adjusted bases. Carcass weights as well as live- and carcass adjusted-ADG of steers fed MED and HIGH treatment barley did not differ from those fed LOW or CON treatment barley. Steers fed segregated barley tended to have heavier carcasses ($P = 0.08$) as well as higher live- and carcass adjusted ADG ($P = 0.09$ and 0.07). There were no differences in F:G on a live basis ($P > 0.05$) of steers across treatments groups. Steers fed the LOW and MED treatment barley had lower conversions ($P < 0.05$) than steers fed the CON and HIGH treatment barley. Barley segregation did not have the same effect on conversion as it did on DMI and ADG as there were no differences in F:G on a live- or carcass adjusted basis due to treatment.

There was a significant gender by treatment interaction in ADG on a live weight basis ($P = 0.04$) and there tended to treatment by gender interactions for F:G on a live basis ($P = 0.09$) and DP ($P = 0.06$). As expected, steers ate more and gained weight faster ($P < 0.05$) than heifers across all treatment groups. No differences ($P > 0.05$) were observed in DMI, HCW, DP, or carcass adjusted ADG or F:G across treatment groups of

heifers. Heifers fed HIGH treatment barley gained less and converted higher ($P < 0.05$) on a live basis than those fed MED treatment barley. Neither HIGH nor MED barley-fed heifers gained at a different rate than cattle fed CON or LOW treatment barley. Heifers fed HIGH treatment barley converted at a similar rate ($P < 0.05$) as heifers fed LOW and CON treatment barley. Heifers fed MED treatment barley had the most efficient feed conversions ($P < 0.05$) of all treatment groups.

Carcass data for steers and heifers are presented in Table 4.8. There were no differences ($P > 0.05$) in likelihood of carcasses grading prime (not shown), Canada AAA, Canada AA, or Canada A (not shown) across treatment groups. There was a significant effect of gender on likelihood of YG1 and YG3 ($P < 0.01$) carcasses and a tendency for gender to have an effect on likelihood of carcasses to be YG2 ($P = 0.08$). Steers fed MED treatment barley had a greater likelihood of Yield Grading 1 than steers fed LOW treatment barley. Conversely, steers fed MED treatment barley were no less likely to Yield Grade 2 or 3 than those fed LOW or MED treatment barley. There were no differences ($P > 0.05$) in likelihood to Yield Grade 1, 2 or 3 of steers fed CON, LOW or HIGH treatment barley.

The performance response observed in both genders at interim was maintained in the steers over the entire feeding period, though it became less extreme as they were on feed longer. Improvement in DMI due to barley segregation dropped from 3.9% at interim to 2.5% by the end of the feeding period. Similarly, improvement in ADG of cattle fed segregated barley was reduced from 16.0% to 5.6% and F:G from 10.0% to 2.7% on a live basis (5.4% on a carcass adjusted basis) by the end of the feeding period. The improvements in DMI, ADG, and F:G observed in cattle of both genders fed

segregated barley over the unsegregated CON treatment barley in the first segment of the feeding period were lost in heifers by the end of the feeding period. Since no interactions were observed between the main effects of barley treatment group and gender at interim, we can assume that heifers were responding to the treatment effect similarly to steers.

ADG of cattle in all groups was reduced with more DOF, but ADG of cattle fed LOW, MED, or HIGH barley was reduced to a greater extent than those in the CON barley. Since performance likely “leveled off” as cattle reached a certain biological endpoint, and cattle fed segregated barley gained at a faster rate earlier in the feeding period, it follows that the reduction in performance seen after the interim weight was greater in those treatment groups that experienced greater improvements early on. Furthermore, Yield Grades of steer carcasses fit targeted ranges, favoring lower numerical Yield Grades, and the improvement in performance was still detectable in cattle fed segregated barley. On the other hand, the more even distribution of Yield Grades of heifer carcasses indicate that an unusually high number of these carcasses were over finished. This could potentially explain the loss in differentiation in performance response of heifers due to barley segregation over the entire feeding period.

The chemical and physical characteristics of the LOW, MED, and HIGH barley are particularly intriguing. The increase in Lag time across LOW, MED, and HIGH treatment groups corresponds to the linear increase in CP content of these samples, and supports the observations reported for corn by Zinn (1992). While we would expect the linear decrease in DM ($P = 0.02$), linear increase in CP ($P < 0.01$), and the tendency for there to be a linear decrease in starch ($P = 0.07$) to ultimately affect performance, they did not appear to. Rather, the improvement in performance appeared to not be isolated to

any one of the treatment groups, so long as the cattle were fed segregated barley. This indicates that minimizing the variation in substrate supplied to microorganisms in the rumen has a greater improvement in animal performance than the DM or CP content of that substrate, as long as nutrient requirements are met.

The significant differences observed across treatment groups in 1,000-kernel weight and kernel diameter would lead us to believe that bushel weight and percent plump should also have been different. The fact that differences in bushel weight and plumpness were not observed across treatment groups indicates that these procedures may not be reflective of what they are designed to measure. This may also explain the lack of consistency in cattle performance response to barley of various bushel weights reported by Mathison et al. (1991) and Grimson et al. (1987). Furthermore, these data indicate that the discounts applied to barley purchases due to lighter volume weight or lower % plump may not be reflected in performance of the cattle.

Conclusion

The interim data indicate that the performance response was greater earlier in the feeding period. An implant-like response (16% improvement in ADG, $P < 0.01$; 10% improvement in efficiency, $P < 0.01$) was observed in the first segment of the feeding trial. Over the entire feeding period, cattle consuming segregated barley had greater intakes ($P = 0.02$), and tended to gain at a faster rate on both a live- and carcass adjusted basis ($P = 0.09$ and 0.07 , respectively). These data indicate that segregation of barley by BSI has the potential to improve cattle production, but additional research needs to be done in order to better understand the performance response related to gender, DOF, and

BW. Furthermore, these data question the ability of the traditional measurements of bushel weight and plumpness to accurately reflect feeding value or subsequent animal performance since these measurements were not affected by BSI but 1,000-kernel weight and kernel diameter were. To the author's knowledge, no other technology available to producers today is able to account for multiple chemical and physical characteristics and generate a single value that is indicative of animal performance.

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Tables and Figures

Table 4.1. NIRS selection criteria for barley grain study population sampling from April to August 2012.

Item	High	Mid	Low
DM, %	> 89.4	87.0 - 85.3	< 83.6
Fat, %	> 3.12	2.19 - 1.60	< 1.08
Starch, %	> 61.5	58.9 - 58.4	< 55.7
CP, %	> 11.3	9.67 - 9.34	< 7.70

Table 4.2. *In vitro* fermentation parameters for all barley grain samples (n = 111).

Item	Mean	SD
IVDMD, %	83.0	4.40
Gas production, mL/g of substrate DM	287	20.2
k, mL /h	20.1	3.13
Lag, h	0.56	0.53

Average *in vitro* fermentation parameters for n = 54 barley grain samples selected from a population representative of the range in nutrient distribution seen in Alberta feedlots.

Table 4.3. *In vitro* fermentation parameters for barley grain samples by treatment group in Experiment 1.

Item	Treatment Group				SEM	P-values			
	CON	LOW	MED	HIGH		Trt	Linear ¹	Quad ¹	CON V. NIRS ²
n	15	9	5	15					
IVDMD, %	82.7 ^a	85.2 ^{ab}	79.7 ^b	81.7 ^{ab}	1.67	0.10	0.15	0.06	0.76
Gas production, mL/g of substrate DM	282	286	291.2	283	6.8	0.61	0.67	0.32	0.48
k, mL /h	20.4 ^a	17.5 ^b	21.0 ^a	21.2 ^a	0.99	0.03	0.01	0.14	0.61
Lag, h	0.549 ^{ab}	0.179 ^b	0.443 ^{ab}	0.715 ^a	0.1583	0.07	0.01	0.98	0.48

^a means within a row with differing superscripts differ at $P < 0.05$

¹Linear and quadratic contrasts for each *in vitro* fermentation parameter were analyzed for the treatments LOW, MED, and HIGH.

²CON v. NIRS contrast was analyzed for each *in vitro* fermentation parameter for the CON treatment versus the LOW, MED, and HIGH treatments.

Experimental groups were defined as LOW < 15th percentile; MED 42.5th < >57.5th percentile; HIGH > 85th percentile; CTRL samples selected across the distribution with same frequency as those occurring in Experiment 2.

Table 4.4. DM formulation of experimental ration for Experiment 2.

Ingredient	DM composition, %
Barley, dry rolled ^a	90.6
Barley silage	7.50
Supplement	1.92

^a LOW, MED, and HIGH BSI barley was utilized in respective experimental diets.

Barley included in CTRL diet was non segregated.

Table 4.5. DM composition of experimental rations.

	Formulated	CON	LOW	MED	HIGH
DM, %	76.1	76.8	77.5	77.2	77.0
CP, %	12.6	10.9	11.4	11.4	11.6
NEm, Mcal/Kg DM	1.98	2.23	2.25	2.25	2.25
NEg, Mcal/Kg DM	1.37	1.54	1.57	1.57	1.57
NEm, Mcal/lb DM	0.90	1.01	1.02	1.02	1.02
NEg, Mcal/lb DM	0.62	0.70	0.71	0.71	0.71

Feed bunk samples were collected weekly from the beginning, middle, and end of three randomly selected pens from each experimental group within a gender. Weekly samples were dried for dry matter determination and a monthly composite of each diet within a gender was analyzed for chemical make-up at Servitech Laboratories (Hastings, NE)

Table 4.6. Interim performance data for steers and heifers.

Item	Treatment Group				SEM	P - values			
	CON	LOW	MED	HIGH		Trt	Linear¹	Quadratic¹	CON vs. NIRS²
DMI, lb	24.7 ^b	25.4 ^a	26.0 ^a	25.7 ^a	0.55	< 0.01	0.38	0.17	< 0.01
ADG, lb	3.25 ^b	3.76 ^a	3.71 ^a	3.83 ^a	0.127	< 0.01	0.61	0.45	<0.01
F:G	7.64 ^a	6.83 ^b	7.04 ^b	6.76 ^b	0.166	< 0.01	0.74	0.17	<0.01

Data analyzed with gender nested within replicate to produce 6 replicates per treatment.

¹Linear and quadratic contrasts for each performance variable were analyzed for the treatments LOW, MED, and HIGH.

²CON v. NIRS contrast was analyzed for each performance variable for the CON treatment versus the LOW, MED, and HIGH treatments.

^{ab}means within a row with different superscripts differ at $P < 0.05$

Table 4.7. Final performance data for steers and heifers.

Item	Steers				Heifers				SEM	Trt	Gen	P – values			
	C	L	M	H	C	L	M	H				Trt* Gen	Lin ¹	Quad ¹	C v. NIRS ²
Initial Wt, lb	1128	1127	1127	1127	1096	1096	1095	1095	49.6	0.11	0.15	0.88	0.07	0.95	0.22
DMI, lb	25.8 ^b	26.6 ^{ab}	26.1 ^{ab}	26.7 ^a	23.2 ^c	23.6 ^c	24.0 ^c	23.6 ^c	0.83	0.14	< 0.01	0.36	0.79	0.71	0.02
HCW, lb	842 ^b	859 ^a	855 ^{ab}	851 ^{ab}	806 ^c	806 ^c	813 ^c	809 ^c	15.7	0.30	< 0.01	0.43	0.64	0.58	0.08
DP, %	58.9 ^c	58.9 ^{bc}	59.5 ^{abc}	59.0 ^{bc}	59.8 ^a	59.8 ^a	59.5 ^{ab}	60.0 ^a	0.38	0.77	< 0.01	0.06	0.52	0.70	0.45
ADG-L ³ , lb	3.43 ^b	3.75 ^a	3.54 ^{ab}	3.58 ^{ab}	2.69 ^{dc}	2.66 ^{dc}	2.88 ^c	2.64 ^d	0.108	0.19	< 0.01	0.04	0.27	0.50	0.09
ADG-C ⁴ , lb	3.11 ^b	3.45 ^a	3.39 ^{ab}	3.27 ^{ab}	2.62 ^c	2.60 ^c	2.76 ^c	2.63 ^c	0.099	0.19	< 0.01	0.39	0.50	0.35	0.07
F:G-L ³	7.52 ^c	7.10 ^c	7.40 ^c	7.46 ^c	8.63 ^{ab}	8.90 ^a	8.36 ^b	8.97 ^a	0.194	0.30	< 0.01	0.09	0.25	0.17	0.77
F:G-C ⁴	8.34 ^{abc}	7.73 ^c	7.74 ^c	8.20 ^{bc}	8.91 ^{ab}	9.13 ^a	8.71 ^{ab}	9.03 ^a	0.423	0.32	< 0.01	0.40	0.46	0.20	0.31

Treatments are abbreviated as: C = CON; L = LOW; M = MED; H = HIGH.

Data analyzed with treatment group (trt) and gender (gen) included as main effects in the model, replicate and shipment day were included as random effects. Replicates 4-6 of each treatment for each gender were shipped 15 d after reps 1-3.

¹Linear and quadratic contrasts for each performance variable were analyzed for the treatments LOW, MED, and HIGH.

²CON v. NIRS contrast was analyzed for each performance variable for the CON treatment versus the LOW, MED, and HIGH treatments.

³average daily gain and feed:gain conversion presented on a live weight basis.

⁴figures adjusted to a common dress of 60% to account for differences in carcass weight that may not be reflected in live weight.

^{ab}means within a row with different superscripts differ at $P < 0.05$.

Table 4.8. Final carcass data for steers and heifers.

Item	Steers				Heifers				SEM	<i>P</i> – values					
	C	L	M	H	C	L	M	H		Trt*	Gen	Trt* Gen	Lin ¹	Quad ¹	C v. NIRS ²
AAA, %	69.2	73.3	66.7	71.7	70.0	70.0	72.5	74.1	4.68	0.87	0.67	0.80	0.78	0.47	0.64
AA, %	30.8	23.3	30.8	26.7	26.7	27.5	25.0	23.4	4.61	0.81	0.48	0.70	0.92	0.41	0.48
YG1, %	70.0 ^{ab}	65.5 ^b	77.5 ^a	70.0 ^{ab}	48.7 ^c	39.2 ^c	48.3 ^c	39.8 ^c	4.62	0.05	< 0.01	0.66	0.54	0.02	0.41
YG2, %	28.3 ^{ab}	26.9 ^b	17.5 ^b	26.7 ^{ab}	29.5 ^{ab}	38.3 ^a	30.6 ^a	32.9 ^a	4.44	0.20	0.08	0.43	0.51	0.06	0.98
YG3, %	1.67 ^b	7.59 ^b	5.00 ^b	3.33 ^b	21.8 ^a	22.5 ^a	21.1 ^a	27.2 ^a	3.33	0.66	< 0.01	0.51	0.94	0.48	0.31

Treatments are abbreviated as: C = CON; L = LOW; M = MED; H = HIGH.

Data analyzed with treatment group (trt) and gender (gen) included as main effects in the model, replicate and shipment day were included as random effects. Replicates 4-6 of each treatment for each gender were shipped 15 d after reps 1-3.

¹Linear and quadratic contrasts for each carcass variable were analyzed for the treatments LOW, MED, and HIGH.

²CON v. NIRS contrast was analyzed for each carcass variable for the CON treatment versus the LOW, MED, and HIGH treatments.

^{ab}means within a row with different superscripts differ at $P < 0.05$.

Cattle were harvested at Cargill Meat Solutions (High River, Ab.) and carcass were analyzed by trained personnel using standard Canadian systems for quality and yield grades.

Table 4.9. Chemical characteristics of barley (dry matter basis).

Item	Treatment Group			SEM	P - values		
	LOW	MED	HIGH		Trt	Linear	Quadratic
n	26	18	27				
DM, %	87.4 ^a	87.2 ^{ab}	86.5 ^b	0.31	0.05	0.02	0.39
CP, %	11.0 ^b	11.8 ^{ab}	12.3 ^a	0.30	< 0.01	< 0.01	0.83
Starch, %	59.5	59.2	58.5	0.45	0.18	0.07	0.76
ADF, %	5.73	5.72	5.74	0.126	1.00	0.95	0.95
Fat, %	1.50	1.51	1.56	0.071	0.75	0.48	0.81
DE, mcal/lb	1.68	1.68	1.68	0.002	0.51	0.28	0.68
ME, mcal/lb	1.38	1.38	1.38	0.002	0.51	0.26	0.79
NEG, mcal/lb	0.635	0.631	0.632	0.002	0.38	0.32	0.33
NEM, mcal/lb	0.941	0.941	0.939	0.002	0.51	0.26	0.79
TDN, %	84.1	84.0	89.9	0.11	0.51	0.28	0.68

^{ab} means within a row with different superscripts differ at $P < 0.05$
Analyses were conducted at Servitech Laboratories (Hastings, Ne.)

Table 4.10. Physical characteristics of barley.

Item	Treatment Group			SEM	P - values		
	LOW	MED	HIGH		Trt	Linear	Quadratic
L (brightness) ¹	63.5 ^a	64.3 ^a	60.6 ^b	1.04	0.01	0.02	0.06
a (red/green) ¹	4.37 ^a	4.38 ^a	4.11 ^b	0.107	0.05	0.04	0.22
b (yellow/blue) ¹	25.8	26.2	25.3	0.47	0.35	0.35	0.27
Hardness ²	50.5	49.9	48.9	2.23	0.84	0.56	0.94
1,000-kernel weight ² , g	44.4 ^b	46.4 ^{ab}	47.9 ^a	0.96	< 0.01	< 0.01	0.79
Kernel diameter ² , mm	2.72 ^b	2.81 ^a	2.72 ^a	0.03	0.04	0.04	0.15
BuWt ³ , lb	49.7	50.6	49.7	0.57	0.46	0.98	0.21
Plumpness ⁴ , %	89.0	90.5	91.5	1.61	0.41	0.19	0.92

¹Brightness is expressed on a scale from 0 (pure black) to 100 (pure white). The red/green scale is a measure of the degree to which each of these pigments are present and is expressed using an index of -60 (pure green) to 60 (pure red). Yellow/green scale is a measure of the degree to which each of these pigments are present and expressed using an index from -60 (pure blue) to 60 (pure blue). The color variables L, a, and b were determined using a Minolta Color Meter (Konica Minolta Sensing Americas, Inc, Ramsey, NJ) at the Kansas State University Wheat Quality Laboratory.

²Hardness is a measure of the force required to crush the kernels and is expressed on an index of -20 to 120. 1,000-kernel weight and kernel diameter measurements are extrapolated/averaged from the individual weights and diameters of 300 kernels. Analyses of kernel hardness, 1,000-kernel weight, and kernel diameter were conducted using the Single Kernel Characterization System (Perten Instruments, Inc, Springfield, Il.) at the Kansas State University Wheat Quality Laboratory.

³Winchester bushel weight was determined using a Cox Funnel and 0.5L cup.

⁴Plumpness was the amount of sample remaining in the top layer of a 3-layer grain sieve (Labtronics Inc., Winnipeg, Mb.) and expressed as a percentage of the total amount of the sample.

^{ab}means within a row with different superscripts differ at $P < 0.05$

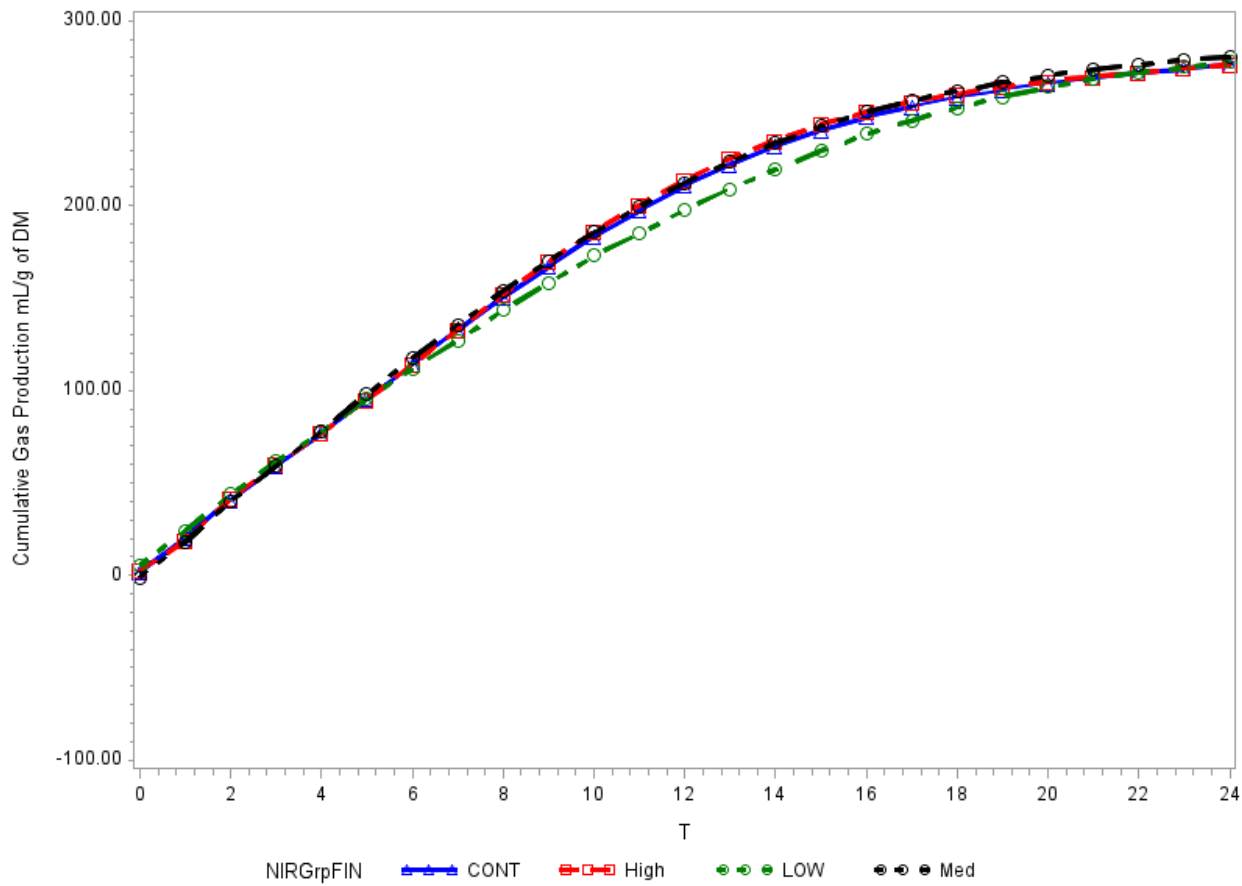


Figure 4.1. Experiment 1: Cumulative gas production of barley samples from 0-24 hours. Incubation was from Time T = 0-24 hours. Samples included LOW (n = 9), MED (n = 15), HIGH (n = 15), and CON (n = 15).

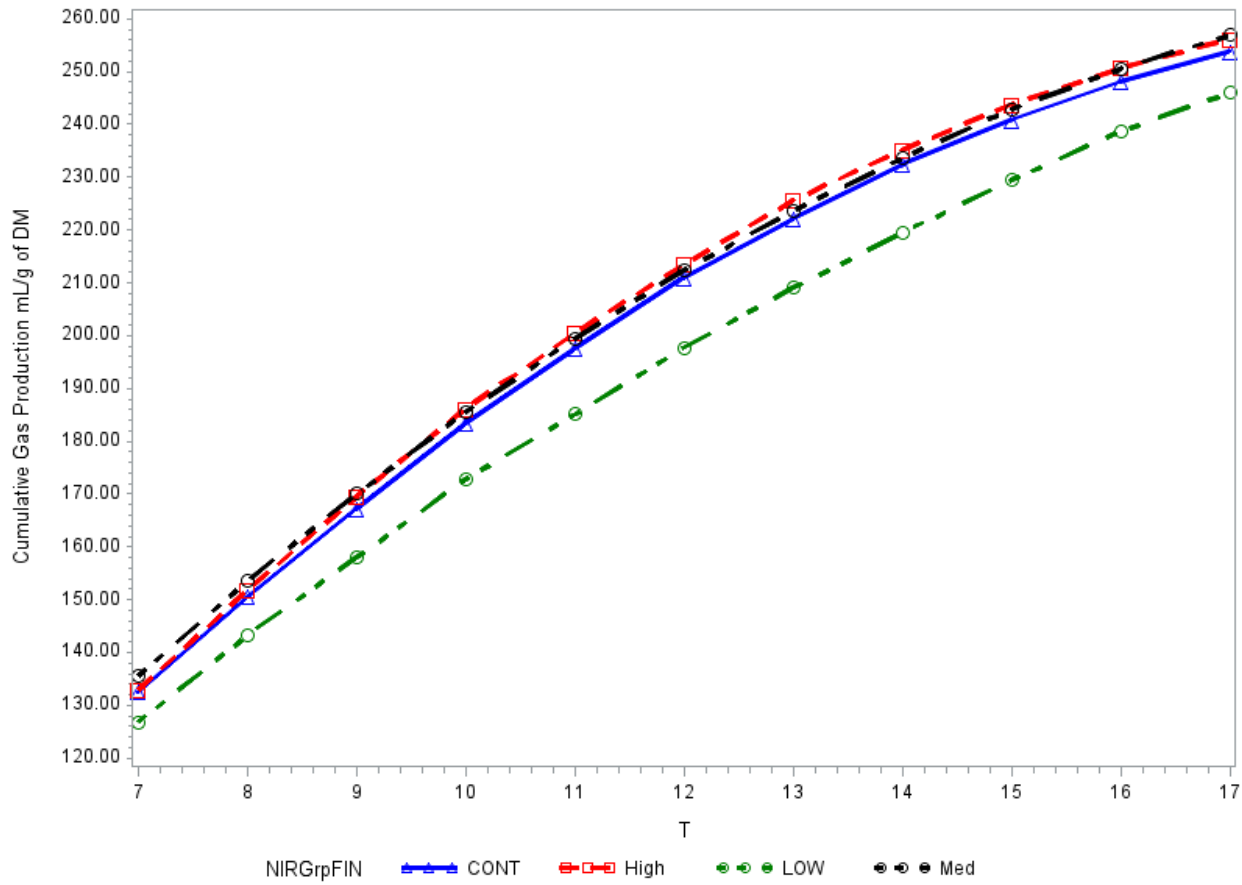


Figure 4.2. Experiment 1: Cumulative gas production of barley samples from 7-17 hours. Samples included LOW (n = 9), MED (n = 15), HIGH (n = 15), and CON (n = 15).

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