USING NEAR INFRARED SPECTROSCOPY OF FECES TO PREDICT GROWTH PERFORMANCE IN FINISHING FEEDLOT CATTLE

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ABSTRACT

The objective of this thesis was to use fecal composition and apparent total tract digestibility (aTTD) as predicted by near infrared spectroscopy (NIRS) to assess growth performance in commercial feedlot cattle. Studies were conducted to 1) develop and validate NIRS calibrations using dried ground feces from cattle, 2) determine the optimal timing of sample collection, 3) compare samples collected from the pen floor versus rectum, 4) determine the optimal number of fecal samples to collect from a pen, 5) determine if NIRS could be used to detect changes in fecal nutrient concentrations and aTTD for a variety of diets, 6) find associations between fecal parameters and growth performance, 7) use NIRS of feed to predict energy content of grain screening pellets (GSP), 8) and assess the ability of fecal NIRS to predict dry matter intake (DMI), average daily gain (ADG), and gain to feed ratio (G:F) of feedlot cattle in a commercial feedlot. Fecal NIRS calibrations yielded accurate predictions ($R^2_{CV} > 0.90$, $SECV < 2.42$) for all fecal constituents except fat, and accuracy of predicting aTTD was high for starch ($R^2_{CV} = 0.84$, $SECV = 1.06$), moderate for DM, OM, CP, and GE ($R^2_{CV} > 0.71$, $SECV < 2.88$), but poor for NDF and ADF ($R^2_{CV} < 0.33$, $SECV > 7.86$). Most fecal nutrients and apparent total tract digestibility (aTTD) predictions varied over 24 h, however spot fecal samples collected at any time point from multiple cattle could be used as predictors of chemical composition and digestibility. Morning samples collected within 0 to 4 h after first feeding are optimal for estimating fecal starch and aTTD of starch. Except for DM, which was higher ($P < 0.01$) in pen floor than rectal fecal samples, there were minimal differences in fecal constituents between collection methods. When diets were fed containing wheat or increasing levels of silage in place of barley, aTTD of GE predicted using NIRS was related to net energy of gain (NEg) of the diets as estimated by performance ($R^2 = 0.58$, $P = 0.03$ and $R^2 = 0.43$, $P < 0.01$, respectively). Similarly, observed ADG could be predicted using NIRS of feces for steers fed wheat ($R^2 = 0.48$, $P = 0.05$) and increasing levels of silage ($R^2 = 0.40$, $P < 0.01$), but not G:F. Compared to measured performance data, NIRS over predicted the energy content of grain screening pellets. When comparing cattle of different sexes, different processing methods and grain types, a quadratic relationship was observed between fecal starch, sex, average BW at time of sampling, and G:F (rho = 0.75, $P < 0.01$). These data indicate that NIRS predictions using dried ground feces collected from the pen floor from multiple feedlot cattle could predict G:F of feedlot cattle with reasonable certitude when variables such as BW and sex were included in the model.

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DEDICATION

To all the amazing animals I had the pleasure of working with through out my PhD program, especially my six heifers.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

Figure number Page number

LIST OF ABBREVIATIONS

ADF	Acid detergent fibre
ADG	Average daily gain
ADL	Acid detergent lignin
aTTD	Apparent total tract digestibility
CP	Crude protein
DDG	Dried distillers grains
DMI	Dry matter intake
EE	Ether extract
F:C	Forage:Concentrate ratio
F:G	Feed:Gain ratio
G:F	Gain:Feed ratio
GE	Gross energy
N	Nitrogen
NIRS	Near infrared spectroscopy
NDF	Neutral detergent fiber
NEg	Net energy of gain
NEm	Net energy of maintenance
OM	Organic matter
PCA	Principle component analysis
R^2	Coefficient of determination
R^2_{cal}	Coefficient of determination of calibration
R^2_{CV}	Coefficient of determination of cross validation

R^2_{val}	Coefficient of determination of validation
SEC	Standard error of calibration
SECV	Standard error of cross validation
SEP	Standard error of prediction
TDN	Total digestible nutrients
TMR	Total mixed ration

CHAPTER 1

1.0 GENERAL INTRODUCTION

Improving the efficiency of feedlot cattle is an important factor in reducing feed costs, as it generally results in lowering the number of days on feed, resulting in overhead costs being spread across more cattle. Feedlot managers are not always aware how their cattle are performing, until after sale. The NASEM (2016) equations to estimate performance require the net energy (NE) concentration of the diet, DMI, final shrunk BW, and adjustments for initial BW and sex (McMeniman et al. 2010; Galyean et al. 2010). The NE concentration of the diet is additonally affected by the types and proportions of dietary ingredients, and the way these ingredients are processed prior to feeding.

Diet digestibility can not be measured accurately or in a timely manner using standard wet chemistry techniques in grazing or commerical livestock systems. Consequently, there has been considerable interest in determing if fecal composition can be used to predict digestibility. Fecal samples contain information relevant to feed digestion, therefore monitoring how efficiently cattle utilize feed by measuring residual nutrients in feces, could directly benefit the efficiency and profitability of livestock production systems. For feedlot cattle in particular, cereal grains are a major dietary ingredient, and given the high proportion of starch in grains, predictions have been developed to estimate starch digestibility using fecal starch concentration (Zinn et al. 2002; Corona et al. 2005; Zinn et al. 2007).

To further facilitate ease and speed of measurement, near infrared spectroscopy (NIRS) of the feces has been employed to predict the chemical composition (Chen et al. 2013), nutrient intake (Dixon and Coates 2009), and the digestibility of a variety of forage (Coleman et al. 1995; Boval et al. 2004), and concentrate diets (de la Roza et al. 2002; Garnsworthy and Unal 2004). Calibrations have also been developed for rapid measurement of starch concentration in feces and to predict starch digestibility by dairy cattle (Fredin et al. 2014).

Previous work using NIRS to predict performance parameters directly has focused mainly on DMI of grazing cattle (Lyons and Stuth 1992; Coates 1998; Garnsworthy and Unal 2004). Fecal NIRS has been also used to predict diet CP and aTTD of OM, coupled with decision support software and nutritional balance calculations to monitor the nutritional status and growth

performance of cattle on pasture (Tolleson and Schaffer 2014). Until now, there has been no attempt to predict the growth performance of feedlot cattle fed high grain diets using NIRS of feces.

The objectives of this literature review are to provide an overview of the commercial feedlot industry including performance measures of interest, and description of grain processing and typical diets fed. Additionally, this review will highlight methods of using the feces of cattle to predict digestibility of nutrients within the diet ingested. Key concepts of NIRS and its application in the field of ruminant nutrition will be discussed, including the past and recent advances of NIRS applied specifically to the feces. The hypothesis and objectives of this thesis will also be presented.

CHAPTER 2

2.0 LITERATURE REVIEW

2.1 Commerical Feedlot Industry

Canada's beef sector is one of the leading producers of beef in the world, steadily contributing around $33 billion annually to the country's economy (Canadian Cattlemans Assocaition 2016). Approximately 3.8 million cattle were raised in Canadian feedlots this year, supplying 1.2 billion kg of beef, over 40% of which is exported to over 70 countries (CanFax 2016). Despite these high numbers, profitability for beef producers is defined by extremely narrow margins, due to the recent economic crisis lowering the demand for beef (CanFax 2016), and the high costs of production, 60-80% of which is accounted for by feed (CanFax 2014).

The combination of high production costs, low profit margins, and consumer demands make the beef industry highly competitive. Competition exists not only between producers who sell similar classes of cattle, but also producers of other sources of animal protein, such as poultry and swine. In order for feedlot operators to gauge their production status relative to their competitors, the industry establishes performance benchmarks and financial goals, which can result in decisions to increase productivity. Average daily gain (ADG), dry matter intake (DMI) and feed conversion efficiency are performance indicators that are closely monitored by feedlots.

2.2 Animal Performance

The simplest measures of animal performance are ADG (kg BW/day) and DMI (kg DM). In a commercial feedlot setting, these indicators represent the average for a group of cattle that have been sorted by age, sex and weight, days on feed, and target to market, and housed within a pen, as opposed to the performance of an individual. Aside from sorting and shipping dates, and when implants or vaccines are administered, even in a high intensity system, it is not realistic to measure BW and as a result values are based on estimates. In Canada, ADG is around 1.50 kg/d, and generally, the greater the ADG, the fewer the days on feed (CanFax 2014). Daily DMI (average 10.7 kg DM/d in Canada, Canfax 2014) is approximated by the amount of feed delivered to a pen and visual estimation of any refusals remaining. Estimates for BW are obtained from the combined knowledge of the feeding value of current ingredients or historical data of feed efficiency, and estimated DMI.

One way to express feed efficiency is as feed-to-gain (F:G), which is a ratio of the kg of feed to the kg of gain over a specific period in time; a value that is also calculated on a pen basis. The F:G can be accurately measured at the end of the feeding period using actual BW data recorded between the time of sorting and shipping. This performance indicator cannot be regularly monitored at any time over the course of the feeding period unless calculated using the estimates of DMI and ADG (carcass gain) as described above. In Canada, the average F:G is 7.14 (SD 1.33) (Beef Cattle Research Council, April 2012), and a lower F:G implies less kg DM is required for the same gain, indicating more efficient cattle. An alternative method of measuring FE is the gain-to-feed ratio (G:F), the inverse of F:G, on a carcass basis.

Factors that influence cattle performance include, but are not limited to, age, sex, breed, diet, nutrition, ingredient processing, environmental conditions, feed additives, growth promotants and hormonal implants, physical activity, and bunk management (NRC 2016; Shenkle et al. 2004; Schwartzkopf-Genswein et al. 2003). Management efforts have been targeted primarily at improving ADG and carcass gain with the use of beta agonists, however, in 2011, Canada had the second highest cost of production globally after Spain, with 76% of these costs being attributed to feed (CanFax 2014). Improving feed efficiency has a larger impact on reducing input costs and increasing overall profitability than simply increasing ADG. Feedlot studies have demonstrated that a 10% improvement in feed efficiency returned a 43% increase in profits, whereas a 10% improvement in ADG increased profits by only 18% (Fox et al. 2001). Basarab et al. (2002) also demonstrated that a 5% improvement in feed efficiency had an economic impact four times greater than a 5% increase in ADG.

2.3 Feedlot Diets

Corn and barley grain are the principal energy sources in the diets of North American feedlot cattle (Owens et al. 1997), but the use of wheat has increased in western Canada. The grain component in a feedlot finishing ration is usually included at 82 to 91% of diet dry matter (DM), with the proportion of forage ranging from 4.5 to 13.5% of diet DM (Vasconcelos and Galyean 2007). Other high-energy by-product feeds such as corn dried distiller's grains (DDG) and grain screenings may also be included, often substituting for up to 20-30% of the cereal grain. Key nutrients in high grain diets are starch (40 to 50% of diet DM), and crude protein (CP; 12-13% of diet DM), with lower contributions resulting from digestible and indigestible fiber

(20-30% of diet DM), and other sugars (10% or diet DM), with values varying with type of grain, by-product, and inclusion level.

Global grain prices have recently been higher than historical averages. In order to remain profitable, feedlot operators have focused on increasing the feeding value of grains to increase feed efficiency. Feed intake in finishing cattle is limited by the energy content of the diet, rather than gut fill (Huntington 1997). Hence, as the energy availability in the diet increases, DMI decreases and feed efficiency increases. Feeding cheaper alternative energy sources to replace a portion of the grain component is another option to lowering production costs, but may have negative effects on performance.

The most common terms for describing the energetic feeding value of ingredients or total mixed rations (TMR) are shown in Table 2.1. These terms are influenced by the chemical composition of the feedstuff, the composition of the total diet, processing, level of feed intake, and the physiological state of the animal (Hall 2002). For grains in particular, feedlot operators have the ability to alter the method or degree of processing, which increases the availability of nutrients for digestion and thus their feed value.

The net energy for maintenance (NEm) content of each diet can also be calculated from BW, DMI, and ADG as described by Zinn et al. (2002) using the estimates of energy gain (EG, Mcal/d) and the maintenance energy (EM, Mcal/d) expended based on growth performance for a medium frame yearling heifer [EG = 0.0557 × (average weight × 478/mature weight) 0.75 × ADG1.0971; where average weight is the mean shrunk weight (full weight × 0.96) and mature weight was 625 kg (defined as the weight at which protein deposition stops and all subsequent gain is fat) and EM = 0.077 × MBW0.75 (NRC 2016)]. The NE values of the diet for maintenance (NEm) can be estimated from the performance and feed intake using the quadratic formula [$x = -b \pm \sqrt{(b2 – 4ac)/2a}$], where a = -0.877DMI, b = 0.877EM + 0.41DMI + EG, and c = -0.41EM (Zinn and Shen 1998). Net energy of maintenance was converted between NEg and TDN using the equations TDN = (NEm + 0.5058)/0.0305 and NEg = 0.877×NEm-0.41 (Zinn et al. 2002; NRC 2016).

2.4 Grain Processing

The major nutrient and energy source in grains is starch, typically comprising over 50% of the dietary chemical composition on a DM basis (Campbell et al. 1995; Ovenell-Roy et al. 1998).

Other nutrients (CP, fiber, and non-structural carbohydrates other than starch including sugar and organic acids), although still important, play a lesser role in supplying energy to the animal. Most of the highly digestible starch granules are contained within the endosperm of the grain, which is embedded in a protective protein matrix. The endosperm is surrounded by a dense multilayered pericarp that is further surrounded by a fibrous husk in hulled barley and oats or by bran in wheat. Corn and sorghum contain dense protein matrices within a vitreous endosperm that surrounds starch granules and impedes the access of amylolytic microbes (McAllister et al. 2011). In contrast, wheat and barley have much more diffuse protein matrices, are similar in their endosperm structure, where the starch is loosely associated with the protein matrix. Even still, differences have been found in the response of barley and wheat to processing (Chapter 5). The pericarp and husk are primarily composed of cellulose and hemicellulose with a small amount of lignin, making them resistant to digestion. Processing reduces the particle size and shatters the pericarp (and husk if present), exposing the highly digestible starch to digestive enzymes in the rumen (McAllister et al. 1994; McAllister 2011). Differences in the response of the two grains to processing could result from many factors including endosperm structure, size and composition of the starch granules (McAllister et al. 2011), kernel hardness (Campbell et al. 2007), moisture content and the size and shape of the kernels (McAllister et al. 2011). The distance between rollers is often left wider for wheat than for barley to avoid shattering of the kernels, but may also result in a greater proportion of intact kernels (McAllister et al. 2011).

Many western Canadian feedlots use barley or wheat that can be sufficiently processed by dry rolling (DR) or temper rolling (TR). Dry rolling consists of passing the grain between two rollers with the distance between the rollers being adjusted to control the degree of fracture of the grain kernel. Temper rolling involves application of water for 8 to 24 h prior to rolling, increasing its moisture level from around 8% to 20%. Tempering enables more control over the degree of fracture due to its malleability thereby reducing fines and helps to control dust. Grain processing methods are used to enhance digestibility with consideration for not detrimentally affecting ruminal pH and causing digestive dysfunction. Under processing has been linked to inefficient starch utilization with a high proportion of intact grain kernels excreted in feces, whereas over-processing can produce a high proportion of fine particles. These fine particles (less than 1 mm diameter) have been shown to lower palatability of the diet, and increase the

Table 2.1. Expressing the energy value of feed or feed ingredients[a]

Term	Definition	Description
DE (Mcal)	Digestible energy[b]	Gross energy of feed – Gross energy in feces. Fails to consider gaseous and urinary losses and losses due to metabolism.
TDN (Mcal/kg)	Total digestible nutrients[c]	Included digestibility of nutreints (NASEM 2016)
ME (Mcal)	Metabolizable energy[b]	DE - (Urinary Energy + Gaseous Energy) also ME = 1.01 × DE – 0.45
NE (Mcal)	Net energy[b]	ME - HE (heat produced during digestion, metabolism and excretion). Energy used for maintenance and growth, gestation, lactation.
NEm (Mcal/kg)	NE for maintenance[d]	The net energy required for maintenance
NEg (Mcal/kg)	NE for gain[d]	The net enrgy required for gain

[a] Adapted from NASEM 2016

[b] derived from in vivo studies

[c] derived from calculations based on nutrient composition or DE

[d] derived from growth studies

occurrence of digestive dysfunction due to starch digestion being too rapid causing accumulation of acid in the rumen (Mathison, 2000).

From an applied measurement standpoint, the degree of processing can be described by the processing index, which is measured as the bushel weight (per unit volume) after processing as a percentage of bushel weight before processing (Koenig and Beauchemin, 2011). Industry standards for PI for dry rolled barley grain ranges between 65 and 82% (Yang et al. 2000), whereas temper rolled barley ranges from 70 to 95% (Beauchemin et al. 2001). Dry rolled wheat, being more susceptible to shattering during processing and having a faster rate of ruminal starch digestion than hulled barley, is typically more coarsely processed.

There has been considerable discussion on the advantages and disadvantages of specific processing methods since the type and severity of grain processing has varying results on feeding value and feedlot cattle performance. Changes in ADG, DMI, and feed efficiency vary from decreases, to no effect, to significant increases, depending on the grain type and processing method (Theurer 1986; Owens et al. 1997; Zinn et al. 2011). Reported improvements in finishing feedlot cattle performance as a result of processing have been attributed to an increase in apparent total tract digestibility (aTTD) of starch; Owens et al. 1997; Theurer 1986; Beauchemin et al. 2001). Improvements in milk and protein yield, and FE in dairy cattle have also been observed due to increases in aTTD of starch (Firkins et al. 2001). Extensive processing maximizes starch digestibility, but is also associated with greater risk for digestive upset and can result in reductions in performance. Rapid starch digestion can cause abnormal rumen function and variable feed intake which can lead to severe health related problems in cattle such as acidosis, bloat, laminitis, and liver abscesses (Nagaraja et al. 2007).

Feedlot operators can control the degree and type of processing; giving them some degree of control over aTTD of starch, and ultimately feed efficiency. Zinn and colleagues (2011) have suggested looking at nutrients in the feces to assess the adequacy of processing corn under feedlot conditions. If a convenient method is developed to predict aTTD of starch in a commercial feedlot setting, it could be used as a means to optimize grain processing on a day-to-day basis. This could lead to potential improvements in growth performance and feed efficiency by regularly monitoring the response of aTTD of starch to processing.

2.5 Measuring Total Tract Starch Digestibility

The most accurate measurements of aTTD of starch involve *in vivo* studies that are specific to each animal and diet. Such studies require cattle to be housed in individual pens or a metabolism stall for a period of dietary adaptation followed by recording intake, collection of diet and ort samples, and total collection of feces for a minimum of 4 d. The time and labour associated with total collection can be reduced using marker based techniques, but both methods require extensive laboratory analysis to determine the starch content in feed ingested and in the feces excreted. *In situ* techniques are much simpler but are only suitable for ranking feeds in terms of actual digestibility because palatability, DMI, and total tract digestibility is ignored; in addition, cattle must be surgically fitted with ruminal fistulae for these procedures. *In vitro* and enzymatic assays can provide estimates of starch content and availability in a feedstuff, but often fail to reflect the form in which the grain is fed to the animal. All of these methods are laborious and expensive, and not practical for implementation at a commercial feedlot.

Sieving of feces collected from individual or pooled fecal pats and visual examination for grain kernels is one method that can be potentially applied in a commercial feedlot (Hall 2002). If the amount of undigested grain in the manure is excessive, it is indicative of a reduction in aTTD of starch. If there is an excessive amount of grain in the feces it may indicate that processing procedures need further refinement. However, this method is subjective, is not quantitative and has low sensitivity and precision. In addition, it is not always obvious whether the kernels appearing in the feces are originating from the grain or the silage. Upon close inspection, it is possible to differentiate between kernel physical attributes if other information is known such as the grain variety, stage of maturity of silage, and if kernel and grain processing was performed.

The ability to quantitatively assess the amount of starch in feces could provide a method of improving the development of a field prediction of aTTD of starch. Several reviews and data from studies where aTTD of starch has been measured have been published examining the relationship between fecal starch concentration and aTTD of starch in dairy cattle and feedlot steers (Table 2.2). Reports have shown that fecal starch content has a modest to strong correlation with aTTD of starch in both lactating cows and feedlot steers with coefficients ranging between 0.64 and 0.97 (Fernandez et al. 1982; Zinn et al. 2002; Owens and Zinn 2005; Corona et al. 2005; Zinn et al. 2007). The dietary starch in these studies ranged from 19% to

62% of diet DM, and at the higher levels of grain where aTTD of starch exceeded 95%, fecal starch more accurately predicted starch digestibility. In higher grain diets, fecal starch in feces of cattle tended to be higher and more easily measured as it was not as diluted by residual fiber (Owens and Zinn 2005). Equations to predict aTTD of starch from fecal starch concentration in cattle were derived by Owens and Zinn from two summaries where data from a large number of metabolism studies were compiled (Figure 2.2). Due to large differences in diet composition and starch digestion, separate prediction equations are required for dairy and finishing feedlot cattle.

Zinn et al. (2002, 2011) demonstrated that the feeding value of corn could also be predicted using fecal starch alone. The feeding value of corn is affected by the intensity of processing during the steam flaking process. Corn and sorghum grains have a different chemical and physical structure within the endosperm than wheat and barley making them notably less digestible without processing. Corn requires softening of the kernel by steaming or tempering prior to rolling in order to enhance the growth responses as a result of increased aTTD of starch (Zinn et al. 2011). It was observed that aTTD of starch was closely associated with the NE value of steam-flaked corn (R^2=0.88), and when corn was the principal dietary source of starch, fecal starch could accurately predict the NE value of flaked corn. Zinn et al. (2002) deduced that the change in NE_m with changes in aTTD of starch was remarkably constant across other grain sources including sorghum, barley, and wheat. These conclusions shed light on the potential value of being able to rapidly and accurately predict aTTD of starch, based on the starch level in fecal samples collected in a commercial feedlot setting.

2.6 Use of Feces to Predict the Digestibility of other Nutrients

Many years ago, fecal nitrogen excretion was observed to be readily predicted from the concentration of dietary N in a high forage diet (Holter and Reid 1959). Lukas et al. (2005) proposed that fecal nitrogen alone, due to its high correlation with digestibility (R^2 = 0.82) and relatively constant output, could be used to calculate the DM digestibility of high forage diets. More recently, researchers have explored the utility of estimating fecal nitrogen in combination with fecal starch to predict aTTD of starch (Zinn et al. 2007). Total tract starch digestion was determined from data sets from 32 reviewed digestibility studies, and 637 individual measurements using concentrations of starch in feed and feces and chromic oxide as a digestibility marker. Diets consisted of a range of grain types and starch and N concentrations

Table 2.2. Correlation of fecal starch to apparent total tract digestibility of starch

Year	Author	Correlation	Cattle type
1982	Fernandez et al.	R^2=0.64	lactating dairy cattle
2002	Zinn et al.	R^2=0.91	feedlot steers
2005	Corona et al.	R^2=0.97	feedlot steers
2005	Owens and Zinn	R^2=0.73	lactating dairy cattle
2007	Zinn et al.	R^2=0.96	feedlot steers
2010	Grant	R^2=0.78	lactating dairy cattle
2012	Ferraretto and Shaver	R^2=0.94	Lactating dairy cattle
2014	Fredin et al.	R^2=0.94	lactating dairy cattle

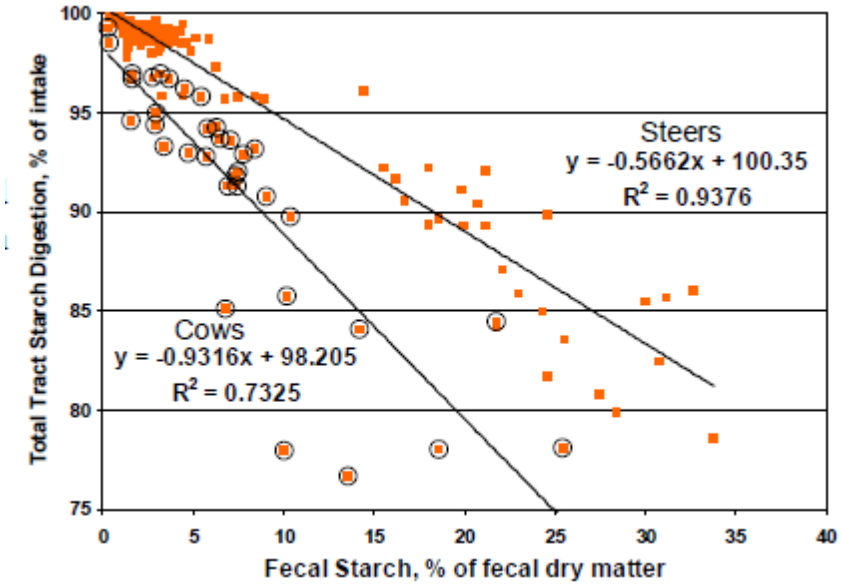


Figure 2.1. Relationship between fecal starch concentration and total tract starch digestibility in feedlot cattle from Owens and Zinn (2005).

(69.8±11.5% grain (DM basis), 25.2 to 61.9% starch and 1.5 to 3.0% N). Fecal N was closely related to N intake (R^2=0.95), however, the relationship only explained 35% of the variation in fecal DM excretion. Nonetheless, including this estimate of fecal DM (using N as a marker), dietary starch, and relative concentrations of starch and N in feces, an equation to predict aTTD of starch was derived. The combination of these three nutrient levels explained 94% of the variability in starch digestion, when in comparison, the much simpler method of using fecal starch alone, explained 96% of the variation in starch digestibility. However, Zinn and colleagues (2007) deduced that when omitting 108 cases in their dataset where starch digestibility was less than 95%, the relationship between fecal starch and aTTD of starch decreased to 0.82, and incorporating fecal nitrogen and dietary starch, a close relationship of 0.92 was maintained. Typically, starch digestibility in feedlot cattle diets is greater than 95% and levels of fecal starch range from 0 to 5%, resulting in most observations occurring at or near the y-intercept (Owens and Zinn 2005; Figure 2.1). The correlation coefficient of fecal starch equations was improved if a broad range in fecal starch (0 to 44.2%) was used to derive estimates as opposed to the mean value (5.9%). From analysis of feed and feces from starch and N, aTTD of starch was predicted more precisely, particularly for lactating cows (R^2 increased from 0.73 to 0.90 in dairy cattle, and from 0.91 to 0.96 for feedlot steers) (Figure 2.2).

It is doubtful that the concentration of other nutrients in feces will correlate with total tract starch digestibility as much as the direct measurement of starch in feces. Due to the contribution of endogenous and microbial synthesis of N and fat, it is likely that total tract digestibility of these two nutrients would be under predicted based on fecal concentrations (Fredin et al. 2014). Between 94 to 95% of fecal nitrogen in feedlot diets is metabolic N (Holter and Reid 1959), whereas fecal starch is of dietary origin. Estimating fecal fiber levels may also be of value considering that fiber is also not endogenously synthesized or produced by microbes (NRC 2001). Fecal neutral detergent fiber (NDF) was examined by Fredin et al. (2014) in lactating dairy cows, and TTD of NDF was not well predicted from fecal NDF concentrations (R^2=0.18). This is likely due to the lower total tract digestibility of NDF as compared to starch, increasing the variation of fecal NDF and reducing it correlation to TTD of NDF.

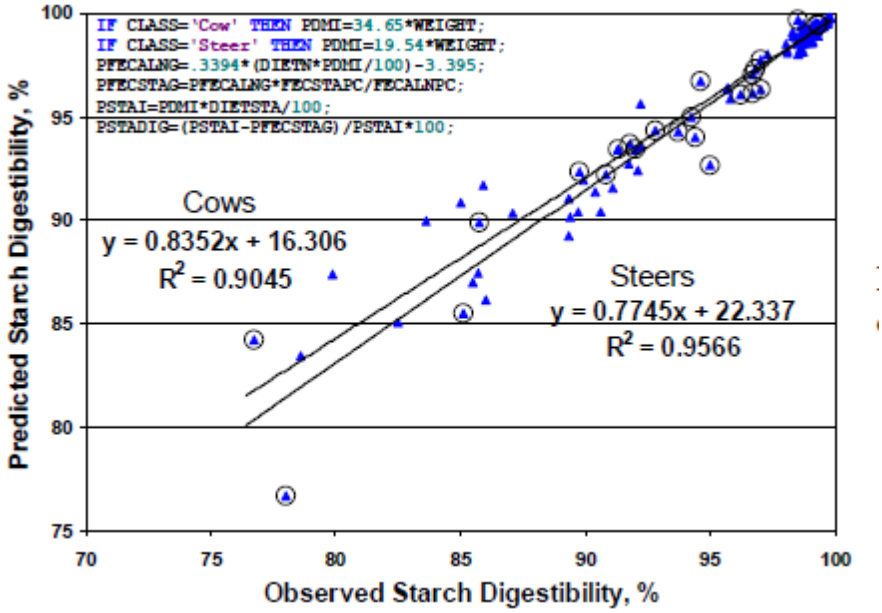


Figure 2.2. Predicted versus observed starch digestibility using equations with fecal starch, fecal nitrogen, and dietary starch as variables. Obtained from Owens and Zinn (2005).

2.7 Techniques to Measure Nutrients in the Feces

Most of the equations derived for aTTD of starch using fecal starch concentration were obtained in digestibility studies using total collections or the use of external (chromium oxide and titanium dioxide) or internal (acid insoluble ash, indigestible NDF, and lignin) markers with continuous sampling of both feed and feces which were subsequently analyzed for starch using wet chemistry (Zinn et al. 2002; Owens and Zinn 2005). These methods use fecal samples that have been pooled over an entire day, over several days, or collected at precise intervals in an effort to represent a full 24-h feeding cycle. Such approaches are impractical in a commercial setting. However, it is not known if daily fecal starch excretion of penned cattle can be estimated from single or pooled fecal samples as accurately as the methods described above.

Grant (2010), quoted J.D. Ferguson (University of Pennsylvania, PA; personal communication) showing that starch and lignin in composite TMR and pen fecal samples could be used to estimate aTTD of starch in lactating dairy cows on commercial farms (R^2=0.73). Using an equation derived from digestibility trials using Holstein cattle, Lidy et al. (2009) estimated aTTD of starch in Holstein dairy herds using fecal starch and fecal lignin content of five fecal pats pooled by pen, along with composite diet samples. Finishing diets for feedlot cattle contain a much lower proportion of forage than dairy diets, resulting in lower levels of lignin in feces, making this technique less likely to be suitable for use in feedlots.

Fredin et al. (2014) conducted a detailed study using lactating dairy cattle and found that differences in fecal sampling time including day within week or by week of sampling did not influence FS concentration. Time of day when fecal samples were taken did influence FS concentration but differences were minimal, suggesting that on-farm collections of feces from individual cows or pens of cows may be adequately reflected by sampling only once per day. These results are not consistent with Leonard et al. (1989), in which diurnal variations in FS were apparent. However, the effect of sampling time within day was not analyzed in that study. Angus steers were fed corn grain and hay once per day and fecal samples were collected over 24 h at 8-h intervals after feeding. The fecal starch concentrations (mean + SE) were 7.8 ± 1.9, 4.8 ± 1.1, and 10.3 ± 2% for collections between 0 to 8, 8 to 16, and 16 to 24 h, respectively. Daily fecal starch concentration in this study averaged 7.6 ± 1.2%. Although both studies fed high forage dairy diets, the proportion of corn was higher in the study by Leonard et al. (1989), which may account for the higher fecal starch and the spike in starch excretion between 16 to 24 h.

Feedlot diets contain a considerably higher proportion of grain and starch than dairy diets and starch digestibility is generally higher in beef cattle than dairy cattle due to a slower passage rate, lower feed intake, and smaller reticulo-omasal orifice in most beef breeds (Welch 1982; 1986). Despite the higher aTTD of starch, fecal starch is expected to be higher in the feces of feedlot cattle than dairy cattle owing to the higher concentration of starch in the diet and the lower levels of fiber in their feces (Owens et al. 2005). Based on variations in dietary ingredients and passage rate, the diurnal excretion rates of fecal starch will likely differ between feedlot and dairy cattle.

Wet chemistry is the standard procedure for the proximate analysis of all common constituents in feedstuffs and feces. Unfortunately, chemical analysis requires significant sample preparation, is expensive and is usually only capable of analyzing one constituent at a time. Analysis of a suite of compositional parameters typically requires at least 3 to 4 d by skilled technicians. Commercial laboratories can measure fecal starch concentrations through wet chemistry; however, starch analysis is associated with a large amount of inter-laboratory error with coefficients of variation as high as 16 percent in a roundtable test among commercial laboratories (Beever et al. 1996). The majority of variation in the procedure arises from incomplete dissolution and/or accessibility of enzymes to starch granules (Mueller-Harvey, 2013). Although still laborious and complex, the Megazyme enzyme kit for starch analysis in cereal grains and animal feeds was accepted as an AOAC method as it was highly correlated with total starch content (Petterson et al. 1999). This method is based on the liberation of glucose after starch is treated with α-amylase and amyloglucosidase, and requires several precise steps prior to extraction and starch hydrolysis.

Near-infrared spectroscopy (NIRS) is a method that uses the near-infrared region (700 to 2400 nm) of the electromagnetic spectrum to predict the concentration of organic compounds. In the past, NIRS has been used to predict the nutrient content of feed ingredients (Givens and Deaville 1999). However, more recently it has been shown to offer valuable information in terms of estimating the nutrient content of feces (Chen et al. 2013; Dixon and Coates 2009) and diet quality of the ingested feed from indirect scanning of the accompanying fecal samples (Dixon and Coaes 2009). Most common applications include prediction using dried and ground fecal samples, but it may also have the potential to predict the composition of fresh feces.

2.8 Electromagnetic Radiation

The electromagnetic spectrum (EMS) extends from the short, high-energy wavelengths of gamma radiation to the long, low energy wavelengths of radio waves (Figure 2.3). All electromagnetic radiation (EMR) can be absorbed, transmitted, and reflected following specific mathematical relationships and properties (Workman and Shenk 2004). Quantum mechanical theory is used to describe the behaviour of the wave-like particles (photons) that are responsible for all electromagnetic interactions. These wave-like particles are emitted when an atomic system shifts from a higher to a lower quantum state, and can also be absorbed when going from a lower to a higher quantum state.

Bonds within molecules oscillate and vibrate constantly in stretching and bending motions, and absorption of radiation takes place when these vibrations occur at the same frequency as the radiation wave (Osborne 2000). The infrared (IR; 2500-16000 nm) part of the EMS is responsible for fundamental vibrational frequencies within molecules, and the near infrared (NIR; 750 to 2500 nm) region is responsible for overtone and combination vibrations. Fundamental vibrations correspond to transitions from orbitals v=0 to v=1, and overtones correspond to transitions from $0 \rightarrow n$, where n>1 (Figure 2.4). When a vibrational mode is excited from v=0 to v=2, this is called the first overtone, from v=0 to v=3 is known as the second overtone, and so on. Combination bands are observed when two or more fundamental vibrations are excited simultaneously.

The energy transfer between light waves and molecules can be measured as a plot of absorption (or transmission in gaseous, liquid or very thin samples) versus wavelength and is called a spectrum (Osborne 2000). Three important parameters of interpreting spectra include the wavelength, the amplitude of the peak, and the width of the peak. The combination of these factors provides a signature of the chemical bonds within a substance, enabling its chemical composition to be predicted (Shenk et al. 1992; Shenk and Westerhaus 1994).

The spectra in the IR and NIR region are the result of vibrations of bonds between polar molecular bonds, predominantly with C-H, N-H, O-H and S-H bonds; the functional groups that are most common in organic substances (Wetzel 1983; Osborne 2000; Pasquini 2003). While absorptions in the IR region are characteristic of sharp distinct peaks, the NIR spectra consist of peaks that lack distinction due to the weaker overtones and combination absorptions (Pasquini

2003). As a result, NIR spectra are identified as a complex pattern or fingerprint of substance as a whole.

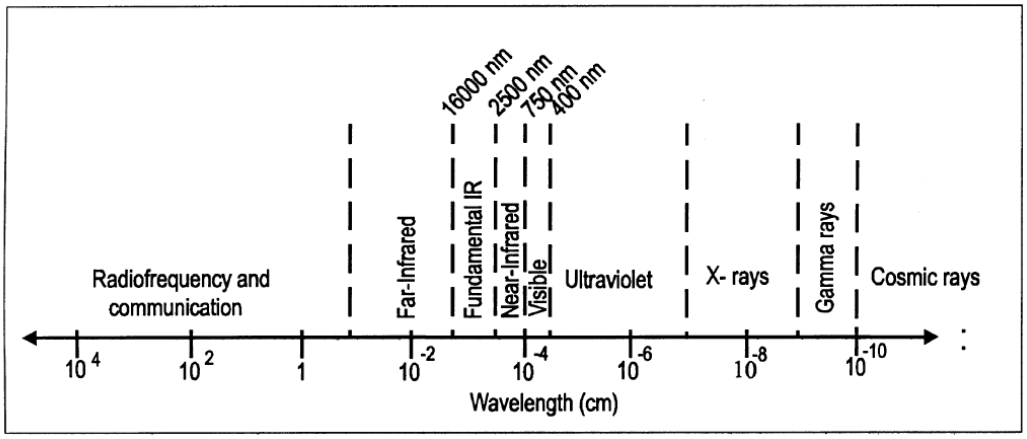


Figure 2.3. The electromagnetic spectrum showing the near infrared region

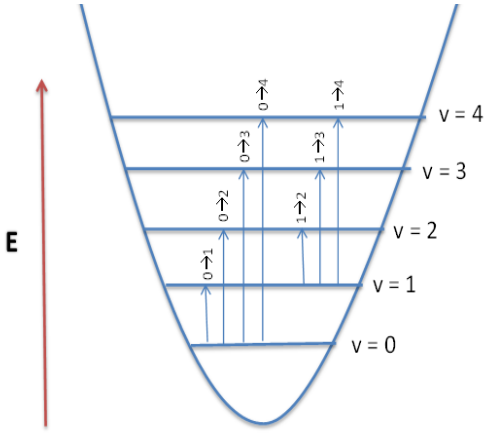


Figure 2.4. Potential energy diagram for a vibrating diatomic molecule

The overtones and combination bands of O-H in water, N-H in proteins, and C-H in carbohydrates readily absorb light in the NIR region (Figure 2.5). For example, the signature for C-H is repeated in an overtone and combination sequence a total of 8 times, with each overtone band appearing further down the spectrum and exhibiting a lower intensity than the preceding band (Osborne 2000). Tables of peak absorption and chemical assignments attributed to those peak absorptions can be found in Williams and Norris (1987), and Osborne et al. (1993). Changes in temperature, interacting molecules, and hydrogen bonding can shift peak location within a spectrum (Lestander, 2003).

The IR region is very useful for the detection of molecules; however, the radiation mainly interacts with the surface of the samples with little interior penetration. The NIR region is more applicable for feed or fecal analysis as the radiation penetrates into the sample (up to 2 cm as opposed to 1 mm for IR) making it more likely to accurately predict the nutrient content of a feed or fecal sample. It is the lower quantum efficiency of overtone and combination vibrations as opposed to fundamental vibrations that gives NIR an advantage over IR (Bokobza, 1998).

2.9 Near Infrared Spectroscopy

In the 1960's, it was discovered that NIRS could be used to predict the moisture content of seeds (Ben-Gera and Norris 1968), and later the protein content in whole grains (Williams et al. 1985). Instead of segregating samples based on N content using the Kjeldahl method, NIRS could be used to predict the N content of grains upon delivery to the grain terminal, reducing labour costs and time required to assess grain quality (Roberts et al. 2004). Eventually, the work by Williams (1975) and Hunt et al. (1977) led to the adoption of NIRS as an official protein testing method for wheat marketing in Canada and the USA. To date, a variety of different grains can be successfully characterized for moisture and chemical constituents including protein, oil, various fibre fractions and structural and non structural carbohydrates using NIRS (Norris and Hart 1965; Ben-Gera and Norris 1968; Campbell et al. 1997; Pazdernik et al. 1997; Velasco et al. 1998). In addition to predicting whole grain constituents, NIRS has been widely applied in the agricultural industry for predicting forage quality (Foley 1998) and the chemical composition of manure (Chen et al. 2013).

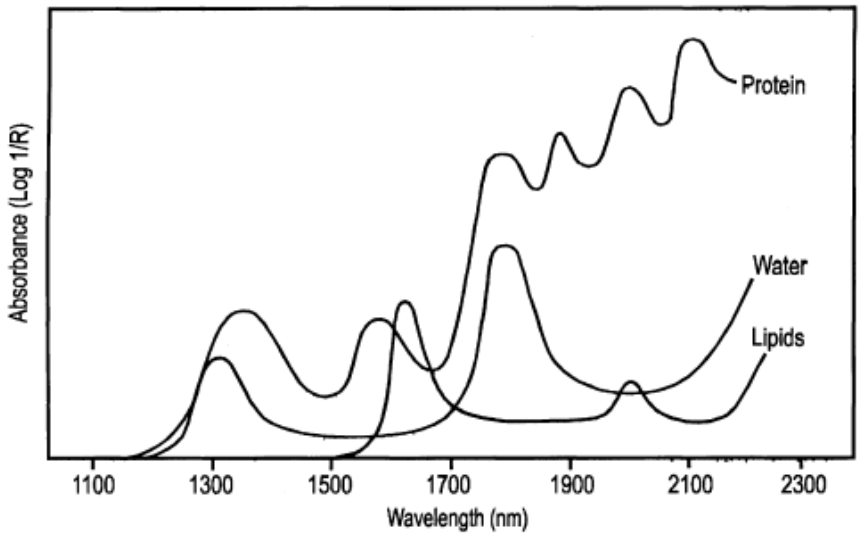


Figure 2.5. Broad and overlapping peaks attributable to different constituent characteristic of the near infrared reflectance spectrum (adapted from Osborne et al. 1993; Givens et al. 1997; Foley et al. 1998)

2.9.1 NIRS Instrumentation

An NIR spectrometer consists of a light source, a means of selecting particular wavelengths (nm), an area for placing the sample, a detector for collecting the reflected radiation, and a computer to process collected signals and data (Figure 2.6). Transmission spectroscopy has the detector positioned behind a sample, and is mostly used for gases or liquids; however, and NIRS measurements on solids or suspensions of solids, are usually made in the diffuse reflection mode. Once a sample is irradiated, the incoming radiation is absorbed and reflected (on the surface and within the sample), and that portion of the diffuse reflected radiation that returns to the detector is measured.

Sources of NIR radiation include tungsten halogen lamps or xenon gas plasma heated to at least 2500 K. Light emitting diodes (LEDs) are also used. Sample cups typically vary from 20 to 55 mm in diameter but can also be larger cups that can hold 50 to 200 g of sample. The larger cups can be used with whole grains and fresh forage, but in most applications samples are dried and ground and measured within smaller cups.

The most common detectors are lead sulphide (PbS), silicon, and indium-gallium arsenide (InGaAs), all of which cover a different spectral range. More than one detector in an instrument must be used if the user wishes to cover a broader spectral range than what is delivered by a single instrument. Early instruments were fixed filter, using selected wavelength bands for moisture, protein, and fiber fractions in wheat and forage (Norris et al. 1976). Later scanning instruments became available permitting the full spectrum (750 to 2500 nm) to be collected over a time interval. These are referred to as grating monochromators and are the most frequently used instruments. The grating mechanism configures light into the appropriate wavelengths. In our studies, a SpectraStar Near-Infrared analyzer (2400 model) Top Window Series (Unity Scientific, Conn, USA) with a grating monochromator was used with an InGaAs detector, covering the spectral range between 1200 and 2400 nm measuring light absorption in 1 nm intervals.

2.9.2 Using NIRS Technology

Near infrared spectroscopy is known for its cost-effectiveness, speed, simplicity, and precision. It is however, a secondary technology that must be calibrated using a reference method, making its accuracy only as good as the reference method that is used. The analysis of chemical constituents in plant and animal tissues is an integral part of agricultural

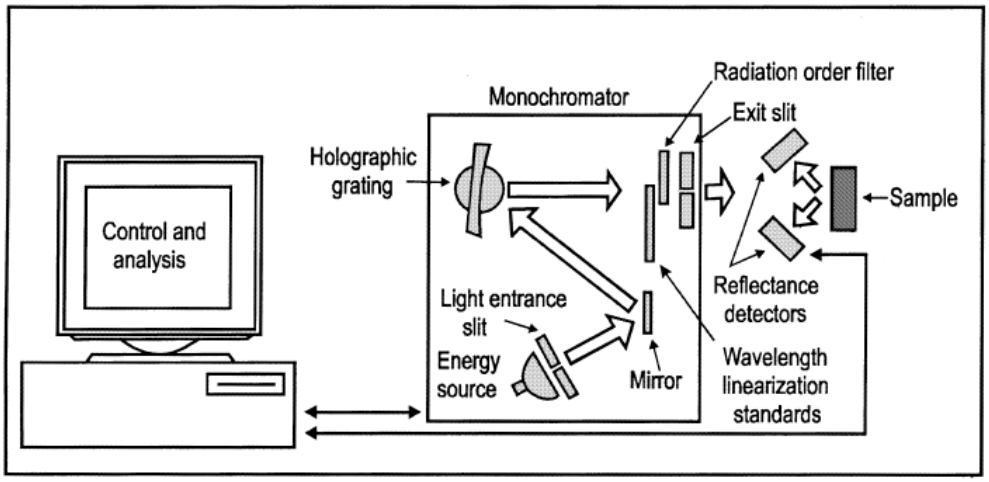


Figure 2.6. Components of a monochromator based near infrared spectrophotometer (derived from Foley et al. 1998)

studies, but wet chemistry methods are expensive, time-consuming and required skilled technicians to complete. The initial process of developing calibration equations for NIRS requires laboratory analysis, but once calibrations are developed, NIRS is capable of the simultaneous estimation of multiple constituents within a sample. In most instances, reproducibility of sample analysis is equal to or at times better than with wet chemistry and in most cases it is non-destructive with little need for sample preparation. Consequently, the sample can be recovered after analysis and used for other purposes.

Near infrared spectroscopy has the capability of predicting the chemical composition of unknown samples of interest using statistical procedures, most commonly multivariate linear regression. A multivariate statistical model is developed which describes the relationship between the NIR spectral absorbance (or transmittance) and the chemical constituents of interest using Beer's Law (Shenk and Westerhous, 1993). The statistical model is then used to predict the composition of unknown samples that belong to the same population. A typical multivariate calibration equation is illustrated below:

$$Y = B_0 + \Sigma B_i (A_i) + \varepsilon \qquad (2.1)$$

Where Y is the concentration of absorber, B_0 is the model intercept (Y value when A_i are 0), Σ is the sum of wavelengths measured from 1 $\rightarrow$ n, B_i is the regression coefficient for the absorbance values at the ith wavelength and is equal to the change in concentration divided by the change in absorbance, n is the total number of wavelengths used for a calibration model, and ε is the experimental model error. The mathematical basis of NIRS calibrations has been described in detail by Martens and Naes (1987).

2.9.3 Sample Selection

Samples used in the development of the calibration model are referred to as a calibration or reference set. When formulating a calibration model, it is always important to use samples that are representative of the target population. Sample type and physical form, chemical composition, as well as environment and seasonal variations must be taken into consideration. If these stipulations are met, any samples that still do not belong to the majority of the sample population are classified as outliers due to differences in spectral characteristics or an error in the

reference method (Pasquini 2003). Accuracy of the reference method is extremely important, as errors in the reference method will be embedded within the calibration equation and all subsequent analysis. Ideally, a calibration model should contain the minimum number of samples that uniformly span the concentration range of interest. Proper sample selection can reduce the number of samples in the calibration set required for wet chemistry, and various sample selection techniques exist.

A common method, Center and Select used in WinISI 1.50 (Infrasoft International, Silver Spring, MD) by Shenk and Westerhaus (1991) uses two algorithms, CENTER and SELECT, for defining the population and selecting samples for calibration. The algorithms establish the variance in the population of samples using a Mahalanobis distance (also known as the H statistic in WinISI and GH in UCAL) from the mean. The Center option in WinISI 1.50 as described by Murray and Cowe (2004) was used to compute principal component scores and Mahalanobis distances for each spectra. Samples are ranked on the basis of their H statistic value from those closest to the population mean to those that were outliers. Samples with an H statistic > 3.0 are defined as global (GH) outliers and may be eliminated as a result of human error due to scanning and flawed or abnormal spectra relative to others. The Select option in WinISI is an algorithm that computes the distance from one sample to another. One sample is chosen to represent a group of samples with similar spectra, and the selected samples that represent the rest of the population are used for reference analysis and for constructing the calibration equation. This ensures that redundant spectra are not used and the error in the calibration set is reduced. When a previously developed calibration is expanded, the same technique is applied, and only selected spectral outliers are added to expand the calibration.

2.9.4 Transformation of Spectral Data

Sample particle size can have a major effect on the NIR spectrum, and differences in particle size alter the effective path length of the sample and the angle of reflected light. Differences in particle size among samples is minimized in order to improve the quality of spectral data before regression models are fitted. The mathematical theory of pre-processing data techniques can be reviewed in several publications in detail (Savitzky and Golay 1964; Geladi et al. 1985; Massart et al. 1988; Barnes et al. 1989; Næs et al. 2002; Rinnan et al. 2009). The most common

techniques include multiplicative scatter correction (MSC), standard normal variate (SNV), detrending, derivatives, and smoothing.

The MSC and SNV procedures use algorithms, followed by detrending, to remove differences in particle size between samples by distinguishing between variations in light scattering and path length from constituent absorption in the spectra (Martens et al. 1983; Geladi et al. 1985; Barnes et al. 1989). Derivatization is used to enhance peak distinction by differentiating and resolving peaks within a complex spectrum. Sharp bands are enhanced at the expense of broad ones, and this may allow for the selection of suitable peaks even when broad bands obscure peak resolution. Derivatives are applied over a selected spectral segment (gap) as a moving window along the entire spectrum, and several combinations of gap sizes (nm) are applied. Smoothing of the spectra is performed prior to derivatization so as to decrease the signal to noise ratio (S/N) and is equivalent to averaging the absorption over a given number of spectral data points (nm). The order of the derivative (usually 1^{st} or 2^{nd}), the gap size, and first smooth over spectral data points are selected and reported with calibration statistics (e.g. 1, 4, 4).

2.9.5 Chemometrics and Multivariate Calibration Techniques

Chemometrics are tools that are applied to extract chemical information from spectra (Pasquini 2003). For NIRS, this is quite challenging because of overtone and combination bands, and the absorbance at a given wavelength contributing to more than one property. Multivariate regression analysis is used in calibration development to describe the relationship between the concentration of an analyte and its spectrophotometric response.

Partial least square (PLS) regression establishes a linear relationship between spectral data and the chemical constituent of interest (Wold et al. 2001). Principle component analysis is a statistica technique that uses orthogonal transformations to reduce a large dataset of correlated variables to linear uncorrelated variables. These linear vactors are orthogonal to eachother and are termed principle components (PCs). This approach is usually applied first to find clustering and similarities within a dataset. Using PLS, the correlation between the spectral data and the component concentration is considered while extracting the PCs. The PCs directly refer to the given component because they are extracted based on the covariance between the spectra and the sample constituent values (Balabin et al. 2007). The “best” calibration equation from which the

constituent of interest can be predicted is selected by statistical evaluations describing the difference between the actual and predicted values at each step of the regression (Osborne 2000).

2.9.6 Statistical Evaluation

Statistics are used to evaluate the performance of an NIRS calibration model. The important terms that should be included when reporting NIRS results are shown in Table 2.3, and these include calibration and validation statistics.

The laboratory standard error (LES) is a good indication of the error of the reference data. The number of samples, outliers, minimum, mean, maximum and standard deviation for a particular constituent in both calibration and validation sets must be included to provide information regarding the distribution of chemistry observed in the samples set of interest. A larger range in chemistry with a uniform distribution will produce a calibration model that is more capable of predicting the constituents within a greater range of samples.

The accuracy of a calibration model in terms of its ability to predict the properties of the target population can best be performed by cross-validation (using samples removed from the calibration (R^2_{CV}, *SECV*)) or by external validation (using a set of independent samples (R^2P, SEP); Bokobza, 1998; Cen & He, 2007). The coefficients of determination (R^2, R^2_{CV}, and R^2P) show the proportion of variance in the reference data explained by the variance in spectral data. The R^2 is generated by using the proposed calibration equation to predict the samples that were used to derive the equation (Williams and Norris 2001). The standard error of calibration (*SEC*) represents the difference between the predicted and the reference values when the equation was developed from the calibration data set (R^2). The error of cross-validation (*SECV*) represents the variability in the difference between predicted and reference values when each equation is applied sequentially to subsets of data from the calibration set (Landau et al. 2006). A calibration model is calculated each time using the remaining samples, and calibration statistics are averaged once each subset has been removed. An NIRS calibration is ideal if the error associated with the prediction of chemical composition is of similar order to that obtained through wet chemistry. The standard error of prediction (*SEP*) represents the variability in the difference between the predicted and reference values when the equation is applied to an external validation data set which was not used in the calibration (Landau et al. 2006). This approach is the most statistically robust at assessing the accuracy of the calibration model (Williams and Norris 2001).

The RPD_C for calibration and cross-validation (RPD_{CV}; Dardenne 2010) are calculated by dividing the SD of the reference values used in the prediction by the SEP, enabling the SEP in terms of the SD of the reference data to be evaluated. The RMSEP also measures the efficiency of a calibration and considers bias error. The residual standard deviation (RSD) indicates the error after bias and slope correction, and should also be reported (Dardenne 2010). These terms indicate the difference between the reference and NIR spectral data and should be as close to zero as possible (Williams and Norris 2001) and account for adjustments made to the regression line from a perfect 45º angle when the relationship is 1:1 (when y=x).

2.9.7 NIRS for Feed Analysis

The work by Williams (1975) and Hunt et al. (1977) led to the first large scale commercial application of NIRS, as an official protein testing method for wheat marketing in Canada and the USA. The use of NIRS analysis in feed production has continued to expand and calibrations are continually being developed and updated for moisture, fat, protein, starch and fiber content of all feed grain species, distiller's grains, grain screenings, and many other by-products. Real-time continuous measurements of whole grains, and by-product feeds upon receipt allow feed mills to monitor the quality of their incoming inventory, intermediate products (during pelleting or mixing), as well as finished products. The technology has significantly lowered feed costs by increasing the precision by which livestock diets are formulated and improved the ability of the industry to predict the feed value of byproduct feeds. Cooperation among NIRS researchers, technicians, and feed mill operators, has enabled current calibrations to be continuously improved by broadening calibration equations to consider more diverse sample populations.

To date prediction of mineral concentration in feedstuffs using NIRS has produced inconsistent results and poor calibration statistics. Due to the chemical nature of heavy metal atoms, and the wavelength region specificity of NIRS, only minerals that form complexes with organic compounds (i.e. Ca, P, Mg, and K) have shown some propensity to be measured by NIRS (Givens and Deaville, 1999).

Feed NIRS analysis is also used for predicting diet quality and DMI based on estimating the nutrient composition of the forage grazed by ruminants. In 1976, Norris et al. demonstrated that forage quality and composition could be predicted by NIRS, including the DMI of sheep fitted with esophageal fistulae. Prediction of voluntary DMI of forages from NIR spectra was possible

based on the fact that those fibre constituents in forage that influenced voluntary intake could be predicted (Gibens and Deville 1999). Subsequently, there were a large number of reports on the use of NIRS to predict various aspects of forage quality and composition. Most of these studies focused on chemical fractions such as CP and NDF (Murray 1993; Shenk et al. 1992), but the DM, OM, ADF, lignin, and starch content of forages and total mixed rations (TMR), were also accurately and precisely predicted by NIRS (Mentink et al. 2006). Many studies have also focused on using NIRS to predict the in vivo digestibility (OMD, DMD) of mixed forage (Lindgren 1983; Coates 2004), grasses (Decruyenaere et al. 2009; Coates 2004) and mixed forage and concentrate diets (Givens et al. 1997) in grazing ruminants. There have also been reports where fecal NIRS was used to predict the digestible energy (DE) content of feeds of non-ruminant species, including the prediction of the digestible energy (DE) content of barley for swine (Zijlstra et al. 2011). Unlike the in-line NIRS which use whole feed samples, the studies reported above were primarily conducted in research settings and used dried-ground feed samples for both calibrations and measurement.

Table 2.3. Important statistics to evaluate the efficiency of a calibration (Dardenne 2010)

Statistics of Calibration	Statistics of Validation
Parameter	Parameter
Units	Units
SEL	
N	N
Min	Min
Mean	Mean
Max	Max
SD	SD
R2	R^2P
SEC	SEP
R^2CV	RMSEP
SECV	RSD
Number of terms	Bias
RPDc	Intercept
RPDcv	Slope
Segments (LOO)	Ave. GH
Wavelength range/step	Ave. NH
Pretreatments	
Regression Method	

SEL=standard error of laboratory; N=number of samples; Min=minimum; Max=maximum; SD=standard deviation; SEC=standard error of calibration; R2=coefficient of determination for calibration; r2=coefficient of determination for validation; RMSEP=root mean square error of prediction; SEP=standard error of prediction; SECV=standard error of cross-validation; R2CV=coefficient of determination for cross-validation; RSD=residual standard deviation; RPDC= Ratio of standard error of Prediction Validation to standard Deviation for calibration; RPDCV= Ratio of standard error of Prediction Validation to standard Deviation for cross-validation; LOO=leave one out (cross-validation)

2.9.8 NIRS for Fecal Analysis

The use of NIRS to predict the chemical composition of feces has a number of important applications. Firstly, since NIRS is capable of predicting many constituents, it has been used to simultaneously predict the nutrient content of forages consumed during grazing (Brooks et al. 1984; Boval et al. 2004; Landau et al. 2006). Secondly, NIRS has been used to predict the composition of feces and manure to enable its distribution to meet the nutrient needs of crops (Chen at al. 2013). Livestock manure contains a variety of chemical constituents that are highly valuable as a fertilizer, including organic matter, N, P, K, and microminerals (Chen et al. 2013; Araji et al. 2001). Thirdly, NIRS has been used to gauge species, gender, and reproductive status of herbivores through compositional analysis of their feces (Dixon and Coates 2009).

The ability to predict the attributes of diet (OMD, DMD, DMI, or nutrients) from fecal samples is possible based on the principle that the spectral information generated from NIR of feces reflects the original composition of the diet (Dixon and Coates, 2009). In herbivores fed high forage diets, the NIR spectra of the diet closely resembles that of the feces, but as the proportion of concentrate in the diet increases more DM is digested and the amount of microbial matter in the feces increases, altering spectral signatures. Sampling of feces has a distinct advantage under circumstances where animals cannot be confined for sampling, or when it is difficult to characterize the nature of the diet that is being consumed. Researchers that have examined the ability of NIRS to predict fecal nutrient profiles have focused on predicting dietary CP, ADF, NDF, DMD and OMD (Lyons and Stuth 1992; Boval et al. 2004; Fanchone et al. 2007; 2009) across a variety of species including; cattle, sheep, goats, elk, deer, kangaroos, and donkeys with a moderate degrees of success ($R^2 = 0.72 – 0.85$) (Dixon and Coates 2009). Digestibility calibrations were developed either using *in situ* nylon bags or *in vivo* procedures in these studies, or using esophageal fistulae to determine actual intake. Fecal NIRS calibrations for indigestible external and internal dietary markers in feces, such as polyethylene glycol (PEG), chromic oxide, *N*-alkanes, and lignin have also been developed (Dixon and Coates 2009).

A constraint in the use of fecal NIR to predict diet attributes lies in that the calibrations have been developed under a limited range of circumstances, and testing the calibrations in new circumstances is difficult animals in pasture and not in research settings.

2.9.9 Using NIRS to Predict Apparent Total Tract Digestibility of Starch

Until recently, using NIRS to predict fecal constituents for purposes other than in grazing systems or for manure management has received little attention. As animal production becomes more intensive, and with the need to increase the feed value of grain in the diet, strategies that would allow for the fine tuning of grain processing methods could result in substantial improvements in starch utilization. NIRS has the potential to rapidly predict fecal starch in an inexpensive manner, an estimate that may correlate with starch digestibility. Fredin et al. (2014) conducted a study predicting aTTD of starch from fecal starch in lactating dairy cattle, and used the samples to develop an NIRS calibration for predicting fecal starch in dried ground fecal samples. The calibration displayed good predictive accuracy when fecal starch was between 0-5% (R^2 = 0.94 *SEC* = 0.45) since a large majority of the reference values were within these limits. However, when fecal starch exceeded 5%, the predictive accuracy of NIRS declined implying that a broader range of samples with higher fecal starch concentrations is required to develop calibration equations that would improve the ability of NIRS to predict fecal starch and consequently aTTD of starch when it deviates from 95% and 0-5% fecal starch (Fredin et al. 2014).

Allen (2010) also attempted to predict fecal starch, in addition to fecal DM, CP, NDF, and ADF, in fresh fecal samples collected from Holstein cattle fed a high grain ration. A portable hand-held NIRS was used to predict nutrient composition in fresh feces of feedlot Holstein cattle. All NIRS calibrations displayed poor validation statistics likely due to high moisture content and narrow range of samples examined. Since hydrogen is a very strong absorber of light, it very easy to determine the moisture content of a sample using NIRS. However, hydrogen can mask absorption peaks of other organic constituents. At high moisture levels pore spaces become filled with water rather than air and peaks in NIRS spectra are primarily due to moisture as opposed to chemical constituents in the sample. Therefore, although samples for NIRS analysis require little preparation, drying (oven or microwave) to remove this interference from hydrogen is recommended when the moisture content of samples exceeds 70% (Duckworth 2004). Portable or hand-held NIRS instruments have been used for obtaining instant results; however their wavelength range is usually limited.

The ability to accurately measure fecal starch in the feces of feedlot cattle could be a significant asset for feedlot cattle producers. High levels of fecal starch could be indicative of

reduced aTTD of starch, and reflect a substantial energetic loss to the animal, resulting in reductions in ADG and feed efficiency.

2.9.10 Using NIRS to Predict Performace in Cattle

Currently most reports for predicting performance of cattle using NIRS are restricted to DMI. Accurate predictions of DMI have been developed (Lyons 1990; Coates 1998; Garnsworthy and Unal 2004) based on spectral changes in both the digestible and indigestible components of feces collected from individual animals. Lyons (1990) and Coates (1998) developed equations to predict DMI directly using NIRS spectra of fecal samples, with moderate success [R^2 = 0.67 and 0.79, respectively]. Garnsworthy and Unal (2004) fed lactating dairy cows a variety of diets ranging from 100% grass hay; to a 60:40 mixture of grass silage and concentrate, and developed NIRS calibrations using fecal spectra to predict DMI (R^2 = 0.97). Huntington et al. (2011) predicted DMI over 4 consecutive years in growing bulls and steers fed a diet of 79% corn silage DM, with R^2 ranging between 0.53-0.71, depending on the year. All of these studies developed calibrations that were specific to a single diet with none being validated using an external dataset.

There have been numerous attempts to predict animal gender and physiological status using fecal NIRS; however, it appears that such differences between groups were confounded with diet (Dixon and Coates 2009). For the same reason, predicting performace such as ADG and G:F may prove to be difficult since ADG is assoicated with diet, as well as DMI. Other methods to predict performance have been applied indirectly. For example, Tolleson and Schaffer (2014) predicted diet quality (CP and digestible OM) using the spectra of the feces, and coupled with decision support software and nutritional balance calculations, monitored the nutritional status and growth performance of cattle on pasture.

2.10 Summary

As the livestock industry continues to intensify, it is becoming more and more important to reduce feed costs, reduce nutrient excretion, and increase feed efficiency without compromising growth performance. Certain farms choose to use precision feeding, which includes weighing animals more often, minimizing size and variation and age of cattle within a group, and ensuring

adequate bunk space, and other practices (PennState Extension, 2016). Collecting fecal samples as an additional tool to monitor nutrient excretion seems only rational. Not only are fecal samples readily available, and do not require contact with the animal, they contain a vast amount of information.

The advent of NIRS now allows us to predict the chemical characteristics of feces, and of the diet ingested indirectly, without requiring intensive laboratory analysis. However for such a technique to be applied, it will need to be simple to use, accurate, and standardized for regular and consistent use.

2.11 Hypothesis and Objectives

Fecal NIRS can be used as a tool for predicting fecal nutrient concentrations and their digestibilities in feedlot cattle, which can then be applied to predict growth performance in commercial feedlots.

The objectives of this research include the following:

1. Develop a broad based fecal near infrared spectroscopy calibration capable of predicting fecal OM, starch, N, NDF, ADF, ADL, EE, and GE contents and apparent total tract digestibility of DM, OM, starch, CP, NDF, ADF, and GE.
2. Use NIRS to evaluate diurnal variation in fecal constituent excretion in cattle fed backgrounding and finishing diets once or twice per day
3. Compare sampling from the pen floor versus the rectum from feedlot cattle. Determine if changes in grain source, processing, and silage inclusion level result in measurable differences in NIRS predicted fecal nutrient composition and aTTD collected over a finishing period, and if fecal measured parameters be used as indicators of increased growth performance
4. Evaluate performance - predicting capabilities of fecal and feed NIRS for cattle fed grain screening pellets
5. Examine fecal nutrient losses in southern Alberta commercial feedlots across a variety of finishing diets, and determine if fecal measured parameters can be used to predict feedlot performance in groups of cattle.

CHAPTER 3

3.0 DEVELOPMENT OF NIRS CALIBRATIONS TO ESTIMATE FECAL COMPOSITION AND NUTRIENT DIGESTIBILITY IN BEEF CATTLE[1]

3.1 Introduction

Traditional laboratory methods for determining the chemical composition of feed or feces for accurate estimation of diet digestibility are impractical in commercial feedlots where results are required immediately for feeding management decisions. Near infrared spectroscopy (NIRS) is a rapid alternative to wet chemistry for predicting the composition of feeds and feces and can be used to estimate the digestibility of diets fed to ruminants (Dixon and Coates 2009).

Traditional methods of estimating diet digestibility require data on feed intake, diet and fecal composition and excretion. The success of using NIRS with feces to predict digestibility relies on the principle that there is sufficient spectral information in feces to describe the composition of the diet ingested, despite the process of removal and addition of organic compounds through digestion and endogenous secretions (Dixon and Coates 2009). Near infrared spectroscopy has been employed to predict the chemical composition of manure (Chen et al. 2013), the nutrient intake of cattle grazing pastures (Dixon and Coates 2009), and the organic matter (OM) digestibility of a variety of forage (Coleman et al. 1995; Boval et al. 2004), and concentrate diets (de la Roza et al. 2002; Garnsworthy and Unal 2004). Calibrations have also been developed for rapid measurement of starch concentration in feces and to predict starch digestibility by dairy cattle (Fredin et al. 2014). Application of NIRS in a similar manner in feedlot cattle production could also have considerable value as levels of fecal starch are highly correlated to starch digestibility (Zinn et al. 2002).

The objective of this research was to generate diverse NIRS calibration models for predicting fecal composition and apparent total tract digestibility (aTTD) of nutrients by feedlot cattle. Digestibility of a range of feedlot diets was estimated using total collection procedures and the data was used to develop NIRS calibrations. The robustness of these calibrations was

[1]A version of this chapter has been published. Jancewicz, L.J., M. L. Swift, G. B. Penner, K. A. Beauchemin, K. M. Koenig, G. E. Chibisa, M. L. He, J. J. McKinnon, W.-Z. Yang, and T. A. McAllister. 2016. Development of NIRS calibrations to estimate fecal composition and nutrient digestibility in beef cattle. Can. J. Anim. Sci. (10.1139/CJAS-2016-0107).

subsequently assessed by collecting feces from feedlot cattle fed in research and commercial production settings.

3.2 Materials and Methods

3.2.1 Origin of Samples

Fecal samples for the development of NIRS calibrations to predict fecal composition (Table 3.1) and apparent total tract digestibility (aTTD; Table 3.2) were obtained from digestibility studies where cattle were fed diets differing in grain type, grain processing, forage type and proportion, and level and type of by-product. Studies were conducted at the University of Saskatchewan, the Lethbridge Research and Development Centre, and commercial feedlots around Lethbridge, Alberta over a 7 year period. Calibrations to estimate fecal composition (Table 3.1) and aTTD (Table 3.2) were validated using samples collected from growth performance feedlot studies.

3.2.2 NIRS Calibrations for Chemical Composition

3.2.2.1 Collection of samples

For digestibility experiments, cattle were housed in individual stalls and samples were collected (≥ 250 g wet weight) either from the rectum or off a clean floor shortly after defecation. Samples were collected from individual animals, and pooled across days (4 to 5 d) within each collection period during the metabolism study. For feedlot experiments, cattle were housed in group pens outdoors, and subsamples from 4 fresh fecal pats were composited equally by wet weight (400 g). For fecal composition, feedlot samples were collected over 1 d, and for aTTD validations, samples were collected at 3 or 4 wk intervals over the feeding period. In addition, 10 fecal samples were collected in a similar manner from one feedlot, and spiked with increasing levels of whole barley grain to expand the range of samples that represented the levels of starch (≥19% of fecal DM) that occur in feces from cattle fed inadequately processed grain (Jancewicz, unpublished results).

All fecal samples were dried at 55ºC and ground through a 1 mm screen using a Wiley Mill (Thomas Scientific, Swedesboro, NJ) or through a 0.75 mm screen using a Retsch grinder (Verder Scientific, Inc, Newton, PA) depending on the location that the original study was

conducted. Ground samples were then packed into quartz ring cups (25 g) and scanned twice (two repacks; where the second scan was a completely different sub-sample from the first) using a SpectraStar Near-Infrared analyzer 2400 RTW (Unity Scientific, Brookfield, CT). Spectral information was collected at wavelengths between 1200 and 2400 nm in 1 nm increments. Duplicate spectra of each sample were averaged. To account for small differences in particle size as a result of using different grinders, standard scatter corrections were applied to the spectra as described below.

3.2.2.2 Reference analysis

Reference analysis was performed first using samples collected from digestibility studies with samples from the feedlots measured after the initial calibration was developed. Fecal samples were analyzed for analytical DM [Association of Official Analytical Chemists (AOAC) 2005, method 930.15] and OM (AOAC 2005, method 942.05). Samples were further ground using a ball mill (Mixer Mill MM2000, Retsch, Haan, Germany) for determination of starch and nitrogen (N). Starch concentration in the compiled studies was determined using two methods depending on the facility that the analyses were conducted; a Megazyme kit (Megazyme International Ireland, Wicklow, Ireland) as described by Rode et al. (1999), or a glucoamylase enzymatic reaction followed by oxidation in a YSI 2700 Biochemistry Analyzer (Yellow Springs, OH) with hydrogen peroxide detection on a platinum electrode surface. Nitrogen was estimated by flash combustion, followed by gas chromatography and thermal conductivity detection (Carlo Erba Instruments, Milan, Italy) or a LECO N analyzer (Joseph, MI) as described by Watson et al. 2003. Neutral detergent fibre and ADF were determined sequentially using an ANKOM fiber analyzer (ANKOM technology Corp. Fairport, NY) or the traditional Van Soest et al. (1991) procedure using filtering glass crucibles. Heat stable α-amylase and sodium sulphite were included during the NDF analysis. Acid detergent lignin (ADL) was extracted using 72% sulphuric acid after the ADF procedure, followed by ashing at 550º C (Van Soest et al. 1991). Crude fat (CF; AOAC 2005, method 2003.05) was extracted by Soxtec HT6 System (Foss, Eden Prairie, MN) using anhydrous diethyl ether, followed by drying.

Table 3.1. Dietary ingredient composition and range in fecal chemical composition of organic matter (OM), starch, nitrogen (N), neutral detergent fiber (NDF), acid detergent fiber (ADF), acid detergent lignin (ADL), and crude fat (CF) from digestibility and feedlot studies with cattle whose fecal samples were used for development of fecal near infrared spectroscopy calibrations.

Dietary ingredients : Grain, Forage, other[z,x] (% of diet DM)	n (nd)	Range in composition (% fecal DM)						
		OM	Starch	N	NDF	ADF	ADL	CF
Digestibility Dataset								
DRC (80), barley silage (10), none	19(1)	88-93	4.3-24	2.4-3.5	30-47	15-24	-	-
DRB (0, 35), barley silage (55), DDGS (37.6, 40)	32(4)	83-89	-	2.4-3.5	49-63	38-44	-	-
DRB (47, 87), barley silage (8), DDGS (37.6, 40)	32(4)	84-90	0.7-14	2.4-3.5	42-58	22-31	-	-
WSC/SRC (0, 43), none, oat hulls (46-49)	13(4)	89-92	0.3-5.0	0.8-1.4	69-82	32-40	-	0.9-2.5
TRB (43-86), barley silage (9), DDGS (20, 40)	137(4)	83-93	3.6-28	2.1-3.9	39-57	19-35	-	1.4-2.4
DRB (44), barley silage (44), none	144(1)	81-85	0.7-4.0	2.3-2.9	55-60	37-40	11-12	1.2-2.8
DRB (80), barley silage (9), none	144(1)	86-90	2.4-11	2.6-3.1	46-61	25-33	7.4-10	1.8-2.7
DRB (68-80), barley silage (0-12), DDGS (15)	32(4)	87-91	2.4-10	2.5-3.5	46-57	18-25	-	-
DRB (0-32), barley silage (36), grass hay (24), wheat bran (0-32)	24(4)	-	-	1.8-2.1	56-67	37-42	-	-
DRB (47-87), barley silage (8), DDGS (20-40)	24(4)	-	-	2.3-4.2	-	-	-	-
none or DRB[y], variety (55-100), variety	41(5)	77-87	0.2-3.5	1.9-2.6	55-63	39-49	10-13	1.3-2.1
Feedlot Dataset								
DRB (56-76), barley silage (9), GSP(0, 20) DDGS(10)	42(3)	82-90	3.7-12	2.2-2.8	47-58	25-34	4.3-8.1	1.3-2.4
DRB (57-87), barley silage (10), DDGS (0-30)	18(4)	-	2.7-12	1.8-2.9	54-65	37-48	-	-
DRB/DRW (89), barley silage (6), none	92(2)	65-85	3.5-33	2.2-2.5	31-56	15-33	4.3-7.7	0.9-2.1
DRB (68-80), barley silage (0-12), DDGS (15)	8 (4)	72-90	3.6-12	2.2-2.5	49-56	24-32	3.7-7.2	0.3-1.8
DRB, variety (7-27), variety	20(4)	88-96	19-53	1.9-2.4	22-41	8.5-22	0.7-3.1	1.5-2.2
DRB/TRB/DRW/TRW variety (7-27), variety	288(24)	70-88	0.7-24	2.0-2.4	20-38	33-61	3.8-5.4	0.4-0.8

Abbreviations :; DRC = dry rolled corn; DRB = dry rolled barely; DDGS = dried distillers grains with solubles (wheat or corn); WSC = whole shelled corn; SRC = steam rolled corn; TRB = temper rolled barley; DRW = dry rolled wheat; TRW = temper rolled wheat; GSP = grain screening pellets; variety = indicates samples obtained from cattle fed a variety of different diets and their type and proportions are not listed; n=number of samples; nd= number of diets of different chemical composition.

[z] List of references in descending order; Digestibility dataset: Vyas et al. 2014; Hunerberg et al. 2013a; Hunerberg et al. 2013b; Davies et al. 2012; Koenig et al, unpublished; Jancewicz et al. 2016; Jancewicz et al. 2016; Chibisa et al. unpublished; Friedt et al. 2014; McKinnon and Walker 2008; Feedlot dataset: Jancewicz et al. unpublished; He et al. 2015; Moya et al. 2015; Koenig et al unpublished; Jancewicz et al. unpublished; Jancewicz et al. unpublished; Jancewicz et al. unpublished. [x]Does not include feed additives or vitamin and mineral or other supplement.

Table 3.2. Dietary ingredient composition and range in digestibility of dry matter (DM), organic matter (OM), starch, crude protein (CP), neutral detergent fiber (NDF), acid detergent fiber (ADF), and gross energy (GE) from digestibility studies with cattle whose fecal samples were used for development of fecal near infrared spectroscopy calibrations and parallel feedlot studies used for evaluation of calibrations.

Dietary ingredients : Grain, Forage, other[z,x] (% of diet DM)	n (nd)	Range in apparent total tract digestibility (% of intake)						
		DM	OM	Starch	CP	NDF	ADF	GE
Digestibility dataset								
DRC (80), barley silage (10), none	19(1)	70-84	71-86	87-99	61-75	32-57	28-53	68-82
DRB (0, 35), barley silage (55), DDGS (37.6, 40)	32(4)	59-75	57-76	-	61-75	30-58	20-47	57-75
DRB (47, 87), barley silage (8), DDGS (37.6, 40)	32(4)	67-88	69-89	-	71-85	26-70	17-72	67-89
WS/SR C (0, 43), none, oat hulls (46-49)	13(4)	59-75	-	95-100	-	27-55	27-57	-
TRB (43-86), barley silage (9), DDGS (20, 40)	32(4)	67-84	69-85	83-98	66-84	38-71	6.1-63	67-84
DRB (44), barley silage (44), none	6(1)	73-78	76-79	98-99	-	50-57	40-49	74-78
DRB (80), barley silage (9), none	6(1)	76-82	81-87	94-98	-	63-71	44-52	76-82
DRB (68-80), barley silage (0-12), DDGS (15)	32(4)	71-90	72-91	94-100	63-87	34-76	8.5-68	-
Validation studies								-
DRB/DRW (89), barley silage (6), none (Study 1)	8(2)	70-73	-	92-94	59-64	49-52	38-39	-
DRB (68-80), barley silage (0-12), DDGS (15) (Study 2)	20(4)	79-86	81-87	97-99	76-82	59-69	40-54	-

Abbreviations :; DRC = dry rolled corn; DRB = dry rolled barely; DDGS = dried distillers grains with solubles (wheat or corn); WSC = whole shelled corn; SRC = steam rolled corn; TRB = temper rolled barley; DRW = dry rolled wheat; TRW = temper rolled wheat; GSP = grain screening pellets; variety = indicates samples obtained from cattle fed a variety of different diets and their type and proportions are not listed; n=number of samples; nd= number of diets of different chemical composition.

[z] List of references in descending order; Digestibility dataset: Vyas et al. 2014; Hunerberg et al. 2013a; Hunerberg et al. 2013b; Davies et al. 2012; Koenig et al, unpublished; Jancewicz et al. 2016; Jancewicz et al. 2016; Chibisa et al. unpublished; Validation dataset: Moya et al. 2015; Koenig et al unpublished. [x]Does not include feed additives or vitamin and mineral or other supplement.

3.2.2.3 Spectral Database

The full database contained spectra for 1110 fecal samples, representing 65 different diets and was divided into 642 spectra associated with the digestibility dataset, and 468 spectra in the feedlot dataset. The digestibility dataset contained the spectra with the corresponding reference analysis for constituents of interest including, OM, starch, N, NDF, ADF, ADL, or CF. However, not all studies had all analytical constituents of interest and as a result the number of samples contributing to the calibration varied among measured nutrients.

3.2.2.4 Fecal NIRS Calibration Development

Partial least squares regression (PLS) in UCal (Unity Scientific, 2010) was used to develop the calibrations. Two mathematical treatments were tested in the development of the calibrations: 1, 8, 8, 1 and 2, 8, 8, 1; where the first digit is the order of the derivative, the second is the gap over which the derivative was calculated, the third is the number of data points used in the running average for smoothing of derivative spectra, and the fourth is the number of data points over which the second smoothing is applied (ISI, 1999). Derivative spectra were used to emphasize small or large absorption peaks, and minimize overlapping peaks and baseline correction (Giese and French 1955). The standard normal variate (SNV) scatter correction was applied, along with the detrend (DT) function (Barnes et al. 1989).

The quality of calibrations was expressed by the coefficient of determination (R^2_{cal}), defined as the proportion of variability in the reference values accounted for by the regression equation (linearity and precision), and by the standard error of calibration (*SEC*) defined as the variability in the difference between reference and predicted values (Landau et al. 2015). As per Landau et al. (2015), the most important estimations of calibration quality were using cross validation, in which a subset (determined by the number of total samples) of the calibration samples were randomly selected and used to validate calibrations calculated with the remaining samples. The optimum math treatment for each constituent was identified on the basis of the greatest coefficient of determination of cross validation (R^2_{CV}), and the minimum standard error of cross validation (*SECV*, calculated as the average *SEC* of every subset). An evaluation based on the *SECV* as an indicator of predictive accuracy may differ for each calibration as it depends on the prediction error that we deem acceptable, while considering the purpose for which the

predictions were to be used, and the probability of a prediction being within the designated error (Coates and Dixon 2011). Based on a normal Gaussian distribution, an *SECV* of 2.00 fecal OM % units (% of fecal DM) would indicate a prediction error of ≤ 2 OM % units for 67% of samples, and ≤ 4 OM % units for 95% of samples. For other constituents, where the units are much smaller such as N, an *SECV* of 0.2 fecal N % units was considered acceptable. For fecal constituents, a R^2_{CV} >0.90 was considered excellent, 0.80 < R^2_{CV} < 0.90, good, 0.70 < R^2_{CV} < 0.80, moderate; and R^2_{CV} < 0.70 poor.

Validation was performed by removing one study at a time to gage how each study affected the calibrations, and also to attempt to remove any large laboratory bias (not shown). The most robust calibrations were used to predict OM, starch, N, NDF, ADF, ADL, and CF in fecal samples collected from feedlots.

3.2.2.5 Sample Selection of Feedlot Dataset for Evaluation of Calibration

To remove repetitious spectra and reduce the large number of samples requiring reference analysis in the feedlot database, samples in the validation set were predicted using the initial NIRS calibration, and ranked from the highest to lowest for each important constituent (starch, NDF, ADF, and ADF, % of fecal DM). From a total of 468 samples, between 45 and 60 samples per constituent were selected to encompass the entire concentration range for all measured constituents, ensuring that at least one sample from every diet was represented in the dataset. Reference analysis was conducted on these samples as described above.

3.2.2.6 Evaluation of Initial Calibration and Expansion of Calibration

Regression standard errors of performance (*SEP*) were used as measures of predictive accuracy in the validation set. As per Coates and Dixon (2011), the coefficient of validation (R^2_{val}) was also used as a measure of linearity, with consideration for the large effect that differences in range of fecal constituents can have on R^2_{val} estimates. The slope and bias were reported, indicating the deviation from a 1:1 relationship, as well as the difference between actual and predicted NIRS values.

The degree that a predicted value matched its reference value was assessed by estimating the T limit (Coates and Dixon. 2011):

[T[x] = (LabResult[x] - PredResult[x] - meanDifference)/(Stdev of Differences)]….(3.1)

where T[x] is the T limit for sample x; LabResult is the reference value determined from wet chemistry; PredResult is the predicted value as determined by near infrared-spectroscopy; meanDifference is the average difference between all reference and predicted values in the dataset; and Stdev of Differences in the standard deviation of all differences in the dataset. Samples with T > 2.5 were designated as outliers that could have arisen as a result of poor NIRS prediction, error in the reference method, or bias in wet chemistry measurements.

Spectra were imported into Unscrambler ® X version 10.3 (CAMO software, Oslo, Norway), and a scatter plot of principle component (PC) scores for each sample within the metabolism and feedlot datasets were plotted along the first two principal components (x axis = PC1, y axis=PC2). Principle component analysis (PCA) is used to visualize the differences in spectral populations similar to Malley et al. (2005), where PCA was used to demonstrate differences in raw, stockpiled or composted manure. A mathematical treatment was applied to the spectra for PCA analysis (1, 8, 8, 1), and the standard normal variate (SNV) scatter correction and detrend (DT) functions. To identify outliers, principle component analysis established if a sample was contained within the calibration spectral population using the Mahalanobis statistic (Maesschalck et al. 2000) or *H* distance (> 3.0). In UCal, the global distance (GD) and the neighbourhood distance (ND) were used to identify outliers based on criteria similar to the Mahalanobis statistic. The GD measured the distance between the spectrum of individual samples and the mean of the full database, with a GD > 5.0 being indicative of a sample that was outside the range of the predictive model. The ND calculated the position of the spectrum of each sample within the database, and assessed the position of the sample of interest relative to all other samples. UCal used the closest ND measurement to differentiate unknown samples from known samples in the calibration set. If the ND was above a threshold of > 0.60, the unknown sample was selected as a candidate to further expand the calibration dataset.

3.2.3 Apparent Total Tract Digestibility

3.2.3.1 Collection of Samples

Fecal samples used to generate digestibility calibrations were collected from individually penned cattle over a 4 to 5 d period after they were adapted to the diets for at least 10 d. Subsamples of feces (400 g) were collected from daily total fecal output from each animal. Dry

matter intake of each animal was estimated and samples of diets and orts were collected for analysis. The majority of the digestibility studies were replicated Latin square designs using 8 cattle. One study was a completely randomized design with 6 cattle fed two diets over the backgrounding and finishing periods (Chapter 4). Fecal samples from digestibility experiments were dried, ground, and scanned as described above. More detailed information regarding these studies can be found in the references listed in Table 3.2.

3.2.3.2 Reference Analysis

Fecal and feed samples from each experiment were analyzed using the same procedures as for the feces and used to estimate aTTD of nutrients and energy. Gross energy (GE) was measured by combustion using a bomb calorimeter (model E2k; Cal2k, Johannesburg, South Africa). For aTTD determination, subsamples of ingredients and any remaining orts were composited over total collection periods, dried (55 ºC for 72 h), and analyzed, and in combination with DMI and total fecal output used to estimate aTTD of DM. Apparent total tract digestibility of OM, starch, NDF, ADF, and GE was calculated from total nutrient (or GE) ingested (taking into account orts) minus the nutrient (or GE) output in feces using the equations of Merchen (1988).

3.2.3.3 Spectral Database

The database contained the spectra of 172 fecal samples, representing 23 diets for the development of NIRS calibrations for predicting aTTD of nutrients and energy of the diets consumed. The spectrum of each sample was associated with the corresponding aTTD coefficient for each nutrient and GE prior to calibration development.

3.2.3.4 Fecal NIRS Calibration Development and Validations

The development of fecal NIRS calibrations for use in the estimation of aTTD coefficients was similar to that described above for chemical composition. A process of external validation was used to determine the influence of various factors on digestibility predictions by removing one study at a time, or classifying studies based on common attributes such as level of grain in the diet (>68% vs <44%) or grain type (barley vs corn). Prediction statistics were computed for

these sub-attributes. The most robust NIRS calibrations based on highest R^2_{CV} and *SECV* were selected to predict aTTD of DM, OM, starch, CP, NDF, ADF and GE in the samples selected from the feedlot database for validation (Table 3.2). For digestibility, previous designations for acceptable calibrations were more lenient, and $R^2_{CV} > 0.70$ was considered good.

Apparent total tract digestibility predictions of feedlot samples from validation studies 1 and 3 were analyzed using the PROC MIXED function for repeated measures since samples were collected at 3 or 4 wk intervals. Study 1 was analyzed as a factorial design, where the main factors included the effect of grain type, processing index, day and associated interactions, and pen as experimental unit. Study 2 was analyzed as a mixed linear model with silage level as a fixed effect, and pen as the experimental unit.

Data for aTTD of nutrients in the metabolism studies were analyzed using the mixed model procedure of SAS 9.1.(SAS,2004) for replicated 2 × 2 (Study 1) or 4 × 4 (Study 2) Latin square design with grain type as the fixed effect and square, animal within square and period within square as random effects for Study 1, and Silage Level was considered a fixed effect for Study 2.

For comparison between aTTD determined in feedlot and digestibility studies, and determined using equations of Zinn (2002, 2007), the MIXED procedure was used with method (NIRS versus Actual or NIRS versus Zinn), diet, and the interaction of method × diet as fixed effects. Differences with $P < 0.01$ were declared significant.

3.4 Results

3.4.1 Calibration for Chemical Composition

The validation steps conducted for the NIRS calibrations using the metabolism dataset resulted in the removal of chemical constituents that were poorly predicted in some of the selected studies, and were reported as missing values in the dataset. Where it was determined that values were deemed as outliers due to laboratory bias, technicians, or techniques, these values were not incorporated in developing the final NIRS calibrations reported. Once removed, additional validations conducted by removing one study at a time indicated that there was no benefit in developing calibrations that were specific for certain diets, grain types or grain processing methods using the current dataset (data not shown).

The 1, 8, 8, 1 spectral transformation was used for all calibrations as it produced calibrations with the highest R^2 and lowest *SEC* values. The calibrations yielded good to excellent linearity between reference and predicted values for OM (R^2_{CV} =0.83), starch (R^2_{CV}=0.87), N (R^2_{CV} =0.97), NDF (R^2_{CV} =0.90), ADF (R^2_{CV} =0.93), ADL (R^2_{CV} =0.85), but were poor for CF (R^2_{CV} =0.43; Table 3.3). Accuracy was excellent to acceptable for all constituents with *SECV* values of 0.96% for OM, 1.38% for starch, 0.108% for N, 2.41% for NDF, 2.28% for ADF, 0.90% for ADL, and 0.27% for CF.

Validations of the metabolism calibrations using the feedlot dataset are reported with the T outliers and the number of samples with ND > 0.6 (Table 3.3). Statistics show estimates with good linearity for OM (R^2_{val} = 0.80), excellent for starch (R^2_{val} > 0.94), moderate for NDF, and N (0.70 < R^2_{val} < 0.79), and poor for ADF, ADL and CF ($R^2_{val} \leq 0.70$). The *SEP* were high for OM, starch, NDF and ADF indicating poor accuracy of prediction. The slopes deviated substantially from 1 for N, NDF, ADF, and CF with OM and ADF exhibiting the greatest biases (-2.97 and -8.62%, respectively). The number of T outliers was high for all constituents, particularly OM, ADF, and CF. The 10 samples with spiked grain that were added to the feedlot dataset fell into the T outlier group (> 2.5) for all constituents. Aside for ADL, all other nutrients exhibited a high number of samples above the ND threshold, suggesting that the additional samples from the feedlot database would strengthen the calibrations. None of the samples displayed a GD > 5.0 (data not presented).

The principle component plot (Figure 3.1A) confirmed that most of the feedlot dataset was within the same population as the digestibility samples, however there were samples which did not overlap with samples in the digestibility dataset, and outliers (fecal samples spiked with barley grain) were evident. Once the datasets were combined by adding the ND outliers for each constituent, the calibrations displayed similar or improved linearity (R^2) and improved accuracy (lower *SECV*) for all constituents except CF (Table 3.3).

3.4.2 Calibrations for Digestibility

The final calibrations for aTTD of DM, OM, starch and GE yielded moderate to good linearity ($R^2_{CV} \geq 0.71$) between reference and predicted values, and high accuracy (*SECV* ≤ 2.88%; Table 3.4). Calibrations for aTTD of CP were moderate ($R^2_{CV} \leq 0.62$) and poor for NDF

and ADF ($R^2_{CV} \leq 0.33$), with low accuracy as indicated by high *SECV* values (3.63, 7.86 and 8.83%, respectively).

3.4.3 NIRS Validations for Digestibility

The validation steps conducted for the NIRS calibrations for aTTD examined the possibility of laboratory bias, but also aimed to describe the influence of certain dietary attributes on predictions. Validations resulted in the removal of 13 samples from one study (Davies et al. 2012) due to laboratory bias as they reduced the predictive statistics for the majority of measured constituents (data not shown). Davies et al. (2012) was the only study where whole shelled or steam rolled corn was fed, and oat hulls replaced barley silage (46-49%). All of the other diets contained barley or corn grain that had been dry- or temper rolled and barley silage. Aside from this study, the validations indicated that there was no benefit in developing calibrations that were specific for dry- or temper rolled barley or corn, nor on the basis of the percentage of grain in the diet.

Comparisons between aTTD determined from NIRS predictions using fecal samples collected from feedlot samples and that determined from parallel digestibility studies using total collection are shown in Table 3.5. The NIRS calibrations over predicted aTTD of DM, starch, N, and NDF, in study 1 (barley or wheat were fed at 89% DM). Aside for aTTD of starch, NIRS was able to predict which diet had higher or lower digestibility coefficients for DM, CP, NDF, and ADF. For aTTD of starch, NIRS predictions were closer to those derived using Zinn (2002; 2007) equations than those determined from total collection. For the diets fed in study 2, NIRS predictions of aTTD of DM, OM, starch, CP, and ADF were close to those determined from total collection and the same dietary effects on digestibility coefficients found using total collection could also be predicted with NIRS. Fecal NIRS predictions of aTTD of NDF were underpredicted compared to those predicted using total collection. The PCA plot confirms that samples from both study 1 and study 2 were represented by the calibration set (Figure 3.1B). The plot also shows that the samples collected from Davies et al. (2012) are outliers within the calibration set.

Table 3.3. Statistics for near infrared spectroscopy calibrations determined by partial least squares regression for fecal constituents including organic matter (OM), starch, nitrogen, neutral detergent fiber (NDF), acid detergent fiber (ADF), acid detergent lignin (ADL) and crude fat (CF) using the digestibility dataset validated with the feedlot dataset and the combined datasets.

Item	Calibration Statistics[z]							Validation Statistics with Feedlot dataset						
	n	Outliers	Range (Mean)	R^2_{cal}	*SEC*	R^2_{CV}	*SECV*	n	Range (Mean)	R^2_{val}	*SEP*	slope	bias	T>2.5	ND>0.6
OM															
Digestibility dataset	244	4	77.5-93.4 (89.2)	0.89	0.81	0.83	0.96	53	69.6-96.4 (85.4)	0.80	3.67	1.03	-2.97	38	42
Combined datasets	283	5	69.6-96.4 (88.5)	0.94	0.79	0.90	1.02								
Starch															
Digestibility dataset	149	7	0.2-24.3 (6.3)	0.93	1.18	0.87	1.38	53	0.7-53.0 (16.0)	0.94	4.00	1.28	1.03	18	28
Combined datasets	197	6	0.2-51.7 (7.8)	0.97	1.49	0.96	1.67								
Nitrogen															
Digestibility dataset	208	0	0.8-4.2 (2.6)	0.98	0.093	0.97	0.108	49	1.8-2.8 (2.3)	0.75	0.24	0.62	-0.16	14	46
Combined datasets	245	1	0.8-4.2 (2.5)	0.98	0.090	0.97	0.100								
NDF															
Digestibility dataset	326	4	30.4-81.5 (51.9)	0.92	2.21	0.90	2.41	49	19.7-61.2 (45.3)	0.73	6.85	1.35	-0.18	19	39
Combined datasets	347	2	19.7-81.5 (51.8)	0.95	1.90	0.92	2.42								
ADF															
Digestibility dataset	179	1	4.9-48.5 (29.2)	0.97	1.53	0.93	2.28	48	8.5-37.8 (25.6)	0.25	11.0	0.71	-8.62	20	44
Combined datasets	225	0	4.9-48.5 (28.4)	0.94	1.85	0.92	1.97								
ADL															
Digestibility dataset	26	0	3.1-13.0 (10.0)	1.00	0.07	0.85	0.90	45	0.70-8.0 (4.9)	0.70	1.57	1.06	-1.00	12	3
Combined datasets	55	0	0.7-13.0 (7.1)	0.95	0.83	0.92	1.00								
CF															
Digestibility dataset	40	2	0.9-2.8 (1.8)	0.70	0.23	0.43	0.27	45	0.3-2.4 (1.5)	0.25	2.82	0.48	-1.96	23	45
Combined datasets	78	9	0.9-2.8 (1.7)	0.53	0.28	0.40	0.32								

Abbreviations: R^2_{cal} = coefficient of determination of calibration; *SEC* = standard error of calibration; R^2_{CV} = coefficient of determination of cross-validation; *SECV* = standard error of cross validation; R^2_{val} = coefficient of determination of validation; *SEP* = standard error of performance; T = T limit; ND = neighbourhood distance.

[z]Math treatments 1, 8, 8, 1, standard normal variate and detrend. Spectra were clipped between 1250-2350 nm for all constituents except for crude fat (1200-2400 nm).

Table 3.4. Statistics for near infrared spectroscopy calibrations determined by partial least squares regression for apparent total tract digestibility of dry matter, (DM), organic matter (OM), starch, nitrogen, neutral detergent fiber (NDF), acid detergent fiber (ADF), and gross energy (GE).

Item	Calibration Statistics[z]						
	N	Outliers	Range (Mean)	R^2_{cal}	*SEC*	R^2_{CV}	*SECV*
Digestibility, % of intake							
DM	156	2	59.4-90.1 (76.5)	0.86	2.18	0.75	2.88
OM	156	2	57.4-91.0 (77.8)	0.88	2.18	0.77	2.88
Starch	92	2	87.2-99.4 (95.9)	0.93	0.74	0.84	1.06
CP	144	2	61.1-86.7 (73.9)	0.73	3.13	0.62	3.63
NDF	154	4	26.4-76.2 (54.3)	0.48	7.10	0.33	7.86
ADF	153	5	6.1-71.9 (43.9)	0.46	7.25	0.21	8.83
GE	124	2	57.4-88.7 (74.8)	0.81	2.34	0.71	2.79

Abbreviations : R^2_{cal} = coefficient of determination of calibration; *SEC* = standard error of calibration; R^2_{CV} = coefficient of determination of cross-validation; *SECV* = standard error of cross validation;

[z]Math treatments 1, 8, 8, 1, SNV and detrend. Spectra were clipped between 1250-2350 nm.

Table 3.5. Digestibility values predicted using NIRS equations in feedlot studies where cattle were fed barley or wheat in study 1 or barley and increasing levels of silage in study 2, as compared to estimates (Actual) derived from total collection in digestibility studies.

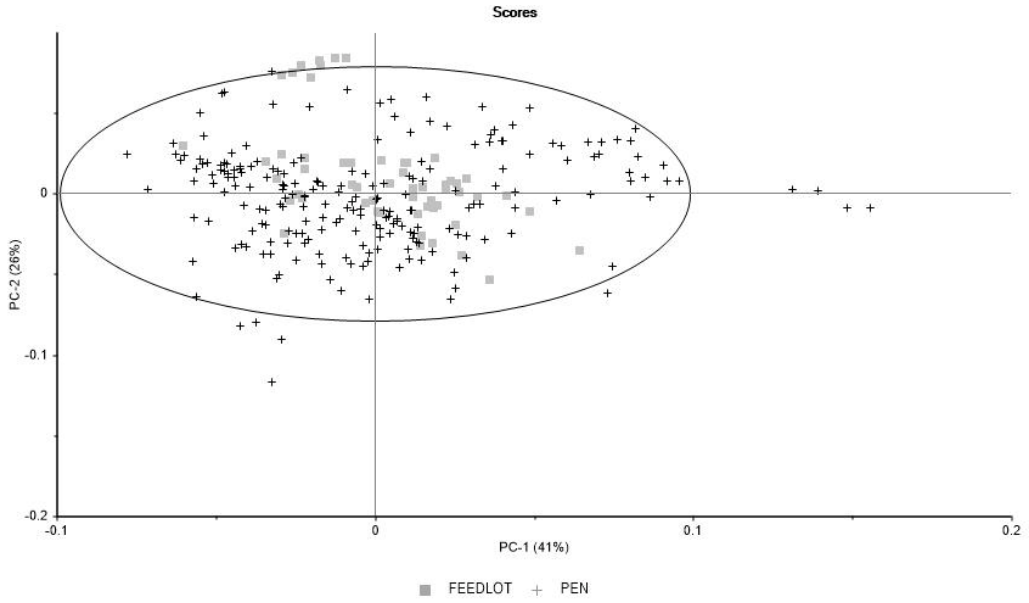
Digestibility Parameter	Study 1			Study 2				
	B79	W80	SEM	SL0	SL4	SL8	SL12	SEM
Dry Matter								
Feedlot study (NIRS)	79.7	80.1	0.76	82.4a	81.0b	79.7c	78.8c	0.357
Digestibility study (Actual)	70.0	73.0	1.26	86.0a	82.1b	81.1bc	79.5c	0.691
P value	<0.01	<0.01		<0.01	0.15	0.08	0.35	
Organic Matter								
Feedlot study (NIRS)	82.0	82.8	0.61	85.1a	83.1b	81.5c	80.5c	0.39
Digestibility study (Actual)	NM	NM	-	87.2a	83.7b	82.4bc	81.1c	0.75
P value	-	-		0.01	0.48	0.25	0.46	
Starch								
Feedlot study (NIRS)	95.3	94.3	0.42	97.5	97.5	97.7	97.8	0.62
Digestibility study (Actual)	91.9	93.1	0.70	98.9a	98.0b	97.9b	96.8c	0.32
P value	<0.01	0.14		0.05	0.54	0.75	0.16	
Feedlot study (NIRS)	95.2	94.1	0.67	97.5	97.5	97.7	97.8	0.42
Zinn et al. 2002[z]	96.0	93.7		96.3	96.3	96.5	96.6	
P value	0.23	0.68		<0.01	<0.01	<0.01	<0.01	
Feedlot study (NIRS)	95.2	94.1	0.59	97.5	97.5	97.7	97.8	0.40
Zinn et al. 2007[z]	96.2	95.2		96.7	96.4	96.4	96.5	
P value	0.13	0.16		0.06	<0.01	<0.01	<0.01	
Crude Protein								
Feedlot study (NIRS)	74.4	74.8	0.85	79.7a	78.4a	76.5b	75.0b	0.58
Digestibility study (Actual)	63.9a	59.1b	1.41	81.7a	76.8ab	77.3ab	75.9b	1.12
P value	<0.01	<0.01		0.12	0.18	0.56	0.50	
NDF								
Feedlot study (NIRS)	57.2	56.4	1.41	59.8a	57.0b	54.7c	52.9c	0.82
Digestibility study (Actual)	51.6	49.5	2.33	68.9a	59.5b	59.3b	58.9b	1.58
P value	0.04	0.01		<0.01	0.16	0.01	<0.01	
ADF								
Feedlot study (NIRS)	43.9	41.7	1.87	51.9a	49.4ab	46.7bc	44.5c	1.11
Digestibility study (Actual)	39.3	37.7	3.11	53.5a	40.2b	41.9b	44.3b	2.16
P value	0.21	0.26		0.65	<0.01	0.05	0.93	

Abbreviations: B79 = barley processed to a PI of 79% where PI is processing index calculated as ratio of the bushel weight after processing over the bushel weight before processing; W80 = wheat processed to 80%; SL = silage level; NIRS = digestibility determined using near infrared spectroscopy calibrations; NM = not measured; NDF = neutral detergent fiber; ADF = acid detergent fiber.

[z] Equations derived by Zinn et al (2002, 2007) to estimated total tract starch digestibility for feedlot samples using NIRS predicted fecal composition.

Italicized letters in each row for each study represent significant differences (P<0.05).

A



B

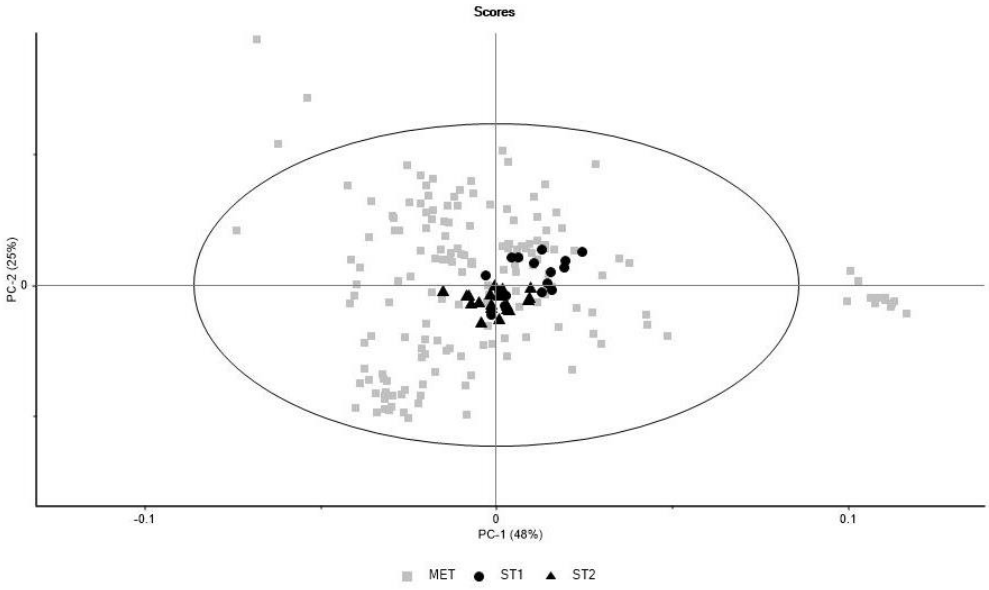


Figure 3.1. Principle component plot showing the two populations of spectra; (A) the feedlot and digestibility (PEN) datasets for the NIRS calibrations for fecal chemical composition, and (B) the metabolism (MET) dataset for the calibrations for apparent total tract digestibilty, and the two external validations for Study 1 (ST1) and 2 (ST2).

3.5 Discussion

3.5.1 NIRS Calibrations for Fecal Constituents

Recently, Landau et al. (2015) developed NIRS calibrations for chemical components of feces using confined beef cows fed 153 forage diets supplemented with barley grain or dried poultry litter, and validated them using samples collected from field studies. They concluded that development of calibrations using confined cattle could be used to predict the CP, ash and NDF content of the feces from grazing cattle (Landau et al. 2015). In an earlier study, Coates and Dixon (2011) found that calibrations developed on fecal samples collected from cattle grazing pastures, *in vivo* metabolism trials, and short term pen trials were not reliable predictors of fecal composition across methods. They attributed this to differences in the ranges and distribution of the reference values observed among each method.

In our study, the range in chemical composition between the digestibility and feedlot datasets differed (Table 3.1), and there were differences noted in their spectral populations (Figure 3.1A). For most fecal constituents, predictions using the digestibility calibration over- or under-estimated values in the feedlot dataset. These over- or under-estimates resulted in deviations in the slope from 1, and bias from 0. In most cases, the spiked fecal samples resulted in these offsets, as they typically deviated the most from the mean of the digestibility samples, indicating that samples collected from controlled experiments were not representative of the fecal samples with high levels of starch (>19% of DM) that were collected from penned feedlot cattle (Jancewicz, unpublished).

Laboratory bias is a type of error that is difficult to avoid and is encountered when developing NIRS calibrations using multiple labs, technicians, and laboratory methods. Laboratory error can be identified as outliers and eliminated during calibration development and manual validation steps (Coates and Dixon 2011). However, if biases are high they can impact calibrations and generate poor calibration statistics. To create our database, the scanning of samples from digestibility studies was carried out after wet chemistry analysis. Therefore, it is possible that the chemical composition of samples could have changed during storage. One example of variation in lab techniques is apparent for ADF, which may have resulted in the large *SEP* and biases observed for this constituent. The two analytical methods used for ADF analysis in our study were sequential (ANKOM) and traditional Van Soest et al. (1991) using filtering

glass crucibles. However, Danelón et al. (2012) reported differences in results obtained using these two methods, with sequential ANKOM ADF values being almost 4% lower for some feeds. When data is collected from multiple laboratories, it can be difficult to ensure that all estimates are generated using only a single method.

Combining the metabolism and feedlot datasets dramatically improved the predictive capacity of NIRS for all constituents, except CF. Improvements in *SEP* were noted due to a greater variety of diets being represented in the full dataset, and this was also observed when Coates and Dixon (2011) combined calibration sets based on fecal samples from cattle grazing pastures, *in vivo* digestibility trials, and short term pen trials. Our calibration and cross-validation statistics for the combined calibrations were comparable to calibrations reported previously for dried ground cattle feces for OM [R^2_{cal} = 0.94 (*SEC* = 0.79) compared to R^2_{cal} = 0.94-0.96 (*SEC* = 4.4-1.29) (Purnomoadi et al. 1996; 1997)], starch [R^2_{CV} = 0.96 (*SECV* = 1.67) compared to R^2_{CV} = 0.91 (*SECV* = 0.57) (Fredin et al. 2014)], N [R^2_{CV} = 0.97 (*SECV* = 0.10) compared to R^2_{CV} = 0.96 (*SECV* = 0.08) (Coates 2004)], NDF [R^2_{CV} = 0.92 (*SECV* = 2.42) compared to R^2_{CV} = 0.86 (*SECV* = 1.40) (Cozzolino et al. 2002)] and ADF [R^2_{CV} = 0.92 (*SECV* = 1.97) compared to R^2_{CV} = 0.92 (*SECV* = 1.2) (Cozzolino et al. 2002)], and ADL [R^2_{CV} = 0.92 (*SECV* = 1.00) compared to R^2_{CV} = 0.94 (*SECV* = 1.1) (Purnomoadi et al. 1996)]. Purnomoadi et al. (1996) also found that calibrations for CF were not predictive for one group of samples. However, other groups provided precise and accurate calibrations for CF (Purnomoadi et al. 1996; 1997). The inability of our study to predict CF likely reflects the narrow range of fat (0.3 - 2.8%) in fecal samples used for calibration development.

The official AOAC method for CF used for the samples in our study [Soxtec (991.36)] is vague with regard to extraction time, with any deviations in extraction time altering CF recovery (Palmquist and Jenkins 2003) and increasing the uncertainty of estimates (Hammond 2001). Also, depending if the fat is of dietary or microbial origin, differences in fatty acid composition may cause shifts in wavelength and absorbance that make it more difficult to quantify (Garrido-Varo et al. 2004).

We also found poor validation statistics for ADL in that the R^2_{val} (0.70) was much lower than the R^2_{CV}. This could also reflect the low range in ADL concentration (0.7 - 13%), and the occurrence of samples with low ADL concentration (≤ 1.0%). Accurate measurement of ADL using NIRS enabled previous researchers to use ADL as a digestibility marker (Purnomoadi et al.

1996, 1997; Chapter 4). In our recent study, ADL analysis was performed by one technician, and the coefficient of variation between replicates was below 2% ensuring excellent precision. Care must be taken when using NIRS predicted ADL for digestibility estimates since errors will be amplified, especially since ADL concentrations are typically low in high grain feedlot diets.

Our calibration statistics compare favourably with previously published reports for OM, starch, N, NDF, and ADF and can be implemented for quantitative measurement of these fecal nutrients in commercial feedlots that feed diets similar to those represented in the calibration.

3.5.2 NIRS Calibrations for Digestibility

To our knowledge, there are only three studies where NIRS calibrations were developed to predict nutrient digestibility based on total collection of feces from cattle that had been fed forage and concentrate diets. Of these three, two developed fecal NIRS calibrations for predicting aTTD of OM (de la Roza et al. 2002) and DM (Garnsworthy and Unal 2004) in dairy cattle and one estimated the aTTD of DM in beef steers (Gibbs et al. 2002). Our results are comparable to those obtained in these studies for aTTD of DM [R^2_{cal} = 0.86, *SECV* = 2.9 compared to R^2_{cal} = 0.87, *SECV* = 3.0 (Gibbs et al. 2002)] and OM [R^2_{cal} = 0.88, *SECV* = 2.9 compared to R^2_{cal} = 0.86, *SECV* = 2.6 (de la Roza et al. 2002)].

The calibrations developed in our study were used in a concurrent study that examined the variation in fecal excretion and digestibility estimates over 24 h, with the calibrations for predicting aTTD of starch being closer to estimates derived by total collection than all other nutrients (Chapter 3). It is not surprising that NIRS is more accurate at predicting aTTD of starch than other fecal constituents, given that other studies have documented the close relationship between fecal starch and starch digestibility in high grain diets (Zinn et al. 2002, 2007; Fredin et al. 2014). The same calibrations for aTTD of starch were not as predictive when grain comprised only 44% of the diet DM as less starch in the diet resulted in less starch in the feces.

Using fecal NIRS to predict digestibility of other nutrients is more difficult than predicting chemical composition directly, explaining the poorer calibration statistics associated with these estimates. Using NIRS for predicting aTTD measures the absorption of the nutrient peaks arising from the feces, and not all changes in fecal composition reflect changes in digestibility. For example, endogenous secretions are composed of N, and they are excreted to a greater extent with more fermentable diets (Oba and Allen 2003). Many of the diets used in calibration

development contained by-products including dried distillers' grains with solubles, grains screenings and added oil, which would have likely increased fecal concentrations of N and CF without directly affecting digestibility. When digestibility is varied by altering dietary proportions of forage and grain, it would be expected to induce greater changes in chemical composition in fecal samples and better predictions (Garnsworthy and Unal 2004). The particularly poor predictions of aTTD of NDF and ADF could also likely be due to their relatively low digestibility compared to the other nutrients. Lower digestibility would result in larger coefficients of variation, reducing the predictive ability of aTTD of NDF and ADF relative to starch (Fredin et al. 2014).

Unlike for chemical composition, validation methods for calibrations for aTTD are impractical as they require performing time consuming and costly digestibility studies. To gage how closely our predictions of aTTD of DM, OM, starch, CP, NDF, and ADF in the feedlot studies were to results from total collection, we compared the predictions to aTTD determined in two parallel digestibility studies (He et al. 2015; Chibisa et al. unpublished). Ideally a greater number of studies would be more informative of the predictability of the calibrations. Predictions for study 2 were much more accurate than study 1 and this is likely because the diets from study 2 were included in the calibration set (Figure 3.1B). Study 1 included wheat in one of the diets, and samples from cattle fed wheat were not included in the calibration set.

The NIRS predicted starch digestibility in study 1 was much closer to starch digestibility estimates generated using the equations of Zinn et al. (2002; 2007; Table 3.5); providing evidence that digestibility may have differed between parallel digestibility and feedlot studies. Similar to our predictions, Koenig and Beauchemin (2011) also failed to find differences in estimated starch digestibility when varying proportions of barley silage was fed to feedlot cattle, a result confirmed in study 2 (Chibisa et al. unpublished).

Landau et al. (2015) and Coates and Dixon (2011) found that predicting *in vitro* DM and OM digestibility was possible if the range in digestibility values encompassed those in the validation sets. However, when considering NIRS aTTD predictions, differences in DMI may affect aTTD. Competition is known to alter intake, which can in turn affect digestibility. Predicting DMI using calibrations developed for confined cattle has not always been successful as many dietary and physiological factors affect intake, particularly of forage diets (Dixon and Coates. 2009; Landau et al. 2015). Intakes for barley and wheat fed steers were greater in study 1

(He et al. 2015) compared to cattle in our feedlot study, and lower in study 2 (Chibisa et al. unpublished). These differences could have led to discrepancies between direct and NIRS predicted estimates of aTTD. Additional discrepancies such as differences in grain processing between feedlot and digestibility studies may have also resulted in the deviations observed.

Calibrations for aTTD of nutrients developed using cattle in metabolism experiments shows potential for predicting samples collected from feedlot cattle, but calibrations are more predictive when the diets are represented in the calibration set. By continuing to expand the calibration by adding more samples, predictability of aTTD could be made applicable to a wider range of diets fed to feedlot cattle. In addition, PCA can be used as an additional technique to rapidly assess whether unknown samples fit within a calibration population.

3.6 Conclusion and Implications

Fecal NIRS calibrations can be applied for use in commercial feedlots for accurate quantitative predictions of fecal chemical composition. However, consistent reference analysis is crucial for developing accurate calibrations. Calibrations for fecal CF proved to lack precision owing to the low CF concentration in the diets and consequently in the feces. Calibration for aTTD of nutrients can be used for qualitative tracking of digestibility when grain type or grain proportion is changed. Additional diets with differing composition should be added to the original database so as to further increase the accuracy of future predictions of those diets that are employed in the Canadian feedlot industry.

3.7 Next Stage

The fecal NIRS calibrations developed in this chapter readily predict the composition of dried ground feces collected from feedlot cattle. Since there is error associated with every analytical method, sampling methods must be standardized to minimize error and to account for variability in samples collected from different animals and at different times in a day. The next chapter focuses on examining the variation in nutrient excretion and digestibility predictions in feedlot cattle fed backgrounding and finishing diets over 24 h. Cattle in this study were fed once or twice per day to account for differences in management practices observed in commercial feedlots. The accuracy of using NIRS calibrations for predicting digestibility at different time

points, pooled over multiple cattle, was also assessed. Both fecal nutrient concentrations and marker methods were examined. From this information, recommendations can be given regarding the appropriate time for sampling feces in commercial feedlots within a 24 h period, and recommendations on suitable methods for predicting digestibility.

CHAPTER 4

4.0 CHARACTERIZATION BY NEAR INFRARED SPECTROSCOPY OF THE VARIATION IN THE DAILY EXCRETION OF FECAL CONSTITUENTS AND DIGESTIBILITY PREDICTIONS IN BEEF CATTLE FED FEEDLOT DIETS[2]

4.1 Introduction

The nutritional value of a diet for cattle is defined by their ability to ingest, digest and metabolize dietary components. To generate accurate estimates of diet digestibility or the nutrients within, typically the daily feed intake and total fecal production from individual cattle must be known. The need to quantify individual feed intake presents a practical challenge for the evaluation of digestibility under commercial settings.

Cereal grains are a major ingredient in many cattle diets, and given the high proportion of starch in grains, predictions have been developed to estimate starch digestibility. For example, fecal starch concentration can be used as a predictor of total tract starch digestion in feedlot (Zinn et al. 2002; Corona et al. 2005; Zinn et al. 2007) and dairy cattle (Fernandez et al. 1982; Fredin et al. 2014). In addition, indigestible markers such as lignin can be used to monitor nutrient digestibility, but knowledge of the concentration of the marker and the nutrient of interest in both the feed and feces is required (Van Soest et al. 1991). Application of indigestible markers has been used to predict total tract starch digestion in lactating dairy cows on commercial farms using starch and lignin concentration in samples of TMR and feces (Lidy et al. 2009; Ferguson 2006).

Near infrared spectroscopy (NIRS) is a rapid method commonly used for predicting chemical constituents in feed and feces, but can also be used for estimating dietary attributes such as nutrient digestibility. Multivariate regression can be used to predict the digestibility of nutrients using fecal samples whose chemistry is unknown, based on their NIRS spectra, and previously generated calibration equations. Predictions of OM and DM digestibility for a variety of ruminant species have been developed, but few have been validated due to the cost and time associated with acquiring spectra for an independent set of samples (Dixon and Coates 2009).

[2]A version of this chapter has been published. Jancewicz, L.J., G. B. Penner, M. L. Swift, J. J. McKinnon, C. L. Waldner, and T. A. McAllister. 2016. Characterization of the variation in the daily excretion of fecal constituents and digestibility predictions in beef cattle fed feedlot diets. Can. J. Anim. Sci. 96:532-549. DOI: 10.1139/cjas-2015-0193.

The methods described for estimating nutrient digestibility generally rely on using fecal samples that are representative of a 24-h period, acquired by either total fecal collection or by compositing fecal samples collected over multiple time points and sourced from a large number of cattle. In production settings, where maximizing nutrient utilization could markedly increase profit margins, it is unrealistic to continuously sample feces from large numbers of cattle. Single sampling times from fewer cattle to generate the same information would be ideal, but there has been limited work to evaluate the variation in fecal nutrient excretion over the duration of a day. In addition, the accuracy of digestibility values obtained using markers requires that markers are distributed within ingesta and that they pass through the digestive tract in a manner similar to that of ingested nutrients (Kane et al. 1952). Considering the practicality of a single sampling approach, it would be useful to describe the 24-h variation in key constituents excreted from cattle, and recognize factors that may alter excretion patterns.

Diurnal excretion of fecal starch has been studied in cattle fed low (≤ 31% diet DM) corn grain diets (Leonard et al. 1989; Caetano 2008; Fredin et al. 2014), with the concentration of starch being lower in feces 12 h after feeding. These data suggest a sample collected at a single point in time could lead to an over or underestimation of starch digestibility. Similar studies have not been conducted with feedlot cattle fed high grain diets, or for other nutrients. Kanani et al. (2012) characterized the excretion pattern of alkaline peroxide lignin and acid detergent insoluble ash in feces every 6 h from cattle fed a forage diet and found no differences in their concentrations over 24 h, suggesting that a single fecal sample may represent the excretion of these markers over the course of a day. In contrast, Kane et al. (1952) found that lignin concentration in feces varied over a 24-h period in dairy cattle fed high forage diets twice daily, and concluded that multiple samples or designated sampling periods are required for accurate estimates of lignin excretion.

The objective of this study was to characterize the diurnal pattern in fecal output of DM and NIRS predicted OM, starch, nitrogen, neutral detergent fiber (NDF), acid detergent fiber (ADF) and acid detergent lignin (ADL) in beef cattle offered low- (backgrounding) and high barley (finishing) diets fed once or twice daily. This information was then used to assess the effect of time of fecal sampling on the predictability of digestibility coefficients using both total collection and that predicted by NIRS.

4.2 Materials and Methods

4.2.1 Animals, Housing and Experimental Design

All heifers were cared for in accordance with the guidelines of the Canadian Council on Animal Care (2009) and the University of Saskatchewan Animal Research Ethics Board (protocol 20100021). The experiment was split into two studies, backgrounding and finishing, using a completely randomized design.

4.2.2 *In Vivo* Studies

Six heifers were housed individually in 13.4 m^2 pens fitted with rubber mats. Upon arrival, heifers underwent a 28-d adaptation. During this time, they were fed a 50:50 grass hay:barley silage diet (DM basis) with continuous access to fresh water, and exposure to 12 h of light from 0830 to 2030 h. These conditions were maintained once experimental diets were provided, and heifers were randomly allocated to 1 of 2 feeding frequencies; once per day (0900 h; FF1), or twice per day (two equal feedings at 0900 and 1700 h; FF2) with each heifer being fed at the same feeding frequency in both the backgrounding and finishing studies. Melengestrol acetate (0.43 mg d^{-1}; Pfizer Inc, New York, NY) and monensin (28 mg/kg DM; Rumensin 80®, Elanco Animal Health, Indianapolis, IN) were included in the supplement. Canola meal was added to ensure adequate protein in both TMR. Barley grain was dry rolled to a processing index of 80%, based on the volume density of the grain before and after processing.

At the start of the backgrounding period, heifers (318 ± 18.8 kg) were adapted for 21 d to a diet fed for ad libitum intake consisting of (DM basis) 44% dry rolled barley, 44% barley silage, 5% canola meal and 7 % supplement (Table 4.1). Total feces were collected for 4 d after adaptation and daily voluntary intake was determined taking into account orts. Bladder catheters (Bardex® Lubricath® Foley catheter, Bard Medical, Covington GA) were inserted 24 h prior to total fecal collection to prevent urine from contacting feces. During fecal collection, heifers were fitted with a halter and tethered. Fecal samples were collected from excreted feces on the pen floor at 4-h intervals corresponding to 0, 4, 8, 12, 16, 20, and 24 h after first feeding. At each 4-h interval, feces were weighed, mixed, and a 375-g subsample was oven dried (55 ºC for a minimum of 72 h). Fecal output (kg; DM basis) was calculated at each 4-h interval for 4 d. Subsamples of ingredients and any remaining orts were collected the day they were fed,

composited over 4 d, dried (55 ºC for 72 h) and in combination with DMI and fecal output over 4 d, used to estimate apparent total tract digestibility of DM as described by Merchen (1988).

Ingredients and fecal samples were ground to pass through a 1-mm screen of a Wiley Mill (Thomas Scientific, Swedesboro, NJ) and retained for chemical analysis. All fecal samples were scanned using NIRS and selected fecal samples (see **selection of samples for reference analysis**) were retained for references analysis and the development of NIRS calibration equations.

At the start of the finishing period, the same heifers (379 ± 31 kg) were transitioned to an 80% barley grain diet over 20 d by increasing the amount of barley in the diet every 5 d (55:45, 65:35, 70:30; 75:25; 80:20). Heifers were fed a finishing diet containing (DM basis) 80% dry rolled barley, 8% barley silage, 5% canola meal, and 7% supplement (Table 4.1). The 4-d total fecal collection followed the same procedures as described for the backgrounding period after 18 d on the final finishing diet.

4.2.3 Selection of Samples for Reference Analysis

Two samples of dried and ground feces (25 g, n = 330) were packed into quartz ring cups and scanned using a SpectraStar Near-Infrared analyzer 2400 RTW (Unity Scientific, Brookfield, CT). Spectral information was collected at wavelengths between 1250 and 2350 nm in 1 nm increments. The two spectra of each sample were averaged.

A subset of samples (day 1, n = 83) was selected for chemical analysis. The export function in Unity Calibration Software (UCAL) (Unity Scientific, 2010) version 2.0.0.31 was used to convert spectra collected during all 4 d into a format that could be imported into Unscrambler ® X version 10.3 (CAMO software, Oslo, Norway). Principle component analysis was used to reduce the full set of spectral data to a smaller set of linearly uncorrelated variables called principle components. The reference data for constituents in day 1 samples were matched to their corresponding spectra, and a plot of principle component scores assigned to each sample and their principle component loadings for selected known constituents was used to identify outliers for chemical constituents in the remaining samples. These outlier samples were determined by the distance (ND) of each sample from neighboring samples, and the samples above the cutoff (ND > 0.6) for particular constituents were set aside for additional reference analysis which

Table 4.1. Ingredient and chemical composition of total mixed diets offered to heifers fed backgrounding and finishing diets once or twice per day

Item	Diet	
	Backgrounding	Finishing
Ingredients (% DM basis)		
Dry rolled barley[z]	44.0	80.0
Barley silage	44.0	8.0
Canola meal	5.0	5.0
Supplement[y]	7.0	7.0
Chemical analysis (%)		
Dry matter	63.7	81.7
Organic matter	92.0	94.9
Starch	33.8	40.8
Crude protein	13.5	14.2
Neutral detergent fiber	31.4	31.1
Acid detergent fiber	17.6	10.8
Acid detergent lignin	4.12	2.50

[z]Processed to 80% as determined from the ratio of the bushel weight of processed grain to the bushel weight of unprocessed grain x 100; [y]Supplement contained (per kg DM) 445.8 g ground barley, 250.0 g corn distillers grains with solubles, 118.4 g limestone, 75.8 g canola meal, 68.5 g Dynamite (K and Mg sulfate), 21.0 g salt, 11.4 g magnesium oxide, 2.82 g MGA 100 (220 mg/kg), 2.01g rumensin (rum/cob) 200, 1.13 g JS-2000 MG semar, 0.99 g ferrous carbonate (38%), 0.60 g vitamin E 50, 0.58 g copper sulphate (25 %), 0.50 g manganese oxide, 0.42 g zinc oxide (72%), 0.04 g Vitamin A 1000, 0.03 g Vitamin D3 500, 0.01 g cobalt carbonate, 0.010 g EDDI (80% iodine).

included OM (n = 53), starch (n = 43), N (n = 7), ADF (n = 13), NDF (n = 13), and ADL (n = 19).The combination of day 1 samples and these selected outliers were used to develop NIRS calibration equations to predict the composition of the remaining fecal samples.

4.2.4 Chemical Analysis for *In Vivo* Studies and NIRS Reference Analysis

Feed ingredients, orts, and selected fecal samples were analyzed for analytical DM [Association of Official Analytical Chemists (AOAC) 2005, method 930.15], OM (AOAC, method 942.05), starch, N, NDF (AOAC method 2002.04) and ADF (AOAC method 973. 18). An ANKOM fiber analyzer (ANKOM technology Corp., Fairport, NY) was used to determine NDF and ADF sequentially, with heat stable α-amylase and sodium sulphite for NDF analysis. Acid detergent lignin was extracted using 72% sulphuric acid, followed by ashing at 550º C (Van Soest et al. 1991). Feed and fecal samples were also ground using a ball mill (Mixer Mill MM2000, Retsch, Haan, Germany) for determination of starch and N. Starch concentration was determined using a Megazyme kit (Megazyme International Ireland, Wicklow, Ireland) as described by Rode et al. (1999), and N was quantified by flash combustion followed by gas chromatography and thermal conductivity detection (Carlo Erba Instruments, Milan, Italy).

4.2.5 Data Processing and Calibration Development

Partial least squares regression (PLS) in UCAL (Unity Scientific, 2010) software was used for development of calibration equations. Several pre-treatments of raw spectral data were performed prior to calibration. Derivative spectra were used to emphasize small or large absorption peaks, and minimize overlapping peaks and baseline correction (Giese and French 1955). Six mathematical treatments were tested in the development of the calibrations, “1, 8, 8, 1” “1, 10, 10, 1” “1, 12, 12, 1” “2, 8, 8, 1” “2, 10, 10, 1” “2, 12, 12, 1”, where the first digit is the order of the derivative, the second is the gap over which the derivative was calculated, the third is the number of data points used in the running average for smoothing of derivative spectra, and the fourth is the number of the second smoothing. Two scatter corrections were applied, standard normal variate (SNV) and detrend (DT) functions (Barnes et al. 1989). For validation, spectra were arranged in order of hour and day of collection, and every third sample was removed. The optimum math treatment for each constituent was “1, 8, 8, 1”, identified on the basis of minimum

standard error of prediction (SEP) and the greatest coefficient of determination of validation (R^2_{val}) (Table 4.2). All fecal samples with unknown chemistry were then predicted using the same math treatment. Any unique samples from the calibration were defined by their global distance (GD), and any samples outside the limit (GD > 3.0) would be considered unique. There were no outliers for any of the constituents predicted.

Fecal NIRS calibrations for digestibility coefficients as described in Chapter 3 were used to predict digestibility coefficients in this study. Validation statistics for digestibility of DM, OM, starch, NDF, and ADF are shown in Table 4.2.

4.2.6 Determination of Total Tract Digestion and Nutrient Digestion Equations

Apparent total tract digestibility (**aTTD**) of nutrients was calculated from total nutrient ingested (taking into account orts) minus the nutrient output in feces for each heifer during the last 4 d of each experimental period. Relationships between nutrient concentration in the feces and aTTD of the respective nutrient were determined using nutrient concentration in each 4-h interval fecal sample as well as in the 24-h composite fecal sample for each heifer. The eTTD of nutrients using ADL as a marker were calculated using the ratios of nutrient and ADL concentration in feces of each heifer and in the TMR (Merchen 1988) using 4-h interval as well as the 24-h composite fecal samples. Estimated fecal output (kg DM/d) was assessed according to the same calculations as Kanani et al. (2012). The recovery rate of ADL was assessed according to Krysl et al. (1988). Total tract digestibility coefficients predicted by NIRS were obtained by scanning each 4-h interval sample as well as the 24-h composite sample from each heifer.

4.2.7 Statistical Analyses

Statistical analyses were performed using SAS v. 9.1. (SAS Inst. Inc., Cary, NC, USA) and the backgrounding and finishing periods were analyzed separately. Before further analyses, the normality of the residues of all the variables was tested using the Shapiro–Wilk test.

Differences in DMI, fecal output, and marker recovery were tested by ANOVA. The model tested was $Y_i = \beta_{0i} + \beta_1 FF_i + \varepsilon_i$, where β_0 was the *y* intercept for feeding frequency, i was heifer,

and FF was the fixed effect of feeding frequency (once versus twice per day), and εi was the residual error term.

Using the 4-h interval samples, the 24-h variation in fecal output and NIRS predicted fecal OM, starch, N, NDF, ADF, and ADL concentrations (% of fecal DM), NIRS predicted aTTD and eTTD of DM, OM, starch, NDF and ADF were analyzed using the PROC MIXED function for repeated measures, based on Kenward–Roger's adjusted degrees of freedom solution. The models included FF for the effect of feeding frequency (fed once versus twice per day), Time for the effect of time after first feeding (i = 0, 4, 8, 12, 16 and 20 h after first feeding), and Day (1 to 4), and the interaction of FF×Time as fixed effects, and the individual animal as the random effect. The model used was $Y_{ijk} = \beta_0 + \beta_1 FF_{1ijk} + \beta_2 Time_{1ijk} + \beta_3 FF{\times}Time_{ijk} + \beta_4 Day_{ijk} + \mu_j + \varepsilon_{ijk}$, where μ_j was the random effect for animal, i was the individual observation of time within day within heifer, j was heifer, β_0 was the average y intercept or overall mean for time and FF, and εijk was the associated error. A first-order autoregressive structure was used to model repeated measures on individual animals within each day. Least square means of the treatments were separated using PDIFF statement, and significances were declared at $P<0.05$.

The difference between total tract digestion determined from total collection (aTTD) or the marker method (eTTD), and mean digestibility coefficients predicted for each 4-h interval were analyzed using PROC MIXED. The model was $Y_{ij} - Y_{pooled} = \beta_0 + \beta_1 Treatment_{1ij} + \beta_2 Time_{1ij} + \beta_3 Treatment{\times}Time_{ij} + \varepsilon_{ij}$. A first-order autoregressive structure was used to model the collection order of mean values for each time within animal. Treatments were separated using the PDIFF statement with effects and their interaction declared significant at $P < 0.05$ and trends when $0.05 < P < 0.10$.

Actual values of fecal ouput were compared by paired *t*-test to estimated values using ADL as a marker. Similar comparisons were carried out for actual and estimated aTTD determined from total collection of feces or by NIRS calibrations for digestibility coefficients by scanning 4-d composite fecal samples.

The relationship between fecal DM, OM, starch, NDF, ADF concentrations, and their digestibility (aTTD) as determined by total collection, and between aTTD or eTTD using total collection or the individual time points was determined using PROC REG in SAS. Pearson's correlation coefficients (r) between actual values and estimates were calculated and coefficients of determination (R^2) and regression equations were declared significant at $P < 0.05$.

Coefficients of variation (C.V.) were calculated to measure variability using the two analytical methods, wet chemistry or NIRS; and also the measure the variability of constituents in the feces for each heifer.

4.3 Results and Discussion

4.3.1 NIRS Calibration Equations

The NIRS calibration and validation statistics for fecal constituent and for digestibility coefficient predictions are presented in Table 4.2. The validation statistics ranged from R^2_{val} of 0.80 (SEP = 0.10) for fecal N to R^2_{val} of 0.92 (SEP = 0.95) for fecal ADF. The validation statistics for digestibility coefficients ranged from R^2_{val} of 0.42 (SEP = 4.65) for ADF to R^2_{val} of 0.88 (SEP = 1.28) for starch.

Most fecal NIRS research has been used to improve the nutritional management of grazing cattle by indirectly predicting the nutrient content of the ingested forage (Dixon and Coates 2009). In the present study, our focus was to characterize the pattern of fecal nutrient excretion in feedlot cattle and to determine the digestibility coefficients of nutrients in the diet. It is well known that prediction by NIRS will be optimal if samples within the calibration set encompass a broad range in constituents to be measured, and the unknown samples are of high spectral and chemical similarity (Shenk et al. 1979). It is also common practice in NIRS studies to use a subset of samples for developing calibration equations to be used to predict remaining samples from the same study (Purnomoadi et al. 1996; Purnomaodi et al. 1997). Considering our samples met these criteria, they were suitable for the development of the calibrations for our study and this was evident in the high R^2_{val} values for fecal constituents and the absence of outliers in the sample set whose constituents were not known. It must be cautioned however that the regressions developed from the NIRS analysis of feces in our study are potentially limited to cattle fed the specific diets at the same level of intake as those used in this study.

Our validation statistics were close to or within the range of those reported previously using dried ground cattle feces for starch [R^2_{val} = 0.89 (SEP = 1.09) compared to 0.91 (SEP = 0.57) (Fredin et al. 2014)], N [R^2_{val} = 0.80 (SEP = 0.10) compared to 0.78 (SEP = 0.08) to 0.83 (SEP = 0.10) (Purnomoadi et al. 1996; Cozzolino et al. 2002)], NDF [R^2_{val} = 0.84 (SEP = 0.98) compared to 0.85 (SEP = 0.80) (Cozzolino et al. 2002)] and ADF [R^2_{val} = 0.92 (SEP = 0.95)

Table 4.2. Fecal near infrared calibration and validation statistics determined by partial least squares regression for fecal organic matter (OM), starch, nitrogen, neutral detergent fiber (NDF), acid detergent fiber (ADF), acid detergent lignin (ADL) and for digestibility coefficients for DM, OM, starch, NDF, and ADF.

Item	Population				Calibration				Validation			
	n	Mean± SD	min	max	outliers	factors	R^2_{cal}	*SEC*	n	Mean±SD	R^2_{val}	*SEP*
Fecal constituent (% fecal DM)												
OM	75	85.8±2.30	81.1	90.3	6	11	0.98	0.33	40	86.0±2.43	0.88	1.10
Starch	81	4.81±3.45	0.74	15.8	0	14	0.98	0.54	43	4.63±3.33	0.89	1.09
Nitrogen	65	2.60±0.23	2.24	3.17	0	5	0.89	0.08	30	2.66±0.22	0.80	0.10
NDF	65	50.5±4.71	40.9	61.2	0	8	0.92	1.33	31	52.2±5.07	0.84	0.98
ADF	65	31.7±5.20	22.9	39.8	0	3	0.96	1.16	31	32.5±5.54	0.92	0.95
ADL	68	14.6±3.25	9.35	20.4	0	4	0.92	0.91	33	13.4±2.95	0.87	0.96
Digestibility (% of intake)												
DM	156	74.3±5.99	59.3	90.1	2	8	0.86	2.18	12	77.4±2.84	0.56	2.63
OM	156	76.1±6.23	57.4	91.0	1	7	0.88	2.18	12	80.4±3.21	0.61	2.56
Starch	92	96.0±2.98	87.2	99.4	0	8	0.93	0.74	12	97.4±1.48	0.88	1.28
NDF	154	50.5±10.5	26.4	76.2	1	5	0.48	7.10	12	60.1±7.66	0.71	4.91
ADF	153	42.1±10.8	6.1	71.9	3	5	0.46	7.25	12	46.4±4.43	0.42	4.65

Abbreviations used: R^2_{cal} = coefficient of determination of calibration; *SEC* = standard error of calibration; R^2_{val} = coefficient of determination of validation; *SEP* = standard error of prediction.

compared to 0.74 (SEP = 0.80) to 0.96 (SEP = 0.12) (Purnomoadi et al. 1996; Cozzolino et al. 2002)]; but poorer for ADL [R^2_{val} = 0.87 (SEP = 0.96) compared to 0.94 (SEP = 0.15) (Purnomoadi et al. 1996)]. The comparatively low R^2_{val} for N (0.80) is likely due to the small variation (2.24 – 3.17%) in reference values for this fecal constituent. We observed low SEP values for N which is desired and indicates predictions were close to reference values.

Two NIRS approaches have been used to predict diet attributes including digestibility coefficients. These include the prediction of lignin concentration in combination with a nutrient of interest in both the feed and feces (Purnomoadi et al. 1997), or the direct method whereby digestibility is estimated using NIRS calibrations developed from in vivo digestibility data (Purnomoadi et al. 1997; Lyons and Stuth 1992; Boval et al. 2004). The direct method has been shown to be reliable for most nutrients only when the diet of interest has also been used for calibration development and validation (Purnomoadi et al. 1997). This is because digestibility is affected by multiple and complex interactions between the feed and the animal, and may not be predicted as well as when a digestibility marker is measured in the feed and feces (Purnomoadi et al. 1997). To our knowledge, there are no studies that have attempted to estimate starch digestibility using the direct method, but our results [R^2_{val} = 0.88 (SEP = 1.28)] show potential for its use with external data sets that were not included in the original calibration. This may reflect the high correlation between fecal starch and starch digestibility (Zinn et al. 2002; Corona et al. 2005; Zinn et al. 2007).

4.3.2 Actual and Estimated Fecal Output

Results relating to DMI and actual and estimated fecal output calculated from NIRS predictions of fecal ADL from the total feces collected during both backgrounding and finishing periods are shown in Table 4.3.

In the backgrounding period, there were no differences in DMI or fecal output at the two feeding frequencies. Recovery of ADL in the feces was only slightly greater than 100%, and estimated fecal output did not differ (t = -0.98, P = 0.37) from actual values. Actual and estimated fecal output were highly correlated (r = 0.94, P < 0.01) indicating similar precision in measurement. The C.V. values calculated to measure variation in the data are not shown,

however the daily variation in ADL for each individual heifer was low (C.V. = 0.047), and the variation between replicates using NIRS was also low (C.V. = 0.018).

Feeding frequency had no effect on DMI or fecal output in the finishing period. There was incomplete recovery of ADL, and therefore estimated fecal output had a tendency ($t = 2.64$, $P = 0.05$) to overestimate the actual values (on average 0.12 kg DM /d higher, SEM = 0.045). Similar to the backgrounding period, actual and estimated fecal output were highly correlated ($r = 0.95$, $P < 0.01$), the average daily variation in ADL for each heifer was low (C.V. = 0.073), as well as for NIRS predicted marker determination (C.V. = 0.026).

It is important to note that although lignin has been used as an internal marker in digestion studies, and has been used for development of fecal NIRS calibrations for predicting digestibility (Purnomoadi et al. 1996; Dixon and Coates 2009), data indicate that difficulties exist with recovery and quantification of this cell wall component (Fahey and Jung 1983). Reports have indicated that non conjugated phenolic units within lignin can bind to low molecular weight sugars or nitrogenous compounds, increasing estimates of lignin recovery (Fahey and Jung 1983). Studies have also indicated that lignin carbohydrate complexes can be solubilized by anaerobic fungi in the rumen (Kajikawa et al. 2000). In addition, the 72% sulphuric acid method used in this study measures cutin and Maillard-type browning products, overestimating true lignin (Goering and Van Soest 1970). Another downside to using ADL is the considerable variation that exists for duplicate determinations within a sample. Cochran et al. (1988) reported the average within-sample C.V. for duplicate marker determinations ranged from approximately 0.02 to 0.12 for feed, orts, and feces, and in concentrate supplements the variability was more erratic and typically larger. The main contributing factor to larger C.V. values is extremely low concentrations of lignin in concentrates, which in predominantly forage diets, fecal output or digestibility predictions would be relatively unaffected, but for high-concentrate diets, even small levels of variation would be magnified.

In our study, these factors did not affect our results in the backgrounding period, as ADL recovery was close to 100%, and we observed low variability in duplicate measurement using NIRS, generating accurate and precise estimates of fecal output. It is important when developing NIRS calibrations to ensure that there is low variability in the reference method as well, and for ADL, the C.V. cutoff for duplicate samples we applied was 0.045.

In the finishing period, the low ADL recovery was likely a result of degradation of lignin within the gut (Fahey and Jung 1983). This lead to the overestimation in fecal output compared to actual values. Cochran et al. (1988) evaluated ADL as a marker for predicting digestibility of warm-season grass diets and the influence of level of sorghum grain supplementation, and found that fecal recovery of ADL declined linearly as level of supplementation increased. They also observed high variability in fecal recovery of ADL, supporting earlier conclusions concerning the inadequacy of ADL as an internal marker (Fahey and Jung, 1983). Although the amount of grain in the diets fed in our study is much higher than the levels tested in Cochran et al. (1988), we noticed a similar decline in ADL recovery from backgrounding to finishing periods. We did not however observe the high degree of variability in our fecal ADL measurements.

Using ADL as a marker resulted in accurate and precise estimates of fecal output for heifers fed a backgrounding diet, however fecal output was overestimated in the finishing period due to incomplete recovery of ADL in the feces. Using NIRS predicted ADL as a marker in commercial feedlots is an attractive possibility for calculating fecal output and digestibility due to its precision we found in measurement. However, when diets are very low in lignin content, discrepancies between actual and predicted fecal output may occur more frequently.

4.3.3 Daily Variation in Fecal Output

In the backgrounding period, a sinusoidal pattern was observed for fecal output (kg DM/4 h) for both feeding frequencies (Figure 4.1A). The excretion varied over 24 h ($P < 0.01$), with the greatest output occurring for the 0- to 4-h and the 16- to 24-h time period relative to feeding. There were no effects of feeding frequency ($P = 0.64$) or any interaction between feeding frequency and time ($P = 0.47$).

For heifers fed a finishing diet, both feeding frequencies exhibited sinusoidal patterns of excretion, and a feeding frequency by time interaction was observed ($P = 0.02$; Figure 4.1B). The greatest output for FF1 heifers occurred in the morning between 0 and 8 h after the first feeding, followed by a reduction between 8 and 24 h after feeding. In contrast, FF2 heifers exhibited multiple peaks in fecal output, at 0 to 4 h, 8 to 12 h, and 20 to 24 h (Figure 4.1B).

The increase in fecal output for all heifers in the study for the 0- to 4-h sampling interval is largely due to the higher incidence of defecation when cattle arise in the morning. The sinusoidal excretion pattern observed over 24 h is likely a reflection of the number of meals consumed over

the day. Passage of digesta through the intestinal tract is triggered by distension or tactile signals that occur several hours after each meal (Sellers and Stevens 1966). When there is minimal competition for feed, pastured, feedlot, group (Ray and Roubicek 1971) and individually fed

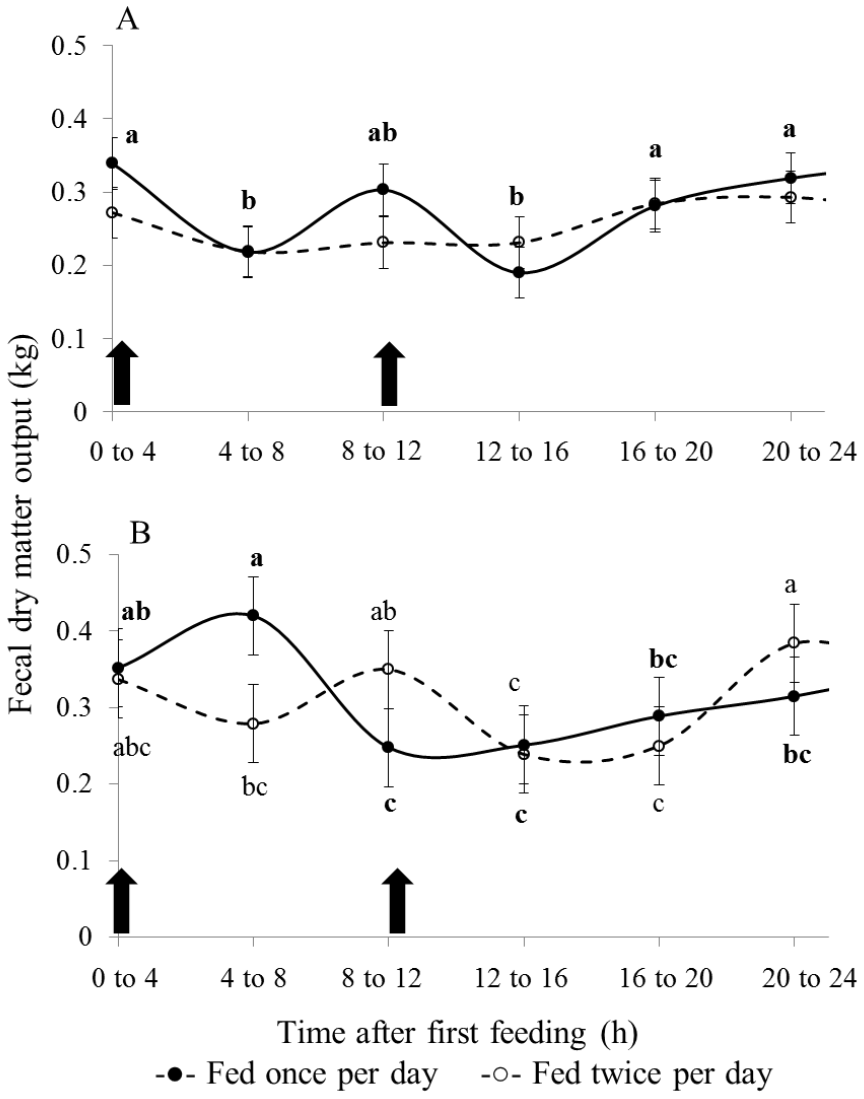


Figure 4.1. Diurnal variation in fecal output of dry matter (DM) in heifers fed once (solid line) or twice per day (dashed line) in (A) the backgrounding period (44% dry rolled barley; 44% barley silage) and (B) the finishing period (80% dry rolled barley; 9% barley silage). A feeding frequency interaction was observed in the finishing period only ($P = 0.02$). Peaks are assigned letters to represent differences that are significant ($P<0.05$) for heifers fed once per day (bold font) and twice per day (regular font). Arrows represent the two feeding times.

(Putnam and Davis 1963) cattle typically exhibit a biphasic feeding pattern over the day (Gibb 2000). The primary meal occurs early to mid-morning, coinciding with the time of first feed delivery (around sunrise), with a second meal being consumed in late afternoon and early evening (Gibb et al. 1998; Schwartzkopf-Genswein 2003).

Increased meal frequency and larger meals in the afternoon occur in cattle that are limit-fed (Gibb et al. 1998) as compared to those that are fed ad libitum (Stricklin 1986). The feeding frequency by time interaction in the finishing period likely occurred due to FF2 heifers consuming less feed in the morning as their meal size was smaller than FF1 heifers. It is also possible that FF1 heifers had increased eating rates in the morning due to the preference for fresh feed, a characteristic associated with peak intake in cattle (Hayton et al. 2012). Differences in fecal excretion patterns in FF1 and FF2 heifers were more evident in the finishing than backgrounding period, likely due to the more rapid consumption and digestion of grain accentuating the impact of time of feeding on fecal excretion patterns.

In the feedlot industry, cattle are typically fed up to 3 times per day in daylight hours, with the first meal delivered shortly after sunrise. Competition for feed can be intense in confined cattle with the first meal of some cattle being delayed if bunk space is limiting. However, assuming that feed is not limited, fecal excretion patterns of these individuals should be similar after they have consumed a meal. From a practical perspective, it would be desirable to collect fecal pats from feedlot cattle early in the morning within 4 h after the first feeding, as large amounts of fresh feces are excreted as cattle rise and approach the feed bunk. Larger samples are less likely to be contaminated with dirt and bedding and provide more accurate predictions of fecal composition and digestibility. This may be most important for cattle fed finishing diets once or twice daily, as fecal output may be reduced in the late afternoon and evening.

Table 4.3. Daily dry matter intake, fecal output, and marker recovery of heifers fed backgrounding and finishing diets once or twice per day

Item	FF		SEM	*P* value
	1x	2x		
Backgrounding				
Dry matter intake (kg/d)	6.68	6.17	0.432	0.45
Fecal output (kg DM/d)				
Actual	1.63	1.51	0.58	0.59
Estimated	1.59	1.49	0.12	0.58
ADL recovery (%)	102.2	101.8	3.38	0.94
Finishing				
Dry matter intake (kg/d)	9.46	8.66	1.100	0.63
Fecal output (kg DM/d)				
Actual	1.86	1.83	0.96	0.94
Estimated	2.01	1.91	0.24	0.79
ADL recovery (%)	91.6	96.0	3.84	0.46

Abbreviations used: FF = feeding frequency.
Estimated fecal output was approximated using the equation: Fecal output = DMI (kg DM/d) × (%ADL in feces/%ADL in diet)
Marker recovery was approximated using the equation: Marker recovery (%) = [%ADL in feces × fecal output (g DM)] / ADL in diet (g, DM).

4.3.4 Diurnal Variation in Fecal Constituent Excretion

For heifers fed a backgrounding diet, fecal DM concentration (%) differed over 24 h (P = 0.01) with DM being the lowest for the 0- to 4-h and 20- to 24-h intervals relative to feeding. Fecal NDF and ADF also showed variation over 24 h, with greatest output occurring between 12 to 16 h. Fecal constituent concentrations were not affected by feeding frequency ($P > 0.05$), and there were no interactions between feeding frequency and time ($P > 0.05$). Measured fecal DM, NDF and ADF concentrations at specific individual sampling times differed ($P > 0.05$) from those calculated using total collection, whereas no differences were observed for the other constituents (Table 4.4).

For heifers fed the finishing diet, greater 24-h variation in fecal constituents was observed, and except for fecal N, all fecal constituent concentrations measured at individual sampling times displayed some differences ($P > 0.05$) from those derived using total collection (Table 4.4). Fecal DM, starch, NDF, ADF, and ADL concentrations differed over 24 h ($P < 0.05$), with a trend (P = 0.05) observed for OM (Table 4.4). Fecal DM (%) was lowest from 0 to 4 h, and highest from 8 to 12 h, and both intervals differed from the average DM concentration determined from total collection. Fecal starch concentration peaked at 4 to 8 h, and was lowest from 16 to 24 h, and differed from the total collection value at these time intervals. Compared to fecal starch, changes in the other fecal constituents were more continuous and did not display as many dramatic increases or decreases. None of the fecal constituent concentrations were affected by feeding frequency, but interactions between feeding frequency and time were observed for fecal N, NDF and ADF. Fecal N, NDF and ADF concentrations were more variable over 24 h in FF1 heifers compared to FF2, and no differences were observed for fecal N excretion in FF2 heifers. When collected from FF1 heifers, fecal NDF and ADF concentrations differed from their total collection value in the evening (8 to 16 h after feeding for NDF and 12 to 16 h for ADF). Fecal ADL also differed from the total collection value in the evening samples, 12 to 20 h after first feeding.

There has been limited work to characterize the diurnal excretion patterns of dietary constituents in ruminants, with most studies describing variation in the excretion of starch (Leonard et al. 1989; Caetano 2008; Fredin et al. 2014) and digestibility markers such as lignin (Kane et al. 1952; Kanani et al. 2012). Fredin et al. (2014) sampled feces from lactating cows fed alfalfa silage and corn silage in equal proportions ad libitum, and two different types of grain

mixes containing dry ground shelled corn either at 15.4% or 24.4% of the diet DM as described in Akins et al. (2014). Fecal samples were collected at 0 and 12 h after feeding, with greater fecal starch concentrations found in the morning (2%) as compared to the evening (1.6%). Caetano (2008) sampled hourly from four Nellore steers fed ground corn as the sole starch source (28.5% corn in the diet DM) once daily. A polynomial pattern of starch excretion (fecal starch = 0.12 - 0.0074 × time + 0.00026 × time^2) was observed, with the greatest fecal starch levels measured in the morning (mean 7.5%), followed by a drop 10 to 18 h after feeding (mean 2.3%). Leonard et al. (1989) measured changes in fecal starch concentration over three 8-h periods from Angus steers offered whole corn (2.27 kg/d) and chopped fescue hay for ad libitum intake once daily. The greatest concentrations of fecal starch were also observed in the morning (21.3% in the first 8 h of sampling) as compared to later in the day (mean of 13.4% in the remaining 16 h). In the same study, grinding corn resulted in more fecal starch being excreted in the evening (10.3% at 16 to 24 h) compared to the morning (7.76% at 0 to 8 h, and 4.83% at 8 to 16 h). These findings suggest that the degree of grain processing affects the optimal fecal sampling time for estimations of starch concentration or digestibility.

In the backgrounding period, fecal starch concentration did not vary significantly over 24 h. Although the level of grain fed in the backgrounding period (44% of dietary DM) was greater than in previous studies with corn (Leonard et al. 1989; Caetano. 2008; Fredin et al. 2014), barley is more digestible than corn, and could explain low fecal starch levels and the absence of diurnal fluctuations. Another possibility for the failure to observe statistical differences could be a result of large residuals of analysis. For low starch values, the average C.V. for duplicate samples were much higher compared to wet chemistry (0.33 versus 0.055), indicating that more replicates should be used when predicting samples where fecal starch is expected to be low (≤ 2% fecal starch). When fecal starch values increased (as observed in the finishing period), the average C.V. for NIRS analysis were only slightly higher than wet chemistry (0.067 versus 0.053). Due to the possibility for variability, closer inspection of statistical differences for feeding frequency and time effects was done. All differences between feeding frequencies and between time of sampling fell well within the 95% confidence intervals, however the PDIFF estimate between 0 to 4 h and 4 to 8 h showed a significant difference (P = 0.02). This inconsistency can occur with small datasets, and although the overall likelihood ratio tests should be more robust than the pairwise comparison tests (Wald tests) (C. Waldner, personal

communication,), it suggests that with less variability in starch measurements, the difference between these two time intervals may be regarded as significant.

In the finishing period, the increase in starch excreted early in the day aligns with the findings of other studies where greater starch concentration in cattle feces was observed in the morning as compared to the evening (Fredin at al. 2014; Caetano. 2008; Leonard et al. 1989). Since grain has a smaller particle size and higher specific gravity as compared to forage particles, it has a lower ruminal retention time, potentially leading to more variable excretion over 24 h. Upon entering the rumen, grain tends to sink and remain in the same location, giving it an early entrance to the reticulum and omasum compared to forage (Schalk and Amadon 1928). Passage of forage is more dependent on contractions of the reticulum and rumen, along with regurgitation and mastication (Schalk and Amadon 1928) and consequently the longer retention time results in a more uniform passage rate and fecal excretion pattern. Upon closer examination of the 95% confidence intervals, it was noted that the difference between the two feeding frequencies was quite large (2.09% greater fecal starch in FF2 heifers), and the confidence intervals were exceptionally broad (-8.09 to 3.91) indicating the possibility of an effect of feeding frequency or an interaction between feeding frequency and time on fecal starch excretion. This enforces the need for standardizing sampling times based on different diets and management practices.

Fecal N originates from dietary, microbial, and endogenous sources, and would not be solely influenced by the passage rate of feed particles. Endogenous N in feces consists of sloughed epithelial tissue and undigested residues of enzyme secretion, and is assumed to also be constant per unit DMI (Strozinski and Chandler 1972). Microbial N is related to the large intestinal fermentation of DMI (Mason 1969; Mason and Frederickson 1979), which is more variable in cattle fed a high fermentable diet once per day, explaining the variation observed in N excretion in heifers fed a finishing diet once per day.

Although there are no studies to compare fecal fiber concentrations over 24 h, our results show significant changes in fecal fiber that are of low magnitude, and ones that vary depending on feeding frequency in the finishing period. Unlike low starch concentrations, the ability to measure NDF, ADF, and ADL using NIR are more precise with C.V. values all below 0.026. The greatest excretion of all fiber fractions and ADL are observed between 8 to 20 h after first feeding, coinciding quite closely to the time of reduction in fecal starch concentration.

High forage diets are consumed more slowly than high concentrate diets with more time spent chewing and ruminating (Bailey 1961; Beauchemin 1991). As a result, excretion of fiber would be expected to be more uniform over the day. High grain diets have a reduced ruminal retention time and are more variable in constituent excretion, particularly of starch. It is likely that in the finishing period, declines in ADF concentration in the morning and its increase in evening reflect the dilution of ADF by starch in the feces in the morning and a reduction in fecal starch in the evening. Since fecal fiber concentrations were more variable over 24 h in cattle fed only once versus twice per day, fecal samples collected from cattle fed multiple times per day at any time point will more accurately represent fiber levels in a 24-h fecal composite.

Kanani et al. (2012) fed cattle bermudagrass hay twice daily with a 9-h interval between meals and measured daily excretion of alkaline peroxide lignin at 6-h intervals. Alkaline peroxide lignin concentrations in fecal samples were similar across sampling times; a result that is consistent with our observations of the excretion of ADL with 4-h intervals in the backgrounding period. The concentration of this constituent is greater in forage than in grain, and as we observed, mirrors the excretion of forage particles. In a separate study, diurnal variation of lignin excretion was measured in three dairy cows fed at 0430 and 1330 h (Kane et al. 1952). The diet consisted of (DM basis) 80% alfalfa hay and corn silage and 20% concentrate. The percentage of lignin in feces differed ($P \leq 0.05$) between the morning and afternoon, but did not appear to be influenced by the time of feeding. The optimal time for sampling was proposed to be between 8.5 h to 10.5 h after the first feeding, as this period most closely resembled the average lignin concentration over 24 h (Kane et al. 1952). In contrast, we did not detect differences in ADL concentration between individual fecal samples and the 24-h composite when heifers were fed the backgrounding diet, but we did observe differences in the finishing period, with samples collected between 0 and 12 h after feeding being similar to total collection. Our results indicate that there is little effect of sampling time on the excretion of fecal constituents in heifers fed the backgrounding diet, and a single sample at any time over 24 h would generate estimates similar to those derived from total collection for OM, starch, N, and ADL. Differences in NDF and ADF were small and may require standardization of timing of sampling if very accurate estimations are required. In contrast to fecal starch, fecal fiber levels have not shown to be of much value for feedlot cattle; hence the differences may be negligible at a commercial setting. Since there were no changes in ADL over 24 h, it is likely that any time

point can be used when applying the marker method to predict digestibility. When fecal starch concentration is expected to be low it is recommended to measure each sample multiple times to generate more accurate and precise results. In contrast, when heifers were fed the finishing diet, different sampling times resulted in different fecal constituent concentrations over 24 h. It is recommended that time of sampling be standardized when collecting fecal samples in order to be representative of total collection, and fecal starch concentration in morning sampling of feces (0-4 h) approximates closely to fecal starch concentrations derived from total collection.

4.3.5 Accuracy of Using Fecal Nutrients to Predict Apparent Total Tract Digestion

In the backgrounding period, the fecal starch concentration of each heifer was linearly related to aTTD when a 24-h composite and the average of individual fecal samples collected at 4-h intervals were used to estimate digestibility (R^2 = 0.80-0.96, $P < 0.05$). The relationship was weaker (R^2 = 0.60, P = 0.07) for samples collected at separate 4-h intervals (Table 4.5). Apparent TTD of starch could be predicted using the 24-h composite sample as [aTTD of starch (%)] = 100 – (0.69 × FS%) (R^2 = 0.96, $P < 0.01$, where FS% = fecal starch concentration as a percentage of DM), with equations being similar using samples collected at 4-h intervals (Table 4.5).

A weak linear relationship between fecal NDF concentration and aTTD of NDF (R^2 = 0.57, P = 0.08) was observed using the 24-h composite fecal sample, with similar linear relationships observed using fecal samples collected between 12 and 24 h after feeding ($0.05 < P < 0.10$). Linear relationships between fecal nutrients and their digestibility were not observed for any of the other nutrients in the backgrounding period.

In the finishing period, fecal starch was linearly related to aTTD of starch using starch concentrations derived from the 24-h fecal composites as well as the fecal samples collected at 4-h intervals (R^2 = 0.84 - 0.98, $P < 0.01$). Apparent total tract digestibility of starch could be predicted from the 24-h composites as [aTTD of starch (%)] = 100 – (0.53 × FS%) (R^2 = 0.98, $P < 0.01$; Table 4.5). However, linear relationships were not observed between fecal concentration and the predicted digestibility of any of the other nutrients. A linear relationship between fecal NDF concentration and aTTD of NDF was observed using only one fecal spot sample (R^2 = 0.70, P = 0.04; data not shown).

Table 4.4. Fecal concentrations of DM and near infrared predicted organic matter (OM), starch, nitrogen (N), neutral detergent fiber (NDF), acid detergent fiber (ADF) and acid detergent lignin (ADL) in a 24 h fecal composite sample and in spot fecal samples collected at 4 h intervals from heifers fed backgrounding and finishing diets once or twice per day

Item	24 h COMP[z]	FF		SEM	FF[y]	Time after first feeding (h)						SEM	*P* value		
		1	2			0 to 4	4 to 8	8 to 12	12 to 16	16 to 20	20 to 24		FF	T	FF×T
Backgrounding															
DM (%)	17.3±1.02	17.8	17.2	0.56	1,2	*17.0*b*	17.5*ab*	*18.0*a*	17.5*ab*	*17.9*a*	17.2*b*	0.45	0.47	0.01	0.83
OM (% DM)	83.7±0.45	83.9	83.3	0.24	1,2	83.8	83.5	83.2	83.5	83.8	83.9	0.23	0.18	0.06	0.80
Starch (% DM)	2.24±0.80	2.33	2.15	0.525	1,2	1.99	2.49	2.42	2.17	2.19	2.16	0.407	0.82	0.29	0.84
N (% DM)	2.48±0.12	2.44	2.54	0.076	1,2	2.51	2.51	2.51	2.45	2.46	2.50	0.057	0.39	0.17	0.62
NDF (% DM)	55.7±1.72	56.3	55.4	1.09	1,2	55.6*abc*	55.5*bc*	55.8*abc*	*56.5*a*	*56.6*ab*	*55.1*c*	0.84	0.56	<0.01	0.99
ADF (% DM)	38.5±1.23	38.4	38.0	0.90	1,2	*37.7*c*	38.0*bc*	38.2*bc*	38.8*a*	38.5*ab*	37.9*c*	0.67	0.76	0.02	0.98
ADL (% DM)	17.2±0.54	17.3	17.1	0.36	1,2	17.1	17.2	17.2	17.4	17.3	17.0	0.28	0.66	0.37	0.90
Finishing															
DM (%)	20.8±1.50	22.0	20.3	0.81	1,2	*19.7*c*	21.3*ab*	*22.0*a*	21.4*ab*	21.4*ab*	21.0*b*	0.65	0.21	<0.01	0.67
OM (% DM)	87.9±1.77	87.6	88.2	0.40	1,2	*88.3	*88.4	87.9	87.7	87.3	87.9	0.35	0.31	0.05	0.65
Starch (% DM)	7.24±2.59	6.29	8.38	1.529	1,2	7.69b	*8.53*a*	7.33*bc*	7.95*ab*	*5.92*d*	*6.58*cd*	1.119	0.39	<0.01	0.70
N (% DM)	2.79±0.14	2.78	2.80	0.088	1	2.84a	2.79ab	2.73bc	2.70c	2.80ab	2.83a	0.091	0.88	0.20	0.02
					2	2.78	2.80	2.84	2.80	2.79	2.80				
NDF (% DM)	49.5±4.78	52.2	47.8	2.55	1	50.2c	51.3bc	*54.1a	*53.4a	52.8ab	51.7bc	2.61	0.28	<0.01	<0.01
					2	48.1ab	46.8b	47.3b	47.6b	49.1a	47.8b				
ADF (% DM)	27.5±1.23	28.3	26.9	0.62	1	27.3c	27.8bc	29.0a	*29.1a	28.5ab	28.2b	0.67	0.17	<0.01	<0.01
					2	26.8abc	26.3c	26.4bc	27.0ab	27.6a	27.1ab				
ADL (% DM)	11.4±0.44	11.8	11.3	0.21	1,2	11.5*abc*	11.3*bc*	11.7*a*	*11.8*a*	*11.7*ab*	11.3*c*	0.21	0.14	0.03	0.11

Abbreviations: FF = feeding frequency; T = time of sampling.
Italicized letters represent significant differences (P<0.05).
[z]24 h COMP = constituent concentrations in the composite fecal samples collected over 24 h.
[y] fecal constituent concentrations are reported separately where a feeding frequency by time of sampling interaction exists.
* indicates the time points that differed in fecal constituent concentration compared to those in the 24 h composites (P<0.05).

Table 4.5. Regression statistics showing linear relationships between fecal starch and apparent total tract starch digestibility using a 24 h fecal composite or spot samples collected at 4 h intervals from heifers fed backgrounding and finishing diets once or twice per day

Item	24 h COMP[z]	Time after first feeding (h)					
		0 to 4	4 to 8	8 to 12	12 to 16	16 to 20	20 to 24
Backgrounding							
R^2	0.96	0.60	0.98	0.80	0.85	0.96	0.96
P value	<0.01	0.07	<0.01	0.02	<0.01	<0.01	<0.01
y=	100-0.69x	99.9-0.73x	99.9-0.58x	100-0.80x	99.5-0.50x	99.6-0.51x	99.7-0.57x
Finishing							
R^2	0.98	0.97	0.88	0.95	0.86	0.89	0.84
P value	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
y=	100-0.53x	99.5-0.40x	100-0.48x	100-0.57x	100-0.46x	100-0.65x	99.6-0.49x

Abbreviations: R^2 = coefficient of determination, [z]24 h COMP = composite fecal samples collected over 24 h. Apparent total tract starch digestion for backgrounding period: % of starch intake = 98.4 ± 0.57%, and for finishing period: % of starch intake = 96.4 ± 1.40%.

Fernandez et al. (1982) predicted aTTD from fecal starch for lactating cows fed high-starch diets (mean = 34% of DM) in which sorghum grain and silage were the only starch sources (aTTD of starch % = 108.8% – (1.82 × FS%); n = 16, R^2 = 0.64). More recently, other linear relationships between fecal starch and aTTD of starch in lactating cows (mean = 27% starch DM) were derived with the following equations : aTTD of starch % = 98.7% – 1.76 × FS%; n = 72, R^2 = 0.73; $P < 0.01$ (Ferguson 2006, as referenced by Grant 2010); aTTD of starch % =100.0% – (1.25 × FS%); n = 16, R^2 = 0.94, $P < 0.01$ (Ferrareto and Shaver 2010); and aTTD of starch % = 100.0% – (1.25 × FS%); n = 564, R^2 = 0.94; $P < 0.001$ (Fredin et al. 2014). The greater slopes in the previous studies compared to our findings for backgrounded heifers reflect the lower estimates of total tract starch digestion or differences in total mean retention time in lactating cows. Some of these differences could arise from differences in grain processing and grain type between these studies and ours. The aTTD of starch in dry-rolled barley is often greater than dry-rolled corn or sorghum (Theurer 1986). Lactating dairy cows would also have higher DMI than the beef heifers in our study, a factor that could lead to more rapid passage rates of the grain and lower starch digestibility. A greater dietary NDF content in the diet of previous studies may have also diluted fecal starch concentrations.

For the finishing period, our results are comparable to estimates of total tract starch digestion in feedlot steers; Corona et al. (2005) aTTD of starch (%) = 102.4 – (0.72 × FS) (n = 16; R^2 = 0.97; $P < 0.01$); and Zinn et al. [2002; 64-trial summary; aTTD of starch (%) = 100.5 – (0.65 × FS); R^2 = 0.91]. The slightly lower slopes in the present study may have resulted from a greater aTTD of barley starch as compared to corn starch.

Our results indicate that with a backgrounding diet, the aTTD of starch can be predicted using spot fecal samples and that this relationship is even more predictive with a finishing diet. Fredin et al. (2014) poorly predicted (R^2 = 0.18, n = 390) total tract NDF digestibility from fecal NDF concentration. In our study, relationships between NDF concentration and digestibility were only observed for a few of the spot fecal samples in the backgrounding period and only for one spot sample during finishing. This suggests that spot fecal sampling is not an effective predictor of NDF digestibility, at least with high grain diets. Compared to starch, NDF is substantially less digestible and changes in its fecal concentrations are less likely to reflect aTTD of NDF. Furthermore, with high grain diets the low NDF concentration may also make the prediction of aTTD of NDF more challenging. The ability to predict the aTTD of starch may be

in part due to low variability in starch digestibility as it was consistently near the y-intercept of 100% digestibility. As NDF is less digestible than starch, the coefficients of variation were larger, reducing the predictive relationship to total-tract digestibility (Fredin at al. 2014).

4.3.6 Accuracy of Predicting Total Tract Digestibility Using ADL

Time of sampling did not affect ($P \geq 0.23$) predictions of eTTD for any of the fecal constituents in the backgrounding period (Table 4.6). Furthermore, eTTD of nutrients estimated using fecal samples collected at 4-h intervals did not differ from that estimated using the 24-h composite sample (Table 4.6). Regression analysis indicated that most spot fecal samples collected from individual heifers could be used to predict the eTTD of all nutrients (Table 4.7). In the backgrounding period, the eTTD of DM and OM could be predicted using fecal samples collected at any time point ($P < 0.05$), and eTTD of starch and NDF were successfully predicted at 5 of the 6 time points ($P \leq 0.01$), and ADF at 4 of the 6 time points ($P \leq 0.01$, and ADF at 16 to 20 h, $P = 0.08$). For these nutrients, fecal samples collected 0 to 4 h after feeding did not provide accurate estimations of their digestibility.

In the finishing period, time of sampling only affected ($P \leq 0.03$) predictions of eTTD of OM and starch, and a trend ($P = 0.06$) for DM (Table 4.6). Aside from starch, the average eTTD of all nutrients did not differ when estimated using spot samples as compared to the 24-h composite fecal sample (Table 4.6). Regression equations showed that the accuracy and precision of measurements varied with time of sampling for predictions of eTTD of DM and OM (Table 4.7). The eTTD of starch, NDF and ADF could be predicted with high accuracy and precision using any of fecal samples collected at 4-h intervals ($P \leq 0.02$), excepting for the sample collected at 20 h to estimate ADF ($P = 0.07$).

Regression analysis measures the linear relationship between individual eTTD based on their 24-h fecal composite sample and each spot fecal sample for each individual heifer, generating 6 equations (Table 4.7). For reasons unknown, linear relationships between eTTD for all nutrients were not observed for samples collected 4 h after feeding. Perhaps after resting overnight, distribution of nutrients is more variable among individual heifers making it difficult to accurately predict digestibility coefficients. It is also possible that variability in the method of measurement affected the regression equations. Despite the lack of linear relationships between individual eTTD derived from 24-h composites and individual eTTD derived from certain time

points, the estimates at each time point are such that when the digestibility coefficients were averaged, no differences were detected. This indicates that eTTD determined from spot samples collected from multiple cattle fed a backgrounding diet may generate values that are close to those determined from a 24-h fecal composite.

In the finishing period, differences in the eTTD of starch can be attributed to the diurnal variation in fecal starch concentration. In the 8-h fecal sample, when starch concentrations were greatest (Table 4.4), eTTD was predicted to be the least relative to other time points (Table 4.6). The reverse relationship was observed when starch concentrations were lowest in the fecal samples collected at 20 h. Differences in fecal ADF concentration (Table 4.4) did not affect the eTTD of ADF as ADL is a constituent of ADF and thus concentrations of these constituents are not independent. Although regression equations showed that predicted eTTD for DM and OM for individual heifers using spot fecal samples would not be accurately predicted, taking an average over multiple cattle approximated the average value determined from the 24-h composite. Our results agree with Kanini et al. (2014), where eTTD of DM using indigestible detergent fiber, acid detergent insoluble ash, and acid peroxide lignin as markers in fecal samples collected four times daily generated estimates of digestibility of bermudagrass hay that were similar to those derived using total collection. Others have found that fluctuations in the fecal concentration of lignin do not preclude its use for estimating eTTD of DM or crude fiber, but it does appear to be less suitable for measuring the eTTD of protein, N-free extract, and fat (Kane et al. 1952).

Our results indicate that when using a single sampling approach and averaging digestibility coefficients from multiple cattle, predictions using ADL closely agree to that determined using a 24-h composite fecal sample collected from heifers fed a backgrounding diet. Results are less reliable when only spot fecal samples are used from individual animals. The results are similar for a finishing diet, aside from starch, in which time of sampling must be considered if comparing eTTD derived at different time points. For other nutrients, using spot fecal samples collected from multiple cattle can be used to estimate digestibility coefficients derived from 24-h composite samples. Morning sampling for predictions of eTTD of starch are recommended to approximate closely with total collection estimates.

Table 4.6. Estimated total tract digestibility coefficients for DM, organic matter (OM), starch, neutral detergent fiber (NDF), and acid detergent fiber (ADF) using the ratio of acid detergent lignin (ADL) in the total mixed diet and in a 24 h fecal composite or in spot fecal samples collected at 4 h intervals for heifers fed backgrounding and finishing diets once or twice per day

Digestibility	24h COMP[z]	FF		SEM	Time after first feeding (h)						SEM	P value		
		1x	2x		0 to 4	4 to 8	8 to 12	12 to 16	16 to 20	20 to 24		FF	T	FF×T
Backgrounding														
DM	76.0±0.77	76.1	75.8	0.52	75.8	76.0	76.0	76.2	76.1	75.7	0.41	0.70	0.35	0.93
OM	78.2±0.75	78.2	78.1	0.53	78.0	78.2	78.3	78.4	78.2	77.8	0.41	0.87	0.23	0.94
Starch	98.4±0.58	98.3	98.4	0.39	98.5	98.2	98.3	98.5	98.4	98.4	0.30	0.84	0.37	0.85
NDF	57.4±1.07	57.3	57.4	0.75	57.4	57.6	57.4	57.3	57.1	57.4	0.59	0.88	0.88	0.98
ADF	48.1±1.06	48.1	47.9	0.70	48.3	48.2	48.0	47.7	47.8	47.9	0.57	0.87	0.83	0.98
Finishing														
DM	78.3±0.81	78.7	77.8	0.39	78.2	77.9	78.4	78.6	78.5	77.8	0.34	0.15	0.06	0.13
OM	79.8±0.75	80.4	79.3	0.33	79.7*abc*	79.4*c*	80.0*ab*	80.2*a*	80.3*a*	79.5*bc*	0.32	0.09	0.03	0.19
Starch	96.1±1.48	96.7	95.4	0.86	95.9*bc*	*95.3*d*	96.1*bc*	95.8*cd*	*96.8*a*	96.4*b*	0.63	0.36	<0.01	0.77
NDF	65.2±2.11	64.5	66.0	1.22	65.7	65.2	65.1	65.5	65.1	64.6	0.91	0.43	0.24	0.79
ADF	44.6±1.49	44.3	44.1	1.44	43.6	44.7	44.9	44.6	44.4	43.3	1.31	0.93	0.78	0.57

Abbreviations: FF = feeding frequency; T = Time.

Italicized letters represent significant differences (P<0.05).

Marker recovery was approximated using the equation: Marker recovery (%) = [%ADL in feces × fecal output (g DM)] / ADL in diet (g, DM).

z24 h COMP = estimated total tract digestibility of nutrients calculated using fecal samples obtained by total collection and ADL.

* indicates the time points that differed in estimated total tract digestibility of nutrients compared to those using the 24 h composites (P<0.05).

Table 4.13. Regression statistics showing linear relationships between estimated apparent total tract digestion of nutrients determined using the ratio of acid detergent lignin in the total mixed diet and in 24 h fecal composites and the ratio of acid detergent lignin in the total mixed diet and spot fecal samples collected at 4 hour intervals in heifers fed backgrounding and finishing diets once or twice per day

Item	Time after first feeding (h)					
	0 to 4	4 to 8	8 to 12	12 to 16	16 to 20	20 to 24
Backgrounding						
Dry matter						
R^2	0.76	0.84	0.76	0.97	0.81	0.86
P value	0.02	<0.01	0.02	<0.01	0.015	<0.01
y=	26.1+0.66x	-8.41+1.11x	0.56+0.99x	14.9+0.80x	30.8+0.59x	12.4+0.84x
Organic matter						
R^2	0.74	0.89	0.84	0.97	0.88	0.89
P value	<0.03	<0.01	<0.01	<0.01	<0.01	<0.01
y=	26.1+0.67x	-19.4+1.25x	-2.65+1.03x	15.7+0.80x	28.7+0.63x	14.4+0.82x
Starch						
R^2	0.63	0.93	0.87	0.87	0.98	0.96
P value	0.06	<0.01	<0.01	<0.01	<0.01	<0.01
y=	-4.12+1.04x	20.9+0.79x	-15.5+1.16x	28.8+0.71x	28.0+0.71x	20.0+0.80x
NDF						
R^2	0.49	0.91	0.88	0.93	0.85	0.92
P value	0.12	<0.01	<0.01	<0.01	<0.01	<0.01
y=	ns	-5.85+1.10x	2.73+0.95x	14.6+0.75x	13.3+0.77x	18.7+0.67x
ADF						
R^2	0.21	0.87	0.94	0.93	0.59	0.85
P value	0.36	<0.01	<0.01	<0.01	0.08	<0.01
y=	ns	7.55+0.84x	13.2+0.73x	13.6+0.72x	16.7+0.65x	13.2+0.73x
Finishing						
Dry matter						
R^2	0.58	0.77	0.27	0.65	0.65	0.70
P value	0.08	0.02	0.29	0.05	0.05	0.04
y=	18.3+0.76x	16.8+0.79x	ns	25.7+0.66x	29.4+0.62x	12.5+0.84x
Organic matter						
R^2	0.45	0.84	0.68	0.61	0.61	0.75
P value	0.15	<0.01	0.14	0.07	0.07	0.03
y=	ns	21.1+0.74x	ns	26.8+0.66x	34.2+0.57x	12.6+0.84x
Starch						
R^2	0.97	0.92	0.92	0.89	0.93	0.86
P value	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
y=	17.3+0.82x	0.48+1.0x	-14.7+1.15x	3.24+0.97x	-35.1+1.35x	-1.48+1.01x
NDF						
R^2	0.96	0.99	0.97	0.97	0.88	0.99
P value	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
y=	9.18+0.85x	7.28+0.89x	4.81+0.93x	-5.04+1.07x	-20.9+1.32x	2.69+0.97x
ADF						
R^2	0.81	0.98	0.91	0.87	0.59	0.79
P value	0.01	<0.01	0.88	<0.01	0.07	0.02
y=	21.5+0.51x	16.6+0.62x	-6.51+1.13x	3.88+0.91x	9.65+0.78x	9.97+0.80x

Abbreviations: R^2 = coefficient of determination, NDF = neutral detergent fiber; ADF = acid detergent fiber; ns = slope or intercept not significant (p>0.05)

Table 4.14. Estimated digestibility coefficients for DM, organic matter (OM), starch, neutral detergent fiber (NDF), and acid detergent fiber (ADF) using NIRS calibrations for digestibility coefficients in a 24 h fecal composite or in spot fecal samples collected at 4 h intervals in heifers fed backgrounding and finishing diets once or twice per day

Digestibility	24 h COMP[z]	24 h NIR COMP[y]	FF		SEM	Time after first feeding (h)						SEM	P value		
			1x	2x		0 to 4	4 to 8	8 to 12	12 to 16	16 to 20	20 to 24		FF	T	FF×T
Backgrounding															
DM	75.2±1.65	75.8±1.79	74.8	76.8	0.86	76.5a	75.9bcd	76.3ab	75.3cd	75.1d	75.7abc	0.65	0.17	<0.01	0.56
OM	77.8±1.26	78.0±1.37	77.2	78.8	0.65	78.4ab	77.9bc	78.8a	77.8bc	77.3c	77.8bc	0.52	0.14	<0.01	0.65
Starch	98.4±0.57	98.4±0.21	98.4	98.4	0.13	98.3	98.3	98.4	98.4	98.5	98.5	0.17	0.69	0.90	0.95
NDF	53.3±3.08	50.5±1.01	50.5	50.6	0.77	51.6a	50.1bc	51.3ab	50.3abc	*49.5c	50.6abc	0.76	0.90	0.02	0.96
ADF	43.3±3.31	*38.3±0.52	38.5	38.2	0.39	*39.2a	*38.3b	*38.6ab	*38.1ab	*37.6b	*38.2ab	0.42	0.53	0.05	0.79
Finishing															
DM	79.6±1.85	79.6±1.30	78.8	80.4	0.63	80.4a	79.3bc	79.3bc	78.9c	79.8ab	79.6abc	0.54	0.14	0.03	0.43
OM	83.0±2.24	83.0±1.11	82.7	83.5	0.68	83.4ab	82.6bc	82.9abc	82.3c	83.8a	83.5ab	0.58	0.47	0.01	0.52
Starch	96.3±1.25	96.4±1.05	96.6	96.3	0.70	96.3cd	95.9d	96.4bc	96.3cd	*97.1a	96.8ab	0.52	0.76	<0.01	0.97
NDF	66.8±3.09	*57.8±2.23	58.9	56.7	1.27	*56.6b	*58.2a	*58.3a	*57.5ab	*57.8a	*58.1a	0.95	0.29	<0.01	0.23
ADF	49.5±2.93	*45.8±0.74	45.4	46.1	0.46	*45.6bc	45.9abc	45.6bc	*45.3c	46.0ab	46.3a	0.38	0.37	0.03	0.86

Abbreviations: FF = feeding frequency; T = Time.

Italicized letters represent significant differences (P<0.05).

[z]24 h COMP = apparent total tract digestibility of nutrients calculated using fecal samples obtained by total collection.

[y]24 h NIR COMP = apparent total tract digestibility of nutrients determined using NIRS calibrations for digestibility coefficients applied to composite fecal samples.

* indicates the time points and composites that differed in NIRS predicted digestibility coefficients from values derived using total collection (P≤0.05).

4.3.7 Accuracy of Predicting Total Tract Digestibility Using NIRS Calibrations

Time of sampling affected ($P \leq 0.05$) predictions of NIRS predicted aTTD for all fecal constituents in the backgrounding period except for starch (Table 4.8). However, aTTD of nutrients estimated using fecal samples collected at 4-h intervals only differed from those estimated using the 24-h composite sample for NDF and ADF (Table 4.8). The aTTD of starch could be predicted using fecal samples collected at any time point.

In the finishing period, time of sampling affected ($P \leq 0.03$) NIRS predictions of aTTD of all nutrients (Table 4.8). Aside for NDF and ADF, and one time interval for starch, the average eTTD of all nutrients did not differ when estimated using spot samples as compared to the 24-h composite fecal sample (Table 4.6).

Near infrared spectroscopy calibrations for digestibility coefficients derived from individual heifers were poor at predicting aTTD of DM ($R^2_{val} = 0.56$), OM ($R^2_{val} = 0.61$), and ADF ($R^2_{val} = 0.42$), and moderate for aTTD of NDF ($R^2_{val} = 0.71$; Table 4.2). The NIRS calibration for predicted aTTD of starch was high ($R^2_{val} = 0.88$; Table 4.2). In the backgrounding period, predictions for starch digestibility were limited using spot fecal samples and were only suitable for the 8-h spot sample ($R^2 = 0.77$, $P = 0.02$; Table 4.7), once again pointing out the low precision when measuring starch in samples were the concentration is low. In the finishing period, aTTD of starch could still be predicted using fecal samples collected at any time point, and the prediction was only compromised for spot fecal samples at 12 h ($R^2 = 0.68$, $P = 0.04$). Otherwise, coefficients of determination (R^2) did not fall below 0.77 and $P \leq 0.02$. Thus, NIRS calibration for aTTD of starch was more suitable for the finishing period, and the R^2_{val} was high when samples from both backgrounding and finishing were plotted together due to the difference in starch digestibility in both periods biasing the relationship ($R^2 = 0.88$; Table 4.2). When averaged together however using all predictions, NIRS predicted aTTD of starch only differed at 16 to 20 h after first feeding from aTTD determined using total collection.

Regression statistics were poor for all other nutrients (not shown) indicating the high variability (low precision and accuracy) in these predictions when using single fecal samples. Once these data points are averaged, the variability is masked, and values come close to the total collection average of all heifers on the same diet.

The use of NIRS to predict aTTD applies multivariate regression, incorporating hundreds of spectral data points. Considering digestibility is affected by many factors including intake

(Leaver et al. 1969), diet (Colucci et al. 1982), animal variation (McDonald et al. 1995), and environment (Christopherson 1976), it is not surprising that validations for constituents other than starch were not successful when using regression analysis. Fecal starch is very closely related to total tract starch digestibility (Zinn et al. 2002; Corona et al. 2005; Zinn et al. 2007), which explains the strong relationship between NIRS predictions and digestibility for this parameter. When dietary starch levels are high, direct measurement of fecal samples is better at predicting total tract starch digestion, a reflection of the strong linear relationship between fecal starch and aTTD of starch. Starch digestibility observed in the backgrounding period was greater (98.4% ± 0.57%) compared to the average in the NIRS reference data set (96.0% ± 2.98%). If more samples had been included in the NIRS data set to encompass a greater range in starch digestibility values, it is likely that validation statistics and predictability would have improved for samples collected in the backgrounding period. Increasing the number of animals used in the regression equations would also potentially improve the observed relationships.

Our starch digestibility calibrations indicate that when used with samples collected from heifers fed a finishing diet, results come close to predicting starch digestibility in individuals using either fecal samples collected at single point in time or with a 24-h composite. This raises the possibility that NIRS could be used to predict aTTD of starch in cattle fed finishing diets within commercial feedlots. For cattle fed backgrounding diets, NIRS predictions can closely approximate actual aTTD of starch if multiple cattle are sampled and pooled together. As for the other digestibility calibrations, aTTD of DM, and OM also show potential for predicting aTTD accurately when using the average of multiple cattle, whereas aTTD of NDF and ADF are under predicted with current calibration equations.

Table 4.15. Regression statistics showing the linear relationships between starch digestibility coefficients predicted using fecal NIRS calibrations applied to a 24 h fecal composite or to spot fecal samples collected at 4 h intervals and apparent total tract starch digestibility determined from total fecal and feed collection from heifers fed backgrounding and finishing diets once or twice per day

Item	24 h COMP[z]	Time after first feeding (h)					
		0 to 4	4 to 8	8 to 12	12 to 16	16 to 20	20 to 24
Backgrounding							
R^2	0.13	0.44	0.77	0.05	0.03	0.22	0.18
P value	0.48	0.15	0.02	0.68	0.73	0.34	0.40
y=	ns	ns	-19.7+1.2x	ns	Ns	ns	ns
Finishing							
R^2	0.97	0.77	0.89	0.68	0.76	0.88	0.94
P value	0.01	0.02	<0.01	0.04	0.02	<0.01	<0.01
y=	-56.8+1.6x	10.1+0.88x	-30.1+1.3x	-34.4+1.3x	-30.2+1.3x	-27.3+1.3x	-75.6+1.7x

Abbreviations: R^2 = coefficient of determination,

[z]24 h COMP = composite fecal samples collected over 24 h. Apparent total tract starch digestion for backgrounding period: % of starch intake = 98.4 ± 0.58%, and for finishing period: % of starch intake = 96.1 ± 1.48%.

4.4 Conclusion and Implications

Since total fecal collection from cattle is impractical under field conditions, there is a need to determine if spot fecal samples are representative of composite fecal samples that are typically used to estimate diet digestibility. This study demonstrated that spot fecal samples collected from penned cattle are similar in most constituents to samples collected from multiple cattle and a 24-h average of constituent concentrations. One important exception is starch, particularly for cattle fed high grain diets, where the timing of fecal collection should be considered, with collection of samples shortly after the first feeding being optimal. When averaged over multiple cattle, total tract digestibility of starch can be predicted directly from spot fecal samples using fecal starch concentration or ADL as a marker, but once again timing of sampling should be within 4 h after feeding. Predictions using NIRS calibrations for starch digestibility can be used when feeding high grain finishing diets, but are less predictive for backgrounding diets which contain higher levels of forage. Predictions of total tract digestion of other nutrients require ADL to be used as a marker in feed and feces, which can be predicted using NIRS. These methods are less accurate than estimates derived from total fecal collection from individual cattle, but if pooled over multiple cattle do not differ from a 24-h fecal composite and consequently may still have merit in predicting nutrient digestibility in cattle housed within commercial feedlots.

4.5 Next Stage

The current study has demonstrated that specific sampling times will result in more accurate estimates of fecal nutrients over 24 hr, and digestibility predictions. Additional factors to consider when sampling from a feedlot include the method of sampling (from the pen floor versus from the rectum) and the ability to detect differences in fecal composition when diets are changed. In the subsequent study, two methods of sampling were compared to find differences between collecting cattle feces from fecal pats off the pen floor or sampling directly from the rectum during restraint in a chute. I also set out to determine if differences in fecal nutrients and digestibility could be detected using NIRS when ingredients and their proportions, and grain processing were altered. If differences were detected, I assessed whether these differences were large enough to be associated with changes in measured performance and net energies.

CHAPTER 5

5.0 PREDICTING FECAL NUTRIENT CONCENTRATIONS AND DIGESTIBILITY AND GROWTH PERFORMANCE IN FEEDLOT CATTLE BY NEAR INFRARED SPECTROSCOPY[3]

5.1 Introduction

Feedlot managers are challenged to make management decisions based on the growth performance of cattle within a feeding period, but are frequently limited to the data obtained after the sale of cattle. The NRC (1996) equations to estimate performance require the NE concentration of the diet, DMI, final shrunk BW, and adjustments for initial BW and sex (McMeniman et al. 2010; Galyean et al. 2010). Efforts have been made to use additional parameters such as body condition, muscle and frame scores, and health records in algorithms to predict performance (Reinhardt et al. 2009). Growth performance is strongly influenced by the energy density of the diet, and the digestibility of ingredients within the feed. Fecal samples contain information relevant to feed digestion as the amount of nutrients excreted in the feces reflect diet digestibility (Zinn et al. 2002; Lukas et al. 2005; Owens et al. 2016), which impacts growth performance (Firkins et al. 2001).

Near infrared spectroscopy (NIRS) is a rapid analytical tool that can predict fecal composition and diet digestibility without requiring samples of the diet or estimates of intake if appropriate calibrations are developed (Chapter 3). Previous work using NIRS to predict performance parameters has focused mainly on DMI of cattle fed primarily forage diets (Lyons and Stuth 1992; Coates 1998; Garnsworthy and Unal 2004), as well as predicting diet quality in free-ranging animals and extended to performance on pasture (Dixon and Coates 2009; Tolleson and Schafer 2014). We are unaware of any attempt to predict the growth performance of feedlot cattle fed high grain diets using NIRS of feces. It has been shown that NIRS of spot fecal samples from cattle over a 4 d duration accurately predicted fecal composition and digestibility in feedlot cattle (Chapter 4). Consequently, NIRS may have merit in predicting growth performance, if relationships among fecal composition, diet digestibility, and performance can be identified.

[3] A version of the chapter has been accepted and is in press. Jancewicz, L.J. G.B. Penner M.L. Swift C.L. Waldner K.M. Koenig K.A. Beauchemin and T.A. McAllister. 2017. Predicting fecal nutrient concentrations and digestibilities and growth performance in feedlot cattle by near infrared spectroscopy. J. Anim. Sci.

The goal of this research was to determine the variation in important measurements within fecal samples collected from individual steers within a pen, and the differences between fecal samples collected from the rectum and the pen floor. The effect of grain type, grain processing, proportion of silage, and day of sampling on NIRS-predicted fecal composition and apparent total tract digestibility (aTTD) was measured, and associations with DMI, ADG, and G:F were then evaluated. Lastly, the ability of NIRS-predicted aTTD of GE to predict observed NEg, ADG, and G:F was assessed.

5.2 Materials and Methods

All procedures and protocols used in this study were approved by the Animal Care Committee at the Lethbridge Research and Development Centre of Agriculture and Agri-Food Canada, with care and management of steers following the guidelines of the Canadian Council on Animal Care (2009).

5.2.1 Feedlot Studies

Two feedlot studies were conducted at the Lethbridge Research and Development Centre Feedlot between May and August 2012 (Study 1; Moya et al. 2015) and April and July 2013 (Study 2; Koenig et al. 2014).

Both studies utilized 160 British crossbred beef steers initially weighing 537.9 ± 35.6 kg BW± SD in study 1, and 349.7 ± 22.3 kg BW± SD in study 2. The steers were fed to a target BW of approximately 650 kg. Steers had continuous access to fresh water, and total mixed diets were delivered using a feed truck once daily between 0800 and 1000 h and were fed to ensure at least a 5% feed refusal.

The DMI, ADG, and G:F data for study 1 are published in Moya et al. (2015), and for study 2, data were derived from Koenig et al. (2014). For both studies, feed offered was recorded daily for each pen over the duration of the experiment; whereas the average DMI of each pen was estimated from the amount of feed offered and refused at the end of each wk. Steers were weighed before feeding on 2 consecutive days at the start and end of each experiment, and at 4 and 3 wk intervals in study 1 and 2, respectively. The G:F was estimated at each weigh day by dividing total gain from the previous weigh day by total DMI determined during the specified

interval, with shrunk BW reported as BW × 0.96. Interim and overall DMI, ADG, and G:F were estimated between d 0 to 28, 28 to 56, 56 to 84, and 0 to 84 in study 1 [interim data extracted from Moya et al. (2015)], and d 0 to 21, 21 to 42, 42 to 63, 63 to 84, 84 to 105, 105 to 126, and 0 to 126 in study 2 [interim data extracted from Koenig et al. (2014)].

5.2.2 Experimental Design, Treatment Structure and Diets

5.2.2.1 Study 1

The first experiment was conducted as a completely randomized design with a 2 × 2 factorial arrangement of diets with 4 replicate pens per diet and 10 steers per pen. Diets contained either barley (89.0% of diet DM) or Canadian Western hard red winter wheat (88.4% of diet DM) processed using a processing index (defined as the bushel weight of the processed grain divided by the bushel weight of the unprocessed grain) of 79 ± 1.5% (more processed) or 88 ± 0.6% (less processed) for barley and 80 ± 0.0% or 86 ± 1.1% for wheat (Table 5.1). Collection of grain samples for determination of the processing indices in the current study was performed independently of the published values in Moya et al. (2015). Both barley and wheat were sourced from a single lot and processed as needed during the experiment. The remainder of the diet consisted of barley silage (6% of diet DM) and supplement (5% of diet DM) containing urea to ensure that diets were isonitrogenous (Table 5.1). Upon arrival at the research center, cattle were tagged, branded, and implanted with Component TE-S with Tylan (Elanco Animal Health, Guelph, ON, Canada). Monensin was included in all diets to achieve a dietary concentration of 25 mg/kg on a DM basis.

5.2.2.2 Study 2

The second experiment was conducted as a completely randomized design using 4 diets, with 5 replicate pens per diet and 8 steers per pen. Total mixed diets consisted of increasing levels of barley silage DM (0, 4, 8, 12%) with silage displacing barley grain. Barley grain was processed to an index of 82 ± 0.9%. The diet also contained corn dried distillers' grains (15% of diet DM) and a mineral/vitamin supplement (5% of diet DM; Table 5.1). Upon arrival at the research center, cattle were tagged, branded, and implanted with a growth promoter (Component

TE-S with Tylan, Elanco Animal Health, Guelph, ON, Canada). Monensin was included in all diets to achieve a final concentration of 32 mg/kg on a DM basis.

5.2.3 Feed Sampling and Analysis

Grain samples were collected weekly before and after processing to determine processing index. Aside for determination of processing index, all other feed and ort sampling and analysis was derived from published work in Moya et al. (2015) for study 1, and unpublished work extracted from Koenig et al. (2014) for study 2. Diet samples were collected weekly for determination of DM by drying in a forced air oven at 55ºC for 48 h, and orts were removed, weighed, and sampled weekly for DM determination. Dried subsamples of the diet were composited every 4 wk in study 1 and every 3 wk in study 2. Subsamples were ground through a 1-mm screen using a Wiley Mill (Thomas Scientific, Swedesboro, NJ) and analyzed for DM, OM, starch, N, NDF, ADF and ADL as described below.

5.2.4 Reference Analysis

All reference analysis for feed samples was derived from Moya et al. (2015) and Koenig et al. (2014). Analytical DM and OM were determined on 1 mm ground samples according to AOAC (2005, method 930.15 and method 942.05, respectively). For determination of starch and N (CP = total N × 6.25), separate samples were ground to 1 mm, followed by grinding using a ball mill (Mixer Mill MM2000, Retsch, Haan, Germany). Starch concentration was determined by enzymatic hydrolysis of α-linked glucose polymers as described by Rode et al. (1999) with the following modifications. Tubes containing samples were initially incubated in a water bath at 90°C and vortexed at 10, 20 and 30 min of incubation without the use of activated carbon. Amyloglucosidase (200 µl; Megazyme, Wicklow, Ireland) was added, tubes were vortexed immediately and twice subsequently at 30 and 60 min during 2 h incubation at 60°C. Samples were centrifuged at 29,000 × g for 15 min at 4°C. Glucose color reagent was added (300 µl; Diagnostic Chemicals, Charlottetown, PEI, Canada), and glucose was determined colorimetrically at 505 nm using a microtiter plate reader. Nitrogen was estimated by flash combustion, followed by gas chromatography and thermal conductivity detection (Carlo Erba Instruments, Milan, Italy). For ADF and NDF, an ANKOM fiber analyzer (ANKOM Technology

Corp., Fairport, NY) was used for sequential determination of NDF and ADF, with heat stable α-amylase and sodium sulfite included in the NDF analysis. Acid detergent lignin was extracted using 72% sulphuric acid after the ADF procedure, followed by ashing at 550ºC (Van Soest et al. 1991).

5.2.5 Fecal Collection

Steers were housed in 21 x 27 m, outdoor pens with dirt floors and 15 m^2 of concrete in front of the feed bunk at least 12.6 m^2 of pen space available for each steer. Pens were enclosed with porosity fencing on two sides. Steers were bedded in the middle of the pen using barley straw as required. Fecal samples were collected from 4 fresh fecal pats removed from the pen floor within 4 h of feeding (0800 h) on d 29, 43, 57, 71, 85, and 99 in study 1, and 4 fresh fecal pats on d 20, 43, 64, 83, 104, and 119 in study 2. All steers within a pen were observed until defecation occurred. Samples (minimum 250 g) were collected from the center of fecal pats from different steers and contamination with dirt or bedding was avoided. Samples were stored in separate plastic bags and kept in coolers with ice until collection from all pens was complete. Once all collections were complete for each sampling day (within 4 h of feeding), samples within the same pen were composited on an equal wet weight basis, resulting in approximately 400 g of feces. Feces were dried in a forced air oven at 55ºC for 72 h, and ground through a 1-mm screen as described for feed samples. Additionally, fecal samples were collected from the rectum of 3 to 7 (average 4.6 ± 0.95 SD) animals per pen before feeding (between 0700 and 0830) during restraint in a chute on d 28, 56, and 84 in study 1, and on d 21, 42, 63, 84, 105, and 120 in study 2. Rectal samples from steers within the same pen were also composited on an equal wet weight basis (400 g) after collections were complete for that sampling day (within 4 h of weighing), followed by drying and grinding. Any remaining individual rectal samples collected from steers in study 2 that were > 80 g wet weight, were dried and ground and used to determine sampling variation within a pen.

Table 5.1. Ingredient and chemical composition of diets fed to feedlot steers in studies 1 (Moya et al. 2015) and 2 (Koenig et al. 2013)

Item	Study 1[1]		Study 2[1]			
	B	W	SL0	SL4	SL8	SL12
Ingredient composition, % DM						
Barley grain	89.0	-	80.0	76.0	72.0	68.0
Wheat grain	-	88.4	-	-	-	-
Barley silage	6.0	6.0	0.0	4.0	8.0	12.0
Corn dried distillers grains	-	-	15.0	15.0	15.0	15.0
Urea	-	0.6	-	-	-	-
Supplement[2, 3]	5.0	5.0	5.0	5.0	5.0	5.0
Chemical composition, % DM						
DM	81.3	82.2	92.0	87.7	83.8	79.5
OM	89.6	89.9	95.3	95.3	95.4	94.4
Starch	50.5	58.9	48.9	46.6	46.7	44.1
CP	13.7	13.4	14.3	14.5	14.6	14.6
NDF	19.6	12.7	21.9	23.5	25.1	26.6
ADF	6.3	4.7	7.5	9.2	9.5	11.1
ADL	2.8	2.4	2.5	2.9	2.5	3.0

[1]B = diets consisting of barley in study 1. Barley grain was processed to 79.3±1.5% and 87.7±0.6%. W = diets consisting of wheat in study 1. Wheat grain was processed to 80.0±0.0% and 86.3±1.1%. SL = silage level in study 2 diets. Barley grain was processed to 81.7±0.87% in all four diets in study 2.

[2]Supplement for study 1 contained (per kg DM) 565 g of ground barley, 250 g of limestone, 30 g of salt, 100 g of canola meal, 0.66 g vitamin E (500,000 IU/kg), 2.5 g Rumensin Premix (200 g monensin/kg; Elanco Animal Health, Guelph, ON), 25 g of molasses, 20 g of urea, and 10 g of trace mineral mix that provided (per kg diet DM) 58 mg of Zn, 27 mg of Mn, 15 mg of Cu, 0.66 mg of I, 0.29 mg of Se, 0.23 mg of Co, 4825 IU/kg of vitamin A, 478 IU of vitamin D, and 32 IU of vitamin E.

[3]Supplement for study 2 contained (per kg DM) 675 g ground barley grain, 260 g limestone, 30 g salt, 1.1 g vitamin E (500,000 IU/kg), 3.2 g Rumensin Premix (200 g monensin/kg; Elanco Animal Health, Guelph, ON), 20 g molasses, 0.5 g flavouring and 10 g trace mineral mix that provided (per kg diet DM) 53 mg of Zn, 14 mg of Cu, 25 mg of Mn, 0.6 mg of I, 0.26 mg of Se, 0.18 mg of Co, 8940 IU/kg of vitamin A, 450 IU of vitamin D and 12 IU of vitamin E.

5.2.6 Predictions of Fecal Composition and Digestibility Coefficients

The following analyses were applied to the composited pen floor and rectal fecal samples from study 1 and 2, and the individual rectal samples from study 2. Fecal DM was determined by drying in a forced air oven at 55 ºC for a minimum of 72 h. Fecal OM, starch, N, NDF, ADF, and ADL concentration, and aTTD of DM, OM, starch, NDF, ADF, and GE were predicted using previously derived NIRS calibrations (Chapter 3).

5.2.7 Statistical Analysis: Fecal Composition and Digestibility Coefficients

Sample size software (EpiTools epidemiological calculators, AusVet 2016) was used to calculate the optimal required sample size (95% confidence interval) for fecal DM, NIRS predicted fecal constituent concentrations and NIRS predicted digestibility coefficients. Variables used in the calculation included the average standard deviation observed per pen, determined at each sampling day (d 21, 42, 63, 85, 105, and 120), and the desired precision. Statistical analyses were performed using SAS v. 9.1. (SAS Inst. Inc., Cary, NC) to identify factors associated with NIRS predictions for fecal composition (except DM) and digestibility coefficients. Differences between sampling method (pen floor vs. rectal) within study were identified using the PROC MIXED function for repeated measures, based on Kenward–Roger's adjusted degrees of freedom.

For study 1, the model included the fixed effect of sampling method, grain type, processing index, day of sampling (i = 28, 56, and 84 for rectal, and 29, 57, and 85 for pen floor), with significant 2-way interactions ($P < 0.05$) in the final model. A compound symmetry structure was used to model repeated measures of individual pens on different sampling days to meet convergence criteria with the small dataset. The model used was $Y_{ijk} = \beta_0 + \beta_1 SM_{ijk} + \beta_2 GT_{ijk} + \beta_3 PI_{ijk} + \beta_4 day_{ijk} + \beta_5 SM \times GT_{ijk} + \beta_6 SM \times PI_{ijk} + \beta_7 SM \times day_{ijk} + \beta_8 day \times GT_{ijk} + \beta_9 day \times PI_{ijk} + \beta_{10} GT \times PI_{ijk} + \varepsilon_{ijk}$, where β_0 was the average y-intercept or overall mean for fecal chemical composition when all terms in the equation are equal to 0, SM was sampling method, GT was grain type, PI was processing index, i was the individual observation of pen within day, j was pen, and ε_{ijk} was the residual error. Least square means of the treatments were separated using PDIFF statement, with significance declared at $P < 0.05$.

For study 2, the model included the effect of sampling method, silage level, day of sampling (i = 21, 42, 63, 85, 105, and 120 for rectal and 20, 43, 64, 83, 104, and 119 for pen floor), and all 2-way interactions. Only significant ($P < 0.05$) interactions were retained in the model. A compound symmetry structure was used to model repeated measures of individual pens on different sampling days. The model used was $Y_{ijk} = \beta_0 + \beta_1 SM_{1ijk} + \beta_2 SL_{ijk} + \beta_3 day_{ijk} + \beta_4 SM \times SL_{ijk} + \beta_5 SM \times day_{ijk} + \beta_6 day \times SL_{ijk} + \varepsilon_{ijk}$, where β_0 was the average y intercept or overall mean for fecal chemical composition and digestibility when all terms in the equation were set to 0, SM was sampling method, SL was silage level, i was the individual observation of pen within day, j was pen, and ε_{ijk} was the residual error. Least square means of the treatments were separated using PDIFF statement, with significance declared at $P < 0.05$.

Fecal composition and digestibility predictions limited only to pen floor samples were then re-examined using the PROC MIXED function for repeated measures using the models described above (without SM as a fixed effect). A first-order autoregressive structure was used to model repeated measures on individual pens on each day, as this produced the best fit. Least square treatment means were separated using PDIFF, with significance declared at $P < 0.05$. Contrast statements were used to test for linear and quadratic responses to day on feed in both studies, and to increasing levels of barley silage in study 2.

5.2.8 Statistical Analysis: Observed DMI, ADG, G:F

Data for DMI, ADG, and G:F data was extracted from previously published data in Moya et al. (2015), and Koenig et al. (2014). The statistical analysis for the current manuscript was performed using performance intervals that corresponded to the intervals around fecal collections and weigh days.

Dry matter intake, ADG, and G:F were calculated for every interval (d 0 to 28, 28 to 56, 56 to 84 for study 1, and d 0 to 21, 21 to 42, 42 to 63, 63 to 84, 84 to 105, 105 to 126 for study 2), and analyzed using the same procedures as fecal composition to determine the effects of treatment and day.

A series of regression models were used to examine the individual associations between each of the fecal nutrients and digestibility measures with DMI, ADG, and G:F. Models using the PROC MIXED function for repeated measures were developed separately for barley

processed to varying degrees, for wheat processed to varying degrees, and for increasing levels of silage.

Because there were fewer weigh days than samples collected in study 1, and there were no pen floor samples collected between d 0 to 28, NIRS-predicted fecal nutrient concentrations and digestibilities of the samples collected between performance intervals for d 28 to 56 and d 56 to 84 were averaged prior to regression. In study 2, there was one fecal sampling day corresponding to each interval of measured performance, therefore averaging was unnecessary.
Processing index (study 1), silage level (study 2), day of sampling, and the interactions of processing index×day (study 1) and silage level×day (study 2) were included as fixed effects, and the individual pen as the random effect. For study 1, the model used was $Y_{ijk} = \beta_0 + \beta_1 X_{1ijk} + \beta_2 day_{ijk} + PI_{ijk} + PI \times day_{ijk} + \mu_j + \varepsilon_{ijk}$, where Y is the dependent variable (DMI, ADG, or G:F), X is the independent variable (processing index, silage level, fecal nutrient concentration, or digestibility), PI is processing index, μ_j was the random effect for pen, i was the individual measurement within day within pen, j was pen, β_0 was the average y intercept or overall mean of Y for each day, and ε_{ijk} was the associated error. For study 2, processing index was replaced with silage level in the model. A first-order autoregressive structure was used to model repeated measures on individual pens on each day, as this was the best fit structure for the model. As study power was limited, models did not examine associations between each predicted fecal constituent concentration or digestibility and performance values at each sampling time.

5.2.9 NEg as Determined from the Chemical Composition of the Diet, Observed Performance, and NIRS

The NEg of the diets was calculated based on chemical composition as described in NRC (2016). Metabolizable energy (ME) values for each grain and processing method were calculated by quadratic procedures from overall DMI and animal performance. The net energy for maintenance (NEm) content of each diet was calculated as described by Zinn et al. (2002) using the estimates of energy gain (EG, Mcal/d) and the maintenance energy (EM, Mcal/d) expended based on growth performance for a medium frame yearling steer. The NEm values were estimated from the performance and feed intake using the quadratic formula [$x = -b \pm \sqrt{(b^2 - 4ac)}/2a$], where a = -0.877DMI, b = 0.877EM + 0.41DMI + EG, and c = -0.41EM (Zinn and Shen, 1998). Net energy of maintenance was converted to NEg using the equation NEg =

0.877×NEm-0.41 (NRC 2016).

Relationships between NIRS predicted aTTD of GE and NEg were determined by linear regression in SAS separated by study and grain type. Changes in diet energy density predicted by NIRS were applied to estimate expected changes in overall ADG and G:F using the above equations.

5.3 Results

5.3.1 Precision of Measurements and Sample Size

The number of samples required per pen to estimate the mean of fecal DM and NIRS predicted fecal constituent concentrations, digestibility coefficients, and observed DMI, ADG, and G:F are displayed in Table 5.2. Fecal starch values were associated with the most variability (9.4 ± 3.28%, C.V. = 35.3), and for a desired precision of ± 2 from the estimated mean, sampling from 11 individual steers would be required. Fecal NDF concentration exhibited the least variability (50.9 ± 2.75, C.V. = 5.40), requiring 8 individual samples per pen for a precision of ± 2. Since digestibility values are generally higher than concentrations of fecal constituents, these measurements were associated with a lesser degree of variation among steers within a pen, with aTTD of starch being the lowest (95.4 ± 1.73, C.V. = 1.81), and aTTD of ADF exhibiting the greatest variation (45.5 ± 4.49, C.V. = 9.87). To achieve a desired precision of ± 2 % on the 95% confidence level for the mean, samples from 20 individual steers would be sufficient for most constituents and digestibility predictions.

5.3.2 Differences in Sampling Method

In studies 1 and 2, DM content was greater ($P < 0.01$) for fecal samples collected from the pen floor as compared to those collected directly from the rectum (Table 5.3). Differences in other fecal nutrient concentrations between sampling methods were small, but estimates of fecal N and ADF concentrations were greater ($P \leq 0.02$) in pen floor compared to rectal samples in study 1, and fecal OM and starch were less ($P \leq 0.03$), and ADL was greater ($P < 0.01$) in pen floor compared to rectal samples in study 2.

Interactions between fecal sampling method and grain type were observed for fecal starch, ADF, and ADL concentrations in study 1 ($P = 0.01$; Table 5.3 and Figure 5.1). For the barley fed

cattle, fecal ADF concentration did not change with sampling method, but it was lower ($P < 0.05$) in rectal samples compared to pen floor samples collected from steers fed wheat. Fecal starch and ADL concentrations were unaffected in the wheat diets, and fecal starch was less ($P < 0.01$), and fecal ADL greater ($P < 0.01$), in the rectal samples compared to pen floor samples from steers fed barley. In study 2, no significant interactions were observed between sampling method and silage level in the diet. Day of sampling affected fecal OM, N, NDF, and ADL concentrations in study 1, and all fecal constituent concentrations in study 2 (Table 5.3). An interaction between sampling method and day occurred for DM, OM, starch, ADF, and ADL in study 2 ($P < 0.01$; Table 5.3), but when graphed, the interactions between sampling method and day did not display any notable patterns (Figure 5.2).

5.3.3 Variations in NIRS-Predicted Fecal Nutrient Concentrations and Digestibility of Pen Floor Samples

5.3.3.1 Study 1

The ADF concentration was lower ($P < 0.01$; Table 5.4) in fecal samples for steers fed wheat as compared to barley, and N and ADF concentrations were lower ($P < 0.01$) with less severe grain processing. Interactions between grain type and processing index ($P \leq 0.05$) were observed for fecal DM, OM, starch, NDF and ADL concentrations. Reductions in DM, OM, and starch, and increases in NDF and ADL concentrations were greater for more severely processed wheat than barley. Day of sampling affected the concentration of most fecal constituents except for DM and starch. Fecal OM linearly decreased ($P < 0.01$) over the feeding period, from 85.9% on d 14 to 81.1% on d 84 (Figure 5.3A). A grain type by day interaction ($P < 0.01$) was observed for fecal N, with no obvious pattern for the barley diets, and a linear decrease ($P < 0.01$) for wheat diets (Figure 5.3B).

Greater ($P < 0.01$) aTTD of NDF and ADF was observed for barley compared to wheat (Table 5.4). More extensive processing increased ($P < 0.01$) the aTTD of all nutrients in both grains. An interaction between grain type and processing index was observed for aTTD of starch and GE ($P < 0.05$), with a greater increase observed for more processed wheat than barley. Day of sampling showed sporadic variations in predicted digestibility of CP, NDF, and ADF, with the

Table 5.2. Sample size to estimate a single mean of fecal composition and digestibility using near infrared spectroscopy of individual dried ground rectal samples, collected from steers in study 2, with specified precision to a 95% confidence level (n=8 steers per pen)

Item	Mean	Average SD	C.V.	Desired precision (± value)						
				0.1	0.25	0.5	1	2	3	4
				Number of samples per pen to estimate the mean						
Composition, % of fecal DM										
DM[1]	17.7	2.33	13.2			85	22	6	3	1
OM	86.9	2.24	2.58				20	5	3	1
Starch	9.3	3.28	35.3				43	11	5	3
N	2.6	0.25	9.61	26	5	2	1			
NDF	50.9	2.75	5.40				29	8	4	1
ADF	28.8	2.62	9.10				27	7	3	1
ADL	5.8	0.71	12.2		32	8	2	1		
Apparent total tract Digestibility, % of intake										
DM	81.7	2.27	2.78				20	5	3	1
OM	80.6	2.74	3.40				29	8	4	1
Starch	95.4	1.73	1.81			46	12	3	2	1
CP	76.0	2.11	2.77				18	5	2	1
NDF	56.2	2.37	4.22				22	6	3	1
ADF	45.5	4.49	9.87				79	20	9	5
GE	83.3	3.22	3.86				40	10	5	3

[1] fecal DM was determined using actual fecal DM and not NIRS calibrations

Table 5.3. Comparisons of fecal composition between pen floor and rectal sampling from feedlot steers as predicted using near infrared spectroscopy of dried ground samples

Item	Sampling Method		SEM	*P* value[1]					
	Pen Floor	Rectal		SM	SM*GT	SM*PI	SM*SL	Day	SM*Day
Fecal constituent, % fecal DM									
Study 1 (n=16 pens)									
DM	22.6	18.3	0.23	<0.01	0.65	0.64		0.94	0.52
OM	84.1	83.6	0.44	0.29	0.07	0.83		<0.01	0.72
Starch	14.3	14.2	0.56	0.79	0.01	0.67		0.39	0.99
N	2.3	2.2	0.02	0.01	0.59	0.55		0.03	0.75
NDF	38.4	39.4	0.39	0.13	0.97	0.77		<0.01	0.29
ADF	25.7	24.1	0.43	0.02	0.01	0.45		0.42	0.06
ADL	5.0	5.2	0.13	0.05	0.01	0.98		0.02	0.68
Study 2 (n=20 pens)									
DM	21.1	17.7	0.21	<0.01			0.52	<0.01	<0.01
OM	85.8	86.6	0.31	0.03			0.51	<0.01	<0.01
Starch	7.0	8.7	0.31	<0.01			0.23	<0.01	<0.01
N	2.5	2.5	0.02	0.18			0.59	<0.01	0.09
NDF	52.3	51.7	0.28	0.09			0.78	<0.01	0.50
ADF	29.7	29.2	0.31	0.09			0.34	<0.01	<0.01
ADL	6.7	6.0	0.06	<0.01			0.20	<0.01	<0.01

[1]SM = sampling method (pen floor versus rectal). GT = grain type (barley versus wheat). PI = processing index of grain in study 1, defined as the bushel weight of the grain after processing divided by the weight before processing). SL = silage level in study 2.

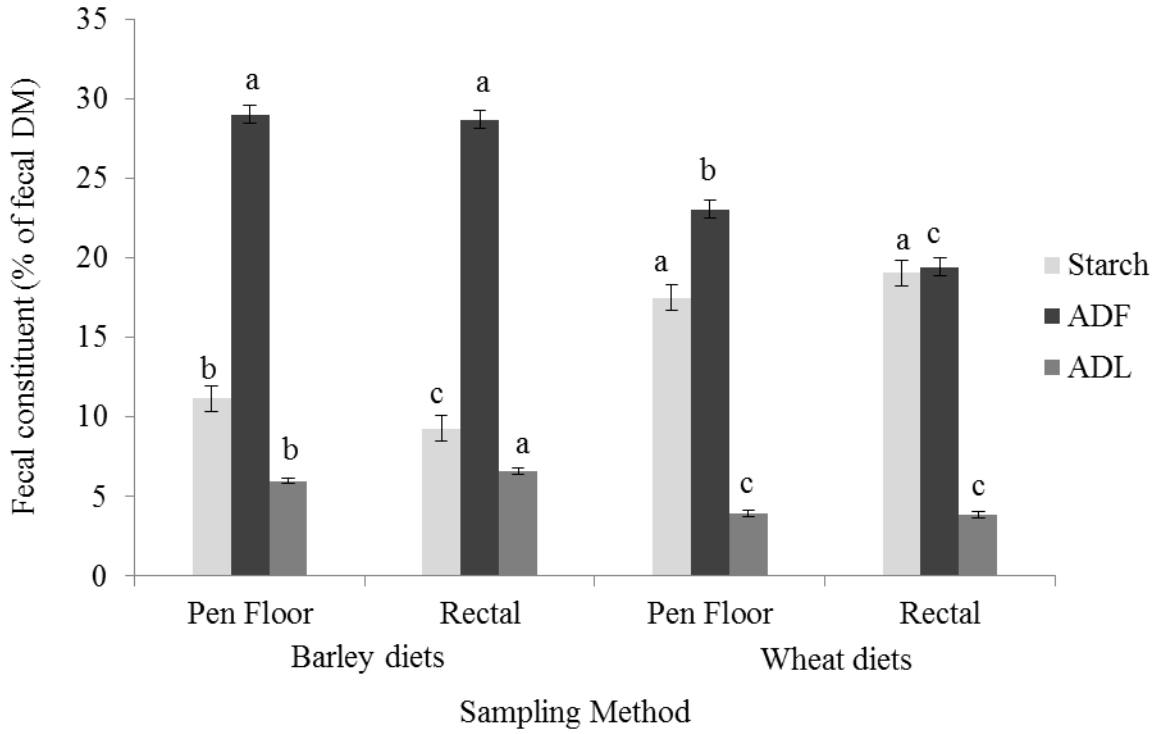


Figure 5.1. Difference in fecal starch, ADF, and ADL concentrations (% of fecal DM) of feces sampled from the pen floor or from the rectum of cattle fed barley and wheat based diets in study 1. Considering each constituent concentration separately, means without a common superscript differ ($P < 0.05$). Error bars represent SEM.

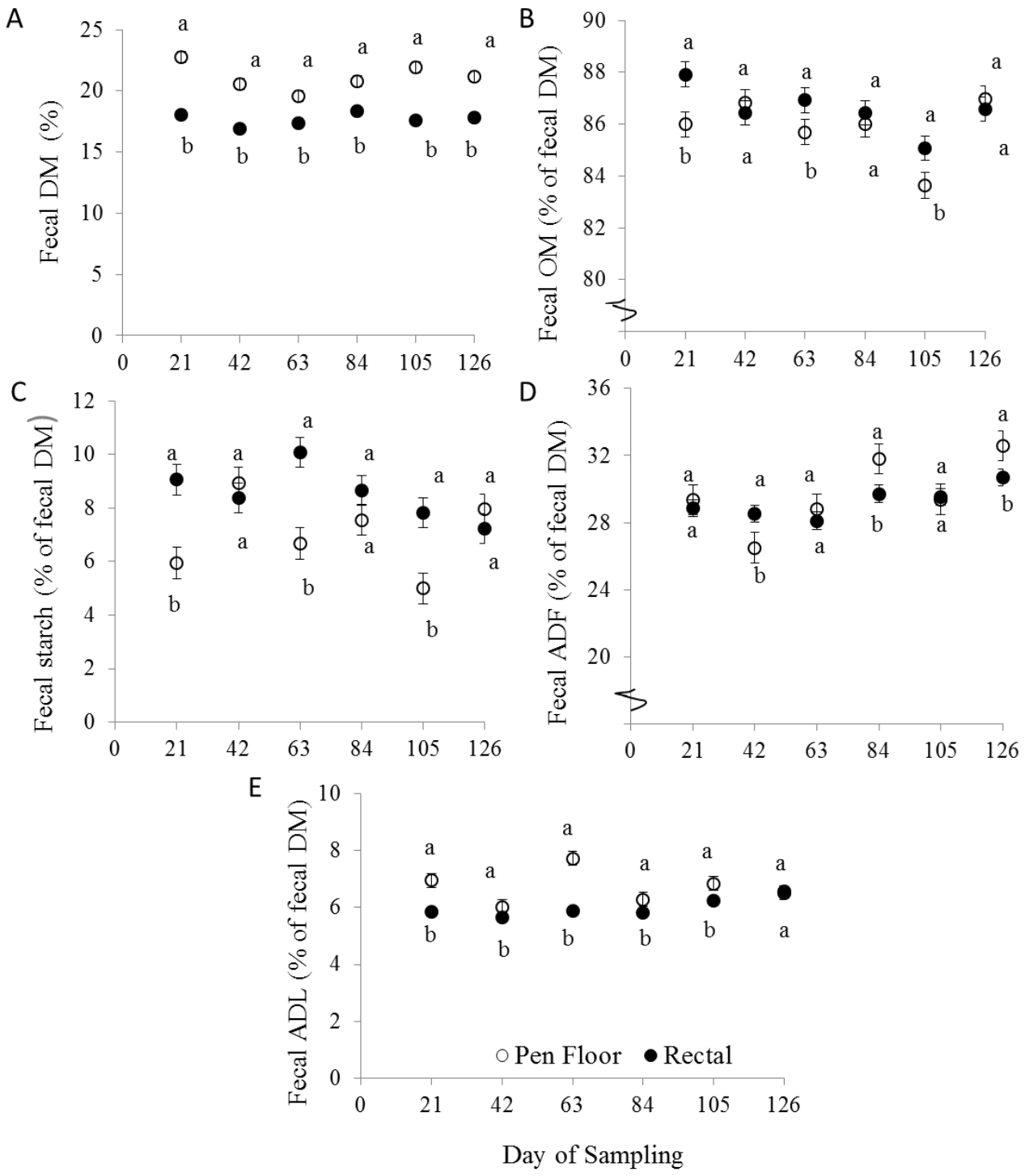


Figure 5.2. Graphs depicting the interaction between sampling method and sampling day on (A) fecal DM ($P < 0.01$, SEM = 0.359), (B) fecal OM ($P < 0.01$, SEM = 0.472), (C) fecal starch ($P < 0.01$, SEM = 0.561), (D) fecal ADF ($P < 0.01$, SEM = 0.504), and (E) fecal ADL concentrations ($P < 0.01$, SEM = 0.127) in study 2. Means without a common superscript differ (P < 0.05). Error bars represent SEM.

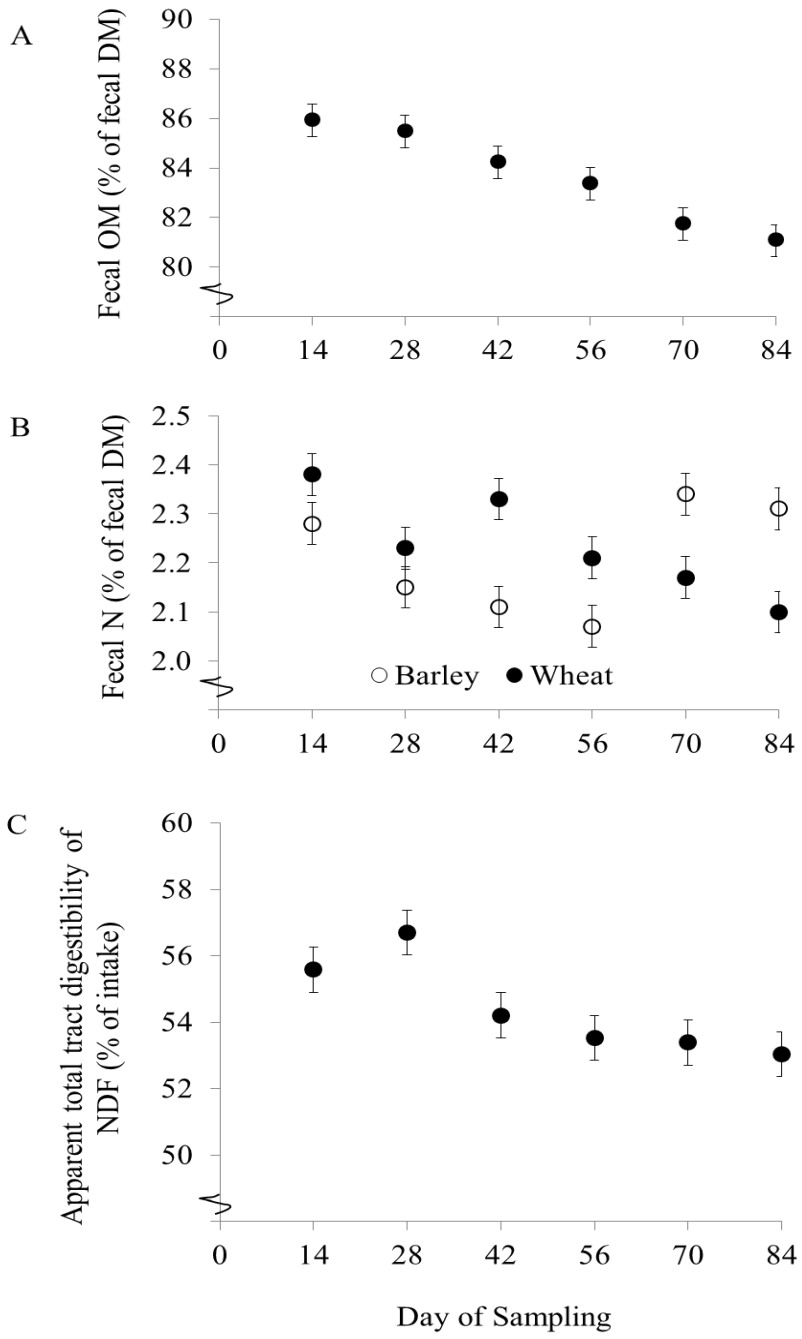


Figure 5.3. Graphs depicting (A) the effect of sampling day on fecal organic matter concentration (linear, $P < 0.01$, SEM = 0.656); (B) the interaction between sampling day and grain type (barley or wheat) on fecal nitrogen concentration ($P < 0.01$, SEM = 0.0425); and (C) the effect of sampling day on the apparent total tract digestibility of NDF (linear, $P < 0.01$, SEM = 0.677) in study 1. Grains were averaged for (A) and (C) as no interactions were observed. Error bars represent SEM.

aTTD of NDF linearly decreasing ($P < 0.01$) over time (Figure 5.3C). There were no grain type by day or processing index by day interactions ($P \geq 0.09$, data not shown).

5.3.3.2 Study 2

Increasing the proportion of silage in the diet linearly reduced ($P < 0.01$) fecal DM and N, and increased ($P < 0.01$) fecal NDF and ADL concentrations in samples from the pen floor (Table 5.5). Sampling day affected the concentration of all fecal nutrients ($P \leq 0.05$) with no obvious patterns. There were no interactions between silage level and day ($P \geq 0.10$, data not shown).

A linear reduction in aTTD of all nutrients and energy was observed with increasing levels of silage in the diet, with starch being the exception (Table 5.5). An effect of sampling day was observed for all digestibility predictions, but the responses were not linear or quadratic. Aside from aTTD of CP and NDF, there were no silage level by day interactions. However, when aTTD of CP and NDF were plotted over time, similar patterns were observed for all four treatments (Figure 5.4A and B).

5.3.4 Observed Performance Data and Variation Among Sampling Dates

5.3.4.1 Study 1

The DMI tended to increase ($P = 0.07$) in steers fed barley and clearly increased ($P = 0.05$) when the severity of grain processing was reduced (Table 5.4; Moya et al. 2015). More processing caused an increase ($P = 0.04$) in G:F, with no effect on ADG. Dry matter intake linearly increased over time ($P < 0.01$), while ADG, and G:F responded quadraticaly ($P < 0.01$; data not shown). Both ADG and G:F were greater ($P < 0.01$) at the d 28-56 interval, compared to d 0-28 and 56-84, which did not differ. There were no interactions between processing index and day; however, a grain type by day interaction ($P = 0.04$) was observed for G:F, with a tendency for an interaction ($P = 0.06$) with ADG (data not shown). Regression equations were developed for each grain type separately; therefore interactions between grain types were not considered.

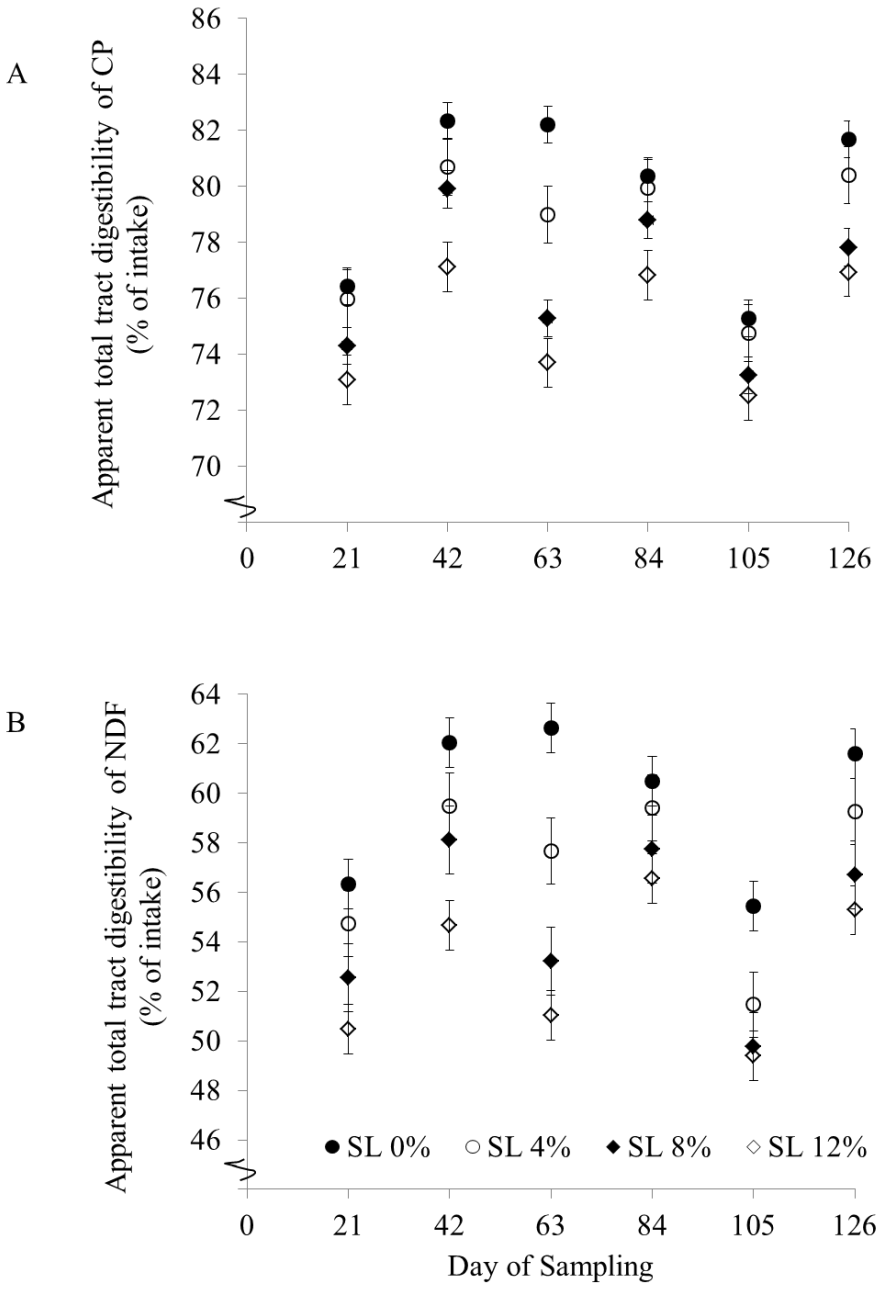


Figure 5.4. Graphs depicting the interaction between sampling day silage level on (A) the apparent total tract digestibility of CP ($P < 0.01$, SEM = 0.660) and (B) the apparent total tract digestibility of NDF ($P = 0.04$, SEM = 1.007) in study 2. Error bars represent SEM.

5.3.4.2 Study 2

Dry matter intake linearly increased ($P < 0.01$), and G:F linearly decreased ($P < 0.01$) with a greater proportion of silage in the diet (Table 5.5; Koenig et al. 2014). All performance measurements were affected by day ($P < 0.01$), with a quadratic response over time for DMI (data not shown). Both ADG and G:F fluctuated with no obvious pattern over the feeding period. There were no silage level by day interactions, indicating steers fed all 4 levels of silage maintained treatment differences, and similar patterns of performance parameters, at each time interval (data not shown).

5.3.5 Associations of NIRS-Predicted Chemical Composition of Feces and Apparent Total Tract Digestibility with Observed DMI, ADG, And G:F

5.3.5.1 Study 1

The regression slopes and standard errors for the individual associations between processing index, fecal nutrients, and aTTD of nutrients and DMI, ADG and G:F are reported in Table 5.6. For the barley diet, only fecal DM and NDF tended to be associated to G:F, but no relationships were identified between the performance parameters of steers and other variables.
For wheat, only fecal NDF (slope = -0.051, $P = 0.05$) was associated with DMI, but the response was not linear. All other measured associations were linear ($P < 0.05$). Near infrared spectroscopy-predicted fecal starch (slope = -0.0069), NDF (slope = 0.012), and ADF (slope = 0.015) were associated with ADG. Apparent total tract digestibility of DM, CP, and starch were also associated with ($P \leq 0.05$) the ADG of steers fed wheat. All fecal nutrients and digestibilities predicted using NIRS, as well as measured processing index of the wheat, were associated with G:F ($P < 0.05$). Only fecal DM, OM, and starch displayed negative slopes when predicting ADG or G:F, indicating that a reduction in these fecal nutrients was associated with greater ADG or G:F in steers fed wheat.

Table 5.4. Chemical composition of feces and apparent total tract digestibility predicted by near infrared spectroscopy using the average of all dried ground fecal samples collected from the pen floor every 14 days over 84 days from feedlot steers fed barley or wheat processed at two different indices, and observed performance parameters measured over three time periods (study 1) (n=16 pens)

Item	Study 1[1]				SEM	*P* value[2]			
	B79	B88	W80	W86		GT	PI	GT*PI	Day
Composition, % of fecal DM									
DM[3]	20.9b	21.5b	21.6b	26.0a	0.46	<0.01	<0.01	<0.01	0.19
OM	81.1c	84.1b	81.9c	87.3a	0.61	0.04	<0.01	0.05	<0.01
Starch	7.3d	13.9b	10.9c	23.9a	0.87	<0.01	<0.01	<0.01	0.25
N	2.3	2.1	2.3	2.2	0.02	0.29	<0.01	0.56	0.04
NDF	43.7a	39.3b	40.1b	32.5c	0.66	<0.01	<0.01	0.02	<0.01
ADF	32.2	27.8	26.8	20.3	0.73	<0.01	<0.01	0.16	0.02
ADL	7.0a	5.8b	5.2c	3.2d	0.19	<0.01	<0.01	0.05	<0.01
Apparent total tract Digestibility, % of intake									
DM	79.9	77.8	80.3	77.5	0.46	0.87	<0.01	0.41	0.67
OM	81.9	79.3	82.7	78.7	0.52	0.88	<0.01	0.17	0.72
Starch	95.3a	92.1b	94.3a	88.1c	0.47	<0.01	<0.01	<0.01	0.63
CP	74.4	72.7	74.8	72.9	0.31	0.30	<0.01	0.65	<0.01
NDF	57.3	53.5	56.5	50.3	0.63	<0.01	<0.01	0.07	<0.01
ADF	43.8	39.1	41.7	35.7	0.69	<0.01	<0.01	0.37	<0.01
GE	86.2b	82.6c	89.6a	82.5c	0.69	0.03	<0.01	0.02	0.19
Performance									
DMI, kg/d	10.4	10.6	9.90	10.3	0.13	0.07	0.05	0.48	<0.01
ADG, kg/d	1.37	1.31	1.34	1.34	0.03	0.71	0.43	0.41	<0.01
G:F, kg/kg DM	0.131	0.123	0.136	0.130	0.003	0.10	0.04	0.63	<0.01

[1]B79 = barley processed to 79%. B88 = barley processed to 88%. W80 = wheat processed to 80%. W86 = wheat processed to 86%.
[2]GT = grain type (barley vs wheat). PI = processing index, defined as the bushel weight of the grain after processing divided by the weight before processing).GT*PI = grain type × processing index.
[3]Fecal DM was determined using actual DM and not NIRS predictions.
Within a row, means without a common superscript differ (P < 0.05).

Table 5.5. Chemical composition of feces and apparent total tract digestibility predicted by near infrared spectroscopy of the average of all dried ground fecal samples collected from the pen floor every 21 days over 124 days from feedlot steers fed increasing levels of barley silage, and observed performance parameters measured over 6 time intervals (study 2) (n=20 pens)

Item	Study 2[1]				SEM	*P* value			
	SL0	SL4	SL8	SL12		SL	SL		Day
							linear	quadratic	
Composition, % of fecal DM									
DM[2]	22.4	21.6	20.9	19.6	0.23	<0.01	<0.01	0.34	<0.01
OM	86.0	85.7	86.1	86.7	0.37	0.32	0.14	0.27	<0.01
Starch	7.2	7.2	6.9	6.7	0.45	0.88	0.44	0.91	<0.01
N	2.6	2.4	2.4	2.4	0.03	<0.01	<0.01	0.03	0.05
NDF	51.3	52.3	52.9	53.8	0.44	<0.01	<0.01	0.94	<0.01
ADF	29.4	29.3	30.1	30.3	0.46	0.32	0.10	0.74	<0.01
ADL	6.4	6.4	6.8	7.2	0.10	<0.01	<0.01	0.09	<0.01
Apparent total tract digestibility, % of intake									
DM	82.4	81.0	79.7	78.8	0.25	<0.01	<0.01	0.32	<0.01
OM	85.1	83.1	81.5	80.5	0.29	<0.01	<0.01	0.09	<0.01
Starch	97.5	97.5	97.7	97.8	0.22	0.76	0.31	0.84	<0.01
CP	79.7	78.4	76.5	75.0	0.34	<0.01	<0.01	0.72	<0.01
NDF	59.8	57.0	54.7	52.9	0.48	<0.01	<0.01	0.32	<0.01
ADF	52.4	49.4	46.7	44.5	0.48	<0.01	<0.01	0.41	<0.01
GE	85.7	84.2	82.0	79.7	0.48	<0.01	<0.01	0.39	<0.01
Performance									
DMI, kg/d	11.1	11.3	11.7	11.8	0.14	0.01	<0.01	0.70	<0.01
ADG, kg/d	1.84	1.84	1.84	1.88	0.029	0.69	0.33	0.59	<0.01
G:F, kg/kgDM	0.165	0.163	0.158	0.160	0.0017	0.04	0.02	0.18	<0.01

[1]SL = Silage level, % DM basis.
[2]Fecal DM was determined using actual DM and not NIRS predictions.

5.3.5.2 Study 2

The regression slopes and standard errors for the individual associations between silage level, fecal nutrients, aTTD of nutrients, and DMI, ADG, and G:F are reported in Table 5.7. The negative slopes for fecal DM (-0.18), NIRS-predicted aTTD of DM (-0.10), of starch (-0.13), and of ADF (-0.027), indicated that increases in these variables were associated with the decreases in DMI ($P \leq 0.03$). Negative associations between aTTD and DMI were expected, and similarly aTTD was expected to decrease as silage replaced grain in the diet. However, positive associations were found between aTTD of CP and DMI ($P < 0.01$) and aTTD of NDF and DMI ($P < 0.01$). Increasing fecal OM, starch, and ADF were associated ($P \leq 0.02$) with increasing DMI. Fecal DM, OM, starch, and ADF, and most digestibility coefficients were ($P < 0.01$) also associated with ADG. Negative slopes were observed for fecal DM (-0.066), ADF (-0.058), aTTD of starch (-0.14), and aTTD of GE (-0.035), and positive slopes were observed for fecal OM (0.086), starch (0.067), aTTD of OM (0.040), of CP (0.045), and aTTD of NDF (0.027), and of ADF (0.024, $P < 0.01$; Table 5.7). The association between fecal OM, starch, and aTTD of ADG with ADG were not linear. Fecal OM, starch, ADF, and all measured digestibility coefficients were associated with G:F ($P < 0.01$). All associations were positive, with the exception of fecal ADF (slope = -0.0054), aTTD of starch (slope = -0.0079), and aTTD of GE (slope = -0.0029). All associations were linear, except for fecal starch and aTTD of ADF with G:F.

5.3.6 NEg as Determined from the Chemical Composition of the Diet, Observed Performance, and NIRS

The relationships between NIRS predicted aTTD of GE (%) and NEg (Mcal/kg) of the diet determined from performance for study 1 and 2 are shown in Figure 5.5. For steers fed barley, there was no relationship between aTTD of GE and NEg, whereas for steers fed wheat, a positive relationship was observed ($R^2 = 0.58$, $P = 0.03$). When combining both barley and wheat diets, a weaker relationship was found than for wheat diets alone ($R^2 = 0.58$, $P = 0.05$). The combined equation was used to calculate NEg as determined by NIRS for steers fed barley, and the equation derived from the study was used to predict the NEg of steers fed wheat (Table 5.8 and Figure 5B). A strong linear relationship was observed between NIRS predicted aTTD of GE and

NEg in study 2 (R^2 = 0.43, $P < 0.01$; Figure 5A). Plotting predicted NEg versus observed NEg from both studies together resulted in a linear relationship ($R^2 = 0.52$, $P < 0.01$; Figure 5B).

Compared to observed ADG (Figure 5.5C) and G:F (Figure 5D), NIRS measured aTTD of GE could only predict ADG for the steers fed wheat in study 1 (R^2 = 0.48, $P = 0.05$), and for steers fed increasing levels of silage in study 2 (R^2 = 0.40, $P < 0.05$).

Table 5.8 reports all NEg calculated either from ingredient composition, performance, and NIRS predictions of aTTD of GE. Based on the NEg content of the diet determined from ingredients, NEg of barley and wheat diets were 1.33 Mcal/kg , 1.38 Mcal/kg for the barley and wheat diets, respectively. Prediction using aTTD of GE by NIRS of the feces was able to identify differences in NEg due to differences in grain processing, and differences were most pronounced when NEg was estimated from direct measurements of DMI and ADG.

When silage replaced barley in the diet, NRC (2016) calculations under predicted the NEg values for each of the diets relative to actual performance estimates. However, predictions based on NIRS were more similar to direct measurements, deviating only by 0.01 to 0.03 Mcal/kg compared to actual NEg (Table 5.9). Both NRC (2016) and NIRS displayed expected decreases in NEg as the silage level decreased, whereas the NEg from actual performance decreased from 1.46 to 1.38 Mcal/kg as silage level increased from 0 to 8%, but then an increase to 1.40 Mcal/kg, when silage level was added at 12%. This was similar to the numerical increase observed for G:F for steers fed 12% barley silage, a direct result of the 0.04kg/d increase in ADG compared to the other three diets.

Table 5.6. Linear regression equation coefficients examining the individual associations between near infrared spectroscopy predicted fecal constituent concentrations, digestibilities, and observed feedlot performance for two time periods in study 1 (n=16 pens).[1]

Equation Variables[2]		Grain type					
		Barley			Wheat		
Dependent	Independent	Coeff/slope (β)	SE	*P*	Coeff/slope (β)	SE	*P*
DMI, kg/d	NDF				-0.051	0.021	0.05
ADG, kg/d	Starch				-0.0069	0.0028	0.04
	NDF				0.012	0.0045	0.03
	ADF				0.015	0.0053	0.02
	aTTD of DM				0.026	0.011	0.04
	aTTD of starch				0.014	0.0056	0.04
	aTTD of CP				0.046	0.016	0.02
G:F, kg/kg	PI75				0.011	0.0035	0.02
	PI85				0	0	0.02
	DM	-0.011	0.0055	0.08	-0.0019	0.00060	0.01
	OM				-0.0017	0.00066	0.03
	Starch				-0.00087	0.00025	<0.01
	N				0.055	0.0200	0.03
	NDF	0.0035	0.0018	0.09	0.0015	0.00044	0.01
	ADF				0.0018	0.00052	0.01
	ADL				0.0052	0.0020	0.04
	aTTD of DM				0.0034	0.00092	<0.01
	aTTD of OM				0.0023	0.00070	0.01
	aTTD of starch				0.0018	0.00048	<0.01
	aTTD of CP				0.0056	0.0016	0.01
	aTTD of NDF				0.0016	0.00058	0.03
	aTTD of ADF				0.0017	0.00050	0.01
	aTTD of GE				0.0014	0.00042	0.01

[1] Processing index, day of sampling, and the interactions of PI×Day were included as fixed effects, and the individual pen as the random effect. Feces from 4 of 10 steers per pen were sampled.
[2] aTTD = apparent total tract digestibility. PI = processing index, defined as the bushel weight of the grain after processing divided by the weight before processing).

Table 5.7. Linear regression equation coefficients examining the individual associations between near infrared spectroscopy predicted fecal constituent concentrations, digestibilities, and observed feedlot performance measured over 6 time intervals in study 2 (n=20 pens).

Equation Variables[2]				
Dependent	Independent	Coeff/slope (β)	SE	*P*
DMI, kg/d	DM[3]	-0.18	0.036	<0.01
	OM	0.076	0.0352	0.03
	Starch	0.092	0.0281	<0.01
	ADF	0.061	0.0268	0.02
	aTTD of DM	-0.10	0.037	<0.01
	aTTD of starch	-0.13	0.027	<0.01
	aTTD of CP	0.10	0.016	<0.01
	aTTD of NDF	0.067	0.0132	<0.01
	aTTD of ADF	-0.027	0.0128	0.03
ADG, kg/d	DM[3]	-0.066	0.0201	<0.01
	OM[4]	0.086	0.0195	<0.01
	Starch[4]	0.067	0.0162	<0.01
	ADF	-0.058	0.0134	<0.01
	aTTD of OM	0.040	0.0149	<0.01
	aTTD of starch	-0.14	0.0158	<0.01
	aTTD of CP	0.045	0.0108	<0.01
	aTTD of NDF	0.027	0.0084	<0.01
	aTTD of ADF[4]	0.024	0.0066	<0.01
	aTTD of GE	-0.035	0.0119	<0.01
G:F kg/kg	OM	0.0067	0.00165	<0.01
	Starch[4]	0.0046	0.00139	<0.01
	ADF	-0.0054	0.00118	<0.01
	aTTD of DM	0.0044	0.00162	<0.01
	aTTD of OM	0.0045	0.00131	<0.01
	aTTD of starch	-0.0079	0.00148	<0.01
	aTTD of CP	0.0030	0.00093	<0.01
	aTTD of NDF	0.0019	0.00072	<0.01
	aTTD of ADF[4]	0.0025	0.00057	<0.01
	aTTD of GE	-0.0029	0.00108	<0.01

[1] Silage level (SL), day of sampling, and the interactions of SL×Day were included as fixed effects, and the individual pen as the random effect. Repeated measures were accounted for with an AR(1) correlation structure. Feces from 4 of 8 steers per pen were sampled.
[2] aTTD = apparent total tract digestibility.
[3] Fecal DM was determined using actual DM and not NIRS predictions.
[4]There was a non-linear association for these variables addressed by the introduction of a squared term into the model

Table 5.8. Net energy values determined from diet chemical composition, observed performance, or predictions of apparent total tract digestibility of GE using NIRS in study 1

Diet[1]	Ingredients (NASEM, 2016)		Performance					NIRS		
	NEm, Mcal/kg	NEg, Mcal/kg	DMI, kg/d	ADG, kg/d	G:F	NEm, Mcal/kg	NEg, Mcal/kg	aTTD of GE[2] (%)	NEm, Mcal/kg	NEg, Mcal/kg
B79	1.97	1.33	10.4	1.37	0.131	2.04	1.38	86.3	2.03	1.37
B88	1.97	1.33	10.6	1.31	0.123	1.95	1.30	82.6	1.99	1.34
W80	2.03	1.38	9.90	1.34	0.136	2.08	1.42	89.6	2.06	1.40
W86	2.03	1.38	10.3	1.34	0.130	2.02	1.36	82.6	1.99	1.34

[1]B79 = barley processed to 79%. B88 = barley processed to 88%. W80 = wheat processed to 80%. W86 = wheat processed to 86%.
[2] aTTD of GE = apparent total tract digestibility of gross energy.

Table 5.9. Net energy values determined from diet chemical composition, observed performance, or predictions of apparent total tract digestibility of GE using NIRS in study 2

Diet[1]	Ingredients (NASEM, 2016)		Performance					NIRS		
	NEm, Mcal/kg	NEg, Mcal/kg	DMI, kg/d	ADG, kg/d	G:F	NEm, Mcal/kg	NEg, Mcal/kg	aTTD of GE[2] (%)	NEm, Mcal/kg	NEg, Mcal/kg
SL0	2.03	1.38	11.1	1.84	0.165	2.13	1.46	85.7	2.12	1.45
SL4	2.00	1.36	11.3	1.84	0.163	2.10	1.43	84.2	2.08	1.42
SL8	1.98	1.33	11.7	1.84	0.158	2.04	1.38	82.1	2.08	1.41
SL12	1.95	1.31	11.8	1.88	0.160	2.06	1.40	79.6	2.04	1.38

[1]SL = Silage level, % DM basis.
[2] aTTD of GE = apparent total tract digestibility of gross energy.

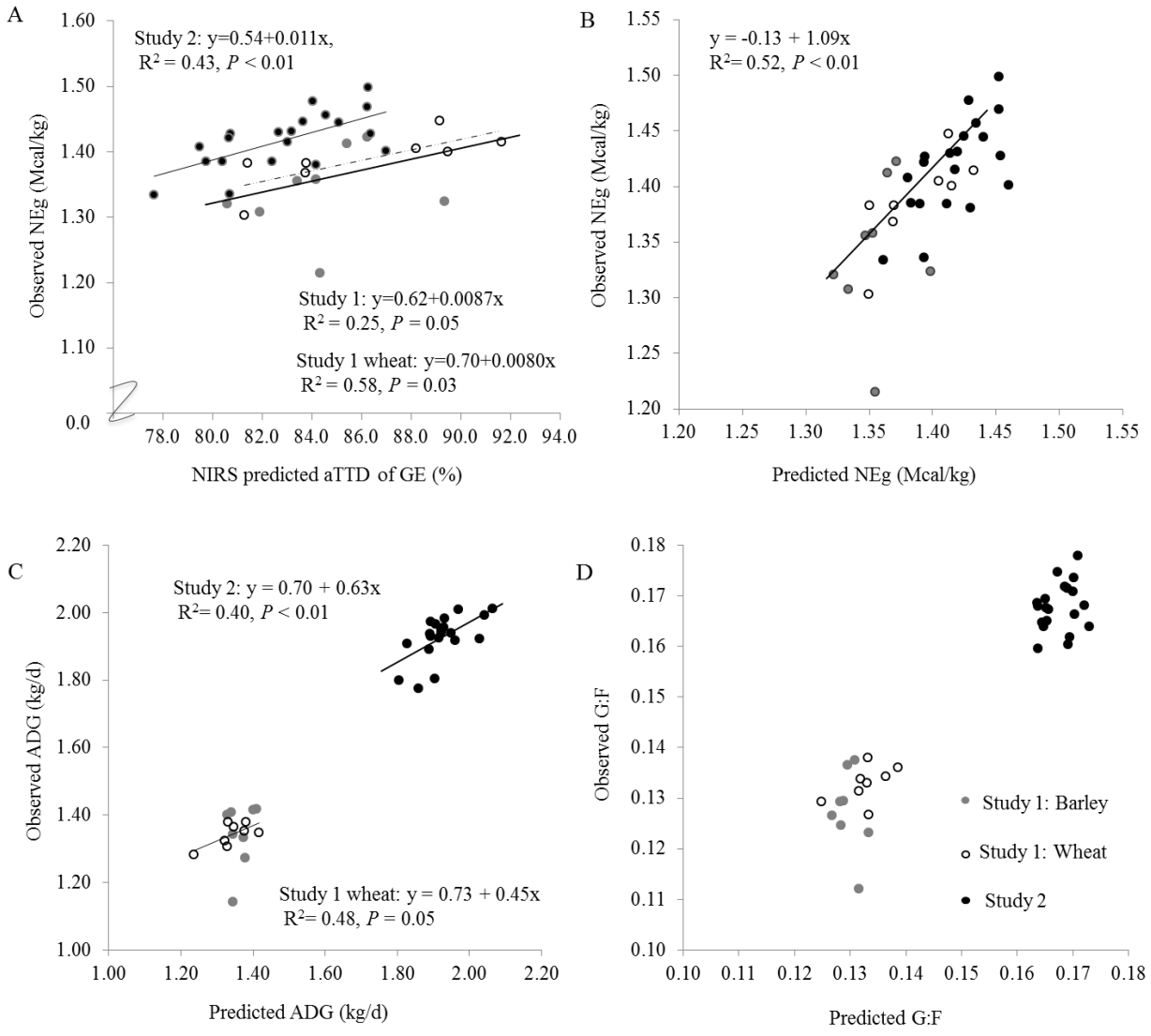


Figure 5.5. Graphs depicting the relationship between (A) apparent total tract digestibility of GE predicted using NIRS and NEg determined from performance where linear relationships are depicted for each study using solid lines, and a dashed line for the wheat diets (B) Observed versus Predicted NEg (C) Observed versus Predicted ADG, and (D) Observed versus Predicted G:F.

5.4 Discussion

5.4.1 Precision of Measurements and Sample Size

In the development of a new technique, an important consideration is the determination of the number of samples necessary to detect differences. Since we sampled from individual steers in study 2, our results are most applicable to samples collected from steers fed these diets, but should still be relevant to cattle fed high-grain feedlot diets. The concentration of starch in feces may be particularly variable, as it can be affected by small changes in grain processing and increasing levels of forage in the diet. Koenig and Beauchemin (2011) found that fecal starch concentrations differed by 7.3% when diets remained constant but grain processing increased from 82% to 87%. In the same study, fecal starch concentration varied by 4.8% when silage level was increased from 3 to 15% of the diet. Starch and N were the only fecal parameters measured in the study, and in both cases, no differences in fecal N concentrations were observed. Although we did not acquire the optimal number of samples for all of the fecal constituents measured, the collection of 4 fecal samples should have generated estimates that were within ± 3% of the mean. The variability in NIRS predicted aTTD of nutrients was not as high as individual constituents, enabling fewer samples to represent the mean of the pen.

The calibrations used to predict the samples within these studies were generated using an initial database of 1110 fecal samples representing 65 diets for fecal chemical composition, and 172 samples representing 23 diets for aTTD of nutrients and GE (Chapter 3). Subsets of fecal samples collected from cattle fed the diets in the current studies were encompassed within the calibration set, increasing the precision and accuracy of prediction. When assessing the predictability of an NIRS calibration, two measures are used, the coefficient of determination of cross validation (R^2_{CV}), for linearity and precision, and the standard error of cross-validation (*SECV*) for accuracy. Cross-validation of calibrations for fecal composition predicted in the current study produced accurate predictions with high linearity ($R^2_{CV} > 0.90$, $SECV < 2.42$) for all fecal constituents, particularly starch ($R^2_{CV} = 0.97$, $SECV < 1.67$), showing that NIRS generates estimates similar to that obtained using wet chemistry. For aTTD of nutrients and GE, accuracy and precision were lower, however acceptable for all variables, aside for aTTD of ADF and NDF. Despite the low predictability of digestibility coefficients, the current method has value in terms of its potential to estimate diet digestibility in group-penned feedlot cattle without using

markers or total collection. Further discussion of the accuracy of the NIRS predictions in the current study is presented in Chapter 3.

These results demonstrate that when fecal samples are collected from a group of individual animals and composited, attention must be given to the number of samples required to estimate the mean. If differences are expected to be large among diets, then fewer samples can be collected to detect differences.

5.4.2 Differences in Sampling Method

In a research setting, fecal samples from performance studies are typically acquired directly from the rectum of feedlot cattle while they are being restrained in a chute. In a commercial feedlot, rectal sampling is impractical as cattle are only handled two or three times during the feeding period. Thus in a commercial setting, collection of fresh fecal pats from the pen floor is a more practical means of obtaining feces as they can be obtained at any time during the feeding period without the need to restrain cattle. Such an approach has been frequently used in studies examining the shedding of *Escherichia coli* O157 in cattle housed in commercial feedlots (Stanford et al. 2013), but care must be taken to ensure that the sample is not contaminated with soil, urine, or bedding.

We deliberately collected fecal samples in the morning as cattle frequently defecate immediately after they rise for the first feeding, and morning samples (0-4 h after first feeding) were found to be representative of the composition of most constituents of feces collected over an entire day (Chapter 4). The greater DM content of feces collected from the pen floor as compared to the rectum was expected owing to greater evaporation upon exposure to air. Additionally, some water may have leached from the bottom of the fecal pat, resulting in a higher DM content of the sample collected from the pat surface. However, on a DM basis, there were only minor differences in the chemical composition of fecal samples collected from the rectum compared to fresh samples collected from the pen floor. Greater fecal N and ADF were observed in pen floor samples in study 1, and lower fecal OM and starch, and greater ADL were observed in pen floor samples in study 2. It is possible that the higher ADL concentration in study 2 pen floor samples may reflect the inadvertent collection of straw bedding with the feces, but could also reflect greater digestion of other nutrients within the feces. However, since these

discrepancies were not consistent between studies, they could be a result of sampling from different steers, with each steer having a unique intake and digestion rate relative to others. The interactions between sampling method and cereal grain type observed within study 1 indicate that the small differences observed between sampling methods were not consistent for wheat and barley, an outcome that may also reflect variation among animals. Another possibility for the discrepancies is that rectal samples were collected an hour or more earlier than pen floor samples. In Chapter 4 I found that the concentration of several nutrients differed when collected 0 to 4 h before feeding, compared to 0 to 4 h after feeding. However, the differences expected according to the earlier study were not confirmed, as the previous findings were based on the average of samples collected over 4 consecutive days from 3 animals. The order of pen sampling in our study may have also played a role in the variation we observed in nutrient concentrations in the feces of the steers at each sampling day. The process of sampling feces from all pens in each study was quite time consuming, with rectal sampling of steers requiring between 2 and 4 h, and pen floor sampling up to 2 h. This may have been a sufficient amount of time to affect excretion patterns in the steers as they vary over 24 h (Chapter 4). This previous study collected fecal samples from individual heifers that were pooled at 4 h-intervals, whereas we collected samples at single time point during the day, possibly resulting in more variation within shorter periods of time.

Sampling method by day interactions were observed for most constituents in study 2, implying that fecal composition varies sporadically over the course of a feeding period. Once again the variation can be partly attributed to pooling samples collected from different steers, or to weather fluctuations. When graphed, the interactions observed between sampling method and day were attributed to fluctuations with no obvious pattern in nutrient concentrations over the course of the feeding period.

Overall, the collection of fresh fecal samples from the pen floor is a practical approach compared to rectal sampling for evaluating changes in the fecal composition of cattle housed in commercial feedlots. Although small, differences were observed between sampling methods for some fecal constituents, but a consistent bias due to collection method only occurred for fecal DM. Variation in predicted chemical composition and digestibility would likely be reduced, and estimates would be closer to the mean, if pen samples were collected from more animals per pen (depending on the measurement of interest), and if samples were pooled over consecutive days.

At a commercial feedlot, stocking density is much greater, and collection of more fecal samples from a suitable number of individuals would be much easier.

5.4.3 NIRS-Predicted Fecal Nutrient Concentration and Digestibility

5.4.3.1 Study 1

Corn and barley grain are the principal energy sources in the diets of North American feedlot cattle (Owens et al. 1997), but the use of wheat has increased in western Canada. Near infrared spectroscopy may provide a simple method for monitoring nutrient utilization and potentially growth performance in commercial feedlots feeding different grain types. Compared to barley, wheat has higher starch and lower fiber concentrations (NRC 1996; McAllister and Sultana 2011), accounting for the greater fecal OM and starch concentrations, and lower NDF, ADF, and ADL concentrations of feces from cattle fed wheat as compared to barley.
Dry rolling disrupts the pericarp / hull and reduces the particle size of grain, increasing the ruminal digestion of all nutrients (McAllister et al. 2011). Disruption of the pericarp further exposes starch within the endosperm to microbial attack, and finer particles present a larger surface area that accelerates microbial colonization and fermentation (McAllister et al. 2011). In our study, more severe processing resulted in a reduction in fecal OM and starch concentrations from cattle fed both wheat and barley. Despite greater digestibility of all nutrients, fecal NDF and ADF concentrations increased, as the increase in starch digestibility was more pronounced than increases in NDF and ADF digestibility. Levels of N in the feces were also greater with more vigorous processing, possibly indicating greater microbial protein synthesis in the rumen and greater excretion of microbial protein in the feces. This is supported by Beauchemin et al. (2001) where microbial protein synthesis in the rumen increased by 50% as barley was processed with increasing severity.

Differences in the response of the two grains to processing could result from many factors including endosperm structure, size and composition of the starch granules (McAllister et al. 2011), kernel hardness (Campbell et al. 2007), moisture content and the size and shape of the kernels (McAllister et al. 2011). Both grains are similar in endosperm structure, where the starch is loosely associated with the protein matrix, and typically require less processing than corn or sorghum for fermentation in the rumen (McAllister et al. 2011). The distance between rollers is

often left wider for wheat than for barley to avoid shattering of the kernels, but may also result in a greater proportion of intact kernels (McAllister et al. 2011).

Fecal composition and nutrient digestibility did not appear to be directly affected by changes in BW or DMI. Instead, variations over the course of sampling were more likely due to differences in digestive processes as a result of sampling different steers. One exception is the linear reduction in fecal OM over time which may reflect the tendency of cattle to consume more soil from the pen floor as the feeding period proceeds. It has been reported that grazing livestock ingest soil as a potential source of essential minerals (Healy 1970; Healy et al. 1970). Cattle located on unpaved lots with sparse vegetation have been shown to ingest soil at rates as great as or greater than animals on pasture (Fries and Marrow 1981). Consumption of soil could account for the reduction in fecal OM over time. Fecal N concentration was found to decrease over the growing period for steers fed wheat, but an explanation for this observation is difficult to formulate because other nutrients did not respond in a similar manner. This may have been due to the occurrence of ruminal acidosis, as suggested by He et al. (2013) and Moya et al. (2015). A decrease in ruminal pH could inhibit growth rates of most ruminal bacteria (Nagaraja and Titgemeyer 2007), thereby lowering fecal N. The decrease in aTTD of NDF over the feeding period could also reflect an increase in the selection of the forage component in the diet or straw bedding in an effort to alleviate discomfort from acidosis.

Fecal OM and N are two variables that are associated with relatively low variability among steers, and had high R^2 and low *SECV* values when predicted using NIRS calibrations (Chapter 3). It is possible that the differences in fecal OM and N that we observed over time were due to our greater precision at measuring these variables as compared to others.

Since we used fecal nutrients collected over the entire feeding period, it was necessary to also describe the fluctuations in performance over time. Beef cattle performance is dependent on many factors including the initial BW, age, sex, animal behaviour, hierarchical behaviour, diet, inclusion of feed additives and anabolic agents, as well as ambient factors [season, temperature (NRC, 2016). Since all other factors were equal, the only elements that could impact the growth of the steers in study 1 was the grain type and processing. Steers fed the more processed grain had lower intakes and increased G:F because the nutrients were more available in the feed. The changes over time we observed (not shown) indicate that DMI increased over the feeding period at the same rate for all diets. Both ADG and G:F varied over the feeding period, with the same

patterns observed for both levels of processing, but these patterns were not the same for wheat vs barley.

5.4.3.2 Study 2

Increasing the proportion of forage in the diet is one approach to alleviating or reducing digestive disorders in cattle (Koenig and Beauchemin, 2011). However, G:F can also be reduced as a result of the higher levels of fiber in the diet (Koenig and Beauchemin, 2011). With more of the diet consisting of silage, the DM content of the diet decreased, and ~~the~~ fiber concentrations increased, resulting in reductions in fecal DM content and increases in fecal NDF and ADL concentrations. Fecal starch concentration remained consistent among diets despite the fact that the level of starch in the diet decreased with increasing silage. Greater silage levels in the diet are associated with increased intake (Koenig and Beauchemin, 2011), which could increase the passage of starch from the rumen and its appearance in feces. This would also explain the linear reduction in other digestibility coefficients over time. However, any increase in passage rate of starch was likely offset by a reduction in the concentration of starch in the total diet and thus no increase in fecal starch concentration was observed. In addition, the barley grain in silage may be less digestible as compared to processed grain as the kernels in silage often remain intact (Koenig and Beauchemin, 2011. Koenig and Beauchemin (2011) who fed increasing levels of silage to feedlot cattle also reported that starch digestibility was not affected by the proportion of silage in the diet as predicted by the equation of Zinn et al. (2007). With increasing levels of silage, a reduction in the grain content and an increase in less digestible forage may have slowed fermentation rates and microbial turnover, accounting for the reduction in fecal N.

The lack of linear or quadratic responses with respect to day, as well as the lack of interactions between silage level and day (apart from aTTD of CP and NDF) suggest that sampling at any time during the feeding period was equally effective at detecting differences among diets. When examined more closely, even the interactions observed between silage level and day for aTTD of CP and NDF were due to only slight variations in the patterns of these measurements over time (Figure 5.4). Models did not examine associations between fecal constituent concentrations or digestibilities and performance at each sampling day due to the limited power of the experiment. Sensitivity to detect statistical differences may have been compromised due to small sample size, and high *SECV* values for digestibility coefficients.

As the silage proportion in the diet increased, and diet digestibility decreased, cattle consumed more feed, and G:F was compromised. Over the course of the feeding period, DMI, ADG, and G:F fluctuated, but the lack of interactions between silage level and day indicated that the steers fed all four diets responded in a similar fashion.

Overall, the results from these two studies indicate that alterations in grain processing, grain type, and silage level have measurable impacts on fecal nutrients and nutrient digestibilities that can be predicted using NIRS. Our data also demonstrate that in certain circumstances (study 1), sampling at any time (any BW) during a feeding period from multiple cattle within a pen will generate similar fecal DM, starch, aTTD of DM, OM, starch, and GE values when predicted using NIRS. For other measured variables, and for all fecal composition, and digestibility predictions in study 2, variations were found with day of sampling. Although we did not statistically compare fecal measurements taken at individual sampling days to the overall averages, it is likely that multiple sampling could approximate overall averages. The minimal occurrences of interactions with sampling day and grain type as well as with processing index or silage level, indicate that the slopes of the regression lines for the predictor variables are also consistent throughout the feeding period. The predictive estimates would certainly be improved if sample sets were broadened either by sampling more cattle within a pen, or collecting fecal samples on more consecutive days all within 4 h of first feeding. Some exceptions include fecal OM and aTTD of NDF that were found to decrease over time in study 1, and fecal N, which decreased over the feeding period in steers fed wheat. When generating predictions using NIRS, loss in accuracy is expected, since some measurements are better predicted than others. One must also consider increasing the sample size to adjust for losses in accuracy due to NIRS.

5.4.4 Associations to DMI, ADF, And G:F, and Predictions Of NEg, ADG, And G:F

The use of NIRS by the livestock industry has permitted nutritional information of the diet (primarily of grazing ruminants) to be obtained from feces, allowing researchers and nutritionists to rapidly improve management strategies. Because measurable changes in fecal composition occur when cattle are fed different diets (Arman et al. 1975, Hinnant 1979, Holloway et al. 1981), it is not surprising that relationships can be found between fecal composition and performance (Holechek et al. 1982). Both dietary and fecal N concentrations were found to be

correlated with ADG in grazing cows (Holechek et al. 1982). Direct relationships have also been found between fecal starch concentration and starch digestibility (Fernandez et al. 1982; Zinn et al. 2002; Owens and Zinn 2005), and between fecal CP concentration and OM digestibility (Lukas el al. 2005), factors that influence G:F (Theurer 1986; Firkins et al. 2001).

Currently most reports for predicting performance of cattle using NIRS are restricted to DMI of free-ranging cattle, but fecal NIRS has been used to predict diet quality (diet CP and digestible OM), coupled with decision support software and nutritional balance calculations to monitor the nutritional status and growth performance of cattle on pasture (Tolleson and Schaffer, 2014). They also demonstrated that fecal NIRS can be useful in projecting body condition score in combination with other information.

In the first stage of exploring this data, we considered the associations between one measure of fecal nutrient composition or digestibility at a time and performance measures after accounting for study design variables. In commercial feedlots, tools are needed to predict performance for the entire feeding period. We developed equations using either pen samples at specific points in time (study 2) or averages of multiple samples at representative intervals (study 1) repeated over the feeding period. Attempts were made to use multiple regression to predict outcome variables with two or more independent variables (data not shown), but unfortunately, the small dataset in our study led to overfitting, an outcome that others have found can result in misleading models (Babyak et al. 2004).

The results of our individual regressions demonstrated that when wheat is processed to an index of 80 and 86, there are a number of significant associations between processing index, fecal composition, digestibility and DMI, ADG, and G:F. For the barley diet, there were no significant associations in this study. For the wheat diets, we found that NDF was associated with DMI, which is consistent with the fact that equations have been developed to predict intake from dietary NDF concentration (Mertens, 1987; Beauchemin, 1996). Poor processing of wheat resulted in changes in fecal composition and G:F, and because many fecal constituents are associated, fecal OM, starch, N, NDF, ADF, and ADL were all indicators of ADG or G:F. We found more associations between fecal parameters and G:F compared to ADG, because G:F was directly associated with processing index. With highly fermentable grain-based diets, changes in ADG of cattle are not as common as changes in G:F, which is often observed with changes in grain type or grain processing (Owens et al. 1997). Unlike in the study of Holechek (1982), fecal

N was not a predictor of ADG, but they examined predominantly forage diets, where small improvements in diet quality can lead to large improvements in ADG. The positive slopes for aTTD of nutrients and GE are expected considering this value is equivalent to DE, and a diet with greater digestible energy, should result in improved performance.

When silage level in the feedlot diets was increased, fecal OM, starch and ADF concentrations were predictors of DMI, ADG, and G:F. However, unlike study 1, greater fecal starch, and lower starch digestibility, resulted in improved ADG and G:F. This is likely a result of similar fecal starch concentrations and digestibility in all four diets, which were numerically greater in fecal starch in the steers fed 0% barley silage. Considering the role of starch in determining the energy density of cereal grains, fecal starch and predicted starch digestibility have potential as indicators of G:F. However, in study 2, fecal starch and starch digestibility no longer fulfilled these expectations. Owens and Zinn (2005) recommended a fecal starch concentration < 5% and TTD of starch > 95% for optimal starch utilization in feedlot cattle, where grain was the primary or sole source of starch. In study 1, fecal starch was above this threshold, and aTTD of starch was below, and in study 2, fecal starch and aTTD of starch were both above the 5% and 95% thresholds, respectively. It is possible that fecal starch measurements are most useful for making changes to management practices when starch digestibility is 95% or lower, as suggested by Owens and Zinn (2005). The diets in study 2, also had lower grain inclusion levels (68 – 80%), which may also compromise the efficacy of using fecal starch as an indicator of starch digestibility or G:F. Owens et al. (2016) stated that the accuracy of predicting starch digestibility from fecal starch is markedly reduced when the starch content of the diet is ignored, and in study 2, the starch content of the four diets was variable, unlike study 1. Additionally, differences in aTTD of DM also add a degree of imprecision to the relationship between fecal starch and starch digestibility (Owens at al. 2016), and likely G:F as well.

With the exception of aTTD of starch and of GE, increases in other digestibilties (aTTD of OM, CP, NDF, and ADF) were associated with greater ADG and G:F. Despite the lack of differences in ADG among all four diets in study 2, fecal predictors of ADG were similar to those for G:F, adding merit to these associations when increasing the experimental power using repeated measures. It is surprising that NIRS predicted aTTD of GE yielded negative associations to both ADG and G:F in study 2. Since this value is comparable to DE (%) since it

is developed from calculating the GE consumed and excreted in cattle undergoing total collection experiments. It is expected that as silage level increases, both DE and aTTD of GE will decrease, but the reason that we did not find a positive association, is because the increase in DMI had the effect of maintaining gains among the four treatments, leading to small reductions in G:F. For this reason, we must factor in the DMI, which affects the metabolizable energy (ME) available to the animal.

Our second approach at predicting performance included predicting the NEg of the diets, as determined from performance using NIRS predicted aTTD of GE. We found that NIRS equations for NEg based on predicted aTTD of GE required unique equations for study 1 and study 2, and for different grain types. One explanation for this is that steers in study 1 differed in age from steers in study 2, hence muscle and fat deposition was occurring at different rates. The initial body weights of steers in study 1 were much higher than those in study 2, yet they were both fed to 650 kg, therefore the NEg was greater overall for steers fed in study 2. In addition, DMI was almost 2% greater for steers in study 1 than study 2. Compared to NRC (2016) calculations, prediction using aTTD of GE by NIRS of the feces was much better at identifying differences in NEg due to differences in grain processing. Using the NRC (2016) method of calculation, we were unable to differentiate the impact of grain processing on the NEg estimate of these diets, therefore NIRS of feces proves to be a much better estimate of these values.

Once NEg were calculated using the individual equations, a linear relationship emerged between observed and predicted NEg, irrespective of study or grain type. Our NIRS predictions of ADG were able to differentiate between the two studies very well, however within study, significant noise was observed, likely due to animal and pen variation, and sensitivity of measurements. This was even more apparent for G:F, likely because dividing by DMI added an additional variable that further increased variability. Using aTTD of GE to predict net energies in feedlot cattle shows promising results in our small dataset. Incorporating DMI and average BW of cattle in future equations would likely increase the accuracy of predictions.

5.5 Conclusions

Fecal sampling from the pen floor is a simple and reliable method to obtain information relating to the chemical composition of feces and the digestibility of the diet. Changes in grain processing and in the forage to concentrate ratio resulted in measurable changes in fecal composition and nutrient digestibility that were predictable by NIRS. Aside for determination of fecal OM, N, and aTTD of NDF, which may decrease over a feeding period, sampling more cattle or over multiple days would likely generate more representative samples of a particular diet, and remove the bias of a single sampling day. Associations between fecal parameters and DMI, ADG, and G:F were found, but they were not consistent across all diets. Our predictions of NEg and ADG using NIRS predicted aTTD of GE demonstrated value, and merit further research. One factor to consider is when developing equations, cattle should be in similar stages of maturity, or separate equations must be derived. Generally, the more consistent the feeding practices, such as grain type, proportion and processing, the more likely it is that predictions will be meaningful. Including additional variables such as breed, stage of maturity, ambient temperature, and growth promotants may also improve predictions in future studies.

5.6 Next Stage

The current study was successful in demonstrating that fecal nutrients and diet digestibility predicted using NIRS are associated to changes in performance. I also showed that digestibility of gross energy shows potential to be used to predict net energy of gain, and growth performance in feedlot cattle fed high grain diets. The subsequent study assessed the ability of NIRS of both feed and feces to predict net energy and growth performance in feedlot cattle when a large proportion of the diet contained by-products, including grain screening pellets and dried distillers grains. The ability to combine fecal and feed NIRS technology to predict growth performance in commercial feedlots is the objective of the next chapter.

CHAPTER 6
6.0 USE OF NEAR INFRARED SPECTROSCOPY TO ASSESS DIGESTIBILITY AND GROWTH PERFORMANCE IN FEEDLOT CATTLE FED PELLETED GRAIN SCREENINGS[4]

6.1 Introduction

The expansion of the bio-fuel industry has increased demand for cereal grains, encouraging producers to seek cheaper alternative by-product feeds for cattle. Grain and pea screenings, dried distillers' grains (DDG), canola meal, and oat hulls are by-product feeds that arise from the industrial processing and cleaning of crops. These ingredients have become common substitutes for cereal grains in beef cattle diets, with grain screenings being the primary by-product arising from the Canadian grain industry. In Saskatchewan, over 600,000 tonnes of grain screenings are produced annually and it is estimated that there are as much as one million tonnes of feed screenings available in western Canada each year (PAMI, 2000).

In Canada, grain screenings consist of mixtures of broken grain, chaff, weed seeds, and dust and are marketed primarily on the basis of bulk density (Marx et al. 2000). The ingredients and chemical composition of screenings varies considerably, making it difficult to predict their feed value (Beames et al. 1986). Typically grain screenings are higher in protein and fibre, but lower in energy than cereal grains (Marx et al. 2000), and can account for up to 50% of the diet DM in barley-based diets without compromising the growth performance of feedlot steers (Pylot et al. 2000).

Considering the compositional diversity of grain screenings, there are obvious advantages in predicting their nutrient content. Grain screenings can be pelleted to increase their bulk density, reduce dust, improve handling, and formulated using known ingredients so as to generate a more defined nutrient composition (Thomas and van der Poel 1996). As screenings are purchased on the basis of bulk density, rapid assessment of their feed value is desired so purchasers can use them to formulate balanced diets for feedlot cattle. Near infrared spectroscopy (NIRS) is an alternative method to wet chemistry that can rapidly predict the nutritional value of dietary ingredients (de Boever et al. 1995). It is currently used by some Canadian feedlot producers and

[4]A version of this chapter has been accepted with minor revisions to the Canadian Journal of Animal Science. Jancewicz, L. J., Gibb, D. J., Swift, M. L., Penner, G. B., and McAllister, T.A. 2017. Use of near infrared spectroscopy to assess digestibility and growth performance in feedlot cattle fed pelleted grain screenings. Can. J. Anim. Sci. XX: YY'ZZ.

feed mills to predict the energy content of grain screenings (Gibb, personal communication). However, predictions of energy content are derived from calculations based on nutrient or anti-nutritional components of the feed (NRC, 2016; Owens et al. 1999; Weiss 1992) rather than actual cattle growth performance.

In addition to monitoring feed, NIRS has been applied to measure fecal composition and nutrient digestibility of grazing (Boval et al. 2004; Dixon and Coates 2009) and feedlot cattle (Chapter 3), as well as the efficiency of feed utilization by feedlot cattle (Chapter 5). In high grain diets, starch is the major source of dietary energy and its concentration in feces has been shown to be a predictor of starch digestibility (Zinn et al. 2002; Zinn et al. 2007; Zinn et al. 2011). Strong negative associations were found between NIRS-predicted fecal starch and ADG and G:F for wheat-based finishing diets that were inadequately processed (Chapter 5), whereas positive associations were found between ADG, G:F and NIRS-predicted fecal NDF and apparent total tract digestibility (aTTD) of DM, starch, and CP. In contrast to cereal grains, grain screenings are typically quite low in starch with a larger proportion of their energy value being associated with the presence of digestible lipids, proteins, and fiber (Marx et al. 2000). Understanding whether fecal nutrient concentration and nutrient digestibility within compositionally diverse ingredients such as grain screenings are related to DMI, ADF, and G:F could be useful information for the nutritional management of feedlot cattle.

The objectives of this research were to assess the extent to which NIRS could predict digestibility and growth performance using the feces of heifers fed grain screening pellets (GSP), as well as the chemical composition and the energy value of GSP as compared to direct measurements of growth performance in feedlot cattle.

6.2 Materials and Methods

6.2.1 Grain Screening Pellets

Two different GSP were manufactured at Hi-Pro Feeds (Lethbridge, AB). On a DM basis, the light screenings pellet (LSP) contained a 60:40 mixture of "heavy" and "light" screenings whereas the heavy screenings pellet (HSP) contained 77.5% "heavy" screenings, 10% millrun, 10% beet pulp, and 2.5% canola oil to raise the levels of crude protein (CP), neutral detergent fiber (NDF) and EE (Table 6.1). Light and heavy screenings were a mixture arising from the

cleaning of predominantly barley grain, lentils and canola. Light screenings contained more chaff and hulls whereas heavy screenings were composed of more broken small grains, weed seeds, and canola. The two lots of GSP were prepared for the entire experiment and transported to the Lethbridge Research Center for use over the duration of the study.

6.2.2 Experimental Design, Animals, Housing, and Diets

The study received institutional animal care approval and was conducted according to the guidelines established by the Canadian Council on Animal Care (2009). A total of 150 yearling British crossbred heifers (445 ± 35.5 kg) were housed in pens, bedded with straw, and provided with free access to water. The experiment was conducted as a completely randomized design with 3 dietary treatments and 5 replicate pens per treatment. Each pen housed 10 heifers, with pens being randomly assigned to diet. Upon arrival at the research feedlot, heifers were ear tagged, implanted with a growth promoter (Component TE-S with Tylan, Elanco Animal Health, Guelph, ON, Canada), and vaccinated (Pyramid 4 modified-live vaccine, Fort Dodge Animal Health, Overland Park, KS; Vision† 7, Intervet Int., Millsboro, DE). Heifers were transitioned from a barley silage diet to their respective high grain diet by increasing the level of barley grain and decreasing the level of silage by 11% every 5 d. The control diet included 76% dry-rolled barley, 10% wheat distillers' grains, 9% barley silage, and 5% supplement (DM basis). Pelleted grain screenings were substituted for 20% of the barley grain in both the LSP and HSP diets at the start of the transition period. Diets were formulated to meet or exceed NRC recommendations (2001). Barley grain was obtained from a single source and was dry-rolled to a processing index of 81%, calculated as the weight of the grain after dry rolling divided by the weight of whole unprocessed grain. Both monensin sodium and tylosin phosphate were included in the diets at 33 and 11 ppm, respectively. Melengestrol acetate was also included in the diet at 0.43 mg d^{-1} to suppress estrus (Table 6.1).

Initial body weight (445 ± 35.5 kg) was determined by weighing the heifers on two consecutive days after they had adapted to the finishing diets and were re-implanted after 50 d on feed. Feed was delivered once daily in the morning and heifers were fed to appetite to minimize orts. Heifers were fed finishing diets for 100 d to a target slaughter weight of 600 kg, before being transported to a commercial abattoir and all slaughtered on the same day.

6.2.3 Feed Sampling and Analysis

Samples of grain, barley silage, TMR and orts were collected weekly and composited after 50 d and 100 d. Sub-samples of the GSP were collected from the bins monthly (500 g). Wheat DDGS and supplement were collected twice throughout the experiment. The processing index of the grain was verified by weighing barley samples every two wks before and after processing. The DM content of ingredients, TMR, and orts was determined by drying (200 g) at 55ºC for 48 h in a forced air oven, followed by grinding through a 0.75-mm screen of a Retsch grinder (Verder Scientific, Inc, Newton, PA) and retained for chemical analysis.

6.2.4 Fecal Collections

Just prior to the morning feeding on d 18, 45, and 78 of the finishing diets, four fecal subsamples (> 200g) were collected from the floor of each pen. The time points were selected so as to represent the full finishing period. Fresh fecal samples were collected from pats in a manner that ensured that they were not contaminated with soil, bedding, or urine as described previously (Chapter 3 and 5). Samples were pooled by pen on an equal wet weight basis (400 g total), generating 45 samples over the feeding period. Samples were dried at 55ºC and ground through a 0.75 mm screen of a Retsch grinder in preparation for scanning using NIRS.

6.2.5 NIRS Analysis of GSP and Feces

Near infrared spectroscopy calibrations for nutrient composition and total digestible nutrients (TDN, %) of GSP were developed prior to the initiation of the feedlot experiment. The calibrations included an independent set of GSP from the current feedlot study and were part of a bigger research study being used for commercial application. The calibrations were derived from 101 samples of GSP collected from feedlots as well as from GSP manufactures (West Central Pelleting, Wilkie, SK and C.B. Constantini, Brooks, AB; Table 6.1). The predicted chemical constituents included OM, starch, N, ADF, NDF, ADL, and EE, and TDN. The TDN value was determined using calculations according to Conrad et al. (1990) with the refinements of Owens et al. (1999): $TDN = 0.98\times(100 - NDF - CP - Ash - EE) + (0.984 - 0.0016\times NDF)\times CP + 2.7\times(EE - 1) + 0.75\times(NDF - ADL)\times(1 - (ADL/NDF)^{0.667} - 7)$ prior to calibration development. The GSP were ground using a heavy duty electric spice grinder (Waring, Stamford, CN) for 3-,

3 sec periods, and scanned in a 250 mL quartz bottom sample cup using a Spectra Star near-Infrared analyzer (2400 model) Top Window Series (Unity Scientific, Brookfield, CT, USA). The scan was conducted between 1250 and 2350 nm in 1-nm increments and scanned samples were retained for chemical analysis and used in the development of the calibrations.

Calibrations were developed using partial least squares regression in Unity Calibration Software (Ucal) version 2.0.0.31. The same mathematical treatment to the raw spectra was applied to each calibration (Unity Scientific, Brookfield, CT, USA), "1, 8, 10, 1", where the first digit was the order of the derivative, the second was the gap over which the derivative was calculated, the third was the number of data points used in the running average for smoothing of derivative spectra, and the fourth was the number of data points over which the second smoothing was applied (Shenk et al. 1989). Derivative spectra were used to emphasize small or large absorption peaks, and minimize overlapping peaks and baseline correction (Giese and French 1955). Both the standard normal variate, scatter correction, and detrend (DT) functions were applied (Barnes et al. 1989).

Principle component analysis (PCA) was used to visualize the differences in spectral populations of the two GSP in the feedlot study and those within the calibration set. The predictive ability of NIRS calibrations strongly depends on the spectral similarities of the unknown samples and the calibration set. The two subsamples of the LSP and HSP fed in the feedlot study were packed into 250-mL quartz bottom cups and scanned as described above. Using the same mathematical treatments as above, a scatter plot of principle component (PC) scores for each sample within the GSP of the feedlot set, and the GSP within the calibration set were plotted along the first two PC factors (x axis = Factor 1, y axis= Factor 2).

For fecal samples, previously developed NIRS calibrations (Chapter 3) were used to predict fecal OM, starch, N, NDF, ADF, ADL, EE, and apparent total tract digestibility (aTTD) of DM, OM, starch, NDF, ADF, and gross energy (GE). Fecal samples were packed into a small quartz ring cup and scanned between 1250 and 2350 nm, in 1-nm increments. Two subsamples were scanned in duplicate. Principle component analysis (PCA) was used to visualize the differences in spectral populations of the fecal samples in the feedlot study and those within the calibration sets for both chemical composition and digestibility.

6.2.6 Chemical Analysis

Ground samples of TMR and ingredients from the feedlot study, and GSP that were used in the development of calibrations were analyzed for analytical DM [Association of Official Analytical Chemists (AOAC) 2005, method 930.15], OM (AOAC, method 942.05), starch, N, NDF, ADF, ADL, EE, and ash (AOAC, method 942.05). Samples were further ground in a ball mill (Mixer Mill MM2000, Retsch, Haan, Germany) for determination of starch and N. Starch was determined using a YSI 2700 Biochemistry Analyzer (Yellow Springs, OH). Nitrogen (AOAC 992.23) was quantified using a LECO N analyzer (Joseph, MI) as described by Watson et al. 2003. The ANKOM fiber analyzer (ANKOM technology Corp., Fairport, NY) was used for sequential determination of NDF and ADF in duplicate, with heat stable α-amylase and sodium sulphite included in the NDF analysis. Acid detergent lignin was extracted using 72% sulphuric acid after the ADF procedure (Van Soest et al. 1991). Ether extract (EE; AOAC 2005, method 2003.05) was extracted in a Soxtec HT6 System (Foss, Eden Prairie, MN) using anhydrous diethyl ether, followed by drying. Ash was determined by subtracting the OM content from 100 (% DM basis). Total digestible nutrients were calculated using wet chemistry values and the equation derived by Owens et al. (1999).

6.2.7 Growth Performance and Carcass Quality

The average DMI of steers in each pen was estimated by subtracting the amount of TMR DM delivered to the pen each week from the amount of orts DM. Average daily gain (ADG) was calculated by subtracting the final weight just prior to slaughter from initial weight both measured on two consecutive days and dividing the result by the number of days on feed (100). Gain:feed was calculated as kg of body weight gained per kg of DMI. Average daily gain and G:F were expressed as shrunk weights (live weight × 0.96)

All heifers were slaughtered at a federally inspected facility at the end of the experiment. The weight of the warm carcass was determined after the hide was removed and the carcass eviscerated (with kidneys removed). Back fat thickness, rib eye area, and percentage of Choice and Prime quality grades were determined by personnel of the Canadian Beef Grading Agency (Canada Beef 2014). Lean meat yield (%) was estimated using the equation: 57.96 – 0.027 × carcass weight + 0.202 × rib eye area – 0.703 × back fat thickness, as described by Basarab et al.

(2003). The percentage of B4 carcasses (dark cutters) was reported, along with the percentage of livers with at least 1 abscess. When the liver had at least 4 small abscesses or at least 1 abscess with diameter greater than 2.5 cm, it was designated as severe (Klinger et al. 2007).

6.2.8 Energy Calculations as Determined from NIRS of the GSP and Measured Performance

The net energy for maintenance (NEm) content of each diet was calculated from BW, DMI, and ADG as described by Zinn et al. (2002) using the estimates of energy gain (EG, Mcal/d) and the maintenance energy (EM, Mcal/d) expended based on growth performance for a medium frame yearling heifer [$EG = 0.0557 \times (\text{average weight} \times 478/\text{mature weight})^{0.75} \times ADG^{1.0971}$; where average weight is the mean shrunk weight (full weight × 0.96) and mature weight was 625 kg (defined as the weight at which protein deposition stops and all subsequent gain is fat) and $EM = 0.077 \times MBW^{0.75}$ (NRC, 2016)]. The NE values of the diet for maintenance (NEm) was estimated from the performance and feed intake using the quadratic formula [$x = -b \pm \sqrt{(b^2 - 4ac)}/2a$], where a = -0.877DMI, b = 0.877EM + 0.41DMI + EG, and c = -0.41EM (Zinn and Shen, 1998). Net energy of maintenance was converted between NEg and TDN using the equations TDN = (NEm + 0.5058)/0.0305 and NEg = 0.877×NEm-0.41 (Zinn et al. 2002; NRC 2016).

The NEm content of the GSP was calculated as described by Zinn et al. (2002); [(NEm of GSP diet – NEm of control diet)/proportion of GSP in diet + NEm of barley grain], where 0.2 is the proportion of GSP in the diet and 2.06 is the NEm of the barley it displaced.

6.2.9 Statistical Analysis

To determine the difference between wet chemistry and NIRS predictions of chemical composition and TDN of the two GSP, the Proc MIXED procedure in SAS (Version 9.2; SAS Inst. Inc. Cary, NC) was used, with Diet and Method and their interaction as fixed effects. Differences among treatments for the NIRS predicted fecal composition and aTTD of nutrients were identified using the PROC MIXED function for repeated measures of SAS. The model included treatment (T), day of sampling (D; i = 1, 2, and 3, representing collection d 18, 45, and 78) and the interaction of treatment × day (T×D). The interaction was only retained if $P < 0.05$.

The model used was $Y_{ijk} = \beta_0 + \beta_1 T_{ijk} + \beta_2 D_{ijk} + \beta_3 T \times D_{ijk} + \varepsilon_{ijk}$, where εijk is the random error associated with each observation. A first-order autoregressive structure was used to model repeated measures on individual pens at each day. Least square means of the treatments were separated using PDIFF statement.

Dry matter intake, ADG, and G:F from the feedlot study were analyzed as a completely randomized design using the mixed procedure of SAS with pen as the experimental unit, and treatment as a fixed effect. The model used for the analysis was $Y_{ijk} = \mu + \alpha i + \varepsilon ijk$, where Y was the observation of the dependent variable, μ is the population mean, αi is the fixed effect of treatment, and εijk is the random error associated with the observation. Carcass characteristics were analyzed as a completely randomized design using Proc MIXED of SAS except for categorical variables such as carcass grade, B4 carcasses, and number of liver abscesses which were analyzed using the Chi square option of Proc GLIMMIX.

To determine associations between the fecal nutrients, digestibility, and DMI, ADG, and G:F, bivariate regression was performed using PROC REG function. The model was $Y_{jk} = \beta_0 + \beta_1 X_{jk} + \mu_{jk} + \varepsilon_{jk}$, where Y is the dependent variable (overall DMI, ADG, or G:F), X is the independent variable (average of fecal nutrient concentration, or digestibility over three times points), μ_j was the random effect for pen, j was pen, β_0 was the average y intercept, and ε_{ijk} was the associated error. For all statistical analyses, significance was declared at $P \leq 0.05$ and trends at $P \leq 0.10$.

6.3 Results

6.3.1 Composition of Grain Screenings Pellets and Diets

Compared to LSP, HSP were greater in starch (23.2 vs 20.5%) and EE (6.7 vs 4.2 %), but after formulating the TMR, differences in EE were much less pronounced (2.8 vs 2.3%) owing to the 20% inclusion rate (Table 6.1). Diets were isonitrogenous, but the control diet was approximately 8% higher in starch, 6% lower in NDF, 4% lower in ADF and 0.5 and 1% lower in EE as compared to the LSP and HSP diets.

Principle component analysis demonstrated that the two GSP from the feedlot study differed from the mean population of the calibration set (Figure 6.1). As only two types of pellets (n=2) were examined, we could not use standard measures for determining the accuracy and precision

of the GSP calibration equations. Instead of using coefficients of determination of calibration (R^2_{cal}), and standard error of calibration (*SEC*), we compared the two methods by mean separation of the diets and the analytical method (wet chemistry versus NIRS; Table 6.2). There was no effect ($P > 0.05$) of method for DM, NDF, and EE, whereas all other constituents varied for NIRS vs wet chemistry. Interactions ($P < 0.03$) between diet and method were found for DM, starch, ADL, and ash.

6.3.2 Fecal DM and NIRS Predicted Nutrient Composition and Digestibility

The DM did not differ ($P = 0.64$) among feces collected from heifers fed the three diets (Table 6.2). Organic matter, N, and EE levels were greater ($P < 0.01$), and NDF was lower ($P < 0.05$) in feces collected from heifers fed the control diet as compared to those fed the LSP or HSP diets. There was also a tendency ($P = 0.09$) for fecal starch to be greater in heifers fed the control diet as compared to the GSP diets. Principle component analysis demonstrated that all fecal samples were within the population of the calibration set for chemical composition (Figure 6.2), which was expected since a subset of these samples was used in calibration development (Chapter 3).

There were no differences in the NIRS predicted aTTD of nutrients or GE among treatments (Table 6.2). It is evident from the PCA graph (Figure 6.3) that the feces collected from the LSP heifers were furthest from the mean population of the NIRS calibration for digestibility, compared to feces obtained from the heifers fed the control and HSP diets.

6.3.4 Performance of Cattle and Carcass Traits

Initial and final BW and DMI of heifers did not differ ($P \geq 0.18$) among diets. The ADG tended to be greater ($P = 0.06$) for heifers fed the control diet as compared to LSP, with no differences observed between control and HSP or LSP and HSP diets (Table 6.3). The G:F was greater ($P = 0.02$) for control heifers than for heifers fed GSP. With the exception of a greater ($P = 0.05$) level of B4 carcasses (dark cutters) in the control group, there were no differences ($P \geq 0.41$) in hot carcass weight, back fat thickness, rib eye area or liver abscesses among heifers fed different diets.

Table 6.1. Ingredient composition of grain screening pellets, and ingredient and chemical composition of total mixed diets fed to cattle with and without grain screening pellets.

Item	Diets		
	Control	LSP	HSP
Pellet ingredient composition % DM basis			
Heavy Screenings		60.0	77.5
Light Screenings		40.0	
Millrun (ground)			10.0
Beet Pulp (ground)			10.0
Canola Oil			2.5
Pellet chemical composition % DM basis			
Dry Matter		91.0	90.8
Organic Matter		91.7	92.0
Starch		23.2	20.5
Crude protein		13.9	13.0
Crude fiber		17.2	17.1
Neutral detergent fiber		38.8	40.0
Acid detergent fiber		24.4	25.7
Lignin		4.5	4.8
Ether extract		4.2	6.7
Ash		8.3	8.0
TDN		69.0	71.0
Diet Ingredient composition, % DM basis			
Dry rolled barley, PI = 81	76.0	56.0	56.0
Barley silage	9.0	9.0	9.0
Wheat dried distillers grain	10.0	10.0	10.0
Supplement[a]	5.0	5.0	5.0
LGSP		20.0	
HGSP			20.0
Diet Chemical Composition, % DM basis			
Dry Matter	84.3	84.9	84.8
Organic Matter	95.0	94.1	93.9
Starch	48.4	40.8	40.3
Crude Protein	14.3	14.6	14.4
Neutral detergent fiber	20.5	26.1	26.5
Acid detergent fiber	9.5	13.3	13.9
Lignin	1.9	2.6	2.6
Ether extract	1.8	2.3	2.8
Ash	5.0	5.9	6.1
TDN	75.1	72.4	72.9

Note : PI, processing index of grain, calculated as the ration of the bushel weight after processing over the bushel weight before processing; LSP, diet fed with light screening pellets; HSP, diet fed with heavy screening pellets; [a]Supplement composition (as fed basis) is 54.5% Barley chop, 10.0% canola meal, 25.0% $CaCO_3$, 2.5% molasses, 3.0% NaCl, 1.0% feedlot premix, 2.0% Urea, 0.07% Vitamin E (50%), 1.0% canola oil, 0.05% flavour, 0.31% rumensin premix, 0.36% melegestrol acetate, 0.23% Tylan 40. TDN calculated by Owens (1999).

Table 6.2. Chemical composition of feces and nutrient digestibility as predicted using near infrared spectroscopy calibrations of feces (n=15) collected at three time points over the feeding period from heifers fed control, light, or heavy screenings diets.

Item	Diets				
	Control	LSP	HSP	SEM	*P* value
Chemical composition (% DM basis)					
Dry matter (%)	19.8	20.2	20.2	0.36	0.64
Organic matter	87.0a	85.3b	85.8b	0.33	0.01
Starch	8.7	6.5	7.2	0.72	0.09
Nitrogen	2.6a	2.4b	2.4b	0.030	<0.01
Neutral detergent fibre	50.3b	53.8a	52.8a	0.69	<0.01
Acid detergent fibre	30.7	31.7	30.9	0.66	0.52
Acid detergent lignin	6.6	7.0	6.8	0.15	0.12
Ether extract	1.7a	1.4c	1.5b	0.03	<0.01
Digestibility (% of intake)					
Dry matter	77.9	77.5	77.5	0.42	0.73
Organic matter	79.1	78.2	78.1	0.45	0.26
Starch	94.3	94.7	95.0	0.32	0.36
Crude protein	73.6	73.7	73.2	0.48	0.70
Neutral detergent fibre	55.8	55.3	56.2	0.38	0.28
Acid detergent fibre	42.4	43.7	41.9	0.68	0.21
Gross energy	79.5	79.9	80.9	0.48	0.14

Note: LSP, diet fed with light screening pellets; HSP, diet fed with heavy grain screening pellets.

Table 6.3. Growth performance and carcass traits of heifers fed control, light or heavy screenings diets.

Item	Diets			SEM	*P* value
	Control	LSP	HSP		
Growth Performance					
Initial BW (kg)	429.4	423.7	429.1	14.31	0.95
Final BW $_{d100}$ (kg)	617.4	601.1	613.3	14.17	0.71
DMI (kg DM/d)	11.8	12.2	12.5	0.24	0.18
sADG (kg/d)	1.88	1.77	1.84	0.03	0.06
sGain:Feed, (kg/kg DM)	0.160a	0.145b	0.148b	0.0032	0.02
Carcass Traits					
Carcass weight (kg)	378.3	372.7	374.9	3.78	0.57
Backfat (mm)	16.2	16.8	16.3	0.62	0.78
Ribeye area (cm^2)	91.6	90.3	91.5	1.27	0.72
Saleable Lean meat yield (%)	54.8	54.3	54.9	0.56	0.74
Choice + Prime (%)	23	29	27		0.41
Chi Square analysis of categories					
B^4 (%)	32	22	12		0.05
No. liver abscess (%)	94	92	94		1
Severe abscess (%)	2	2	2		1

Note: LSP, diet fed with light screening pellets; HSP, diet fed with heavy screening pellets; B^4, grading is assigned to those carcasses that have dark purple rather than bright red meat. sADG and sGain:Feed correspond to shrunk weights (live weight*0.96)

6.4.5 Prediction of Energy Values Using NIRS of GSP and Growth Performance

Predicted performance parameters (NEm and NEg) determined from the feedlot study indicated that the control diet was 8.0% and 10.6% greater in NEm and NEg than the LSP and 6.84% and 8.41% higher than HSP, respectively (Table 6.4). The NEm and NEg were calculated to be 1.31 and 0.74 for the LSP, and 1.39 and 0.81 for the HSP. Predictions using NIRS of the TDN value of both GSP using Owens et al. (1999) resulted in values similar but slightly higher than those calculated directly from measured growth performance (62.3 vs 59.6% for LSP and 66.7 vs 64.3% for HSP; Table 6.4).

6.4.6 Associations between NIRS Predicted Fecal Composition, Digestibility, and Growth Performance

We were unable to find associations between growth performance and fecal nutrient composition or aTTD of nutrients in heifers fed the two GSP diets, except for a negative association between ADG and fecal ash ($R^2 = 0.45$, $P = 0.03$; data not shown). When the control diet was included in the regression equation, digestibility of GE was positively associated to DMI ($R^2 = 0.31$, $P = 0.03$). Positive associations were also found between ADG and fecal N ($R^2 = 0.30$, $P = 0.03$), and fecal starch ($R^2 = 0.26$, P = 0.05), and negative associations between ADG and fecal NDF ($R^2 = 0.46$, $P = <0.01$), and fecal ADL ($R^2 = 0.61$, $P < 0.01$). There were tendencies for G:F to be positively associated to fecal OM ($R^2 = 0.25$, $P = 0.06$), fecal N ($R^2 = 0.25$, $P = 0.05$), fecal starch ($R^2 = 0.20$, $P = 0.09$) and fecal EE ($R^2 = 0.23$, $P = 0.07$) and negatively associated with fecal NDF ($R^2 = 0.23$, $P = 0.07$; data not shown).

6.5 Discussion

6.5.1 Composition of Grain Screenings Pellets and Diets

In Canada, grain screenings mainly arise as a result of the cleaning of barley, wheat and canola for export, and consist of contaminants such as broken grains, weed seeds, hulls, chaff and dust (Beames et al. 1986). They are classified based on the level of uncleaned grain, and the major categories include No. 1 feed screenings, No. 2 feed screenings; refuse screenings, and uncleaned screenings (Beames et al. 1986). The screenings used in this study were refuse

screenings, which contain on average 70% chaff plus dust as well as grain and weed seeds (Tait et al. 1986). The merit of feeding grain screenings to livestock is in cost savings, as they are considered a waste by-product as opposed to an actual grain. However, the downside is their reduced starch and greater structural carbohydrate content as compared with grains (Marx et al. 2000; Mustafa et al. 2000). This typically results in a reduction in G:F when screenings are added to the diets of beef cattle (Górka et al. 2013; Joy et al. 2015). Due to the lower energy content of screenings, oil is often added to screenings to increase their energy density (Górka et al. 2013; Górka et al. 2015; Joy et al. 2015).

In the current study, two GSP were manufactured with the assumption that heavier screening would generate GSP with a higher energy density. In addition, millrun, beet pulp, and canola oil were added to the HSP. Although the classification and exact parent grain composition of our GSP were unknown, the starch content of both GSP was lower than GSP reported by Marx et al. 2000 (26.2% DM) who fed sheep a mixture (85:15) of refuse screenings and lentil screenings. The NDF and ADF values for both LSP and HSP were higher than the NDF (33.7%) and ADF (20.9%) values reported by Marx et al. (2000). The CP, ADF, EE, and ash content of both GSP were all within the ranges of refuse screening pellets as reported by Beams et al. (1986; 10.6-19.3% CP, 22.8-37.2% ADF, 3.2-8.6% EE, and 7.6-12.8% ash).

Considering the diversity in the composition of screenings that make up GSP, a rapid analytic technique such as NIRS to evaluate chemical composition is highly advantageous. Although the predictability of our GSP calibrations cannot be assessed properly with so few samples, our data show that NIRS estimates were close to those estimated by wet chemistry, demonstrating the potential to use NIRS to estimate the feed value of GSP (Table 6.6). The interactions we observed indicate that for some constituents, NIRS over- or under-predicted their presence in LSP or HSP, but without R^2_{cal} or *SEC* values, we cannot measure the accuracy or precision of the calibrations. However, NIRS calibrations did under predict CP, ADF, and ash for both GSP. Since the completion of the current study, additional sources of GSP have been continually collected to increase the sample size of the calibration set. For any NIR work, ensuring that the population and range of unknown samples is encompassed by the calibration set will improve the predictability of calibrations.

6.5.2 Fecal DM and NIRS Predicted Nutrient Composition and Digestibility

Consistent with the fact that by-products such as grain screenings are typically higher in fibre and lower in starch content relative to grains (Marx et al. 2000; Mustafa et al. 2000), our findings demonstrated the same trends in the feces of GSP fed cattle relative to control. Contrary to this, fecal N and EE concentrations did not match the differences in dietary levels. Despite the diets being isonitrogenous, fecal N was lower in both GSP diets, and EE in the feces was lower with GSP despite having higher levels of dietary EE than the control diet. These results are likely due to greater microbial turnover and excretion of microbial protein and lipids in the control diet, an observation that is frequently associated with more fermentable diets (Beauchemin et al. 2001).

Marx et al. (2000) found that increasing levels of GSP in place of barley grain resulted in a reduction in DM digestibility in sheep, a response attributed to the greater fibre content of GSP (Marx et al. 2000). Similarly, NDF and GE digestibility were found to decrease with increasing GSP, with no differences in CP or ADF digestibility observed (Marx et al. 2000). Górka et al. (2015) fed increasing levels of high-lipid, high-fiber pellets and observed linear decreases in organic matter and NDF digestibility. Using NIRS, we did not predict differences in digestibility between the two GSP diets and the control diet. Lipid supplementation is known to directly reduce ruminal fibre digestibility (Hess et al. 2008) and shift the site of starch and fibre digestion to lower parts of the gastrointestinal tract of finishing cattle (Plascencia et al. 2003). Pylot et al. (2000) reported that apparent NDF digestibility increased with inclusion of increasing rates of canola screening despite an increase in dietary lipid content from 6.7 to 16.2% of diet DM. Similar to Górka et al. (2015), it is unlikely that lipid inclusion negatively affected digestibility in the present study since ether extract content did not exceed 6% of diet DM (Zinn and Jorquera 2007).

The dissimilarity between the present study and previous studies may be due to the lower (20% of diet DM) inclusion of GSP compared to Marx et al. (2000; 25 to 100% of diet DM) and Górka et al. (2015; 30 to 90% of diet DM). Aside from starch, our predicted digestibilities were greater than those reported by Górka et al. (2015), even for the control diets, a result that may reflect the lower DMI in our study. Additionally, Górka et al. (2015) predicted digestibility using Yb and Cr as markers as opposed to NIRS predictions derived from total collection methods. Our digestibility predictions were comparable for DM, CP, and GE digestibility to those reported

by Marx et al. (2000), when GSP were included at 25% of the diet DM for sheep. However, NDF and ADF digestibility were 15% higher, a result that may reflect the inclusion of beet pulp in the HSP. Additionally, the NIRS calibrations for NDF and ADF digestibility that we used have substantially lower R^2cal and SEC values compared to other nutrient digestibility calibrations, hence the risk for error is greater (Chapter 3). Since there were no pelleted ingredients in diets used for the development of the digestibility calibrations, it is likely that the feces of GSP fed cattle are not adequately represented by the calibration set, and this was shown in particular for the LSP diet.

6.5.3 Performance of Cattle and Carcass Traits

A second objective of this study was to determine the effects of feeding the two GSP on performance measures and carcass quality. Certain characteristics of GSP have been found to reduce feedlot cattle performance. Firstly, by-products such as grain, pea, canola screenings and oat hulls are typically high in fiber, and are often lower in dietary energy than grains (Marx et al. 2000). This energetic deficiency must be limited or they will lower the energy density of the diet (Marx et al. 2000), unless energy dense lipids are added (Górka et al. 2013; 2015). Secondly, the fine physical structure of most by-products and the reduction in particle size associated with grinding prior to pelleting reduces fibre retention time in the rumen, digestibility, and G:F (Abouheif et al. 2012). Although we did not find any detrimental effects on nutrient digestibility upon feeding the GSP or any difference in DMI, we did find that ADG tended to be lower for LSP compared to control heifers, and a reduction in G:F of heifers was observed for both GSP diets as compared to the control diet. Joy et al. (2015), fed high-fat by-product pellets to finishing steers at 30% of diet DM for 120 d, and although there was no impact on DMI or ADG, the G:F was reduced. Górka et al. (2013) fed a similar high-fat by-product pellet as a partial replacement for barley grain and canola meal in finishing diets for steers at 30% and 60%. In their study, ADG did not differ among treatments, but DMI was higher and G:F was lower for both pelleted diets as compared to the control diet. Pylot et al. (2000) concluded that canola screenings can be included as a source of fibre in barley-based diets for feedlot cattle, however, levels in excess of 50% of diet DM, compromised both ADG and G:F. Our study shows however that even at an inclusion rate of 20%, reductions in G:F occurred when feeding LSP.

Table 6.4. Total digestible nutrients and energy content of light or heavy screening pellets as estimated by actual performance of heifers or as predicted by near infrared spectroscopy.

Item	LSP		HSP	
	Energy values			
	Performance	NIRS	Performance	NIRS
Energy Value				
TDN (%)	59.6	62.3	64.3	66.7
NEm (Mcal kg^{-1}) GSP	1.31	1.39	1.45	1.52
NEg (Mcal kg^{-1}) GSP	0.74	0.81	0.86	0.82

Note: LSP, diet fed with light screening pellets; HSP, diet fed with heavy screening pellets; TDN, total digestible nutrients; NEm, net energy of maintenance; NEg, net energy of gain. Calculated from TDN = Nem+0.5058/0.0305; Calculated from NEg = 0.877*Nem-0.41; Performance calculated based on average animal weights, DMI, ADG as .0557*(AVG BW*478/mature weight)0.75*ADG, where mature weight of 600 kg was used NEm of GSP diet - NEm of control diet/0.2-2.06, where 0.2 = proportion of GSP in diet and 2.06 = NEm of the barley it displaced.

Table 6.5. Range, mean, and standard deviation of chemical composition of grain screening pellets used for the development of near infrared spectroscopy calibrations.

Item	n	Range	Mean±SD
Chemical composition (% DM basis)			
Dry matter	95	86.3-96.1	91.10±2.39
Starch	98	7.1-47.8	20.8 ± 6.1
Crude protein	101	9.3-23.8	18.1 ± 2.7
Neutral detergent fibre	100	21.6-52.4	33.5 ± 6.9
Acid detergent fibre	97	9.8-39.9	23.1 ± 5.8
Acid detergent lignin	100	2.3-12.3	6.6 ± 1.8
Ether extract	98	4.3-23.2	10.0 ± 2.5
Ash	95	3.2-9.6	6.4 ± 1.1

Table 6.6. Differences between chemical composition of light and heavy grain screening pellets fed to heifers as estimated by either chemical analysis or near infrared spectroscopy (n=5).

Item	LSP		HSP		*P* value			
	Chemical	NIRS	Chemical	NIRS	SEM	Diet	Method	Method × Diet
Composition (% of DM)								
DM	91.0ab	90.5b	90.8ab	91.3a	0.21	0.12	0.86	0.04
Starch	23.6a	22.5b	20.5c	23.5a	0.26	<0.01	<0.01	<0.01
CP	13.9a	12.7b	13.0b	12.2b	0.27	0.02	<0.01	0.45
NDF	38.8	38.9	40.0	37.8	0.84	0.95	0.29	0.25
ADF	24.4ab	22.7b	25.7a	23.1b	0.66	0.23	<0.01	0.58
ADL	4.5b	7.2a	4.8b	4.7b	0.58	0.09	0.05	0.03
EE	4.2b	4.4b	6.7a	7.0a	0.16	<0.01	0.17	0.60
Ash	8.3a	5.9b	8.0a	4.8c	0.09	<0.01	<0.01	<0.01
TDN	69.0ab	62.3c	71.0a	66.7b	0.83	<0.01	<0.01	0.18

Note: LGSP, diet fed with light grain screening pellets; HGSP, diet fed with heavy grain screening pellets; NIRS, near infrared spectroscopy

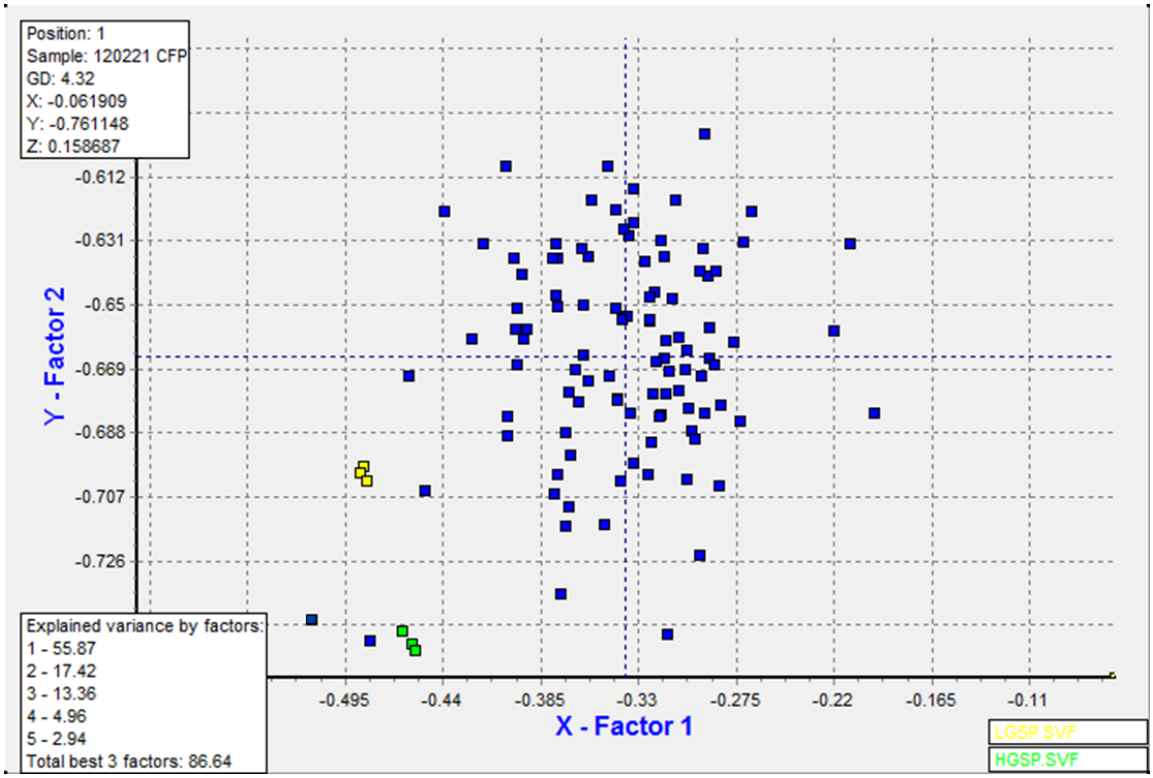


Figure 6.1. Principal component graph displaying the spectra of the light (yellow) and heavy (green) grain screening pellets manufactured for the feedlot study in relation to the pellets in the NIRS calibration set for GSP composition (blue).

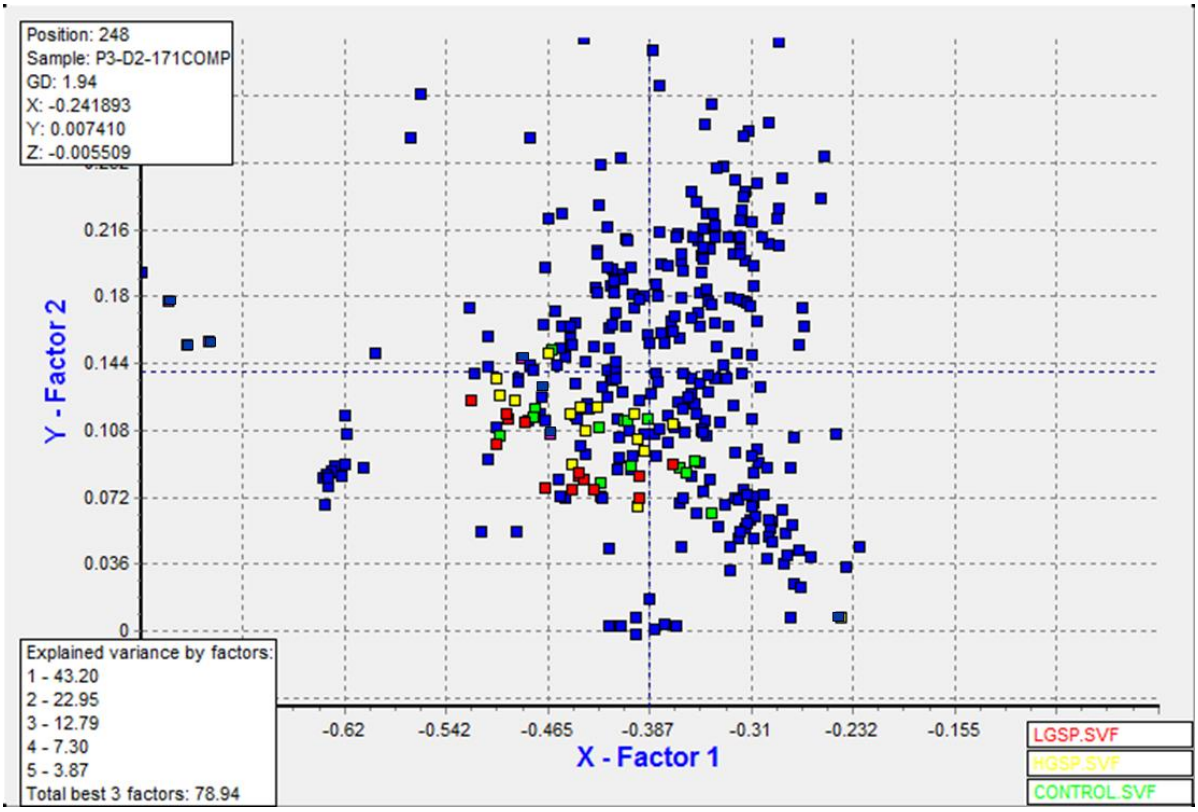


Figure 6.2. Principal component graph displaying the spectra of fecal samples collected from heifers fed the control diet (green), LGSP diet (yellow) and HGSP diet (red) in relation to the fecal samples in the NIRS calibration set for chemical composition (blue).

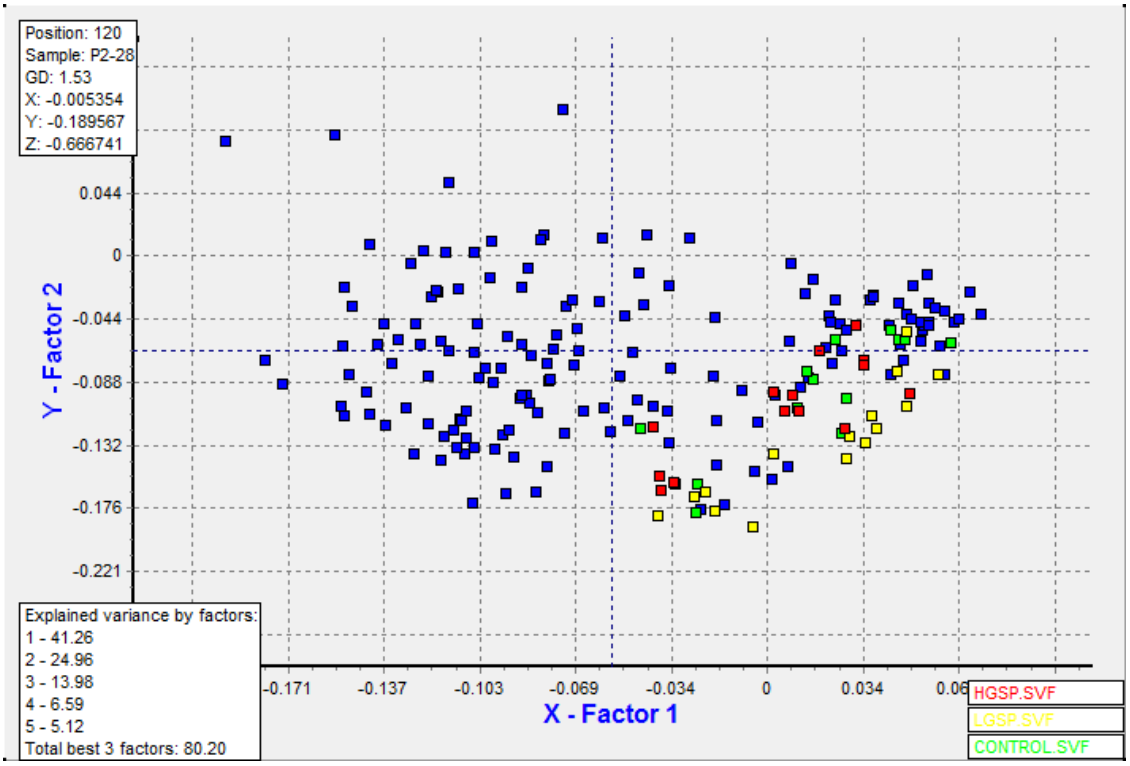


Figure 6.3. Principal component graph displaying the spectra of fecal samples collected from heifers fed the control diet (green), LGSP diet (yellow) and HGSP diet (red) in relation to the fecal samples in the NIRS calibration set for digestibility (blue).

Feeding GSP has not had consistent effects on carcass traits. Positive effects on carcass yield were reported when high lipid by-products were fed in the latter 60 d of the finishing period, but negative effects when fed for 120 d (Joy et al. 2015). Others have reported no effect of GSP on carcass characteristics (Górka et al. 2013). In the present study, we did not observe any differences among treatments, however, we did note low lean meat yields and high B4 carcasses for both control and GSP diets, and a tendency for higher B4 carcasses in the control fed heifers. The meat yield reported is slightly lower than those reported in Joy et al. (2015; 60%), but greater than Moya et al. (2015; 48.5%) where an 89% grain diet was fed to feedlot cattle. The extremely high B4 cutters can be due to multiple factors including sex of the cattle, the use of growth promotants, and stressful events. Feedlot data were compiled over a 3 yr period from nine commercial feedlots, and a higher percentage of dark cutters were found in heifers than steers (Scanga et al. 1998). This may be related to an increase in mounting activities, but also the lighter weight of the dark cutters may reflect the predisposition of heifers to this trait (Tarrant 1989). Implants and beta-agonists have been considered to increase the risk of dark cutters (Tarrant 1989; Grandin 1992) since they modify growth curves, rates of gain, and nutrient requirements of beef cattle through hormonal changes (Scanga et al. 1998). Fasting for a considerable time during long haul road transport would also increase stress in the cattle and raise the final pH of meat, producing more dark cutters (Tarrant 1989). Shipping in the winter can also increase the occurrence of dark cutting beef, since cold weather elicits body-heat loss (Grandin 1992), a possible factor as these heifers were shipped in November.

6.5.4 Prediction of Energy Values Using NIRS of GSP and Growth Performance

Once the chemical composition of GSP is predicted using NIRS, and appropriate equations to calculate energy are applied, we do not always know how close these values reflect growth performance. Considering the success of NIRS in predicting nutrient content of feed ingredients (de Boever et al. 1995) it is not surprising that NIRS is able to predict the TDN of GSP, especially when calculated based on chemical composition. Near infrared spectroscopy is currently being used to predict the value of GSP using equations based on their chemical composition (Gibb, personal communication). Although our predicted values overestimated those calculated from actual performance, NIRS was able to differentiate TDN between LSP and

HSP. Many factors must be considered when measuring animal performance, including breed, sex, animal behaviour, diet, use of growth promotants, and climate. Therefore, there is a great chance of over or under predicting actual values when only using ingredient composition. Our small margin of error and the ability to rapidly differentiate between two GSP using NIRS at the time of purchase will allow producers to assess the value of GSP and their expected effect on the growth performance of feedlot cattle.

6.5.5 Associations Between NIRS Predicted Fecal Composition, Digestibility, And Growth Performance

Aside for fecal ADL, the lack of associations between growth performance and fecal nutrient composition or aTTD of nutrients in heifers fed the two GSP diets was likely due to there being no statistical differences in fecal composition in aTTD, or DMI, ADG, and G:F for the GSP diets. Had the differences in composition of the two GSP been greater, this may have translated into differences in fecal composition and nutrient digestibility and enabled us to test for such associations. We can however conclude, that the greater G:F we observed in control cattle was associated with greater fecal OM, starch, N, and EE, and lower NDF compared to the GSP diets, and similar conclusions can be made for ADG. The greater OM, starch, and lower NDF in feces is related to greater performance since the control diet consisted of a greater concentration of high energy nutrients, and was lower in NDF. Greater fecal N and EE possibly indicate greater microbial protein synthesis in the rumen and greater excretion of microbial protein in the feces, a possibility supported by Beauchemin et al. (2001) where microbial protein synthesis in the rumen increased by 50% when barley starch was more accessible after more severe processing. Due to low statistical power, and the differences observed in the PCA graph between the feces of GSP fed heifers in the current study and the calibration set for digestibility, some associations may not be applicable.

6.6 Conclusion

Feedlot cattle diets and ingredients are highly variable as they are dependent on fluctuations in feed costs. Producers choose to feed by-product feeds when the price of grain is high, with an understanding of the costs and benefits. Considering the compositional diversity of GSP,

implementing NIRS in feedlots is a practical tool to immediately evaluate their feed value. Additionally, NIRS of the feces also shows potential in predicting the performance of feedlot cattle. Replacing 20% barley grain with GSP in our study did not affect nutrient digestibility or DMI, but ADG and G:F were reduced, and this was evident by changes in the fecal composition. Near infrared spectroscopy can predict the energy content of GSP similar to performance estimates, and be applied in commercial feedlots to assess pellet quality at time of purchase and diet formulation for feedlot cattle.

6.7 Next Stage

The previous chapters described studies and techniques developed under research settings where most variables could be controlled. Dietary and management practices for each group of cattle on the same treatment were constant. The following chapter examines the impact of feedlot industry variables on NIRS predicted fecal nutrients and digestibilities. These variables include location, sex, frequent changes in dietary ingredients and proportions, changes in grain processing, and average BW of cattle at time of sampling, Finally, the ability of NIRS of feces to predict DMI, ADG, and G:F within groups of feedlot cattle was assessed for its accuracy and suitability under various commercial settings.

CHAPTER 7

7.0 PREDICTABILITY OF COMMERCIAL FEEDLOT CATTLE GROWTH PERFORMANCE USING FECAL NEAR INFRARED SPECTROSCOPY

7.1 Introduction

Cereal grains are a major ingredient in diets for finishing cattle, and given the high proportion of starch in grains, predictions have been developed to rapidly estimate starch digestibility using fecal starch concentration (Zinn et al. 2002; Corona et al. 2005; Zinn et al. 2007). Research by Owens and Zinn has shown that accuracy of starch digestibility predictions can be improved by including additional variables such as fecal N (Zinn et al. 2011), DM or OM digestibility, and starch intake (Owens et al. 2016). Methods to predict NDF digestibility based on fecal concentration have been examined (Chapter 4; Fredin et al. 2014; Owens et al. 2016), and the relationship between fecal CP and OM digestibility have been explored (Lukas et al. 2005). Since fecal samples contain information relevant to feed digestion, monitoring how efficiently cattle are utilizing nutrients could directly benefit the efficiency and profitability of feedlot cattle production. For such predictive approaches to be commercially applied, they need to be practical and accurately reflect impacts on performance efficiency, rather than just digestibility.

Near infrared spectroscopy (NIRS) is a rapid alternative to wet chemistry and can successfully predict nutrient composition and diet digestibility using fecal samples collected from the pen floor of feedlot cattle (Chapter 3 and 5). The calibrations have been directly applied to identifying associations between measured fecal parameters and performance, and to predicting ADG and G:F using small datasets where grain type, processing, or grain proportion were deliberately altered (Chapter 5). These results suggest that the use of NIRS may have merit in predicting growth performance in commercial feedlot cattle, if calibrations are relevant to the samples collected. However, the commercial feedlot industry encounters many challenges that do not occur in typical feedlot research studies. Feedlot managers are faced with implementing management decisions that require changes in the type and proportion of ingredients in the diet, grain processing, sorting of cattle into production lots for marketing and adjusting the shipping dates prior to slaughter. All of these actions introduce variables that are not easily anticipated, potentially making equations developed for specific diets less reliable.

The first objective of this study was to use previously developed NIRS calibrations to predict fecal composition and digestibility in dried ground fecal samples collected from commercial feedlots. Principal component analysis was used to qualitatively visualize the differences in the spectral populations of samples in the calibration sets and those to be analyzed. Secondly, the impact of variables including grain type, grain inclusion rate, processing method, processing index, forage to concentration ratio (F:C; included grain and other concentrates), sex, season, and average BW of the cattle at the time of sampling on NIRS predicted fecal nutrients and digestibility was examined. Lastly, NIRS of feces was assessed for its ability to predict DMI, ADG, and G:F within groups of commercial feedlot cattle.

7.2 Materials and Methods

All sampling procedures used in this study were approved by the Animal Care Committee at the Lethbridge Research Centre of Agriculture and Agri-Food Canada, which operates under the guidelines of the Canadian Council on Animal Care (2009).

7.2.1 Description and Development of Feedlot Fecal Database

A database was constructed by collecting and analyzing monthly fecal samples from 6 feedlots from the floor of pens over a 1-yr period. The feedlots in the database were located in southern Alberta. Cattle were housed in outdoor pens with dirt floors. All pens were enclosed with porosity fencing on at least two sides. Cattle were bedded by placing barley straw in the middle of the pen as required. Pens were either steers (228 pens) or heifers (54 pens) of primarily British or Continental cross breeds. Steers and heifers were separated by sex, and housed at an average density of 167 ± 71.6 hd/pen, with a range of 26 to 438 hd/pen. Pen areas also varied, but provided approximately 13.8 to 16.6 m^2/hd, and 22 to 26 cm of bunk space/hd.

Each month, four fecal pats from four pens were composited by pen on an equal wet weight basis, resulting in a total of 292 samples. Each composited fecal sample was associated with appropriate linked measurements including: feedlot identification (1 to 6), diet (ingredients, grain type, dietary grain inclusion rate, grain processing, processing index, forage to concentrate [F:C] ratio), average BW of cattle, sex, season (Summer; June to August; Fall; September to November; Winter; December to February; and Spring; March to May), fecal DM, NIRS predicted fecal composition, and NIRS predicted nutrient and GE digestibility.

Grain type included barley or a mixture of barley and wheat, depending on the feedlot. Grain was processed on site in a feedmill located at each feedlot. The processing methods included grain that was either dry- or temper rolled, and the method varied based on feedlot and season. For example, one feedlot used both processing methods with dry rolling used in the winter and temper rolling in other seasons. The processing index was calculated as the bushel weight of the grain after dry rolling or temper rolling divided by the bushel weight of the whole grain before processing. Temper-rolled grain was dried prior to this measurement. Among feedlots, the amount of grain in the diet ranged from 52% to 90% of DM, and the processing index from 52% to 97%. Two feedlots fed potato waste at 3% to 15% of diet DM as an additional source of starch. None of the feedlots that fed wheat or dry rolled grain fed waste potatoes. Therefore, additional dietary starch from potatoes was only relevant to feedlots that fed temper-rolled barley. The F:C accounted for concentrates other than grain included in the diet, such as dried distillers grains, potato waste, grain screenings, and supplement. Samples were collected from both heifers and steers in four feedlots, and from only steers at the remaining two. The diet composition, processing methods, and overall performance for each feedlot at the end of the 1-yr eriod are summarized in Table 7.1.

7.2.2 Feed Offered and Sampling

Feed was delivered in the morning at all feedlots using a feed truck with a mixer, in 2 primary passes (twice/day) with pens of cattle being fed to appetite. In some feedlots, three feedings per day occurred occasionally, but was rare. Only cattle that had been fed a finishing diet for a minimum of 5 d or the final step-up diet for a period of 3 wks were sampled. In all 6 feedlots, a supplement was included at 2.3% of diet DM and provided 33 - 48 ppm monensin (Rumensin 200®, Elanco Animal Health, Indianapolis, IN) and 11 ppm tylosin phosphate (Tylan 40; Elanco Animal Health, Indianapolis, IN). The heifer supplement also contained MGA 100 (Zoetis Inc, Parsippany, NJ) targeted at delivering 0.4 mg of melengesterol acetate hd/d. Optaflexx (Elanco Animal Health, Indianapolis, IN) was provided through a separate supplement during the final 20 to 40 days of the feeding period targeting an intake of 200 to 300 mg of ractopamine hd/d. Ingredients and their proportions in the finishing diet were changed based on pricing, availability, and manager preference. Diet adjustments were made as frequently as weekly, or infrequently with ingredients remaining the same for a month or more.

Table 7.1. Summary of variables observed with fecal samples collected from pens of cattle housed in six commercial feedlots over a 1 year period

Item[1]	Feedlot[2]					
	1	2	3	4	5	6
No samples	52	52	46	51	39	42
Management[3]						
Sex	Steers	Steers and Heifers	Steers	Steers and Heifers	Steers and Heifers	Steers and Heifers
Grain	Barley	Barley	Barley and Wheat	Barley and Wheat	Barley and Wheat	Barley
Processing	Dry rolled	Temper rolled	Temper rolled	Dry and Temper rolled	Dry rolled	Temper rolled
Grain percent	58.5-73.7 (69.2±5.08)	65.1-86.5 (78.0±5.94)	52.5-88.7 (81.2±11.62)	54.7-89.7 (81.3±11.48)	62.8-85.9 (80.0±6.67)	56.7-73.7 (64.7±5.10)
F:C	0.10-0.38 (0.16±0.090)	0.08-0.19 (0.11±0.025)	0.09-0.11 (0.10±0.009)	0.07-0.11 (0.10±0.012)	0.11-0.24 (0.11±0.021)	0.10-0.23 (0.11±0.042)
PI	63.6-82.6 (70.6±6.12)	58.9-69.4 (64.7±3.48)	51.7-76.1 (66.1±5.42)	65.8-74.8 (69.5±2.63)	71.2-96.9 (84.3±8.27)	58.7-71.0 (65.8±3.52)
Ingredients[4]						
Dry rolled barley	58-74 [12]			60-85 [4]	51-86 [12]	
Tempered barley		65-85 [12]	27-89 [12]	15-88 [8]		57-74 [12]
Dry rolled wheat				10 [4]	11-17 [8]	
Tempered wheat			25-43 [6]	33-40 [4]		
Corn DDGS	12-15 [12]	7-20 [8]	10-20 [3]	20 [2]		12-20 [12]
Corn silage		7-16 [12]	9-10 [6]	4-10 [10]		5 [12]
Barley silage			6-9 [6]	4-10 [3]	10-19 [12]	
Wheat silage			10-12 [4]			
Grass silage	9-27 [12]					
Triticale silage						2.3 [12]
Straw			2 [1]	3 [1]		
Grain screenings			15 [2]	15 [2]		
M.Sprouts/Millrun				11-23 [6]		
Potatoes		3-6 [3]				5-15 [12]
Performance[3]						
BW	474-680 (628±37.9)	421-687 (546±77.5)	363-636 (487±73.3)	364-614 (494±61.0)	474-740 (586±63.3)	462-599 (519±45.0)
DMI	9.0-12.7 (10.7±1.05)	8.7-11.1 (10.0±0.98)	8.0-11.3 (9.5±1.04)	8.0-10.9 (10.0±0.66)	9.4-11.7 (10.7±0.78)	8.7-11.0 (9.7±0.86)
ADG	1.41-1.91 (1.66±0.145)	1.36-1.74 (1.51±0.102)	1.42-1.74 (1.60±0.104)	1.24-1.78 (1.52±0.181)	0.86-1.64 (1.19±0.212)	1.43-1.76 (1.56±0.125)
G:F	0.14-0.17 (0.16±0.009)	0.13-0.16 (0.15±0.010)	0.14-0.18 (0.17±0.012)	0.13-0.19 (0.15±0.014)	0.08-0.16 (0.11±0.026)	0.14-0.18 (0.16±0.013)

[1] F:C = forage to concentrate ratio; PI = processing index of grain.
[2] A total of 292 fecal samples are represented in the table. Data is missing for F:C (n = 269), BW (n = 242), and grain percent (n = 272).
[3] Numbers in brackets represent the mean ± standard deviation
[4] Numbers in square brackets represent the frequency out of 12 months that each ingredient was fed.

Grain samples (4 L) were collected before and after processing the same day as feces were collected from the pen floors at each feedlot, ensuring that the grain was from the same original bin.

7.2.3 Fecal Collection and Analysis

Chapter 3 and 5 describe the method of fecal collections in detail. Each month, fecal samples (minimum 250 g) were collected from four fresh fecal pats on the pen floor, from four pens within each feedlot. Each pen was associated with performance measures at the end of the feeding period for the particular lot of cattle housed within that pen. One monthly collection was missed for two of the feedlots. Cattle within a pen were observed until defecation occurred so that fresh samples could be collected from different cattle. Samples were collected from the center of four fecal pats and contamination with dirt or bedding was avoided, as described in Chapter 5. Samples were collected between 0800 to 1300 h, and were stored in separate plastic bags and kept in coolers until collection from all pens was complete. Samples within the same pen were composited on an equal wet weight basis (≈100 g each). Composites were dried at 55ºC for a minimum of 72 h, followed by DM determination, and grinding through 0.75 mm screen using a Retsch grinder (Verder Scientific, Inc, Newton, PA).

7.2.4. Analysis using NIRS

Dried ground samples were packed into small quartz ring cups (25 g) and scanned in duplicate (two repacks; where the second scan was a different subsample from the first) using a SpectraStar Near-Infrared analyzer 2400 RTW (Unity Scientific, Brookfield, CT). Spectral information was collected at wavelengths between 1100 and 2400 nm in 1-nm increments, and trimmed between 1250 to 2350 nm to reduce noise peaks above and below this range. Duplicate spectra of each sample were averaged, and fecal OM, starch, N, NDF, ADF, ADL, ether extract (EE) and aTTD of DM, OM, starch, CPD, NDF, ADF, and GE were predicted using previously derived NIRS calibrations as developed by Chapter 3.

In order to expand the original calibration to encompass samples from the current study, all commercial feedlot samples predicted using the initial NIRS calibrations were ranked from the highest to lowest for each constituent (% of fecal DM). Samples were selected to include the

entire concentration range of each constituent of interest, while ensuring that representative samples from each feedlot were included in the dataset. Validation of the calibration included not only samples from the current study, but also from other feedlot studies as described in Chapter 5. Fecal starch, NDF, and ADL were selected as the key nutrients of interest based on results from regression models (described below), and previous studies (Chapter 5; Owens et al. 2016). Reference analysis consisting of wet chemistry for OM, starch, N, NDF, ADF, ADL, and EE (AOAC 2005), was conducted on a subset of fecal samples from each feedlot (24 from the current study) and the samples whose neighborhood distance (ND) was above a certain threshold (0.60) were used to expand the original calibration. A similar approach could not be used for calibrations for digestibility as this parameter could not be realistically measured for the commercial feedlot samples. Details of calibration development for digestibility are described in Chapter 3.

Principal component analysis (PCA) was used in Unscrambler® X version 10.3 (CAMO Software, Oslo, Norway) to visualize the differences in spectral populations of the commercial feedlot dataset and the calibration set. The export function in Ucal was used to convert spectra into a JCAMP format that could be imported in Unscrambler® X to generate PCA graphs. The full spectra were used with no trimming (1100 to 2400 nm). Raw spectra were transformed using a standard normal variate and detrending procedure, followed by first derivatization using Savitsky Golay with three points. A scatter plot of principal component (PC) scores for each sample from the feedlot dataset, and within the calibration dataset were plotted along the first two PC factors (x axis = Factor 1, y axis= Factor 2). Outliers were visually identified using the Hotelling's T^2 distribution as per its application in identifying differences in population means in multivariate statistics. The samples outside of the defined Hotelling's T^2 ellipses were considered strong outliers from the population at a confidence level of 95%.

7.2.5 Performance Measurements

For this study, close out performance data were obtained for groups of cattle that remained together as a single lot from arrival until slaughter. Lots of cattle were weighed together on trucks, at the start and end of the finishing feeding period. The fecal samples (pool of 4 as described above) were collected from a total of 292 pens corresponding to 90 lots of cattle where DMI, ADG, and G:F could be obtained. Of these lots, 70 contained steers, and 20 contained

heifers. Measures of interest (i.e. fecal composition, digestibility, grain percent, F:C, grain processing) were averaged if samples were collected from the same lot more than once. The frequency of fecal collections within a feeding period for each lot was recorded.

Close out DMI was estimated by the amount of feed offered to a lot (adding the total feed offered to each pen making up a lot) over the full feeding period, divided by the number of days on feed and the number of cattle in the lot. Close out ADG and G:F were calculated assuming 4% shrink. Close out data were calculated by subtracting average initial weight from the average final shrunk body weight with dead animals removed. This difference was divided by the number of cattle, and the days on feed for each pen, as described by Gaylean et al. (2010). The pen G:F was calculated as ADG divided by DMI.

7.2.6 Statistical Analysis

The Mixed procedure in SAS v. 9.1. (SAS Inst. Inc., Cary, NC, USA) was used to determine the differences in management strategies (grain percent, F:C, processing index), in NIRS predicted fecal composition and digestibility, and performance, observed among the six feedlots. Pen was used as the experimental unit, but the number of repetitions varied because of frequent shipping of cattle and because the distribution of cattle within pens varied among feedlots and with month. A compound symmetry structure was used to model repeated measures on individual pens sampled on more than one day as it exhibited best fit for convergence. In addition to fecal starch and fecal NDF, aTTD of DM, OM, and starch were selected as key interests for digestibility based on previous investigations (Chapter 5; Owens et al. 2016). The aTTD of GE (%) was also selected because of its relationship to net energy of gain (Chapter 5). The model used was $Y_{ij} = \beta_{0ij} + \beta_1 feedlot_{ij} + \beta_2 day_{ij} + \mu_j + \varepsilon_{ij}$, where β_0 was the average y-intercept or overall mean for management factors, fecal chemical composition, digestibility, BW, DMI, ADG, or G:F when all terms in the equation are equal to 0, i was the individual observation of day within pen, j was feedlot, μ_j was the residual associated with feedlot and ε_{ij} was the residual error associated with each observation. Least square means of the treatments were separated using PDIFF statement, with significance declared at $P < 0.05$.

The Mixed procedure was then used to determine the effect of grain type, processing method, and sex on NIRS predicted fecal composition, digestibility and growth performance, adjusting for differences among feedlots as a random intercept. Pen was used as the experimental

unit, and a compound symmetry structure was used to model repeated measures on individual pens. Interactions were not examined in this observational study because of the large number of variables examined, unequal numbers of pens per lot and feedlot, and missing data. The model used was $Y_{ij} = \beta_{0ij} + \beta_1 \text{grain type}_{ij} + \beta_2 \text{processing method}_{ij} + \beta_3 \text{sex}_{ij} + \beta_4 \text{season}_{ij} + \beta_5 \text{day}_{ij} + \mu_j + \varepsilon_{ij}$, where β_0 was the average y-intercept or overall mean for fecal chemical composition, digestibility, DMI, ADG, or G:F when all terms in the equation are equal to 0, i was the individual observation of day within pen, j was feedlot, μ_j was the residual associated with feedlot and ε_{ij} was the residual error associated with each observation. Least square means of the treatments were separated using PDIFF statement, with significance declared at $P < 0.05$.

A third series of regression models were used to examine the bivariate associations between each of fecal nutrients and digestibility measures, and each measure of performance, including DMI, ADG, and G:F at each lot. Lot of cattle was considered the experimental unit for this analysis, and if multiple fecal collections occurred within the same lot of cattle, fecal nutrients and digestibility measures were averaged before inclusion in the regression. Manual backward stepwise regression was used to generate multivariable equations to predict DMI, ADG, or G:F for each lot of cattle using information on fecal nutrients and NIRS predicted digestibility, with a p-value for bivariate association of < 0.20 suggesting a potential association. The mixed linear regression model included a random effect for feedlot and fixed effects for grain type, grain percent, processing method, processing index, F:C, sex, season, and average BW of the cattle at the time of sampling.

The correlations between all pairs of variables that were significantly associated with an outcome of interest were assessed using PROC CORR. Where variables were highly correlated ($r \geq 0.90$, $P < 0.01$), the variable from the pair with the greatest *P* value was removed before consideration in building the final multivariable model. The linearity assumption was examined for each of the continuous measures of fecal nutrient concentrations and digestibilities in these models. Differences were declared significant at $P < 0.05$, and trends at $P < 0.10$ for all models.

The concordance correlation coefficient (CCC) (rho; Lin 1989; 2000) was used to calculate the concordance between observed and predicted DMI, ADG, and G:F. The CCC reflects the agreement between two sets of results with a value of 1 indicating perfect agreement between the results. Differences were declared significant at $P < 0.05$, and trends at $P < 0.10$ for all models.

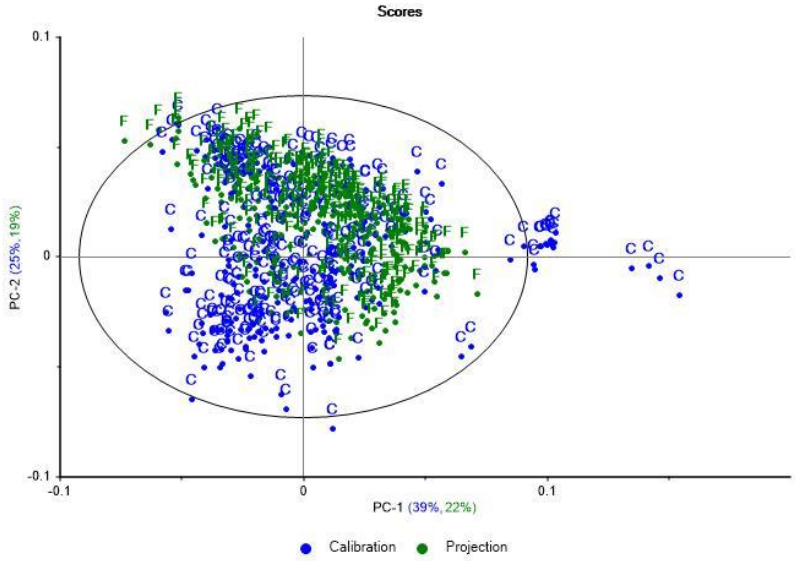
7.3 Results and Discussion

7.3.1 Accuracy of NIRS Predictions of Fecal Samples

A pre-requisite for successful NIRS of feces is that the spectral variability of samples to be analyzed is encompassed by the calibration database (Landau et al. 2015), a requirement satisfied by the calibrations in the present study. Calibrations should not be static, but rather continuously evolve as new datasets become available. To expand previous calibrations, data selection methods such as random selection, manual selection, and discriminant analysis by wavelength selection or PCA can be used (Westerhaus et al. 2004). These methods recognize outlier samples that are identified using *H* (or Mahalanhobis) distances, Hotelling's T^2 distribution (WinISI 1.50, Infrasoft International, Silver Spring, MD), Global and Neighborhood distances (Ucal, Unity Scientific, 2010), and *T* statistics (Ucal), depending on the type of software employed. In the current study, we used manual selection to expand the original calibration by including a subset of commercial feedlot samples that were selected based on NIRS predicted constituent concentrations. We then examined ND in the validation set, and added samples with ND > 0.60. Prior to adding the subset of samples into the initial calibration for chemical composition, the coefficients of determination of validation (R^2_{val}) was 0.94 for fecal starch, $0.70 \leq R^2_{val} \leq 0.80$ for fecal OM, N, NDF, ADL, and $R^2_{val} = 0.25$ for fecal ADF, and EE (Chapter 3). We did not perform a second validation or additional reference analysis after initial calibration expansion, but it is likely that this step improved predictability. The coefficients of determination of cross-validation increased, or remained high (except for EE), and the standard errors of cross validation did not dramatically change (Chapter 3). Obviously, total tract digestibility could not be measured in commercial feedlot cattle. Consequently, all samples in the calibration set used to predict digestibility were collected from cattle housed indoors during metabolism experiments (Chapter 3). As a result, none of the commercial feedlot samples could be used to expand the digestibility calibration set.

Discriminant analysis (PCA) was used to visualize and compare spectral populations as a whole using the Hotelling's T^2 distribution. Samples outside of the defined Hotelling's T^2 ellipses were identified as outliers from the population to a confidence level of 95%. Principal component analysis demonstrated substantial overlap along PC 1 and PC 2 between the calibration set for fecal chemical composition and the samples collected from the commercial

A



B

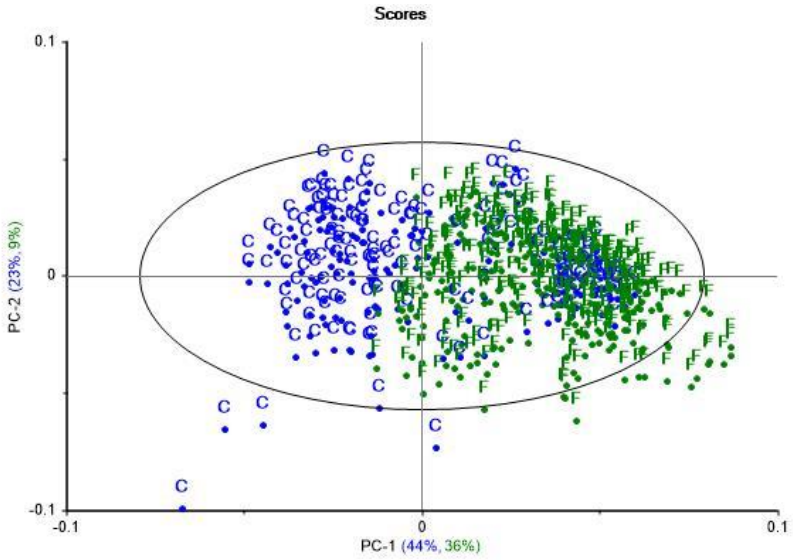


Figure 7.1. Principal component graph displaying (A) the spectra of fecal samples collected from commercial feedlots over a 1 year period in relation to the fecal samples in the NIRS calibration set for chemical composition along the first two principal components, and (B) the spectra of fecal samples collected from six commercial feedlots over a 1 year period in relation to the fecal samples in the NIRS calibration set to estimate digestibility along the first two principal components. Samples outside of the defined Hotelling's T^2 ellipses are considered outliers from the population to a confidence level of 95%.

feedlots (Figure 7.1A). Principal components 1 and 2 explained 64% of the variation in the calibration samples, and 41% in the feedlot samples. The only samples from the feedlot dataset that were considered outliers according to the Hotelling's T^2 distribution were samples collected in January. There was much less overlap between the calibration sets for digestibility and those collected from the commercial feedlots, and two separate populations were evident (Figure 7.1B).

The first two PCs still explained a large proportion of the spectral variability in both datasets (67% for the calibration samples, and 44% for the feedlot samples) and feedlot samples collected in January were again identified as outliers, as well as a few samples collected in November and December. It is possible that the cold temperatures that cattle were exposed to during this period may have altered the composition of feces as compared to those used in the development of digestibility equations which were all collected indoors at room temperature. This is consistent with previous work by Coates and Dixon (2012) where calibrations for predicting diet digestibility in ruminants were developed using samples collected over 10 years using various sampling methods. They demonstrated that experimental site and sampling method often had important effects on calibration statistics and performance. Landau et al. (2015) also developed NIRS calibrations of feces for predicting dietary composition in beef cattle in east Mediterranean rangelands, and found that seasonal trends in pasture quality and responses to management practices impacted estimates. This would explain the differences shown in the PCA graphs between spectra collected from metabolism studies in Chapter 3, and those collected from the commercial feedlots in the current study.

7.3.2 Characterization of Fecal Samples

To our knowledge, this study is the first to examine the extent to which NIRS can be used to predict the composition of feces, diet digestibility and growth performance of commercial feedlot cattle. The average fecal DM, and NIRS predicted fecal composition and digestibility estimates (Table 2) were comparable to estimates from a previous study (Chapter 3). The previous datasets were compiled from fecal samples representing over 60 diets from both research feedlot and metabolism studies. Although none of the average fecal concentrations reported in the commercial feedlot samples appeared unusual, the largest discrepancy identified occurred with fecal NDF, which was on average 3% lower than previous estimates from research

feedlot studies. This is not likely due to an error with NIRS as the average fecal NDF in a subset of these samples that were analyzed using wet chemistry was also lower than reported in previous studies (46.0 ± 8.02%; Chapter 4). As we did not analyze the diets that were fed to the commercial feedlot cattle, it was impossible to determine if the lower fecal NDF was a result of lower NDF intake.

The commercial feedlot fecal samples possessed individual samples with unusually low and high estimates of starch concentration. For example, 5 fecal samples had predicted starch concentrations of less than 1% starch. These samples came from separate pens collected within the same month from two feedlots that were feeding temper-rolled barley. The standard error in the calibration for starch was 1.67%, which indicates an error of ±1.67% fecal starch DM in 68% of samples (based on a normal Gaussian distribution), and ±3.34% in 95% of the samples. In a previous study, NIRS was used to predict starch in fecal samples collected from backgrounding cattle, and if starch levels in feces were low (≤ 2%), the average C.V. for duplicate samples were much higher than wet chemistry estimates (0.33 versus 0.055; Chapter 4). In the finishing period, fecal starch concentrations were higher and the average C.V. for NIRS analysis were only slightly higher than wet chemistry (0.067 versus 0.053; Chapter 4). All of the fecal samples with starch values > 16% originated from a single feedlot that exhibited the highest processing index.

The nutrient and GE digestibility values in the 6 commercial feedlots were higher than those reported in previous studies (Chapter 3) and unrealistically high (up to 100%) estimates in aTTD of GE were observed (Table 2). The reason for this high variability in prediction in field as compared to our previous research studies is unknown. In commercial feedlots, competition among cattle for feed can be intense if meal delivery is delayed or if bunk space is limiting. Competition is known to increase consumption rates and reduce eating time (Olofsson 1999), factors that can affect digestibility. Furthermore, because dominant and subordinate cattle are penned together, eating behaviours of these cattle differ from the individually housed cattle (Striklin and Gonyou 1981) that were used in metabolism studies to develop the NIRS calibrations to predict digestibility. Strengthening of the calibration equations through the addition of data from a broader range of digestibility studies may help improve these estimates.

Since the PCA graphs show different populations, it is also possible that the NIRS predictions lacked accuracy, and were over-predicting actual values. Despite the ability of NIRS

Table 7.2. Simple statistics of fecal DM and NIRS predicted fecal composition and apparent total tract digestibility derived from pen composite fecal samples collected from feedlot cattle fed in six commercial feedlots (n=282 pens) over a 1 year period

Item	Measurement[1]			
	Mean±SD	C.V. (%)	Min	Max
Fecal composition, % of fecal DM				
Dry matter	18.9±2.31	12.2	13.6	27.0
Organic matter	83.8±3.58	4.27	67.5	89.4
Starch	7.0±3.87	55.3	0.00	25.1
Nitrogen	2.36±0.215	9.11	1.83	3.00
NDF	50.4±4.78	9.48	33.2	60.5
ADF	29.3±3.18	10.8	19.9	36.2
ADL	5.41±0.939	17.3	3.29	8.03
Ether extract	1.50±0.183	12.2	0.849	1.912
Digestibility, % of intake				
Dry matter	82.0±3.28	4.00	70.4	89.2
Organic matter	81.6±3.53	4.32	70.0	89.3
Starch	95.0±2.14	2.25	86.2	99.5
Crude protein	77.6±3.04	3.92	68.4	85.0
NDF	62.0±3.56	5.74	53.5	73.5
ADF	46.9±5.10	10.9	27.8	63.6
Gross energy	88.0±5.19	5.90	74.6	100

[1]SD = standard deviation; C.V. = coefficient of variation (SD/mean)

to be less predictive of digestibility as compared to the chemical composition of feces, the current method has value in terms of its potential to estimate diet digestibility in group-penned feedlot cattle without using markers or total collection techniques. Chapter 3 reported that even though predictions of certain digestibility coefficients using NIRS were not identical to estimates obtained from total collection, expected changes in digestibility in response to dietary changes could be predicted, illustrating the merit of this approach.

Variation in fecal chemical composition and diet digestibility among different individual cattle is important to address when attempting to predict outcomes from a population of cattle without sampling all of them. Consistent with Chapter 5, where fecal starch values within a pen varied substantially, a high degree of variation was also observed in fecal starch (C.V. = 55%) in the commercial feedlot samples. In contrast, all other fecal constituents were reported to have C.V. of 12% or less (Table 2). The greatest variability in digestibility was observed for aTTD of ADF, an outcome that was attributed to the relatively poor linearity and accuracy of predicting this fecal constituent (Chapter 3).

7.3.3 Differences between Management Practices, Fecal and Performance Measures

Beef cattle performance is dependent on many factors including the initial BW, age, sex, body condition, health record (Reinhardt et al. 2009; McMeniman et al. 2010; Galyean et al. 2010), animal behaviour, hierarchical behaviour, diet, inclusion of feed additives and anabolic agents, as well as season and temperature (NASEM, 2016). Our sample size was limited (6 feedlots and 4 pens per feedlot) and therefore we did not have sufficient statistical power investigate interactions among production parameters. The factors that we could account for such as grain type, grain processing method, sex, season, and day were adjusted for in the analysis. Other factors such as the effects of grain inclusion rate, F:C ratio, and diet ingredients were reported as well. For example, grain percent was quite low for feedlots 1 (69.2 ± 5.08) and 6 (64.7±5.10) in relation to others (all ≥ 77.6%), with feedlot 1 also having the highest F:C ratio (0.16 versus ≤ 0.11 for the remaining five feedlots; Table 3). Without any additional information, we could expect that cattle in this feedlot would have the poorest performance because of the lower energy density of these diets. However, this was not the case as the DMI was the greatest for feedlot 1, resulting in increased energy intake. Also, the degree of grain processing and method utilized must also be considered as the least vigorous grain processing occurred in

feedlot 5 (84.3±8.27; Table 3). This likely explains why cattle in feedlot 5 exhibited the lowest ($P \leq 0.01$) ADG (1.19 kg BW/d) and G:F (0.110), the highest ($P \leq 0.01$) fecal starch (11.6% of fecal DM) and the lowest ($P \leq 0.01$) fecal NDF (45.7% of fecal DM) concentration of the feedlots examined (Table 3).

To clarify other discrepancies, despite feedlot 1 having the lowest percentage of grain in the diet, and greatest F:C, performance was not compromised. In times of poor weather or low DMI as a result of rapid diet changes, feedlot 1 fed cattle a finishing diet with lower levels of grain for long periods of time. This occurred over the course of several sampling times, and would have resulted in the lower grain percentage and higher F:C ratio reported. Feedlot 6 always temper-rolled grain and included potatoes in the diet, both of which would increase the energy content of the diet. Feedlot 5 was the most obvious outlier, where processing index and fecal starch concentration were exceptionally high, and digestibility coefficients low, factors that likely contributed to the reduced growth performance of cattle at this location.

Many western Canadian feedlots use barley or wheat that is processed by dry or temper rolling (McAllister et al. 2011). When grain is dry-rolled, the degree of fracturing of the grain kernel is dependent on the adjustable distance between two rollers. This processing method is sufficient for barley and wheat, but can result in greater variability in particle size including the generation of fine particles and dust (Wang et al. 2003; McAllister et al. 2011). Fines (particles less than 1 mm diameter) have been shown to increase the occurrence of digestive dysfunction due to starch digestion being too rapid acid accumulating in the rumen (Mathison, 2000). Temper rolling involves application of water for 8 to 24 h prior to rolling, enabling more control over the degree of fracture of the kernel and reducing the generation of fine particles. Industry standards for processing index of dry rolled barley grain ranges between 65 and 82% (Yang et al. 2000), whereas temper rolled barley ranges from 70 to 95% (Beauchemin et al. 2001). Over the 1 yr period, only feedlot 5 exceeded the upper range of the recommended PI for dry rolling.

Cereal grain processing acts to increase ruminal digestion of all nutrients, especially starch, by first disrupting the pericarp (and hull in hulled grains) and secondly by reducing the grain particle size, accelerating microbial colonization and fermentation (McAllister et al. 2011). If grain is processed more vigorously, measured as a lower processing index, exposure of the starch to microbes will increase. Our results show that compared to temper rolled grain, dry rolled grain was processed less vigorously (75.4 ± 9.33 versus 66.0 ± 4.08). However, temper rolled grain

Table 7.3. Differences in NIRS predicted fecal starch and NDF, digestibility of DM and OM, performance, and management strategies in six commercial feedlots over a 1 year period (n=282)

Item[1]	Feedlot[2]						*P* value
	1 (n=52)	2[3] (n=51)	3 (n=46)	4 (n=51)	5 (n=39)	6[4] (n=42)	
Management							
Grain %	69.2 (1.209)c	77.6 (1.317)b	80.7 (1.363)ab	81.4 (1.26)a	80.7 (1.49)ab	64.6 (1.63)d	<0.01
F:C	0.16 (0.006)a	0.11 (0.005)b	0.096 (0.0066)b	0.10 (0.006)b	0.11 (0.008)b	0.11 (0.007)b	<0.01
PI	70.7 (0.77)b	64.6 (0.89)c	65.4 (0.90)c	69.4 (0.82)b	84.2 (0.98)a	65.6 (0.98)c	<0.01
Processing methods	Dry rolled	Temper rolled	Temper rolled	Dry/Temper rolled	Dry rolled	Temper rolled	n/a
Composition, % of fecal DM							
Starch	7.90 (0.449)b	4.84 (0.494)c	5.46 (0.509)c	7.53 (0.471)b	11.60 (0.555)a	5.20 (0.544)c	<0.01
Neutral detergent fiber	50.6 (0.63)ab	51.0 (0.72)ab	51.5 (0.73)ab	52.4 (0.67)a	45.7 (0.80)c	50.1 (0.79)b	<0.01
Digestibility, % of intake							
Dry matter	79.9 (0.41)c	82.4 (0.44)b	83.8 (0.46)a	82.9 (0.43)ab	79.7 (0.51)c	82.7 (0.49)b	<0.01
Organic matter	79.4 (0.47)c	81.4 (0.51)b	83.4 (0.53)a	83.1 (10.49)a	80.9 (0.58)b	80.9 (0.56)b	<0.01
Starch	94.3 (0.30)bc	95.4 (0.33)a	95.6 (0.34)a	95.5 (0.31)a	93.8 (0.37)c	95.1 (0.37)ab	<0.01
Gross energy	86.9 (0.66)c	88.8 (0.67)b	90.9 (0.71)a	87.1 (0.67)bc	83.7 (0.78)d	90.5 (0.73)a	<0.01
Performance							
BW, k5[d]	629.9 (9.15)a	550.3 (10.59)c	491.7 (10.54)d	489.9 (9.69)d	583.2 (12.05)b	520.0 (20.60)cd	<0.01
DMI, kg/d	10.7 (0.14)a	10.1 (0.17)bcd	9.69 (0.170)d	9.96 (0.152)cd	10.5 (0.18)ab	9.94 (0.184)cd	<0.01
ADG, kg/d	1.66 (0.022)a	1.52 (0.027)bc	1.60 (0.027)ab	1.51 (0.025)c	1.19 (0.029)d	1.56 (0.030)bc	<0.01
G:F, kg/kg DM	0.155 (0.0022)bc	0.151 (0.0026)bc	0.167 (0.0026)a	0.151 (0.0023)c	0.115 (0.0028)d	0.159 (0.0028)b	<0.01

[1] F:C = forage to concentrate ratio; PI = processing index of grain. Values with lowercased letters represent significant differences ($P < 0.05$).

[2]A total of 292 fecal samples are represented in the table. Data is missing for F:C (n = 269), BW (n = 242), and grain percent (n = 272). Numbers in brackets represent the standard error of the mean.

[3]Included potatoes as a source of starch during 3 of the sampling days

[4]Included potatoes as a source of starch all year

[5]Average body weight of cattle in pens at time of sampling

Table 7.4. Differences in NIRS predicted fecal starch and NDF, digestibility of DM, OM, performance, and management strategies for dry rolled grain versus temper rolled, barley versus barley and wheat diets, and heifers versus steers, (n=282)

Item[1]	Processing Method		
Management	DR (n=107)	TR (n=174)	*P* value
Grain %	75.6 ± 8.39	76.4 ± 11.35	
F:C	0.134 ± 0.0675	0.106 ± 0.0247	
PI	75.4 ± 9.33	66.0 ± 4.08	
Composition, % of fecal DM			
Starch	10.4 (0.64)	6.5 (0.55)	<0.01
NDF	46.5 (1.25)	49.2 (1.09)	0.04
Digestibility, % of intake			
DM	80.0 (0.58)	82.9 (0.50)	<0.01
OM	80.1 (0.75)	82.4 (0.64)	0.01
Starch	93.4 (0.39)	94.9 (0.33)	<0.01
GE	85.8 (1.05)	89.3 (0.91)	<0.01
Performance			
BW, kg	593.7 ± 68.82	511.2 ± 74.64	
DMI, kg/d	10.2 (0.25)	10.0 (0.21)	0.50
ADG, kg/d	1.47 (0.074)	1.53 (0.070)	0.22
G:F, kg/kg DM	0.146 (0.0079)	0.150 (0.0076)	0.32
	Grain type		
Management	B (n=202)	BW (n=79)	*P* value
Grain %	75.0 ± 9.59	78.8 ± 11.40	
F:C	0.123 ± 0.0563	0.103 ± 0.0125	
PI	67.7 ± 5.64	74.5 ± 10.74	
Composition, % of fecal DM			
Starch	7.2 (0.50)	9.4 (0.66)	<0.01
NDF	47.6 (1.03)	48.1 (1.15)	0.54
Digestibility, % of intake			
DM	80.8 (0.50)	82.1 (0.57)	0.02
OM	80.3 (0.63)	82.1 (0.73)	<0.01
Starch	94.2 (0.35)	94.2 (0.39)	0.89
GE	87.6 (0.87)	87.5 (0.99)	0.92
Performance			
BW, kg	562.2 ± 81.42	510.9 ± 75.10	
DMI, kg/d	10.1 (0.20)	10.1 (0.22)	0.83
ADG, kg/d	1.46 (0.068)	1.54 (0.071)	<0.01
G:F, kg/kg DM	0.144 (0.0075)	0.151 (0.0077)	<0.01
	Sex		
Management	Heifer (n=53)	Steer (n=228)	*P* value
Grain %	76.5 ± 9.47	76.0 ± 10.48	
F:C	0.114 ± 0.0318	0.118 ± 0.0518	
PI	73.5 ± 10.10	68.7 ± 7.17	
Composition, % of fecal DM			
Starch	8.9 (0.65)	8.0 (0.50)	0.07
NDF	47.4 (1.14)	48.3 (0.98)	0.20
Digestibility, % of intake			
DM	81.0 (0.59)	81.9 (0.45)	0.05
OM	80.9 (0.74)	81.5 (0.57)	0.29
Starch	94.0 (0.42)	94.4 (0.31)	0.21
GE	86.9 (0.99)	88.1 (0.81)	0.07
Performance			
BW, kg	530.8 ± 82.96	549.4 ± 82.64	
DMI, kg/d	10.0 (0.21)	10.2 (0.19)	0.20
ADG, kg/d	1.45 (0.070)	1.55 (0.068)	<0.01
G:F, kg/kg DM	0.142 (0.0077)	0.153 (0.0075)	<0.01

[1] F:C = forage to concentrate ratio; PI = processing index; B = barley; BW = barley and wheat.
[2] A total of 292 fecal samples are represented in the table. Data is missing for F:C (n = 269), BW (n = 242), and grain percent (n = 272). Numbers in brackets represent the standard error of the mean. Diets containing potatoes as a source of starch were only included barley and temper rolled diets. Different letters across each row represent significant differences $P < 0.05$.

has a higher moisture content (17 to 25%) and greater malleability, allowing for less severe processing. Therefore, the processing indices for the two methods should not be directly compared. As expected, cattle fed dry rolled grain had greater ($P < 0.01$) fecal starch concentrations, and lower ($P \leq 0.04$) fecal NDF, and lower ($P \leq 0.01$) aTTD of DM, OM, starch and GE (Table 4). However, those fed temper rolled grains did not differ in DMI or growth performance compared to those fed dry rolled grain. The lack of effect on performance is consistent with Bradshaw et al (1996), who found no difference in ADG and G:F of growing and finishing feedlot cattle fed tempered vs dry rolled barley. This confirms that many other factors, in addition to nutrient digestibility impact ADG and G:F. Had we examined the interactions between feedlot, grain processing, processing method, and grain type, we may have identified other factors that contributed to differences in the growth performance of feedlot cattle.

When processing grains, we must also consider grain type as differences have been found in the responses of barley and wheat to processing (Chapter 5). Size and composition of the starch granules, moisture content, size and shape of the kernels (McAllister et al. 2011), and kernel hardness (Campbell et al. 2007) can all influence the efficiency of grain processing. When barley and wheat were fed together, we observed a 2.2% increase ($P < 0.01$) in fecal starch, which may have been due to the 3.8% greater grain proportion, and 6.8% greater processing index of the grain. Despite the increase in fecal starch, and less vigorous processing, there was still an increase ($P \leq 0.02$) in aTTD of DM, OM, ADG, and G:F for cattle fed barley and wheat. Compared to barley, wheat has higher starch content [on average 10% more; based on a range of 56.5-65.6% starch in barley (Engstrom et al. 1992), and 61.6-73.9% in wheat (McAllister and Sultana 2011)], and requires less vigorous processing to expose the endosperm (McAllister et al. 2011;Chapter 5). This may have contributed to the higher aTTD of DM that we observed when wheat and barley grain were fed together (Table 4). We cannot attribute increases in performance to be solely due to feeding wheat, when in fact cattle in feedlot 5 were fed both wheat and barley, but had the numerically lowest ADG and G:F.

We also found differences in fecal starch, digestibility and growth performance based on the sex of the cattle. Several studies have documented differences in fecal starch excretion (Caetano 2008) as well as in intake and feeding patterns (Owens et al. 1985; Hicks et al. 1990; Schwartzkopf-Genswein et al. 2002) between heifers and steers. In contrast to Caetano (2008), who found higher fecal starch in steers compared to heifers, we found a tendency ($P = 0.07$) for

greater fecal starch in heifers compared to steers. We also found greater ($P = 0.05$) aTTD of DM in steers ($P \leq 0.05$), and a tendency ($P = 0.07$) for greater aTTD of GE in steers compared to heifers (Table 4). Eating behavior can affect nutrient excretion and digestibility. For example, higher intake, rate of intake, and lower feeding frequency are associated with lower digestibility. Schwartzkopf-Genswein et al. (2002) reported that heifers visited the feed bunk more frequently and spent more time there than did steers, however, Chirase et al. (1991) found that steers spent more time eating than heifers, but with a similar visitation frequency. Since we did not record feeding patterns, stocking density, or hierarchical behaviour in the current study, we can only attribute differences to data that was measured. For example, small dietary variances may have biased our results, particularly the higher processing index of grain for heifers than steers (73.5 ± 10.10 versus 68.7 ± 7.17; ignoring processing method), and the lower proportion of pens of heifers fed temper rolled grain compared to steers (59% versus 64%).

Using pen-averaged data, Owens et al. (1985) and Hicks et al. (1990) reported that steers consumed up to 3% more DM than heifers, but we found no differences ($P = 0.20$) in DMI, despite a 18.6 kg greater average BW of steers. Once again, the less vigorous processing of grain may have contributed to heifers consuming more feed so as to increase their energy consumption. As expected, steers exhibited greater ADG and G:F than heifers (Table 4). Differences in fattening patterns among sexes reflect the fact that heifers exhibit a more rapid rate of fat deposition and fatten at a lighter weight than steers (Berg et al 1979). Even when at the same weight, heifers display a much greater percent body fat and a lower percent body protein then steers (NASEM 2016), resulting in lower gains and G:F.

7.3.4 Relationships between fecal starch, processing index, and G:F among feedlots

When averaged by feedlot, processing index predicted fecal starch concentration using the equation: fecal starch (%, DM) = 0.36 × processing index - 18.38 ($R^2 = 0.97$, $P < 0.01$; Figure 7.2A). A weaker relationship ($R^2 = 0.79$, $P < 0.01$; Figure 7.2B) was found between fecal starch concentration and G:F. This strong linear relationship was only identified when data were averaged across feedlots, confirming that factors other than processing index and fecal starch concentration affected these predictions. Considering some feedlots feed up to 15,000 kg of grain

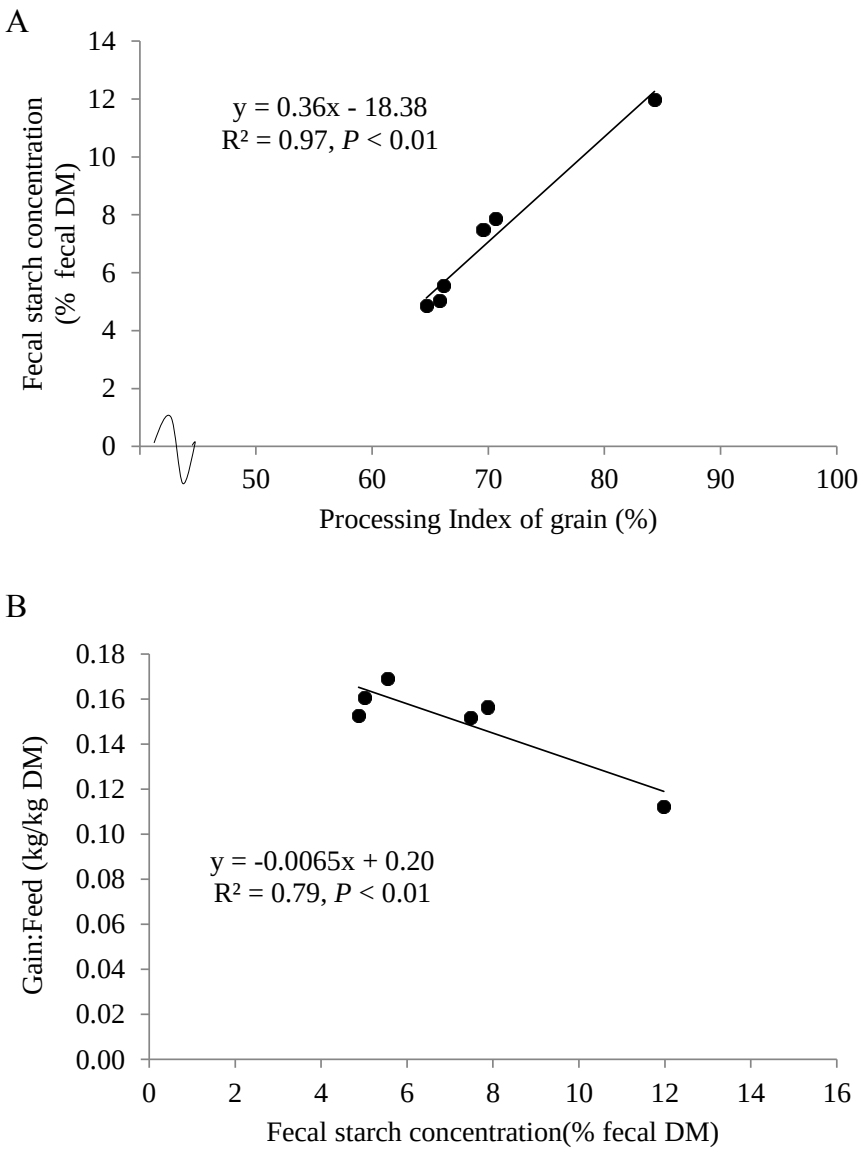


Figure 7.2. Relationship between A) processing index and fecal starch concentration, and B) fecal starch concentration and gain:feed, when fecal samples collected monthly from six feedlots are averaged over a year.

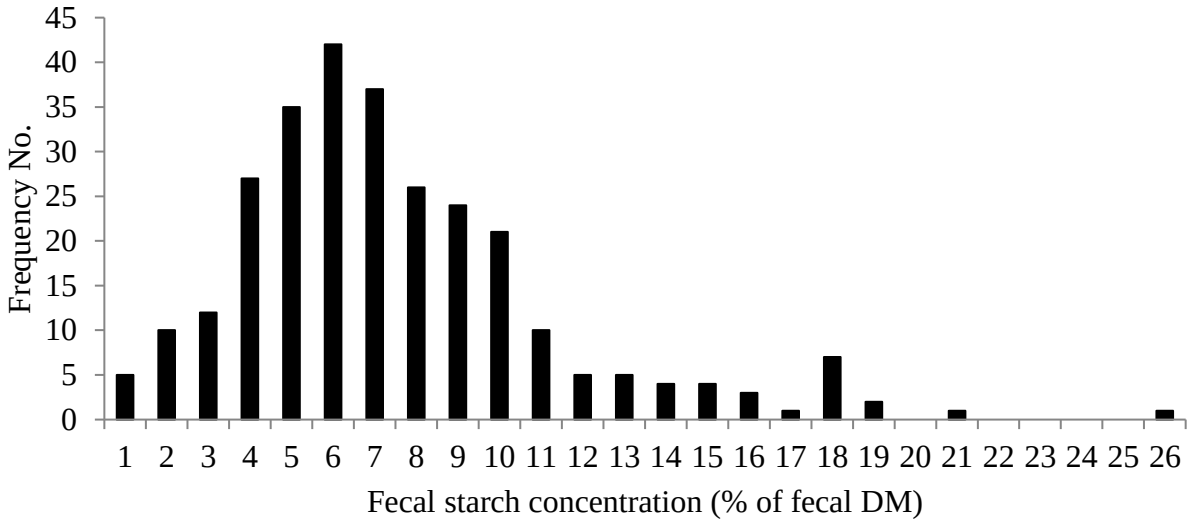


Figure 7.3. Histogram of NIRS predicted fecal starch concentration found in pooled feces collected monthly from 4 animals per pen from 6 commercial feedlots over a 1 year period. The mean and standard deviation was 7.0 ± 3.86%.

per day, it would be difficult to always sample the exact grain at the feed mill that was fed to a particular pen of cattle. In the present study, we assumed that the grain variety and processing method would not change drastically within 1 to 2 d. The histogram of fecal starch concentration as predicted in all samples indicated that fecal starch concentration ranged from < 1 % to > 25%, with 40% of the fecal samples containing between 5 and 7% starch. If feedlot 5 was excluded from the data set due to its high processing index, the average processing index of feedlots declined from 69.6 ± 8.01% to 67.2% ± 4.96% and average concentrations of fecal starch declined from 7.0 ± 3.87% to 6.2 ± 2.96% (Figure 7.3). These results provide processing indices and associated fecal starch levels that can be used as a benchmark for well-processed grains in barley-based finishing diets.

7.3.5 Associations between and Predictions of Performance in lots of Cattle Using NIRS

The use of NIRS by the livestock industry has permitted nutritional information of the diet (primarily of grazing ruminants) to be obtained from feces, allowing researchers and nutritionists to rapidly improve management strategies. Currently most reports for predicting performance of cattle using NIRS are restricted to free-ranging cattle (Tolleson and Schaffer 2014), but recently fecal NIRS has been used to predict NEg and ADG in feedlot cattle (Chapter 5). Near infrared spectroscopy calibrations have also been directly applied to finding associations between measured fecal parameters and performance, and to predicting ADG and G:F using small datasets where grain type, processing, or grain proportion were deliberately altered (Chapter 5). Consistent with previous work, many associations were found between DMI, ADG, and G:F. The regression slopes and standard errors for the individual associations between cattle BW, grain percentage in the diet, fecal nutrients, aTTD of nutrients, and DMI, ADG, and G:F for lots of cattle are reported in Table 5. Increasing values of grain percent, NIRS-predicted ADL, aTTD of DM, OM (aTTD of DM and OM had correlation coefficients of r = 0.99, $P < 0.01$), and GE, were associated with decreasing values of DMI ($P \leq 0.04$). Increasing values of BW, fecal DM, NDF, and ether extract (EE) were also associated with increasing DMI ($P \leq 0.04$). For ADG, a tendency ($P = 0.08$) for a negative association was observed with fecal starch, and positive associations with fecal NDF, ADF, aTTD of CP, NDF, and GE ($P \leq 0.05$). Average BW, fecal OM, starch, and EE were negatively associated with G:F ($P \leq 0.04$). Fecal ADF, ADL, ash,

Table 7.5. Linear regression equation coefficients examining the individual associations between near infrared spectroscopy predicted fecal constituent concentrations, digestibilities, and observed growth performance of feedlot cattle (n=90 groups) housed within 6 commercial feedlots

Equation Variables[2]				
Dependent	Independent	Coefficient/slope (β)	SE	*P*
DMI, kg/d	BW	0.0091	0.00098	<0.01
	Grain%	-0.025	0.0107	0.02
	DM	0.105	0.0565	0.06
	NDF	0.057	0.0279	0.04
	ADL	-0.33	0.121	<0.01
	EE	1.60	0.705	0.03
	aTTD of DM	-0.11	0.037	<0.01
	aTTD of OM[5]	-0.094	0.0340	<0.01
	aTTD of GE	-0.051	0.0246	0.04
ADG, kg/d	Starch[4]	-0.010	0.0058	0.08
	NDF	0.012	0.0048	0.01
	ADF	0.016	0.0073	0.03
	aTTD of CP	0.023	0.0071	<0.01
	aTTD of NDF[4]	0.012	0.0057	0.04
	aTTD of GE	0.0087	0.00433	0.05
G:F kg/kg	BW	-0.00010	0.000030	<0.01
	OM	-0.0012	0.00052	0.03
	Starch[4]	-0.0014	0.00057	0.02
	ADF	0.0021	0.00070	<0.01
	ADL	0.0043	0.00204	0.04
	EE	-0.030	0.0117	0.01
	aTTD of DM	0.0021	0.00063	<0.01
	aTTD of OM	0.0012	0.00058	0.04
	aTTD of starch	0.0017	0.00101	0.09
	aTTD of GE	0.0015	0.00040	<0.01

[2] aTTD = apparent total tract digestibility.
[3] Fecal DM was determined using actual DM and not NIRS predictions.
[4] There was a non-linear association between independent variable and performance addressed by the introduction of a squared term into the model.
[5]correlated with aTTD of DM r=0.99.

Table 7.6. Multivariable regression equation coefficients examining the joint associations between near infrared spectroscopy predicted fecal constituents, digestibilities, and observed performance of feedlot cattle (n=45 pens) in 6 commercial feedlots

Equation Variables[2]				
Dependent	Independent	Coefficient/slope (β)	SE	*P*
DMI, kg/d	Intercept	21.6	7.94	0.04
	BW	0.0096	0.00127	<0.01
	NDF	0.10	0.038	0.01
	aTTD of OM	-0.23	0.094	0.02
ADG, kg/d	Intercept	0.22	0.396	0.60
	Heifer	-0.18	0.064	<0.01
	Steer	0		
	NDF	0.026	0.0077	<0.01
G:F, kg/kg	Intercept	0.21	0.017	<0.01
	BW	-0.00010	0.00029	<0.01
	Heifer	-0.019	0.0053	<0.01
	Steer	0		
	Starch	0.0028	0.00167	0.10
	Starch*Starch[3]	-0.00032	0.000095	<0.01

[2] aTTD = apparent total tract digestibility.

[3] There was a non-linear association between Starch and performance in two of the models addressed by the introduction of a squared term into the model.

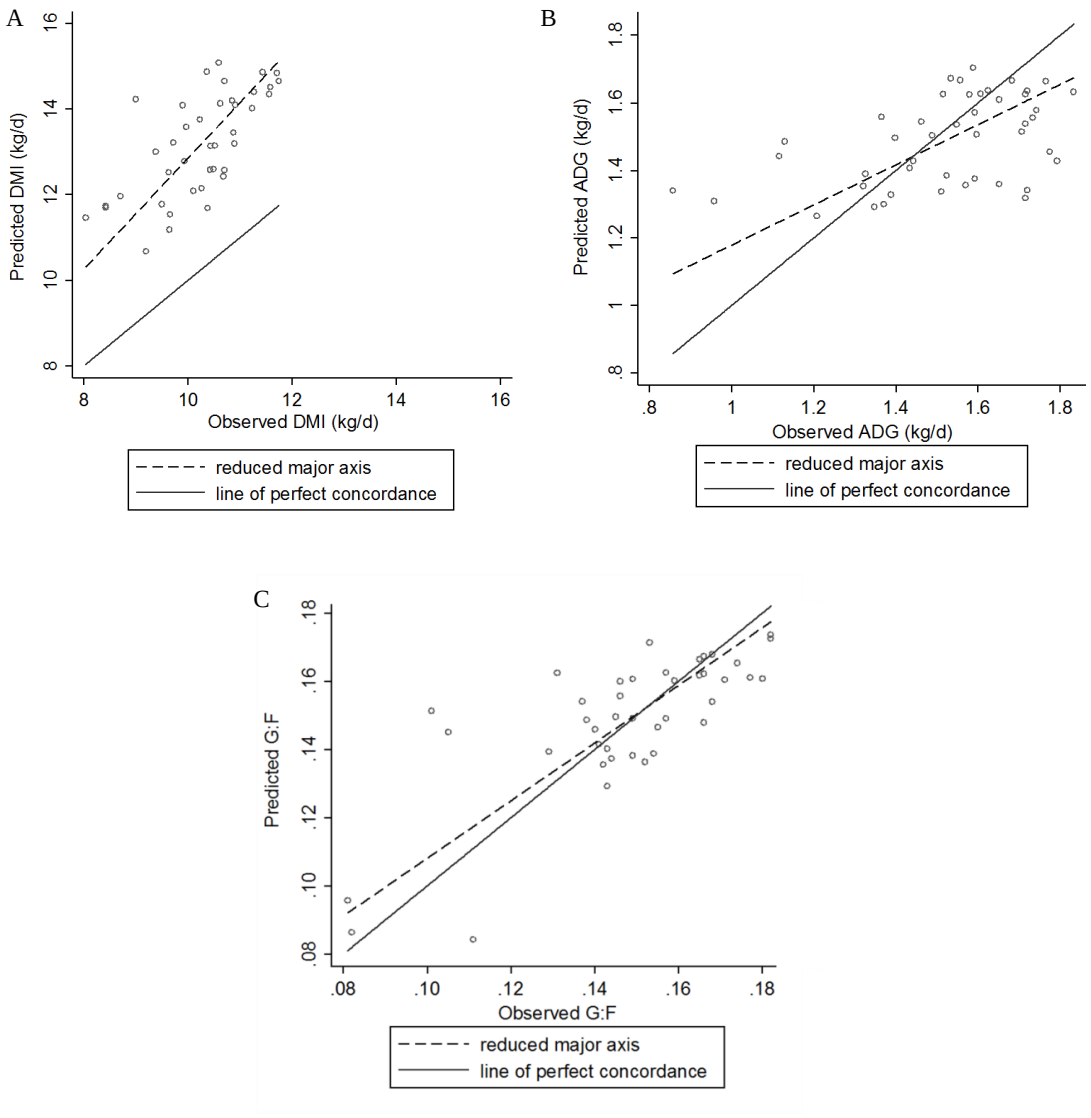


Figure 7.4. Observed versus predicted graphs for A) DMI, B) ADG, and C) G :F. Concordance correlation coefficients (Lin, 1989, 2000) are rho=0.145 (SEM = 0.036), P<0.01, Pearson's r = 0.702. Average difference=2.94 for DMI, rho=0.416 (SEM = 0.106), P<0.01, Pearson's r = 0.481. Average difference=-0.032 for ADG, and rho=0.753 (SEM = 0.067), P<0.01, Pearson's r = 0.764. Average difference=0.001 for G :F.

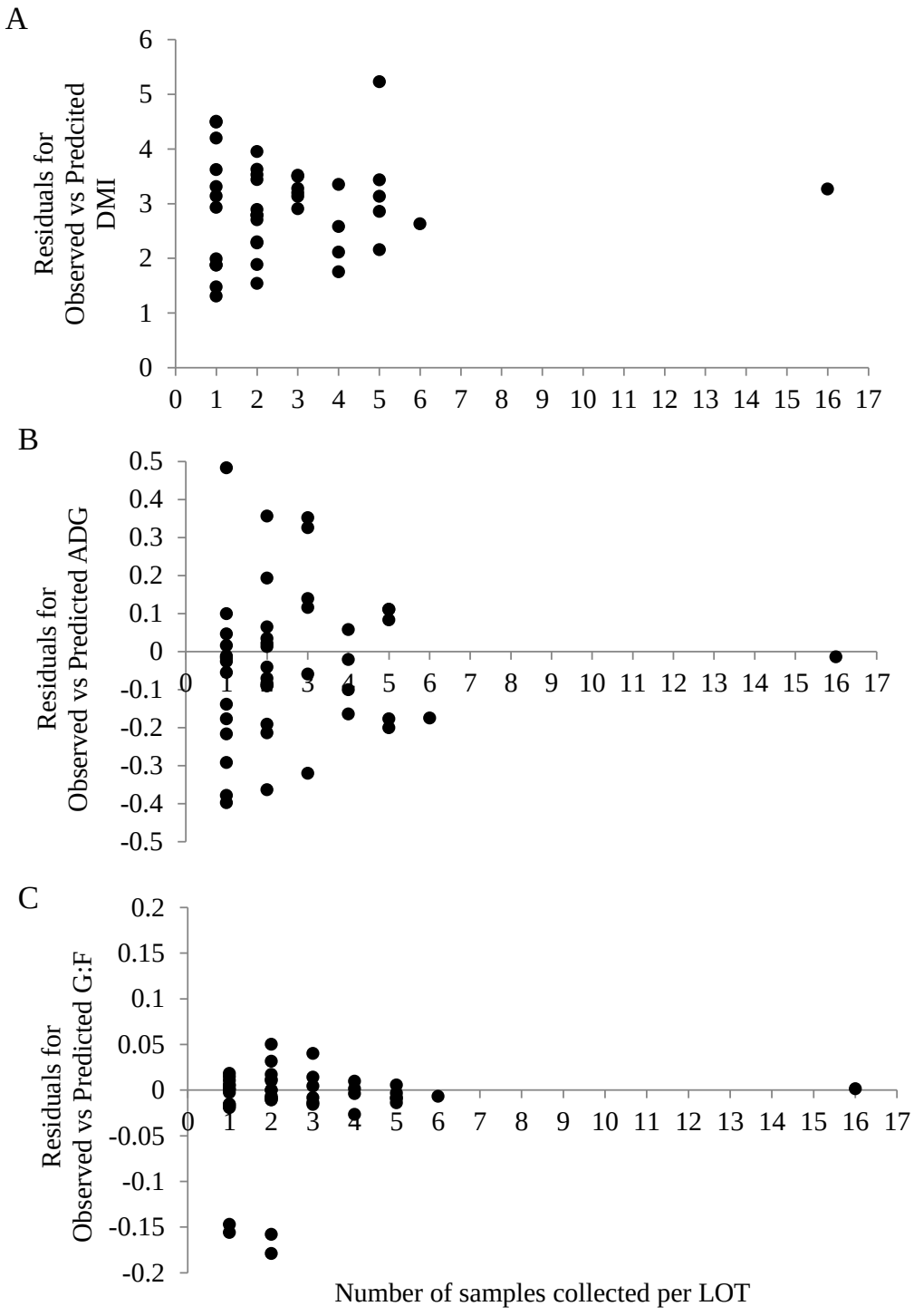


Figure 7.5. Residuals (Predicted – Observed; y-axis) for each group of cattle with the number of samples collected per group displayed on the x-axis for A) DMI, B) ADG, and C) G:F.

aTTD of DM, OM, and GE were positively associated ($P \leq 0.04$) with G:F and there was a tendency ($P = 0.09$) for a positive association between G:F and aTTD of starch. All associations were linear, except for those between fecal starch and ADG and G:F, and between aTTD of NDF and ADG.

All digestibility coefficients that were significant, were negatively associated to DMI (aTTD of DM, OM, and GE), and positively associated with ADG (aTTD of CP, NDF, and GE) and G:F (aTTD of DM, OM, starch, and GE). This result is expected, since greater digestibility would indicate more energy available to the cattle, and gains could increase with lower feed consumption. Similar to previous work, a non-linear association was found for fecal starch and G:F, and a positive association was observed between fecal NDF and ADG (Chapter 5). The reason for greater fecal NDF being associated with greater ADG is unknown; however, it may simply reflect an increase in fecal NDF as the contribution of starch to fecal DM decreases.

Multivariable models including fecal chemical composition and digestibility were developed for DMI, ADG, and G:F, after randomly splitting the data set in half, ensuring equal number of heifers and steers lots were represented in each, and accounting for study design variables (Table 6). Average BW at the time of sampling was included in the regression models for both DMI and G:F, and sex was accounted for in predictions of ADG and G:F. Greater fecal NDF resulted in an increase in both DMI and ADG. The concordance between observed versus predicted DMI and ADG were poor (rho = 0.14, P < 0.01 for DMI, Figure 7.4A; and rho = 0.41, P < 0.01 for ADG, Figure 7.4B) relative to G:F (rho = 0.75, P < 0.01; Figure 7.4C). The final model for G:F incorporated BW, sex, and a quadratic term for fecal starch.

Unlike G:F, multivariable models between predicted and observed DMI and ADG displayed poor concordance or agreement. A positive linear relationship was observed for DMI, but the predicted values were about 2% greater than observed. Average daily gain depends on the energy density of the diet, and DMI, therefore, without considering these factors the prediction would be expected to be poor. It is likely that the improved prediction of G:F is a reflection that DMI and ADG are both taken into account in the formula for G:F. Consequently, having ADG and DMI in the numerator and denominator of G:F permits us to consider both values at the same time. As reported in other work, the stage of maturity of the cattle resulted in different equations for predicting net energy, ADG, and G:F (Chapter 5). In the current study, the prediction of G:F includes the average BW at the time of sampling, as well as sex, as reported in equations 1 and

2. The quadratic relationship for fecal starch implies that the maximum G:F obtained for steers and heifers weighing 550 kg, would be obtained when fecal starch levels were 4.4%. Unlike G:F, multivariable models between predicted and observed DMI and ADG displayed poor concordance or agreement. A positive linear relationship was observed for DMI, but the predicted values were about 2% greater than observed. Average daily gain depends on the energy of the diet, and DMI, therefore without directly incorporating these factors; we would expect predictions to be poor. The better success of the prediction of G:F is that this variable is a measure of feed conversion, and the impact of DMI would be considered in both the numerator and denominator. As reported in other work, the stage of animal maturity resulted in different equations for predicting net energy, ADG, and G:F in cattle (Chapter 5). In the current study, the prediction of G:F includes the average BW at the time of sampling, as well as the sex of the cattle, as shown in equations 1) and 2). The quadratic relationship for fecal starch implies that the maximum G:F obtained for steers and heifers weighing 550 kg, would be 4.4%.

$$G:F = 0.21-0.00010\times BW+0.0028\times fecalstarch - 0.00032\times fecalstarch^2 \text{ for steers} \quad \ldots\ldots\ldots\ldots\ldots\ldots (7.1)$$

$$G:F = 0.19-0.00010\times BW+0.0028\times fecalstarch - 0.00032\times fecalstarch^2 \text{ for heifers} \quad \ldots\ldots\ldots\ldots\ldots\ldots (7.2)$$

We have shown that fecal starch is dependent on dietary starch concentration, source of starch, degree of grain processing and DMI. Thus, a lower fecal starch concentration does not always indicate increased G:F. In fact, highly fermentable diets and extensive processing of grain increases the risk of bloat and acidosis by allowing for too rapid fermentation and increased acid production in the rumen and lower tract (Wang et al. 2012; Aschenbach et al. 2011). Currently we do not know the detailed nutrient characteristics of fecal samples collected from acidotic cattle, but fecal pH is depressed, and volatile fatty acid concentrations increased (Gressley et al. 2011; Mao et al. 2012;). As a result of damage to the gut epithelium, watery or foamy feces that contain mucin casts are typical for identifying hindgut acidosis (Gressley et al. 2011). It is likely that fecal starch concentration will be lower than normal in these abnormal fecal samples.

Starch digestibility predicted using NIRS did not generate strong associations to ADG and G:F as fecal starch measurements, despite their close relationship. This is likely because large changes in fecal starch do not result in as large differences in aTTD of starch. Our results show that in addition to measuring fecal starch, NIRS predicted aTTD of DM or OM, and DE

could also be monitored, since these values were also associated with improved growth performance.

The residual graphs depict a cone shape (Figure 7.5) for all performance parameters. This indicates that as more samples are collected per lot of cattle, the difference between predicted and observed performance measurements becomes less. The greatest differences between predicted and observed estimates occurred when pooled samples were collected once or twice over the feeding period from the same lot of cattle. Residuals are almost 0 when 16 pooled samples were collected over the full feeding period. It is difficult to assess results from the residual graphs since many sampling points are missing; however, the graphs shows that as more samples taken over a feeding period, the less likely that predicted values will differ from observed. We must consider that as sampling frequency increases, the practicality of our approach decreases, especially if samples are collected at the end of the feeding period, when predictions of G:F are no longer useful in terms of being used for immediate management decisions.

7.4 Conclusions

I have found that the composition of fecal samples can predict ADG and G:F under some circumstances, but is not a universal predictor due to the myriad of factors that can influence feedlot cattle production. Increasing the frequency of collection during the feeding period and number of fecal samples collected per pen, could be strategies to improve the predictive abilities of NIRS. However, it may not be reasonable to collect fecal samples this frequently under commercial production conditions. For feedlots that choose to keep diets and practices fairly constant, there is more value in using NIRS for predictive purposes. Using the recommendations for processing index and maximum fecal starch concentrations, combined with monitoring digestibility of DM, OM or GE and the predictive equation for G:F, it may be possible to identify poor management practices that can be alleviated so as to improve production outcomes.

CHAPTER 8

8.0 GENERAL DISCUSSION

Feedlot operators make daily management decisions to increase profitability by strategically selecting feed ingredients, varying diet composition, and grain processing, and scheduling shipping dates for cattle. Decisions are made with the help of nutritionists, veterinarians, and those directly involved in production economics, and are based on up-to-date knowledge of nutrition, current feed costs and availability, and historic growth performance data. The primary driver of animal performance is the digestible energy (DE) of feed, which can be converted to net energy of gain (NE_g) for predicting animal gain, from which economic returns can be more accurately estimated. It is known however, that expected DE values based on ingredient composition, or those based on actual digestibility studies, are not always consistent with those observed for cattle housed in feedlots. Beef feedlots try to ensure that nutrients are in excess of animal requirements as it is not possible to precisely predict how well the cattle are digesting nutrients in feed. The current thesis shows that examination of the feces using near infrared spectroscopy (NIRS), which contains information relevant to feed digestion as it reflects the nutrients excreted, can be used to predict the digestibility of dietary nutrients within an individual or group of cattle and provide an additional means for making management decisions in commercial feedlots.

Near infrared spectroscopy is a rapid alternative to traditional wet chemistry methods for predicting the organic content of feed or feces, and can be used to estimate digestibility in a much more practical and cost-effective manner. Previous NIRS research using feces has been focused on predicting dry matter and organic matter digestibility of free ranging ruminants, and thereby the growth performance of cattle on pasture (Dixon and Coates 2009; Tollensen and Schafer 2014). It has also been used to predict the nutrient content of manure for its use as an effective organic fertilizer (Chen et al. 2013). This study is the first attempt to predict both nutrient composition of feces and digestibility in feedlot cattle fed high grain diets, and to find associations to performance parameters based on these data.

The project was initiated by compiling dried ground fecal samples and their associated composition and digestibility data collected from previous and on-going metabolism and feedlot studies to develop NIRS calibrations. The calibrations that were developed were used in

subsequent studies for three purposes; first to test and validate their predictability and to expand current calibrations, second, to rapidly predict nutrient composition or digestibility values in the studies within the project, and third, to reduce the number of samples required for wet chemistry. Qualitative analysis using principle component analysis proved to be invaluable in identifying populations of feces that differed from each other, and for selecting unique samples for analysis. Calibrations for chemical composition were found to be comparable to or higher (except for crude fat) in accuracy and precision to those developed in previous publications (Dixon and Coates, 2009), and despite low accuracy for ADF and NDF digestibility, all calibrations were capable of predicting changes in digestibility, suggesting their potential for application in commercial feedlots.

In order to implement the use of NIRS in commercial feedlots, fecal sampling methods required standardization, and this project assessed differences between sampling from the rectum versus the pen floor, and the impact of sampling at different times within a day. Fecal sampling by pooling multiple samples from the pen floor was a simple and reliable method to obtain information relating to the chemical composition of feces. Aside from higher fecal DM cotent of pen floor samples compared to rectal samples, discrepancies for other constituents were minor and did not show any obvious patterns. When sampling from individual animals within a pen, spot fecal samples collected from multiple cattle fed the same diet between 0 and 4 h after first feeding resulted in samples that were representative of fecal starch concentration and most constituents. These samples also generated digestibility predictions that were comparable to that obtained using a 24 h composite fecal sample. Morning collections also resulted in the collection of sufficient amounts of feces to make the desired predictions. Although starch digestibility can be predicted directly from fecal starch, accurate predictions of dry matter, organic matter, NDF or ADF digestibility required using acid-detergent lignin (ADL) as a fecal marker. If pooled over multiple cattle, direct NIRS digestibility estimates for dry matter and starch did not differ from 24-h fecal composites using spot samples collected within 0 to 4 h after feeding. Consequently, NIRS of feces would be best suited for on-farm prediction of DM and starch digestibility in cattle housed within commercial feedlots. When cattle were fed a backgrounding diet, or were fed more than once per day, it appeared that there was less daily variation in nutrient excretion, suggesting that timing of sampling may result in less variation in fecal composition in cattle fed diets high in forage, or cattle housed in feedlots that feed multiple times per day. It should be

noted that NIRS calibrations for starch digestibility were less predictive for backgrounding diets which contain less starch and higher levels of forage.

This project also assessed the effects of dietary changes in nutrient composition of feces and digestibility, and then applied those changes to expected versus observed digestibility and performance. Digestibility calibrations were effective at demonstrating expected changes in digestion. Grain processing was found to have a greater impact on fecal nutrient excretion and digestibility in wheat- as compared to barley-fed cattle. High fecal starch and low digestibility coefficients proved to be indicators of lower feed efficiency in wheat-fed cattle for the two processing levels measured. However, for barley, the two levels of processing were not different enough to alter performance. Altering silage level also had an impact on fecal nutrient concentration and digestibility, confirmed by wet chemistry and predictions using NIRS. Day of sampling within a feeding period did not influence fecal nutrient excretion; therefore, sampling any day within the feeding period (at any BW) would generate similar predictions. My results suggested that due to variability in digestion among individual animals, predictions would be more reliable if a greater number of animals per day were sampled on multiple days. Sampling more cattle in a pen would increase the likelihood that the average of the pen would be represented. Sampling twice within the same week would also enable one to better account for the impact of variability in climate on intake and fecal output. Sampling should not take place on days where there are dramatic changes in climate (storm or excessively hot days), because such events cause short-term changes in behavior and intake.

My fourth study showed that NIRS calibrations for predicting the energy content of grain screening pellets (GSP) were capable of predicting differences in animal growth performance. In fact, NIRS was able to distinguish between low and high quality GSP, and current efforts are being made to continue to expand these calibrations and use them for on-farm application for rapidly predicting the energy content of GSP.

Finally, when compiling data from 6 commercial feedlots over one year, grain processing varied significantly among feedlots and within feedlots, as did fecal starch concentration. Processing plays a major role in increasing starch availability in the digestive tract of cattle, and reducing fecal starch losses. Strong correlations were found between processing index and fecal starch concentration, and G:F and fecal starch concentration. Average fecal starch concentration was found to be 7%, with an average processing index of 69%. When a group of cattle were

sampled between 1 and 16 times throughout their finishing period, and averaged by lot, predictive equations (blocking by feedlot) were developed to predict G:F. Fecal starch concentration was found to be an important predictor of G:F using the quadratic equations (rho=0.75, P<0.01):

$$G:F = 0.21-0.00010\times BW=0.0028\times FS-0.00032\times FS^2 \text{ for steers} \quad (7.1)$$

$$G:F = 0.19-0.00010\times BW=0.0028\times FS-0.00032\times FS^2 \text{ for heifers} \quad (7.2)$$

In order to strengthen these findings for their use in the commercial feedlot industry, further research would be beneficial. Firstly, for a feedlot operator to utilize this technology, it should be simple and practical and not require much expertise. The operator must also be suited to the technology, as it would be most useful to a feedlot where the diet ingredients and proportions are not altered often. Secondly, real time monitoring of animal performance and digestibility is desired, therefore instead of using dried ground feces, fresh, intact feces, or rapid and simple drying procedures could be employed. There are difficulties associated with NIRS scanning of wet feces, primarily because of their high moisture content (75 to 88%). Water creates large moisture peaks in the spectra that masks peaks due to other compounds. Additionally, the pathlength in NIR reflectance spectroscopy is not long enough to measure all the substances contained within intact-large volume samples. However, large volumes are required to generate representative samples. One option would be to use a microwave to dry the feces, followed by grinding. Grinding samples acts to reduce the particle size and make the sample more homogenous. This becomes important when measuring specific compounds within a representative sample. For example, fecal starch, which is usually contained within whole or broken grain kernels, will not be distributed evenly throughout a large intact sample that is not ground. My study was successful in demonstrating that NIRS of 1 mm dried ground samples was capable of generating predictions of the composition of feces with only slightly less precision and accuracy than wet chemistry for most constituents. The next step in this research area could be to determine how much accuracy is lost using wet, intact, microwave dried, and coarsely ground feces (i.e., using a coffee grinder).

Another consideration before commercial feedlot application would be to strengthen the current calibrations for predicting digestibility. It would be my recommendation to focus on

starch digestibility, due to the obvious role of starch in feedlot diets, and GE digestibility, because of its relationship to NEg, a value that applies to estimations of ADG for a specific DMI based on NRC equations. By including more samples from on-going digestibility studies, a greater range in digestibility values could be incorporated into current calibrations, increasing robustness. I found that starch digestion was the most accurate when starch digestibility was high because of the low coefficients of variation close to the y intercept that are typically observed for finishing feedlot cattle fed high grain diets. By incorporating more samples with lower starch digestibility, calibrations that are more accurate at predicting the extent to which starch digestion has been compromised by inadequate grain processing could be developed. Our current NIRS calibrations for starch digestibility did not prove to be as useful as fecal starch concentration. It may also be valuable to develop NIRS calibrations using feces to predict NEg of the diet using calculations based on actual animal performance. Because ADG is a function of energy intake, calibrations that use energy intake (DMI* ME content of the diet) may have been more successful rather than directly attributing fecal composition to ADG and G:F. In feedlot settings, the DMI of each pen can be estimated on a daily basis, therefore incorporating DMI into prediction equations may be of value, but once again, sampling frequency may need to increase to account for the great variability of intake of animals within a pen. Additionally, we can not assume that the average intake of a pen is the same as that for an individual animal, and studies have shown considerable variability among animals (Gibb et al. 1998; Schwartzkopf-Genswein et al. 2004).

Establishment of an online NIRS network that could be accessed by producers or nutritionists would also help to expand the use of this technology. Because factors such as season, climate, sample handling, and composition have such a large effect on NIR spectra, it may be most suitable to have NIRS networks that consist of a small region with similar climate, common feed ingredients, and close proximity so that collection and sample handling procedures can be standardized and monitored. In order to fund such a network, it may be required that feedlot producers pay a fee for accessing up-to-date calibrations and to be provided with technical support. The fee would include information and updated calibrations that are being validated and expanded continuously by a research group. A nutritionist with NIR knowledge could work closely with both the feedlot and the research group.

As mentioned numerous times within this work, when developing NIRS calibrations we are constantly faced with variation within analytical lab procedures. Near infrared spectroscopy can only be as accurate as the data derived from wet chemistry, therefore poor accuracy and precision in wet chemistry leads to less accurate NIRS calibrations. It should be mandatory for the purpose of NIRS calibration development that one laboratory be used, or selected labs who have had their analytical capabilities tested in round table evaluations be employed. Additionally, lab analysis should always be performed in replicates, and the C.V. value should be included with the final result. Only C.V. values deemed acceptable (decided by NIRS technicians) for each constituent should be used when developing calibrations. The purpose of the C.V. is to help make decisions about rejecting bad data. For simplicity, the first approach could be to focus only on fecal starch concentration, a nutrient that is known to have a high level of predictability (>0.91) using NIRS. Operators of the NIRS network would validate new data as it was submitted to ensure that it was encompassed within the calibration set. Outliers could be flagged and analyzed using wet chemistry, thereby expanding the calibration data set.

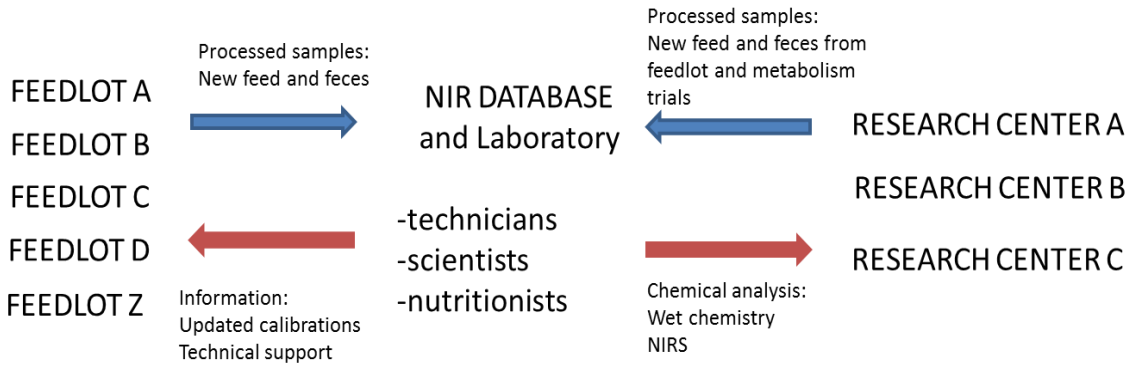


Figure 8.1. Schematic diagram showing the NIR network with cooperating feedlots and research centers where the NIR database and laboratory are responsible for all wet chemistry related to calibration development and validations so no external laboratory is required.

Finally, it may be possible that our NIRS calibrations could be used to identify acidotic animals using NIRS of their feces rapidly upon onset. Currently we associate runny feces (low DM) with acidotic cattle, but we do not know other compositional characteristics of these fecal samples. Near infrared spectroscopy has been applied to monitor fermentation broth from

anaerobic digestion processes (Spanjers et al. 2006) and the anaerobic digestion of a mixture of cellulose, albumin, and minerals (Nordberg et al. 2000). When manure was spiked with corn silage, NIRS could be satisfactorily applied for monitoring the chemical concentration of volatile fatty acids including acetic, propionic, butanoic, iso-butanoic, valeric, and iso-valeric acids produced from anaerobic digestion (Lomborg et al. 2009). Because digestion proceeds too quickly in acidotic cattle, it is likely that fecal starch concentration will be lower than normal, and lactic acid concentrations may be high. Once these characteristics are identified, NIRS can be used as a method to access the degree of acidosis. Feedlot studies using feed intake monitoring (e.g., Grow safe technology) and rumen pH meters to obtain data for individual animals would be required for validating such studies.

9.0 GENERAL CONCLUSION

This research has shown that fecal NIRS has many advantages for monitoring nutrient and energy within the feces of feedlot cattle. Changes in the composition of the feces can also be related to digestibility of nutreints and energy within the diet, which are related to ADG and G:F. Once calibrations are strengthened and larger datasets are tested, fecal NIRS could be implemented for routine use at commerical feedlots whose goal is to use precision feeding techniques to reduce feed costs, reduce nutrient excretion, and improve feed efficiency without growth performance being compromised.

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