

USE OF A PORTABLE NEAR-INFRARED SPECTROPHOTOMETER TO PREDICT
NUTRIENT COMPOSITION OF FECES FROM FEEDLOT HOLSTEIN CATTLE
AND ITS APPLICABILITY FOR ON-SITE RESEARCH AND INDUSTRY USE

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Honestly . . .

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LIST OF ABBREVIATIONS

ADF – Acid detergent fiber
ADG – Average daily
ADL – Acid detergent lignin
BW – Body weight
CP – Crude protein
CV – Coefficient of variation
d – day
DM – Dry matter
DMI – Dry matter intake
G:F – Gain to feed ratio
MIR – Mid-infrared
MLR – Multiple linear regression
NDF – Neutral detergent fiber
NIR – Near-infrared
NIRS – Near-infrared spectrophotometer
PCA – Principle components analysis
PCR – Partial coefficient regression
PLS – Partial least squares regression
R - Reflectance
 R^2 – Coefficient of determination
RPD – Ratio of predictive deviations
SD – Standard deviation
SEC – Standard error of calibration
SECV – Standard error of cross-validation
SEL – Standard error of laboratory
SEP – Standard error of prediction
USD – United States dollar
VFA – Volatile fatty acid

ABSTRACT

Two studies were performed to investigate the ability of a portable near-infrared spectrophotometer (**NIRS**) for on-site analysis of nutrient components in feces from cattle. In trial 1 of study 1, growing dairy steers were fed diets containing either 86 or 90% concentrate. Regression values from a calibration set of 56 samples were promising for CP, DM, and NDF, but not for ADF or starch. In trial 2 of study 1, finishing dairy steers were fed diets containing either thick (512 g/L) or thin (460 g/L) steam-flaked corn. Regression values from a calibration set of 126 samples were poor for all nutrients. Both studies showed statistically valid NIRS calibrations, but further validation was required to make regression values acceptable ($R^2 > 0.80$) for all fecal nutrient components.

In study 2, NIRS analysis was employed on novel research. Young dairy bull calves were fed diets containing either whole or steam-flaked corn from pre-weaning until 8 weeks post-weaning when the first animal was heavy enough for inclusion into a commercial feedlot. Again, although statistically valid, regression values from a calibration set of 220 samples were promising for CP and ADF, but not predictive for DM, NDF, ash, and starch. Growth performance parameters were similar between diets, with starch digestibilities diverging after weaning and changing to a Holstein starter diet.

These 2 studies show that commercial and research application of a portable NIRS for on-site analysis of the nutrient composition of feces from young, growing, and finishing dairy steers statistically possible but requires further validation research. Also, results from the second study imply that there is no advantage in feeding steam-flaked

corn to dairy calves from pre-weaning to 8 weeks post-weaning or until reaching feedlot weight. However, starch digestibility begins to improve for steam-flaked corn to whole corn once the animal has been weaned.

INTRODUCTION

Dairy beef production provides a unique feeding opportunity where calves are weaned prior to 10 weeks of age (BAMN, 2003). Because of this quick-weaning practice, rumen development in the dairy calf begins earlier compared to the traditional beef calf. During this transitional period, it has been reported that there is no advantage to corn processing in the growth performance of young dairy cattle (Leismaister and Heinrichs, 2004). However, digestibility parameters, especially for starch, may begin to differ according to type of corn processing. The differentiating feeding practices of dairy steers requires longer confined feeding, and the nutritional management of dairy steers must be diligent from pre-weaning through finishing (Duff and McMurphey, 2007; Maas and Robinson, 2007).

One way nutritional management may remain attentive is through sample analyses. Nutritionists routinely analyze diets, feedstuffs, and fecal samples to provide indices of nutrient digestibility and chemical composition. These analyses are often performed by commercial laboratories which may be costly. Also, results may not be returned within the time period when they would be most helpful. Quick and immediate analysis of fresh fecal samples would help improve nutritional management.

Near-infrared spectrophotometer (NIRS) technology has been used in research and commercial settings to diminish costs and time spent between sampling and analysis (Marten et al., 1985). Current NIRS technology has been reported as applicable for on-site analysis for feedstuffs (Perez-Marin et al., 2004; Berzaghi et al., 2005). However,

while portable technology is capable for on-site feedstuff analysis, it has yet to be validated on fecal samples.

Currently, there has been no report on the cattle fecal analysis using a portable NIRS for both commercial and novel research. The two hypotheses of this dissertation is that current portable NIRS technology is capable of accurately predicting nutrient components in cattle feces and that fecal components from dairy calves fed either whole or steam-flaked corn from pre-weaning to feedlot weight will change over time. The objectives of this dissertation, therefore, is to validate the on-site use of portable NIRS technology for cattle fecal analysis and to determine the effect of corn processing on the growth performance and nutrient digestibility in young dairy calves fed to feedlot weight.

CHAPTER ONE: LITERATURE REVIEW

Dairy Beef

Dairy beef history. Dairy cattle breeds have a longer existence of recognition as compared to beef cattle breeds (Tyler and Ensminger, 2006). As such, cattle in the dairy industry have had a longer time to be selectively bred for production purposes: milk production. Selectivity has focused on a single goal: increased volume output per cow lactation cycle (Tyler and Ensminger, 2006). As a result, it is suggested that 30 percent of the U.S. Holstein herd gene pool is genetically related to 2 particular bulls and 20 percent of the Canadian Holstein herd gene pool can be related back to 1 bull (Eng, 2005). This intensive breeding selection within the dairy population has given rise to a large number of cattle that are genetically similar with predictable production.

Calves that are bred for milk production, however, create a problem in the dairy industry. Not all calves born to dairy cows are female. Anatomically, dairy bulls do not have the equipment necessary to produce milk, the focus of the dairy industry. As such, dairy producers have little need for male calves that are not desired as future breeder bulls. To complicate the matter, all calves are removed from the dam quickly so that dams can be placed into the milk production line. Calves have little choice but to become dependent on artificial rearing practices.

Prior to the 1960's, unwanted dairy bull calves were either sold at local sale barns or given away by the dairies (Eng, 2005). Interest in using dairy calves as an extra source of beef was not realized until a father-son company invested in the first Holstein steer feeding operation in the mid-1960's (Eng, 2005). As dairy operations have started to

relocate to traditional beef production areas such as the Texas panhandle and the Midwest, availability of dairy calves for inclusion into feedlot production has become more convenient (Duff and Anderson, 2007). Demand from consumers for lean, high quality beef renewed interest in Holstein beef production (Apple et al., 1991; Chester-Jones et al., 1991). Today, Holstein steer production has grown into a substantial provider of beef in the United States, comprising 8 to 8.5 percent of finished cattle (Cheatham and Duff, 2004; Schaefer, 2005).

Considering that over 92 percent of the U.S. dairy herd is of Holstein origin, bull calves from other dairy breeds are often lost in the numbers. The Brown Swiss, for instance, was bred for dual purpose in milk and beef production, and the bull calves are usually sold for beef production. On the other hand, the smaller Jersey breed is still considered as having little or no value as the Holstein bull calf was once deemed decades ago, and unwanted Jersey bull calves are often sold for veal production or euthanized on site (Arnett, 2010). Today, results from research identifying possible feeding practices to boost beef production in Jersey steers are mixed (Cole et al, 1963; Arnett, 2010). However, because Jersey beef complements consumer demand in palatability characteristics, interest in using Jersey cattle for low input beef production remains (Alberti et al., 2008).

Dairy beef production. Several attempts have been made to quantify dairy steer populations in the United States. Because of its uniqueness, national statistics performed by the USDA is limited and quantification is derived from general numbers. However, Cheatham and Duff (2004) estimated a population of 4.1 million dairy bull calves

available for beef production each year using 2001 data (9,141,000 cows) from Hoard's West. Recent population data from the USDA reports similar dairy cow numbers around 9.1 million (USDA, 2010). Using the general formula employed by Cheatham and Duff (2004), the total population is multiplied by the portion of cows that are Holstein (90.1 percent; USDA, 2007). This leaves 8.2 million Holstein cows in production. With a statistical chance of 50 percent of calves born as bulls, approximately 4.1 million bull calves are born each year in the U.S. With an estimated 7.8 percent mortality rate (USDA, 2007), a probable 3 quarters of 79,800 calves fed and harvested for veal (NASS, 2009), and bull retention (unknown estimate), a final 3.79 million Holstein bull calves are potentially sold into beef production operations each year.

On the other hand, Jersey cattle comprise only 5.3 percent of the total U.S. dairy herd population (USDA, 2007). Using the same standard numbers employed for Holstein steer numbers, approximately 482,000 Jersey cows are present, 241,000 Jersey bull calves are born each year, and a final estimate of 202,000 bull calves are available for beef production.

Since dairy beef production is relatively new as compared to traditional beef and dairy production, limited peer-reviewed data is available on the management practices and population numbers of dairy steers. Several extension papers from across the United States have provided various suggestions as to proper production practices for both pre- and post-weaned dairy steers, and some general conclusions can be made. However, because of the climate diversity accounted for by these papers (i.e. Wisconsin vs. Arizona) various diversions of core production practices exist. Production practices

mentioned in this dissertation are focused mainly on Holstein steer production, with Jersey steer practices included as needed.

Production segments between the dairy and beef industry vary greatly. Beef production segments are often separate from each other, and segment integration is not as consistent as poultry or swine production where animals are often kept in the same operation through all production segments. Seedstock cattle producers provide breed-specific heifer replacements and bull services to cow-calf producers (Damron, 2003). In turn, cow-calf producers provide 6-10 month-old weaned calves to either the stocker/backgrounder for low-input growth production until they are 8-14 months old (275-350 kg/animal) or the feedlot producer for finishing cattle at 12 to 22 months old (400-650 kg/animal; Lowe and Gereffi, 2009). In contrast, the primary segment of the dairy industry is the dairy farm itself. However, because dairy operations hold primary focus on milk production, interest in sending replacement heifer calves to another operation to be raised until first parturition is increasing (Tyler and Ensminger, 2006). The advantage of consigning calves to a heifer or calf ranch also opens space for more producing cows on the dairy.

Dairy bull calves are situated between the two industries, and segments from both industries must cooperate together in order to establish functional dairy beef production. Chester-Jones and DiCostanzo (1996) describe 3 distinct segments: pre-weaning (up to 60 days of age), weaning through growing (150-300 kg/animal), and finishing. Unlike the beef industry, dairy calves are weaned early in life and can be sold at smaller weights. They can be sold as fresh calves (3-14 days of age), freshly weaned calves (90-120

kg/animal), light feeders (150-200 kg/animal), feeders (225-350 kg/animal), heavy feeders (450-500 kg/animal) and finished steers (>525 kg/animal; Chester-Jones and DiCostanzo, 1996; Siemens, 1996).

The variety of marketable categories is due to differing growing options for dairy steers. Historically, calves would be backgrounded on a low-input feeding regime after weaning until they have reached 400-500 kg/animal, where they would be placed on a high-concentrate diet until finished (Burdine, 2005). However, a shift to newer production methods place newly-weaned calves directly on high concentrate feed until they reach 150-200 kg/animal, a weight acceptable to southwestern feedlot operators (Duff and McMurphey, 2007; Maas and Robinson, 2007). In the Midwest, it is common to background cattle on wheat pasture or another cheap-gain program until they reach ~260 kg/animal before placing them on a high-concentrate feeding regime until finished (Duff and Anderson, 2007).

Regardless of segment production, calf production practices are fairly similar. Once born, calves are removed from the dam after receiving 1 to 2 feedings of colostrum (Duff and Anderson, 2007; Maas and Robinson, 2007), usually by bottle for accurate measurement (Burdine, 2003). After removal from the dam, calves can be placed in group or individual housing. The typical housing method is the calf hutch, where calves are placed into individual stalls that are at least 24 inches wide (Duff and Anderson, 2007; Comerford et al., 2008). Calves are left in these hutches until they are weaned, which usually occurs between 28 and 60 days of calf age (Chester-Jones and DiCostanzo, 1996; Siemens et al., 1996; Duff and Anderson, 2007).

There are several parameters in which to determine time to wean, including body weight, age, and feed intake. One survey of dairy producers reported calves are most often weaned by age (43%), while the rest were evenly weaned either by feed intake (26.9%) or body weight (26.4%; BAMN, 2003). A more recent survey on dairy heifer management lists the average age at weaning as 8.2 weeks (USDA, 2007). The use of age over feed intake and body weight may be explained by Quigley (1996) who suggests that feed intake can be unpredictable, leading to variable weight gain and late weaning and higher cost. Bascom (2006) reported that Jersey calves should be weaned according to 2 parameters: at least 6 weeks of age and consistently eating 0.7 kg feed/day.

After weaning, calves can be housed in group pens of less than 25 cattle (Maas and Robinson, 2007; Comerford et al., 2008) where they are fed a high concentrate starter ration until they reach feedlot weight (275 or 570 lbs; Duff and Anderson, 2007). Comerford and others (2008) also suggested an “all in/all out” protocol to help prevent disease outbreak. Calves can also be placed on pasture at anytime during the growing phase, which would help reduce feed cost (Comerford et al., 2001; Lehmkuhler, 2005). Once heavy enough for introduction, dairy steers can be finished at a commercial feedlot. Overall, cattle may spend approximately one year on concentrate feed before reaching harvest weight if placed in the feedlot at the minimum weight requirement (Cheatham and Duff, 2004; Eng, 2005). Peters (2005) breaks the time on feed according to the body weight in which the cattle are introduced into the feedlot. Dairy steers less than 250 kg average 327 days on feed, steers between 250 and 400 kg average 205 days on feed, and steers greater than 400 kg average 151 days on feed.

Young dairy beef nutrition. While housed in the hutches, calves are fed some type of milk replacer as their primary source of nutrition. Calves can be dependent on liquids up to 2 weeks of age (BAMN, 2003). While several dairies use waste or hospital milk (milk unfit for human consumption) as the primary Siemens et al. (1996) suggest feeding a commercial milk replacer twice daily (2 L/feeding) until one week prior to weaning when slow reduction of milk fed is reduced. Others have suggested abrupt weaning from milk replacer (Leismeister and Heinrichs, 2004). This replacer should contain 20-28 percent crude protein and 10-22 percent fat (Maas and Robinson, 2007). A high-quality dry starter feed can be offered to the calves immediately or by 3-4 days of age (Maas and Robinson, 2007; Comerford et al., 2008). One survey reported most dairy operations offering a starter feed before 14 days of age (BAMN, 2003).

Because dairy steers typically remain on high-concentrate feed for over 300 days, rumen development during pre-weaning and post-weaning up to 300 lbs is critical to overall growth performance. Generally, calves are born as functional monogastrics, having little capacity for rumen fermentation (Berge, 1991). Also, calves lack sucrase and have limited amylase capacity, making simple monosaccharides such as glucose the primary energy nutrient requirement (Van Soest, 1994). At birth, the relative size of the reticulorumen as compared to the entire four-chambered stomach is smaller compared to the reticulorumen of a fully functioning bovine stomach; eventually the reticulorumen will proportionately grow from 35 percent of total stomach size to 62 percent (Lyford, 1988). Rumen development correlates to increased surface area and nutrient absorption. Calves that are kept on milk diet, as in the case of veal calves, have diminished rumen

development compared to calves that are consuming dry feed, whereas the presence of volatile fatty acids (VFA) at the rumen wall, VFA particularly from grain and starch fermentation, greatly improved rumen epithelial surface area (Van Soest, 1994). Butyrate, a product of sugar fermentation, is the major VFA responsible for rumen development, specifically in rumen papillary development (Stobo et al., 1966).

With the introduction of dry feed, nutrient production from rumen microbial fermentation and rumen development begins. This is considered the transitional phase. This period in the development of rumen function is characterized by the dual feeding of both a liquid milk replacer and dry feed. Supplemental energy from dry feed rumination on a primary milk diet will transition to supplemental energy from milk on a primary dry feed diet. Energy from VFA production in the rumen becomes more prominent than energy from the glucose and lactose from milk replacer.

After weaning, when the animal becomes solely dependent on solid feed, the calf becomes a functional ruminant (BAMN, 2003). Duff and Anderson (2007) further distinguish stomach function according to age of animal: primary monogastric up to 3 weeks of age, transitional ruminant from 3 to 8 weeks of age, and operational ruminant after 8 weeks of age (the maximum recommended age at weaning).

Compared to beef calves, dairy calves are placed in intensive programs to quickly develop rumen function. The reason for quick weaning falls mainly on economics. Milk replacers typically cost more than feed (BAMN, 2003). Having to feed twice-daily fresh-made milk replacer is also labor intensive (Chester-Jones and DiCostanzo, 1996), one

reason dairy operations have increased interest to send calves off-site to heifer ranches where calves are raised to production age (USDA, 2007).

Feeding Corn to Cattle

The practice of feeding grain to cattle in America started prior to the introduction of the Angus or Hereford breed into the country. The first report of feeding heavy grain to beef cattle was reported in Ohio in the early 1800's (Matsushima, 2006). With the invention of grain processing machinery and the formation of a commercial feed manufacturing industry by 1885, the use of corn in cattle feeding increased. As the commercial cattle feeding business began increasing during the 1940's, corn-fed cattle become more prevalent. The invention of steam-flaking for better utilization of milo in cattle in the 1960's was eventually determined practical for use on corn (Hales et al., 1966; Matsushima, 2006).

Grain processing effects on digestion. Overall, starch digestion efficiencies in the ruminant have been reported to reach 80 percent (of intake) in the rumen, 97 percent (of starch flow) in the small intestine, and 62 percent (of starch flow) in the large intestine (Harmon and McLeod, 2001). However, the rate and extent of starch digestion are dependent on several factors, including dietary starch, diet composition, dry matter intake over time, grain processing alterations, and ruminal bacteria adaptation (Huntington, 1997). For instance, starch digestion is directly proportional to the rate of digesta passage, which in turn is proportional to dry matter intake (DMI) and diet composition (Van Soest, 1994; Huntington, 1997). Muntifer and others (1981) reported the addition of monensin, a feed-grade antibiotic, decreased the amount of starch digested in the rumen

while not affecting total starch digestion in cattle fed a whole corn-based diet. Huntington (1997) reviewed several starch digestion trials and reported different starch digestibilities among several corn processing methods as related to DMI. Owens (2005) also reported that whole corn diets, because of the higher density than steam-flaked diets, are retained longer in the rumen and are exposed to further rumination.

However, the benefits of grain processing over feeding whole corn are debatable, with the economic value of feeding whole corn being the major issue. When dealing with economic value of a feedstuff, both digestibility and growth performance as a result of the digestibility must be considered. As mentioned previously, over 75 percent of operation costs are due to feed cost (Richards and Hicks, 2007). Therefore, the benefit of processing grain must outweigh the cost of not processing. Comparisons among processing methods and whole corn should be addressed.

Starch digestion has been the major initial factor in corn processing. In a review of 48 feedlot trials, total starch digestion for steam-flaked corn (99.1%) was higher than whole corn (83.6%; Owens, 2005). However, the author does concede that digestibility will vary with same factors mentioned above by Huntington (1997). Also, another review reported that dry processing (i.e. rolled or ground) had no difference in total starch digestibility (Owens et al., 1986). Owens and Zinn (2005) also reviewed 48 digestibility trials and reported that starch digestion – rumen, post-rumen, and total – were all superior ($P < 0.05$) for steam-flaked corn at 84.2, 94.1, and 99.1 percent respectively. Whole corn digestion in the rumen, however, was higher than rolled corn (74.3 vs. 60.6%), but total digestion was in the favor of rolled corn (83.6 vs. 89.3%). Trial results summarized by

Loerch and Gorocica-Buenfil (2006) contradict Owens and Zinn (2005). The authors reported post-ruminal starch digestion to be slightly in favor of whole corn over steam-flaked corn (19.8 vs. 16.9%) and that total starch digestion for whole corn was above 90 percent. Compared to ground corn fed to beef steers, whole corn had 17 percent more ruminal energy (Sharp et al., 1982). It would appear that while whole corn is less degradable to steam-flaked, digestibility parameters are comparable to both rolled and ground corn.

Arguable results between dry processing and whole corn can be explained by either mastication or rumen retention. In one trial feeding an 86 percent grain diet, ingesta collected from full-grown beef cows was reported to contain better-masticated corn with less rumination (Beauchemin et al., 1994). The authors also incubated unmasticated whole corn in the rumens of the cows for 96 hr and reported that less than 30 percent (DM basis) of the kernels remained. They concluded that since mastication of the whole corn reduced particle size, released corn solubles, exposed kernel interior, and hydrated the feed, grain processing by mechanical means could be reduced. However, the trial by Beauchemin et al. (1994) did not evaluate total starch digestion, making it difficult to extrapolate anything other than the effectiveness of mastication and a longer-than-normal incubation on the digestibility of whole corn. To support the results of Beauchemin et al., (1994), others have reported that mastication, which occurs more in younger animals compared to older animals, improves the digestibility of unprocessed grain (Nicholson et al., 1971; Panichnantakul and Stanton, 1998; Owens, 2005, Loerch and Gorocica-Buenfil, 2006).

The physical character of whole corn may also explain its comparative digestibility to rolled corn. Owens and Zinn (2005) have reported that the density of whole corn causes longer rumen retention and increased rumination when compared to rolled corn. Whole corn may have a higher effective fiber component, which is linked to increased rumination (Owens et al., 1997). Also the rigidity of the pericarp may slow acid production in the rumen, ultimately reducing subclinical acidosis and preventing a decrease in feed efficiency and intake. Production of acid and pH from whole corn diets are higher than ground corn diets and remain steady for longer periods of time (Panichnantakul and Stanton, 1998). Any negative affects associated with roughage level may also be less with whole corn due to this greater ruminal stability (Murphey et al., 1994).

Starch escaping to the small intestine may also support the use of whole corn in cattle feed. As reported above, starch digestion in the rumen is higher for processed corn, but whole corn remains above 90 percent total starch digestion. Starch digestion in the small intestine is more efficient (Owens et al., 1986). This suggests that although total starch digestion may be lower compared to processed corn, higher post-ruminal digestion prevents total digestion from being significantly lower than dry processed corn.

Starch digestibility within a specific processing method can also vary. Zinn (1990) reported results from a feeding trial using three steam-flaked diets that differed only by flake density. A positive correlation between flake density and rumen pH as well as a negative correlation between flake density and post-rumen and total starch digestion were reported. It was concluded the lower flake density (300 g/L) was the most

digestible. More recent reports have suggested flake densities below 488 g/L are best to insure at least 95 percent starch digestion (Zinn et al., 2002; Owens and Zinn, 2005), and that the feedlot industry standard was approximately 360 g/L (Vasconcelos and Galyean, 2007). Huntington (1997) reported ground corn diets to be slightly more digestible (93.5 vs. 92.2%) compared to rolled corn. When feeding processed corn, the extra quality control on particle size, including flake thickness, steaming period and temperature, and bulk density, must be taken into account when measuring cost effectiveness.

Grain processing effects on growth performance. Performance parameters across processing methods tend to mirror digestibility results. A recent study comparing a whole corn-no roughage finishing diet to a traditional steam-flake corn-based finishing diet fed to beef steers showed a tendency ($P < 0.10$) for higher body weight and average daily gain in the traditionally fed steers (Turgeon et al., 2010). This was partly caused by the lower feed intake in the experimental steers, which in turn improved feed efficiency above the traditional steers. In contrast, Owens (1997) reported that most research showed no difference in feedlot performance – average daily gain, dry matter intake, and feed efficiency – between steers fed steam-flaked or whole corn; dry matter intake and feed efficiency were higher for rolled corn diets.

Within processing method, Hales and others (2010) reported improved feed efficiency for cattle fed steam-flaked corn with a 335 g/L density over cattle fed corn with a 386 g/L density. Regardless, over the 125 d trial, no difference in average body weight was detected.

Studies are lacking when attempting to measure starch digestibility alongside growth performance. In one study by Corona et al (2005), steers (273 kg initial BW) were fed for 112 d on a 75 percent grain diet consisting of either ground, rolled, steam-flaked or whole corn. Cattle fed steam-flaked corn had higher average daily gain, improved feed efficiency, and increased carcass yield over other diets. Whole corn cattle also tended to have lower average daily gain and carcass yield to the dry processed cattle. Despite the lower performance parameters, final weights for all diets were not significantly different, with whole corn cattle being numerically (26 kg) smaller. Fecal starch was greater in the whole corn cattle while steam-flaked cattle had the highest total starch digestion. The authors concluded that steam-flaked corn was superior to dry processed and whole corn.

Corn processing effects on young Holstein cattle. As mentioned earlier, the unique production setting that young dairy bull calves are placed requires these animals to become fully functioning ruminants several months earlier in age compared to beef cattle. Most of the trials investigating corn processing effects on digestion and performance including those mentioned above have used older (> 300 kg body weight) beef breeds and crossbreeds as the animal model. Results from these trials, while helpful in delineating overall differences among different processing methods, should not be indicative of possible results when using cattle unique to dairy-beef production. As a complement to the beef studies, studies using younger dairy cattle are included.

While data comparing whole vs. steam-flaked corn digestibility is lacking in cattle less than 200 kg body weight is lacking, some research has examined differences between steam-flaked and dry processed corn. Diets consisting of steam-flaked, rolled, or ground

corn (73 percent corn diet) fed to Holstein steers (142 kg initial body weight) had no differences in ruminal pH or rumen VFA production (Plascencia and Jose Maria, 2007). Rumen starch and organic matter digestion as well as total starch and organic matter digestion tended to be increased for steam-flaked corn above dry processed corn. While the results imply that steam-flaked corn was more efficient, no significant differences were seen, and the trial was focused solely on digestion parameters, being only 14 d in length.

Blood VFA concentration was higher in dairy calves (42 d of age) fed a calf starter diet with steam-flaked corn compared to whole corn (Leismeister and Heinrichs, 2004). Diets consisted of only 33 percent corn and 15.5 percent oats. The authors also reported increased rumen wall thickness. In comparison, calves fed whole corn and oats (25 and 25% of starter diet) had thinner rumen wall thickness, greater duodenal epithelium thickness, and greater jejunal mucosal layer when compared to calves fed a control diet containing ground barley and triticale (33 and 30% of diet; Strusinska et al., 2009). A higher prevalence in rumen wall keratinization was also reported in control calves. Fine feed particles have been shown to keratinize rumen wall, which increases wall thickness as well as reduces nutrient absorption (Greenwood et al., 1997). Advanced keratinization has been linked to metabolic disorders in growing cattle (Cozzi et al., 2002), and rumen papillae growth may be altered by keratinization decreasing metabolic activity (McGavin and Morrill, 1976). In contrast, larger particles such as whole corn influence epithelial development by scratching or abrading the wall lining, which prevents keratinization (Beharka et al., 1998). To further support this idea, Leismeister

and Heinrichs (2004) reported papillae length similar for calves in the whole and steam-flaked corn treatments. Calves in the control steam-flaked group of Strusinska et al. (2009) had irregular villi shape in the small intestine, whereas the whole corn-fed calves had normally developed villi.

In relation to growth performance, calves fed whole grain diets had higher end body weight (10 kg, $P < 0.01$) and average daily gain as well as lower dry matter intake when compared to control calves (Strusinska et al., 2009). This data may seem misleading since the control diet contained two different grains, albeit ground, than did the experimental diet. In contrast, when only corn grain and processing is compared, Chester-Jones and others (1991) reported equal or better growth efficiency in Holstein calves fed either a whole or rolled corn diet (39 to 127 d of age). Calves fed the whole corn starter had increased overall body weight (9 kg) and average daily gain (0.07 kg/d). In research involving heavier post-weaned Holstein steers (177 kg initial BW), animals were fed either whole or steam-flaked corn to a harvest endpoint of 545 kg body weight (Reinhardt et al., 1998). Steers fed whole corn had significantly lower ($P < 0.05$) average daily gain and feed efficiency only during the finishing phase; while whole corn-fed cattle stayed in the feedlot 14 d longer, differences in growth performance prior to the finishing phase were not seen. To further complement the findings of Reinhardt et al. (1998), Leismeister and Heinrichs (2004) reported similar growth performance parameters, including weight gain, average daily gain, and feed efficiency, in dairy calves fed a starter diet containing either steam-flaked or whole corn through 42 d of age. The only difference found was in dry matter intake, which was higher in calves fed whole

corn. Others have also reported similar results in calves fed a calf starter containing 40 percent rolled, whole, or steam-flaked corn through 56 d of age (Batemen et al., 2008). Together these reports suggest that feeding whole corn to Holstein calves is digestively efficient and may diminish feed processing costs until the animal has reached approximately 300 kg body weight.

Cost of feeding processed grains. The possible effectiveness of feeding whole corn to young dairy steers without loss to production compared to processed corn leads into the possible management benefits as well. Whole corn requires no processing prior to feeding, so several facility factors are kept out of the cost for diet mixing. The use of processing machinery as well as the extra labor required for processing corn is no longer necessary. However, calculating management costs alone does not effectively evaluate the total value of feeding processed corn over whole corn. In order to understand the economic value of feeding processed grains, costs of processing and value of feeding must be considered together.

Reports identifying the total costs of processing grains for cattle have existed for nearly 2 decades. Schake et al. (1981) compared the processing costs of 5 different corn processes, specifically dry processed (rolled) and steam-flaked corn, using dry processed corn as the comparative basis. The authors assumed several fixed factors across processing method, including feedlot capacity (90%), interest applied on storage of processed grains, and total amount of grain consumed by cattle over the entire feeding period (1180 kg for 204 kg body weight gain). Variable energy costs included electricity required to run the hammermill/rollermill and natural gas required to produce steam.

After calculating total costs, the price of processing steam-flaked corn per animal was nearly twice the amount required for processing rolled corn and 3.75 times greater for each ton of grain processed. With the added cost of natural gas for steam production, energy cost per ton of processed grain was 45 times greater for steam-flaked corn than rolled corn. The authors concluded that steam-flaking was energy intensive when compared to rolling (Schake et al., 1981). Healy and others (1994) concur with the evaluation reported by Schake. The authors reported that as the milling input increased to decrease particle size, milling costs (energy and labor) also increased. As processing method is intensified, cost of processing becomes more expensive.

While Schake et al. (1981) and Healy et al. (1994) provide a fundamental cost comparison across different processing methods, the authors did not include within their evaluation the value added from the different forms of corn produced. Peters (2006) described the value added factors as the increase in feed efficiency which, in growing and finishing beef cattle, is greater in cattle fed steam-flaked corn compared to cattle fed rolled or whole corn.

More recent evaluations of processing economics has helped update the need to include the value of processing. Macken et al. (2006) first reported the general initial costs for equipment used in rolling or flaking corn. Initial costs for a working roller mill average around \$75,000, whereas initial costs for a steam-flaking processor average around \$210,000. In evaluating processing costs, fixed costs included management costs (depreciation and interest, insurance, and tax) and variable costs included labor, maintenance and repair, and energy. Total costs per metric ton of corn processed were

averaged to \$1.20 for rolled corn and \$7.90 for steam-flaked corn. The authors concluded that natural gas inputs were the greatest insult to the total cost for steam-flaked corn (~\$4.00/ton; Macken et al., 2006).

Following along with report from Macken et al. (2006) with the inclusion of efficiency comparisons reported by Zinn et al. (2002), Peters (2006) further establishes the value of feeding steam-flaked corn. With the inclusion of feed efficiency into Macken's model and using whole corn as the basis, the net value of steam-flaked corn (per bushel) was highest, with ground corn, rolled corn, and whole corn following in value. While dry processing (grinding or rolling) costs were lower, the added feeding value of steam-flaked improved overall value. Within these reports, however, it is imperative to understand that the efficiencies used (Zinn et al., 2002) were for growing or finishing beef cattle and not dairy steers.

Laboratory Analysis

In order to understand nutrient digestion, several laboratory methods are employed to determine nutrient content of a sample. Samples commonly analyzed in cattle production are feedstuffs (i.e. diet and forage) and manure. In comparing nutrient composition of feces to the diet fed, nutritionists can better evaluate the digestibility of the diet and can make appropriate adjustments in feeding. The following methods were employed in the studies of this dissertation.

Laboratory methods. While the accepted range of temperature for determining dry matter is wide (50°C to 100°C; Kellems and Church, 2010), only 1 temperature point is required and provides consistently of method. In the current studies, the samples were

dried at 90°C until reaching a constant weight (24-48 hr). After drying, samples were ground in a Wiley mill (Model #4, Thomas Scientific, Swedesboro, NJ) and passed through a 1mm screen.

Although the Kjeldahl method has been used in the past, newer technology has made the older Kjeldahl method less practical. Samples were analyzed using a nitrogen analyzer (TC400, Leco Corp., St. Joseph, MI). Crude protein was calculated as the nitrogen concentration multiplied by a factor of 6.25.

Fiber fractions (neutral and acid detergent fiber) were analyzed with a fiber digester (Ankom 200, Ankom Technologies, Macedon, NY). The Ankom neutral detergent method is derived from the Van Soest detergent fiber method which uses a sodium lauryl sulfate (pH 7) to remove the cell contents from the cell wall components, cellulose, hemicellulose, and lignin (Goering and Van Soest, 1975). The newer method also employs a sealable porous polymer bag for each sample and an enclosed, heated liquid chamber (2 L volume) to limit deviation within a run. Samples (500 mg/bag) were placed in the chamber with the solution and 4 mL heat-stable amylase (eliminates starch contamination) and boiled for 1 hr. After boiling, bags were washed 3 times in agitating hot water for 5 minutes each with 4 mL of amylase being added to the first 2 washes. Bags were then subjected to a 10-minute soak in acetone to remove any residual fats or organic contaminants, air dried for at least 3 hr, and placed in a drying oven for 2 hr at 90°C before obtaining final weight. Neutral detergent fiber was calculated as the remaining sample weight divided by the initial sample weight.

Acid detergent fiber was also determined using a modified method of Van Soest (Goering and Van Soest, 1975). Samples having already been run through a neutral detergent analysis were placed in the Ankom digester with 2 L of a cetyltrimethylammonium bromide and 1 N sulfuric acid solution and boiled for 1 hr. After digestion, bags were washed 3 times in agitating hot water followed by a 10-minute soak in acetone. Bags were then air dried for at least 3 hr and placed in a drying oven for 2 hr at 90°C before obtaining final weight. Acid detergent fiber was calculated as the remaining sample weight divided by the initial sample weight.

Ash was determined as the residual weight of a sample after subjecting the dried, ground sample (1 g/sample pan) to a heat of 500°F for 4 hr in a muffle furnace. Ash was calculated as the residual weight divided by the initial sample weight.

Starch analyses methods differ depending on the laboratory performing the analyses (MacRae and Armstrong, 1968; Fleming and Reichert, 1980; Zinn, 1990). The method developed by Zinn (1990) was used. However, modifications by the Zinn lab and the current laboratory were also employed. Samples (250 mg/vial; 2 vials/sample) were placed in a 15 mL scintillation vial with 5 ml deionized water and 1 drop toluene, the screw cap was tightly closed, and the vial was incubated for at 100°C in a water bath for 3 hr. Vials were then cooled in an ice bath for 10 min, and 5 mL of enzyme solution [0.55 mg amyloglucosidase (~70 units/mg) per sample in solution containing 10% anhydrous sodium acetate (wt/vol) and 7.27% glacial acetic acid (vol/vol) in deionized water] was added; the vials were capped, vortexed, and incubated for 2 hr in a 39°C shaking water bath for 2 hr. After digestion, vials were removed and cooled to room temperature. One

mL of 15% zinc sulfate solution was added to each vial and allowed to settle for 10 min. The supernatant was recovered with filtering through a Whatman #4 filter paper.

In 4 ml culture tubes containing 2 mL of a 6% o-toluidine/94% glacial acetic acid, 50 μ L of sample filtrate was added. Tubes were lightly covered with a plastic cap and incubated in 100°C water for 10 min. Tubes were removed and cooled in a cold water bath for 5 min. Reaction solution was pipetted into a 96-well plate, and absorption was read at 620 nm by a microplate reader (Multiskan Ascent, Thermo Scientific, Waltham, MA). One blank and 2 concentrations of both glucose and commercial corn starch were included in each run to measure digestion and starch concentration.

Analyses issues. Livestock nutritionists depend on accurate sample analyses to accurately calculate diet formulations and predict digestibility. However, there is concern with the method accuracy within and among laboratory, specifically the nutritionist's ability to match predicted response with a balanced ration calculated from laboratory analyses (Undersander, 2000). In a survey of 86 laboratories certified by the National Forage Testing Association, the author reported larger standard deviations from the testing laboratories compared to the reference laboratory deviations for dry matter, crude protein, and acid and neutral detergent fiber. Laboratories that used near-infrared spectroscopy as the primary analysis method had lower deviations than those that used wet chemistry for analysis. Undersander argued that the demand on higher production has increased the need for greater precision concluded that wet chemistry methods may vary across laboratories. Flawed methods may also affect accuracy (Undersander, 2000).

Another major issue with wet chemistry analyses is the variable analyses and costs included by commercial laboratories. In a dated survey, Coppock and others (1981) surveyed 32 commercial feed laboratories. The price of the “standard” package from 32 responding labs ranged from \$6 to \$35 (mean = \$12.42). Analyses offered in these packages varied by laboratory, with dry matter, acid detergent, and calculated total digestible nutrients being the most often analyses included. Crude fiber and calculated net energy for lactation were less often included and neutral detergent fiber and crude protein being included more sparsely. While a basic minerals analysis were included by some laboratories, others charged mineral analyses separately and at nearly the same price as the standard package (Coppock et al., 1981).

A short survey of current fee schedules and offered analyses from 5 commercial laboratories across the United States reveals the same amount of variation (J.D. Allen, unpublished data). Basic packages analyzed mainly dry matter and crude protein with a few laboratories offering extra fiber analyses for extra cost (\$13.50-\$23.00). Standard packages ranged from \$25.00-\$35.50, but varied in offered analyses. Main analyses included dry matter, crude protein, fiber fractions, and calculated total digestible nutrients and net energy (lactation and gain). Basic mineral analyses were usually included but also offered as optional. One laboratory, although not specifically identifying individual analyses, offered spectrophotometer analyses exclusively and for less than the lowest wet chemistry package offered by the other laboratories. Another laboratory offered either wet chemistry or spectrophotometer analyses of nutrients, the spectrophotometer covered

extra nutrient analyses (including starch) at half the price of the wet chemistry cost. All 5 laboratories did not offer fecal analyses.

According to the fee schedules in the survey, wet chemistry analyses could take over a week for results. Timely analysis of feedstuffs and feces is important in nutrient management. Results from samples taken a week prior may not be relevant. Feedstuff batches or diet composition may have been changed during that span of time. Therefore, quicker analytical results that are also accurate are required to better manage the nutrition of production livestock.

Spectroscopy

One way to reduce time between collection and analysis is through near-infrared spectroscopy (NIRS). Although discovered over 200 years ago, the potential for using near-infrared light as a tool in determining chemical composition and physical characteristics of organic samples was not fully realized until the late 1950's with even more applicability in the agriculture arena beginning in the 1970's with Norris' research and the acceptance of NIRS technology in feedstuff analysis (NFTA, 2010). Today, with the advent of high-speed computers and advanced chemometric algorithms, NIRS technology is used worldwide in all aspects of organic qualitative and quantitative analyses, from fecal nutrient composition to pharmaceutical quality control.

Near-infrared history. Prior to 1800, little research had been done examining different forms of wavelengths and light radiation. However, at the beginning of the 19th century, Frederick William Herschel conducted an experiment to examine the variation of energy from the sun within the range of visible light (Blanco and Villarroya, 2002). The

development of his discovery of infrared light was merely to answer phenomena that he had noticed while doing work with telescopes. In his experience with blocking various light wavelengths to focus far-seeing telescopes, Herschel had noticed that in some cases where he had little light in which to see, the sensation of intense heat was apparent. However, in some cases where he used greater amounts of light to see, the heat intensity was unnoticeable (McClure, 1994).

These apparently related peculiarities lead him to hypothesize that different types of light (i.e. radiation wavelengths) varied in capacity to produce heat. His experiment was relatively basic, which included a darkened wooden bench top, a prism to separate the different color bands of sunlight (Davies, 1998). By passing sunlight through the prism and onto the bench top, Herschel was able to determine relative heating potentials of all visible colored light gradients or bands, with heat increasing from the blue band to the red. As Herschel moved slightly beyond the red band where there was no visible light, however, he discovered that heat continued to rise on the bench top. Since this heat was produced near the red band, this “invisible” light was deemed the infrared region (Davies, 1998).

Unfortunately, the use of the near-infrared region as an analytical tool was deemed by other scientists as not important (Blanco and Villarroya, 2002). Research involving the use of NIRS was minimal until the early 1920’s, when technology had improved but was not practical until the 1950’s and 60’s when computer technology and mathematical algorithms that complemented NIRS analysis were also being developed and improved (Reich, 2005).

By the 1970's, the advent of NIRS analysis in agriculture had begun. Particularly, the work of Karl Norris and co-researchers helped verify its applicability in analyzing important nutrient concentration (i.e. protein and digestible organic matter) of agriculture food samples (Blanco and Villorroya, 2002). Still, NIRS technology was considered inferior to other spectroscopic analysis methods, being called by Wetzel (1983) as the “sleeper among spectroscopic techniques” because of minimal research usage but considerable potential.

A little over a decade passed with advancements in computer technology when McClure (1994) declared NIRS technology was “running strong”. This may be attributed to the agriculture research and industry sector that championed NIRS technology as a viable analytical tool. During the 1970's and 80's, Karl Norris' agriculture feed analysis group had contributed several research papers involving NIR analysis and encouraged the application of multivariate analysis in spectroscopic studies (McClure, 1994). In 1974, the Canadian Grain Commission replaced the traditional Kjeldahl laboratory protocol with NIRS technology as the preferred and primary method for analyzing nitrogen content in cereal grains (Blanco and Villarroya, 2002).

Today, research using NIRS technology as the sole analysis technique has been used by several environmental and clinical disciplines, including agriculture, medicine, chemistry, and physics (Davies, 1998). To complement these disciplines, NIRS technology has improved to include portable units capable of on-site (*in situ*) forage analysis (Guo et al., 2010). Also, industry professionals in various fields including food production, natural and textiles, pharmaceuticals, and petroleum have adopted this

technology for everyday quality control directly on the production line (Davies, 1998; Blanco and Villarroya, 2002).

NIRS positives and negatives. Near-infrared spectroscopy is essentially the use of the non-visible NIR spectrum of light to analyze constituents and chemical structures of organic samples both directly (nutrient concentration) and indirectly (physical structure of sample, digestibility, etc). Coupled with a particular group of mathematical algorithms (chemometrics) performed by high-speed computers, analysis is quick, noninvasive, and can analyze for several constituents with only one reading (Norris, 1989a; Foley et al., 1998; Blanco and Villarroya, 2002). Traditional laboratory methods call for time consuming and expensive protocols which require the destruction of the sample used (i.e. acids, detergents, assays, furnace ashing, etc.). As an alternative method, NIRS does not require the destruction of a sample; at most, a sample needs only to be dried and ground (bench top reading). There is minimal sample preparation leading to higher throughput; in a study analyzing pasture samples to assess forage quality for the wombat, Woolnough and Foley (2002) were able to analyze up to 140 ground samples in one day for a total of 8 components. Allen and others were able to analyze approximately 200 unprocessed fecal and diet samples for 6 components in less than 4 hr (J.D. Allen, unpublished). Sample sizes can be as low as 200 mg (if dried and ground), no chemical waste is produced, and NIRS can save up to 80 percent of laboratory costs (Foley et al., 1998). Because a smaller amount of sample is required, less time is spent in sample collection. Other advantages include the capability of determining physical characteristics, comparable accuracy to laboratory techniques, high precision, high performance sensing

capability (fiber optics), and suitable ruggedness for use in industrial plants. (Blanco and Villarroya, 2002). Near-infrared spectroscopy provides many advantages over laboratory methods of analysis.

The advantage of NIRS analysis is further touched upon by Reeves (2007) using a dairy operation as a template. At this typical dairy, several analyses is required by several agencies, including the United States Department of Agriculture (USDA), the cattle nutritionist, the milk buyer, and the manure manager. Quick analyses on soil carbon for carbon sequestration, feed nutrient composition and digestibility, milk nutrient composition, and manure composition are needed and at times required. However, laboratory analysis of all of these sectors requires time and money to obtain the sample and then have it analyzed by an off-site laboratory. Time is wasted waiting for results, and if the results do not return in an adequate amount of time, data received may be useless for timely management. While some federal and local regulations mandate frequent analysis of some, especially soil composition for environmental impact assessment and milk composition for grading the quality of a bulk tank, the dairy is liable to default testing on non-regulated items (Reeves, 2007). Overall, the author is concerned that analytical testing on farms is low because of the number of tests that are required along with the loss in time and revenue obtaining the analysis results. As such, NIRS technology is capable of producing quicker results in order for any operation to assess environmental impacts and comply with mandatory regulations.

Reeves (2007) further applauds the NIRS affordability. Whereas benchtop models can reach up to \$100,000 USD, portable models range between \$20-50,000 USD.

Although this may seem high, the fact that either model type is capable of analyzing all of the samples mentioned above at the dairy farm as well as any other organic-based sample. Also, compared with other spectroscopic technologies such as mid-infrared (MIR) spectrophotometers, the NIRS models are more adaptable to on-site analysis, are less expensive, and are more commercially available while sustaining robust analysis capability (Reeves, 2007).

Even with these advantages over traditional analytical methods, NIRS technology still has its pitfalls. Near-infrared analysis is dependent on the environment. Both temperature and physical structure or particle size of the sample can interfere with spectral reading (Foley et al., 1998). With traditional NIRS procedures involving dried and ground samples, analysis is dependent on particle size, which may vary between different grinding machines. Also, heat energy interferes with molecular vibrations, which in turn shifts expression of absorption bands in NIR spectra (Stuth et al., 2003).

Because NIRS uses light for analysis, spectral analysis is required. This involves the use of a calibration sample set to create statistical models of detecting nutrient components, which, if not done, renders the spectral reading useless (Walker, 2010). Therefore, NIRS analysis is dependent on the laboratory analysis of the calibration samples which also leads to the idea that imprecise and faulty laboratory analyses can influence NIRS accuracy and precision (Foley et al., 1998). Regardless, analytical techniques must initially be performed, and each constituent requires a separate statistical model for proper analysis, thus increasing initial lab costs as well as time spent creating the prediction models (Blanco and Villarroya, 2002). Certainly, with the purchase of

advanced machinery, the cost of NIRS training, and the cost of calibrating the NIRS machine, initial costs can be pricey, but overall costs, as stated above, is economical.

Also, the NIRS is not very sensitive for constituents that are minor in the sample (i.e. specific sugars in manure) nor as selective in spectral measurement as compared to other spectroscopic disciplines such as MIR; since analysis is only direct on organic components, NIRS must rely on indirect analysis of inorganic components such as metals or physical characteristics (Blanco and Villarroya, 2002).

Despite these shortcomings, however, most of these issues can be resolved with precise and accurate laboratory analyses of calibration samples, refined statistical models, and consistent adherence to a set and specific NIRS analysis protocol (Foley et al., 1998). Near-infrared technology remains economically practical compared to other disciplines, and demand for NIR analysis technology is expected to increase as units become even more cost-effective (Reeves, 2007). Too, the advantage of having one machine to rapidly analyze different organic samples for a variety of constituents and characteristics may far outweigh the initial cost. Virtually any characteristic that is suspected of being influenced by the organic chemistry is a sample (plant or animal) could theoretically be modeled by NIRS technology (Foley et al., 1998). Overall, NIRS technology can provide a capable and relevant alternative to laboratory analysis.

To improve upon this, NIRS machinery is now portable and does not require drying and grinding of samples prior to analysis (Guo et al., 2010). This adds to NIRS attractiveness two-fold. First, it allows for analysis of samples within their own environment, promoting first-hand and pristine data collection without the risk of

disturbing or destroying the sample prior to analysis. Second, it eliminates the amount of time traveling between stations. Particularly, samples can be analyzed on location rather than having to travel between sampling locations, drying and grinding stations, storage facilities, and NIRS analysis laboratory. With portable units, the sampling location eliminates the need for the other locations. Although still not considered as robust compared to stationary units (D. Tolleson, personal communication), portable NIRS technology has expanded NIRS capabilities.

NIRS procedures. Sample analysis using NIRS technology follows a main stepwise pattern:

1st: Obtaining single or group samples. This primary step is universal. In the case of NIRS, location and physical nature of the sample is dependent on the type of NIR spectrophotometer used. Due to transportation logistics, bulky, heavy bench top units need samples to be brought to them. Portable units, on the other hand, can be taken to the samples in the field.

2nd: Preparing the samples for NIRS scanning. Usually the samples are dried and ground prior to NIR scanning, a practice meant to homogenize the physical nature of all samples and limit water content, 2 characteristics of sampling that can interfere with NIRS spectra (Foley et al., 1998). This makes plausible sense considering the variable time that can be spent bringing fresh samples to the laboratory for scanning. Dry, homogeneous samples may reduce sample degradation via wilting or mold. Even so, bench top units are capable of producing statistical data without sample manipulation

(Perez-Martin et al, 2004). In the case of portable units, fresh sampling is the main focus, and samples are left as they are in their environment.

3rd: NIRS scanning. This step replaces laboratory analysis. Spectral data is collected from the sample through the detection of reflected NIR wavelengths. Reflected light is then quantified and converted to absorbance values for each wavelength used (Reich, 2005).

4th: Calibrating the NIRS unit. As laboratory analyses such as assays use controls and standards to calculate accurate constituent results and regression models, NIRS spectra need to be calibrated to known constituents from samples with known composition. Once statistically-valid regression models have been validated by chemometrics, this step can be omitted.

5th: Analyzing the NIRS data. After calibrating the NIRS unit to known compositions, non-calibration samples can be analyzed using the calibrated regression models for each desired constituent.

All 5 of these steps are important to produce accurate results and are discussed in greater detail below.

NIRS instrumentation and presentation. Near-infrared technology is available in all shapes and sizes. As described above, they can be bulky bench top machines or lightweight, handheld units that can be placed in a backpack and carried into the field. This reduction in size and improvement on motility has occurred due to advancements in technology (Davies, 1998). Computer chips have been miniaturized while increased processing capacity, and fiber optic technology has replaced older sensor components.

These and other advancements have also contributed to the accessibility and economic feasibility of NIRS machinery (Davies, 1998).

To start, a sample, traditionally dried and ground to promote homogeneity within a sample and consistency among a sample set, is placed in a sampling non-NIR-absorbing container (Foley et al., 1998). A container that has one side consisting of quartz or silica is often used because it is inorganic (does not absorb or deflect NIR radiation) and transparent to insure homogeneity in the sample. In the case of field collection where silica sample cups are not practical in carrying, plastic polymer bags with guaranteed consistency in chemical makeup and bag wall thickness can be used (D. Tolleson, personal communication). Before starting the sample reading, a container with a blank (white ceramic that does not absorb NIR radiation) is needed to eliminate any noise created by the containers. After blanking the machine and preparing the sample container, the first sample is ready to be irradiated.

The light filter or chromator differs on whether analysis will be discrete or whole spectrum detection (Blanco and Villarroya, 2002). Essentially, chromators are dependent on when the emitted light is changed from monochromatic (i.e. white light) to polychromatic (i.e. blue light). Here only the basic principles will be discussed. Older NIRS machines disperse and filter light prior to irradiating a sample (D. Tolleson, personal communication); dispersal is performed by broadband, discrete filter photometer, or light-emitting diode (LED)-based components (Reich, 2005). These types of dispersive components limit the frequencies (50-100 nm sections) allowed to be emitted upon the sample. In contrast newer models disperse light after irradiating a

sample through the use of diffraction grating, interferometer, or diode array (Reich, 2005). These types of post-dispersive chromators allow for full spectrum analysis and have displaced the need for pre-dispersive machines.

After light has both irradiated the sample and passed through the chromator (not necessarily in that order), reflected wavelengths are detected by semiconductors. These semiconductors, or wavelength detectors, also vary by machine and purpose (Reich, 2005). In older models, silicon or lead sulfide semiconductors are used. Silicon detectors, which are fast with low noise, are highly sensitive to wavelengths from visible light to 1100 nm, whereas lead sulfide detectors are slower and are sensitive from 1,100 to 2,500 nm (Reich, 2005). Differing sensitivities are congruent with wavelength intensities: wavelengths below 1,200 nm are weak enough to render difficulty in calculating accurate quantitative measurements, and wavelengths above 2,500 nm are strong enough to create the same problem (Norris, 1989b). The most versatile detector is the indium-gallium-arsenic (InGaAs) semiconductor because it is small, comparatively fast, and is also sensitive from 1,100 to 2,500 nm, the NIR section where most constituent analysis occurs (Blanco and Villarroya, 2002; Reich, 2005). All detectors are capable of simultaneous detection of all wavelengths within their respective sensitivity.

To end, detection of reflected NIR wavelengths by the semiconductors is interpreted by a computer in spectral form (Figure 1.1). The spectrophotometer emits a known quantity of NIR light and records the reflectance for a given sample (Stuth et al., 2003). Then, the computer equates reflectance wavelengths into absorption values for each wavelength. Further description of NIR analysis is discussed in a later section.

Spectroscopy basics. After mentioning previously about wavelength reflectance, the photometric intricacies of NIR analysis should be discussed. To keep in context, wavelength and energy are convertible one with the other (Walker, 2010). Spectroscopy, in which NIRS is included, is the study of interaction between electromagnetic radiation and matter as a function of wavelength (Walker, 2010). The electromagnetic spectrum consists of photons which carry different levels of energy (Stuth et al., 2003). This spectrum ranges from short (< 1 pm), high frequency (10^{24} Hz) wavelengths (gamma rays) to long (> 1 mm), low frequency ($< 3 \times 10^{11}$ Hz) wavelengths (radio waves). Visible light wavelengths extends from 400 nm (violet) to 750 nm (red), whereas NIR wavelengths extend from above 750 nm to 2500 nm (Fig. 2; Foley et al., 1998). Although this review is focused on the NIR region of the electromagnetic spectrum, examples using other sectors of the electromagnetic spectrum are included.

There are several fates of photon radiation as it emitted upon a sample. It can be reflected, transmitted, or absorbed by chemical bonds of molecules within the sample (Walker, 2010). Since NIR technology measures via transmittance or reflectance (Walker, 2010), absorption is essentially the inverse of either measure (i.e. low reflectance is relative to high absorption of a wavelength). Transmittance is radiation that passes through a sample; it is important for liquid, transparent, or extremely thin samples, and can be recorded by detectors placed on the backside of the sample. Transmittance detection is not important for solid or sludge-like samples since the probability of radiation passing through the sample is minimal. Radiation reflectance and absorption are the primary NIR measures in agriculture samples. Reflectance is recorded as a variable

(R), and the absorption value is expressed as the logarithm of the inverse of R ($\log 1/R$; Dryden, 2003). An absorption value is calculated for each wavelength reflectance value, creating a spectrum for each sample.

Not all reflected radiation is helpful in obtaining $\log 1/R$ values. Reflectance directly off the surface of the sample is called specular reflectance and is considered more of an interference in NIRS spectral analysis; the noise created by specular reflectance can be corrected or reduced with chemometrics (Dryden, 2003). The radiation relevant to NIRS analysis is diffuse reflectance, radiation that enters a sample and is deflected at an angle to the semiconductor detector (Walker, 2010). Diffuse reflection contains a number of variations due to non-specific scatter, variable path length, and path distortion created by the chemical composition of the sample (Dryden, 2003). Because of these variations, diffuse reflectance is significant and unique for each sample, regardless of the number of similarities to other samples.

Near infrared wavelengths have low absorption coefficients, allowing radiation to penetrate deeper (up to 2 mm) into substances (Dryden, 2003). A deeper penetration capability leads to more effective analysis of highly heterogeneous samples (i.e. solids, total mixed rations, feces) without pretreatment or homogenization (drying and grinding) of the sample (Reich, 2005). The low absorption coefficient of NIR wavelengths creates a problem in spectral analysis, which will be discussed later.

Although indirectly quantified by reflectance by NIRS analysis, the basics of absorption is needed to explain how matter is capable of trapping or rejecting wavelength radiation. Absorption occurs only if the wavelength is equal to the energy difference

between the vibrational and rotational states of the bond (Walker, 2010). Vibration is identified as the stretching (moving along axes) of a bond as atoms move closer and farther together, and rotation is identified as the revolution of a bond about its molecular axes (Foley et al., 1998). Fast wavelengths such as gamma rays can cause electron shifts whereas slow wavelengths such as NIR cause molecular vibrations (Stuth et al., 2003). Wavelength absorption is further complicated by bond strengths that influence the length and geometry of other bonds within a molecule as well as bonds from one molecule influencing the frequency state of a chemical bond in an adjacent molecule. Since bonds held by a molecule already vibrate at differing but specific frequencies according to geometry and spatial arrangement to other bonds as well as atom repulsion and bond dissociation within a molecule, energy levels across a molecule are not equally spaced (Blanco and Villarroya, 2002). This complexity of a molecule's energy absorption capacity individualizes each molecule within a sample. Essentially, molecules each have their own unique wavelength (Foley et al., 1998). Radiation that is not absorbed by a molecule is reflected elsewhere within or out of the sample (Stuth et al., 2003). Therefore, each different molecule has its own reflectance fingerprint to separate itself from other chemically dissimilar molecules within the sample.

To put this into perspective, the idea of visible color is used (Stuth et al. 2003). White or visible light is made of all color wavelengths and can be detected by the human eye. Surfaces such as a yellow tabletop are perceived as yellow because that tabletop is absorbing all color wavelengths except yellow. The yellow wavelength is being reflected by the tabletop to the eye and is interpreted as yellow by the brain.

Near-infrared technology can be interpreted in the same way, only it does not deal with visible light. As with color wavelengths in the visible spectrum, differing NIR wavelengths are absorbed by molecules while other wavelengths are reflected, creating a specific “color” perceived by NIRS. For NIR wavelengths, the “color” is more a perception of organics. The infrared wavelength region is known as the organic region because chemical bonds between carbon, hydrogen, nitrogen, and sulfur (i.e. C-H, O-H, C-S, C-C, etc.) all absorb wavelengths within infrared frequency range (Foley et al, 1998). All organic matter consists mainly of carbon, nitrogen, oxygen, and hydrogen. Much like a human cannot see wavelengths outside of the visible light range, NIRS technology cannot perceive or interpret wavelength reflectance that does not exist within the established 750 – 2500 nm region (or smaller sector within the region as is mentioned for wavelength detectors).

However, near-infrared absorption is not solely an organic region and has some extra inclusions. Although organic chemical bonds can be absorbed, other non-organic molecules can be read, mainly due to hydrogen and hydrogen bonding. Hydrogen is the lightest element and exhibits greater vibration states and greater deviation from harmonic (normal) behavior (Blanco and Villarroya, 2002). This makes hydrogen bonds to other molecules stronger than some unstable organic bonds. Hydrogen is susceptible to ion presence and temperature, causing hydrogen bonds to alter the length of other chemical bonds (Davies, 1998). Hydrogen bonding to inorganic molecules (i.e. phosphate) and metals allows for analysis of inorganic substances that are associated with organic samples. Also, water by its chemical nature is not technically organic. However, the H-O-

H bond chemistry is highly susceptible to absorbing NIR radiation (Dryden, 2003). If kept at high concentration within a sample, water retains a high capacity to distort spectra, which is a reason some laboratories continue the protocol of drying samples prior to NIR analysis (Abrams et al., 1988).

Calibration and multivariate regression. Near-infrared analysis by itself is useless. Because of overtones and combinations, spectral data give a mottled, indistinct view of the fundamental organic bonds and related inorganic constituents within a sample, but do not give concise quantitative analysis of specific constituents. A need for comparative analysis against a calibration sample set with known composition is required to bring functionality to NIRS technology.

Calibration is the use of NIRS (or any other spectroscopic region) spectra as multivariate descriptors to predict nutrient concentrations or physical characteristics by using statistical analysis (Walker, 2010). It is the creation of a spectro-chemical prediction model (Stuth et al., 2003). Samples in a calibration set have desired component analyses performed through traditional laboratory methods. Then, through the use of multivariate statistical modeling, regression equations are created for each desired component (Foley et al., 1998). Regression models are not interchangeable one constituent to another. Once regression models are perfected, calibration models can continue to be improved upon with the inclusion of more samples into the calibration set. Periodic calibration updating is recommended (Reeves, 2007). In some circumstances, however, the cost of added laboratory analyses may outweigh the plan for recalibration. In the case of digestibility studies, both diet and fecal samples need to be analyzed to

determine digestibility parameters, which can be time consuming and costly when dealing with variations of forage due to season, location, fertilizer use, weather, and plant maturity (Walker, 2010).

Just like NIRS analysis, calibrating NIRS analysis also has a general stepwise procedure (Blanco and Villarroya, 2002; Reich, 2005). First, calibration samples are chosen. This requires some tactical expertise. In order for a calibration model to be effective, samples need to represent the entire concentration range of the desired constituent expected in any future samples (Stuth et al., 2003). Also, the sample set should be well-distributed to reduce the chance of skewing the regression model toward one end of the constituent range and to represent a defined degree of spectral variation; samples with extreme or duplicative spectra should also be avoided for this reason (Foley et al., 1998).

Calibration models are unique to the machine in which they are developed. Although identical machines may be able to share models, non-identical machines have been proven incapable, so it is generally considered to treat each machine as its own regression model creator (Shenk et al., 1985; Williams and Krischenko, 1986; Dryden, 2003).

When dealing with range and distribution, calibration size must also be considered. In general statistics, a higher number of observations is correlated to more robust predictive regression models (Ott and Longnecker, 2001). Still, calibration sets may vary in size according to the ease of statistical modeling and the predictive statistical values that are accepted by the operator. In consideration of these variables, a universal

optimum calibration size cannot be established (Dryden, 2003). Researchers have reported calibration sizes from less than 50 to well over 100 samples while interpreting predictive statistics as acceptable (Dryden, 2003; Perez-Martin et al., 2004). It is generally considered to have at least 50 calibration samples if partial component regression (PCR) is used in modeling (Dryden, 2003). With calibration having an open-ended time frame, weak regression models can be shored up and made more robust with the continued addition of calibration samples.

Once the calibration samples have been selected, the next step is to determine the target parameter or constituent in the calibration samples. Obviously, the desired constituent or constituents have already been chosen prior to the initiation of a research trial or the use of a NIR spectrophotometer on the production line. Specific target parameters should be analyzed according to precise laboratory protocol. Since spectroscopy analysis is reliant on laboratory analysis, NIRS predictions can only be as accurate and precise as the data that is used in the calibration modeling. As such, NIRS technology has helped to improve laboratory techniques, encouraging analysts to evaluate the effectiveness of the procedures used by identifying and correcting sources of error (Foley et al., 1998). In circumstances where the cost for improving laboratory protocols is unfeasible, it is suggested to add more samples to the calibration set (Dryden, 2003).

Next, calibration samples are scanned according to procedures described previously. Again, meticulous adherence to a NIRS scanning procedure is advised to avoid misleading and inaccurate spectral data (Reich, 2005).

Spectral bands are not selective because of the variety of absorption potentials among the composition of molecules within a sample. Spectral bands are influenced by physical, chemical, and structural characteristics of the sample, environment, and the NIRS machine (Blanco and Villarroya, 2002). Specifically, light scatter by specular reflectance, path length variations, and environmental and instrumental interference create irrelevant information, or spectral “noise”, in the spectra which makes direct analysis of constituents difficult, especially if water and particle size varies across samples (Blanco and Villarroya, 2002; Reich, 2005).

The next step in calibration is to eliminate, standardize, or reduce the irrelevant information interfering with calibration modeling (Reich, 2005). Chemometric pretreatment of the spectra deals with this issue by helping to differentiate overlapping band peaks (Walker, 2010). Various mathematical techniques are often used including first and second derivatives or smoothing, multiplicative scatter correction (MSC), standard normal variate (SNV), and detrending (Blanco and Villarroya, 2002; Reich, 2005). Each technique targets a specific noise component, so combining 2 or 3 pretreatment methods is common; combinations are dependent on the acceptance of the predictive statistics that each combination is able to produce.

Once the spectra have been pretreated, regression fitting is performed. In terms of NIR spectra, wavelength bands represent fundamental bonds and not whole molecules, and their intensities can be derived from various molecules containing those fundamental bonds. To accommodate multiple absorption values for constituent molecules, multivariate analysis is used.

Three general types of multivariate analysis have been used in spectral analysis, multiple linear regression (MLR), PCR, and partial least squares (PLS; Lipp, 1996). The first, MLR, has been used longer than the other 2. The latter 2, in fact, are sequential analytical improvements upon MLR. Thus, MLR is the least often used, especially in agriculture analysis, because it assumes that absorbance is equal to concentration, which as discussed earlier is not always the case (Blanco and Villarroya, 2003). The MLR method also uses a few wavelengths for regression analysis, regardless of whether a better fit regression using a greater number of wavelengths can be used.

An improved method of multivariate analysis over MLR is the PCR method. The main feature of PCR is its use of principal component analysis (PCA). In this method, principal components are identified by using wavelengths having the most variability, reducing them by relevancy, and then orthogonally relating them for the best fit (Blanco and Villarroya, 2002). The use of PCA resolves the spectral data in to orthogonal components whose linear combination approximates the original data (Reich, 2005). Figure 1.3 helps to visualize the process.

The PLS method takes PCR in a slightly different direction. Instead of using PCA to identify principle components with the largest variations, PLS identifies principle components that have the most relevant variations while maintaining the best correlation between components (Reich, 2005). Since its development by Wold in the 1960's, PLS, including its variants, remains the most popular form of chemometrics. It provides a high degree of correlation between similar spectra and is capable of maintaining orthogonality while optimizing relevant wavelength factors (Walker, 2010).

Qualitative calibration or pattern-recognition methods use variant forms of the multivariate regression. However, instead of identifying constituent concentrations, qualitative analyses group samples according to the specified classification through correlation coefficients between samples or as simple distance measurements (Blanco and Villarroya, 2002). Separate sample groups (i.e. whole corn vs. steam-flaked corn, levels of parasite infestation, etc.) are determined according to specific spectral characteristics (Reich, 2005).

Validation. The final step in calibration is validation of the regression model. Individual constituent regression models are tested on non-calibration samples or samples from the calibration set that have been removed from the set to determine the accuracy, precision, and statistical validity of the model (Stuth et al., 2003). This procedure involves further chemometrics, comparing standard errors and coefficients among the calibration, validation, and laboratory sample sets. It is used often to avoid statistical problems, including over-fitting and colinearity. Validation, therefore, tells the analyst if the model is satisfactory in its predictive capabilities, or robustness, according to the analyst's preferences and limits of determination.

There are several statistical parameters used in validating regression models, and a portion of those are calculated during other procedures. The lone laboratory measure needed for validation statistics is the standard error of laboratory values (SEL). This parameter is usually reported as the standard error of the mean (SEM) in traditional laboratory statistics.

Calibration statistics include a coefficient of determination (R^2) and a standard error of calibration (SEC). The R^2 value reports the proportion of variability in the calibration set that is accounted for by the regression equation (Mark and Workman, 1991; Walker, 2010). Its value lies within a range between 0 and 1.0. A value greater than 0.8 is considered acceptable, but a value above 0.9 is considered more robust. The SEC is the variability between the predicted and laboratory values of the calibration set when the calibration regression model is the equation used (Dryden, 2003). A low SEC value is an indication of accuracy.

The validation set will also have a R^2 . Again this is an indication of how well the calibration equation accounts for the variability of the sample set. The indicator of accuracy is the standard error of prediction (SEP), which should be relatively low and have a similar value to the SEC (Stuth et al., 2003). It reports the variability between predicted and laboratory values when the calibration model is applied to the validation set (Dryden, 2003).

The method of validation is just as important as the calibration or laboratory procedures used in NIRS analysis. Validation statistics can be deceiving. For instance, during PCA analysis and subsequent validation, a failure in focusing correlative behaviors among principle components increases the risk of over-fitting the regression model (Boulesteix and Strimmer, 2006). Too many components – or factors – will lead to a high R^2 and a low SEC, but will also create a high SEP, a combination that is not considered very robust (Reich, 2005). Over-fitting can also be identified with a SEC much less than the SEL, a large difference between laboratory and NIR values, or a SEP

greater than twice the SEC and/or less than twice the SEL (Dryden, 2003). Essentially, over-fitting is committed when too many wavelengths are used to create a highly accurate regression equation that fails to accurately predict the constituent in samples that are not in the calibration.

The most popular way of avoiding over-fitting is through cross-validation. This method involves dividing a sample set into groups (usually 4 or more) and creating a calibration model using all but one group, which is used as the validation set (Foley et al., 1998). After the calibration model has gone through validation, another group is chosen as the validation set and the remaining groups are used to create another calibration model. Calibration and validation is performed until all groups have been used as the validation set (Stuth et al., 2003). Using the entire set to create capable regression parameters helps to reduce the risk of over-fitting (Reich, 2005).

One advantage of cross-validation is that all laboratory analyses can be used for the calibration modeling without separating validation and calibration sets (Foley et al., 1998). Also, another validation statistic, the standard error of cross validation (SECV) – a measurement of accuracy – is created. The production of SECV also gives rise to another validation statistic: the ratio of SEL to SECV or RPD (Perez-Marin et al., 2004). The RPD should ideally be greater than 3, which allows for evaluation of the SECV in relation to the standard error of laboratory samples (Walker, 2010). Cross-validation validation is considered the best estimate of predictive capability of a NIRS equation (Stuth et al., 2003). Also, cross validation helps reduce the risk of over-fitting.

NIRS practice. The application of NIRS technology has been reported in several disciplines. Analysis by NIRS has proven versatile in both research and industry arenas. As such, a copious number of science papers have been published over the past several decades. McClure (1994) reported 30 different sciences reporting use of NIRS technology. Other than agriculture and pharmaceutical, reports varied from other science disciplines (biology, chemistry, astronomy, physics, geology) to industry (cosmetics, polymers, textiles) and technology advancement (chemometrics, sensors, software). With its quantitative and qualitative capabilities, NIRS technology is only expected to increase in use and distribution across disciplines.

In non-agriculture disciplines, a mix of qualitative and quantitative NIRS analyses is employed. Pharmaceutical production focuses NIRS technology on quality control issues to avoid drug adulteration and uniform products (Reich, 2005). Samples are often analyzed for particle size of ingredients, surface area of pills, crystal content for absorption effectiveness, viscosity in liquids, and gel strength as a drug release parameter (Ciurczak et al., 1986; Buckton et al., 1998; Storz et al., 2002). Since adulteration of pharmaceuticals is an important aspect of drug production, NIRS inspection can occur on raw materials through packaging, on intermediates such as active ingredients and carriers, and on finished products in their various forms to determine acceptability (Blanco and Villarroya, 2002).

In soil and manure management science, monitoring can be done with NIRS analysis on the carbon and organic fraction of the sample (Cozzolino and Moron, 2006). The carbon fraction of soil is vital to the growing potential of plants as it affects soil

quality, agriculture sustainability, soil stability, and crop yield. Because of the direct and easy analysis, carbon, nitrogen, and pH are commonly analyzed with excellent regression results ($R^2 > 0.90$), but mineral (calcium, potassium, magnesium, copper, iron) content has been reported with acceptable regression values (Cozzolino and Moron, 2003). In a study involving various sources of compost (i.e. wine waste, sewage, animal manure, citrus and nut waste), NIRS predictive R^2 values for pH, electrical conductivity, total organic matter, nitrogen, and several minerals were acceptable for all constituents except phosphorus and potassium, 2 minerals that have produced better R^2 values in other reports (Galvez-Sola et al., 2010).

Along with manure and soil management, the greatest area in which NIRS has excelled is in agriculture (Foley et al., 1998). Qualitative and quantitative analyses of feedstuffs and meat products are common. Baeten and others (2005) showed the potential for NIRS technology to detect meat and bonemeal adulteration in livestock feeds at level lower than 0.05 percent of sample mass ($R^2 = 0.87$). Near-infrared analysis of fishmeal reported an 80 percent detection capability to determine source of fishmeal source by fish specie (Cozzolino et al., 2005). Perez-Marin and others (2004) accurately ($R^2 > 0.80$) predicted concentrations of various feedstuff ingredients such as barley and corn in animal feed. Meat products analyses have proven excellent ($R^2 > 90$) predictability for the major components of fat, protein, and moisture in sausage but poor ($R^2 < 0.60$) for quality factors such as pH, drip loss, cooking loss, Warner-Bratzler shear force (Ortiz-Somovilla et al., 2007; Prieto et al., 2008).

In animal agriculture, the primary foci have been in the nutrient composition of feedstuffs and the nutrition and health management of foraging animals. Acceptable ($R^2 > 0.80$) NIRS qualitative analyses on fecal samples have been reported for various health status parameters: species and sex determination of red and fallow deer (Tolleson et al., 2005), tick prevalence on cattle and horses (Tolleson et al., 2007), and pregnancy status of dairy cows (Tolleson et al., 2001). Reported qualitative nutrient analyses of feedstuffs, forage plants, and fecal samples have included total nitrogen (which is often converted to CP), fiber components (NDF, ADF, ADL), moisture content, starch, sugar, amino acid profile, and tannins (Foley et al., 2003; Mika et al., 2003; Li et al., 2007; Landau et al., 2008). When analyzing fecal constituents, older reports often analyzed strictly for crude protein and digestibility parameters, although newer research has reported other constituents (Lyons and Stuth, 1992; Lyons et al., 1995; Landau et al., 2006; Landau et al., 2008).

Animal agriculture has also led the research in eliminating the need for processing samples (drying and grinding) before NIRS scanning. In a study using sample sets of 433 and 178 feedstuff samples containing up to 60 ingredients and varying in presentation form (pellet, fines, meal), Perez-Marín and other (2004) reported similar SECV and acceptable ($R^2 > 0.80$) prediction values between processed and unprocessed samples. Unprocessed corn silage samples were accurately ($R^2 > 0.80$) analyzed for DM and NDF and adequately ($R^2 > 0.70$) analyzed for CP (Berzaghi et al., 2005).

The most reported foraging animal constituent is crude protein or total nitrogen, with R^2 values greater than 0.95 and similar SEC, SEP, and SEL values (Stuth et al.,

2003). This is attributed to the strong N-H bond. Fiber is the second most reported constituent. Fiber components such as NDF, ADF, and ADL are more difficult to analyze because of their varied and large molecules that they contain. For instance, NDF excludes non-cell wall carbohydrates while estimating cellulose, hemicellulose, and lignin concentration while ADF is the measurement of cellulose and lignin alone (Van Soest, 1994). Acid detergent lignin is a measurement of the portion of lignin that remains in an ADF sample and does not measure whole lignin content of a sample.

Lipid and ash content are least to be reported. In forages, lipid content is minimal, and forage reports have had varied calibration success (Stuth et al., 2003). Ash content, as described previously, also has its unique issues with NIRS. Not only is ash inorganic and cannot be detected without association to detectable or organic molecules, it also is minimal in concentration of plant material, often less than 5 percent in forage (DM basis; Van Soest, 1994). Regardless, detection of macrominerals (potassium, phosphorus, calcium, sulfur, and magnesium) has been reported (Clark et al., 1987; Clark et al., 1989, and Saiga et al., 1989). A recent article reported that validation of NIRS predictability for the determination of microminerals (sodium, copper, iron, manganese, zinc, boron) varied ($0.50 < R^2 < 0.85$ for calibration set) by mineral and pretreatment used (Cozzolino and Moron, 2004). Foley et al. (1998) warns of the calibration failure that occurs because ash composition can vary in plants across season and species.

To date, NIRS estimation of starch concentration in fecal samples has not been reported. Most starch NIRS work has been performed on feedstuffs and cereal grains. In particular, Hicks and others (2002) applied NIRS technology to measure CP, *in vitro*

digestibility (IVDMD), crude fat, and starch content in both whole and ground sorghum. Using 184 samples, predictive quality was statistically valid but predictive accuracy was low ($R^2 = 0.45$, RPD = 1.32) for ground sorghum and even worse ($R^2 = 0.35$, RPD = 1.23) for whole sorghum. On the other hand, Batten et al. (1993) reported high correlation ($R^2 = 0.98$) in predicting non-structural carbohydrates (with starch and fructans as the primary components) in rice and wheat shoots. Starch content in unprocessed whole corn was adequately ($R^2 = 0.77$; SEP = 1.06; RPD = 2.1) predicted using 940 calibration and 304 validation samples (Paulsen et al., 2003).

Summary and Objectives

Dairy beef production provides a unique sector for quick weaning and subsequent concentrate feeding of young dairy steers. However, the cost of corn processing may have a detrimental impact on dairy beef economics. Digestion in young animals may be similar for diets containing processed or unprocessed grains, particularly corn.

In the past 30 years, reports involving feces, cereal starch, starch digestion, and portable NIRS analyses have been published. However, the combination of the fecal nutrient analysis, including starch, with the use of portable NIRS technology in an on-site industry setting has yet to be tested.

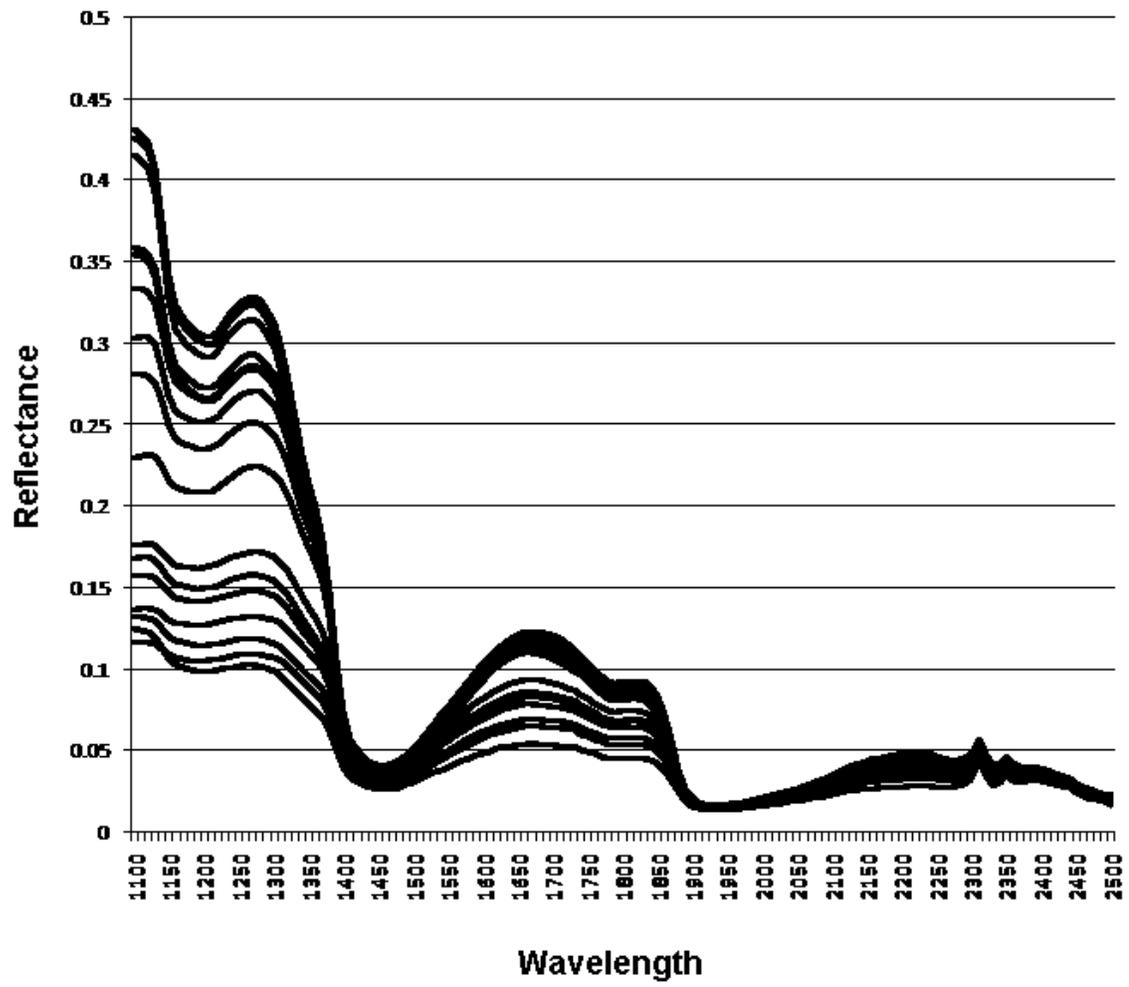


Figure 1.1. Example of spectral output comparing 16 different samples.

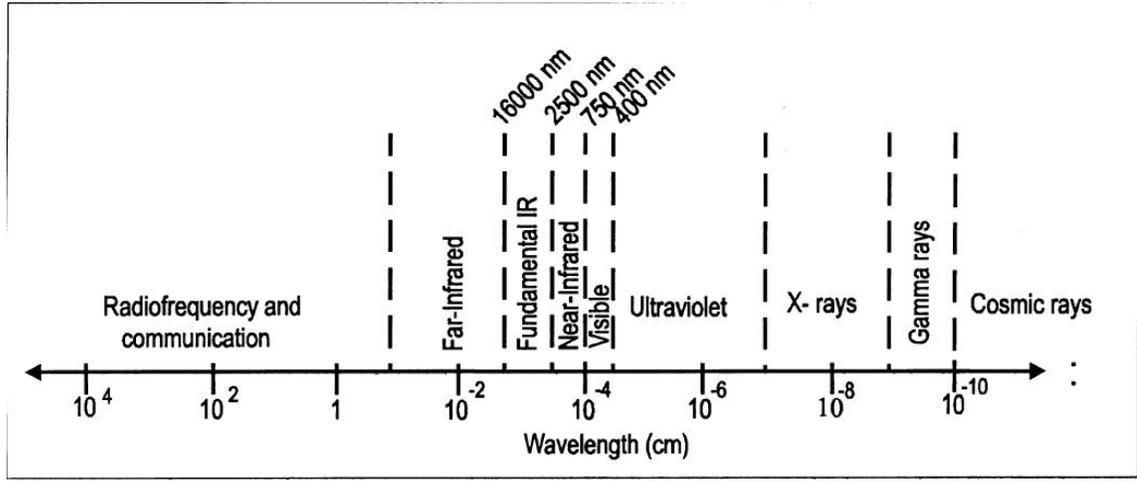


Figure 1.2. Radiation wavelength spectrum. From Foley et al. (1998).

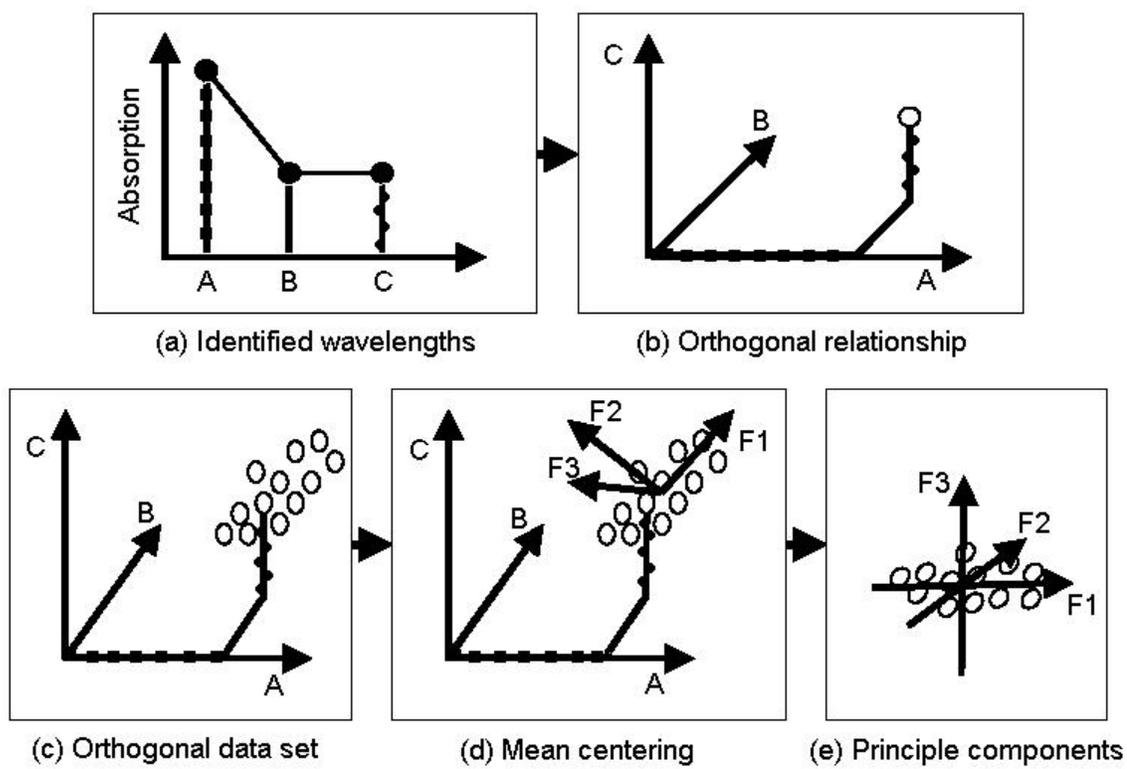


Figure 1.3. Orthogonal development of 3 principal components from wavelengths (a) to a system with one axis per wavelength (b), introduction of all sample spectral data using the 3 wavelengths (c), mean centering (d), and determination of principle components F1, F2, and F3 (e). Adapted from Reich (2005).

CHAPTER TWO: USE OF A PORTABLE NEAR-INFRARED
SPECTROPHOTOMETER TO PREDICT NUTRIENT COMPOSITION OF FECES
FROM HOLSTEIN CATTLE FED HIGH-CONCENTRATE DIETS DIFFERING IN
CONCENTRATE LEVEL OR GRAIN PROCESSING

Abstract

Our objective was to evaluate a chute-side infrared spectrophotometer (NIRS) analysis to predict nutrient composition of feces from Holstein cattle. In trial 1, growing Holstein cattle ($n = 44$; 219 ± 37.2 kg BW) were fed either 86 or 90% steam-flaked corn-based concentrate diets (3 pens/diet; $n = 6$). Fecal samples were collected in plastic bags and scanned within 2 h after collection using an ASD Field Spec NIRS unit (Boulder, CO). Spectra were collected under ambient conditions using a contact probe. Samples were then analyzed for DM, CP, NDF and ADF. Calibrations were developed using samples collected on d 0 and 28 with log 1/R spectra in the 1,100 to 2,400 nm range. Partial least squares (PLS) regression in SAS was used to develop calibrations. Simple regression was used to evaluate the relationship between observed and predicted constituent values. Although regression values were moderate for predicting CP ($R^2 = 0.88$) and fair for DM ($R^2 = 0.68$) and NDF ($R^2 = 0.62$), prediction regression values for ADF were statistically significant ($P < 0.01$) but not predictive ($R^2 = 0.34$). In trial 2, finishing Holstein cattle ($n = 44$; 490 ± 22.5 kg BW) were fed a 90% concentrate diet containing either thick (412 g/L) or thin (360 g/L) steam-flaked corn (3 pens/diet; $n = 6$). Fecal samples were collected once weekly over a 4-week period, frozen after collection, and thawed and scanned using the ASD Field Spec NIRS unit. Calibrations were

developed using samples from the first 3 collections. Regression values for all components analyzed were fair ($P < 0.01$) for CP ($R^2 < 0.61$) and DM ($R^2 < 0.62$) but poor ($P < 0.01$; $R^2 < 0.35$) for fiber components and starch. Regression validation values were all insignificant ($P > 0.10$). Discriminant values were higher (> 75% correct within group) within collection dates than between groups (< 61% correct within treatment). Our data indicate that while our calibrations were variably successful, validations were mostly unsuccessful ($R^2 < 0.4$; $P =$ not statistical). Lack of validation success is most likely due to narrow variance and limited range of values. However, this project has illustrated a relationship between NIR spectra and the observed laboratory values for these constituents, and that the use of a portable NIRS on-site may improve the nutritional management of a commercial feedlot.

Introduction

Feedlot nutritionists routinely analyze diet samples for nutrient composition including DM, CP, NDF, ADF as well as estimates of starch availability. Besides nutrient composition of diets, fecal analyses can provide indices for nutrient utilization (Hussein and Berger, 1995; Bradshaw et al., 1996). Samples are often analyzed at commercial laboratories using wet chemistry procedures. However, commercial analyses may be expensive and time consuming. The time period between sampling and notification of results may extend beyond the time period in which results would be most beneficial for decisions involving nutrient management. Aside from the continuous cost and time spent waiting for results, fecal samples are susceptible to continued degradation if not properly

stored. Therefore, quick analysis of fecal components is important for the nutritional management of cattle.

The use of near infrared spectroscopy in animal agriculture has been used for over 20 years (Marten et al., 1985), but research has recently studied its possible application in on-site testing of animal feedstuffs (Perez-Marin et al., 2004; Berzaghi et al., 2005). However, the use of a bulky and heavy near infrared spectrophotometer (NIRS) on-site at a commercial feedlot would require accommodations such as laboratory space and labor spent transporting samples to the laboratory. Portable NIRS technology is currently available that can perform analyses without these accommodations (Berzaghi et al., 2005).

Currently, there has been no report using NIRS analysis of unprocessed cattle feces in real-time. We hypothesize that accurate fecal analyses can be obtained with a portable NIRS. Therefore, the objective of this experiment was to evaluate a chute-side portable NIRS analysis of nutrients from fecal samples from differently-fed Holstein cattle in a feedlot setting.

Materials and Methods

Trial 1. Procedures were approved by the University of Arizona Institutional Animal and Care Use Committee. In trial 1, 44 growing Holstein cattle (42 steers and 2 freemartin heifers; 219 ± 37.2 kg) housed at the University of Arizona feedlot were randomly allotted into 6 pens (8 to 9 cattle/pen). Pens were assigned 1 of 2 diets: 86% or 90% concentrate (DM basis; Table 2.1; 3 pens/diet). Animals were provided free access to water and adequate shade. Cattle were previously on a growing diet, so diet

acclimation was not necessary. Cattle were fed respective diets for 91 days. Initial and final unshrunk BW were collected, with respective ADG calculated.

On d 0, 28, 56, and 91, fecal samples were collected. Diet samples were obtained on the last 3 collection dates. Feces were collected by running cattle through a squeeze chute and either collecting identified piles on the ground or by rectal retrieval. Feces contaminated with dirt were not sampled. All samples were placed in 13 x 19 cm plastic bags (Nasco; Whirl-Pak; Modesto, CA). After all samples were collected for that day, the outside of the bags were cleaned with running water at ambient temperature to avoid contamination of the NIRS probe.

Samples were scanned within 2 h after collection using an ASD Field Spec NIRS unit (Boulder, CO). Spectra were collected under ambient conditions using a contact probe upon each sample bag. Samples containing small amounts of feces (< 13 g wet basis) were rejected due to the inability of the NIRS to read samples that could not produce a scanning area of 15 cm² with a depth of 0.67 cm.

All wet laboratory analyses were performed at the University of Arizona cattle nutrition laboratory. Samples were dried at 60° C and ground in a Wiley mill to pass through a 1 mm screen. Ground samples were analyzed for all or part of the following: ADF and NDF using an Ankom system (Macedon, NY) and CP using a nitrogen analyzer (TC400; Leco Corp.; St. Joseph, MO). Briefly, starch was analyzed by gelatinization, followed by amyloglucosidase digestion, and finished with measurement of glucose concentration (Zinn, 1990, with modifications per R.A. Zinn, personal communication).

Trial 2. Forty-four (44) finishing Holstein cattle (42 steers and 2 freemartin heifers; 490 ± 22.5 kg BW) were randomly assigned 1 of 2 diets: a 90% concentrate diet containing either thick steam-flaked corn (412 g/L) or thin steam-flaked corn (360 g/L; 3 pens/diet; Table 2.1). Cattle were housed as described in trial 1, but cattle in the second trial were fed respective diets for 28 days, with fecal collections on d 7, 14, 21, and 28. Diet samples were obtained on the last 3 collection dates.

All samples were placed in previously described plastic bags and cleaned. After all samples were collected for that day, bags were frozen until all samples for the trial could be collected and analyzed by the NIRS at the same time. On the designated scanning date, samples were thawed to room temperature and scanned with the NIRS procedure used in trial 1. Wet laboratory analysis for all samples was performed as described in trial 1.

Statistics. Growth performance and diet and fecal data from wet chemistry were analyzed as a completely random design using the Proc Mixed procedure in SAS (Cary, NC). Pen was considered the experimental unit.

Spectrophotometer calibrations were developed with $\log 1/R$ spectra in the 1,100 to 2,400 nm range using samples collected on d 0 and 28 ($n = 56$) for trial 1 and d 7, 14, and 21 ($n = 126$) for trial 2. Partial least squares (PLS) regression in SAS was used to develop calibrations. Cross validation was employed to avoid over-fitting. Simple regression was used to evaluate the relationship between observed and predicted constituent values. Discriminant validation was employed in trial 2 to determine the

ability of the NIRS calibration to correctly distinguish samples according to treatment or collection date.

Results

Trial 1. Sample number, mean, SD, and data ranges of the calibration set (d 0 and 28) are shown in Table 2.2. The range and SD for starch concentrations were numerically highest (31.9 and 8.86% respectively) with NDF having the second highest parameters. Not all calibration samples yielded enough DM for all constituent analysis.

Although not pertinent to the objective of this project, growth performance of the cattle and statistical analysis of wet chemistry is provided (Table 2.3) to verify differences between the 86 and 90% concentrate diets. Growth performance characteristics were statistically equivalent ($P > 0.10$) between the 2 diets. However, chemical analysis of feces collected on all 4 dates reveal differences between the diets. Dry matter and CP were not different ($P > 0.10$), but starch tended ($P < 0.10$) to be higher in feces sampled from cattle receiving the 86% concentrate diet. In contrast, NDF tended ($P < 0.10$) to be higher and ADF was higher ($P < 0.05$) in feces from cattle receiving the 90% concentrate diet.

Calibration values for the NIRS were statistically significant ($P < 0.01$; Table 2.4) for all components. Calibration regression values were fair to good for fecal CP ($R^2 = 0.89$), DM ($R^2 = 0.69$) and NDF ($R^2 = 0.62$) but not predictive for ADF ($R^2 = 0.34$) and starch ($R^2 = 0.31$). Regression values for validation of the calibration set were lower. All regression validation values were both not predictive ($R^2 < 0.35$; Table 2.4) and statistically invalid ($P > 0.07$).

Trial 2. Chemical composition of the first three collection dates was used for NIRS calibration (Table 2.5). Neutral detergent and starch were widest in range (26.8 and 25.6%) and SD (5.36 and 4.58%), while CP had the narrowest and SD (2.25%) and range (10.9). Starch had the lowest mean percent (6.5%); neutral detergent fiber was highest in mean percent (34.4%).

Growth performance and fecal constituents are recorded in Table 2.6. Just as trial 1, statistical analysis was performed to verify previously reported effects of bulk density on fecal nutrient composition (Zinn, 1990). All growth parameters were similar ($P > 0.10$), with both groups gaining approximately 30 kg BW during the trial at 1 kg ADG. The thinner (360 g/L) flake diet tended ($P < 0.10$) to have higher fecal NDF and was lower ($P \leq 0.05$) in fecal DM and starch content. Starch was double in concentration for cattle fed the thicker flake diet, while a difference in DM was only slightly above 1 percent. Fecal protein and ADF content was similar ($P > 0.10$) across treatment.

Predictive NIRS regression values are given in Table 2.7. Both DM and CP regression values were fair ($R^2 > 0.60$), but validation statistics were not significant. Fiber and starch calibration regression values were statistically poor ($R^2 < 0.40$) with validation statistics also insignificant. Discriminant values are given in Table 2.8. Placement of fecal samples by treatment were poor (< 60% correct placement), while higher success (> 80% placement) was seen in determining samples by collection date.

Discussion

Recent research has reported the use of NIRS technology in determination of unprocessed samples (i.e. “as is” vs. dried and ground), including dairy silage (Berzaghi

et al., 2005). Perez-Marin et al. (2004) reported workable predictive regressions ($R^2 > 0.84$) on various feedstuffs, including ruminant pellets and companion animal feedstuffs. To further justify calibrating to unprocessed samples, these various feedstuffs were also dried and ground to 2 different particle sizes and reanalyzed by the NIRS. The authors reported some constituents to have higher predictive regression values when left unprocessed when compared to the further processed counterparts. Altogether, it was reported that regardless of processing, predictive analyses of all 3 sample forms were accurate (Perez-Marin et al., 2004). This suggests that processing samples prior to NIRS analysis is not necessary, saving cost and time that would be spent to have samples processed. In a feedlot setting, this would result in quicker analysis of fecal and diet components and improved nutritional management.

Research involving NIRS analysis of feces of feedlot cattle is minimal or focused on manure management involving processed samples contaminated with soil and bedding (Malley et al., 2005). However, calibration of any spectrophotometer, regardless of organic material involved, relies upon 5 major factors: the number of samples in the calibration set, the range within specific component values, the range of the constituent SD, the accuracy and precision of the wet chemistry, and the consistency of NIRS protocol and calibration statistics. Sample numbers used in calibration of past research that analyzed feedstuffs have varied from over 600 to less than 200 (Perez-Marin et al., 2004). The current study had only 58 samples used for calibration, which may have played part in the failed validation regression values. Calibration sets can be strengthened as wet chemistry results for validation and future samples are included. Adding more

samples has the potential to create more robust regression values for calibration and validation, making NIRS predictive values more accurate (Reich, 2005). Failure in the current study, however, cannot be fully explained by calibration number, since the lower regression values were obtained from larger calibration set of trial 2.

Although calibration number is important, the other 4 factors should also be evaluated. After a calibration set consisting of 111 feedstuff samples, Xiccato et al. (2003) considered their validation regression values for some of the constituents to be low ($R^2 < 0.70$). While disregarding the importance of calibration sample numbers, Perez-Marin et al. (2004) attributed the low values of Xiccato et al. (2003) to the limited range and narrow SD of the individual constituents. Range and SD values for fecal components in the current study could also be considered limiting, especially when compared to the diverse calibration samples used by others (Perez-Marin et al., 2004). Reich (2005) further expressed the importance for any calibration set to contain samples that expressed the wide range and variability that was to be expected in future sampling. The use of only 2 diets in each trial to calibrate the NIRS in the current study may have led to the limited range and SD of fecal constituents. Furthermore, the narrow divergence of between diet in each trial may also have limited fecal nutrient variation between diets.

Based on the previous discussion, it is counter-intuitive to see a lower R^2 value for starch, which is the component with a wider range and SD as compared to the other fecal constituents in each trial. This may be explained by the nature of NIRS calibration. Predictive values from NIRS analysis are derived from sample spectra calibrated to wet chemistry analysis of the same sample (Reich, 2005). Essentially, the NIRS predictive

capability is only as accurate as the chemistry that the calibration is based. In the current study, starch assay values were allowed with minimal variation (sample CV values were < 0.10), but a more confident regression value may have been obtained if sample CV values were kept to less than 0.05 (R.A. Zinn, personal communication). Researchers have reported accurate results with this particular starch assay (Zinn, 1990; Zinn et al. 2007), suggesting our NIRS-predicted starch variability may be overcome with a greater number of calibration samples and a lower CV value tolerance for sample wet chemistry.

Furthermore, calibrating NIRS spectra to fecal samples with high moisture content may be harder considering the high infra-red absorbance capacity of the O-H bonds in water (Blanco and Villaroya, 2002). Water interference in the spectra may have complicated statistical calibration. Statistical methods employed in NIRS spectral calibration are constantly being developed and improved, allowing further refinement in NIRS predictive accuracy (Reich, 2005).

Discrimination results in Trial 2 were better and possibly valuable for determining collection date compared to determining diet. Others have reported valid NIRS fecal discrimination for determining gender, species, reproductive status, and tick infestation (Tolleson et al., 2001; 2005; 2007). Failure for discrimination between diets may be explained by the initial failed validation and that changes within digestion parameters as the animals aged were greater than differences between diet treatments.

Although validation of predicted NIRS values in the current study is not as accurate as has been reported by others (Xiccato et al., 2003; Perez-Marin et al., 2004), it should be noted that the potential for NIRS analysis on fresh cattle feces is still viable.

Calibration regressions in the current study were statistically significant, suggesting that NIRS analysis of constituents of unprocessed bovine feces is possible, and calculated regression formulas can be strengthened. The failed validation regressions merely reinforce the need for calibrating NIRS spectra according to the 4 factors described above. Samples for all collection dates in the current study were analyzed with wet chemistry, allowing for inclusion into the calibration set and creating a more robust regression potential in predicting fecal constituents.

Implications

The results from the current study imply that on-site NIRS-predicted values of fecal constituents from Holstein cattle are possible. Furthermore, a portable NIRS may be used for quick on-site feedlot fecal analysis without sample processing. Faster determination of these parameters from a portable NIRS may help improve the nutritional and health management of cattle in commercial feedlots by decreasing time spent in performing wet chemistry analysis and quickly and accurately predicting digestibility of feedlot diets. Although NIRS prediction of fecal composition is possible, future research utilizing a larger calibration data set is needed to verify the predictive accuracy of on-site NIRS analysis of fecal composition.

Table 2.1. Dietary ingredient and chemical composition of experimental diets (DM basis)¹

Item	Trial 1		Trial 2	
	86%	90%	360	412
<i>Ingredient, %</i>				
Corn	72.5	76.5	76.5	76.5
Alfalfa hay	13.5	9.5	9.5	9.5
Molasses	5.2	5.2	5.2	5.2
Fat	4.4	4.4	4.4	4.4
Mineral mix ²	2.2	2.2	2.2	2.2
Urea	1.2	1.2	1.2	1.2
Rumensin premix ³	1.0	1.0	1.0	1.0
<i>Chemical composition, %</i>				
DM	83.7	83.9	88.6	88.4
CP	16.7	15.6	13.3	12.7
NDF	13.6	14.2	17.6	16.4
ADF	8.5	8.7	8.6	7.6
Starch	36.0	36.5	29.9	28.8

¹Diets in Trial 1 designated according to concentrate level and in Trial 2 designated according to bulk density (g/L) of steam-flaked corn portion.

²Composition (% mix DM): Limestone (46), Ground corn (20), Salt (20.2), Potassium Cl(7.8), Ammonium sulfate (6.5), Magnesium oxide (3.4), Dicalcium P (1), Zinc sulfate (0.83), Vitamin E (0.54), Manganese sulfate (0.45), Vitamin A (0.27), Copper sulfate (0.15), Iron sulfate (0.13), Selenium (0.12), Calcium iodate (0.003) and Cobalt carbonate (0.002).

³Mixed to provide 300 mg·animal⁻¹·d⁻¹ of Rumensin 90 and 100 mg·animal⁻¹·d⁻¹ of Tylan 40 (Elanco Animal Health, Greenfield, IN).

Table 2.2. Chemical composition of fecal samples from Holstein cattle in Trial 1 used as the initial calibration set¹

Item	Sample #	Mean, %	SD, %	Range, %
DM	57	22.1	2.77	15.6 – 27.4
CP ²	56	20.7	3.34	15.1 – 30.2
NDF	56	33.5	6.64	21.5 – 48.1
ADF	56	20.9	4.82	10.7 – 34.3
Starch	56	15.5	8.86	1.2 – 33.1

¹Samples are from d 0 of trial when animals (n = 44) were receiving same diet and d 28 when animals were receiving either an 86 or 90% concentrate diet. Calibration set used for prediction of fecal composition by a portable NIRS.

²CP, ADF, NDF and starch reported on a DM basis.

Table 2.3. Growth performance of Holstein cattle and wet chemistry of fecal samples for 2 diets in Trial 1¹

Item	Diet		SEM	P value
	86	90		
<i>Growth Performance</i>				
Initial BW, kg	223.2	215.5	19.92	0.80
Final BW, kg	335.8	328.0	17.43	0.77
ADG, kg/d	1.78	1.79	0.084	0.98
<i>Fecal Composition, %²</i>				
DM	23.1	21.9	0.54	0.18
CP	17.6	19.1	0.53	0.12
NDF	31.9	36.0	1.13	0.06
ADF	21.3	25.2	0.73	0.02
Starch	16.8	13.5	1.08	0.09

¹Diets consisted of either 86 or 90 % concentrate, DM basis. Pen (n = 6) was the experimental unit.

²Components other than DM expressed on DM basis. Fecal samples (n = 116) from d 28, 56, and 91 were analyzed.

Table 2.4. Predictive NIRS regression values of fecal constituents from Holstein cattle in Trial 1¹

Item:	Calibration Set		Validation set	
	R ²	<i>P</i> value	R ²	<i>P</i> value
DM	0.69	0.0001	0.31	NS ²
CP	0.89	0.0001	0.15	NS
NDF	0.62	0.0001	0.13	NS
ADF	0.33	0.0002	0.004	NS
Starch	0.31	0.003	0.21	NS

¹Cattle (n = 44) fed either an 86 or 90% concentrate diet. Fecal samples for calibration (n = 58) and validation (n = 7) sets collected on d 0 and 28 after incorporation of diets.

²NS = Not statistically relevant.

Table 2.5. Chemical composition of fecal samples from Holstein cattle used as the initial calibration set in Trial 2¹

Item	Sample #	Mean, %	SD, %	Range, %
DM	126	20.9	2.94	12.1 – 31.0
CP ²	122	17.0	2.25	11.4 – 22.3
NDF	122	34.4	5.36	20.9 – 47.7
ADF	122	19.3	3.44	10.1 – 28.8
Starch	122	6.5	4.58	0.5 – 26.1

¹Samples are from d 7, 14, and 21 of trial. Calibration set used for prediction of fecal composition by a portable NIRS.

²CP, ADF, NDF and starch reported on a DM basis.

Table 2.6. Growth performance of Holstein cattle and wet chemistry of fecal samples for 2 diets in Trial 2¹

Item	Diet		SEM	P value
	360 g/L	412 g/L		
<i>Growth Performance</i>				
Initial BW, kg	493.3	487.4	22.65	0.87
Final BW, kg	523.3	518.1	23.10	0.89
ADG, kg/d	1.07	1.10	0.141	0.91
<i>Fecal Composition, %²</i>				
DM	20.3	21.6	0.33	0.05
CP	17.2	16.7	0.57	0.54
NDF	35.4	32.8	0.80	0.09
ADF	20.0	18.6	0.51	0.15
Starch	4.4	9.0	0.50	0.01

¹Diets consisted of a 90.5 % concentrate (as fed) containing either thick (412 g/L) or thin (360 g/L) steam-flaked corn. Pen (n = 6) was the experimental unit. Fecal samples (n = 116).

²Components other than DM expressed on DM basis.

Table 2.7. Predictive NIRS regression values of fecal constituents from Holstein cattle in Trial 2¹

Item:	Calibration Set		Validation set	
	R ²	<i>P</i> value	R ²	<i>P</i> value ²
DM	0.606	0.01	NS	NS
CP	0.617	0.01	NS	NS
NDF	0.343	0.01	NS	NS
ADF	0.276	0.01	NS	NS
Starch	0.245	0.01	NS	NS

¹Diets consisted of a 90.5 % concentrate (as fed) containing either thick (412 g/L) or thin (360 g/L) steam-flaked corn. Calibration set = 126 samples (n = 126).

²NS = Not statistically relevant.

Table 2.8. Discriminant equation validation results within groups in Trial 2

Comparison	Group	#Correct/Total
Diet ¹	412 g/L	33/61
	360 g/L	45/74
Collection Date	d 7	18/19
	d 14	34/38
	d 21	29/38
	d 28	33/40

¹Diets consisted of a 90.5 % concentrate (as fed) containing either thick (412 g/L) or thin (360 g/L) steam-flaked corn.

CHAPTER THREE: USE OF A PORTABLE NEAR-INFRARED
SPECTROPHOTOMETER TO PREDICT NUTRIENT DIGESTIBILITY
PARAMETERS IN FECES COLLECTED FROM YOUNG DAIRY STEERS FED
DIETS CONTAINING WHOLE OR STEAM-FLAKED CORN FROM PRE-
WEANING TO FEEDLOT WEIGHT

Abstract

A trial was performed to investigate the effect of corn processing on nutrient digestion and growth performance in dairy bull calves fed from pre-weaning to feedlot weight. The trial also focused on the on-site applicability of predicting nutrient composition in feces from these animals using a portable near-infrared spectrophotometer. Twenty dairy bull calves (10 Holstein and 10 Jersey; average initial BW = 34.2 ± 8.13 kg) were separated within breed into 1 of 2 treatments: diets containing either steam-flaked or whole corn. Fecal samples were collected once weekly from arrival until the first steer reached feedlot weight (125 kg). Thawed samples were scanned at room temperature using an ASD Field Spec NIRS unit (Boulder, CO). Spectra were collected under ambient conditions using a contact probe. Calibrations were developed from sample analyses of DM, CP, NDF, ADF, and starch. Growth performance, including BW (initial, weaning, and final), ADG (pre- and post-wean), pre-wean DMI, and feed efficiency were similar ($P > 0.10$), with animals fed steam-flaked corn tending ($P < 0.10$) to have higher overall DMI and lower post-wean feed efficiency ratio. Post-weaning DMI was greater ($P < 0.05$) for calves consuming steam-flaked corn. For NIRS calibration, regression values were good for CP ($R^2 = 0.84$) and fair for ADF

($R^2 = 0.72$). Although statistically valid ($P < 0.01$), calibrations for DM, NDF, Ash, and starch were not predictive ($R^2 < 0.60$). All validation attempts were not statistically valid. Discriminant values were higher (> 70% correct within time period) than between whole and steam-flaked groups (< 55% correct within group). Failure for validation and discrimination between treatments may likely be due to an insufficient number of samples used for calibration as well as homogeneity of fecal samples between groups. This project shows that corn processing does not effect dairy calf performance through 8 weeks post-weaning. However, starch digestibility between whole and steam-flaked corn begins to diverge once the dairy calf has been weaned.

Introduction

The effect of feeding whole or steam-flaked corn to cattle in regards to starch digestion and utilization has been debated for several years. Both practices have their advantages above the other (Loerch and Gorocica-Buenfil, 2006). While most agree that whole corn digestion is comparatively lower to steam-flaked corn in finishing feedlot cattle, there remains a window of uncertainty in younger ruminants. Several authors have reported no difference in growth parameters in young dairy calves that were fed either whole or processed corn (Leismeister and Heinrichs, 2004, Strusinska et al., 2009). However, no report has established the effect of corn processing on starch digestion in neonatal calves fed through weaning to feedlot weight. This may be due to amount of time and cost of sampling feces and diets and having them analyzed at a commercial laboratory.

Agriculture research employing near-infrared spectroscopic nutrient analysis in agriculture research has been relevant for several decades (Marten et al., 1985). Near-infrared spectrophotometer (NIRS) technology has also been used in commercial settings as a means for quality control (Davies, 1998, Perez-Martin et al., 2004). Use of a NIRS is non-invasive, quick, universal for all organic sampling (i.e. feedstuff, fecal, etc.), and involves less sample handling and processing (Davies, 1998). One major advantage for NIRS analysis over traditional wet chemistry analysis is the overall economic return on investment for operations (commercial or research) that employ high-volume sampling. Recent NIRS technology has produced portable NIRS units capable of accurately analyzing several agriculture-related samples on-site (van Vuuren et al., 2006). Current research has attempted to create accurate portable NIRS calibrations for on-site analysis of nutrient components of feces from feedlot cattle (Allen et al., 2010). While the authors were not able to produce acceptable calibration statistics, they did conclude that on-site analysis of cattle feces with a portable NIRS was statistically possible.

Several projects have applied NIRS technology for commercial compatibility. However, direct application of portable NIRS technology for on-site calf fecal analysis has not been established. We hypothesize that current portable NIRS technology is capable of producing statistically accurate nutrient analyses on novel research. Our objective, therefore, was two-fold: statistically-validate the use of on-site fecal analyses with a portable NIRS, and determine the effect of corn processing on starch digestion in neonatal Holstein bulls fed to feedlot weight.

Materials and Methods

Animals, Facilities, and Diet. Procedures were approved by the University of Arizona Institutional Animal and Care Use Committee. Twenty dairy bull calves (10 Holstein, 10 Jersey; 34.2 ± 8.13 kg BW) housed at the University of Arizona Agriculture Research Center were individually placed into modified California hutches one wk (d 7) after arrival. Animals were randomly assigned within breed to 1 of 2 treatments: calf diets containing either steam-flaked or whole corn (Table 3.1; 5 Holstein and 5 Jersey calves per treatment). Animals were provided free access to water and adequate shade. Calves were bottle fed 227 g of a 22/22 milk replacer (mixed with 1.9 L water; Lawley's Inc., Stockton, CA) twice daily, then once daily during weaning (~d 60). All calves were offered the calf starter diet one wk (d 7) after arrival.

Once weaned, calves were transitioned to a Holstein starter diet (Table 3.1). Weaned calves were relocated to the University of Arizona West Agriculture Center feedlot, processed [castration, vaccination, dehorned, and implanted (Synovex C, Pfizer, New York, NY)] and placed in individual pens containing adequate water and shelter. Animals were fed to satiety (as fed basis) up to 5 kg feed/day as suggested by Maas and Robinson (2007). The trial ended when the first animal had reached 125 kg BW.

Growth performance parameters collected included initial, weaning, and final BW, daily DMI, and calculated pre- and post-weaning ADG, DMI, and feed efficiency. Birth dates could not be verified for the Jersey calves, whereas Holstein calves were acquired within 24 hr of birth. Therefore, all growth parameters were adjusted to weaning date.

Collections. Diet samples were taken at least once weekly ($n > 5/\text{treatment}/\text{diet}$). Fecal samples were collected for each week that the animals were separately housed. During the pre-weaning phase, fresh fecal samples were collected by placing collection plates underneath the slatted floors of the hutches or by grabbing fresh samples that were still in the hutches. Samples were not collected from calves experiencing diarrhea. During the post-weaning phase, fecal samples were collected from the pen floor. During both phases, care was taken to collect samples that did not contain portions exposed to environment (i.e. dried, dirty) or was contaminated with urine or predated by birds. Single weekly samples, especially during the pre-weaning phase, required at times an amalgamation of several collections to produce enough sample for analysis. All fecal samples were placed in 13 x 19 cm plastic bags (Nasco Whirl-Pak; Modesto, CA).

After each full sample was collected, the exterior of each bag was cleaned with a wet rag to avoid contamination of the NIRS probe. Bags were then frozen until all samples for the trial could be collected and analyzed by the NIRS at the same time.

Sample Analysis. Samples were scanned after thawing to room temperature using an ASD Field Spec NIRS unit (Boulder, CO). Spectra were collected under ambient conditions using a contact probe upon each sample bag. Samples containing small amounts of feces (< 13 g wet basis) were rejected due to the inability of the NIRS to read samples that could not produce a scanning area of 25 cm^2 with a depth of 0.67 cm.

For wet laboratory analysis, samples were dried at 60°C and ground in a Wiley mill to pass through a 1 mm screen. Ground samples were analyzed for all or part of the following: DM, ash, ADF and NDF (Ankom 200 fiber digester, Ankom, Macedon, NY),

and CP(TC400; Leco Corp.; St. Joseph, MO). Briefly, starch was analyzed by gelatinization, followed by amyloglucosidase digestion, and finished with measurement of glucose concentration (Zinn, 1990, with modifications per R.A. Zinn, personal communication and J.D. Allen).

Statistics. Spectrophotometer calibrations were developed using 220 samples with log 1/R spectra in the 1,100 to 2,400 nm range. Partial least squares regression in SAS was used to develop calibrations. Cross validation was employed to avoid over-fitting. Simple regression was used to evaluate the relationship between observed and predicted constituent values. Samples that had missing component values were not used for calibration.

Growth performance data, including pre-and post-weaned fecal components, were analyzed as a completely random design using the Proc Mixed procedure in SAS (Cary, NC) with the LSMEANS option. Animal was considered the experimental. Fecal samples were also analyzed as a completely random design using the Proc Mixed procedure, with the week from weaning as the repeated measurement and animal within treatment as the subject. Statistics were best fit as autoregressive(1). The LSMEANS and PDIFF options were employed for treatment, week, and treatment \times week effects. Animal (n = 16) was considered the experimental unit. Three (3; 1 Holstein-steam-flaked, 1 Jersey- and 1 Holstein-whole) calves died prior to weaning, and 1 Jersey steer (whole) was removed due to excessive diarrhetic episodes throughout the trial.

Results

Chemical composition of samples used in the calibration set are recorded in Table 3.2. Dry matter, CP, and NDF had a SD greater than 10 percent; however, only DM and ADF had a range greater than 50 percent. Starch had the lowest mean (4.1%) and range (25.9%) values. Calibration regression values (Table 3.3) for each component analyzed were statistically valid ($P < 0.001$) but generally poor except for CP ($R^2 = 0.84$) and ADF ($R^2 = 0.72$). Discriminant values between treatments were also relatively poor (< 55% correct discrimination; Table 3.4). However, when collection dates were grouped in 4-week periods, discrimination across time was more successful (> 70% correct discrimination across time).

No differences ($P > 0.10$; Table 3.5) were observed between diets for BW and ADG parameters. However, there was a tendency ($P = 0.06$) for calves fed whole corn to have higher feed efficiency post-weaning. In contrast, pre-wean and overall G:F ratios were equal ($P > 0.10$) between diets.

Fecal constituents are shown for pre- and post-weaning in Table 3.6. Ash content was the only component different ($P = 0.03$) before calves were weaned, with whole-corn fed calves having a 1.7 percent greater ash content. After weaning, calves fed steam-flaked corn had higher ($P = 0.01$) fecal fiber components (NDF and ADF) but lower ($P = 0.01$) fecal starch when compared to calves fed whole corn.

Change in fecal starch content across time is shown in Figure 3.1. A diet \times week effect was observed for starch content ($P < 0.01$), with significant differences ($P < 0.05$) occurring post-weaning between week 1 and 6.

Discussion

NIRS. The development of reliable portable NIRS technology has coincided along its acceptance within commercial and research areas (Chauchard et al., 2004; Malley et al., 2005). However, research using newer, portable NIRS units has focused primarily on validating NIRS predictive capability rather than actual analyses of novel experimentation. This may be explained partly because reliable predictions from portable NIRS technology has yet to be established and that commercial use of NIRS units have little need for portable units when desktop units capable of analyzing unprocessed samples can be placed on production lines or within reasonable distance of the sampling area (Perez-Marín et al., 2004). However, locales where immediate analysis is required still exist, such as in a range setting or where samples are prone to rapid degradation (i.e. feces).

Although calibration for DM, NDF, ash and starch failed, predictive capacity for all constituents were statistically valid. This suggests that on-site prediction of fecal samples from cattle using a portable NIRS is possible, but further calibration is required. Calibration regressions appear slightly lower to previous work on fresh cattle manure (Allen et al., 2010). However, validation failure by Allen and others (2010) were attributed to specific aspects: low sample number of calibration set, narrow SD and range of the calibration samples, and precision of the chemical analyses. In the current study, both calibration number and SD and range values are noticeably greater, leaving only a problematic chemical analysis as the source of calibration failure.

The higher predictive capacity reported by Allen et al. (2010), however, does not support this conclusion. The authors were able to produce higher regression values for constituents with only a quarter ($n = 56$) of the samples included in the calibration set. Their calibration set also contained narrower ranges and lower SD as compared to the current study. One possible explanation might be in the experimental diets used. Allen et al. (2010) fed 2 diets that were different in corn and alfalfa concentration, which may have provided a higher variation in the physical nature of fecal samples between diet groups. Diets fed in the current study differed only by corn processing, and may have been comparatively similar in physical nature when excreted as feces.

Validation failure can be overcome by addressing these causal issues. Various statistical techniques are currently used and are constantly being refined for NIRS calibration (Blanco and Villaroya, 2002). However, reanalyzing the calibration set does not insure that regression values will become more accurate, but improved accuracy may be possible for values that are near or above acceptable. The addition of samples to the calibration is commonly used to create more robust regression values (Reich, 2005). Perez-Marin et al. (2004) reported acceptable regression values ($R^2 < 0.80$) using a calibration set containing more than 400 samples. Similarly, Xiccato et al. (2003) reported lower regression values ($R^2 < 0.80$), but the authors used less than 200 samples for the calibration set. Further addition of samples with wider SD and ranges, therefore, may improve regression values in the current study.

Discriminant values are means of using spectra to qualitatively separate samples according to set criteria. In the current study, it was easier for the NIRS calibration to

distinguish samples by 4-week periods than by treatment, suggesting the calibration is not robust enough to distinguish the differing physical characteristics in feces of calves fed either whole or steam-flaked corn. This further supports the reasoning behind the NIRS calibration failure, considering that physical characteristics of fecal matter from young calves can be better grouped according to age of animal rather than by diet fed to animal. Others have reported higher discriminant values using either processed or unprocessed samples (Perez-Marin et al., 2004; Tolleson et al., 2005; Tolleson et al., 2007).

Calves. Growth performance values in the current study are similar to previous work investigating the effect of corn processing. Leismeister and Heinrichs (2004) reported no difference in BW gain, ADG, and feed efficiency of young calves fed diets containing whole, rolled, roasted-rolled, or steam-flaked corn up to 2 weeks post-weaning. Although the calves were weaning at 28 days of age, the authors reported only a slightly better feed efficiency in calves fed whole corn after weaning. Chester-Jones and others (1991) compared weaned dairy calves fed diets containing either whole or rolled corn. The authors reported no difference in BW or ADG until after the eighth week post-weaning. Others have reported no differences in growth performance in calves fed diets with differing processed corn during similar age parameters (Bateman et al., 2008). Results from the current study also showed a slight advantage in feed efficiency in calves fed whole corn without an observed difference in other growth parameters, pre- or post-weaning, up to 8 weeks post-weaning. Although the value of feeding steam-flaked corn to whole corn has been established in cattle heavier than 200 kg (Zinn, 1990; Huntington,

1997), the current findings along with supporting literature show little advantage for feeding processed grains to young dairy animals.

Although effects of corn processing on gastrointestinal physiology and blood metabolites have been reported (Leismeister and Heinrichs, 2004; Strusinska et al., 2009), no literature could be found that focused on the effect of corn processing on fecal composition and nutrient digestibility in young calves. Since a trending diet \times week effect was observed in fecal starch content only, pre- and post-weaning values will be discussed. Prior to weaning, digestibility parameters appear equal between diets except for ash content. Since all other components are statistically similar, the difference in ash is considered coincidental. Differences in digestibility can be seen once calves were weaned. During the week of weaning, calves were transitioned from the pellet diet to the starter diet (Table 3.1), decreasing CP density and increasing fiber content. Higher NDF and ADF content in the feces of calves fed steam-flaked corn compared to whole corn is seen as direct and negative correlation to the lower starch content. Starch digestion was increased in calves fed steam-flaked corn, a concept reported by Leismeister and Heinrichs (2004). The authors reported higher blood VFA concentration and increased rumen wall thickness in calves fed steam-flaked corn compared to whole corn, suggesting that processed corn had a higher digestibility factor in the developing rumen. As seen in Figure 3.1 and Tables 3.5 and 3.6, the digestibility advantage of steam-flaked corn is observed after the animal is weaned but is not statistically effective until after the animal is heavy enough to be sold to a commercial feedlot. Coinciding with the higher starch digestion capacity, a higher DMI in weaned calves fed steam-flaked corn is observed

(Figure 3.2; Table 3.5). A combination of greater DMI and starch digestion in steam-flaked corn fed calves, while not statistically valid prior to weaning, may eventually improve growth performance over whole corn fed animals after feedlot incorporation.

Implications

The current study has shown that on-site prediction of fecal components by a portable NIRS is statistically possible, but currently requires further calibration efforts. Also, feeding diets containing steam-flaked corn will have no effect on growth performance in young dairy bull calves younger than 8 weeks post-weaning. However, the digestibility advantage of steam-flaked corn over whole corn can be observed once the animal has been weaned. Further research is needed to determine when the digestibility advantage of feeding steam-flaked corn correlates with improved growth performance in the dairy steer.

Table 3.1. Dietary ingredient and chemical composition of experimental diets (DM basis)

Item	Diet ¹	
	Calf Pellet	Holstein Starter
<i>Ingredient, %</i>		
Corn	59.0	63.1
Calf Pellet	25.0	-
Alfalfa Hay	-	16.2
Molasses	6.0	7.8
Beet pulp	10.0	-
Fat	-	2.6
Soybean meal	-	6.8
Mineral mix ²	-	2.2
Urea	-	0.3
Rumensin premix ³	-	1.0
<i>Chemical composition, %</i>		
DM	91.2	88.5
CP	19.3	14.7
NDF	10.1	15.0
ADF	8.4	7.5
Ash	5.6	6.1
Starch	29.4	36.5

¹Diets fed to calves (n = 17): Calf pellet diet from receiving to weaning; Holstein starter after weaning to feedlot weight. One week of a transition diet (not included) was fed during weaning to acclimatize animals to a non-pellet and non-beet pulp diet.

²Composition (% mix DM): Limestone (46), Ground corn (20), Salt (20.2), Potassium Cl(7.8), Ammonium sulfate (6.5), Magnesium oxide (3.4), Dicalcium P (1), Zinc sulfate (0.83), Vitamin E (0.54), Manganese sulfate (0.45), Vitamin A (0.27), Copper sulfate (0.15), Iron sulfate (0.13), Selenium (0.12), Calcium iodate (0.003) and Cobalt carbonate (0.002).

³Mixed to provide 300 mg·animal⁻¹·d⁻¹ of Rumensin 90 and 100 mg·animal⁻¹·d⁻¹ of Tylan 40 (Elanco Animal Health, Greenfield, IN).

Table 3.2. Chemical composition of fecal samples from Holstein cattle used as the initial calibration set.¹

Item	Sample #	Mean, %	SD, %	Range, %
DM	220	40.0	12.51	9.5—73.7
CP ²	220	28.3	11.32	13.3—58.2
NDF	220	31.9	11.22	1.4—70.1
ADF	220	16.0	8.42	0.2—54.3
Ash	220	14.7	4.12	5.8—41.9
Starch	220	4.1	4.9	0.2—26.1

¹Calibration set (n = 220) used for prediction of fecal composition by a portable NIRS.

²CP, ADF, NDF and starch reported on a DM basis.

Table 3.3. Predictive NIRS regression values of fecal constituents from Holstein cattle.¹

Item:	Calibration Set		Validation set	
	R ²	<i>P</i> value	R ²	<i>P</i> value
DM	0.36	0.0001	NS	NS ²
CP	0.84	0.0001	NS	NS
NDF	0.57	0.0001	NS	NS
ADF	0.72	0.0001	NS	NS
Ash	0.32	0.0001	NS	NS
Starch	0.25	0.0001	NS	NS

¹Cattle (n = 16) fed diets containing either steam-flaked or whole corn. Fecal samples for calibration (n = 220).

²NS = Not statistically relevant.

Table 3.4. Discriminant equation validation results within groups

Comparison	Group	#Correct/Total
Diet ¹	Whole Corn	53/100
	Steam-Flaked	61/116
Collection Period (4 wk intervals)	Period 1	50/61
	2	37/49
	3	43/59
	4	39/53

¹Diets contained either whole or steam-flaked corn.

Table 3.5. Growth performance of young Holstein cattle fed a whole or steam-flaked corn based diet from pre-weaning to feedlot¹

Item	Treatment		SEM	P value
	Steam-flaked	Whole		
<i>Growth Performance</i> ²				
Initial BW, kg	33.1	35.5	2.93	0.57
Wean BW, kg	56.2	56.1	3.72	0.99
Final BW, kg	93.5	96.4	6.02	0.93
Overall BW gain, kg	60.5	61.0	4.07	0.93
Pre-wean ADG, kg	0.38	0.34	0.038	0.45
Post-wean ADG, kg	0.58	0.63	0.047	0.45
Overall ADG, kg	0.50	0.51	0.037	0.82
Pre-wean average DMI, kg	0.96	0.95	0.053	0.84
Post-wean average DMI, kg	3.09	2.73	0.110	0.04
Overall average DMI, kg	2.02	1.84	0.072	0.09
Pre-wean G:F	0.40	0.36	0.035	0.33
Post-wean G:F	0.19	0.23	0.016	0.06
Overall G:F	0.30	0.30	0.017	0.95

¹Diets formulated with either steam-flaked or whole corn. Animal (n = 16) was the experimental unit.

²Animals (n = 16) were weaned at approximately 56 d from receiving and fed until at least one animal had reached feedlot weight (125 kg; approximately 60 days post-weaning).

Table 3.6. Fecal components from calves fed diets differing corn processing¹

Item:	Pre-wean Diet				Post-wean Diet			
	SFC	WC	SEM	<i>P</i> <	SFC	WC	SEM	<i>P</i> <
DM	38.0	36.8	1.36	0.52	39.1	43.5	3.42	0.35
CP	36.5	37.9	1.50	0.48	22.0	19.2	2.41	0.39
NDF	27.3	24.0	1.92	0.23	41.7	35.4	0.98	0.01
ADF	10.7	9.5	0.99	0.42	24.3	20.6	0.72	0.01
Ash	15.3	17.0	0.53	0.03	14.3	12.5	0.78	0.11
Starch	1.4	1.5	0.28	0.73	4.3	7.7	0.83	0.01

¹n = 16. Diets contained either steam-flaked (SFC) or whole (WC) corn. Pre- and post-wean period comprise 8 weeks each.

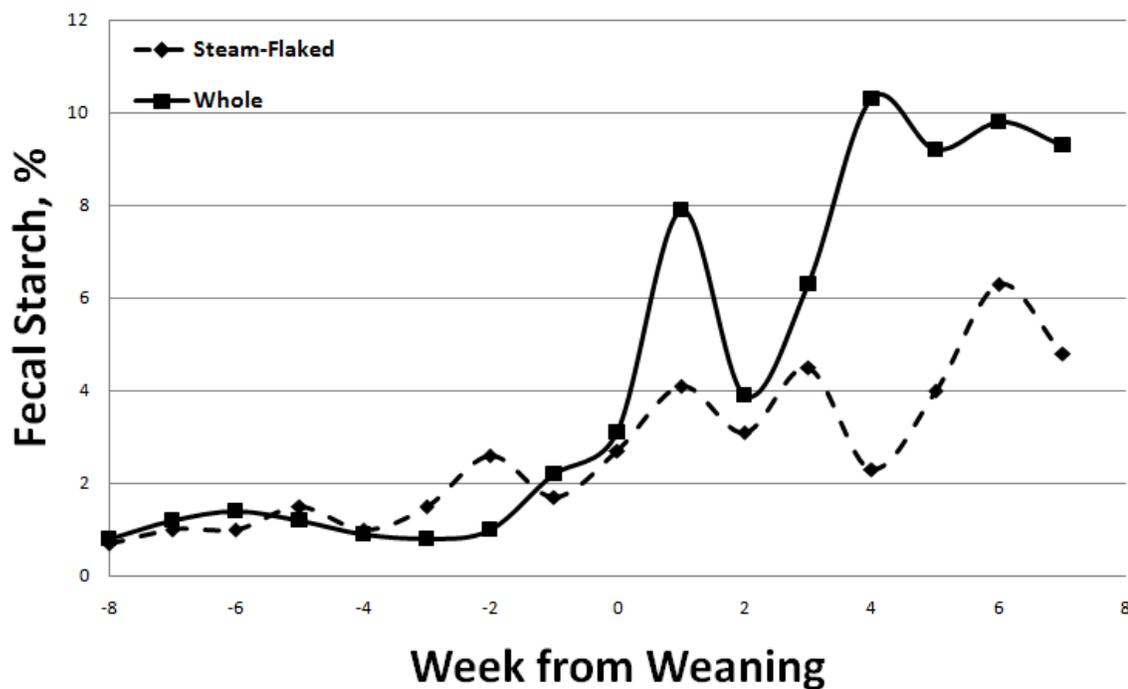


Figure 3.1. Change in fecal starch content across time. Calves ($n = 16$) were fed diets differing in corn processing. A diet \times week effect is observed ($P < 0.01$), with individual differences ($P < 0.05$) beginning at week 1 post-weaning.

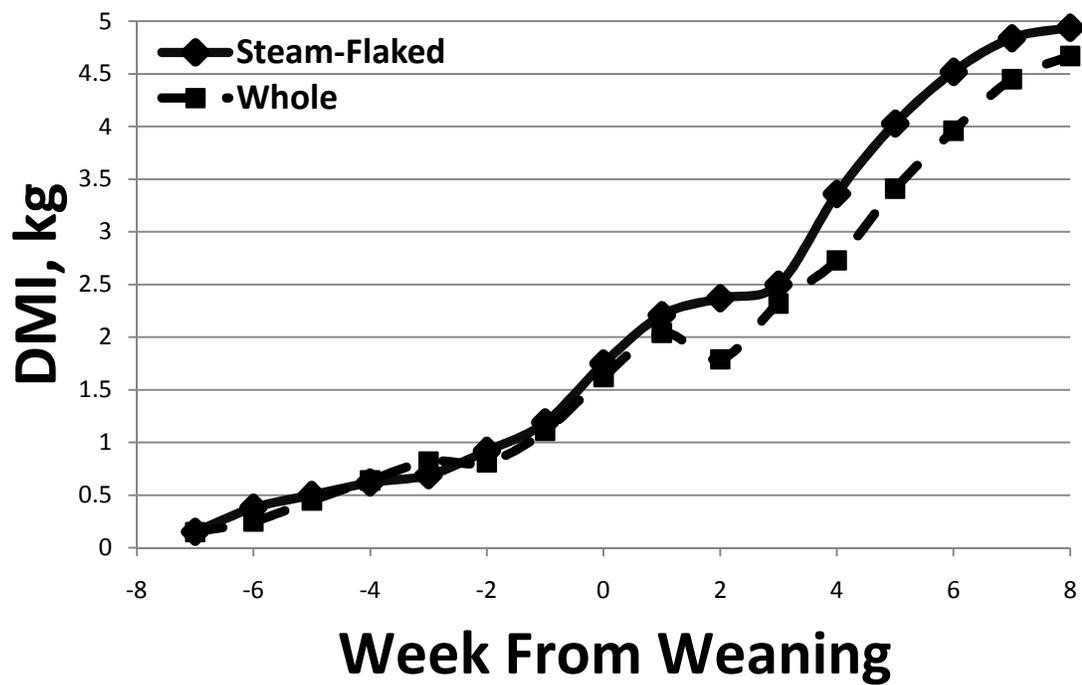


Figure 3.2. Change in DMI across time. Calves ($n = 16$) were fed diets differing in corn processing. A diet \times week effect is observed ($P < 0.01$), with individual differences ($P < 0.05$) between weeks 1 through 6 post-weaning.

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