APPLICATION OF FECAL NEAR INFRARED REFLECTANCE SPECTROSCOPY AND N-ALKANE LABELED SUPPLEMENTATION TECHNIQUES TO PREDICT VOLUNTARY INTAKE IN BEEF CATTLE

A Thesis

by

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Submitted to the Office of Graduate and Professional Studies of
Texas A&M University
in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

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August 2014

Major Subject: Animal Science

ABSTRACT

The objectives of this study were to evaluate the use of fecal NIRS profiling and the n-alkane labeled supplementation method for the prediction of voluntary intake in beef cattle for the identification of animals divergent in feed efficiency. Additionally, the use of fecal NIRS profiling technology was evaluated for the prediction of diet characteristics.

To examine the use of fecal NIRS profiling to estimate diet quality and dry matter intake (DMI), fecal samples and phenotype data were collected from 14 beef cattle trials that utilized Calan-gate feeders (American Calan, Northwood, NH) or electronic GrowSafe™ feedbunks (GrowSafe™ DAQ 4000E; GrowSafe™ system Ltd., Airdire, AB, Canada) to measure individual animal feed intake.

The coefficient of determination for calibration (R^2_c) and cross-validation (R^2_{cv}) of combined trial equations to predict diet characteristics were least accurate for the prediction of NDF using composite fecal samples ($R^2_c = 0.85$; $R^2_{cv} = 0.82$), and most accurate for the prediction of CP using individual-day fecal samples ($R^2_c = 0.94$; $R^2_{cv} = 0.91$). For the prediction of DMI, R^2_c and R^2_{cv} ranged from 0.49 and 0.42 for the prediction of average-trial DMI using individual-day fecal samples to 0.76 and 0.73 for the prediction of fecal-collection-period DMI using composite fecal samples. While the values obtained for the prediction of DMI were inferior to those obtained for the prediction of diet quality or digestibility, fecal NIRS prediction equations for DMI were

successful in predicting the mean DMI of groups as well as predicting individual-animal DMI for the evaluation of divergent RFI groups.

To evaluate the use of an n-alkane labeled supplement for the prediction of intake, 24 mid-gestation heifers, previously identified as having divergent postweaning RFI, were fed chopped sorghum hay and an n-alkane labeled supplement, ad libitum in electronic GrowSafe™ feedbunks (GrowSafe™ DAQ 4000E; GrowSafe™ system Ltd., Airdire, AB, Canada). In this study, accurate intake predictions were not obtained as 6-d forage intake was overestimated by 73.0% when using C_{31}:C_{32} alkane pairs and by 38.9% when using C_{33}:C_{32} alkane pairs. However, inaccurate measures of supplement and forage intake by the GrowSafe™ system, as well as large between-animal variation in supplement intake, feeding behavior, and digestibility may have greatly influenced the accuracy of these results.

Results from this study indicate that fecal NIRS profiling can be used to predict dietary characteristics and DMI for the identification of animals divergent in feed efficiency. Conversely, the n-alkane labeled supplement technique was inaccurate in predicting forage intakes in this trial.

DEDICATION

I would like to dedicate this thesis to my family. To my loving father who first introduced me to the agriculture industry, and provided me with many opportunities that have influenced where I am today. Though he is no longer with us physically, he has continued to watch over me from heaven and has given me the strength and courage necessary to succeed. To my mother who has provided unconditional love and support and has pushed me my entire life to go above and beyond to reach my goals. To my grandparents who have been there for me through the good and bad times. They have been amazing role models and have helped me to pursue my dreams.

ACKNOWLEDGEMENTS

I would like to thank my committee chair, Dr. Gordon Carstens, as well as my committee members, Dr. Forbes and Dr. Jason Sawyer, for their continued support and guidance throughout the course of this research. You are all responsible for increasing my knowledge and providing me with unique experiences that will remain with me throughout my career.

I would also like to thank my coworkers and friends that have helped me with my research even when things got messy. Also for their continued entertainment that was always available to cheer me up after a long day.

Finally, I would like to thank my husband Cameron. You are always there to listen to my daily highs and lows, and have been so forgiving on those not so good days. I am so lucky to have such an amazing person in my life that I can always turn to for love and support.

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CHAPTER I

INTRODUCTION AND LITERATURE REVIEW

Introduction

The agriculture industry is currently challenged by rapidly growing per capita incomes and increasing populations that have begun encroaching upon agricultural land and resources (Herrero et al., 2009). By the year 2050, demand for animal protein sources is expected to nearly double, owing to a global population expected to reach 9.5 billion people (US Census Bureau, 2008), and increased per capita incomes driving consumers demand to purchase high-quality food products (Rosegrant et al., 2009). The livestock industries will need to adapt by increasing production in the face of rising input costs as competition for land, energy, and water supplies is also expected to double if not triple by 2050. Furthermore, growing societal concerns about the environment will challenge future livestock production as the US beef system's sustainability has been questioned by social and political agendas opposed to animal agriculture (Nierenberg, 2005; Capper et al., 2011). Consequently, agricultural researchers and producers will be faced with the challenge of developing and implementing new technologies that will allow for more economically and environmentally sustainable beef production systems.

In order to improve the efficiency of beef cattle production systems, input costs must be reduced per unit of output, since producers have minimal control of market prices for their products (Herd et al., 2003). Given that feed accounts for the single

largest variable input cost of production, research regarding the selection for efficiency of feed utilization could have profound impacts on increasing the overall efficiency of beef production. Research in this area will also have a favorable impact on environmental sustainability as efficient cattle have reduced feed intakes while maintaining production, resulting in less manure and methane emissions per unit of product produced (Nkrumah et al., 2004; Hafla et al., 2012; Basarab et al., 2012; Basarab et al., 2013). Accordingly, a great deal of research has recently been conducted to determine the appropriate methods to select beef cattle with favorable genotypes for feed efficiency.

Historically though, this type of research has concentrated on young growing cattle, and has often failed to emphasize the importance of the cow herd, even though maintenance requirements for the cow herd can account for approximately 50% of the total beef production system (Arthur et al., 2004; Montano-Bermudez et al., 1990). It has also been estimated that the cow herd is responsible for about 80% of GHG emissions from the production of beef in North America (Beauchemin et al., 2010). This large percentage of beef GHG emissions attributed to the cow herd is due to the increased proportion of total feed consumed by cows within a calf-to-beef system, and the increased proportion of roughage in their diets compared to feedlot progeny (Basarab et al., 2013; Capper, 2011).

Currently, favorable selection for ratio-based traits (F:G) will result in cows with greater mature body size and maintenance energy requirements due to their strong association with growth traits (Herd and Bishop, 2000; Arthur et al., 2001). In order to

reduce feed input cost and GHG emissions, selection for lower maintenance energy requirements and(or) improved efficiency of feed utilization is necessary. Residual feed intake (RFI) is a trait that accounts for the variation between individual animals' feed consumption, independent of growth and production, and may be a more appropriate selection trait for the breeding herd (Hafla et al., 2012; Herd and Arthur, 2009). Previous studies have reported that low-RFI growing calves consumed 15 to 21% less feed (Herd et al., 2002; Lancaster et al., 2009) and produce 24 to 28% less methane (Nkrumah et al., 2006; Hegarty et al., 2007) compared to high-RFI calves with no impact on performance.

Research reporting on favorable selection for RFI has been limited, however, by the absence of an affordable method to accurately measure individual animal intake, especially for grazing cattle or confined cattle on high roughage diets. While there are currently multiple techniques used to estimate DMI, none accurately account for individual animal variation without the use of expensive equipment or complex sampling schemes. An estimation of DMI is necessary to evaluate the nutritive value of feed and the nutritional status of the animal, and therefore for the reduction of GHG emissions by favorable selection for feed efficiency (Ferreira et al., 2004). For these reasons, the industry must recognize the implications for research concerning individual animal intake determination methods for grazing or confined animals fed high roughage diets.

Methodology for reducing methane emissions

The accumulation of GHG, especially carbon dioxide (CO_2), methane (CH_4), and nitrous oxide (N_2O), has become an increasing concern due to the global warming phenomenon (Boadi et al. 2004). International organizations such as the Intergovernmental Panel on Climate Change (IPCC) have determined the primary sources of GHG emissions, and are working to develop and implement mitigation strategies to reduce GHG emissions. Based on recent reports, the livestock industry contributes 18% of total global GHG (FAO, 2006), 9% of total CO_2, 17-37% of total CH_4, and 65% of total N_2O emissions (Steinfeld and Wassenaar, 2007; Lassey, 2008). While an in-depth scientific review by Pitesky et al. (2009) found significant flaws in the methods used to generate previously reported GHG emission data, many continue to use the FAO (2006) report to support claims that the animal agricultural industry should be curtailed in order to decrease GHG emissions (Steinfeld et al. 2006; Deutsch, 2007). Regardless, the livestock sector is facing increasing pressures to become more environmentally sustainable, and governments have discussed implementing policies such as carbon taxes to reduce GHG emissions from animal agriculture. Therefore, the livestock industry will need to develop and implement new strategies to evaluate and reduce future GHG emissions.

Within the beef industry, the cow-calf herd is responsible for 80% of total GHG emissions, with 53% resulting from enteric CH_4, 20% from manure N_2O, 3% from energy use of CO_2, 2% from manure CH_4, and 2% from soil N_2O (Beauchemin et al.,

2010; Basarab et al., 2013). Based on these proportions, enteric CH_4 from the cow-calf herd represents the largest source of GHG emissions from the beef industry. Within the cow-calf system, 79% of enteric CH_4 produced is accounted for by the cow herd, with bulls and calves representing only 5%. Given that the largest source of GHG emissions within the beef production cycle emanates from the cow herd in the form of enteric CH_4, increased emphasis should be placed on mitigation strategies aiming to reduce enteric CH_4 emissions from the cow herd.

During ruminal fermentation, excess metabolic H_2 is produced from microbial metabolism, and oxidized primarily by methanogens during the regeneration of NAD^+, resulting in the reduction of CO_2 to CH_4 (McAllister and Newbold, 2008). If H_2 accumulates in the rumen, the reoxidation of NADH to NAD^+ will be restricted, thus inhibiting the degradation of dietary carbohydrates, production of ATP, and subsequent microbial growth (Cottle et al., 2011). Therefore, methods to reduce enteric CH_4 emissions must involve direct inhibition of methanogens, or indirect alterations that affect the availability of substrates for methanogenesis (Hook et al., 2010), and may include diet manipulation, rumen manipulation, or animal manipulation strategies (Boadi et al., 2004; Herrero et al., 2009; Eckard et al., 2010).

Effect of diet

Factors that affect the availability of substrates for methanogenesis, and therefore the production of CH_4 may include factors such as intake levels relative to maintenance (Hunter and Neithe, 2009), proportion of grain in the diets (Johnson and Johnson, 1995;

DeRamus et al., 2003), quality of forage consumed (Benchaar et al., 2001), or any other factors altering the balance of methanogenic and other species present in the rumen (Martin et al., 2010). Sauvant and Giger-Reverdin (2007) examined diets with varying proportions of concentrates, and found a reduction in CH_4 production with increasing proportions of concentrate. Alterations in methanogenesis have also been found in response to dietary starch levels (Van Kessel and Russell, 1996), as starch components promote propionate formation, decreasing available H_2, thereby reducing CH_4 production. Additionally, reducing dietary fiber levels may result in decreased CH_4 production, as the digestion of cellulose produces 3 times more CH_4 than digestion of hemicellulose (Moe and Tyrrell, 1979). While diet manipulation strategies have strong implications for the reduction of CH_4 emissions in cattle fed mixed rations, its' application may be limited within grazing cow herds, as feeding more grain is not suitable on pasture, and improving quality of forage available is not always feasible.

Rumen manipulation

Direct inhibition of methanogens can be accomplished through antibiotics, feed additives, vaccinations, or through the elimination of protozoa present in the rumen (Cottle et al., 2011). Thornton and Owens (1981) examined the effect of monensin, a commonly used ionophore, on methanogenesis in growing steers fed low, medium, or high roughage diets. Monensin increased the proportion of propionate produced, thereby decreasing acetate and associated H_2 production available for methanogenesis, resulting in 16 and 24% reductions in methane for cattle fed low and high roughage diets,

respectively. Monensin has also been shown to decrease methane emissions by a reduction in voluntary DMI (Goodrich et al. 1984), and increased selection for succinate-forming *Bacteroides* and *S. ruminatium* (Chen and Wolin, 1979). However, the use of monensin has failed to reduce CH_4 production in grazing cattle (Grainger et al., 2008; Grainger et al., 2010), and may consequently have limited use in reducing enteric CH_4 emissions from the cow herd.

Early research, investigating the use of feed additives for the purpose of reducing CH_4 emissions, focused primarily on the use of synthetic chemicals such as halogens or nitrate (Bauchop, 1967; Clapperton, 1974; Allison et al., 1981; Allison and Reddy, 1984). Pioneering research by Bauchop (1967) discovered the use of halogenated CH_4 analogues for the direct inhibition of CH_4 production. Since, chloral hydrate (Van Nevel et al., 1996), 2-chloro-6-(trichloromethyl)pyridine (Salvas and Taylor, 1980), bromoethanesulfonic acid (BES) (Balch and Wolfe, 1979), chloro-substitued benzo-1,3-dioxins (Stanier and Davies, 1981), and dimethyldiphenyliodonium chloride (Chalupa, 1980) have been found to also inhibit CH_4 production. However, the use of these compounds in commercial practices may not be practical, as researchers have found accumulations of hydrogen (Demeyer and Van Nevel, 1975), depression of substrate degradation (Marty and Demeyer, 1973; Mathers and Miller, 1982), depression of intake (Chalupa, 1980), and toxicity issues (Lanigan et al., 1978) related to the use of these compounds. Nitrate additives may provide a better alternative, as they have been found to reduce CH_4 production (Allison and Reddy 1984; Martin and Macy, 1985; Guo et al., 2009) without altering feed intake, digestibility, or growth rate (Le Thi Ngoc Huyen et

al., 2010). However, their future application in commercial practices will rely on the presence of new cost incentives for the reduction of CH_4 emissions as nitrate-N is currently more expensive than urea-N.

More recently natural compounds such as proanthocyanidins (Carulla et al., 2005; Tiemann et al., 2008), saturated fatty acids (Odongo et al., 2007), and oils and fats (Blaxter and Czerkawski, 1996; Dohme et al., 2000; Jordan et al., 2006; Beauchemin et al., 2008; Moate et al., 2010) have been investigated to determine their role in the reduction of CH_4 emissions. Carulla et al. (2005) and Tiemann et al. (2008) assessed the effects of condensed tannin supplementation, and found a 13% reduction in CH_4 emissions, with no effects on whole-animal nitrogen or energy retention. While these results appear promising, considerations must be made as increasing the concentration of tannins in legumes may have negative effects on DMI and subsequent production (Cottle et al., 2011). The inclusion of oils and fats in the diet has been found to reduce CH_4 production by 5.6 to 18% (Jordan et al., 2006; Beauchemin et al., 2008; Moate et al., 2010) in cattle. Given that intake is maintained and performance is enhanced during oil and fat supplementation, this method may prove to have practical application in commercial practices.

Wedlock et al. (2010) set out to provide proof that harnessing the immune system of ruminants may provide a viable approach for the mitigation of GHG emissions from agriculture. In this study, evidence was provided to indicate that the antigenic fractions of methanogen microbes are immunogenic in sheep, and that providing antibodies inhibits the production of CH_4. These results suggest that development of vaccines

targeting key antigens crucial for methanogenesis may have implications for reducing CH_4 production in livestock. Successful vaccination formulations have yet to be produced (Wright et al., 2004; Williams et al., 2009), and therefore, reduction of CH_4 by vaccinations is not applicable to current production systems.

Kreuzer et al. (1986) evaluated adult wethers, and reported that defaunation reduced CH_4 emissions and increased the energetic efficiency of feed. Hegarty (1999) reviewed the protozoa-methanogen relationship, and concluded that the reduction of CH_4 emissions by protozoa-free rumen may be due to a reduction in ruminal dry matter digestion, a decreased methanogen population, an altered pattern of volatile fatty acid production and hydrogen availability, or an increased partial pressure of oxygen in the rumen. While others have investigated the use of defaunation to reduce CH_4 emissions (Stumm et al., 1982; Newbold et al., 1995; Eugene et al., 2004; Hegarty 2004), the effectiveness of defaunation strategies to reduce CH_4 emissions in commercial situations is still very uncertain.

Interest regarding the use of DFM in beef production has grown in recent years due to the growing public concerns regarding the use of antibiotics and other growth stimulants. Direct fed microbials are live, naturally occurring microorganisms (Yoon and Stern, 1995), and include viable cultures of fungi, bacteria, and yeast. Research involving DFM has led to implications for their use in neonatal and stressed calves (Abu-Tarboush et al., 1996; Beeman, 1985), feedlot cattle (Swinney-Floyd et al., 1999; Ohya et al., 2000), and mature cows (Jaquette et al., 1988; Ware et al., 1988; Gomex-Basauri et al., 2001). Gomez-Basauri et al. (2001) evaluated the effect of lactic acid

producing DFM on DMI and milk yield in lactating dairy cows. The authors did not directly investigate the use of DFM to mitigate CH_4 emissions, but they did report increased efficiencies (kg milk/kg DMI) for cows fed DFM. Other research has found similar results (Komari et al., 1999; De Ondarza et al., 2009), and suggested that DFM increase efficiency by increasing the production of propionate, which decreases the amount of available H_2 for methanogenesis. While few studies have investigated the effect of DFM on CH_4 emissions, Newbold and Rode (2006) suggested that the development of a commercial yeast product may be applicable for the reduction of CH_4. Further research is necessary, but proposed mechanisms indicate the potential use of DFM for reduction of CH_4 emissions in beef and dairy cows.

Animal manipulation

Variation in CH_4 production between animals has been substantially investigated with between-animal variations ranging from 11.5% (Lassey et al., 1997) to 25% (McNaughton et al., 2005). Research regarding the selection for low CH_4 producing animals has therefore been examined (Pinares-Patino et al., 2003; Goopy and Hegarty, 2004). However, the results to date have been inconsistent, possibly due to high within-animal variation in daily CH_4 emissions (Vlaming et al., 2008), and long term effects on animal production are greatly unknown. Genetic selection based solely on the production of CH_4 also serves to be impractical, due to difficulties and high costs associated with measuring individual animal CH_4 production for extended periods (Cottle et al., 2011). A more practical approach may involve the indirect reduction of CH_4 emissions through

animal breeding and genetic selection for feed efficiency as it is permanent, cumulative, and compatible with existing breeding objectives (Alford et al., 2006; Eckard et al., 2010).

Traditionally, genetic selection for feed efficiency has focused on favorable selection for feed to gain ratios (F:G) or feed conversion ratios (FCR). Favorable selection for ratio-based traits such as F:G, will result in cows with greater mature body size and maintenance energy requirements due to their strong association with growth traits (Herd and Bishop, 2000; Arthur et al., 2001). Increases in maintenance energy requirements may increase intake, consequently increasing CH_4 production, as DMI and CH_4 production are highly correlated (Shibata and Terada, 2010). Therefore, reductions in CH_4 emissions may not be achieved through genetic selection for F:G. Residual feed intake (RFI) is a measure that accounts for the variation between individual animals' feed consumption, independent of growth and production, and may be a more appropriate selection trait for the breeding herd (Herd and Arthur, 2009; Hafla et al., 2012).

Koch et al. (1963) was the first to propose RFI after recognizing that feed could be adjusted based on BW and weight gained. They effectively partitioned feed into 2 proportions, an expected portion based on body size and a given level of production, and a residual portion, the amount that an individual animal actually consumed, above or below that of what they were predicted to have consumed. This residual portion allows for the identification of animals that consume less feed than expected (low RFI) or more feed than expected (high RFI), independent of body size and growth (Herd and Aurthur,

2009). Therefore, RFI is a feed efficiency trait that can account for between-animal variance in feed intake unexplained by variations in BW and ADG (Arthur et al., 2001).

Herd et al. (2002) and Okine et al. (2003) reviewed the use of favorable RFI selection on CH_4 production and hypothesized that low RFI animals would produce decreased levels of CH_4 compared to high RFI animals, given that low RFI animals have reduced DMI with similar levels of production, resulting in increased efficiency of feed utilization, compared to high RFI animals. More recently, Nkrumah et al. (2006) evaluated feedlot steers to determine the relationship between feed efficiency, performance, and feeding behavior with metabolic rate, digestion, and energy partitioning in beef cattle ranked by RFI. The authors found CH_4 production of low RFI animals to be 28 and 24% less compared to high- and medium-RFI animals, respectively. Similar results have also been reported by Hegarty et al. (2007) and Jones et al. (2011) with CH_4 reductions of 25 and 26% for low RFI animals compared to high RFI animals.

Arthur and Herd (2008) evaluated low- and high-RFI cattle that had been divergently selected for 5 years (approximately 2 generations). After five years of divergent selection, the direct response was -0.54 ± 0.18 kg/d in the low RFI-line and 0.70 ± 0.17 kg/d in the high RFI-line (Arthur et al., 2001). Selection for low RFI was accompanied by corresponding reduction in daily feed intake (9.4 ± 0.3 vs. 10.6 ± 0.3 kg/d) and reduced (improved) F:G (6.6 ± 0.2 vs. 7.8 ± 0.2 kg/kg; Herd et al., 2003). Richardson et al. (1998) reported on Angus and Angus-crossbred steers that were born following a single generation of divergent selection for postweaning RFI. The authors

reported a reduction in daily intakes (9.2 ± 0.2 vs. 9.8 ± 0.2 kg/d) and F:G (7.0 ± 0.2 vs. 7.6 ± 0.2 kg/kg) with no significant differences found between low and high RFI steers for IBW, FBW, or ADG. These results indicate that the selection for low RFI post-weaning may lead to progeny that consume less feed and still maintain similar performance compared to the cattle selected for high RFI (Arthur and Herd, 2008; Arthur et al., 1999). Since RFI has displayed moderate heritability (0.39 to 0.45; Arthur et al., 2001; Schenkel et al., 2004; Berry and Crowley, 2012), the establishment of divergently selected lines for low and high RFI may be applicable (Hegarty et al., 2007), aiding in the reduction of CH_4 emissions by livestock.

In a recent estimate, Alford et al. (2006) projected that for an individual beef cow herd, selection for reduced RFI is expected to reduce GHG emissions by 15.9% after 25 years. The authors concluded that favorable selection for RFI will result in substantial and lasting CH_4 abatement, mainly as a consequence of its application as a breeding objective for grazing beef herds. Genetic selection for favorable RFI will also result in cows with improved forage utilization, consequently increasing the overall efficiency of beef production, which will be necessary for meeting future livestock product demands.

Long term impacts on cow productivity and reproductive performance from selection based on RFI is still not fully understood, and may limit commercial acceptance without further research. Research reporting on favorable selection for RFI has been limited however, by the absence of an accurate and affordable method for determining individual animal intake, especially for grazing or confined cattle on high roughage diets. While there are currently multiple techniques used to estimate DMI,

none accurately account for individual animal variation without the use of expensive equipment or complex sampling schemes. An estimation of DMI is necessary to evaluate the nutritive value of feed and the nutritional status of the animal, and therefore for the reduction of GHG emissions by favorable selection for feed efficiency (Ferreira et al., 2004).

Summary

Current mitigation strategies involving the use of rumen manipulation, when applicable can provide substantial benefits in the reduction of enteric CH_4 emissions in cattle. Rumen manipulation techniques may be limited however, in their application for cow herds as feeding additives, supplements, and high quality forages is not always feasible for grazing animals (Basarab et al., 2013). The use of animal manipulation strategies, specifically genetic selection for favorable RFI, may prove to have larger implications in grazing cow herds as results are permanent, cumulative, and compatible with existing breeding objectives. However, the impact of selection for RFI on economically relevant traits is still not fully understood, and further research and application may be limited by the absence of an accurate and affordable method for determining individual animal intake.

Methodology for predicting individual-animal consumption

For over 50 years, researchers have been attempting to develop techniques which accurately estimate DMI of grazing animals (Schneider et al., 1955; Langlands, 1975; Holloway et al., 1981). Research in this area has a long history since quantifying DMI is

necessary for further improvements in management and efficiency of cattle production. For confined animals, accurate and reliable measures of direct individual animal intake can be achieved through individual pen feeding or the use of specialized feeding systems such as Calan-gate feeders or the GrowSafe^{TM} system. While these methods are unable to measure the intake of animals on pasture, they have been used to validate forage intake estimation techniques, and are considered to be the most accurate methods for measuring intake. The inability to measure intake of grazing animals has limited research, and been characterized as being inaccurate, expensive, laborious, and highly sensitive to bias by numerous researchers (Langlands, 1975; Holloway et al., 1981; Ungar, 1996; Cappers, 2011). Current techniques for estimating DMI of grazing animals can include measurements of herbage disappearance, use of prediction models, use of internal and external markers, and use of fecal near infrared reflectance spectroscopy (fecal NIRS; Macoon et al., 2003; Undi et al., 2008). All methods are estimates of intake with variable degrees of error, and each provides unique advantages and disadvantages (Macoon et al., 2003).

Herbage disappearance

An early technique developed to measure DMI of grazing animals was based on herbage disappearance. This method requires accurately and representatively measuring herbage mass, the total mass of herbage per unit area of ground, before and after grazing (Meijs et al., 1982; Burns et al., 1994; Undi et al., 2008). The difference between the two measurements gives an estimate of herbage disappearance. To calculate herbage

consumption, a correction for herbage growth is applied to the herbage disappearance estimates to account for herbage growth during the grazing period (Walters and Evans, 1979). For estimating herbage intake per animal per day, the herbage consumption per unit area is divided by the number of animal days per unit (Meijs et al., 1982). Individual animal intake is therefore calculated using the following equation:

((Herbage mass before grazing – herbage mass after grazing) + (herbage growth correction)) ÷ (number of animals x number of days)

While this method can give estimates of animal intake, individual differences in animal intake can only be calculated for animals kept on individual plots. Due to the increased labor requirements and lack of normal grazing patterns associated with keeping animals on individual plots, studies estimating intake using the herbage disappearance technique typically involve the use of groups instead of individual animals. Therefore, a huge limitation in using this method for estimating intake is the inability to calculate individual animal intake variations. Other limitations resulting from intake estimations based on the herbage mass technique include intense sampling requirements, inaccurate estimations of herbage growth during the period, increased ability of animals to selectively graze, and disappearance of forage from trampling and other losses not associated with consumption (Smit et al., 2005; Undi et al., 2008). Therefore, accurate measurements of herbage intake are best achieved when using short grazing periods on pastures with high grazing pressures (Walters and Evans 1979; Meijs et al., 1982). Under these conditions, the amount of herbage growth will make up only a

small proportion of total herbage consumption, reducing the inaccuracies associated with estimating herbage growth (Meijs et al., 1982).

When average intake estimations for groups of animals are acceptable; the herbage mass technique for estimating intake can provide advantages such as providing information on the herbage allowance, the efficiency of grazing, and the pasture quality of a particular area (Meijs et al., 1982; Meyer et al., 2008). Meyer et al. (2008) used the herbage disappearance technique to estimate average forage intake of grazing beef cows that were identified to have divergent phenotypes of RFI as growing heifers. Although not found to be statistically significant, the authors reported a 21% reduction of forage intake by low RFI animals compared to high RFI animals during mid-late gestation with no impact on gain or BCS. These results agree with Hafla (2012) who found a 17.3% reduction of intake by low RFI cows compared to high RFI cows, using direct measures of intake. These results suggest that the herbage disappearance technique may be an acceptable method for estimating average intake of groups of defined animals in order to evaluate differences in feed. The herbage disappearance technique also offers a quick estimate of intake without the need for elaborate laboratory analysis (Undi et al., 2008). Thus, the herbage disappearance technique may be advantageous when estimating intake of groups of animals in order to provide information on pasture quality, pasture efficiency, or herbage allowance when time and equipment limitations are present.

Prediction models

In the early 1960s, Conrad et al. (1964) determined DMI and digestibility in 114 trials with lactating dairy cows. Using a multiple regression analysis they reported that digestibility, fecal dry matter per 1,000 lb body weight per day, and BW accounted for 99.5% of the variation in feed intake for animals consuming roughage diets that were 52 to 66% digestible. Since their early research, models spanning from simple regression equations to interacting sets of differential equations, have been used to predict intake in cattle (Conrad et al., 1964; Oltjen, 1986; Minson and McDonald, 1987; Macoon et al., 2003; Smit et al., 2005). This method of estimating intake involves the use of predictive models, and relies on the presence of repeatable correlations between predictor variables and DMI. To insure that developed prediction models are applicable to the industry, researchers have focused predictor variables on animal factors that can be easily measured or quantified (NRC, 2001; Halachmi et al., 2004). Common predictor variables may include elements relating to animal performance, forage composition, fecal chemistry, or the environment.

The use of predictive models may be advantageous for examining intake responses over an entire period, or for the development of efficient feeding management strategies (Macoon et al., 2003). Their simplicity and ease of application offer up unique advantages for estimating intake of grazing cattle. The limitations of predictive models relate to their inability to accurately quantify intake of individual animals. While these models may provide reasonable estimates of intake for populations of cattle, their ability

to measure between-animal variance is limited (Reeves et al., 1996). Predictive models also fail to accurately account for many of the physiological, environmental, and management factors that affect intake, limiting further insight into understanding the basic biology of the animal (Burns et al., 1994; NRC, 1996; Undi et al., 2008).

Internal and external markers

In the late 1980s, researchers began developing a new technique for estimating DMI involving the use of long-chain hydrocarbons in plant cuticular wax, especially n-alkanes, as fecal markers (Mayes et al. 1986; Dove and Mayes, 1991; Dillon, 1993; Smit et al., 2005; Keli et al 2008). The alkanes occurring naturally in the plant cuticular wax, odd-chained alkanes, can be used along with synthetic, even-chained alkanes to estimate DMI as they are both non-toxic and primarily indigestible. Early studies demonstrated the incomplete recovery of n-alkane in the feces, and led to the concurrent use of both odd- and even-chained alkanes in order to provide unbiased estimations of intake (Mayes and Lamb, 1984; Mayes et al., 1986). A known amount of even-chained, synthetic alkane was administered to the animal through an intra-ruminal controlled-release device (CRD; Berry et al., 2000; Dove et al., 2002; Boland et al., 2012), by daily dosing (Mann and Stewart, 2003), or by providing a labeled concentrate supplement (Unal and Garnsworthy, 1999; Charmley and Dove, 2007). Intake is then estimated from the ratio of even-chained, administered alkane to the naturally occurring, odd-chain alkane. Dry matter intake can be calculated with the following equation (Undi et al., 2008):

$$DMI(kg\ d^{-1}) = F_i/F_j \times D_j/(H_i\text{-}F_i/F_j \times H_j)$$

where H_i and F_i are the herbage and fecal concentrations of an odd-chain n-alkane; and H_j and F_j are herbage and fecal concentrations of an even-chained alkane, D_j is the amount of dosed even-chain alkane released per day.

The use of both odd- and even-chained alkanes allows for the simultaneous computation of digestibility (odd-chain n-alkanes) and fecal output (even-chained n-alkanes), which will remove errors associated with the incomplete fecal recovery of n-alkanes reported in earlier studies (Bezabih et al., 2012).

Accurate intake estimates using the n-alkane method have been reported (Table 1.1), but results are based largely on the fecal recovery rates for adjacent alkanes, dosing precision, sampling of feces, and sampling of forage. Bezabih et al. (2012) measured fecal recovery rates of n-alknaes and evaluated the use of molasses-based alkane boluses to estimate feed intake and digestibility in bulls. They fed 4 experimental diets and found that the diet type affected the fecal recovery rate of odd-chain n-alkanes. Observed error in intake estimations increased as differences in recovery rates of adjacent n-alkanes increased ($R^2 = 0.75$, $P < 0.01$).

Table 1.1. Summary of studies that evaluated the use of the n-alkane marker method for the prediction of intake in cattle

Diet	Dosing procedures	Intake	N	Mean		Diff.[1]	R^2	References
				Observed	Estimated			
Estimated by C_{33}:C_{32} ratio								
Forage + supplement	CRC	kg DM d^{-1}	4	12.7	12.7	-0.20	0.72	Berry et al. (2000)
Lowland grass	CRC	kg DM d^{-1}	6	16.0	15.6	-2.50	---	Estermann et al. (2001)
Kikuyu grass	Xantham gum	kg DM d^{-1}	9	6.28	6.21	-1.11	---	Man and Stewart (2003)
Meadow hay	CRC	kg DM d^{-1}	4	6.70	7.92	+18.3	---	Ferreira et al. (2004)
Fresh forage	CRC	kg DM d^{-1}	6	8.32	8.03	-3.49	0.54	Molina et al. (2004)
Chopped hay	CRD	kg DM d^{-1}	6	6.33	6.14	-3.00	---	Premaratne et al. (2005)
Forage	Paper pellets	kg DM/100 kg BW	3	0.96	1.22	+27.1	---	Ferreira et al. (2007)
Lucerne hay	Paper pellets	kg DM d^{-1}	11	1.06	0.86	-18.9	0.18	Olivan et al. (2007)
Forage + concentrate	CRC	kg DM d^{-1}	6	4.90	5.00	+2.04	0.66	De Oliveira et al. (2008)
Tropical grass	Paper pellets	kg DM d^{-1}	8	4.24	3.11	-26.7	---	Morais et al. (2011)
Tropical roughage	Boluses	kg DM d^{-1}	8	2.78	2.64	-5.04	---	Bezabih et al. (2012)
Forage	Boluses	kg DM d^{-1}	32	10.38	10.43	+0.48	0.61	Hafla (2012)
Estimated by C_{31}:C_{32} ratio								
Forage + supplement	CRC	kg DM d^{-1}	4	12.7	11.2	-11.8	0.77	Berry et al. (2000)
Lowland grass	CRC	kg DM d^{-1}	6	16.0	15.8	-1.25	---	Estermann et al. (2001)
Meadow hay	CRC	kg DM d^{-1}	4	6.70	7.55	+12.7	---	Ferreira et al. (2004)
Fresh forage	CRC	kg DM d^{-1}	6	8.32	7.81	-6.13	0.23	Molina et al. (2004)
Chopped hay	CRD	kg DM d^{-1}	6	6.33	5.65	-10.7	---	Premaratne et al. (2005)
Forage	Paper pellets	kg DM/100 kg BW	3	0.96	1.01	+5.21	---	Ferreira et al. (2007)
Lucerne hay	Paper pellets	kg DM d^{-1}	11	1.06	0.80	-24.5	0.61	Olivan et al. (2007)
Tropical grass	Paper pellets	kg DM d^{-1}	8	4.24	2.97	-29.9	---	Morais et al. (2011)
Tropical roughage	Boluses	kg DM d^{-1}	8	2.78	2.55	-8.27	---	De Oliveira et al. (2008)
Forage	Boluses	kg DM d^{-1}	32	10.38	9.89	-4.72	0.63	Hafla (2012)

[1] Diff. = ((Estimated - observed) ÷ observed) × 100.

Bezabih et al. (2012) concluded that known fecal recovery rates of adjacent n-alkanes will improve the reliability of intake predictions. In agreement with these results, Charmley and Dove (2007) reported that intake of up to five diet components can be more accurately estimated when feeding a known amount of supplement labeled with beeswax and synthetic C28 alkane, if fecal n-alkane recovery rates are known. Since animal species, physiological status, and diet type have been shown to affect fecal recovery rates, accuracy of intake estimations across trials has been variable. Reported intake estimates have been accurate when fecal recovery rates of adjacent alkanes were similar (Hameleers and Mayes, 1998; Estermann et al., 2001; Bezabih et al., 2012), and inaccurate when fecal recovery rates of adjacent alkanes were dissimilar (Berry et al., 2000; Keli et al., 2008).

Mann and Stewart (2003) reported that intake of tropical forage measured using the Calan-gate feeders was comparable to estimated intake calculated by paired alkanes when a mean of morning and afternoon fecal samples were used (6.28 ± 0.24 vs. 6.21 ± 0.15 kg/d, respectively). They found however, significant variation in the fecal concentration of dosed n-alkanes, and corresponding variation in the intake estimations based on morning or afternoon fecal samples (5.61 ± 0.17 vs. 6.81 ± 0.22 kg/d, respectively). Berry et al. (2000) found similar results, but used intra-ruminal controlled-release capsules (CRC) to estimate intake of 4 cows, attempting to reduce diurnal variability of maker excretion to improve the validity of grab-fecal sampling. Fecal grab samples were collected 3 times daily, on days 8 through 14 following the administration of the CRC. Intake estimates exposed variability in the fecal odd-chain n-

alkane concentrations based on both time of sampling and number of days post sampling. Fecal grab samples collected at 0630. resulted in the most accurate intake estimations within a day, with estimated intake of 10.35 ± 0.28 kg DM/d compared to actual intake of 10.35 ± 0.18 kg DM/d. While the diurnal excretion of odd-chained alkanes is typically consistent, this study shows that variability can exist, and concluded that fecal grab samples from a 7-d period provide precise estimates of herbage intake compared to actual (12.67 vs. 12.70 kg DM/d, respectively). Dependent upon the dosing procedures, fecal grab sampling may not provide the most accuracy in analyzing fecal samples for alkane concentrations. However, due to the laborious nature of total fecal collections, fecal sampling is typically done once or twice a day for a defined number of consecutive days, which can be a limitation to the use of this method. Therefore, precise dosing based on fecal sampling procedures must be maintained in order to minimize the diurnal variation in excretion of both even- and odd-chain alkanes.

Another source of variation in the use of the n-alkane method to estimate intake is the method of forage sampling. The animal's ability to selectively graze forage will affect the amount of n-alkane consumed, as certain plant species and plant parts have different concentrations of n-alkanes present (Dove et al., 1996). The n-alkane technique relies on distinct differences in the n-alkane profiles to be present in the forage consumed by the animal (Bugalho et al., 2002), and in the forage sampled for analysis. Sampling of forage when animals are grazing pastures consisting multiple species of plants may therefore prove to be a laborious task. Thus, the ability of the alkane method to accurately predict intake will decrease as the number of different plant species

consumed increases (Mayes and Dove, 2000). Regardless of the diet, a forage sample that is representative of the plant species and plant parts that an animal has selected to consume is necessary, in order to obtain accurate estimations of intake using this technique.

When these potential sources of variation are minimized, the n-alkane method may provide several advantages for estimating DMI of grazing animals, including low invasiveness, accuracy, and ability to estimate diet composition (Mayes, et al., 1986; Dove and Mayes 1991; Hameleers and Mayes, 1998; Olivan et al., 1999; Mayes and Dove 2000). The n-alkane method allows for the estimation of diet composition when between-species differences in n-alkane profiles are present (Dove, 1992), and accommodates individual animal differences in digestibility. Since alkanes are chemically discrete components, this method also allows for alkanes to be easily analyzed by gas chromatography (Bezabih et al., 2012). Therefore, the n-alkane method may be valuable when individual animal intake, digestibility, or diet composition is required for grazing animals, and extensive fecal and forage sampling is acceptable.

Fecal NIRS

The use of NIRS technology to evaluate the nutritional characteristics of forage has been investigated for over 20 years. Norris et al. (1976) sparked interests in this area after demonstrating the potential of NIRS profiling of forage to predict chemical composition and animal response variables of intake and digestibility in sheep (R^2 = 0.64 and 0.78, respectively). Following these results, researchers such as Ward et al. (1982)

and Lippke and Barton (1988) were able to further demonstrate the use of NIRS profiling of forage to predict responses of animals fed forage. However, obtaining a representative diet sample can be challenging as grazing animals selectivity is not always represented in a pasture sample. A forage sample will likely be of little use when determining the diet of grazing livestock when a representative diet sample is not obtained. Fecal samples contain a wide array of information about the diet, physiology, and ecology of the animal due to undigested residues of the forage consumed (Dixon and Coates, 2009), and may consequently be more useful for determining characteristics of the diet and animal response variables. Holloway et al. (1981) reported that 70% of the between-animal variation in intake could be explained through a diverse array of chemical components found in fecal material. They used 37 composited 5-d fecal samples and multiple reference values from a variety of chemical analysis, to estimate intake of steers with an R^2 and RSD of 0.69 and 0.46, respectively. While this research was not conclusive enough to provide an industry applicable equation for measuring intake or diet characteristics, it did provide evidence for potential application of this technology.

Research by Lyons and Stuth (1992) evaluated the accuracy of fecal NIRS to predict forage crude protein (CP) and digestible organic matter (DOM) concentrations in free ranging cattle grazing diverse rangelands. The authors reported calibration equations with accuracies (R^2 and SEC) of 0.92 and 0.89 for CP and 0.80 and 1.66 for DOM. The calibration and validation values obtained were comparable to the standard error of laboratory methods (SEL) to quantify CP (0.44) and DOM (1.68; Awuma, 2003). The

authors concluded that fecal NIRS technology may have the potential for nutritional profiling of grazing cattle and other herbivores on rangelands.

Later research by Lyons et al. (1995) was conducted to further validate forage CP and DOM predictions of free ranging cattle using previously developed fecal NIRS equations. Seven trials were conducted on 5 different pastures representing a wide range of forage quality. The calibration statistics (R^2 and standard error of prediction; SEP) reported for this study were reported 0.98 and 0.49 for CP and 0.87 and 1.12 for DOM. Results from this study provided further support for the use of fecal NIRS profiling to monitor forage quality and digestibility of grazing cattle.

Results of studies that used fecal NIRS profiling to predict diet characteristics (CP, NDF, and DMD) in ruminant animals are presented in Table 1.2. Studies included in this summary were limited to those that evaluated forage characteristics in ruminant animals with sample sets involving at least 30 animals. The use of fecal NIRS for the prediction of diet characteristics such as CP, dry matter digestibility (DMD), and neutral detergent fiber (NDF) has been used as a method to monitor forge quality, providing insight to further improve animal management practices (Cook, 1999; Dixon and Coates, 2005; Kneebone, 2011).

More recently, fecal NIRS has been examined for potential application in estimating intake of confined or grazing animals. Garnsworthy and Unal (2004) estimated DMI for group-fed dairy cows with varying diets and levels of intake by

Table 1.2. Summary of studies that evaluated the use of fecal NIRS profiling to predict diet nutritional characteristics

					Calibration[1]		Validation[2]		Validation[3]				
Attribute	Animal species	Diet	N	Mean	SEC	R^2_c	SECV	R^2_{cv}	SEV	R^2_v	Bias[4]	RPD[5]	Reference
CP	Cattle	Forage	98	---	0.89	0.92	0.86	0.93	---	---	---	---	Lyons & Stuth (1992)
CP	Cattle	Forage	77	11.5	---	---	---	---	0.49	0.98	---	2.91	Lyons et al. (1995)
CP	Cattle	Forage	630	8.08	1.26	0.90	---	---	2.70	0.59	0.57	---	Ossiya (1999)
CP	Cattle	Forage	156	---	0.90	0.92	1.12	0.87	---	---	---	---	Awuma (2003)
CP	Cattle	Forage	86	10.5	0.33	0.98	0.50	0.95	---	---	---	---	Boval et al. (2004)
CP	Sheep	TMR	116	18.4	0.19	0.95	0.24	0.92	---	---	---	---	Decandia et al. (2007)
CP	Sheep	Forage	78	166	7.90	0.88	10.3	---	---	---	---	---	Decruyenaere et al. (2009)
CP	Cattle	TMR	1322	13.3	1.15	0.81	---	---	1.18	0.84	---	2.22	Tran et al. (2010)
DMD	Cattle	Forage	30	56.5	5.20	0.91	---	---	4.70	0.91	---	---	Purnomoadi et al. (1997)
DMD	Cattle	TMR	30	69.7	2.60	0.64	---	---	3.20	0.56	---	---	Purnomoadi et al. (1997)
DMD	Cattle	TMR	31	56.5	2.89	0.95	---	---	3.50	0.89	---	2.92	Purnomoadi et al. (1998)
DMD	Cattle	Forage	313	---	3.90	0.80	4.10	---	---	---	---	---	Coates (2005)
DMD	Cattle	TMR	44	---	0.03	0.68	0.03	0.53	---	---	---	---	Garnsworthy and Unal (2004)
DMD	Cattle	TMR	1322	63.6	1.91	0.88	---	---	3.07	0.87	---	1.74	Tran et al. (2010)
NDF	Cattle	Forage	87	75.5	0.96	0.88	0.50	0.95	---	---	---	---	Boval et al. (2004)
NDF	Sheep	TMR	115	38.6	1.92	0.96	2.70	0.93	---	---	---	---	Decandia et al. (2007)
NDF	Sheep	Forage	84	---	1.54	0.51	1.64	0.45	---	---	---	---	Fanchone et al. (2007)
NDF	Cattle	TMR	1322	45.4	3.58	0.83	---	---	3.57	0.80	---	2.47	Tran et al. (2010)

[1] Calibration included 100% of the samples in the data set.
[2] Validation accomplished using cross validation.
[3] Validation accomplished using test set validation.
[4] Bias: mean difference between observed and NIR predicted data; bias = ($\sum$ reference data/N) – ($\sum$ predicted NRS data/N).
[5] RPD: ratio performance deviation; the ratio of SEV to SD of the reference data; RPD = SD of reference data ÷ SEV.
SEC: standard error of calibration; R^2_c: coefficient of determination for calibration; SECV: standard error of cross validation; R^2_{cv}: coefficient of determination for cross validation; SEV: standard error of validation; R^2_v: coefficient of determination for validation.

means of fecal NIRS. The validation accuracies (SE of cross validation; SECV and R^2) of the equation for DMI were 0.64 and 0.99. Their results demonstrated that direct prediction of DMI by fecal NIRS was more accurate (SEP = 0.51; R^2 = 0.99) than indirect predictions via fecal NIRS predicted alkanes (SEP = 1.42; R^2 = 0.95), concluding that fecal NIRS could be used in place of the alkane method for intake predictions if a suitable calibration set is available. Boval et al. (2004) investigated the potential of fecal NIRS profiling for the prediction of various parameters including intake. With the use of 11 individually housed steers, 88 fecal samples (11 steers x 8 weeks) were scanned and calibration equations were developed using the modified partial least squares (MPLS) technique. Their results, which were similar to other reports (Olson, 1984; Flinn et al., 1992; Forbes and Coleman, 1993; Coates, 2005), did not provide an accurate calibration for intake (Calibration R^2 and Validation R^2, 0.61 and 0.52, respectively).

Huntington et al. (2010) evaluated the application of the fecal NIRS technique to predict intake using data collected from multiple metabolism trials with growing Angus bulls. Direct measurements of intake were obtained for all animals, and 4 spectral libraries (1 for each of 4 years) of fecal samples were created using MPLS regression on spectra obtained from a Model 5000 NIR spectrometer. A calibration equation was developed using fecal samples from the first metabolism trial, then expanded upon in a year-by-year, chronological sequence resulting in four calibration equations. The calibration accuracies (R^2) ranged from 0.71 for the equation developed using fecal samples from the first metabolism trial to 0.53 for the equation developed with fecal

samples from each of the four trials. Huntington et al. (2010) concluded that year of trial was a significant source of variation, which affected the accuracy of the calibration equation and the robustness of this technique. These outcomes illustrate current disadvantages involving the use of fecal NIRS profiling and demonstrate a need for further research.

Recently, Tran et al. (2010) evaluated the use of global and local calibration techniques to predict DMI in dairy cows using fecal NIRS. The global calibrations technique used the full spectrum of all samples for equation development, while the local calibration procedure used spectrally similar samples to develop specific calibration equations as described by Shenk et al. (1997). Fecal samples were collected from 537 dairy cows in France and 785 dairy cows in Vietnam, to develop local and global fecal NIRS calibrations. Results from this study demonstrated improved calibration accuracies (R^2 and SEC) of 0.87 and 1.37 with the use local equations, compared to 0.84 and 1.53 with the use of global equations for the prediction of DMI. While the use of local equations provided more accurate predictions, the global equation developed in this study was still fairly accurate in predicting DMI, demonstrating the potential for future development of robust calibration equations for applications in predicting intake across multiple forage types and production systems.

Table 1.3 summarizes results from experiments that reported fecal NIRS calibrations for the prediction of direct and indirect measurements of voluntary intake in ruminants. While previous studies have demonstrated the potential of the fecal NIRS

Table 1.3. Summary of studies that evaluated the use of fecal NIRS profiling to predict voluntary intake in ruminants

Animal species	Diet	Intake	N	Mean	Calibration[1]		Validation[2]		Validation[3]				References
					SEC	R^2_c	SECV	R^2_{cv}	SEV	R^2_v	Bias[4]	RPD[5]	
Direct intake measured													
Cattle	Tropical grass	g/kg $BW^{0.75}$	87	76.2	4.62	0.61	0.5	0.52	---	---	---	---	Boval et al. (2004)
Sheep	Barley grain and straw	g/kg $BW^{0.75}$	15	---	1.96	0.83	3.58	0.45	---	---	---	---	Valiente et al. (2004)
Sheep	Pelleted diet	g/kg $BW^{0.75}$	117	72.2	11.8	0.90	15.6	0.83	---	---	---	---	Decandia et al. (2007)
Sheep	Lucerne and ryegrass	g/kg $BW^{0.75}$	15	48.2	9.71	0.44	11.6	0.2	---	---	---	---	Keli et al. (2007)
Sheep	Fresh grass	g/kg $BW^{0.75}$	84	56.5	6.64	0.77	11.0	0.45	---	---	---	---	Fanchone et al. (2007)
Sheep	Lucerne and ryegrass	g/kg $BW^{0.75}$	15	46.5	1.37	0.99	4.52	0.90	---	---	---	---	Keli et al. (2008)
Sheep	Fresh grass	g/kg $BW^{0.75}$	936	51.2	4.28	0.83	4.56	---	---	---	---	---	Decruyenaere et al. (2009)
Cattle	Corn silage	g/kg BW	407	23.4	3.00	0.53	3.2	0.23	---	---	---	---	Huntington et al. (2010)
Cattle	Fresh grass	kg d^{-1}	1322	17.1	1.53[6] 1.37[7]	0.84[6] 0.87[7]	--- ---	--- ---	1.97[6] 1.82[7]	0.58[6] 0.67[7]	--- ---	1.97[6] 2.13[7]	Tran et al. (2010)
Indirect intake measured													
Cattle	Mixed	kg d^{-1}	70	19.4	0.44	0.99	0.64	0.98	---	---	---	---	Garnsworthy and Unal (2004)
Cattle	Forage	g/kg BW	472	---	2.17	0.79	2.42	---	---	---	---	---	Coates (2005)

[1] Calibration included 100% of the samples in the data set.
[2] Validation accomplished using cross validation.
[3] Validation accomplished using test set validation.
[4] Bias: mean difference between observed and NIR predicted data; bias = (∑ reference data/N) – (∑ predicted NRS data/N).
[5] RPD: ratio performance deviation; the ratio of SEV to SD of the reference data; RPD = SD of reference data ÷ SEV.
[6] calibration (n = 1322) and validation (n = 75) for global calibration.
[7] calibration (n = 1322) and validation (n = 75) for local calibration.
SEC: standard error of calibration; R^2_c: coefficient of determination for calibration; SECV: standard error of cross validation; R^2_{cv}: coefficient of determination for cross validation; SEV: standard error of validation; R^2_v: coefficient of determination for validation.

technique to predict intake, these results have also revealed some of the limitations associated with this method. A major limitation reported by Huntington et al. (2010) and Tran et al. (2010) was the significant effect of trial. Factors such as time of year, diet, breed type, age, fecal sample handling, or environment likely contribute significant sources of variation due to trial and limit the robustness of prediction equations. Further research with expanded data sets will be required to determine the specific cause and effects of such factors.

Tran et al. (2010) also found a reduction in the predictability of intake when various combinations of data from multiple trial locations and/or climates were used to develop calibration equations. The inability to accurately combine data sets encompassing multiple trials and varying locations will limit the future application and robustness of calibration equations. Another limitation impacting the robustness of past calibrations is the limited number of samples used to develop calibration equations. In order to develop an industry applicable calibration equation, a large sample set, representative of forages used in a defined geographic region will need to be developed. To date, few studies have compiled data sets (> 2000) of sufficient size to generate accurate prediction equations for estimating intake across multiple forages and production systems. With further research requirements, and these limitations aside, the use of fecal NIRS offers several advantages for the prediction of intake over standard methods, such as a non-destructive, low cost, and quick analysis, with minimal labor requirements to prepare and analyze fecal samples. Fecal NIRS may also provide individual intake estimations that account for variation in diet selection and digestibility

across individual animals, without the need for extensive forage sampling. For these reasons, fecal NIRS may be a preferred method when individual animal estimates of intake, diet characteristics, or digestibility are required for cattle grazing pastures.

Summary

While accurate and reliable methods are available to directly measure individual-animal feed intake in confinement situations, there are few methods available to accurately measure DMI of grazing animals. Current DMI estimation techniques based on predictive models or measurements of herbage mass disappearance have been demonstrated to be relatively effective in prediction DMI for groups of animals. These methods have facilitated advancements in diet formulation and management practices, but have limited ability to accurately estimate forage intake of individual animals, and consequently have limited value to identify animal phenotypes with improved feed efficiency or to evaluate effectiveness of management practices to mitigate GHG emissions. Implications for future research regarding the use of fecal-marker and NIRS techniques exist, as further refinement of these techniques may provide more accurate predictions of individual-animal intake to improve production efficiency of beef cattle systems.

Conclusion and objectives

As rising global populations and per capita incomes lead to increased demand for animal protein foods, the beef industry will need to adapt by increasing production efficiency in the face of increasing input costs and societal concerns regarding the

sustainability of animal agriculture. One strategy to improve the efficiency of feed utilization in beef cattle is through selection programs that focus on reducing maintenance energy requirements relative to size and productivity (e.g., RFI). Residual feed intake is a moderately heritable feed efficiency trait that accounts for between-animal variations in maintenance requirements, independent of growth and BW. Favorable selection for RFI has been shown to reduce feed inputs and GHG emissions in cattle, with minimal impact on performance. However the absence of accurate and affordable methods to measure individual-animal intake, especially for grazing or confined cattle on high roughage diets, has limited further widespread application of this method to improve feed efficiency and(or) mitigate GHG emissions in the beef industry.

Of the current methods available for predicting DMI of grazing animals, the n-alkane marker and fecal NIRS profiling techniques have the greatest potential to accurately account for between-animal variation in DMI of grazing animals. Therefore, the objectives of this study were to evaluate the use of fecal NIRS profiling technology to estimate diet characteristics and voluntary intake in beef cattle, and to evaluate the use of an n-alkane labeled supplement to estimate forage intake in mid-gestation heifers selected for divergent post-weaning RFI.

CHAPTER II

APPLICATION OF FECAL NEAR-INFRARED REFLECTANCE SPECTROSCOPY PROFILING FOR THE PREDICTION OF DIET CHARACTERISTICS AND VOLUNTARY INTAKE IN BEEF CATTLE

Introduction

Near infrared reflectance spectroscopy (NIRS) technology has become a well-established method to predict chemical composition and digestibility of forages. This technology has been extensively reviewed (Norris et al., 1976; Shenk and Westerhaus, 1985; Coleman et al., 1999; Kitessa et al., 1999; Landau et al., 2006), and has become an acceptable alternative to laboratory chemical procedures. Researchers have also evaluated the use of NIRS to predict voluntary intake of forages (Norris et al., 1976; Ward et al., 1982; Redshaw et al., 1986), however, this technique has not become an accepted method for estimating forage intake of grazing animals, as obtaining representative diet samples from grazing livestock can be challenging. Additionally, grazing animals' ability to selectively graze has limited the application of NIRS technology to predict diet quality and digestibility of forage consumed by individual animals.

Holloway et al. (1981) determined that 70% of the between-animal variation in intake and digestibility could be explained through a diverse array of chemical components found in fecal material. Following these results, the application of NIRS technology was developed to predict diet characteristics and intake on the basis of fecal

NIRS spectra. This application, known as fecal NIRS profiling, has been evaluated for its use in predicting diet quality (Lyons and Stuth, 1992; Purnomaodi et al., 1997; Givens and Deaville, 1999; Boval et al., 2004; Tran et al., 2010), digestibility (Lyons and Stuth, 1992; Ossiya, 1999; Awuma, 2003; Coates, 2004; Garnsworthy and Unal, 2004; Tran et al., 2010), and intake (Boval et al., 2004; Garnsworthy and Unal, 2004; Coates, 2005; Huntington et al., 2010; Tran et al., 2010) in beef and dairy cattle.

The application of fecal NIRS profiling to predict diet characteristics such as CP, DMD, and NDF is being used as a method to monitor forage quality, and improve animal management practices (Cook, 1999; Dixon and Coates, 2005; Kneebone, 2011). However, results have been variable across studies, and databases available for the development of globally robust calibrations are limited.

The application of fecal NIRS profiling to predict intake is in its' infancy. While past research has shown the potential of this technology to predict intake (Boval et al., 2004; Decruyenaere et al., 2009; Huntington et al., 2010; Tran et al., 2010), studies are limited, and results have not always been consistent. Additionally, further research is necessary, as few studies have compiled data sets of sufficient size (>2000) to generate accurate prediction equations for estimating forage intake across multiple forages and production systems, possibly limiting the accuracy and application of this technology. Fecal NIRS profiling offers many advantages over standard methods used for predicting diet characteristics and intake, as it provides a non-destructive and low-cost analysis that doesn't require laborious fecal sample preparation. Fecal NIRS technology also allows for the potential to estimate between-animal variation in diet quality, digestibility, and

intake. Therefore, implications for further research evaluating the use of fecal NIRS profiling to predict diet characteristics and intake exist.

The objectives of this study were to evaluate the use of fecal NIRS profiling to predict diet characteristics and voluntary DMI in growing and pregnant cattle of various breeds.

Materials and methods

Experimental animals and design

For this research study, fecal samples and phenotype data were collected from 14 trials utilizing Santa Gertrudis steers (n = 57), Brangus heifers (n = 60), Bonsmara heifers (n = 58), Bonsmara pregnant females (n = 30), Santa Gertrudis heifers (n = 95), Angus bulls (n = 60), *Bos taurus* heifers (n = 80), and *Bos taurus* pregnant cows (n = 60) fed roughage based or forage only diets at the O.D. Butler Jr. Animal Science Complex (ASTREC; Texas A&M University College Station, TX), the Beef Cattle Systems Research Center (BCSR; College Station, TX), the University of Manitoba's Glenlea Research Station (UMGR; St Adolphe, Manitoba), or the Lacombe Research Centre (LRC; Lacombe, Alberta). All procedures were approved by the University Laboratory Animal Care committee of Texas A&M University. A description and summary of data collected for each of these trials is presented in Table 2.1.

Santa Gertrudis Steers (Trial 1). Performance and feed intake was measured for 77 d on one hundred sixteen sire-identified Santa Gertrudis steers from the King Ranch (Kingsville, TX) at the O. D. Butler Jr. Animal Science Teaching Research and

Extension Center in College Station, TX. Steers (initial BW = 296.6 ± 33.6 kg; age = 10 to 11 mo) were blocked by BW and sire progeny group, randomly assigned to one of twenty pens (6 steers per pen) equipped with Calan-gate feeders (American Calan, Northwood, NH) and adapted to a roughage diet for 28 d. During the 77 d feeding period, steers were fed ad libitum twice daily a diet (2.13 Mcal ME/kg DM and 11.2% CP DM, Table 2.2) consisting of chopped alfalfa, alfalfa pellets, cottonseed hulls, cracked corn, molasses, and premix. On d 70 of the postweaning trial, steers were ranked by RFI and the lowest (n = 18), middle (n = 20), and highest (n = 19) 16% were identified for subsequent fecal sampling. Fecal samples were collected once daily at 0700 for 10 consecutive d starting on d 70 of the study for the 57 identified heifers. Feed ingredients and orts were weighed and sampled daily during the fecal collection period and stored at -20º C for subsequent analysis. Feed, ort, and fecal samples were dried at 60º C in a forced air oven, ground through a 1-mm screen in a cyclone sample mill, and composited by combining equal amounts of sample from each of the individual day samples available per animal.

Table 2.1. Description and summary of data collected from animal trials used to evaluate the ability of fecal NIRS profiling to predict diet characteristics and intake in cattle

Trial	Year	Animals	Location[1]	N	Intake method	Fecal sampling protocol	Fecal chemistry
1	2004	Santa Gertrudis steers	ASTREC	57	Calan gates	1X sample/d; 10 d	DMD, NDF, CP
2	2004	Brangus heifers	ASTREC	40	Calan gates	1X sample/d; 10 d	DMD, NDF, CP
3	2005	Brangus heifers	ASTREC	20	Calan gates	1X sample/d; 7 d	DMD, NDF, CP
4	2009	Bonsmara heifers	ASTREC	18	Calan gates	1X samples/d; 5 d[2]	DMD, NDF, CP
5	2010	Bonsmara heifers	ASTREC	20	Calan gates	2X samples/d; 4 d[2]	DMD, NDF, CP
6	2011	Bonsmara heifers	ASTREC	20	Calan gates	2X samples/d; 4 d	NDF, CP
7	2010	Pregnant Bonsmara females	BCSR	30	GrowSafe system	2X samples/d; 5 d[2]	NDF, CP
8	2012	Santa Gertrudis heifers	BCSR	46	GrowSafe system	1X samples/d; 6 d[2]	NDF, CP
9	2013	Santa Gertrudis heifers	BCSR	49	GrowSafe system	1X samples/d; 6 d	NDF, CP
10	2012	Angus bulls	UMGR	30	GrowSafe system	1X samples/d; 5 d	NDF, CP
11	2013	Angus bulls	UMGR	30	GrowSafe system	1X samples/d; 5 d	NDF, CP
12	2012	*Bos taurus* heifers	LRC	80	GrowSafe system	1X samples/d; 5 d	NDF, CP
13	2003	*Bos taurus* pregnant cows	LRC	31	GrowSafe system	1X sample/d; 1 d	NDF
14	2004	*Bos taurus* pregnant cows	LRC	29	GrowSafe system	1X sample/d; 1 d	NDF

[1] ASTREC = O.D. Butler Jr. Animal Science Complex, Texas A&M University, College Station, TX; BCSR = Beef Cattle Systems Research Center, College Station, TX; UMGR = University of Manitoba's Glenlea Research Station, St Adolphe, Manitoba; LRC = Lacombe Research Centre, Lacombe, Alberta.

[2] A fecal composite sample and an individual-day sample from each animal were used to form calibration equations.

Table 2.2. Summary of ingredient and chemical composition of diets used in Trials 1-14

	Trial number													
Items	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Ingredient composition, % DM basis														
Grass hay											29.6			
Chopped sorghum							70.0							
Chopped alfalfa hay	35.0	35.0	35.0	35.0	35.0	35.0	30.0	35.0	35.0	50.7				
Pelleted alfalfa	15.0	15.0	15.0	15.0	15.0	15.0		15.0	15.0					
Cracked corn	19.5	19.5	19.5	19.5	19.5	19.5		19.5	19.5					
Corn silage										49.0	69.5			
CSH	21.5	21.5	21.5	21.5	21.5	21.5		21.5	21.5					
Barley grain												10.0		
Barley silage												90.0	40.0	40.0
Chopped barley straw													56.6	57.1
Beef Supplement[1]													3.40	2.90
Molasses	7.0	7.0	7.0	7.0	7.0	7.0		7.0	7.0					
Premix[2]	2.0	2.0	2.0	2.0	2.0	2.0		2.0	2.0					
Chemical composition														
DM, %	87.1	87.5	89.4	90.0	90.0	90.0	92.0	87.1	86.7	48.2	50.2	39.0	52.1	51.9
ME, Mcal/kg DM	2.13	2.03	2.00	1.99	1.99	1.99	2.11	1.94	1.88	2.28	2.66	2.27	2.42	2.35
CP, % of DM	11.2	12.6	13.2	13.0	13.0	13.0	12.3	11.6	10.8	11.8	13.2	10.5	11.2	9.68
NDF, % of DM	63.6	43.0	43.8	44.8	44.8	44.8	68.5	51.7	56.0	51.1	39.9	---	52.9	60.1

[1] Beef supplement contained 32% crude protein, 1.5% crude fat, 7.0% crude fiber.

[2] Premix for study 2 contained 1.66 g/kg monensin, 0.55 g/kg tylosin, 6.5% CP, 675 mg/kg Cu, 1050 mg/kg Mn, 2850 mg/kg Zn, 15 mg/kg Se, 35 mg/kg I, 7.5 mg/kg Co, 132,300 IU/kg vitamin A, and 3308 IU/kg vitamin E.

Brangus heifers (Trials 2 and 3). Two postweaning test were conducted over 2 consecutive years at the O. D. Butler Jr. Animal Science Teaching Research and Extension Center in College Station, TX to measure performance and feed intake for 70 d on two hundred twenty nine (n = 114 in year 1, n = 115 in year 2) purebred Brangus heifers from Camp Cooley ranch. Heifers from year 1 (Trial 2; initial BW = 285.1 ± 28.0 kg; age = 225.8 ± 9.1 d) and year 2 (Trial 3; initial BW = 268.5 ± 23.8 kg; age = 236.0 ± 10.7 d) were blocked by BW, randomly assigned to one of twenty pens (6 heifers per pen) equipped with Calan-gate feeders (American Calan, Northwood, NH) and adapted to a roughage diet for 24 d. During the 70 d feeding period, heifers were fed ad libitum twice daily a diet (2.03 Mcal ME/kg DM and 12.6% CP DM in year 1; 2.00 Mcal ME/kg DM and 13.2% CP DM in year 2, Table 2.2) consisting of chopped alfalfa, alfalfa pellets, cottonseed hulls, cracked corn, molasses, and premix. On d 56 of the postweaning trials, heifers were ranked by RFI and in year 1 20 heifers with the lowest and 20 heifers with the highest RFI were identified for subsequent fecal sampling, and in year 2 11 heifers with the lowest and 9 heifers with the highest RFI were identified for subsequent fecal sampling. In year 1, fecal samples were collected once daily at 0700 for 10 consecutive d starting on d 59 of the trial, and in year 2 fecal samples were collected once daily for 7 consecutive d starting on d 62 of the trial. Feed ingredients and orts were weighed and sampled daily during the fecal collection period and stored at -20º C for subsequent analysis. Feed, ort, and fecal samples were dried at 60º C in a forced air oven, ground through a 1-mm screen in a cyclone sample mill, and composited by

combining equal amounts of sample from each of the individual day samples available per animal.

Bonsmara heifers (Trials 4, 5, and 6). Three postweaning test were conducted over 3 consecutive years at the O. D. Butler Jr. Animal Science Teaching Research and Extension Center in College Station, TX to measure performance and feed intake for 70 d on one hundred seventy five (n = 62 in year 1, n = 53 in year 2, n = 60 in year 3) Bonsmara heifers from the Texas Agrilife Research and Extension Center, in Uvalde Texas. Heifers from year 1 (Trial 4; initial BW = 306.5 ± 39.16 kg; age = 276.0 ± 19.9 d), year 2 (Trial 5; initial BW = 275.0 ± 24.6 kg; age = 284.9 ± 18.8 d), and year 3 (Trial 6; initial BW = 270.6 ± 33.9 kg; age = 280.9 ± 24.4 d) were blocked by BW, randomly assigned to one of twenty pens (6 heifers per pen) equipped with Calan-gate feeders (American Calan, Northwood, NH) and adapted to a roughage diet for 24 d. During the 70 d feeding period, heifers were fed ad libitum twice daily a diet (1.99 Mcal ME/kg DM and 13.0% CP DM, Table 2.2) consisting of chopped alfalfa, alfalfa pellets, cottonseed hulls, cracked corn, molasses, and premix. On d 56 of the postweaning trials, heifers were ranked by RFI and the lowest (n = 9 in year 1, n = 10 in year 2 and 3) and highest (n = 9 in year 1, n = 10 in year 2 and 3) 16 to18% were identified for subsequent fecal sampling. Fecal samples were collected twice daily at 0700 and 1800 for 5 d in year 1 and 4 d in year 2 and 3, starting on d 65 of the trials. Feed ingredients and orts were weighed and sampled daily during the fecal collection period and stored at -20º C for subsequent analysis. Feed, ort, and fecal samples were dried at 60º C in a forced air oven, ground through a 1-mm screen in a cyclone sample mill, and composited by

combining equal amounts of sample from each of the individual day samples available per animal.

Bonsmara pregnant females (Trial 7). Performance and feed intake was measured for 77 d on 23 1st -parity pregnant Bonsmara heifers and 19 2nd -parity pregnant Bonsmara cows, previously identified as having divergent postweaning RFI, at the Beef Cattle Systems Research Center in Millican, TX. Females were assigned to one of two pens (based on age), each equipped with 4 electronic GrowSafe™ feedbunks (GrowSafe™ DAQ 4000E; GrowSafe™ system Ltd., Airdire, AB, Canada) and adapted to the experimental diet consisting of chopped sorghum and chopped alfalfa (2.11 Mcal ME/kg DM and 12.3% CP DM, Table 2.2). On d 49 of the trial, a preliminary RFI was computed in order to identify 30 pregnant females for determination of predicted intake using n-alkanes. Fecal samples were collected twice daily at 0700 and 1800 for 5 d starting on d 56 of the trial for the 30 identified pregnant females. Feed ingredients and orts from each pen (1st –parity heifers vs 2nd –parity cows) were weighed and sampled daily during the fecal collection period and stored at -20º C for subsequent analysis. Feed, ort, and fecal samples were dried at 60º C in a forced air oven, ground through a 1-mm screen in a cyclone sample mill, and composited by combining equal amounts of sample from each of the individual day samples available per animal. Processed forage and fecal samples were then used for extraction and analysis of alkanes, as described by Hafla (2012). A gas chromatography system (Agilent 6890N, Santa, Clara, CA, USA) with an auto sampler and Chemstations software (Agilent Technologies, Santa Clara, CS, USA) was used to determine n-alkane concentration in the feces and diet

components, and the alkane procedure described by Dove and Mayes (1991) were used to estimate intake with the following equation:

$$\text{Intake} = \left(\frac{Fi}{Fj}\right) D_j / (H_i - \frac{Fi}{Fj} H_j)$$

where H_i and F_i are the herbage and fecal concentrations of an odd-chain n-alkane; and H_j and F_j are herbage and fecal concentrations of an even-chained alkane, D_j is the amount of dosed even-chain alkane released per day.

Santa Gertrudis heifers (Trials 8 and 9). Two postweaning test were conducted over 2 consecutive years at the Beef Cattle Systems Research Center in Millican, TX to measure performance and feed intake for 70 d on two hundred sixteen (n = 108 in year 1, n = 108 in year 2) Santa Gertrudis heifers from King ranch. Heifers from year 1 (Trial 8; initial BW = 293.5 ± 32.6 kg) and year 2 (Trial 9; initial BW = 281.2 ± 28.9 kg) were blocked by BW, randomly assigned to one of four pens (27 heifers per pen) each equipped with 4 electronic GrowSafe™ feedbunks (GrowSafe™ DAQ 6000E; GrowSafe™ system Ltd., Airdire, AB, Canada) and adapted to a roughage diet for at least 14 d. During the 70 d feeding period, heifers were fed ad libitum twice daily a diet (1.94 Mcal ME/kg DM and 11.6% CP DM in year 1; 1.88 Mcal ME/kg DM and 10.8% CP DM in year 2, Table 2.2) consisting of chopped alfalfa, alfalfa pellets, cottonseed hulls, cracked corn, molasses, and premix. Fecal samples were collected from pens 1 and 3 (n = 46 in year 1; n = 49 in year 2) once daily at 0800 for 6 consecutive d starting on d 47 in year 1 and d 60 in year 2. Fecal samples were dried at 60º C in a forced air oven,

ground through a 1-mm screen in a cyclone sample mill, and composited by combining equal amounts of sample from each of the individual day samples available per animal.

Angus bulls (Trials 10 and 11). Performance and feed intake was measured for 82 d and 78 d on Angus bulls in two consecutive year tests at the University of Manitoba's Glenlea Research Station in St Adolphe, Manitoba. Bulls from year 1 (Trial 10; initial BW 309 kg; age = 280 d) and year 2 (Trial 11; initial BW = 312 kg; age = 249 d), were blocked by BW, placed in a large pen equipped with electronic GrowSafe™ feedbunks (GrowSafe™ DAQ 6000E; GrowSafe™ system Ltd., Airdire, AB, Canada), and adapted to a diet (2.28 Mcal ME/kg DM and 11.8% CP DM in year 1; 2.66 Mcal ME/kg DM and 13.2% CP DM in year 2, Table 2.2) consisting of chopped alfalfa hay and corn silage in year 1, and grass hay and corn silage in year 2 for at least 35 d. Fecal samples were collected one time per day for 5 consecutive days starting on d 82 in year 1 and d 80 in year 2. Feed samples were collected and analyzed weekly. Fecal samples were dried at 60º C in a forced air oven, ground through a 1-mm screen in a cyclone sample mill, and composited by combining equal amounts of sample from each of the individual day samples available per animal.

Bos taurus heifers (Trial 12). Performance and feed intake was measured for 74 d on eighty Hereford x Aberdeen Angus and Charolais x Red Angus heifers at the Lacombe Research Centre in Lacombe, Alberta. Heifers (initial BW = 313.5 ± 38.9 kg; age = 316 ± 20 d) were placed in a large pen equipped with electronic GrowSafe™ feedbunks (GrowSafe™ DAQ 6000E; GrowSafe™ system Ltd., Airdire, AB, Canada), and adapted to a diet (2.27 Mcal ME/kg DM and CP DM = 10.5%) consisting of barley grain and

barley silage for 14 d. Fecal samples were collected one time per day for 5 consecutive days starting on d 70. Feed samples were collected weekly and composited monthly for subsequent analysis. Fecal samples were dried at 60º C in a forced air oven, ground through a 1-mm screen in a Wiley Mill, and composited by combining equal amounts of sample from each of the individual day samples available per animal.

Bos taurus pregnant cows (Trials 13 and 14). As described by Basarab et al., (2007), performance and feed intake was measured for 113 d on pregnant *Bos taurus* cows in two consecutive year tests at the Lacombe Research Centre in Lacombe, Alberta. At the end of the fall grazing period, cows were selected based on progeny RFI (n = 31 in year 1, n = 29 in year 2), placed in a large pen equipped with electronic GrowSafe™ feedbunks (GrowSafe™ DAQ 6000E; GrowSafe™ system Ltd., Airdire, AB, Canada), and adapted to a diet (2.42 Mcal ME/kg DM and 11.2% CP DM in year 1; 2.35 Mcal ME/kg DM and 9.68% CP DM in year 2, Table 2.2) consisting of barley silage, chopped barley straw, and beef protein supplement. One fecal sample was collected for each animal on either d 54, 84, or 112 in year 1 and on d 55 or 112 in year 2. Feed samples were collected weekly and composited monthly for subsequent analysis. Feed and fecal samples were dried and ground through a 1-mm screen in a Wiley mill, and composited by combining equal amounts of sample from each of the individual day samples available per animal.

Chemical analysis

Acid insoluble ash (AIA) was used as an internal marker to estimate digestibility coefficients for trials 1, 2, 3, 4, and 5 using fecal and ort composite samples for individual animals, and feed ingredient composite samples for each experimental diet. Acid insoluble ash was determined according to Van Keulen and Young (1977) using 2 N HCL digestion and ashing, and was analyzed according to Van Soest et al. (1991) using the ADF procedure and subsequent ashing. Neutral detergent fiber (trial 1 to 14) and ADF (trials 1 to 5) were determined using an ANKOM Fiber Analyzer F200 (ANKOM Technology Corporation, Fairport, NY.) according to manufacturer's protocol. Nitrogen was determined using a LECO FP2000 nitrogen analyzer (trials 1, 2, and 3) and an Elementar Rapid N Cube (Elementar, Switzerland; trials 4 to 12) with 6.25 used as a conversion factor to calculate CP (LECO Corporation, St. Joseph, MI).

Calculations of digestibility and intake

Acid insoluble ash was used as an internal marker to determine digestibility using the following equation:

$$\text{Digestibility (DMD), \%} = \left(1 - \frac{Ci}{Cf}\right) \times 100$$

where C_i is the concentration of the internal marker in the diet and C_f is the concentration of the internal marker in the feces. The equation was corrected for the DM concentration of the orts.

Two average DMI values were used for the development of fecal NIRS calibrations, an average DMI corresponding to the fecal collection period (fecal-

collection-period DMI) and an average DMI for the trial (trial DMI). The average collection period DMI (g/$BW^{0.75}$) used for the development of fecal NIRS calibrations, with the exception of trials 13 and 14, was calculated as the average g of DMI during fecal collections, starting 1 d prior to the first fecal collection and ending 1 d prior to the last fecal collection (5 to 10 d average), per kg of average $BW^{0.75}$ during the fecal collection period (5 to 10 d), which was calculated from a linear regression of serial BW data for each trial. For trials 13 and 14 the average fecal intake (g/$BW^{0.75}$) was calculated as the average g of DMI from the 5 d prior to the first fecal collection, per kg of $BW^{0.75}$ on the day of the fecal collection, calculated from a linear regression of serial BW data for each trial.

The average trial DMI (g/$BW^{0.75}$) was also used for the development of fecal NIRS calibrations, and was calculated as the average g of DMI during a trial (63 to 113 d average), per kg of average $BW^{0.75}$ for a trial, which was calculated as the average of the model predicted IBW and FBW.

Statistical analysis

Residual feed intake (RFI) was calculated as the difference between actual and expected DMI from a phenotypic regression model of actual DMI on ADG and mid-test $BW^{0.75}$ (Koch et al., 1963). To further characterize RFI, standard deviations above and below the mean were used to group animals into high (> 0.5 SD), medium (± 0.5 SD), or low RFI (< 0.5 SD; Nkrumah et al., 2004). To evaluate the effect of RFI classification on observed and fecal NIRS predicted DMI, MIXED procedure of SAS was used.

To examine the effect of postweaning RFI classification on predicted DMI, individual trial calibrations were used to predict DMI for trials 1, 2, 3, 4, 5, 6, 7, 8, and 9 using fecal composite samples. Trials 1, 2, 3, 4, 5, 6, 8, and 9 were compiled and examined collectively as each of these trials were comprised of growing animals consuming the same ration, and trial 7 was examined individually to evaluate the effect of postweaning RFI classification of fecal NIRS predicted and n-alkane predicted intakes. The mixed procedure of SAS was used for each of these data sets.

Fecal NIRS analysis

A dried and ground fecal composite sample from each animal in trials 1 through 12, and one dried and ground individual-day sample from each animal in trials 4, 5, 7, 8, 13, and 14 were subjected to fecal NIRS analysis. The individual-day fecal samples analyzed by fecal NIRS in trials 4, 5, 6, and 8 were those collected on the third consecutive day of fecal sampling. Before scanning, the dried and ground fecal samples (individual-day fecal samples = 174, composite fecal samples = 438) were placed in coin envelopes and oven dried at 60ºC for a minimum of 4 h to eliminate any recaptured moisture. The dried samples were then placed in a desiccator for 1 h in order to cool to ambient temperatures (Lyons et al., 1995), prior to being packed into sample cups that had quartz lenses. After sample cups were packed, they were stored in a desiccator, and immediately scanned using a Foss NIRS 6500 scanning monochromator at the Grazingland Animal Nutrition Laboratory (GANLAB). Reflectance energy (log 1/R)

was measured and recorded at 2-mm intervals from 400 to 2,498 nm, and stored using Infrasoft International software, version 1.5 (Win ISI Port Matilda, PA).

Twenty one spectral libraries were created from fecal spectra, 1 library for each of the 12 individual trials using fecal composite samples (trials 1 through 12), 1 library in which 11 individual growing cattle trials were compiled to form 1 data set using fecal composite samples (trials 1, 2 ,3, 4, 5, 6, 8, 9, 10, 11, and 12; n = 408), 1 library for each of the 6 individual trials using individual-day fecal samples (trials 4, 5, 7, 8, 13, and 14), 1 library containing samples from 1 pregnant female and 3 growing heifer trials using individual-day fecal samples (trials 4, 5, 7, and 8; n = 114), and 1 library combining all trials in which individual-day fecal samples were used (trials 4, 5, 7, 8, 13, and 14; n = 173).

Spectral pretreatment and fecal NIRS calibration development

Prior to calibration, fecal spectra were corrected for scatter using a standard multiplicative scatter correction (MSC), which corrects for the mean and standardization at each wavelength. Fecal spectra were also subjected to a second derivative transformation of the spectral data, with a gap and smooth of 4. Modified partial least squares regression (MPLS) approach was then used for the development of calibration equations for diet characteristics and DMI using stored NIRS spectra from fecal samples as the independent variable, and diet characteristics or DMI as the dependent variable. A total of 256 wavelengths were used for calibration development (400-2,498, 8), and two

outlier elimination passes were used to identified and eliminated outliers based on a Mahalonobis distance (GH) ≥ 8 and a critical 'T' statistic ≥ 2.5.

To evaluate the performance of calibrations, the standard error of calibration (SEC) and the coefficient of determination for calibration (R^2_c) were used. The SEC defines how well the calibration samples fit the reference data (Westerhaus, 1989), and can be examined against the standard laboratory error (SEL; SE of the lab means) to evaluate the efficiency of NIRS equations. According to the recommendations by Westerhaus (1989) and Li et al. (2007) acceptable equations must have SEC values lower than two times the SEL, and R^2 values greater than 0.80. However, Williams (2005) considers NIRS equations usable with caution if the standard error of cross-validation (SECV) is close to the SEC and R^2 values are greater than 0.83.

Fecal NIRS equation validation

For each of the individual trial calibrations, which included 12 trials that involved composite fecal samples (trials 1-12), and 6 trials that involved individual-day samples were used (trials 4-5; 7-8; and 13-14), validation of spectral libraries was accomplished using cross-validation. The cross-validation procedure as described by Williams (2005) used the same samples for validation as were used for calibration development. To accomplish cross-validation, a random group of samples is removed at a time, with the remaining samples used for calibration. The samples from the removed group are then predicted using the developed calibration, and the residuals are recorded. Those samples are then placed back into the original data set to be used for subsequent

calibration, and another group is removed and predicted. This procedure is repeated until all samples within a data set have been used in the development of the calibration and validation. The number of groups used for cross-validation is dependent upon the number of samples within a spectral library, therefore the number of groups varied across calibrations for this study. The statistical parameters used to evaluate the prediction accuracy of the calibration equations were standard error of cross-validation (SECV), which is the standard deviation of differences of the residuals between NIRS predicted values and reference data, and the coefficient of determination for cross validation (R^2_{cv}).

For the remaining calibrations, which include the combined trials involving growing cattle that included composite fecal samples (trial 1-6; 8-12; n = 408),and the combined trial data sets where individual-day samples were used (trials 4-5; and 7-8; n = 114; and trials 4-5; 7-8; and 13-14; n = 174), validation of spectral libraries was accomplished using test-set validation in addition to the previously described cross-validation procedures. For test-set validation, independent validation sets are developed, therefore, samples used for validation are not used for calibration development. For this study, independent validation sets were built by randomly selecting 20% of the samples from a data set. The remaining 80% of samples were used to develop a calibration equation, which was then used to predict the independent validation set. For test-set validation, the statistical parameters used to evaluate the prediction accuracy of the calibration equations were standard error of validation (SEV), which is the standard deviation of differences between NIRS predicted and reference data, the coefficient of

determination for validation (R^2_v), ratio performance deviation (RPD), which is the ratio of SEV to SD of the reference data, bias, which is the mean difference between the observed and NIR predicted data, and the percent difference between the predicted and observed data (Diff). Differences between observed and fecal NIRS predicted values were evaluated by T-tests using the PROC TTEST procedures in SAS.

Results and discussion

Prediction of crude protein by fecal NIRS profiling

Summary statistics of fecal NIRS prediction equations for CP (% DM) obtained from 11 individual growing cattle trials with composite fecal samples are presented in Table 2.3. The calibration statistics (SEC and R^2_c) of individual trial equations for CP with composite fecal samples ranged from 0.84 and 0.24 in Trial 10 to 0.08 and 0.98 in Trial 4. The calibration accuracies (SEC and R^2_c) for Trials 3, 4 and 11 were within the ranges recommended by Westerhaus (1989) and Li et al. (2007; $R^2 > 0.80$ and SEC < 2.0 × SEL). However, the equations for Trials 1-2, 5-6, 8-10, and 12 were considered unacceptable based on these recommendations for either exceeding an SEC > 2.0 × SEL or an $R^2 < 0.80$. The cross validation accuracies (SECV and R^2_{cv}) ranged from 1.03 and 0.09 for Trial 10 to 0.34 and 0.80 for Trial 3. The range in calibration and validation accuracies across the individual trial equations was likely due to insufficient population sizes (n = 18 to 80), as accuracies were substantially improved when individual trial data sets were compiled (n = 408).

Table 2.3. Summary statistics for calibration and cross-validation of fecal NIRS prediction equations for crude protein (CP, % DM) based on composite fecal samples from growing cattle (Trials 1-6; 8-12)

		CP, % DM				Calibration[2]		Validation[3]	
Trial	N	Range	Mean	SEL	Outliers[1]	SEC	R^2_c	SECV	R^2_{cv}
Individual trial calibrations									
1	57	11.9-15.2	13.78	0.12	2	0.46	0.73	0.68	0.43
2	40	14.3-17.0	15.55	0.10	0	0.45	0.49	0.51	0.34
3	20	13.7-16.6	15.28	0.18	2	0.27	0.87	0.34	0.80
4	18	13.7-15.6	14.46	0.13	0	0.08	0.98	0.31	0.68
5	20	14.8-18.8	15.58	0.19	1	0.24	0.79	0.51	0.10
6	20	14.6-16.8	15.59	0.15	1	0.37	0.68	0.62	0.16
8	46	11.7-16.6	13.45	0.14	1	0.61	0.43	0.68	0.30
9	49	9.26-12.8	10.99	0.12	3	0.28	0.87	0.64	0.40
10	30	9.03-12.7	10.06	0.18	0	0.84	0.24	1.03	0.09
11	30	10.5-16.5	12.58	0.23	6	0.18	0.96	0.48	0.76
12	80	10.3-16.4	12.18	0.12	2	0.74	0.44	0.89	0.19
Combined trial calibration									
1-6; 8-12	408	9.03-18.8	13.14	0.10	22	0.61	0.90	0.67	0.88

[1] Outliers were identified as having a "GH" statistic > 8.0 or a "T" statistic > 2.5 and were not included in the calibration equation.

[2] Calibration included 100% of the samples in the data set.

[3] Validation accomplished using cross-validation.

SEL = standard laboratory error; SEC = standard error of calibration; R^2_c = coefficient of determination for calibration; SECV = standard error of cross validation; R^2_{cv} = coefficient of determination for cross validation.

Calibration and cross-validation summary statistics of the combined trial fecal NIRS equation for CP (% DM) in growing cattle with composite fecal samples is presented in Table 2.3. This calibration was developed with 100% of samples in the data set; therefore cross-validation was used to evaluate the performance. The calibration accuracies (SEC and R^2_c) for this equation were 0.61 and 0.90. The R^2_c value for CP reported in this study compares favorably with values reported by Lyons and Stuth (0.92; 1992), Awuma (0.92; 2003), Boval et al. (0.98; 2004), and Decandia et al. (0.95; 2007), and is considered to be an indication of exceptional quantitative information by Shenk and Westerhaus (1996; $R > 0.90$). In contrast, the reported SEC value in this study, while low compared to values reported by Lyons and Stuth (0.89; 1992), Ossiya (1.26; 1999), Awuma (0.90; 2003), Decruyenaere et al. (7.90; 2009), and Tran et al. (1.15; 2010), exceeds the range recommended by Westerhaus (1989) and Li et al. (2007; $SEC < 2.0 \times SEL$), and may indicate the presence of outliers. However, according to Williams (2005) this equation may be suitable for some applications, due to the similarity of SEC and SECV (0.61 vs. 0.67) and the reported value for R^2_{cv} (0.88).

Further validation of this data set was completed through the prediction of an independent validation set, as previously described for test-set validation procedures. The calibration (SEC and R^2_c) and validation accuracies (SEV, R^2_v, bias, and RPD) for this equation are reported in Table 2.4. The R^2_v reported for CP in this study was lower than the value reported by Lyons et al. (0.98; 1995), comparable to values reported by Tran et al. (0.84; 2010), and higher than the value reported by Ossiya (0.59; 1999). While the reported R^2_v value indicates a closeness of fit between the NIRS predicted and

Table 2.4. Summary statistics for calibration and test-set validation of fecal NIRS predictive equations for crude protein (CP, % DM) based on composite fecal samples from the combined growing cattle data set (Trials 1-6; 8-12)

		Calibration[1]			Validation[2]				Mean ± SD			
Item	Trials	N	SEC	R^2_c	N	SEV	R^2_v	RPD[3]	Observed	Predicted	Bias[4]	Diff[5]
CP, % of DM	1-6; 8-12	327	0.66	0.89	81	0.89	0.79	2.10	13.2 ± 1.87	13.0 ± 1.83	0.16	-1.52

[1] Calibration developed with 80% of the samples in the data set.
[2] Validation was accomplished using test set validation with the remaining 20% of the data set.
[3] RPD: ratio performance deviation; the ratio of SEV to SD of the reference data; RPD = SD of reference data ÷ SEV.
[4] Bias: mean difference between observed and NIR predicted data; bias = (∑ reference data/N) – (∑ predicted NRS data/N).
[5] Diff. = ((Predicted ÷ Observed) - 1) × 100.
SEC = standard error of calibration; R^2_c = coefficient of determination for calibration; SEV = standard error of validation; R^2_v = coefficient of determination for validation.

observed data, alone it does not adequately indicate the efficiency of the prediction equation. Therefore, the RPD and bias should be evaluated along with the validation R^2_v to determine the true efficiency of a predictive equation. For this study, the reported RPD value (2.10) was comparable to the value reported by Tran et al. (2.22; 2010), but based on recommendations by Williams (RPD > 3; 2005), the reported RPD was low, possibly resulting from a low SEL. While the R^2_v and RPD for this equation were moderate, the equation did succeed in predicting the mean CP for this data set, as no significant difference (P = 0.58; bias = 0.16; Diff. = -1.52) was found between the observed and NIRS predicted means. The relationship between observed and fecal NIRS predicted CP values for the growing cattle validation (n = 81) data set is illustrated in Figure 2.1.

The capacities of individual trial equations for CP using composite fecal samples reported for this study were limited as R^2_{cv} ranged from 0.09 to 0.80. The lower values reported for R^2_{cv} suggests that future validation of independent data sets with these equations may not be applicable. However, significant cross-validation improvements were made upon the compilation of individual trials into one larger growing cattle data set. Reported cross-validation accuracies (SECV and R^2_{cv}) for this equation of 0.67 and 0.88 indicated that with caution, this equation may provide reasonable predictions with further validation of independent data sets. When this data set was validated using the test-set validation procedures, this indication was confirmed as the equation had a low bias, moderate R^2_v, and a slightly low RPD. Therefore, the calibration equation developed using the larger data set of composite fecal samples has potential application

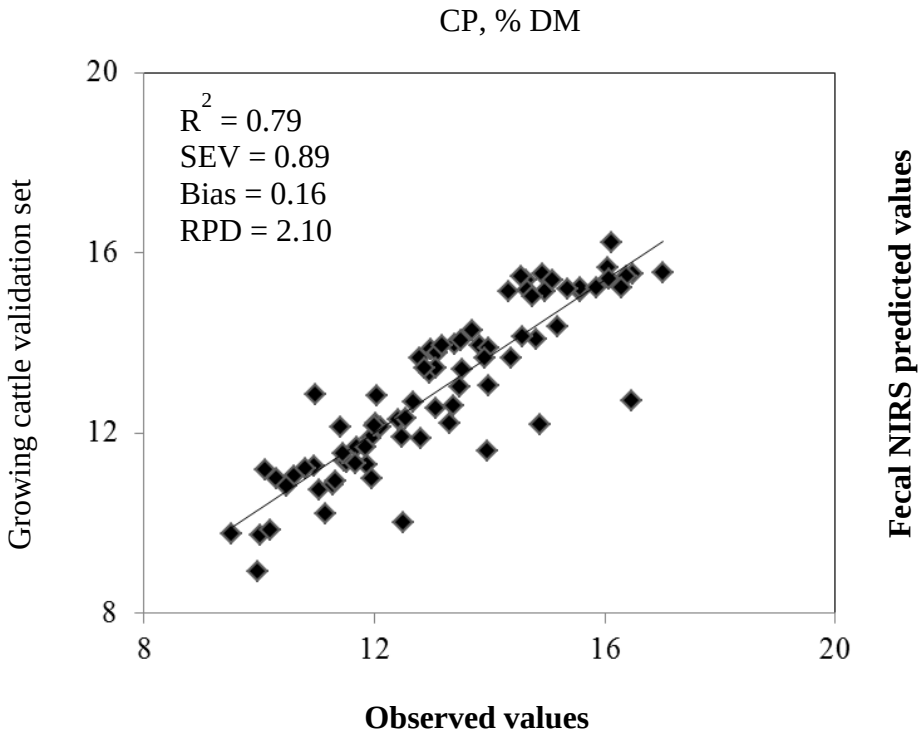

Figure 2.1 Observed values vs. fecal NIRS predicted crude protein (CP, % DM) for the growing cattle validation set that utilized composite fecal samples.

to evaluate diet CP of growing cattle as the calibration represented a diverse population of cattle.

Fecal NIRS equations were also developed to predict CP using individual-day fecal samples from growing heifers (Trials 4, 5, and 8) and pregnant females (Trial 7). Individual trial summary statistics of fecal NIRS equations for CP (% DM) from these growing heifer and pregnant female trials are presented in Table 2.5. The calibration accuracies (SEC and R^2_c) of individual trial equations for CP using individual-day fecal samples ranged from 0.41 and 0.32 in Trial 5 to 0.15 and 0.92 in Trial 4. These results are comparable to the reported values for composite fecal samples, in that validation accuracies were inconsistent with R^2_{cv} ranging from 0.04 to 0.79.

In order to increase the robustness of predictive equations for CP using individual-day fecal samples, the growing heifer and pregnant female trials were combined. The calibration (SEC and R^2_c) and cross-validation (SECV and R^2_{cv}) accuracies for the combined data are presented in Table 2.5. The R^2_c value reported for this equation is considered to be an indication of exceptional quantitative information by Shenk and Westerhause (1996; $R^2 > 0.90$), and would indicate that this equation is usable for most applications based on the recommendations by Williams ($R^2 > 0.92$; 2005). The SEC for this equation slightly exceeds the range recommended by Westerhause (1989) and Li et al. (SEC < 2.0 × SEL; 2007), but is lower than the SEC reported for the equation using composite fecal samples to predict CP. To further

Table 2.5. Summary statistics for calibration and cross-validation of fecal NIRS prediction equations for crude protein (CP, % DM) based on individual-day fecal samples from growing heifers (Trials 4, 5, and 8) and pregnant females (Trial 7)

		CP, % DM				Calibration[2]		Validation[3]	
Trial	N	Range	Mean	SEL	Outliers[1]	SEC	R^2_c	SECV	R^2_{cv}
Individual trial calibrations									
4[4]	18	13.7-15.6	14.46	0.13	1	0.15	0.92	0.26	0.79
5[4]	20	14.8-18.8	15.58	0.19	1	0.41	0.32	0.52	0.04
7[4]	30	10.0-12.5	10.96	0.10	0	0.38	0.41	0.43	0.30
8[4]	46	11.7-16.6	13.45	0.14	4	0.24	0.92	0.73	0.19
Combined trial calibration									
4-5;7-8	114	10.0-18.8	13.33	0.17	6	0.44	0.94	0.51	0.91

[1] Outliers were identified as having a "GH" statistic > 8.0 or a "T" statistic > 2.5 and were not included in the calibration equation.
[2] Calibration included 100% of the samples in the data set.
[3] Validation accomplished using cross-validation.
[4] Individual-day fecal samples analyzed by fecal NIRS were those obtained on the third consecutive day of fecal collections.
SEL = standard laboratory error; SEC = standard error of calibration; R^2_c = coefficient of determination for calibration; SECV = standard error of cross validation; R^2_{cv} = coefficient of determination for cross validation.

evaluate the predictive capacities of the growing heifer and pregnant female data set, test-set validation was completed. The summary statistics for calibration (SEC and R^2_c) and test-set validation (SEV, R^2_v, bias, and RPD) for this equation are reported in Table 2.6. The R^2_v and RPD for this equation were higher than the values reported in this study for the prediction of CP with composite fecal samples, and the SEV and Bias were lower, indicating that this equation has an increased capacity for predicting CP of independent data sets. According to Williams (2005), this equation would be acceptable based on having an $R^2 > 0.92$, RPD > 3.0, a low bias, and a SEV close to the SEC. These results suggest that this equation has potential to predict CP of individual animals, as well as predict the mean CP of the validation set ($P = 0.77$; bias = 0.18; Diff = -1.45). The relationship between fecal NIRS predicted and observed CP values for the growing heifer and pregnant female validation set is illustrated in Figure 2.2.

The capacities of individual trial predictive equations for CP using individual-day fecal samples reported for this study were comparable to those reported using composite fecal samples, and were considered limited as R^2_{cv} ranged from 0.04 to 0.79. Significant cross-validation improvements were made upon the compilation of individual trials into one larger data set, and reported calibration and cross-validation accuracies were again comparable to those reported for the equation developed with composite fecal samples from growing cattle. When this data set was validated using the test-set validation procedures, a low bias, high R^2_v, and acceptable RPD were achieved, indicating that individual-day fecal samples may be used to develop an accurate equation for the prediction of CP in growing heifers and pregnant females.

Table 2.6. Summary statistics for calibration and test-set validation of fecal NIRS predictive equations for crude protein (CP, % DM) based on individual-day fecal samples from the combined growing heifer and pregnant female data set (Trials 4-5; 7-8)

		Calibration[1]			Validation[2]				Mean ± SD			
Item	Trials	N	SEC	R^2_c	N	SEV	R^2_v	RPD[3]	Observed	Predicted	Bias[4]	Diff[5]
CP, % of DM	4-5;7-8	92	0.47	0.93	22	0.55	0.92	3.24	13.8 ± 1.78	13.6 ± 1.82	0.18	-1.45

[1] Calibration developed with 80% of the samples in the data set.
[2] Validation was accomplished using test set validation with the remaining 20% of the data set.
[3] RPD: ratio performance deviation; the ratio of SEV to SD of the reference data; RPD = SD of reference data ÷ SEV.
[4] Bias: mean difference between observed and NIR predicted data; bias = (∑ reference data/N) – (∑ predicted NRS data/N).
[5] Diff. = ((Predicted ÷ Observed) - 1) × 100.
SEC = standard error of calibration; R^2_c = coefficient of determination for calibration; SEV = standard error of validation; R^2_v = coefficient of determination for validation.

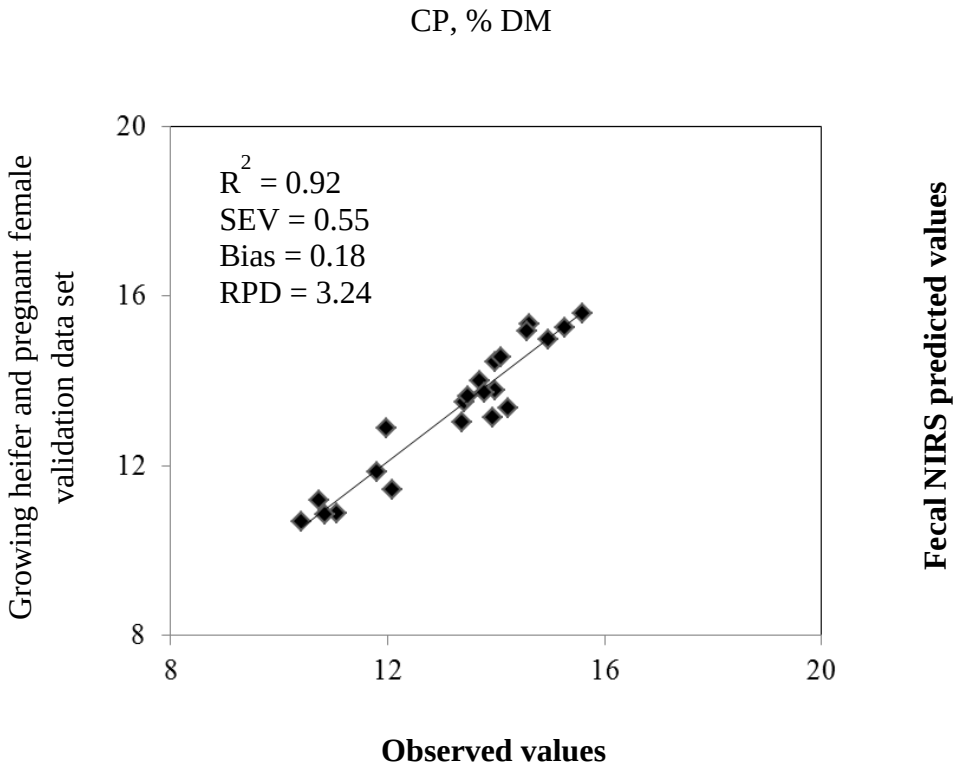

Figure 2.2 Observed values vs. fecal NIRS predicted crude protein (CP, % DM) for the growing heifer and pregnant female validation set that utilized individual-day fecal samples.

In this study, prediction of CP by fecal NIRS was most accurately achieved using individual-day fecal samples from growing heifers and pregnant females. While it appears fecal spectra from individual-day fecal samples were more correlated with CP, further research is necessary to determine if other factors affected the outcome of these results such as diet, stage of production, or population size. However, based on this study, it can be concluded that fecal spectra from both composite and individual-day fecal samples may be used to form equations to predict CP in growing cattle and pregnant females on roughage or forage based diets.

Prediction of neutral detergent fiber by fecal NIRS profiling

Summary statistics of fecal NIRS prediction equations for NDF (% DM) from 11 individual growing cattle trials with composite fecal samples are presented in Table 2.7. The calibration accuracies (SEC and R^2_c) of individual trial equations for NDF with composite fecal samples ranged from 1.85 and 0.24 in Trial 12 to 0.47 and 0.98 in Trial 6. The cross-validation accuracies (SECV and R^2_{cv}) ranged from 2.33 and 0.02 in Trial 10 to 1.26 and 0.72 in Trial 2. The calibration accuracies (SEC and R^2_c) for Trials 5, 6, and 11 were within the ranges recommended by Westerhaus (1989) and Li et al. (2007; $R^2 > 0.80$ and SEC < 2.0 × SEL). However, the equations for Trials 1- 4, 8-10, and 12 were considered unacceptable based on these recommendations for either exceeding an SEC > 2.0 × SEL or an $R^2 < 0.80$. The cross-validation accuracies (SECV and R^2_{cv}) for all trials were poor, indicating that these equations are not applicable for predicting NDF of growing cattle.

Table 2.7. Summary statistics for calibration and cross-validation of fecal NIRS prediction equations for neutral detergent fiber (NDF, % DM) based on composite fecal samples from growing cattle (Trials 1-6; 8-12)

		NDF, % DM				Calibration[2]		Validation[3]	
Trial	N	Range	Mean	SEL	Outliers[1]	SEC	R^2_c	SECV	R^2_{cv}
Individual trial calibrations									
1	57	56.3-67.1	61.5	0.28	0	1.63	0.41	1.97	0.16
2	40	46.5-57.3	51.9	0.38	0	1.12	0.78	1.28	0.72
3	20	56.2-65.1	61.4	0.53	0	1.22	0.73	1.49	0.63
4	18	56.1-63.9	60.1	0.45	0	1.05	0.69	1.81	0.12
5	20	50.4-63.9	55.3	0.76	0	0.91	0.89	2.12	0.46
6	20	53.0-65.7	59.0	0.79	1	0.47	0.98	1.71	0.63
8	46	41.2-54.2	50.6	0.43	0	1.54	0.71	2.30	0.37
9	49	33.0-51.8	43.7	0.57	2	2.99	0.37	3.44	0.18
10	30	53.2-62.4	57.7	0.43	1	1.03	0.81	2.33	0.02
11	30	47.6-60.8	53.7	0.56	1	0.64	0.95	2.81	0.05
12	80	49.1-63.3	57.6	0.27	3	1.85	0.24	2.00	0.10
Combined trial calibration									
1-6; 8-12	408	33.0-67.1	55.3	0.31	15	2.35	0.85	2.46	0.82

[1] Outliers were identified as having a "GH" statistic > 8.0 or a "T" statistic > 2.5 and were not included in the calibration equation.

[2] Calibration included 100% of the samples in the data set.

[3] Validation accomplished using cross-validation.

SEL = standard laboratory error; SEC = standard error of calibration; R^2_c = coefficient of determination for calibration; SECV = standard error of cross validation; R^2_{cv} = coefficient of determination for cross validation.

To further evaluate the capacities of these equations, 11 growing cattle trials were combined to form 1 data set that utilized composite fecal samples. Calibration and cross-validation summary statistics of the combined trial fecal NIRS equation for NDF (% DM) in growing cattle with composite fecal samples is presented in Table 2.7. This calibration was developed with 100% of samples in the data set; therefore cross-validation was used to evaluate the performance. The calibration accuracies (SEC and R^2_c) for this equation are 2.35 and 0.85. The R^2_c value for NDF reported in this study compares favorably with values reported by Boval et al. (0.88; 2004), and Tran et al. (0.83; 2010), is lower than the value reported by Decandia et al. (0.96; 2007), and is higher than value reported by Fanchone et al. (0.51; 2007). The SEC value reported for this study is higher than the values reported by Boval et al. (0.96; 2004), Decandia et al. (1.92; 2007), and Fanchone et al. (1.54; 2007), and lower than the value reported by Tran et al. (3.58; 2010).The calibration (SEC and R^2_c) and cross-validation (SECV and R^2_{cv}) accuracies for this equation are slightly lower than the reported values for CP in this study, but indicate that this equation may be suitable for some applications as SEC and SECV are similar (2.35 vs. 2.46) and R^2_c and R^2_{cv} are greater than 0.80.

To further investigate the predictive capacities of these calibrations, test-set validation was completed on the combined growing cattle data set. The summary statistics for calibration (SEC and R^2_c) and test-set validation (SEV, R^2_v, bias, and RPD) for this equation are reported in Table 2.8. The R^2_v and RPD values reported for NDF in this study are comparable to the values reported by Tran et al. (R^2_v = 0.80 and RPD = 2.47; 2010), but based on recommendations by Williams (RPD > 3; 2005), the reported

Table 2.8. Summary statistics for calibration and test-set validation of fecal NIRS predictive equations for neutral detergent fiber (NDF, % DM) based on composite fecal samples from the combined growing cattle data set (Trials 1-6; 8-12)

		Calibration[1]			Validation[2]				Mean ± SD			
Item	Trials	N	SEC	R^2_c	N	SEV	R^2_v	RPD[3]	Observed	Predicted	Bias[4]	Diff[5]
NDF, % of DM	1-6; 8-12	327	2.24	0.85	81	3.42	0.76	1.89	54.1 ± 6.94	54.4 ± 6.46	-0.30	0.55

[1] Calibration developed with 80% of the samples in the data set

[2] Validation was accomplished using test set validation with the remaining 20% of the data set

[3] RPD: ratio performance deviation; the ratio of SEV to SD of the reference data; RPD = SD of reference data ÷ SEV

[4] Bias: mean difference between observed and NIR predicted data; bias = (∑ reference data/N) – (∑ predicted NRS data/N)

[5] Diff. = ((Predicted ÷ Observed) - 1) × 100

SEC = standard error of calibration; R^2_c = coefficient of determination for calibration; SEV = standard error of validation; R^2_v = coefficient of determination for validation.

RPD was low. While the R^2 and RPD for this equation were moderate, the equation did succeed in predicting the mean NDF for this data set, as no significant difference (*P* = 0.78, bias = -0.30, Diff = 0.55) was determined between the observed and NIRS predicted means. The relationship between observed and fecal NIRS predicted NDF values for the growing cattle validation set is illustrated in Figure 2.3.

Overall fecal NIRS equation accuracies for the prediction of NDF with fecal composite samples were comparable to those reported for CP in this study. Individual trail calibrations for NDF using fecal composite samples failed to provide acceptable equations as R^2_{cv} ranged from 0.02 to 0.72. However, significant improvements were reported upon the compilation of individual trials into one larger growing cattle data set, with reported calibration and cross-validation accuracies indicating the potential of these equations for the prediction of NDF in growing cattle data sets. Validation of this data set using the test-set validation procedures further indicated the application of fecal NIRS of composite fecal samples for the prediction of NDF in growing cattle.

Fecal NIRS equations were also developed to predict NDF using individual-day fecal samples from growing heifers (Trials 4, 5, and 8) and pregnant females (Trial 7, 12, and 13). Individual trial summary statistics of fecal NIRS equations for NDF (% DM) from these growing heifer and pregnant female trials are presented in Table 2.9. The calibration accuracies (SEC and R^2_c) of individual trial equations for NDF using individual-day fecal samples ranged from 1.97 and 0.29 in Trial 8 to 1.27 and 0.94 in Trial 13. The reported cross-validation accuracies (SECV and R^2_{cv}) for these equations

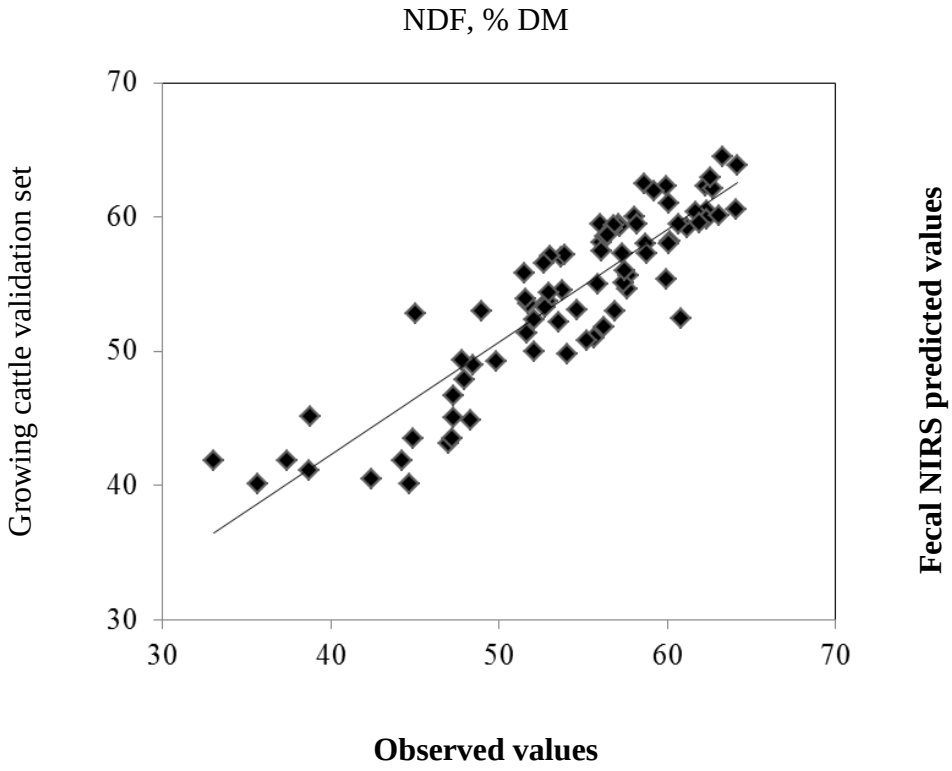

Figure 2.3 Observed values vs. fecal NIRS predicted neutral detergent fiber (NDF, % DM) for the growing cattle validation set that utilized composite fecal samples.

Table 2.9. Summary statistics for calibration and cross-validation of fecal NIRS prediction equations for neutral detergent fiber (NDF, % DM) based on individual-day fecal samples from growing heifers (Trials 4, 5, and 8) and pregnant females (Trials 7, 13, and 14)

		NDF, % DM				Calibration[2]		Validation[3]	
Trial	N	Range	Mean	SEL	Outliers[1]	SEC	R^2_c	SECV	R^2_{cv}
Individual trial calibrations									
4[4]	18	56.1-63.9	60.1	0.45	0	1.26	0.56	2.05	0.06
5[4]	20	50.4-63.9	55.3	0.76	1	1.64	0.63	2.81	0.07
7[4]	30	44.4-55.3	49.9	0.51	0	1.63	0.66	2.46	0.23
8[4]	46	41.2-54.2	50.6	0.43	3	1.97	0.29	2.22	0.12
13	31	51.2-76.4	64.7	0.67	0	1.27	0.94	2.29	0.79
14	29	58.8-68.0	63.2	0.40	1	1.71	0.37	2.65	0.43
Combined trial calibration									
4,5,7,8,13,14	174	41.2-76.4	56.6	0.52	7	2.33	0.88	2.67	0.85

[1] Outliers were identified as having a "GH" statistic > 8.0 or a "T" statistic > 2.5 and were not included in the calibration equation.

[2] Calibration included 100% of the samples in the data set.

[3] Validation accomplished using cross-validation.

[4] Individual-day fecal samples analyzed by fecal NIRS were those obtained on the third consecutive day of fecal collections.

SEL = standard laboratory error; SEC = standard error of calibration; R^2_c = coefficient of determination for calibration; SECV = standard error of cross validation; R^2_{cv} = coefficient of determination for cross validation.

are comparable to the reported values for individual trial equations using composite fecal samples, and prove to also be limited in their validation abilities as R^2_{cv} ranges from -0.06 to 0.79.

In order to increase the robustness of the predictive equation for NDF using individual-day fecal samples, the growing heifer and pregnant female trials were combined into one larger data set. The calibration (SEC and R^2_c) and cross-validation (SECV and R^2_{cv}) accuracies for this data set are presented in Table 2.10. The accuracies reported for this equation are comparable to the values reported in this study for the equation developed with composite fecal samples of growing cattle, and indicate that this equation may be cautiously used for some applications as it has a moderate R^2_c and R^2_{cv}, and SEC and SECV are similar.

Further validation of this data set was completed through the prediction of an independent validation set, and summary statistics of calibration (SEC and R^2_c) and test-set validation accuracies (SEV, R^2_v, bias, and RPD) for this equation are reported in Table 2.10. The R^2_v and RPD values for this equation were higher than the values reported in this study for the prediction of NDF with composite fecal samples, and the SEV and Bias were lower, indicating that this equation has a higher capacity for predicting NDF of independent data sets. Additionally, this equation was successful in predicting the mean NDF of the validation set (P = 0.86, bias = -0.29, Diff = .52). The relationship between observed and fecal NIRS predicted NDF values for the growing heifer and pregnant female validation set is illustrated in Figure 2.4.

Table 2.10. Summary statistics for calibration and test-set validation of fecal NIRS predictive equations for neutral detergent fiber (NDF, % DM) based on individual-day fecal samples from growing heifers (Trials 4, 5, and 8) and pregnant females (Trials 7, 13, and 14)

		Calibration[1]			Validation[2]				Mean ± SD			
Item	Trials	N	SEC	R^2_c	N	SEV	R^2_v	RPD[3]	Observed	Predicted	Bias[4]	Diff[5]
NDF, % of DM	4,5,7,8,13,14	140	2.51	0.86	34	2.34	0.88	2.92	58.1 ± 6.84	58.4 ± 6.70	-0.29	0.52

[1] Calibration developed with 80% of the samples in the data set.
[2] Validation was accomplished using test set validation with the remaining 20% of the data set.
[3] RPD: ratio performance deviation; the ratio of SEV to SD of the reference data; RPD = SD of reference data ÷ SEV.
[4] Bias: mean difference between observed and NIR predicted data; bias = (∑ reference data/N) – (∑ predicted NRS data/N).
[5] Diff. = ((Predicted ÷ Observed) - 1) × 100.
SEC = standard error of calibration; R^2_c = coefficient of determination for calibration; SEV = standard error of validation; R^2_v = coefficient of determination for validation.

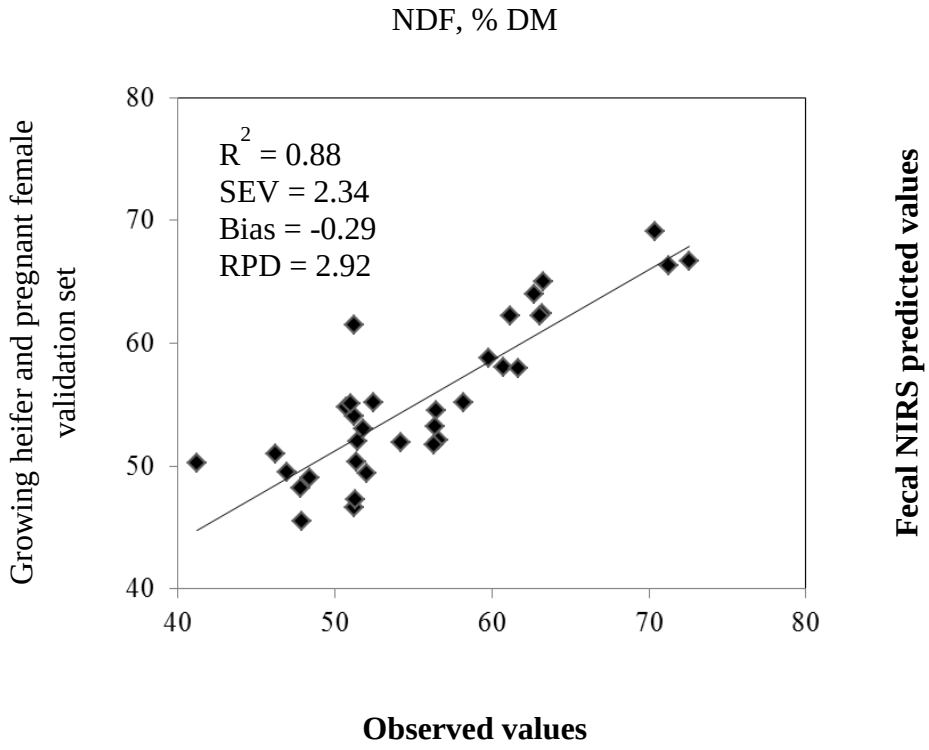

Figure 2.4 Observed values vs. fecal NIRS predicted neutral detergent fiber (NDF, % DM) for the growing heifer and pregnant female validation set that utilized individual-day fecal samples.

The capacities of individual trial equations for NDF using individual-day fecal samples reported for this study were comparable to those reported using composite fecal samples, and were considered limited as R^2_{cv} ranged from 0.06 to 0.79. Significant cross-validation improvements were made upon the compilation of individual trials into one larger data set, and reported calibration and cross-validation accuracies were higher than those reported for the equation developed with composite fecal samples from growing cattle. When this data set was validated using the test-set validation procedures, a low bias, moderate R^2_v, and slightly low RPD were achieved. These results indicate that there is a correlation between spectra from individual-day fecal samples and NDF, and the equation developed may cautiously be used to evaluate the NDF of growing heifers and pregnant females.

In this study, prediction of NDF by fecal NIRS was most accurately achieved using individual-day fecal samples from growing heifers and pregnant females. While it appears fecal spectra from individual-day fecal samples were more correlated with NDF, further research is necessary to determine if other factors affected the outcome of these results. Based on this study, it can be concluded that fecal spectra from both composite and individual-day samples have a lower ability to predict NDF compared to CP, and that further expansion of data sets are necessary to develop an equation that is applicable to the industry.

Prediction of dry matter digestibility by fecal NIRS profiling

Summary statistics of fecal NIRS prediction equations for DMD (%) from 5 individual growing cattle trials with composite fecal samples are presented in Table 2.11. The calibration accuracies (SEC and R^2_c) of individual trial equations for DMD ranged from 5.45 and 0.38 in Trial 2 to 2.81 and 0.85 in Trial 3. Cross-validation was performed to test the predictive capabilities, and cross-validation accuracies (SECV and R^2_{cv}) ranged from 3.67 and 0.07 in Trial 4 to 2.91 and 0.61 in Trial 5. The calibration accuracies (SEC and R^2_c) for Trial 3 were within the ranges recommended by Westerhaus (1989) and Li et al. (2007; $R^2 > 0.80$ and SEC < 2.0 × SEL), but cross-validation accuracies (SECV and R^2_{cv}) for all trials indicate that these equations may not be applicable for the prediction of DMD with independent data sets.

To further evaluate the capacities of these equations, 5 growing cattle trials were combined to form 1 data set that utilized composite fecal samples. The calibration and cross-validation summary statistics of the combined trial fecal NIRS equation for DMD (%) in growing cattle with composite fecal samples is presented in Table 2.11. This calibration was developed with 100% of samples in the data set; therefore cross-validation was used to evaluate the performance. The calibration accuracies (SEC and R^2_c) for this equation are 3.74 and 0.87. The R^2_c value reported for DMD compares favorably with values reported by Purnomoadi et al. (0.91; 1997) and Tran et al. (0.88; 2010), is lower than the value reported by Purnomoadi et al. (0.95; 1998), and is higher than values reported by Coates (0.80; 2005) and Garnsworthy and Unal (0.68; 2004).

Table 2.11. Summary statistics for calibration and cross-validation of fecal NIRS prediction equations for dry matter digestibility (DMD, %) based on composite fecal samples from growing cattle (Trials 1-5)

		DMD, %				Calibration[2]		Validation[3]	
Trial	N	Range	Mean	SEL	Outliers[1]	SEC	R^2_c	SECV	R^2_{cv}
Individual trial calibrations									
1	57	53.5-82.6	83.95	0.99	2	2.03	0.71	3.39	0.21
2	40	47.4-79.2	68.45	1.21	1	5.42	0.38	6.32	0.24
3	20	54.9-77.4	71.78	1.62	0	2.81	0.85	7.39	0.11
4	18	56.5-70.4	60.10	0.45	0	1.76	0.79	3.64	0.07
5	20	48.3-65.9	58.26	1.23	2	2.30	0.76	2.91	0.61
Combined trial calibration									
1-5	155	47.4-82.6	73.67	0.58	11	3.74	0.87	4.31	0.82

[1] Outliers were identified as having a "GH" statistic > 8.0 or a "T" statistic > 2.5 and were not included in the calibration equation.
[2] Calibration included 100% of the samples in the data set.
[3] Validation accomplished using cross-validation.
SEL = standard laboratory error; SEC = standard error of calibration; R^2_c = coefficient of determination for calibration; SECV = standard error of cross validation; R^2_{cv} = coefficient of determination for cross validation.

The SEC value reported for this study is lower than the value reported by Purnomoadi et al. (5.20; 1997), comparable to the value reported by Coates (3.90; 2005), and higher than the values reported by Purnomoadi et al. (2.89; 1998) and Tran et al. (1.91; 2010).The calibration (SEC = 3.74; R^2_c = 0.87) and cross-validation (SECV = 4.31; R^2_{cv} = 0.82) accuracies for this equation are comparable to the values reported in this study for NDF, and are slightly lower than the values reported in this study for CP, similarly indicating that this equation may be suitable for some applications as SEC and SECV are similar (3.74 vs. 4.31) and R^2 and R^2_{cv} are greater than 0.80.

To further investigate the predictive capacities of these calibrations, test-set validation was completed on the combined trial data set. The calibration (SEC and R^2_c) and test-set validation (SEV, R^2_v, bias, and RPD) summary statistics for this equation are reported in Table 2.12. The R^2_v value reported for DMD in this study is lower than the values reported by Purnomoadi et al. (0.91; 1997), Purnomoadi et al. (0.89; 1998), and Tran et al. (0.87; 2010). The RPD reported for this equation is also low compared to the value reported by Purnomoadi et al. (2.92; 1998). While the R^2_v and RPD for this equation were low, the equation did succeed in predicting the mean DMD for this data set, as no significant difference (P = 0.82, bias = -0.60, Diff = 0.83) was determined between the observed and NIRS predicted means. The relationship between observed and fecal NIRS predicted DMD values for the growing cattle equation validation set is illustrated in Figure 2.5.

Table 2.12. Summary statistics for calibration and test-set validation of fecal NIRS predictive equations for dry matter digestibility (DMD, %) based on composite fecal samples from the combined growing cattle data set (Trials 1-5)

		Calibration[1]			Validation[2]				Mean ± SD			
Item	Trials	N	SEC	R^2_c	N	SEV	R^2_v	RPD[3]	Observed	Predicted	Bias[4]	Diff[5]
DMD, %	1-5	128	3.73	0.86	31	6.24	0.68	1.79	72.1 ± 11.2	72.7 ± 9.17	-0.60	0.83

[1] Calibration developed with 80% of the samples in the data set.
[2] Validation was accomplished using test set validation with the remaining 20% of the data set.
[3] RPD: ratio performance deviation; the ratio of SEV to SD of the reference data; RPD = SD of reference data ÷ SEV.
[4] Bias: mean difference between observed and NIR predicted data; bias = (∑ reference data/N) – (∑ predicted NRS data/N).
[5] Diff. = ((Predicted ÷ Observed) - 1) × 100.
SEC = standard error of calibration; R^2_c = coefficient of determination for calibration; SEV = standard error of validation; R^2_v = coefficient of determination for validation.

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DMD, %

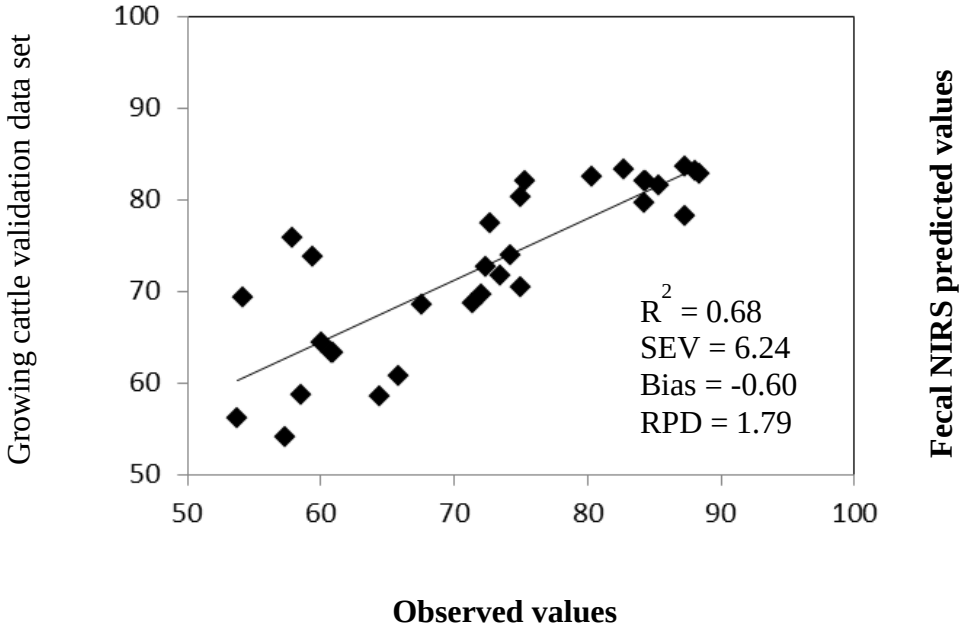

Figure 2.5 Observed values vs. fecal NIRS predicted dry matter digestibility (DMD, %) for the growing cattle validation set that utilized composite fecal samples.

The capacities of fecal NIRS equations for the prediction of DMD in this study were limited. While the reported calibration accuracies for the combined data sets were similar to those reported for CP and NDF, validation accuracies exposed inferior predictions of DMD by fecal NIRS in this study, which can likely be attributed to inadequate population sizes. Additional data sets are needed for further investigation. While the reported validation accuracies were limited in their abilities to predict individual animal DMD, the equations developed from the combined data sets were able to accurately predict the mean DMD of the validation set. The inclusion of more data sets will be necessary to improve the robustness of the DMD equations developed in this study.

Prediction of voluntary intake by fecal NIRS profiling

To determine which single-day of intake was most correlated to the spectra of individual-day fecal samples in this study, calibration equations were developed using the growing heifer (Trials 4, 5, and 8) and pregnant female (Trials 7, 13, and 14) data set. Summary statistics of fecal NIRS equations to predict DMI 1 to 5 d prior to the fecal collection d are presented in Table 2.13. The calibrations were developed with 100% of samples in the data set, and cross-validation was used to evaluate the performance of each equation. The calibration and cross-validation accuracies (R^2_c and R^2_v) ranged from 23.5 and 0.53 for intake 2 d prior to 18.0 and 0.68 for intake 1 d prior. While these

Table 2.13. Summary statistics for calibration and cross-validation of fecal NIRS prediction equations for single-day DMI ($g/BW^{0.75}$) 1 to 5 d prior to fecal collection in growing heifers (Trials 4, 5, and 8) and pregnant females (Trials 7, 13, and 14) with individual-day fecal samples

			DMI, $g/BW^{0.75}$		Calibration[2]		Validation[3]	
Item	Trials	N	Mean ± SEL	Outliers[1]	SEC	R^2_c	SECV	R^2_{cv}
DMI 1 d prior	4,5,7,8,13,14		146.3 ± 3.59	10	18.0	0.68	20.6	0.58
DMI 2 d prior	4,5,7,8,13,14		143.0 ± 2.95	6	23.5	0.53	26.9	0.39
DMI 3 d prior	4,5,7,8,13,14	173	139.7 ± 3.19	5	21.3	0.60	24.0	0.50
DMI 4 d prior	4,5,7,8,13,14		129.2 ± 2.96	11	17.8	0.61	20.3	0.50
DMI 5 d prior	4,5,7,8,13,14		128.9 ± 2.98	8	23.2	0.63	26.7	0.51

[1] Outliers were identified as having a "GH" statistic > 8.0 or a "T" statistic > 2.5 and were not included in the calibration equation.

[2] Calibration included 100% of the samples in the data set.

[3] Validation accomplished using cross validation.

SEL = standard laboratory error; SEC = standard error of calibration; R^2_c = coefficient of determination for calibration; SECV = standard error of cross validation; R^2_{cv} = coefficient of determination for cross validation.

equations have limited accuracies of prediction, the results suggest that individual-day fecal samples obtained 1 day after a single-day measurement of intake was most predictive, suggesting that for these animals, rate of passage was approximately 24 h, or that the excretion levels were highest around 24 h. In support of this observation, Putnam et al. (1967) found that animals with non-restricted intakes had peak excretions of Cr_2O_3 between 24 and 30 h. However, the results reported in this study for the prediction of intake 2 to 5 d prior to the d of fecal collections do not agree with this study. Putnam et al. (1967) found excretion rates to decrease linearly as time increased up to 96 h, and the results from this study show lower correlations for intake 2 d prior than for intake 5 d prior to the fecal collection d. However, the equations developed in this study were not based on a single dietary marker, so correlations may be affected by day to day variations in individual animal consumption. While no accurate equation was developed for the prediction of single-day intake in this study, the results were used along with knowledge from previous studies to determine which intake d to average for the prediction of DMI by fecal composite and individual-day samples.

To evaluate the ability of fecal NIRS equations to predict fecal-collection-period and trial DMI, calibration equations were developed for 11 growing cattle trials with fecal composite samples (Trials 1-6; 8-12), and 7 growing heifer and pregnant female trials (Trials 4-5, 7-8, and 13-14) with individual-day fecal samples. Summary statistics of fecal NIRS prediction equations for fecal-collection-period DMI ($g/BW^{0.75}$) are presented in Table 2.14, and summary statistics of fecal NIRS prediction equations for

Table 2.14. Summary statistics for calibration and cross-validation of fecal NIRS prediction equations for fecal-collection-period DMI ($g/BW^{0.75}$) based on composite fecal samples from growing cattle (Trials 1-6; 8-12) and individual-day fecal samples from growing heifers (Trials 4, 5, and 8) and pregnant females (Trials 7, 13, and 14)

		Fecal-collection-period DMI ($g/BW^{0.75}$)				Calibration[2]		Validation[3]	
Trial	N	Range	Mean	SEL	Outliers[1]	SEC	R^2_c	SECV	R^2_{cv}
Composite fecal samples									
1	57	78.5-162.2	122.1	2.26	1	4.25	0.93	12.7	0.35
2	40	45.1-142.0	113.2	2.71	0	5.98	0.88	15.5	0.20
3	20	89.5-154.7	125.9	3.69	0	9.98	0.63	13.8	0.35
4	18	103.8-165.8	133.4	3.53	1	4.33	0.91	9.44	0.61
5	20	119.4-175.6	145.5	3.25	0	2.33	0.97	12.6	0.12
6	20	84.7-167.9	130.7	4.30	0	9.83	0.73	26.6	0.47
8	46	77.9-145.9	117.1	2.38	2	8.11	0.72	11.9	0.43
9	49	66.0-154.7	111.8	2.70	3	15.4	0.21	17.8	0.04
10	30	75.6-110.2	90.89	1.40	1	0.87	0.99	5.67	0.49
11	30	68.9-107.4	94.00	1.52	2	1.55	0.95	5.46	0.31
12	80	57.6-94.9	80.17	0.80	2	2.61	0.87	5.57	0.51
Individual-day fecal samples									
4[4]	18	103.8-165.8	133.4	3.53	1	2.53	0.97	17.03	0.24
5[4]	20	119.4-175.6	145.5	3.25	0	11.1	0.39	13.7	0.09
7[4]	30	52.6-153.9	106.5	4.66	0	19.5	0.50	22.7	0.35
8[4]	46	77.9-145.9	117.1	2.38	2	13.8	0.23	16.4	0.07
13	31	109.5-293.6	180.7	6.03	2	26.7	0.45	32.5	0.20
14	29	86.1-212.9	139.2	4.41	0	14.9	0.41	18.7	0.11

[1] Outliers were identified as having a "GH" statistic > 8.0 or a "T" statistic > 2.5 and were not included in the calibration equation.
[2] Calibration included 100% of the samples in the data set.
[3] Validation accomplished using cross validation.
[4] Individual-day fecal samples analyzed by fecal NIRS were those obtained on the third consecutive day of fecal collections.
SEL = standard laboratory error; SEC = standard error of calibration; R^2_c = coefficient of determination for calibration; SECV = standard error of cross validation; R^2_{cv} = coefficient of determination for cross validation.

trial DMI (g/$BW^{0.75}$) are presented in Table 2.15. For equations developed for the prediction of fecal-collection-period DMI with composite fecal samples, calibration accuracies (SEC and R^2_c) ranged from 15.4 and 0.21 in Trial 9 to 0.87 and 0.99 in Trial 10, with Trials 1, 2, 4, 5, 10, and 11 having accuracies within the ranges recommended by Westerhaus (1989) and Li et al. (2007; $R^2 > 0.80$ and SEC < 2.0 × SEL) for acceptable predictive equations. The equations developed for the prediction of fecal-collection-period DMI with individual-day fecal samples had calibration accuracies (SEC and R^2_c) ranging from 13.8 and 0.23 in Trial 8 to 2.53 and 0.97 in Trial 4. Calibration accuracies (SEC and R^2_c) for equations developed for the prediction of trial intake ranged from 5.33 and 0.12 to 3.23 and 0.96 in trials where composite fecal samples were used, and 13.6 and 0.19 to 3.77 and 0.93 in trials where individual-day fecal samples were used. Calibration and cross-validation accuracies (SECV and R^2_{cv}) for the equations developed for the prediction of fecal-collection-period DMI and trial DMI were poor ($R^2_{cv} < 0.80$), indicating that these equations have limited capacities to predict DMI of independent data sets.

To further evaluate the capacities of these equations, 11 growing cattle trials were combined to form 1 data set that utilized composite fecal samples, and 7 growing heifer and pregnant female trials that utilized individual-day fecal samples were combined to form another data set. Calibration equations were developed to predict fecal-collection-period DMI and trial DMI for each of these data sets using MPLS regression. Calibration and cross-validation summary statistics of the combined trial fecal NIRS equations for fecal-collection-period DMI (g/$BW^{0.75}$) and trial DMI

Table 2.15. Summary statistics for calibration and cross-validation of fecal NIRS prediction equations for trial DMI ($g/BW^{0.75}$) based on composite fecal samples from growing cattle (Trials 1-6; 8-12) and individual-day fecal samples from growing heifers (Trials 4, 5, and 8) and pregnant females (Trials 7, 13, and 14)

		Trial DMI, $g/BW^{0.75}$				Calibration[2]		Validation[3]	
Trial	N	Range	Mean	SEL	Outliers[1]	SEC	R^2_c	SECV	R^2_{cv}
Composite fecal samples									
1	57	96.7-157.7	125.9	1.76	1	10.5	0.36	12.4	0.17
2	40	94.5-149.4	123.3	2.62	3	3.23	0.96	10.3	0.61
3	20	104.0-151.5	128.8	3.40	0	11.8	0.40	15.6	0.02
4	18	84.6-137.8	108.3	3.56	0	5.23	0.88	16.9	0.17
5	20	103.3-143.7	126.3	3.13	0	9.28	0.56	13.3	0.08
6	20	109.4-147.2	128.3	2.49	1	5.97	0.70	11.5	0.05
8	46	89.3-142.6	118.8	1.95	2	6.47	0.71	10.4	0.29
9	49	101.7-137.9	118.7	1.16	0	7.19	0.24	7.89	0.11
10	30	77.3-108.4	89.01	1.36	0	3.99	0.72	6.56	0.26
11	30	84.1-109.6	97.76	0.99	0	4.67	0.26	5.73	0.10
12	80	65.5-99.5	86.72	0.69	0	5.33	0.12	5.81	0.01
Individual-day fecal samples									
4[4]	18	84.6-137.8	108.3	3.56	0	13.6	0.19	18.6	0.43
5[4]	20	103.3-143.7	126.3	3.13	0	3.77	0.93	13.2	0.13
7[4]	30	56.7-161.4	112.9	4.37	0	13.7	0.68	15.6	0.61
8[4]	46	89.3-142.6	118.8	1.95	2	7.24	0.68	11.7	0.19
13	31	109.8-174.9	141.2	2.42	0	15.6	0.21	19.2	0.16
14	29	116.9-205.6	150.1	3.91	1	17.4	0.31	21.2	0.00

[1] Outliers were identified as having a "GH" statistic > 8.0 or a "T" statistic > 2.5 and were not included in the calibration equation.
[2] Calibration included 100% of the samples in the data set.
[3] Validation accomplished using cross validation.
[4] Individual-day fecal samples analyzed by fecal NIRS were those obtained on the third consecutive day of fecal collections.
SEL = standard laboratory error; SEC = standard error of calibration; R^2_c = coefficient of determination for calibration; SECV = standard error of cross validation; R^2_{cv} = coefficient of determination for cross validation.

($g/BW^{0.75}$) are presented in Table 2.16. The calibrations were developed with 100% of samples in each data set, and cross-validation was used to evaluate their performance. The calibration and validation accuracies (R^2_c and R^2_{cv}) ranged from 0.49 and 0.42 for the equation developed to predict trial DMI with individual-day fecal samples to 0.76 and 0.73 for the equation developed to predict fecal-collection-period DMI with composite fecal samples. The reported R^2_{cv} for the equation developed to predict fecal-collection-period DMI with composite fecal samples was higher than the values reported by Boval et al. (0.52; 2004), Valiente et al. (0.45; 2004), and Keli et al. (0.20; 2007) where composite fecal samples were also used to predict an average intake surrounding fecal collections in cattle or sheep. When composite fecal samples were used to predict trial DMI, this study reported a higher R^2_{cv} value compared to a previous study completed by Fanchone et al. (0.45; 2007) where composite fecal samples were used to predict an average trial DMI in sheep. For trials where individual-day fecal samples were used to predict DMI, R^2_{cv} values reported in this study were higher than the value reported by Huntington et al. (0.23; 2010) where one individual-day sample was used per animal to predict a single-day DMI in growing bulls, and lower than the value reported by Decandia et al. (0.83; 2007) where multiple individual-day samples were used per animal to predict a 6-d average DMI in sheep.

To further investigate the predictive capacities of these calibrations, test-set validation was completed on each of the data sets. The calibration (SEC and R^2_c) and validation accuracies (SEV, R^2_v, bias, and RPD) for these equations are reported in Table 2.17. The R^2_c values ranged from 0.52 for the prediction of trial DMI with

Table 2.16. Summary statistics for calibration and cross-validation of NIRS prediction equations for dry matter intake (DMI, g/BW$^{0.75}$) based on composite and individual-day fecal samples from the combined growing cattle data set (Trials 1-6; 8-12) and the combined growing heifer and pregnant female data set (Trials 4, 5, 7, 8, 13, and 14)

					Calibration[2]		Validation[3]	
DMI, g/BW$^{0.75}$	Trials	N	Mean ± SEL	Outliers[1]	SEC	R^2_c	SECV	R^2_{cv}
Composite fecal samples								
Trial DMI	1-6;8-12	408	111.0 ± 1.06	5	10.9	0.69	11.1	0.67
Fecal-collection-period DMI	1-6;8-12	408	109.1 ± 1.18	20	11.3	0.76	11.8	0.73
Individual-day fecal samples								
Trial DMI	4,5,7,8,13,14	174	126.8 ± 1.75	5	14.9	0.49	15.9	0.42
Fecal-collection-period DMI	4,5,7,8,13,14	174	135.2 ± 2.71	6	17.8	0.67	20.7	0.56

[1] Outliers were identified as having a "GH" statistic > 8.0 or a "T" statistic > 2.5 and were not included in the calibration equation.
[2] Calibration included 100% of the samples in the data set.
[3] Validation accomplished using cross validation.
SEL: standard laboratory error; SEC: standard error of calibration; R^2_c: coefficient of determination for calibration; SECV: standard error of cross validation; R^2_{cv}: coefficient of determination for cross validation.

Table 2.17. Summary statistics for calibration and test-set validation of fecal NIRS predictive equations for dry matter intake (DMI, g/$BW^{0.75}$) based on composite and individual-day fecal samples from the combined growing cattle data set (Trials 1-6; 8-12) and the combined growing heifer and pregnant female data set (Trials 4, 5, 7, 8, 13, and 14)

		Calibration[1]			Validation[2]				Mean ± SD			
DMI, g/$BW^{0.75}$	Trials	N	SEC	R^2_c	N	SEV	R^2_v	RPD[3]	Observed	Predicted	Bias[4]	Diff[5]
Composite fecal samples												
Trial DMI	1-6;8-12	327	10.9	0.68	81	11.8	0.69	1.81	108.9 ± 21.3	108.9 ± 18.1	0.01	0.00
Fecal-collection-period DMI	1-6;8-12	327	10.5	0.78	81	15.1	0.65	1.78	112.0 ± 25.4	110.8 ± 18.5	1.20	-1.07
Individual-day fecal samples												
Trial DMI	4,5,7,8,13,14	140	15.8	0.52	34	15.1	0.40	1.30	124.8 ± 19.6	123.4 ± 13.3	1.40	-1.12
Fecal-collection-period DMI	4,5,7,8,13,14	140	18.2	0.68	34	32.9	0.48	1.42	140.6 ± 45.1	133.9 ± 28.5	6.72	-4.76

[1] Calibration developed with 80% of the samples in the data set.
[2] Validation was accomplished using test set validation with the remaining 20% of the data set.
[3] RPD: ratio performance deviation; the ratio of SEV to SD of the reference data; RPD = SD of reference data ÷ SEV.
[4] Bias: mean difference between observed and NIR predicted data; bias = (∑ reference data/N) – (∑ predicted NRS data/N).
[5] Diff. = ((Predicted ÷ Observed) - 1) × 100.
SEC = standard error of calibration; R^2_c = coefficient of determination for calibration; SEV = standard error of validation; R^2_v = coefficient of determination for validation.

individual-day fecal samples to 0.78 for the prediction of fecal-collection-period DMI using composite fecal samples. The test-set validation accuracies (R^2_v, and RPD) reported for this study were higher than the values reported by Tran et al. (0.58 and 1.97; 2010) where validation was completed on an independent data set with individual-day fecal samples in cattle. However, the current study is not directly comparable to Tran et al. (2010), as calibration was completed with 1,332 individual-day samples, and the validation data set consisted of only 75 individual-day samples.

Overall the equations developed in this study were limited in their abilities to predict individual animal DMI, as R^2_v and RPD values were low, but the developed equations did succeed in predicting the mean DMI ($g/BW^{0.75}$), as no significant differences were found between the observed and fecal NIRS predicted DMI values, as *P* was > 0.05 for all equations. The relationship between observed and fecal NIRS predicted fecal-collection-period and trial DMI values for the growing cattle and pregnant female validation sets are illustrated in Figure 2.6.

For this study, equations developed with composite fecal samples showed improved validation accuracies (R^2_v) compared to those developed with individual-day fecal samples for the prediction of trial DMI (0.69 vs. 0.40; respectively) and fecal-collection-period DMI (0.65 vs. 0.48; respectively). Additionally, while the reported R^2_{cv} demonstrated an increased ability of composite fecal samples to predict fecal-collection-period DMI compared to trial DMI (0.73 vs. 0.68; respectively) the R^2_v accuracies were conflicting as the reported R^2_v for trial and fecal-collection-period DMI were

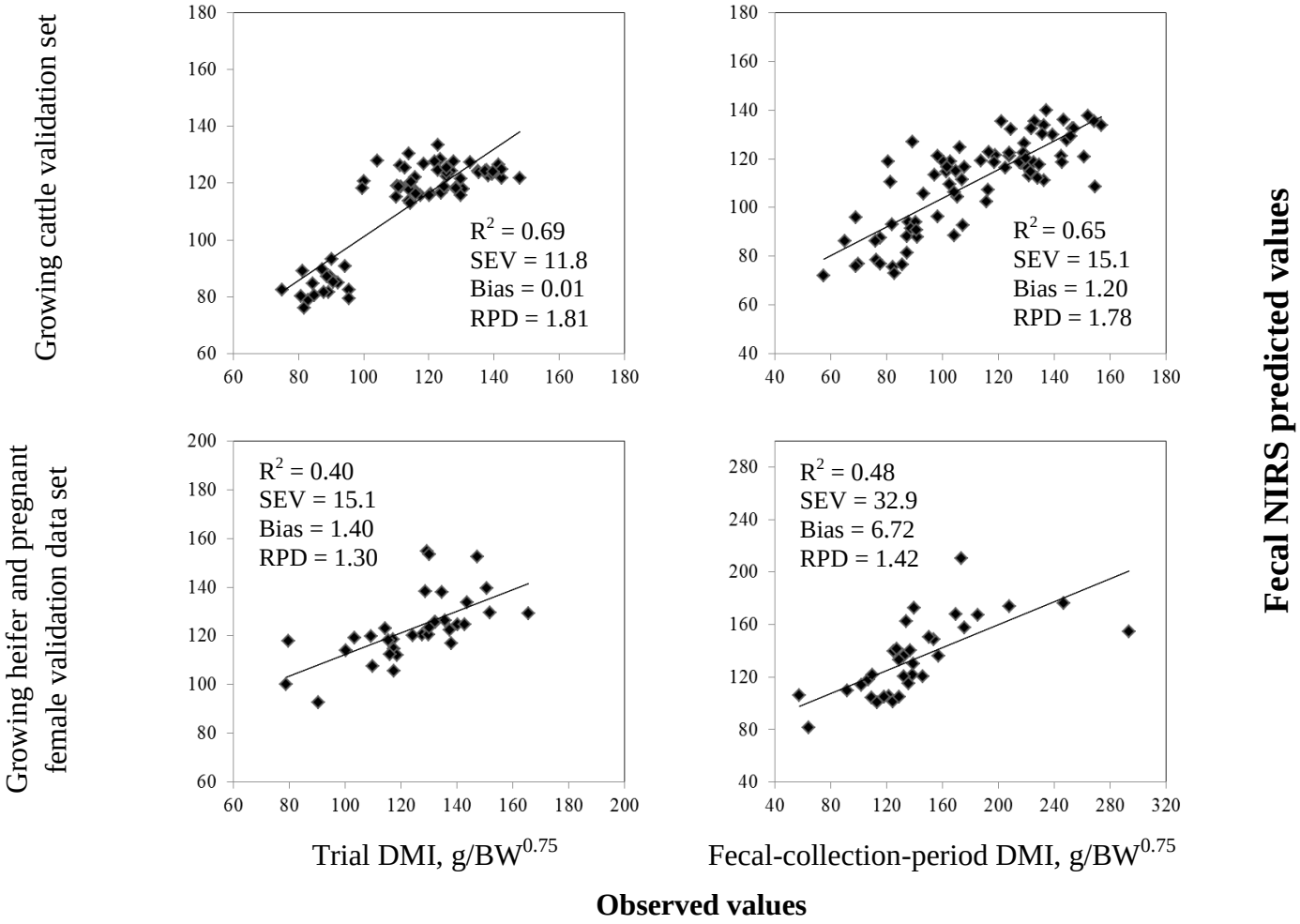

Figure 2.6 Observed values vs. fecal NIRS predicted values for trial DMI and fecal-collection-period DMI ($g/BW^{0.75}$) from the growing cattle validation set that utilized composite fecal samples, and the growing heifer and pregnant female validation set that utilized individual-day fecal samples.

0.69 and 0.65, respectively. These results suggest that composite fecal samples have the highest capacity for predicting DMI of independent data sets, but do not indicate the number of individual-day samples necessary for a composite to provide the most robust equation as composite size varied across trials.

To evaluate the effect of composite size on the performance of fecal NIRS equations, individual-day fecal samples from trials 4, 5, 7, and 8 were used. Calibration equations were developed by mathematically averaging the spectra from 2, 3, 4, or 5 individual days for each animal to develop 2-d, 3-d, 4-d, and 5-d composite samples. The mathematically averaged composites were then used to develop equations for the prediction of fecal-collection-period DMI. The summary statistics of fecal NIRS equations for the prediction of fecal-collection-period DMI with mathematically averaged composites are presented in Table 2.18. The calibrations were developed with 100% of samples in each data set, and cross-validation was used to evaluate their performance. The calibration and cross-validation accuracies (R^2_c and R^2_{cv}) ranged from 0.45 and 0.36 for the 2-d composites to 0.79 and 0.55 for the 4-d composites. There was a significant improvement found in the calibration and validation accuracies (R^2_c and R^2_{cv}) between the 3-d and 4-d composites, which may indicate that a 4-d composite is sufficient for predicting the fecal-collection-period DMI, but due to a limited sample size, these results are inconclusive.

While results from this study were comparable, if not superior, to previously reported data, the equations developed for the prediction of DMI failed to provide an

Table 2.18. Summary statistics for calibration and cross-validation of fecal NIRS prediction equations for fecal-collection-period DMI (g/$BW^{0.75}$) in growing heifers and pregnant females with mathematically averaged composite fecal samples

			Fecal-collection-period DMI		Calibration[2]		Validation[3]	
Item	Trials	N	Mean ± SEL	Outliers[1]	SEC	R^2_c	SECV	R^2_{cv}
2-d composite	4,5,7,8	114	130.9 ± 2.03	7	15.8	0.45	16.9	0.36
3-d composite	4,5,7,8	114	130.9 ± 2.03	6	13.8	0.52	14.7	0.46
4-d composite	4,5,7,8	114	130.9 ± 2.03	3	10.5	0.79	15.3	0.55
5-d composite	4,5,7,8	114	130.9 ± 2.03	6	13.8	0.55	14.7	0.49

[1] Outliers were identified as having a "GH" statistic > 8.0 or a "T" statistic > 2.5 and were not included in the calibration equation.
[2] Calibration included 100% of the samples in the data set.
[3] Validation accomplished using cross validation.
SEL = standard laboratory error; SEC = standard error of calibration; R^2_c = coefficient of determination for calibration; SECV = standard error of cross validation; R^2_{cv} = coefficient of determination for cross validation.

acceptable predictive equation based on the recommendations by Westerhause (1989), Williams (2005), or Li et al. (2007), as R^2_v values were < 0.80, RPD values were < 3.0, and SEC values were > than 2 × the SEL values. However, these recommendations may not be applicable to fecal NIRS equations for the prediction of DMI as variations in intake can be caused by factors that are unobservable in fecal samples. Therefore, fecal NIRS equations may never be able to predict DMI with the same accuracy as equations developed for the prediction of diet characteristics such as CP or NDF.

When comparing the results from this study to those reported for mean DMI estimations based on the n-alkane technique, the Diff. values reported in this study were more accurate than the values reported by Ferreira et al. (18.3; 2004), Ferreira et al. (27.1; 2007), and Olivan et al. (-22.3; 2007), comparable to the values reported by Estermann et al. (-2.50; 2001), Mann and Stewart (-1.11; 2003), Premaratne et al. (-3.00; 2005), De Oliveira et al. (2.04; 2008), and Bezabih et al. (-5.04; 2012), and slightly less accurate than the values reported by Berry et al. (-0.20; 2000) and Hafla (0.48; 2013). These results indicate that current fecal NIRS prediction equations may be able to predict mean DMI with similar accuracy as the n-alkane technique.

This study also found similar accuracy of individual-animal DMI predictions with the use of fecal NIRS profiling compared to the n-alkane technique. To examine this, observed, fecal NIRS predicted, and n-alkane predicted DMI from Trial 7 were compared. The relationship between observed and fecal NIRS predicted DMI, and observed and n-alkane predicted DMI from Trial 7 are illustrated in Figure 2.7. The

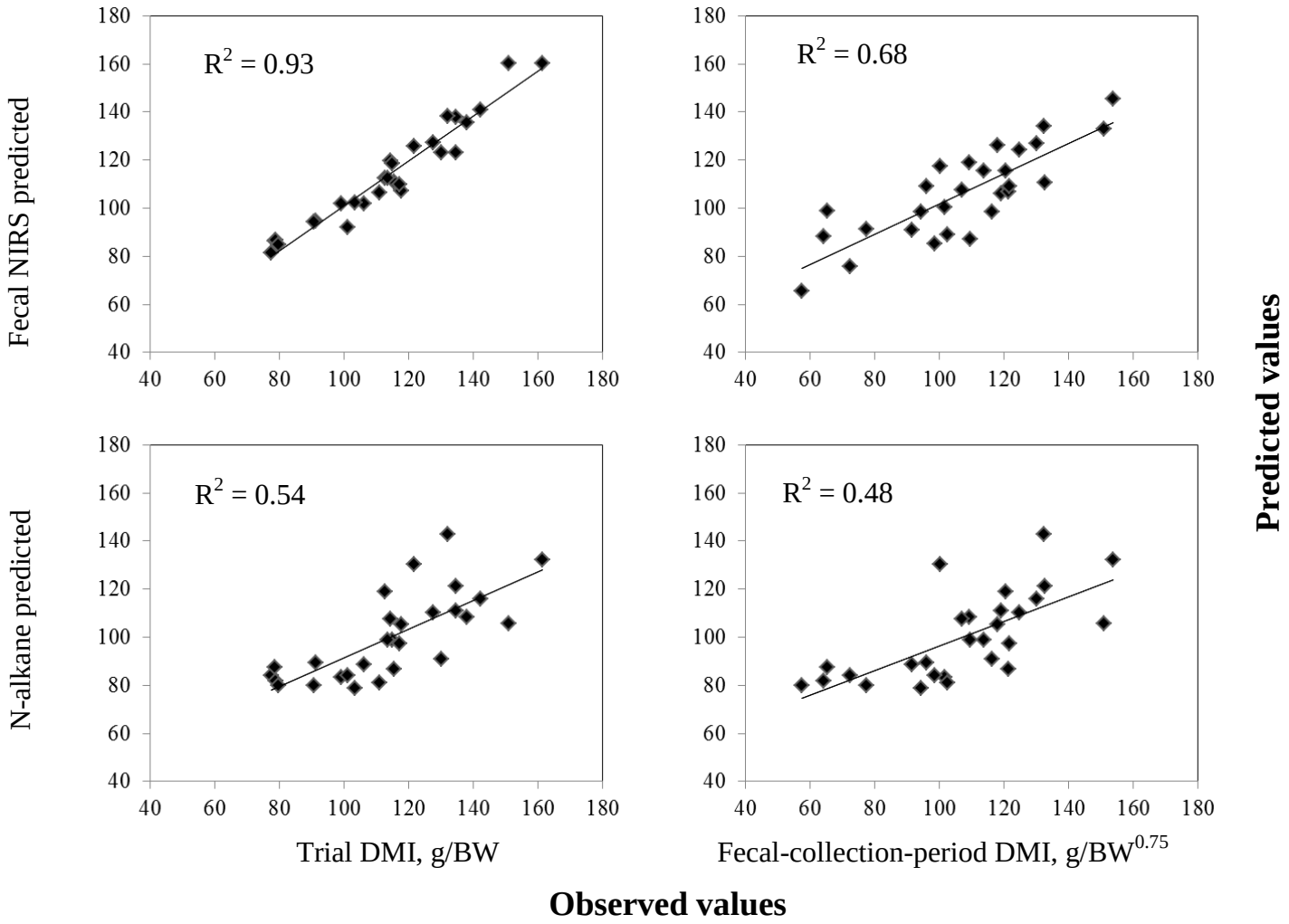

Figure 2.7 Observed vs. fecal NIRS predicted values and observed vs. n-alkane predicted values for trial DMI and fecal-collection-period DMI from pregnant females in Trial 7, based on composite fecal samples.

reported R^2 values suggest that for this trial, fecal NIRS predicted DMI were more capable of predicting individual-animal DMI compared to the n-alkane method. While the data set evaluated here is limited in terms of sample size (n = 30), the reported R^2 value for fecal-collection-period DMI is higher than the R^2 values reported by Molina et al. (0.54; 2004) and Olivan et al. (0.18; 2007), comparable to the value reported by De Oliveira et al. (0.66; 2008), and lower than the value reported by Berry et al. (0.72; 2000), where intakes were estimated with the n-alkane method. The R^2 reported for trial DMI by fecal NIRS in this study was higher than all of these trials, further indicating that fecal NIRS profiling is a comparable technique to the n-alkane method for determining individual-animal intake of cattle.

Overall, the capacity of fecal NIRS equations for the prediction of individual-animal DMI are limited, as the equations developed in this study reported calibration and validation R^2 values less than 0.90. However, the accuracies of the developed equations were comparable to results from the n-alkane technique for the prediction of group and individual-animal DMI. Furthermore, the full capacity of fecal NIRS profiling for the prediction of intake has yet to be obtained. Current calibrations have failed to compile large data sets (n > 2000), limiting the robustness of the calibration equations. Also, the enhancement of calibrations may be achieved through the use of software that will allow for trial effects to be blocked, as over 90% of the variation in fecal spectra is accounted for by trial effects. The results from a discriminate analysis, illustrating the variation in fecal spectra from trials 4, 5, and 6, due to the 1^{st}, 2^{nd}, and 3^{rd} principle components are presented in Figure 2.8. These trials were chosen for analysis as they represent 3

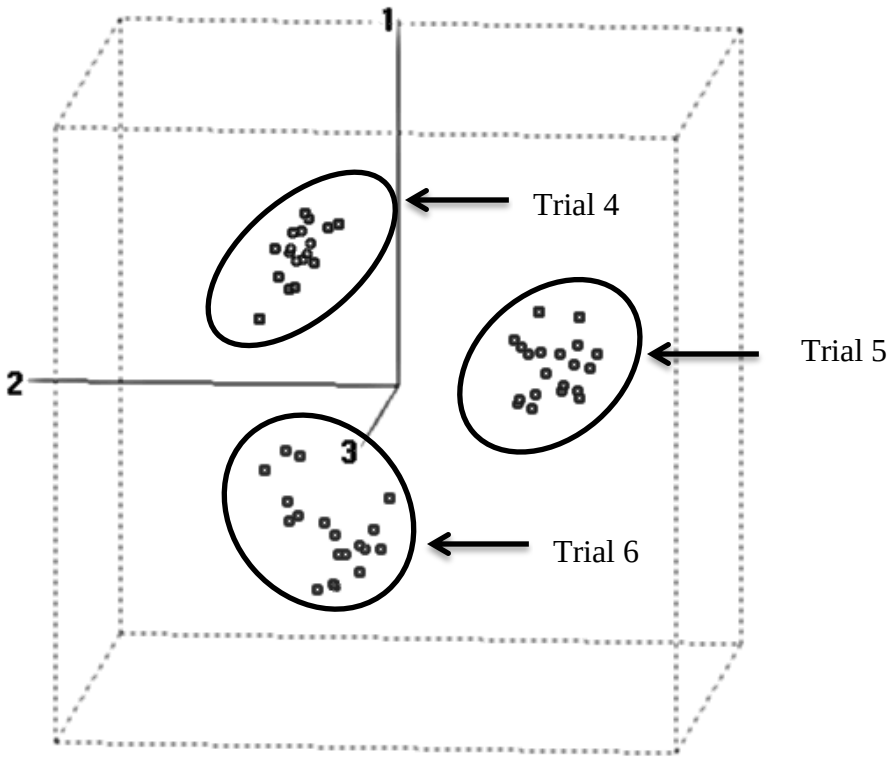

Figure 2.8 Discriminate analyses of fecal spectra from Trials 4, 5, and 6, representing the variation in fecal spectra resulting from the 1st, 2nd, and 3rd principle components, which represent 90% of the variation found between the fecal spectra across these trials. The observed variation is unrelated to breed, stage of production, ration, location, or season as these factors were unchanged across trials.

consecutive trials where breed of cattle, stage of production, ration, location, and season were similar across years. Therefore, the discriminate analysis emphasizes the variations existent in the fecal spectra of these animals unrelated to breed, stage of production, ration, location, or season, indicating that trial effects represent the largest variation in fecal spectra. Thus, an increased performance of calibration equations may be accomplished if trial effects were blocked, allowing the software to amplify the remaining 10% of variation in fecal spectra that would more likely be associated with individual animal variance in feed intake.

Prediction of residual feed intake by fecal NIRS profiling

To evaluate the ability of fecal NIRS equations to predict RFI of individual animals, calibration equations were developed using 11 growing cattle trials that utilized composite fecal samples, and 7 growing heifer and pregnant female trials that utilized individual-day fecal samples. For these trials, summary statistics of fecal NIRS prediction equations for RFI are presented in Table 2.19. The calibration and cross-validation accuracies (R^2_c and R^2_{cv}) for the equations developed for the prediction of RFI were poor ($R^2 < 0.80$), indicating that these equations have limited capacities to predict individual-animal RFI.

To improve the capacities of these equations, 11 growing cattle trials were combined to form 1 data set that utilized composite fecal samples, and 7 growing heifer and pregnant female trials that utilized individual-day fecal samples were combined to

Table 2.19. Summary statistics for calibration and cross-validation of fecal NIRS prediction equations for residual feed intake (RFI, kg d^{-1}) based on composite fecal samples from growing cattle (Trials 1-6; 8-9; 12) and individual-day fecal samples from growing heifers (Trials 4, 5, and 8) and pregnant females (Trials 7, 13, and 14)

		RFI, kg d^{-1}				Calibration[2]		Validation[3]	
Trial	N	Range	Mean	SEL	Outliers[1]	SEC	R^2_c	SECV	R^2_{cv}
Composite fecal samples									
1	57	-2.11-2.02	0.00	0.11	1	0.65	0.42	0.75	0.23
2	40	-2.01-1.89	0.08	0.18	0	0.53	0.78	0.91	0.36
3	20	-1.35-1.69	-0.13	0.23	0	0.79	0.43	1.14	0.17
4	18	-2.09-1.86	-0.09	0.27	0	0.42	0.86	1.31	0.24
5	20	-1.78-1.42	-0.03	0.23	0	0.67	0.57	0.95	0.12
6	20	-2.95-1.90	-0.32	0.33	1	0.72	0.75	1.34	0.24
8	46	-1.63-1.59	0.00	0.10	1	0.55	0.39	0.66	0.12
9	49	-1.07-1.32	0.02	0.08	4	0.42	0.40	0.48	0.22
10	30	-1.08-0.98	0.00	0.07	0	0.20	0.77	0.34	0.37
11	30	-1.21-0.81	0.00	0.08	0	0.38	0.29	0.48	0.08
12	80	-0.80-0.80	0.02	0.04	6	0.32	0.30	0.44	0.27
Individual-day fecal samples									
4[4]	18	-2.09-1.86	-0.09	0.27	0	0.99	0.23	1.33	0.28
5[4]	20	-1.78-1.42	-0.03	0.23	0	0.22	0.95	0.85	0.31
7[4]	30	-3.99-5.80	-0.14	0.41	0	1.71	0.47	2.14	0.23
8[4]	46	-1.63-1.59	0.00	0.10	2	0.40	0.67	0.66	0.12
13	31	-8.27-9.33	0.21	0.48	2	1.97	0.54	2.41	0.34
14	29	-4.07-3.96	-0.17	0.30	1	1.27	0.40	1.72	0.05

[1] Outliers were identified as having a "GH" statistic > 8.0 or a "T" statistic > 2.5 and were not included in the calibration equation.
[2] Calibration included 100% of the samples in the data set.
[3] Validation accomplished using cross validation.
[4] Individual-day fecal samples analyzed by fecal NIRS were those obtained on the third consecutive day of fecal collections.
SEL = standard laboratory error; SEC = standard error of calibration; R^2_c = coefficient of determination for calibration; SECV = standard error of cross validation; R^2_{cv} = coefficient of determination for cross validation.

form another data set. Calibration and cross-validation summary statistics of the combined trial fecal NIRS equations for RFI are presented in Table 2.20. The calibrations were developed with 100% of samples in each data set, and cross-validation was used to evaluate their performance. The calibration and cross-validation accuracies (R^2_c and R^2_{cv}) ranged from 0.05 and 0.03 for the equation developed with individual-day fecal samples to 0.15 and 0.07 for the equation developed with composite fecal samples. These accuracies further indicate that for this study, fecal NIRS was not successful in predicting RFI of individual-animals. Due to the low accuracies obtained for this data set, further validation was not completed. The calibration and cross-validation accuracies reported for the prediction of RFI in this study suggest that individual-animal RFI is not significantly correlated with the spectra from fecal samples.

While fecal NIRS prediction equations in this study were unable to provide accurate predictions of individual-animal RFI, an effect of RFI group on fecal NIRS predicted intakes was observed. To examine this, individual trial predictions of DMI for Trials 1-6, and 8-9 were combined to form a data set which consisted of growing animals fed similar rations. The MIXED procedure of SAS was used to evaluate the effect of RFI classification on observed and fecal NIRS predicted trial and fecal-collection-period DMI for this data set. The results from this analysis are presented in Table 2.21. Based on the reported values, fecal NIRS was able to identify significant differences in trial ($P = 0.03$) and fecal-collection-period ($P = 0.04$) DMI across low and high RFI groups. However, there was less divergence in fecal NIRS predicted DMI between RFI groups compared to observed DMI.

Table 2.20. Summary statistics for calibration and cross-validation of NIRS prediction equations for residual feed intake (RFI, kg d^{-1}) based on composite and individual-day fecal samples from the combined growing cattle data set (Trials 1-6; 8-12) and the combined growing heifer and pregnant female data set (Trials 4, 5, 7, 8, 13, and 14)

					Calibration[2]		Validation[3]	
Item	Trials	N	Mean ± SEL	Outliers[1]	SEC	R^2_c	SECV	R^2_{cv}
Composite fecal samples								
RFI, kg d^{-1}	1-6;8-12	408	0.00 ± 0.04	12	0.69	0.15	0.73	0.07
Individual-day fecal samples								
RFI, kg d^{-1}	4,5,7,8,13,14	174	-0.03 ± 0.18	15	1.19	0.05	1.22	0.03

[1] Outliers were identified as having a "GH" statistic > 8.0 or a "T" statistic > 2.5 and were not included in the calibration equation.
[2] Calibration included 100% of the samples in the data set.
[3] Validation accomplished using cross validation.
SEL = standard laboratory error; SEC = standard error of calibration; R^2_c = coefficient of determination for calibration; SECV = standard error of cross validation; R^2_{cv} = coefficient of determination for cross validation.

Table 2.21. Effect of RFI classification on observed and fecal NIRS predicted DMI of growing animals with composite fecal samples from Trials 1-6 and 8-9

Item	Low RFI	High RFI	SE	*P*-value
No. animals	95	100	---	---
Observed intake, g/BW$^{0.75}$				
Trial DMI	110.8	135.9	0.91	0.01
Fecal-collection-period DMI	115.4	133.3	1.75	0.01
Fecal NIRS predicted DMI[1], g/BW$^{0.75}$				
Trial DMI	121.9	124.6	0.84	0.03
Fecal-collection-period DMI	123.2	126.8	1.20	0.04

[1] Individual trial calibration equations were used to predict DMI from Trials 1-6 and 8-9.

To further examine this, fecal NIRS predictions of DMI from Trial 7 were evaluated along with observed and n-alkane predicted DMI. The effect of RFI classification on observed, fecal NIRS predicted, and n-alkane predicted DMI are presented in Table 2.22. For this trial, significant differences were found for observed, fecal NIRS predicted, and n-alkane predicted DMI, with observed and fecal NIRS predicted DMI demonstrating the most significance across RFI groups. These results indicate that fecal NIRS technology may be a useful tool for identifying groups of animals with divergent RFI, with results comparable to the n-alkane method.

Conclusion

Results from this study provide further evidence that fecal NIRS profiling is a capable tool for the prediction of diet quality and digestibility. While the reported calibration equations were not robust enough for the accurate validation of independent data sets, they do illustrate the existence of significant correlations between diet characteristics and fecal spectra. The prediction of voluntary DMI in this study proved to be less accurate as calibration and validation statistics were inferior to those obtained for the prediction of CP, NDF, or DMD. However, the ability of fecal NIRS equations to predict individual-animal DMI and mean DMI of groups was comparable to results reported for intake predictions by the n-alkane technique. Further research will be necessary to develop larger, more robust calibration equations for the prediction of

Table 2.22. Effect of RFI classification on observed, fecal NIRS predicted, and n-alkane predicted DMI of pregnant females in Trial 7 with composite fecal samples

Item	Low RFI	High RFI	SE	P-value
No. animals	13	15	---	---
Observed intake, g/BW$^{0.75}$				
Trial DMI	98.8	127.5	4.41	0.01
Fecal-collection-period DMI	92.9	120.5	5.10	0.01
N-alkane predicted DMI	92.9	106.2	4.36	0.05
Fecal NIRS predicted intake, g/BW$^{0.75}$				
Trial DMI	99.5	126.9	4.30	0.01
Fecal-collection-period DMI	98.1	115.6	4.38	0.01
N-alkane predicted DMI	93.1	106.2	4.21	0.04

intake, but the results obtained in this study show evidence that improved fecal NIRS calibrations may be used as an alternative to the n-alkane technique for the prediction of intake for individual-animals and groups of animals. This study also found fecal NIRS profiling to be capable of predicting DMI in growing cattle and pregnant females for the evaluation of divergent RFI groups. Although, the ability of fecal NIRS to directly predict RFI appears limited as no accurate equation was developed in this study.

CHAPTER III

LABELED SUPPLEMENT N-ALKANE PREDICTED INTAKE OF MID-GESTATION HEIFERS WITH DIVERGENT POSTWEANING RESIDUAL FEED INTAKE

Introduction

Of the quantifiable factors pertaining to animal productivity, intake is the most variable and difficult to accurately determine. For confined animals, accurate and reliable measures of direct individual animal intake can be achieved through the use of specialized feeding systems such as Calan-gate feeders™ or the GrowSafe™ system. However, for grazing animals, direct individual animal intake cannot be measured, and must instead be estimated. Current techniques for estimating DMI of grazing animals can be based on herbage disappearance, prediction models, the use of internal and external markers, or the use of fecal near infrared reflectance spectroscopy (fecal NIRS; Macoon et al., 2003; Undi et al., 2008).

Long chain hydrocarbons in plant cuticular wax, especially n-alkanes, have been examined for their use in predicting intake of animals since the late 1980s (Mayes et al. 1986; Dove and Mayes, 1991; Dillon, 1993; Smit et al., 2005; Keli et al 2008). Accurate intake estimates using the n-alkane method have been reported for groups of ruminants (Mann and Stewart, 2003; Premaratne et al., 2005). However, results for individual animal intake prediction are less accurate due largely to variations in fecal recovery rates for adjacent alkanes and dosing precision. Intra-ruminal control release devices (CRD)

were developed to increase the accuracy of individual animal intake estimations by providing a device that allows for a single dose rate to be delivered continually for all animals. The use of these devices increased the dosing precision, which reduced the diurnal variability in the ratio of adjacent alkane concentrations, allowing for improved intake estimations without the need for total fecal collections or laborious dosing procedures. However, the production of intra-ruminal CRD was ceased by its manufacturer (Captec, Auckland, New Zealand) in 2008. Therefore, alternative methods are now used and being developed to accurately administer n-alkanes to animals.

An alternative approach involving the use of n-alkane labeled supplements has recently been examined, as supplements are often fed and could possibly be fed in known amounts to grazing livestock, which would eliminate the need for separate dosing of alkanes to individual animals (Charmley and Dove, 2007). When supplement intake is known, the n-alkane method can be used to estimate forage intake by evaluating the n-alkanes in the roughage, supplement, and feces of an animal. Previous studies have demonstrated that when the amount of supplement intake is known, the proportion of supplement in the diet can be estimated, and roughage intake can be estimated by multiplying the total intake by the estimated roughage proportion of the diet (Dove et al., 2002; Charmley and Dove, 2007). However, for this approach to work, supplements must contain a distinct alkane profile, or be labeled with an alkane source, and fecal recovery rates must be known or estimated in order to correct for incomplete recoveries.

While the feeding of a supplement is more convenient then daily dosing, the laborious nature of total fecal collections may limit the application of this technique as

they are not applicable to large scale operations. For commercial operations, spot sampling of feces once or twice daily would be more likely. However, for spot sampling to be used, estimates of intake must be achieved without corrections for incomplete fecal recoveries, or general sets of fecal alkane recoveries must be made available so that separate measurements of alkane recoveries are not needed for each experiment.

Additionally, methods for administering known amounts of supplement to grazing cattle must be evaluated. Therefore, the objectives of this study were to evaluate the accuracy of the GrowSafe™ system in measuring supplement intake, and to evaluate the use of an n-alkane labeled supplement for the prediction of forage DMI in pregnant females without the use of total fecal collections.

Materials and methods

Animals and experimental design

Performance and feed intake was measured for 56 d on 12 Angus steers at the Beef Cattle Systems Research Center in Millican, TX during a preliminary trial to evaluate the accuracy of the GrowSafe™ system to quantify supplement intakes. During this trial, steers (initial BW = 318.0 ± 28.0 kg) were randomly assigned to 1 of 2 pens (6 steers per pen) each equipped with 4 electronic GrowSafe™ feedbunks (GrowSafe™ DAQ 4000E; GrowSafe™ system Ltd., Airdire, AB, Canada), and fed ad libitum chopped bermudagrass hay (1.96 Mcal ME/kg DM and 7.1% CP DM) in 2 feedbunks per pen, and ad libitum supplement containing 75% dried distillers grain and 25% salt in 1 feedbunk per pen. Hay supply weights were measured using feed truck load cells, and

supplement supply weights were measured using an Ohaus Defender™ 5000 scale. Supply weights were measured daily, and orts measured weekly throughout the 56 d trial. The daily hay and supplement supplies were used along with weekly ort measurements to calculate intake in order to evaluate the accuracy of the GrowSafe™ system in measuring hay and supplement intake.

Following the preliminary trial, performance and feed intake was measured for 70 d on 120 Nellore cross heifers at the McGregor Research Center in McGregor, TX. Heifers (initial BW = 228.6 ± 29.3 kg) were blocked by BW and randomly assigned to 1 of 2 pens (60 heifers per pen) each equipped with 10 electronic GrowSafe™ feedbunks (GrowSafe™ DAQ 4000E; GrowSafe™ system Ltd., Airdire, AB, Canada) and adapted to a roughage diet for 7 d. During the 70 d feeding period, heifers were fed ad libitum twice daily a diet (2.15 Mcal ME/kg DM and 13.5% CP DM) consisting of 35% chopped alfalfa, 22% cottonseed hull, 20% cracked corn, 15% alfalfa pellets, 7% molasses, and 2% premix. Body weight and temperament were measured at 14 d intervals.

At the conclusion of the 70 d postweaning trial, heifers were ranked by RFI and bred by natural serve at the McGregor Research Center in McGregor, TX. Following rectal palpation to determine pregnancy, mid-gestation heifers with the lowest (n = 13) and highest (n = 12) RFI were identified for use in the subsequent study. The identified heifers were blocked by BW and RFI and randomly assigned to one of two pens (n = 12 in pen 1, n = 13 in pen 2) each equipped with 10 electronic GrowSafe™ feedbunks (GrowSafe™ DAQ 4000E; GrowSafe™ system Ltd., Airdire, AB, Canada). For each pen, heifers were fed ad libitum sorghum hay (1.94 Mcal ME/kg DM and 9.20% CP

DM; Table 3.3) in 6 feedbunks and ad libitum supplement (2.66 Mcal ME/kg DM and 16.6% CP DM) in 2 feedbunks. For the first 27 d of the 56 d trial, a non n-alkane labeled supplement was fed, and for d 28 through 56 an n-alkane labeled supplement was fed. Forage and supplement intake data was collected daily, and orts and BW were measured at 7 d intervals during the 56 d trial.

Preparation of labeled supplement

During the pregnant heifer trial, n-alkanes were administered to the animals using the labeled supplement approach. Since animals were fed ad libitum, the labeled supplement was formulated to restrict intakes and consisted of 35.0% corn meal, 27.8% wheat middling, 17.2% CSM, 15% malic acid, 4% molasses, 1% beeswax, 0.03% synthetic C32, and 0.013% Rumensin (2.66 Mcal ME/kg DM and 16.6% CP DM). To label the supplement, beeswax was frozen with liquid nitrogen, finely ground, and combined with synthetic C32 and a small proportion of corn meal. The corn meal was used as a carrier to increase the uniformity of beeswax and synthetic C32, and was therefore, mixed with the ground beeswax and C32 prior to being combined with the remaining ingredients in a premix mixer.

Collection and preparation of fecal and forage samples

Starting on d 44 of the 56 d pregnant heifer trial, fecal samples were collected by rectal palpation at 0800 daily for 6 consecutive d from each of the 25 experimental animals, and immediately frozen at -20 ºC. Fecal samples were then dried at 60 ºC in a forced air oven, ground through a 1-mm screen in a cyclone sample mill, and

composited by combining equal amounts of sample from each of the individual-day samples available per animal. Forage, supplement, and ort samples were collected weekly from each pen. The forage samples were composited by weight, resulting in 1 sample for the trial.

N-alkane analysis

To determine the n-alkane concentrations in the feces, forage, and labeled supplement, a gas chromatography system (Agilent 6890N, Santa Clara, CA, USA) with auto sampler and Chemstation software (Agilent Technologies, Santa Clara, CA, USA) was used. To increase the accuracy of extractions, 0.20 g of fecal sample was used for animals that consumed low amounts of labeled supplement (< 2.0 kg), while the conventional 0.10 g of fecal sample was used for animals that consumed higher amounts of labeled supplement (> 2.0 kg).

Calculations of intake and digestibility

A 56 d average DMI was calculated for forage and supplement consumption by the 12 Angus steers in the preliminary trial. For the pregnant female trial, 2 average DMI values were used, an average DMI corresponding to the fecal collection period (6-d DMI) and an average DMI corresponding to the feeding period where labeled supplement was being fed (28-d DMI). The 6-d forage and supplement DMI was calculated as the average kg of DMI per d from d 43 to 48 of the pregnant heifer trial, and 28-d DMI was calculated as the average kg of DMI per d from d 30 to 56 of the pregnant heifer trial. To calculate the 6-d forage and supplement DMI average, the 6-d

forage DMI and 6-d supplement DMI were added together. The 6-d ME intake was calculated as the average Mcal of ME consumed per d based off of the 6-d forage DMI and 6-d supplement DMI averages for each individual animal.

Estimates of forage intake were made using alkane pairs and the equation described by Dove and Mayes (1991):

$$\text{Intake} = \left(\frac{Fi}{Fj}\right) D_j / (H_i - \frac{Fi}{Fj} H_j)$$

where H_i and F_i are the herbage and fecal concentrations of an odd-chain n-alkane; and H_j and F_j are herbage and fecal concentrations of an even-chained alkane, D_j is the amount of dosed even-chain alkane released per day.

Estimates of supplement intake were made using alkane pairs as described by Dove and Mayes (1991), with modifications:

$$\text{Intake} = \left(\frac{Fi}{Fj}\right) D_j / (C_i - \frac{Fi}{Fj} C_j)$$

where C_i and F_i are the concentrate and fecal concentrations of an odd-chain n-alkane; and C_j and F_j are concentrate and fecal concentrations of an even-chained alkane, D_j is the amount of dosed even-chain alkane released per day.

Estimates of digestibility were determined from the relative concentrations of the 2 endogenous alkanes (C_{31} and C_{33}) in the feed and feces using the following equation:

$$\text{Dry matter digestibility (DMD)}, \% = (1 - \left(R_i \left(\frac{Hi}{Fi}\right)\right)) \text{ x } 100$$

where H_i and F_i are the herbage and fecal concentrations of an odd-chain n-alkane and R_i is the fecal recovery rate of the odd-chained alkane. Fecal recovery rates 0.75 and 0.73 for C_{31} and C_{33}, respectively were obtained from a previous study where cattle were fed tropical forages (Bezabih et al., 2012).

Feeding behavior traits

Feeding behavior traits were measured daily for each individual animal and averaged across all 56-d during the preliminary trial and across the 28-d period corresponding to the feeding period where labeled supplement was fed during the mid-gestation heifer trial. A subroutine of the GrowSafe™ system (DAQ 4000E; version 9.25), Process Feed Intakes (v. 7.29) was used to calculate feed intake and bunk visit data. The bunk visit frequency, bunk visit duration, mean DMI, and eating rate were then evaluated for both trials. The bunk visit frequency was defined as the number of bunk visit events recorded daily for an animal regardless of whether feed was consumed or not, and bunk visit duration was defined as the length summation of all the bunk visit events per day. Eating rate was calculated as the average amount of DM consumed per minute within a meal event.

Statistical analysis

Residual feed intake (RFI) was calculated as the difference between actual and expected DMI from a phenotypic regression model of actual DMI on ADG and mid-test $BW^{0.75}$ (Koch et al., 1963). To further characterize RFI, standard deviations above and below the mean were used to group animals into high (> 0.5 SD), medium (± 0.5 SD), or low RFI (< 0.5 SD; Nkrumah et al., 2004). To evaluate the effect of RFI classification on performance and feed efficiency, the mixed procedure of SAS was used. To evaluate phenotypic correlations between RFI, performance, and feed efficiency, the CORR procedure of SAS was used.

For the pregnant heifer trial, the PROC TTEST procedure of SAS was used to evaluate significance between observed and n-alkane predicted intakes, and the mixed procedure of SAS was used to evaluate the effect of postweaning RFI classification on measured and n-alkane estimated DMI.

Evaluating the accuracy of GrowSafe™ measured intakes

To evaluate the accuracy of the GrowSafe™ 4000E system in measuring forage and supplement intakes, mean GrowSafe™ and calculated forage and supplement DMI were compared. The calculated forage and supplement DMI were calculated as the total dry matter feed supplied minus the total dry matter orts. The percentage difference between the two was then calculated with the following equation:

$$\text{Difference, \%} = \frac{\text{GrowSafe}^{\text{TM}}\ \text{measured} - \text{calculated}}{\text{GrowSafe}^{\text{TM}}\ \text{measured}}$$

The accuracy of GrowSafe™ intake measurements were also evaluated with data quality measurements provided by the GrowSafe™ 4000E system. These quality measurements include assigned feed disappearance (AFD), assigned feed supply (AFS), and accounted feed balance (AFB). Accounted feed disappearance is the percentage of feed that disappears from the bunks and is assigned to an animal. The AFS is the percentage of scale weight increases that are associated with the feed supply events, and the AFB is a ratio comparing the amount of assigned feed disappearances (total intakes) of all the animals in a pen and the total amount of feed that disappeared from the bunks in that pen. For GrowSafe™ intake measurements to be considered accurate, AFD should be greater than 90.0%, AFS should be greater than 85.0%, and AFB should be between 0.90 and 1.10.

Results and discussion

Preliminary feeding trial performance, efficiency, and GrowSafe™ measured intake

Summary statistics for data collected during the preliminary feeding trial are presented in table 3.1. The initial BW of steers at the start of the trial was 318 ± 28.0 kg, and ADG for the steers was 0.90 ± 0.12. The average 56-d forage DMI was 6.30 ± 0.77, and average 56-d supplement DMI was 2.23 ± 0.72.

Table 3.1. Summary statistics of performance, feed efficiency, and feeding behavior traits of Angus steers during the preliminary feeding trial

Trait[1]	Mean	Minimum	Maximum	SD	CV[2], %
No. of steers	12	-	-	-	
Performance					
Initial BW, kg	318	290	388	28.0	8.81
Final BW, kg	368	344	433	25.0	6.79
ADG, kg d^{-1}	0.90	0.73	1.10	0.12	13.3
56-d forage DMI, kg d^{-1}	6.30	4.81	7.21	0.77	12.2
56-d supplement DMI, kg d^{-1}	2.23	0.92	3.56	0.72	32.3
Feed efficiency					
F:G ratio, kg/kg	9.23	6.62	12.3	1.72	18.6
Feeding behavior					
Forage intake					
Bunk visit frequency, events d^{-1}	103	55.8	143	21.0	20.5
Bunk visit duration, min d^{-1}	184	142	233	31.7	17.3
Eating rate, g/min	37.4	28.5	47.2	5.13	13.7
Supplement intake					
Bunk visit frequency, events d^{-1}	25.5	15.9	43.0	8.64	33.9
Bunk visit duration, min d^{-1}	23.2	8.69	56.8	13.9	59.9
Eating rate, g/min	120	61.9	205	48.4	40.4

[1] Initial traits measured at d 0 of feeding trial, final traits measured on d 56 of feeding trial.
[2] Coefficient of variation = (SD ÷ Mean) × 100.

For the preliminary feeding trial, descriptive statistics of mean GrowSafe™ measured and mean calculated forage and supplement DMI are presented in Table 3.2. Based on the reported results, the GrowSafe™ 4000E system was able to accurately measure forage and supplement intake as the percent difference between mean GrowSafe™ measured and mean calculated DMI was 6.35% for forage DMI and 4.46% for supplement DMI. The AFD and AFB reported for this trial further indicated that the GrowSafe™ 4000E system was accurate in measuring forage and supplement intakes for the 56-d trial as the reported AFD values were greater than 95% and the AFB values were between 0.90 and 1.10 (Table 3.3).

While the AFS values were low for this trial, they do not indicate inaccurate measurements of intake, as the feeding supply events were not always registered by the GrowSafe™ 4000E system due to the slow rate of speed that feed was delivered to the bunks. For the GrowSafe™ system to accurately record feed supply events, feed must enter the bunk at a rate of 200 g per s, which was not accomplished during this trial resulting in the low AFS estimates. Therefore, based on the reported results, the GrowSafe™ 4000E system at the Beef Cattle Systems Research Center was able to accurately measure forage and supplement intake of Angus steers.

Table 3.2. Descriptive statistics of mean GrowSafe™ measured and mean calculated forage and supplement DMI of Angus steers at the Beef Cattle Systems Research Center during the preliminary feeding trial

	Mean, kg d^{-1}		
Item	GrowSafe™ measured	Calculated[1]	Difference[2], %
56-d Forage DMI	6.30	5.90	6.35
56-d Supplement DMI	2.24	2.34	4.46

[1] Calculated = (Total feed supplied – total orts) ÷ total number of animals.
[2] Difference = ((GrowSafe™ measured – calculated) ÷ GrowSafe™ measured) × 100.

Table 3.3. Summary statistics of data quality measurements for the GrowSafe™ measured forage and supplement intake of Angus steers at the Beef Cattle Systems Research Center during the preliminary feeding trial

Item	AFD[1], %	AFS[2], %	AFB[3]
56-d Forage intake	97.8	38.4[4]	1.09
56-d Supplement intake	97.5	30.9[4]	1.06

[1] AFD: Accounted feed disappearance is the percentage of feed that disappears from the bunks and is assigned to an animal; should be > 90.0%.
[2] AFS: Assigned feed supply is the percentage of scale weight increases that are associated with the feed supply events; should be > 85.0%.
[3] AFB: Accounted feed balance is a ratio comparing the amount of assigned feed disappearances (total intakes) of all the animals in a pen and the total amount of that disappeared from the bunks in that pen; should be between 0.90 and 1.10.
[4] AFS values are low for this trial because forage and supplement were not supplied at a fast enough rate, or in large enough quantities to be registered as feeding supply events by the GrowSafe™ system.

Heifer postweaning trial performance and efficiency

Summary statistics for data collected during the postweaning heifer feeding trial are presented in table 3.4. The initial BW of heifers at the start of the trial was 229 ± 28.4 kg, and the final BW of heifers at the conclusion of the 70-d trial was 286 ± 33.6. Average daily gain of heifers was 0.80 ± 0.20 and the average DMI was 6.80 ± 1.10. For this trial, phenotypic RFI ranged from -2.40 for the most efficient heifer to 2.10 for the least efficient heifer with a mean RFI of 0.00 ± 1.00.

The heifers with the lowest (n = 13) and highest (n = 12) postweaning RFI were identified for the subsequent pregnant heifer trial. Effects of postweaning RFI classification on performance and feed efficiency of growing heifers identified for subsequent pregnant heifer trial are presented in table 3.5. The low RFI heifers consumed 31.8% less feed compared to the high RFI heifers (7.06 vs. 10.35 kg d^{-1}; P = 0.01) and had 29.8% lower F:G (8.64 vs. 12.3 kg/kg; P = 0.01), while maintaining similar BW and ADG. Lancaster et al. (2009) and Hafla (2012) reported similar results with low RFI growing heifers consuming 15 to 19% less feed compared to high RFI heifers with no impact on performance.

Pregnant heifer feeding trial performance, efficiency, and GrowSafe™ measured intake

Summary statistics for data collected during the pregnant heifer feeding trial are presented in table 3.6. The initial BW of heifers at the start of the trial was 229 ± 28.4 kg, and the final BW of heifers at the conclusion of the 70-d trial was 286 ± 33.6. Average daily gain of heifers was 0.80 ± 0.20 and the average DMI was 6.80 ± 1.10. For

Table 3.4. Summary statistics of performance and feed efficiency of heifers during the postweaning heifer feeding trial

Trait[1]	Mean	Minimum	Maximum	SD	CV[2], %
No. of heifers	120	-	-	-	
Performance					
Initial BW, kg	229	171	297	28.4	12.4
Final BW, kg	286	217	383	33.6	11.8
ADG, kg d^{-1}	0.80	0.40	1.30	0.20	19.2
DMI, kg d^{-1}	6.80	4.10	9.20	1.10	16.2
Feed efficiency					
RFI, kg d^{-1}	0.00	-2.40	2.10	1.00	---
F:G ratio, kg/kg	8.60	5.10	16.2	1.90	21.8

[1] Initial traits measured at d 0 of feeding trial, final traits measured on d 56 of feeding trial.
[2] Coefficient of variation = (SD ÷ Mean) × 100.

Table 3.5. Effects of postweaning residual feed intake classification on performance and feed efficiency of growing heifers identified for subsequent pregnant heifer trial

Trait[1]	Low RFI	High RFI	SE	*P*-value
No. of heifers	13	12	-	-
Performance				
Initial BW, kg	234.6	227.4	8.37	0.54
Final BW, kg	292.3	287.9	9.69	0.74
ADG, kg d^{-1}	0.82	0.86	0.04	0.47
DMI, kg d^{-1}	7.06	10.35	0.15	0.01
Feed efficiency				
RFI, kg d^{-1}	-1.44	1.75	0.13	0.01
F:G ratio, kg/kg	8.64	12.3	0.41	0.01

[1] Initial traits measured at d 0 of feeding trial, final traits measured on d 70 of feeding trial; RFI = residual feed intake.

Table 3.6. Summary statistics of performance and feeding behavior traits of mid-gestation heifers during the pregnant heifer feeding trial

Trait[1]	Mean	Minimum	Maximum	SD	CV[2], %
No. of steers	25	---	---	---	---
Performance					
Initial BW, kg	351	298	419	34.7	9.88
Final BW, kg	395	330	466	41.2	10.4
ADG, kg d^{-1}	0.70	0.20	1.20	0.22	29.8
28-d forage DMI, kg d^{-1}	6.75	4.99	8.39	0.97	14.4
6-d forage DMI, kg d^{-1}	6.30	4.41	8.14	1.04	16.5
28-d supplement DMI, kg d^{-1}	2.11	0.17	4.09	1.05	49.8
6-d supplement DMI, kg d^{-1}	1.89	0.14	3.58	0.99	52.7
Feeding behavior					
Forage intake					
Bunk visit frequency, events d^{-1}	90.5	54.3	159	23.1	25.5
Bunk visit duration, min d^{-1}	187	113	265	44.4	23.8
Eating rate, g/min	41.9	31.8	62.8	7.93	18.9
Supplement intake					
Bunk visit frequency, events d^{-1}	29.4	3.05	67.1	14.6	49.7
Bunk visit duration, min d^{-1}	59.4	3.11	100	24.1	40.6
Eating rate, g/min	40.7	7.47	73.8	15.7	38.6

[1] Initial traits measured at d 0 of feeding trial, final traits measured on d 56 of feeding trial.
[2] Coefficient of variation = (SD ÷ Mean) × 100.
[3] F:G ratio = (6-d forage and supplement DMI) ÷ ADG.

this trial, phenotypic RFI ranged from -2.40 for the most efficient heifer to 2.10 for the least efficient heifer with a mean RFI of 0.00 ± 1.00.

The accurate measurement of forage and supplement intake reported in the preliminary trial provided confidence that the GrowSafe™ 4000E system could be used to measure forage and supplement intake. However, similar results were not obtained with the GrowSafe™ 4000E system located at the McGregor Research Center during the pregnant heifer feeding trial. Descriptive statistics of mean GrowSafe™ measured and mean calculated DMI for this trial are presented in Table 3.7. Based on the reported results, the GrowSafe™ 4000E system was not accurate at measuring 28-d forage and supplement intake during this trial as mean GrowSafe™ measured and mean calculated DMI was 13.3% different for 28-d forage DMI and 15.6% different for 28-d supplement DMI. While the GrowSafe™ 4000E system was more accurate in measuring the 6-d forage and supplement intake, overall the accuracy of the measured intakes for this trial were poor as AFS values were below 85% or AFB values were greater than 1.10, as presented in Table 3.8.

The inaccuracy of the GrowSafe™ 4000E system to measure forage and supplement intakes at the McGregor Research Center was likely due to several factors. One factor that may have impacted the variable results obtained across trial locations is the weather, as wind and rain can heavily influence the accuracy of data collected by the GrowSafe™ system. This affect was magnified across the trial locations for this study as the GrowSafe™ feedbunks are enclosed at the Beef Cattle Systems Research Center,

Table 3.7. Descriptive statistics of mean GrowSafe™ measured and mean calculated forage and supplement DMI of mid-gestation heifers at the McGregor Research Center during the pregnant heifer feeding trial

	Mean, kg d^{-1}		
Item	GrowSafe™ measured	Calculated[1]	Difference[2], %
28-d Forage DMI	6.75	5.85	13.3
6-d Forage DMI	6.30	5.58	11.4
28-d Supplement DMI	2.11	1.78	15.6
6-d Supplement DMI	1.89	1.76	6.88

[1] Traditional = (Total feed supplied – total orts) ÷ total number of animals.
[2] Difference = (GrowSafe™ measured – calculated) ÷ GrowSafe™ measured.

Table 3.8. Data quality statistics of the GrowSafe™ measured forage and supplement intake of mid-gestation heifers at the McGregor Research Center

Item	AFD[1], %	AFS[2], %	AFB[3]
28-d Forage intake	96.9	78.1	1.16
6-d Forage intake	96.8	86.2	1.13
28-d Supplement intake	94.4	60.5	1.19
6-d Supplement intake	95.0	67.3	1.08

[1] AFD: Accounted feed disappearance is the percentage of feed that disappears from the bunks and is assigned to an animal; should be > 90.0%.
[2] AFS: Assigned feed supply is the percentage of scale weight increases that are associated with the feed supply events; should be > 85.0%.
[3] AFB: Accounted feed balance is a ratio comparing the amount of assigned feed disappearances (total intakes) of all the animals in a pen and the total amount of that disappeared from the bunks in that pen; should be between 0.90 and 1.10.

while the feedbunks at the McGregor Research Center are not. Therefore, the GrowSafe™ system at the McGregor Research Center is more exposed to inclement weather, which may have had a negative impact on the accuracy of GrowSafe™ measured intakes.

Another factor that may have influenced the accuracy of intake measured by the GrowSafe™ 4000E system is the intake and feeding behavior of the mid-gestation heifers compared to that of the Angus steers in the preliminary trial. For forage intake, there was no numerical difference found between Angus steers and mid-gestation heifers for the intake or feeding behavior traits evaluated for this study. However, the mid-gestation heifers displayed increased between-animal variation for all traits associated with forage intake, which may have affected the accuracy of the GrowSafe™ measured forage intakes.

For supplement intake, the between-animal variance in bunk visit duration and eating rate associated with supplement intake was lower for Angus steers, but the mid-gestation heifers displayed an increased amount of between-animal variance in supplement intake. While the increased variance in supplement intake could be related to breed type differences, it is likely a consequence of using malic acid compared to salt for limiting supplement consumption. Regardless, the increased variance found may have had a negative impact on the accuracy of GrowSafe™ measured supplement intakes.

Additionally, a numerical difference was found between the bunk visit duration and eating rate of Angus steers and mid-gestation heifers. These results may indicate that mid-gestation heifers spent an increased amount of time sorting through the supplement

compared to the Angus steers in the preliminary trial. If this is the case, it would help to explain the decreased accuracy reported in GrowSafe™ measured supplement intakes during the mid-gestation heifer feeding trial, as the sorting of a supplement would increase the number of bunk disturbances that occur when an animal alters the scale weight by coming into contact with the feedbunks. The scale weight will increase when the animals are pushing down onto the feedbunks then decrease when the animal releases the pressure. When the scale weight decreases the GrowSafe™ 4000E system will record it as a consumption of feed, thus the sorting of feed will lead to an increased occurrence of inaccurate intake measurements. Additionally, the scale weight increases caused by the animals pushing down onto the feedbunks will increase the percentage of scale weight increases that are not associated with the feed supply event, therefore lowering the AFS value.

Predicted forage and supplement intake of pregnant heifers

The chemical composition and concentration of n-alkanes in the forage and labeled supplement fed to mid-gestation heifers during the pregnant heifer trial are presented in table 3.9. The concentrations of C_{31} and C_{33} of the sorghum hay in this study were similar to the concentrations reported by Hafla (2012). For the supplement in this study, the C_{31} concentration was similar to that reported by Elwert and Dove (2005), although lower compared to the labeled supplement used by Dove et al. (2002).

Table 3.9. Chemical composition and concentration of n-alkanes of forage and n-alkane labeled supplement of mid-gestation heifers

Item	Sorghum	Labeled supplement[1]
Chemical composition		
DM, %	94.0	90.3
ME, Mcal/kg DM	1.94	2.66
CP, % DM	9.20	16.6
NDF, % DM	52.2	15.4
ADF, % DM	37.6	6.20
Concentration of n-alkanes		
C31, mg/kg DM	48.9	131.8
C33 mg/kg DM	22.5	30.2

[1] Labeled supplement contained 35.9% corn, 27.8% wheat middling, 17.2% CSM, 4% molasses, 15% malic acid, 0.013% Rumensin, 1% beeswax, 0.03% synthetic C32.

The descriptive statistics for measured DMI of forage and supplement, and n-alkane predicted DMI of forage are presented in Table 3.10. Mean forage DMI predicted by C_{33}:C_{32} was 29.6% higher than measured 28-d forage DMI and 38.9% higher than measured 6-d forage DMI. The forage DMI predicted from C_{31} gave even greater overestimations, as it was 61.5% higher than measured 28-d forage DMI and 73.0% higher than measured 6-d forage DMI. The R^2 between measured forage DMI and forage DMI estimated by C_{31} and C_{33} were 0.16 and 0.01 for measured 28-d forage DMI, and 0.28 and 0.04 for measured 6-d forage DMI. The R^2 values reported in this study were low compared to values reported by Berry et al. (0.72; 2000), Molina et al. (0.54; 2004), De Oliveira et al. (0.66; 2008) and Halfa (0.61; 2013), and suggest that little to no correlation was found between measured and predicted forage intake in this study.

The effect of postweaning RFI classification on measured forage, supplement, and n-alkane predicted forage DMI of mid-gestation heifers is presented in table 3.11. Based on the reported values, significant differences were not observed across RFI groups for measured or predicted DMI. These results contrast those reported by Halfa (2012), as a significant difference between measured and n-alkane predicted forage DMI was found between pregnant Bonsmara females with divergent postweaning heifer RFI classification. However, for the pregnant Bonsmara female study animals consumed forage only, whereas for this study, animals consumed supplement and forage.

Discrepancies found between measured and n-alkane predicted DMI, and the lack of significance found between RFI groups in this study may have resulted from

Table 3.10. Descriptive statistics for measured intake of forage and supplement, and n-Alkane predicted DMI of forage in pregnant females

			Range		Difference[1], %	
Item	Mean	SD	Min	Max	28-d forage	6-d forage
No. animals	25	---	---	---	---	---
Measured DMI						
28-d forage DMI, kg d^{-1}	6.75	0.97	4.99	8.39	---	---
6-d forage DMI, kg d^{-1}	6.30	1.04	4.41	8.14	---	---
28-d supplement DMI, kg d^{-1}	2.11	1.05	0.17	4.09	---	---
6-d supplement DMI, kg d^{-1}	1.89	0.53	0.14	3.58	---	---
Alkane predicted forage DMI						
C_{31}:C_{32} Predicted forage DMI, kg d^{-1}	10.9	2.49	4.85	14.2	61.5	73.0
C_{33}:C_{32} Predicted forage DMI, kg d^{-1}	8.75	1.67	4.93	11.4	29.6	38.9

[1] Difference = percentage difference to measured forage intake; ((Alkane predicted intake – measured intake) ÷ measured intake) × 100.

Table 3.11. Effect of postweaning RFI classification on measured forage, supplement, and n-Alkane predicted forage DMI of pregnant females

	Postweaning RFI classification			RFI
Item	Low RFI	High RFI	SE	P-value
No. females	13	12	---	---
Measured DMI				
28-d forage DMI, kg d^{-1}	6.82	7.25	0.29	0.28
6-d forage DMI, kg d^{-1}	6.60	6.82	0.33	0.62
6-d supplement DMI, kg d^{-1}	1.75	1.93	0.28	0.65
Alkane Predicted DMI				
C_{31} Predicted DMI, kg d^{-1}	10.9	10.8	0.73	0.90
C_{33} Predicted DMI, kg d^{-1}	8.90	8.61	0.49	0.69

many factors. While the inaccurate measurements of forage intake by the GrowSafe™ could have been a factor, the mean calculated forage intake was lower than the mean GrowSafe™ measured intake. Consequently, it appears that more accurate measures of forage intake would have yielded even larger discrepancies between n-alkane predicted intakes. While this would alter the reported results, it is not likely a significant factor influencing the overall poor accuracy of intake predictions found for this study.

The inaccurate measures of supplement intake by the GrowSafe™ 4000E system may have had a greater influence on the accuracy of intake predictions as the daily dosing rate was calculated based on the average daily supplement consumption. While the mean GrowSafe™ measured 6-d supplement consumption was only 6.88% different compared to calculated 6-d supplement DMI, this may not be representative of individual animal 6-d supplement DMI. Based on feeding behavior data, it appeared that animals spent an increased amount of time sorting through the supplement bunks in this trial. However, the large between-animal variance found for bunk visit duration suggest that there was large between-animal variance in the amount of sorting of the supplement. While this could not be confirmed, it would lead to an increased variance in the accuracy of GrowSafe™ measured supplement intake for individual animals.

Additional factors appear to be related to the digestibility and recovery rate of alkanes, which are associated with the large between-animal variation observed in supplement intakes in this study. Figure 3.1 illustrates the relationship between measured 6-d supplement intake and the difference between measured 6-d and n-alkane predicted

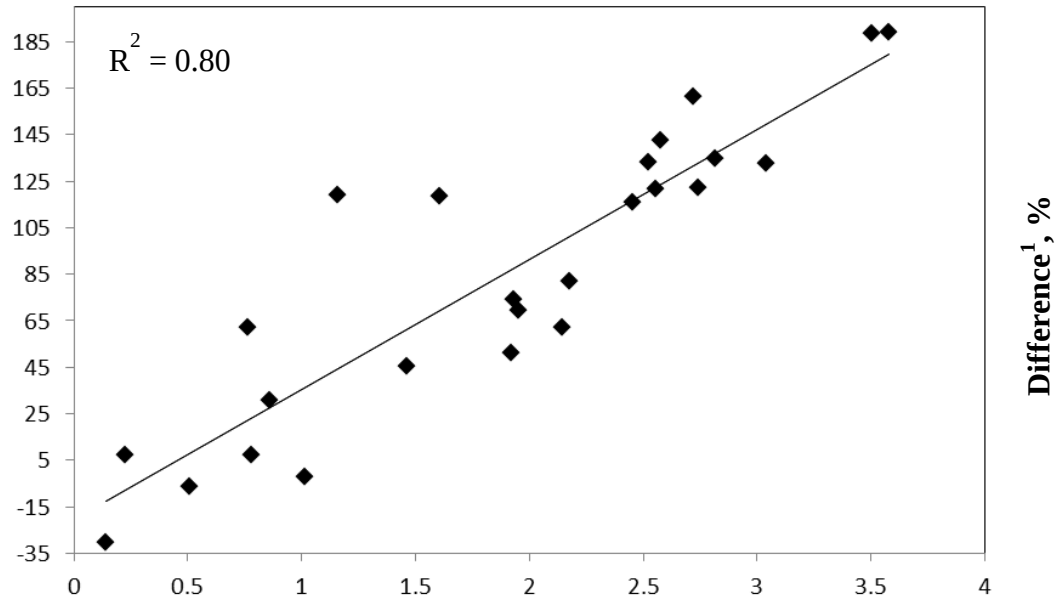

Figure 3.1 Relationship between measured 6-d supplement DMI and the difference between measured 6-d and n-alkane predicted forage DMI for individual animals.
[1] Difference = percentage difference to measured 6-d forage intake; ((Alkane predicted forage intake – 6-d measured forage intake) ÷ 6-d measured forage intake) × 100.

forage intake for individual animals. As illustrated, supplement intake was significantly correlated ($R^2 = 0.80$) with the accuracy of forage intake predictions by the n-alkane method in this trial. The decreased accuracy of intake predictions associated with increasing levels of supplement intake may be due to a variety of factors.

In this study, relationships were found between individual animal digestibility of C_{31} and both the proportion of supplement in the diet ($R^2 = 0.88$) and the accuracy of intake predictions ($R^2 = 0.70$). These results suggest that the increased supplement intake led to increased digestibility which influenced the accuracy of intake predictions in this study. However, the correlation between digestibility and the accuracy of intake predictions was not perfect, indicating that there were other factors influencing the results.

In a study be Elwert and Dove (2005), a linear relationship between the proportion of supplement in the diet and fecal recovery of alkanes was observed. The authors suggested that the beeswax alkanes present in the supplement had lower recoveries compared to the native Trifolium subterraneum hay. These results may suggest that fecal alkane recoveries were variable across animals in this study due to between-animal variations in supplement intake, with increased supplement intake resulting in lower fecal alkane recoveries. However, this relationship could not be evaluated in this study since total fecal collections were not accomplished.

Conclusion

The GrowSafe™ 4000E system was able to accurately measure forage and supplement intakes during the preliminary feeding trial. However, during the mid-gestation heifer feeding trial, accurate measurements of forage and supplement intakes were not obtained by the GrowSafe™ 4000E system. While there is no conclusive answer as to why the GrowSafe™ 4000E system failed to accurately measure intakes at the McGregor Research Center, the results do suggest that weather, supplement formulation, and feeding behavior may have affected the accuracy of intake measurements.

The inaccurate measurements of supplement and forage intake by the GrowSafe™ system in the pregnant heifer trial, along with the large between-animal variation found in supplement intake, appear to have influenced the accuracy of intake predictions by the n-alkane method in this trial. However, since individual pen feeding and total fecal collections were not accomplished, the exact influences cannot be determined. In conclusion, the labeled supplement method was not capable of accurately predicting forage intake of mid-gestation heifers for this study.

Further research would be necessary to determine if the GrowSafe™ 4000E system is capable of providing consistently accurate forage and supplement intake measurements, as well as to determine if the labeled supplement method is applicable for use in predicting forage intake with spot fecal samples and unknown fecal recovery rates.

CHAPTER IV

SUMMARY

The livestock industry continues to be challenged to meet the demand for animal protein sources in a cost effective and environmentally sustainable manner. Therefore, selection for lower maintenance energy requirements and(or) improved feed efficiency is necessary to reduce feed input cost and GHG emissions for beef production. Residual feed intake is a feed efficiency trait that if favorable selected for may decrease input cost and GHG emissions as previous studies have reported that low-RFI growing calves consumed 15 to 21% less feed (Herd et al., 2002; Lancaster et al., 2009), and produce 24 to 28% less methane (Nkrumah et al., 2006; Hegarty et al., 2007) compared to high-RFI calves with no impact on performance. However, adoption of technology to enable favorable selection for RFI has been limited by the absence of an affordable method to accurately measure intake of grazing animals. This study examined the use of two intake determination techniques, fecal NIRS profiling and n-alkane labeled supplementation, to predict individual animal DMI in order to identify animals with divergent RFI.

Results from the first study found that fecal NIRS profiling can be used for the prediction of dietary characteristics (R^2_c and $R^2_{cv} > 0.80$) , and while predictions of DMI were less accurate (R^2_c and $R^2_{cv} < 0.80$) , prediction equations were able to accurately predict mean DMI ($P > 0.05$) and individual-animal DMI for the identification of groups divergent in RFI ($P < 0.05$). Additionally, the reported accuracies for the prediction of individual-animal DMI by fecal NIRS were at least similar to those reported using the n-

alkane marker method. The accuracy and robustness of fecal NIRS profiling technology to predict intake can likely be improved with further calibration using larger data sets (n < 2000) and the use of statistical software capable of partitioning variation in fecal spectra attributable to trial effects..

In the second study, accurate DMI predictions were not obtained as 6-d forage DMI was overestimated by 73.0% when using C_{31}:C_{32} alkane pairs and by 38.9% when using C_{33}:C_{32} alkane pairs. Inaccurate measurements of supplement and forage DMI by the GrowSafe^{TM} system in this study, as well as large between-animal variation in supplement DMI, feeding behavior, and digestibility likely influenced the results of this study.

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