USE OF *N*-ALKANE AND FECAL NEAR INFRARED REFLECTANCE SPECTROSCOPY (fNIRS) METHODS AND TRADITIONAL PREDICTION EQUATIONS TO ESTIMATE INTAKE OF RFI-DIVERGENT BEEF CATTLE GRAZING ANNUAL AND PERENNIAL PASTURES

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By

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DEDICATION

This thesis is dedicated to my Papa, who shared Farmer Brown stories with us as kids and sparked a love for storytelling and the simpler days of back porch rocking chairs overlooking the farmyard. This man, along with my Mama, raised a family of five amazing, supportive and caring children, one who I am proud to call mom. While he wasn't necessarily a "career man", he was a jack of many trades from farming, to building vet clinics throughout the province, to starting a successful transportation company, and selling AI, and continued to volunteer on many committees into his retirement. His stories have inspired my will to continuously learn, and I think my ability to roll with the punches. I can only hope to build half the life he has.

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Je dois aussi remercier mon Marcel: thank you for telling me to eat or go outside when I became cranky or restless at my laptop, and for reminding me when it was time to get back to work. Thank you for patiently waiting for me to come home after long days on campus or at work, for taking care of me when I was exhausted or sick, and for keeping me sane. Thank you for putting our dream life on hold so that I could science. Here's to whatever is next!

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LIST OF ABBREVIATIONS

Abbreviation	Definition
1-VR	Variance ratio
AARD	Alberta Agriculture and Rural Development
ADF	Acid detergent fiber
ADG	Average daily gain
AM	Ante meridiem (before midday) Also refers to sample collected prior to grazing in Manuscript II
AOAC	Association of Official Analytical Chemists
BF	Backfat
BW	Body weight
$BW^{0.75}$	Metabolic body weight
C31	*n*-hentriacontane, $C_{31}H_{64}$
C32	*n*-dotriacontane, $C_{32}H_{66}$
C34	*n*-tetratriacontane, $C_{34}H_{70}$
CCAC	Canadian Council on Animal Care
CI	Confidence interval
CP	Crude protein
CRD	Controlled release device
d	Day
DDGS	Distiller's dried grains with solubles
Diff.	Difference; Percent difference between predicted and observed data
DM	Dry matter
DMSI	Standardized dy matter intake
DMY	Dry matter yield
DMI	Dry matter intake
EFI	Expected feed intake
EPD	Expected progeny difference
F:G	Feed to gain ratio
FAO	Food and Agriculture Organization
FCR	Feed conversion ratio
FE	Feed efficiency
fNIRS	Fecal near infrared reflectance spectroscopy
GC	Gas chromatography
GF	GreenFeed
GH	Global H statistic (Mahalonobis distance)
h	Hour(s)
h^2	Heritability
ha	Hectare
HD	Hand-dosed
HP	Heat production
KC	Kinsella Composite
KIN	Heifer from KRR
kg	Kilogram
KRR	Roy Berg Kinsella Research Ranch

LAC	Heifer from LRDC
LRDC	Lacombe Research and Development Centre
m	Meter
MBV	Molecular breeding value
Mcal	Megacalorie
ME	Metabolizable energy
MEI	Metabolizable energy intake
MIDWT	Body weight at the mid-point of the test period
MJ	Megajoule
mg	Milligram
mm	Millimeter
MPLS	Modified partial least squares
MSC	Multiplicative scatter correction
NDF	Neutral detergent fiber
NE	Net energy
NE_m	Net energy for maintenance
NIRS	Near infrared reflectance spectroscopy
nm	Nanometer
NRC	National Research Council
OMD	Organic matter digestibility
OMVI	Organic matter voluntary intake
P	P-value
PM	Post meridiem (after midday) Also refers to sample collected 7 hr into grazing in Manuscript II
r	Correlation coefficient
R^2	Coefficient of determination
R^2cv	Coefficient of determination for cross validation
R^2v	Coefficient of determination for test-set validation
RE	Retained energy
RFI	Residual feed intake
RFID	Radio frequency identification
RFI_{fat}	Residual feed intake adjusted for backfat
r_g	Genetic correlation coefficient
r_p	Phenotypic correlation coefficient
SAS	Statistical Analysis Software
SDMI	Actual standardized dry matter intake
SEC	Standard error of calibration
SECV	Standard error of cross validation
SEL	Standard error of the laboratory reference method
TDN	Total digestible nutrients

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ABSTRACT

Byron, Brittany Leigh. M.Sc, The University of Manitoba, 2018. Intake determination of RFI-divergent grazing beef cattle using the *n*-alkane and fecal near infrared reflectance spectroscopy (fNIRS) methods and traditional prediction equations.

Advisors: K.H. Ominski and J.A. Basarab.

The objectives of this research were to i) compare pasture intake of individual beef heifers via *n*-alkane, fecal near infrared reflectance spectroscopy (fNIRS; directly, or indirectly via fecal C31 and C32) and three equation-based methods; ii) examine the relationship between individual-animal pasture intake estimates and residual feed intake adjusted for fat (RFI_{fat}), as measured in a previous drylot test period; iii) compare dry matter intake (DMI) of low- and high-RFI_{fat} heifers as estimated using the aforementioned six intake methods, and iv) assess the suitability of swath-grazed triticale for *n*-alkane studies by examining its *n*-alkane and starch profiles, and morphology (via plant part weights). This knowledge is critical to understanding individual animal intake in non-confinement environments.

Methodology to assess perennial forage pasture intake is described in Manuscript 1. Groups of 20 pregnant heifers from two locations, Roy Berg Kinsella Research Ranch (KIN heifers) and Lacombe Research and Development Centre (LRDC; LAC heifers), ranked for RFI in a previous drylot period, grazed daily allocations of meadow bromegrass pasture at LRDC in 2015 and 2016. From Day 0 to 12, a C32-dosed pellet was offered to each heifer and on Days 0, 8 to 12 a fecal grab sample was collected. In addition, daily forage and pellet samples were collected for *n*-alkane analysis. Fecal subsamples from Day 8 to 12 were pooled for fNIRS analysis. Individual DMI was estimated via equation-based methods using forage quality data as

well as heifer weights recorded on Days 0, 8, 12 and end of test (approximately Day 59 of the pasture intake trial). DMI_{NRC} was least variable and greater in magnitude than DMI_{Alkane}, DMI_{fNIRS} and $DMI_{fNIRSC31C32}$. While RFI_{fat} was correlated with direct or indirect fNIRS methods ($P < 0.05$) for LAC 2015 heifers only, DMI_{Alkane} was correlated to RFI_{fat} in 2015 ($P < 0.05$) but not in 2016. However, none of the equation-based DMI methods differed significantly between RFI_{fat} groups, suggesting that these methods are not able to differentiate between animals when intake varies based on factors other than BW or forage quality. The *n*-alkane method shows most promise to estimate DMI of RFI-divergent grazing animals.

The potential to estimate annual forage swath intake was assessed in Manuscript 2. Groups of 18 or 12 beef cows grazed triticale swaths over 15 days in each of two years (Trial 1 and Trial 2), respectively. Daily forage samples were collected in both trials, and in Trial 2 additional triticale samples were collected and separated into plant parts for *n*-alkane analysis. Trial 1 starch and C31 concentrations were lower in PM compared to AM samples ($P \leq 0.0002$), suggesting selective grazing. In Trial 2, weight of plant parts (head, leaf/stem and head, leaf and stem) as well as C31 and C32 concentrations in plant parts differed ($P < 0.0001$). Collectively, these results suggest that differences in *n*-alkane profile between plant parts paired with selective grazing of plant material may limit the ability to estimate DMI of cattle grazing swathed triticale forage.

Keywords: Residual feed intake, RFI, intake, GreenFeed, *n*-alkane, pasture, extended grazing swath, beef heifers and cows

FOREWARD

This thesis is written in manuscript style, with each manuscript having its own abstract, introduction, materials and methods, results, discussion and conclusion. There is also a general introduction, literature review, discussion and conclusions, followed by the literature cited. None of the manuscripts have been submitted for publication at the time of thesis completion.

1. GENERAL INTRODUCTION

As global population increases, the global demand for animal protein is expected to increase by up to 70% (Food and Agriculture Organization, 2009), while agricultural land area decreases (Herrero et al., 2009), and consumers and governments demand higher environmental standards. The agriculture industry, in particular the beef industry, which produces over 70% of the total Canadian agricultural methane emissions (Environment Canada, 2014), is faced with the challenge of increasing production while improving both economic and environmental sustainability. As input costs are expected to rise along with competition for valuable resources such as land, energy and water, producers must adopt new management practices in order to improve sustainability of beef production systems.

One avenue by which sustainability may be improved is via selection for efficiency of feed utilization through genetics (Basarab et al. 2013; Durunna et al. 2011b). Progress in this area is expected to decrease the beef sector's environmental impact, as efficient cattle have reduced feed intake (15-21%; Herd et al. 2002; Lancaster et al. 2009) while maintaining productivity (Herd and Bishop 2000; Wang et al. 2012; Basarab et al. 2013; Manafiazar et al. 2014) and producing less manure (Basarab et al. 2002) and less methane per unit of product (Nkrumah et al. 2006, 2014; Hegarty 2007; Basarab et al. 2013). Many studies have focused on improved feed efficiency and its impact in growing animals (Arthur et al. 2001; Basarab et al. 2003; Crowley et al. 2010). However, understanding the impact of selection on the performance of the cow herd, which accounts for 60-65% (Kaliel 2004) of maintenance requirements from the total beef production system, is imperative (Crowley et al. 2014). Previously, selection of replacement animals focused on maximizing outputs, through means such as maintaining fertility as well as increased meat yield and quality. More recent efforts have shifted toward reduction of

inputs, particularly feed costs, while maintaining production. This strategy may be more advantageous as Basarab et al. (2002) proposed a 5% improvement in feed efficiency had an economic impact four times greater than that of a 5% increase in average daily gain (ADG).

A key component to measuring feed efficiency in individual animals is accurate measurement of an animal's feed intake. Feed efficiency, when calculated using individual animal intake and paired with an understanding of nutrient utilization, will enable producers to more aptly meet the nutritional requirements of the animal, and thus optimize production. Feed costs, which are the largest variable input cost of production in Canada (Figure 1.1) may also be reduced through an improved understanding of feed intake and efficiency, thus improving the economic viability of the beef sector. Measurement of dry matter intake (DMI) of beef cattle on pasture is necessary to evaluate nutrient balance, understand grazing animal behavior, and improve pasture management, ultimately optimizing pasture production systems. Accurate measurement of individual animal intake, particularly on pasture, is essential for improved

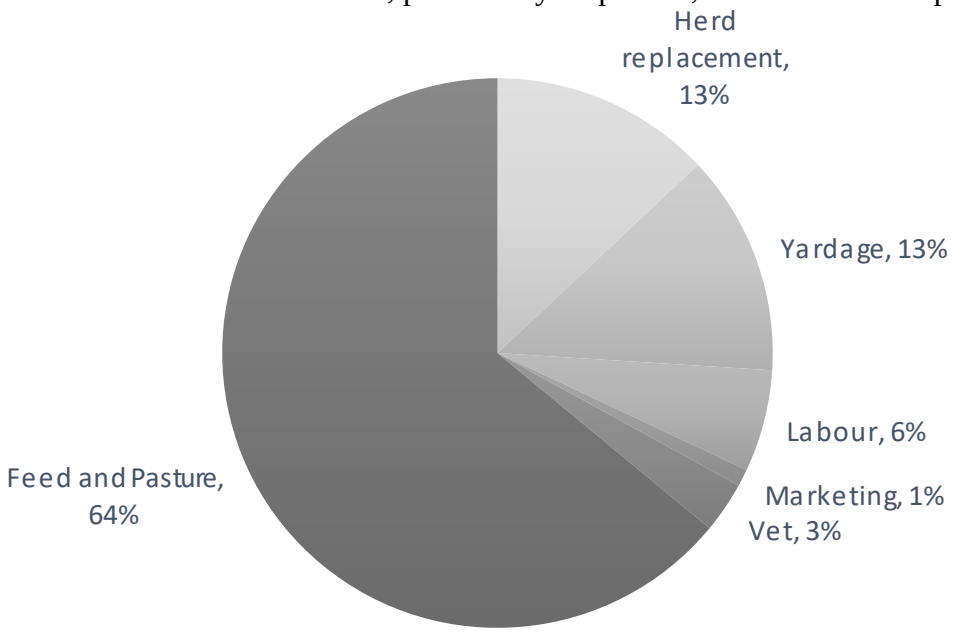

Figure 1. 1 Cow-calf cost of production, average 2012-2015 in Canada. Modified from CanFax, 2017.

efficiency in the beef sector, and particularly for improved economic and environmental sustainability.

Selection for lower maintenance energy requirements or improved efficiency of feed utilization will likely prove to be the most successful strategy in reducing overall feed costs. Residual feed intake (RFI) is a technique to measure feed efficiency that has received much attention as it is based on the difference between an animal's actual feed intake and its expected feed requirements for maintenance and production (Koch et al. 1963). Selection for low RFI has potential to improve livestock efficiency, as RFI is positively correlated with DMI and metabolizable energy (ME) intake and is independent of ADG and body weight (BW; Archer et al. 1997; Arthur et al. 2001; Nkrumah et al., 2004; Crowley et al. 2011). However, evaluation of RFI and thus favorable selection for the trait have been limited by the inability to accurately measure individual animal intake, especially in pasture-based systems, without the use of expensive equipment and complex sampling and analytical procedures. Development of accurate methods to measure DMI in pasture-based feeding systems will enable the industry to assess RFI in grazing animals, in the environment in which they spend a significant portion of their lives, increasing the likelihood of adoption by the cattle industry.

2. LITERATURE REVIEW

2.1 Predicting feed intake

Researchers have been attempting to develop accurate techniques for predicting DMI in grazing animals for many decades. Quantifying DMI is required for future progress in management and feed efficiency of cattle production. For animals in confinement, individual animal feed intake is relatively easy to measure using innovations such as Calan-gate feeders, GrowSafe Feed Intake™ and Insentec™ systems. These methods typically consist of a head gate to allow entry of a single animal into a trough-type feeder, and a scale to record feed disappearance. While these systems provide accurate measurements of total feed intake in confinement, such as a feedlot or group housing pen, they are not suitable for measuring forage intake on pasture due to the nature of the equipment and infrastructure necessary for their operation. Furthermore, the diet available and the ability of animals to acquire feed differ between confinement and pasture-based systems. Prediction methods utilized on pasture include measurement of herbage mass disappearance through the use of grazing cages (Macoon et al. 2003; Smit et al. 2005), use of external (ie. chromium oxide; Berry et al. 2000; Ferreira et al. 2004) or internal (ie. acid-insoluble ash, lignin; Lawrence et al. 2011; Kanani et al. 2014) markers, use of prediction models (Coleman 2005; Anele et al. 2014), use of fecal near infrared reflectance spectroscopy (fecal NIRS; Keli et al. 2008; Undi et al. 2008; Johnson 2014), use of the ratio technique (Lippke 2002) and monitoring of grazing behaviour and animal performance (Macoon et al. 2003). Each of these methods provides an estimate of intake with varying degrees of error, and has unique advantages and disadvantages which will be discussed further in the following subsections.

Herbage mass disappearance

Prediction of grazed intake via estimation of herbage mass disappearance is a direct method which is fairly easy and low-cost, however it has several shortcomings. Rising plate meters or sward height meters can be used to determine the difference in herbage prior to and following grazing, however reliability of this method is poor unless the grazing period is short and stocking density is high, in order to avoid error associated with non-uniform grazing and regrowth. Grazing cages, or exclusion cages, can also be employed, however this method does not account for urinary or fecal restitution, trampling, or other losses, and may overestimate intake, above the biological capacity of the animal (Undi et al. 2008). Successive cuttings can be taken in order to simulate grazing, however the challenge of accurately and representatively measuring herbage mass before and after grazing limits widespread utilization of this method (Smit et al. 2005). Though herbage disappearance estimates are corrected for herbage growth, this technique simply calculates average disappearance for a group of animals, rather than providing an accurate estimate of individual-animal intake where

$$\text{herbage disappearance} = \frac{\text{herbage mass before grazing - herbage mass after grazing } + \text{herbage growth correction}}{\text{number of animals} \times \text{number of days}}$$

This method fails to account for individual differences in animal intake, unless animals are kept on individual plots, which increases labor requirements and alters normal grazing behaviour.

Meyer et al. (2008) used the herbage disappearance technique to estimate average forage intake of grazing beef cows previously identified as low- and high-RFI as heifers. Although not statistically significant, a 21% reduction in forage intake by low-RFI animals was reported compared to their high-RFI counterparts, with no effect on gain or BCS during mid-late gestation. This technique might therefore be acceptable for estimating average intake of groups of animals, without the need for elaborate laboratory analysis (Undi et al, 2008).

Monitoring animal behaviour

Grazing time, biting rate and bite mass measured via observation and weigh-graze-weigh technique have also been used to estimate individual animal intake (Erlinger et al. 1990). Although this method requires minimal cost and equipment, forage samples which represent consumption by the grazing animal are required. Furthermore, presence of the observer may disturb grazing behaviour and thus bias efforts. Simultaneous measurement of movement while grazing, rumination and grazing time is another option, however these approaches require expensive recording equipment such as GPS collars, as well as the harnessing of animals with this equipment which may disturb grazing behaviour. It is difficult to predict individual animal intake from individual animal grazing behaviour, but may be more practical for group feeding management (Undi et al. 2008).

Prediction models

Prediction models, ranging from simple regression equations to more complex differential equations, have been used to predict intake in cattle (Macoon et al. 2003; Smit et al. 2005; Anele et al. 2014). This method of intake estimation relies on correlations between predictor variables and DMI. Generally, predictor variables can be easily measured or quantified [National Research Council (NRC) 2001], such as animal performance, forage composition, fecal chemistry or the environment. An example of these are the NRC equations which, like all prediction equations, provide an estimate of intake developed using specific criteria which must be met in order to apply the equations to a given production situation. These models are typically based on research data which fails to account for all variables within field conditions. Prediction equations serve therefore as a guideline rather than an absolute prediction of intake (NRC 1996). Because NRC equations are based on dietary NE_m concentrations and average $BW^{0.75}$, multiple

authors (Patterson et al. 2000; Block et al. 2001; Anele et al. 2014) have reported under- and over-prediction of DMI depending on dietary and animal conditions when using the NRC 1996 intake equation. Patterson et al. (2000) examined seven growing studies including 54 diets and found that NRC equations 7-a and 7-1 (NRC 1996) resulted in DMI estimates which were −2.1 to +0.6 kg from actual DMI, depending on dietary NE levels.

Though NRC equations cannot serve to differentiate low- and high-efficiency individuals within the herd, a prediction of average intake for all animals may be obtained using the following equations (Equation 10-1; NRC 2016):

$$\textit{Forage } NE_m \textit{ intake, Mcal } d^{-1} = BW^{0.75} * (0.2435 * NE_m - 0.0466 * NE_m^2 - 0.0869)$$

$$\textit{Forage DMI, kg DM } d^{-1} = \textit{forage } NE_m \textit{ intake (Mcal } d^{-1})/NE_m$$

Where BW = average body weight (kg) over the feeding period and NE_m = standing forage net energy for maintenance (Mcal kg^{-1} DM).

In addition to the NRC equations, other equations have also been used to predict individual animal intake. For example, Minson and McDonald (1987) published an equation which was validated by comparing predicted intake with previously-published observed intake, measured using chromic oxide and herbage disappearance, for beef steers grazing temperate forages. Mean predicted and observed intake differed by 0.6%, leading the authors to conclude that forage intake may be predicted with a coefficient of variation of 8.7% using the following equation:

$$\textit{Forage DMI, kg } d^{-1} = (1.185 + 0.00454BW - 0.0000026BW^2 + 0.315ADG)^2$$

Where BW = average BW over the feeding period (kg) and ADG = average daily gain (kg d^{-1}).

The Mertens equation (Mertens et al. 1987), validated for use in lactating dairy cattle, predicts intake using BW and forage characteristics. NRC (2016) suggests that the Mertens equation may also be of use in beef cattle:

$$Forage\ DMI,\ kg\ d^{-1} = ((120/NDF)/100) * BW$$

Where BW = average BW over the feeding period (kg) and NDF = forage neutral detergent fibre (% DM).

It is evident that predictive models offer a simple method for the development of feeding management strategies (Macoon et al. 2003) for groups of cattle. However, their use is limited by the inability to accurately estimate individual animal intake. Furthermore, these models cannot account for many of the physiological, environmental and management factors that affect intake (NRC 1996; Undi et al. 2008).

The ratio technique (Lippke 2002) is another method used to predict intake based on determination of forage digestibility and fecal output. Accuracy of this method is dependent on precise estimation of digestibility and fecal output. In determining forage digestibility, it is imperative that forage sampled is representative of that ingested by the animal, usually by hand plucking forage, although this is difficult to achieve. The ratio technique also requires total fecal collection, which is difficult to apply to grazing animals, particularly cattle. Cattle can be harnessed with a fecal collection bag; however this disturbs grazing behaviour and can be a source of error if feces escape the collection bag.

Internal and external markers

Although the use of internal and external markers dates back many years, there are several challenges associated with this methodology, preventing widespread use of any one method as a simple, accurate prediction of intake. For example, pinitol (Smith and Phillips 1980; Smith

1982), present in legumes but not in grasses, herbage calcium content (Playne et al. 1979) or acid insoluble ash (Kanani et al. 2014), have been proposed as markers and subsequently refuted, due to difficulty with analysis or inability to completely extract the marker. The ideal fecal marker, as described by (Kotb and Luckey, 1972), must reach steady state in the rumen, be non-toxic, chemically discrete and inert, with no effect on digestion, be completely recovered in feces, and have physical characteristics, such as gut passage rate and density, similar to the other feed material in the digestive tract.

Markers may be internal (endogenous) to the feedstuff, or external: added to the feedstuff or dosed separately to the animal as either a single large dose, daily pulse doses, or through a controlled release device (CRD). Chromium sesquioxide (Cr_2O_3; chromic oxide) and *n*-alkane markers are the most common, and each of these has been administered through various techniques in attempt to reach steady-state in the rumen, a condition which is necessary to obtain representative fecal samples. For example, *n*-alkanes have been dosed using shredded paper pellets, gelatin capsules, *n*-alkane boluses, intra-ruminal CRDs (Berry et al. 2000) as well as concentrate supplements (Unal and Garnsworthy 1999; Charmley and Dove 2007). While CRDs were effective at reducing diurnal variation previously seen with shredded paper dosing (Ferriera et al. 2004), as well as eliminating the need for laborious daily dosing procedures, production ceased in 2008 (Cottle 2013*).* The use of concentrate supplements has potential as a valid dosing technique, however sufficient marker intake must be ensured, as well as accurate measurement of supplement intake. Greenfeed systems (C-Lock, Inc., Rapid City, USA), for example, may serve as a novel on-pasture delivery system for C32 pellets, also collecting methane data without needing to move cattle off pasture for daily dosing, assuming sufficient intake can be achieved to reach steady state.

2.2.1 *n*-alkane technique

Since its development in the late 1980s (Mayes et al. 1986), the *n*-alkane method has received increasing acceptance (Keli 2008) as these saturated hydrocarbons are widespread and easy to analyze (Dove and Mayes 1991; Moshtaghi-Nia and Wittenberg 2002). The cuticular wax of grasses contain both odd- and even-numbered *n*-alkanes in the range of C24 to C36, with odd-numbered *n*-alkanes present in much higher concentrations, particularly C29, C31 and C33 (Dove and Mayes 1991). Boloventa et al. (1994) estimates odd-numbered *n*-alkanes make up 94.4 to 96.6% of total C27-C35. Therefore, the odd-numbered *n*-alkane is used as the internal marker, while a synthetic, even-numbered *n*-alkane is added to the supplement and serves as the external marker. Alkanes are non-toxic and primarily indigestible, serving as an ideal marker substance. When using the *n*-alkane technique, it is assumed that the concentration of both the internal and external markers collected in the fecal sample is representative of that in the total fecal output (Mayes et al. 1986), and therefore total fecal collection is not required (Olivàn et al. 2007).

Analysis of *n*-alkanes has the potential to simultaneously obtain an accurate estimate of forage intake and digestibility for individual animals (Dove and Mayes 1991, 1996; Boadi et al. 2002) consuming both forage and concentrate (Moshtaghi-Nia and Wittenberg, 2002; Dove and Mayes 2005), using the following formula:

$$Intake\ (kg\ d^{-1}DM) = \frac{\frac{F_i}{F_j} \times \left(D_j + IS \times S_j\right) - IS \times S_i}{H_i - \left(\frac{F_i}{F_j}\right) \times H_j}$$

Where F_i = natural alkane concentration in feces (mg kg^{-1} DM), F_j = dosed alkane concentration in feces (mg kg^{-1} DM), D_j = dose rate of synthetic alkanes (mg d^{-1}), IS = intake of supplement (kg d^{-1} DM), S_j = dosed alkane concentration in supplement (mg kg^{-1} DM), S_i = natural alkane concentration in supplement (mg kg^{-1} DM), H_i = dosed alkane concentration in forage (mg kg^{-1} DM), H_j = natural alkane concentration in forage (mg kg^{-1} DM).

This technique also accounts for differences in digestibility between individual animals, thus providing an estimate of individual-animal intake (Dove and Mayes 1996), assuming forage samples are representative of the animal's ingested diet. Dove and Mayes (1996) have also suggested that the *n*-alkane method would be suitable for use in genetic studies examining the differences between individual animal intake, digestibility and feed conversion efficiency. In a comparison of intake-determination techniques, Undi et al. (2008) preferred the use of the *n*-alkane technique over prediction equations as this method accounts for environmental conditions under which animals are grazing. Further to these advantages, the *n*-alkanes are chemically discrete compounds, therefore both plant and dosed (internal and external) markers are determined simultaneously using gas chromatography, thus limiting analytical error and bias. Bovolenta et al. (2012) report high repeatability ($r > 0.85$) for the *n*-alkane method, and a review of many studies by Dove and Mayes (1991) reports acceptably accurate estimates of intake.

While the *n*-alkane technique appears promising in its ability to accurately estimate intake on pasture, some disadvantages do exist. The technique requires consistent intake of the marker in order to reach steady-state in the rumen, which is difficult to achieve in pasture-based systems. Depending on the method of administration, time to reach steady state can take between four (using paper pellets – Ferreira et al. 2007; using molasses-based *n*-alkane boluses - Bezabih et al. 2012) and 14 days (using *n*-alkane CRD capsules - Undi et al. 2008). Generally, an adaptation period of five to seven days is recommended (Mayes et al. 1986). Equally challenging is collection of a forage sample representative of the ingested diet (Dove et al. 1996; Smit 2005; Decruyenaere et al. 2009a), as animals will tend to selectively graze, and *n*-alkane profiles differ between plant parts and species (Dove and Mayes 1996). Because of the repeated handling necessary to dose animals and collect fecal samples, as well as the complex laboratory analysis

required, the *n*-alkane method is both challenging and costly to use for long periods of time (Mayes and Dove 2000; Decruyenaere et al. 2009a). Researchers may also see changes in animal behaviour related to the dosing and fecal collection procedures, with interrupted grazing resulting in altered voluntary feed intake. Cattle must be accustomed to the system used to deliver supplements prior to the data collection period in order to reduce the effects of stress on intake.

The *n*-alkane method is independent of digestibility, making it more accurate than chromium and NE methods (Smit et al. 2005). Yet there are many aspects to consider when measuring intake using the *n*-alkane method in order to ensure accuracy. Some studies have shown an under- (Bezabih et al. 2012) or over- (Smit et al. 2005; Keli et al. 2008) estimation of herbage intake compared to animal's energy requirements. Many variables including animal species (Keli et al. 2008; Ferriera et al. 2009), physiological status and diet type may affect fecal recovery rates, providing variable intake estimates across trials (Olivàn et al. 2007; Elwert et al., 2008). The carrier material used, as well as frequency of dosing and fecal sampling schedules may also influence fecal excretion patterns of dosed *n*-alkanes (Bezabih et al. 2012). Although Unal and Garnsworthy (1999) have shown incomplete recovery of *n*-alkanes, Mayes et al. (1986) postulated that simultaneous determination of digestibility (using odd-chain *n*-alkanes) and fecal output (using even-chain *n*-alkanes) would remove any error associated with incomplete fecal recoveries. In addition, correcting for differences in fecal recovery may improve intake estimates; a difference in fecal recoveries of as little as 3% can lead to a variation of up to 13% if digestibility is 0.8 (Dove and Mayes 1991). In order to reduce error associated with incomplete fecal recoveries, an *n*-alkane marker of appropriate chain length should be chosen based on forage species and composition (Unal and Garnsworthy 1999; Dove and Mayes 2005; Smit et al.

2005), and actual *n*-alkane fecal recoveries for diet type and experimental conditions should be calculated prior to application of this method (Dove et al. 1989; Berry et al., 2000; Lippke 2002; Olivàn et al., 2007; Undi et al. 2008; Bezabih et al. 2012). It is typically recommended to choose *n*-alkanes of adjacent chain length, as these tend to have similar recoveries (Mayes et al. 1986; Dove and Mayes 1991, 2005). Generally, fecal recovery of dosed *n*-alkanes is better than that of natural *n*-alkanes (Berry et al. 2000; Hendricksen et al. 2002; Bezabih et al. 2012). This is likely due to the varying passage rate of solid (associated with herbage *n*-alkanes) and liquid (associated with dosed *n*-alkanes) phases of digesta (Dove and Mayes 1991). Although Olivàn et al. (2007) have suggested that feeding level may affect fecal recoveries, and therefore estimates of intake, Unal and Garnsworthy 1999 found that fecal recoveries were not affected by diet or feeding level.

As fecal recovery of n-alkanes improves with increasing chain length (Dove and Mayes 1991; 2005; Lippke et al. 2002; Olivàn et al. 2007), C33 is commonly used to estimate digestibility. Olivàn et al. (2007) have suggested the use of C31/C32 *n*-alkanes to determine intake, however other researchers (Dove and Mayes 1986, 1991, 2006; Gordon et al. 1995; Lippke 2002; Moshtagia-Nia and Wittenberg 2002; Smit et al. 2005; De-Stefani Aguiar et al. 2013) have recommended the use of C33/C32 *n*-alkanes as markers. It is important to choose the form of administration by taking into account the digestion kinetics of the diet in order to pair synthetic and natural *n*-alkanes based on their behaviour within the gut (Sibbald et al. 2000). Intake estimates may be biased in cattle consuming forages with odd-chain *n*-alkane concentrations below 50 mg kg^{-1} (Casson et al. 1990; Laredo et al. 1991; Boadi et al. 2002) and familiarity with the *n*-alkane profile of a given forage is thus an important consideration when designing an intake study using the *n*-alkane method. This must also be considered if the forage

to be grazed varies in maturity or has multiple plant parts which may differ in *n*-alkane profile. While little research in this area has been conducted on Canadian forages, multiple authors in other countries have found differences in *n*-alkane profiles of the same plant species based on age, stage of development (Dove and Mayes 1991; Cortes et al. 2005) and between plant parts (wheat – Tulloch 1973; barley – Valiente et al. 2003). Dove et al. (1996) reported differences in *n*-alkane concentrations and ratios between plant parts of six pasture species. Dove and Mayes (1996) found significant differences in *n*-alkane concentrations between leaf lamina, leaf sheath, stem and flower spike in annual ryegrass. Smith et al. (2001) also found highly significant differences in *n*-alkane concentrations between flower head, leaf and stem in common grass species in South Africa and concluded that there is "less similarity between plant parts of the same species than between whole plant samples of different species".

Dose interval (once- or twice-daily dosing) may also impact *n*-alkane kinetics. While it is accepted that diurnal variation must be avoided (Dove and Mayes 1991; Unal and Garnsworthy 1999), the main factor responsible for fluctuations in fecal *n*-alkane concentrations may be incomplete mixing of the marker with ruminal contents (Dillon and Stakelum, 1989), and therefore with correct dosing, once-daily fecal collection may be sufficient (Olivan et al. 2007). Using paper pellets for once-daily dosing of *n*-alkanes, Olivàn et al. (2007) and Mayes et al. (1986) documented no diurnal variation in fecal *n*-alkane ratios C31/C32 and C33/C32 in cattle and in sheep, respectively. Richmond et al. (2014) conducted studies on beef cattle using two 500 mg boluses of synthetic C32 alkanes and concluded that once-daily dosing and fecal sampling is a valid alternative to the commonly used (Mayes et al. 1986; Malossini et al. 1996; Lippke 2002; Bezabih et al. 2012; Basarab et al. 2013; De-Stefani Aguiar et al. 2013) protocol of twice-daily dosing and fecal sampling.

Multiple studies have explored single (once daily), composite (AM and PM), or separate AM and PM fecal samples to provide the most accurate estimate of intake. A study of beef steers grazing tall fescue by Stewart et al. (2006) reported no differences in DMI estimation (P = 0.88) using fecal samples from morning- and afternoon-collected samples, or daily composite samples. Olivàn et al. (2007) found diurnal variation in *n*-alkane ratios between alkane pairs C23/C24, C25/C24 and C35/C36, though not for C31/C32 and C33/C32 in cattle (CI = 95%). Similar findings have also been reported by Mayes et al. (1986) in sheep. Furthermore, when comparing fecal samples taken every 8 h throughout an 88-h period, Olivàn et al. (2007) found that fecal grab samples collected once every 24 h, at the time of dosing, may be representative of the total fecal n-alkane concentration and could thus be used for accurate estimates of intake. It is clear that a change in the accepted protocol from twice- to once-daily dosing and fecal sampling would allow for reduced labor, cost and disruption of grazing behavior, thus facilitating more widespread adoption of the *n*-alkane technique, however based on contradictory evidence between studies, further research is needed before a change in protocol is implemented.

2.2.2 Near infrared reflectance spectroscopy

Near infrared reflectance spectroscopy (NIRS) has been employed as a method of predicting chemical composition and digestibility of forages (Stuth et al. 2003). It is based on the creation of robust calibration databases which link NIRS spectra to values, such as chemical or biological composition. A known quantity of NIR light is projected onto a substance, and the reflectance from that substance is recorded, providing information based on the interaction between the radiation and the biological material, whether forage or feces. NIRS has become an acceptable alternative to traditional laboratory chemical procedures to determine both nutrient profile and digestibility of feedstuffs, and has been evaluated for its potential to predict voluntary intake.

Several research groups have reported that voluntary DMI could be measured by NIRS analysis of forage samples, with standard error of calibration (SEC) between 7 to 9 g/kg $BW^{0.75}$ (Norris et al. 1976), and 9.6 g/kg $BW^{0.75}$ (Ward et al. 1982). However, further research by Decruyenaere et al. (2009b) found it was not possible to estimate organic matter voluntary intake using forage NIRS with sufficient accuracy ($R^2 = 0.30$). The use of forage NIRS to estimate intake may be limited by our ability to obtain forage samples representative of the diet of grazing animals.

Although it has proven difficult to accurately estimate intake from forage using NIRS, predictions are more accurate from feces, with improved R^2 and standard error of cross validation (SECV) (Garnsworthy and Unal 2004; Decruyenaere et al. 2009b). Furthermore, fecal samples provide information about diet, physiology and ecology of a given animal as a consequence of undigested residues of the consumed forage (Dixon and Coates 2009). In fact, Holloway et al. (1981) attributed 70% of between-animal variation in intake and digestibility to properties detectable in feces, providing evidence for potential prediction of intake based on fecal NIRS spectra.

Lyons and Stuth (1992) found that estimates of in vivo digestibility of grass in grazing cattle using fecal NIRS (fNIRS) and conventional wet chemistry methods were both acceptably precise, and that fNIRS provided more accurate estimates of digestible organic matter and CP when compared to forage NIRS predictions. Mayes and Dove (2000) demonstrated that estimate of forage intake by fNIRS may be as accurate as the *n*-alkane technique. More recently, Johnson et al. (2017) reported accuracies for the prediction of individual-animal DMI ($n = 327$) by fNIRS comparable to those reported using the *n*-alkane technique, with coefficient of determination for test-set validation (R^2v) ranging from 0.65 to 0.69 and differences (Diff.) ranging from −1.07 to 0.00. These results are comparable to previously published results for the prediction of DMI

using the *n*-alkane technique and CRD or paper pellets (R^2v 0.18 to 0.72, Diff. 27.1 to 0.48, $4 \leq n \leq 11$; Berry et al. 2000; Ferreira et al. 2007; Oliván et al. 2007) indicating that fNIRS and *n*-alkane methods for prediction of mean and individual-animal DMI have similar capacities. Decruyenaere et al. (2009b) found that estimation of organic matter digestibility (OMD) by fNIRS provided similar or improved accuracy compared to traditional wet chemistry methods. Although the authors were unable to estimate organic matter voluntary intake (OMVI) with sufficient accuracy with forage NIRS ($R^2 = 0.30$), fecal spectra greatly improved prediction models for OMVI ($R^2 = 0.80$ to 0.90). This is likely due to the fact that OMD and OMVI also depend on physiologic and metabolic parameters, such as digestion rate in the rumen, plant characteristics, and animal behaviour. These are difficult, if not impossible, to quantify using only forage samples. However, feces reflect both the biological and chemical characteristics of the ingested forage, as well as the physiological status of the animal, thereby making fNIRS much more efficient as an indicator compared to forage NIRS (Decruyenaere et al. 2009b). Fecal NIRS technology has the ability to account for the entire chemical composition of feces (Fanchone et al. 2009), however its application is in its infancy. While initial research (Boval et al. 2004; Decruyenaere et al. 2009; Tran et al. 2010; Johnson et al. 2014) appears promising, further studies are required to confidently use this technology to its fullest potential for estimation of intake. Results are generally variable across studies as robust calibrations are limited. Data sets of > 2000 (Johnson 2014), which include diversity of field conditions (Decruyenaere et al. 2015), are necessary to formulate robust and accurate prediction equations to reduce or eliminate the effect of trial seen in previous studies. Factors such as diet, breed, age, environment and sample handling within a trial can be largely influential on fecal spectra (Huntington et al. 2010; Tran et al. 2010), thus limiting the robustness of current prediction

equations. Future development of calibration equations may be limited by the inability to accurately combine data sets from multiple trials and locations, as Tran et al. (2010) reported a reduction in predictability of intake when data sets were combined to develop calibration equations. An industry-applicable calibration equation will contain a large sample set representative of all forages within a defined region (area and climate). To date, few studies have compiled data sets of sufficient size to provide accurate prediction equations which could be applied across multiple forages and production systems.

In summary, fecal NIRS technology boasts many advantages over traditional wet chemistry methods of analysis, and may be a useful tool for estimation of diet quality, digestibility and individual-animal intake. While start-up costs are high, NIRS analysis is relatively inexpensive long-term (Stuth et al. 2003). In addition, analysis is rapid, non-destructive, requires no reagents or labor-intensive sample processing, animal manipulation or heavy analytical procedures (Decruyenaere et al. 2012), making NIRS an attractive method for intake prediction as long as accuracy can be validated though larger data sets. Calibration is required for different physiological states and diets (Gordon 1995; Stuth et al. 2003; Decruyenaere et al. 2009b), including a large diversity of samples representing temporal, spatial, biological, species, environmental and landscape conditions which might be experienced by the animals (Stuth et al. 2003).

Accurate estimate of DMI continues to challenge the research community, however several promising techniques are currently under investigation. The *n*-alkane and fNIRS techniques, though both appear promising, do have some obstacles to overcome, such as confirmation of an acceptable sampling protocol and development of a robust calibration

database. Ultimately, accurate measurement of DMI is essential for the selection of feed efficient animals.

2.2 Animal efficiency

Early work measured efficiency as a ratio of inputs (i.e. feed) to outputs (i.e. weight gain) through feed conversion (FCR) or feed:gain (F:G) ratios. While selection for improved (decreased) FCR resulted in less feed required for gain, a strong genetic correlation with growth traits also led to increased mature size and thus increased maintenance requirements and associated costs (Herd and Bishop 2000; Arthur et al. 2001; Crews 2005). Increases in maintenance requirements of the breeding herd must offset the gains in efficiency of market progeny in order to make progress in total system efficiency (Crews 2005). As a consequence, the concept of RFI has been explored extensively in the last several decades.

2.2.1 Residual feed intake

Residual feed intake was first proposed by Koch et al. (1963). It is the difference between an animal's actual intake and its expected intake, independent of body size and production. Animals that consume less than expected have a low-RFI while those that consume more than expected have a high-RFI. Therefore, RFI is a feed efficiency trait which accounts for between-animal variation in feed intake unexplained by differences in metabolic BW and ADG (Arthur et al. 2001). RFI is calculated as the difference between actual standardized DMI (SDMI) and expected feed intake (EFI), with SDMI summarized using the following model:

$$Y_i = \beta_0 + \beta_1 ADG_i + \beta_2 MIDWT_i^{0.75} + e_i,$$

where Y_i is the SDMI for animal *i*, β_0 is the regression intercept, β_1 is the partial regression coefficient of SDMI on ADG, β_2 is the partial regression coefficient of SDMI on metabolic MIDWT, and e_i is the random error term.

2.2.1.1 RFI and efficiency

Nkrumah et al. (2004) observed that DMI was positively correlated (r = 0.75) with RFI (P < 0.0001). Further, low RFI animals have been shown to consume 10-12 % less feed, have greater efficiency of feed utilization (Basarab et al. 2013; Manafiazar et al. 2015) and have lower maintenance energy requirements (Herd and Bishop 2000) compared to their mid- or high-RFI counterparts at equal body weight, growth and fatness. Researchers have hypothesized that selection for low RFI animals has a greater potential to improve overall production efficiency compared to other measures of energetic efficiency (Nkrumah et al., 2004; Lancaster et al. 2009, 2014) such as G:F or FCR. Nkrumah et al. (2014) suggested that low RFI animals have improved crude protein (CP; r = −0.34) and dry matter (DM; r = −0.33) digestibility (P < 0.10).

Biological and physiological mechanisms that control RFI are still unknown, however, several have been proposed (Herd and Arthur 2009), including differences in physical activity, protein turnover and overall tissue metabolism, more efficient digestion, thermoregulation or lower heat increment of fermentation, differences in body composition or composition of gain, and feeding behavior (reduced feeding duration and frequency; Basarab et al. 2013). Richardson et al. (1996) also observed increased DM digestibility in low-RFI steers and suggested that this small variation in digestibility results in a large improvement in feed efficiency. A study by Basarab et al. (2003) found that steers with low RFI (adjusted for body composition, i.e. off-test ultrasound backfat thickness) had 9.3 % lower heat production (HP), 12.0 % less retained energy (RE) and 10.2 % lower metabolizable energy intake (MEI) than their high-RFI counterparts (P < 0.01). Nkrumah et al. (2014) also found a correlation between RFI and HP (r = 0.68; P < 0.001) and retained energy (r = −0.67; P < 0.001) in feedlot steers. This may be due to the presence of a rumen bacterial profile which improves rumen fermentation in these animals (Basarab et al. 2013), more efficient biogenesis of energy and/or to changes in the chemical composition of

gain, as low RFI steers may have slightly less marbling, intermuscular fat and body cavity fat (Basarab et al. 2003). Another likely cause for reduced heat production in low RFI animals however is a decrease in DMI, which is generally correlated with a decrease in the size of visceral organs (Ferrel and Jenkins 1998; Basarab et al. 2003). Basarab et al. (2003) have suggested that increased heat production in high RFI steers may be attributed to a decrease in the metabolizability of diets at high levels of DMI, or to increased maintenance cost or heat increment of feeding at high levels of feed intake and heavier organ weights.

In addition to reduced DMI, manure production and methane emissions are also reduced by 15-20% (Basarab et al. 2002) and 15-30%, respectively (Nkrumah et al., 2006, 2014; Hegarty, 2007; Basarab et al. 2013), resulting in more economically and environmentally efficient animals (Herd and Bishop 2000). In Australia, the cumulative reduction in methane emissions from beef cattle over 25 years as a consequence of selection for low RFI is predicted to be $8.5 million per year in carbon credits (Alford et al. 2006). Beef producers also stand to benefit from offsetting methane emissions via selection for RFI through improved environmental stewardship and as additional revenue gained from selling carbon credits (AAF 2017).

2.2.1.2 RFI and its relationship with other traits

RFI has been shown to be moderately heritable (h^2 = 0.26 to 0.43; Crews 2005; Basarab et al. 2013). Basarab et al. (2013) predicted that selection for RFI "or its component traits such as DMI, BW, ADG and backfat in a multi-trait selection index will result in slow incremental improvement in feed efficiency". Hence the establishment of breeding goals which include selection for low-RFI may improve both economic and environmental efficiency, with few or no negative effects on economically important traits. However, selection for RFI and associated breeding decisions may be hindered by the cost and ability to accurately rank animals for RFI. To date, no major gene has been associated with RFI (Moore et al. 2014); as feed efficiency, like

most quantitative traits, is likely under the control of many genes, each with a small effect (Arthur and Herd 2008). Thus to avoid undesirable correlated responses, it is important to study the effects of selection for RFI on other traits (Moore et al. 2014).

Performance traits

There is a need for improved understanding of genetic and phenotypic relationships between RFI and other production traits such as locomotion, disease and other metabolic processes (Herd and Arthur 2009). To date, selection for RFI has been found to have minimal or no deleterious effects on performance traits such as body weight, growth rate or body composition (Crews 2005; Wang et al. 2012; Manafaziar et al. 2014). Richardson et al. (1998) reported a small reduction in subcutaneous fat thickness in response to a single generation of selection against RFI, and Herd and Bishop (2000) also found slight genetic and phenotypic correlations between RFI and carcass leanness. Basarab et al. (2003) found that low RFI steers had reduced carcass fat compared to their high-RFI counterparts (9.9% vs. 11.3%, $P < 0.05$), particularly marbling, intermuscular fat and body cavity fat. Robinson and Oddy (2004) found correlations in both steers and heifers between RFI and rib fat ($\hat{r}_g = 0.48$) and rump fat ($\hat{r}_g = 0.72$). However, these correlations are higher than those reported by Arthur et al. (2001), Carstens et al. (2002) and Nkrumah et al. (2004), which predict correlation with 12th rib fat thickness of 0.14 to 0.25. Furthermore, carcass fat composition traits explained 9% of variation in unadjusted RFI (RFI) in finishing steers (Basarab et al. 2003) and 5% in growing bulls (Richardson and Herd 2004; Schenkel et al. 2004). Crews et al. (2003) found a genetic correlation of $r = -0.44$ between finishing period RFI and carcass marbling score in steers, concluding that selection for improved RFI may be associated with a favorable response in carcass quality grade. For these reasons, RFI is typically adjusted for off-test ultrasound backfat thickness (RFI_{fat}).

Multiple researchers have investigated potential relationships between RFI_{fat} and meat quality and yield, end product palatability (including tenderness, juiciness, flavor or off-flavor and overall like or dislike) and retail attributes (including fat content and color). In all studies, low-, moderate, and high-RFI beef cattle were not significantly different between groups for growth, feed intake, other efficiency measures and body composition (Basarab et al. 2003; Arthur et al. 2001a, b; Baker et al. 2006; Nkrumah et al. 2007; Basarab et al. 2011; Crowley et al. 2010; Ahola et al. 2011).

Reproductive traits

Fertility and reproductive traits must also be considered when making breeding decisions. In a study of 190 beef heifers, Basarab et al. (2011) reported a delay in age at puberty as well as a lower pregnancy rate in low-RFI animals compared to their high-RFI counterparts ($P = 0.09$). This effect was removed however when adjusted for end of test ultrasound backfat thickness ($RFI_{fat)}$ and off-test ultrasound backfat thickness and feeding event behaviour ($RFI_{fat\&activity}$). No differences were observed between RFI rankings for calving difficulty, age at first calving, calf birth weight, calf weaning weight and heifer productivity, although high-RFI heifers had higher calf death loss. It was proposed that higher survivability in low-RFI_{fat} heifers could be due to their decreased maintenance requirements, a hypothetically better uterine environment, increased availability of nutrients for accumulation of body fat, or possible improved calf passive immunity status (Basarab et al. 2007; 2011). Arthur et al. (2001) and Schenkel et al. (2004) found no correlation between selection for RFI and scrotal circumference, semen concentration, sperm motility and abnormalities, and overall breeding soundness of bulls. Further, Hafla et al. (2015) reported a weak association between RFI and sperm morphology ($r = 0.13$) while Wang et al. (2012) found that progressive sperm motility was greater ($P < 0.05$) in high-RFI (85%) than low-RFI (80%) bulls. However, breeding soundness exams and overall fertility are impacted by

many factors, and these associations may be attributable to differences in maturation rate and age at puberty between low- and high-RFI groups. Despite differences in sperm morphology and motility, low-RFI bulls had a significantly greater ($P < 0.05$) mean number of progeny as well as a positive effect on reproductive performance and bull fertility (Wang et al. 2012).

2.2.1.3 Repeatability of RFI rankings

Another challenge in selecting for low-RFI cattle is the observation of genotype × environment interactions which exist for DMI and RFI, as reported by Durunna et al. (2011). Differences in the RFI classification of animals as a consequence of diets and environmental conditions may necessitate selection of animals which can perform efficiently on a range of diets, in a range of environmental conditions. There is also a question of the consistency of feed efficiency measures throughout the production cycle. While the ultimate goal is to breed cattle which are efficient at all stages of production regardless of diet type, it may be necessary to make distinct selections for the cow herd, where selection of animals that are efficient on pasture, and for their progeny, efficient on both forage and concentrate-based feedlot diets is optimal. Typically, low-RFI animals are selected when they are young and fed a diet with sufficient energy to enable them to express their genetic potential for growth (11 – 12 MJ kg^{-1} ME; Basarab et al. (2003, 2007)). However, replacement animals are generally fed a diet lower in energy as they mature and utilize nutrients differently as their needs shift from growth to maintenance and reproduction (NRC 2000). As such, multiple studies have evaluated RFI in animals as they mature (Arthur et al. 1999; Archer et al. 2002; Herd et al. 2006; Black et al. 2013; Halfa et al. 2013). A study by Durunna et al. (2011) examined 190 replacement heifers over a three-year period, fed the same diet (90% barley silage and 10% rolled barley grain) and ranked for RFI (low, medium or high) over two consecutive periods. They found that while 49% of heifers maintained the same RFI class throughout both periods, 51% had a different RFI class,

suggesting that re-ranking does occur despite having the same diet and similar environmental conditions, and may be due to differences in maturity. Manafiazer et al. (2015) found a positive correlation (r_p = 0.30) between RFI_{fat} measured in 171 crossbred beef heifers when in a drylot and grazing tame pasture using the *n*-alkane technique to estimate intake approximately two months after the initial RFI ranking. Basarab et al. (2013) also reported moderate repeatability of RFI measurements across diets (r_p = 0.33 to 0.67) and within-animal repeatability of feed intake measurements (0.29 to 0.49). There is sufficient evidence to conclude that differences in maturity or physiological status can impact RFI when measured at different stages of production. This may be due to differences in maintenance requirements, fat deposition, conceptus growth, lactation, activity and thermoregulation. These results suggest that feed efficiency is not one trait, but several traits such as RFI under higher energy diets, and RFI in mature cows on lower energy diets.

2.2.1.4 Measuring RFI

Currently, an accepted protocol for measurement of DMI is over 45 to 50 days, while a 63-day test period is necessary to accurately measure ADG when BW is measured weekly (Wang et al. 2014). In many previous studies, RFI has been measured in young, growing cattle (7 to 10 months of age, maximum age difference of 60 days) in confinement using feeding stations (such as GrowSafe Systems Ltd, Airdrie, AB) which monitor individual animal intake, requiring a 21- to 28-day adjustment period followed by a minimum 63-day test period with weekly cattle weigh-ins (Wang et al. 2014), though many Western Canadian studies have used longer (≥ 76−day) test periods with weights measured at 14- to 28-d intervals (Basarab et al. 2007, 2011; Wang et al. 2006). Further, at the start and end of the test period, cattle are weighed on two consecutive days and once for ultrasound backfat thickness at end of test. More recently, Manafiazer et al. (2017) successfully (R^2 = 0.78, P < 0.0001) shortened the typical DMI data

collection period to 42 days with only 7% of predicted values outside the predicted range and 12% loss of precision. This protocol allows for twice the number of animals to be tested at a reduced cost.

Given the cost and complexity of measuring feed intake in order to select for feed efficiency, several other methods have been proposed to measure RFI. Both Durunna et al. (2011) and Nkrumah et al. (2007) suggest the use of feeding behaviour as an indicator trait for RFI may be useful. Basarab et al. (2013) have found that inefficient (high-RFI) cattle have 14 to 22% more daily feeding events and expend 2 to 5% more energy on feeding activities. Researchers in multiple countries have identified moderate to strong positive correlations (r = 0.08 to 0.62; Robinson and Oddy 2004; Basarab et al. 2007; Nkrumah et al. 2007; Kelly et al. 2010; Durunna et al. 2011) of RFI and RFI_{fat} to feeding duration, frequency and eating rate; efficient (low-RFI) steers consistently have fewer observations of feeding behaviour (lower feeding frequency, shorter feeding duration and shorter head-down time). Furthermore, these feeding behaviours are moderately repeatable (r = 0.37 to 0.67; Kelly et al. 2010) and heritable (h^2 = 0.28 to 0.38; Nkrumah et al. 2007).

The absence of an accurate, affordable method for determining individual animal intake is a barrier to widespread adoption of RFI as a tool for selection of efficient cattle. Arthur and Herd (2005) predicted that though feed costs are reduced, it would take many years to see an economic return when selecting bulls tested for RFI. Although RFI testing is estimated to cost between $50 and $100 per animal under GrowSafe's contract pricing, molecular breeding values (MBV) now have increased accuracy (> 35% for RFI) and each bull purchased with an RFI estimated progeny difference (EPD) will sire 20-30 offspring annually, greatly improving the speed of genetic progress and thus economic returns (Basarab, J., personal communication). For

example, a bull with an EPD of −0.5 bred to a dam with an EPD of 0.0 can net a −0.05 kg DM d^{-1} improvement in progeny RFI for an annual rate of genetic progress of approximately 0.5 %. While selection for low RFI cattle would lead to greater efficiency and sustainability in the beef industry, its adoption has been restricted by the cost and difficulty to measure the trait. This is especially true of cattle raised on pasture-based systems where intake is difficult to estimate accurately.

2.3 Summary

While accurate methods are available to measure individual-animal feed intake in confinement situations, to date no method has been widely accepted as a gold standard to accurately measure DMI of grazing animals. While current DMI estimation techniques based on predictive models or herbage mass disappearance are fairly effective for prediction of average DMI for groups of animals, they are limited in their ability to accurately estimate forage intake of individual animals. As such, they are of limited value to identify grazing animals with improved feed efficiency. Further research to refine the use of *n*-alkane and fNIRS techniques may provide more accurate predictions of individual-animal intake, thus facilitating improved production efficiency of beef cattle systems. As RFI is a moderately heritable trait which accounts for between-animal variability, independent of growth and BW, selection for this trait will lead to reductions in feed inputs.

3. RESEARCH HYPOTHESES AND OBJECTIVES

3.1 Hypotheses

Intake on pasture as measured by direct and indirect fNIRS and *n*-alkane methods will differ in their predictions of individual-animal pasture intake compared to traditional prediction equations. Using previously-measured RFI rankings as a reference, fNIRS and *n*-alkane methods will be suitable for predicting individual animal intake on pasture as these methods account for individual-animal biological variations not related to BW or ADG.

The *n*-alkane profiles of several plant species have been characterized in the published literature, with many species' *n*-alkane concentrations varying with maturity and between plant parts. Therefore, it is logical to hypothesize that the *n*-alkane profile of swath-grazed triticale will also differ significantly between plant parts. However, using plant part weights as indicators of morphological variation, there will be no significant morphological differences between samples of swath-grazed triticale that would limit the use of triticale as a forage in *n*-alkane intake studies. Therefore, despite differences in *n*-alkane profile between plant parts, grazing management to encourage uniform consumption of plant parts will allow for prediction of intake using the *n*-alkane technique.

3.2 Objectives

The overall objectives of this study were to 1) characterize and compare pasture intake of individual beef heifers using the *n*-alkane method, fNIRS (used either to generate a direct estimate of intake, or to estimate fecal C31 and C32) and traditional equations-based predictions; 2) correlate estimates of intake on pasture to previously-measured RFI rankings; 3) compare DMI of low- and high-RFI_{fat} heifers as estimated using the six aforementioned intake methods; and 4) assess the suitability of swath-grazed triticale for *n*-alkane intake studies, by examining i)

its *n*-alkane profile, ii) its morphology (through the measure of plant part weights), and iii) residual starch content to estimate uniformity of grazing.

4. MANUSCRIPT I

Intake of RFI-divergent grazing beef heifers using the *n*-alkane and fecal near infrared reflectance spectroscopy (fNIRS) methods and traditional prediction equations, and relationship between intake on pasture and heifer RFI

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4.1 ABSTRACT

Many Canadian beef producers maintain their reproductive herd on pasture throughout much of the year and must budget for feed which comprises a significant portion of annual input costs. Residual feed intake (RFI) has been explored as a means of improving efficiency and decreasing feed costs, however most research to date has been conducted in confinement. The objectives of this study were to i) characterize and compare pasture intake of individual beef heifers using the *n*-alkane method, fNIRS (used either to generate a direct estimate of intake or to estimate fecal C31 and C32) and traditional equations-based predictions ii) correlate estimates of intake on pasture to previously-measured RFI rankings and iii) compare DMI of low- and high-RFI_{fat} heifers as estimated using the six aforementioned intake methods. Twenty beef heifers from two locations (KIN and LAC) were selected for RFI divergence in each of two years (2015 and 2016). Heifers grazed meadow bromegrass pastures located at the Lacombe Research and Development Centre (LRDC) allocated *ad libitum* daily and were offered 1000 ± 0.5 g of an *n*-alkane marked pellet for 12 days. Beginning on the eighth day, a fecal grab sample was also collected twice daily from each animal for later *n*-alkane analysis. Heifers were weighed on Days 0, 8, 12 and at end of test (Day 52 to 64, depending on the Herd and Year) and ultrasound backfat was measured on Day 0. Dried and ground forage, pellet and fecal samples were analyzed using the *n*-alkane method (Moshtaghi-Nia and Wittenberg, 2002 with slight modification) to estimate DMI_{Alkane}. Five-day fecal composites were analyzed via NIRS to determine DMI_{fNIRS} and C31 and C32 *n*-alkane profile, in order to estimate $DMI_{fNIRSC31C32}$ indirectly. The NRC (National Research Council 2016), Minson (Minson and McDonald 1987) and Mertens (Mertens et al. 1987) equations were also used to estimate DMI via forage and animal measures.

Dry matter intake as measured by direct and indirect fNIRS and *n*-alkane methods differed in individual-animal pasture intake compared to traditional prediction equations. For each herd in both years, the NRC equation provided the least variable estimates of intake and over-estimated DMI compared to the *n*-alkane, fNIRS and $fNIRS_{C31C32}$ techniques, while the Minson equation provided the lowest DMI estimates. The $fNIRS_{C31C32}$ method resulted in the most variable estimates of DMI. However, neither direct nor indirect fNIRS methods were significantly correlated with previously-measured RFI_{fat} except in the LAC 2015 group ($P < 0.05$). While DMI_{Alkane} was correlated to RFI_{fat} rankings for both Herds in 2015 ($P < 0.05$), this was not repeated in 2016. None of the equation-based DMI prediction methods provided estimates which differed significantly between RFI_{fat} groups, confirming that these methods are unable to account for biological differences between animals.

In summary, the *n*-alkane technique provided the most reliable estimates of individual-animal DMI given its ability to provide statistically significant differences between high- and low-RFI_{fat} heifers in 2015. However, further research is warranted as findings from this study were not repeated in both years.

4.2 INTRODUCTION

The agricultural sector is evolving in response to an ever-changing consumer landscape and increasing demand for both crops and food-producing animals. One strategy to provide more food beyond the sector's current capacity is to improve efficiencies in production while concurrently improving economic and environmental sustainability. Within the livestock sector, selection for lower feed to gain ratio (F:G) has been used to improve efficiency of beef cattle. However, more recently, residual feed intake (RFI) is being used by many countries (Australia – Herd and Arthur 2009; Canada – Basarab et al. 2013; Ireland – McDonnell et al. 2016; UK –

Herd and Bishop 2000; USA – Hafla et al. 2014) as an improved selection tool as it is independent of body weight (BW) and average daily gain (ADG). Thus, selection for low-RFI animals as part of a multi-trait selection index may improve cattle efficiency without increasing maintenance costs.

Most Canadian cattle producers maintain their breeding herd on pasture in the summer months, where cattle will graze forage ad libitum. There is currently no widely-adopted method to measure intake on pasture of grazing animals, and there is no true means of evaluating these methods in foraging conditions except by comparison with other, usually inferior, methods (Mayes and Dove, 2000). This limits the use of RFI as a selection tool for animals, particularly the reproductive herd, which must be efficient on grazed forage, compared to the standard RFI ranking environment in a confined feeding system. Although a study by Manafiazar et al. (2015) found that RFI_{fat} measured under drylot conditions using a silage-based diet in growing heifers was positively correlated to grazed RFI_{fat} determined in pregnant heifers ($p = 0.04$), Durunna et al. (2014) found RFI was affected by diet and therefore it may be ideal to improve our understanding of intake on pasture when considering efficiency measures for cattle who will consume primarily forage-based diets. The use of *n*-alkane markers and fNIRS techniques have shown promise (Johnson 2014) however they require further investigation within a Canadian climate and in non-confinement systems.

The objective of this study was to evaluate six techniques to estimate dry matter intake (DMI) in pasture-based systems. More specifically: 1) to characterize and compare pasture intake of individual beef heifers using i) the *n*-alkane method, ii) fNIRS as a direct estimate of DMI, iii) fNIRS estimates of fecal C31 and C32 used within the *n*-alkane equation to estimate DMI, and iv) traditional equation-based predictions (NRC, Mertens, Minson), 2) to correlate

estimates of intake on pasture using the *n*-alkane and direct fNIRS techniques to previously-measured RFI rankings, and 3) to compare DMI of low- and high-RFI_{fat} heifers as estimated using the aforementioned six intake methods.

4.3 MATERIALS AND METHODS

4.3.1 Test pasture preparation

In June 2001, six 1.3 ha paddocks at the Agriculture and Agri-Food Canada Lacombe Research and Development Centre (LRDC; Lacombe, AB) of orthic black chernozem soil were seeded with a monoculture of Fleet meadow bromegrass (*Bromus riparius Rehm)* at a rate of 17 kg ha^{-1} using a broadcast seeder. This forage species was selected for its regrowth, composed almost entirely of leaf sheath and blade tissue, which is of more consistent *n*-alkane profile than the first growth which contains more leaf and stem. In addition, available forage was of sufficient quantity to provide *ad libitum* intake, and the Fleet variety was agronomically acceptable. Paddocks were managed using fertilizer and intermittent grazing in the years leading up to this study.

Mean monthly temperatures varied (-0.2°C to 1°C in 2015; 0.0 to 0.3°C in 2016) from the long-term (1908- 2008) average of 16.1, 14.9 and 10.1°C, respectively in July, August and September (Environment Canada). Rainfall from May to the end of August in 2015 and 2016 was 241.6 and 292.5 mm, respectively compared to a long-term average of 278.7 mm.

4.3.2 Animal management

All procedures involving animals were reviewed and approved by the University of Alberta Animal Care and Use Committee (Livestock) and the LRDC Animal Care Committee, and all animals were cared for in accordance with the guidelines of the Canadian Council on Animal Care (CCAC).

Animals were selected for the grazing trial based on three criteria. The primary selection tool was residual feed intake (RFI) divergence as yearling heifers, followed by frequency of GreenFeed (GF; GreenFeed systems (C-Lock, Inc., Rapid City, USA) visitation, recorded prior to the study period and, when possible, confirmation of pregnancy. Each of these selection methods are described in further detail below.

Replacement heifer selection

Kinsella Composite (KC) crossbred heifers (KIN) from the Roy Berg Kinsella Research Ranch (KRR; Kinsella, AB, Canada) were predominantly Aberdeen Angus (mean=70%) with smaller percentages of Hereford, Simmental, Charolais, Limousin and Gelbvieh, and were born between March 19 and June 1, 2014 and March 18 and June 5, 2015. Crossbred heifers from LRDC (LAC) were Hereford-Aberdeen Angus, and Charolais-Red Angus-Hereford and were born between February 1 to May 16, 2014 and February 26 to May 7, 2015. Cow/calf pairs grazed meadow bromegrass pastures from early June to weaning in mid-October (LRDC) or mid-November (KRR). Calves were selected to become replacement heifers at weaning based on body weight (BW), frame size, temperament, calving ease and dam performance.

RFI test

Selected replacement heifers were housed in a feedlot pen with access to shelter, water and a barley silage-based diet for the RFI test period as outlined in Table 4.1. Individual-animal feed intake (kg DM d^{-1}) data were collected from automated GrowSafe feeding stations (GrowSafe Systems Ltd., Airdrie, AB, Canada). Animals were ranked for residual feed intake (RFI) in each year over a 76- or 92-d period, as previously described by Basarab et al. (2002, 2003). In brief, heifers were weighed prior to morning feeding on two consecutive days at the start and end of the test period, as well as at monthly intervals (Wang et al. 2006). Ultrasound backfat thickness (mm) was measured between the 12^{th} and 13^{th} ribs at the end of each test

period by a certified ultrasound technician using an Aloka Echo Camera SSD-210 DXII (Aloka Co., Ltd., Tokyo, Japan) as described by Brethour (1992).

Table 4. 1 Summary of RFI test period including ingredient and nutrient composition of diet delivered to KIN and LAC heifers in 2015 and 2016.

	2015		2016	
	KIN	LAC	KIN	LAC
Ingredient composition (%, as fed basis)				
Barley silage	55	90	55	100
Barley grain		10		
Whole oats	27		27	
Canola meal	13		13	
Rumensin pellet	5		5	
Nutrient composition (%, DM basis)				
CP	19.0	12.5	19.4	11.4
ADF	24.2	29.1	22.6	30.85
NDF	35.8	44.5	35.1	46.65
Ca	1.20	0.60	0.93	0.46
P	0.51	0.34	0.51	0.29
TDN	67.13	63.3	70.10	63.6
Test period summary				
Number of animals	274	86	145	103
Test period date start date	05-01-2015	19-02-2015	15-12-2015	22-12-2015
Test period date end date	23-03-2015	05-05-2015	29-02-2016	22-03-2016
Number of days in test period	78	76	77	92

GreenFeed delivery system

Subgroups of the RFI-tested heifers described above were selected for adaptation to GreenFeed stations and increased visitation frequency. The GF system includes a radio frequency identification (RFID) reader, gas measurement equipment, and software which transmits data to an online interface. As seen in Figure 4.1, the GF system allows one animal at a time to place its head into a hood to acquire a pelleted feed, which is delivered from a hopper by

intermittent cup drops into a feed pan. GreenFeed pellet delivery was controlled by setting the maximum allowable number of cup drops per animal per visit and per day, drop frequency and visitation frequency. Individual animal GF visitation frequency including time, duration and number of cup drops delivered was recorded during each visit. The GF system also allows for collection of gas emissions data, as described by Hammond et al. (2015). Frequency of GF visitation was included as a selection criterion to identify those animals with greatest visitation frequency to increase the likelihood of successful delivery of the *n*-alkane marked pellet through this system.

Figure 4. 1 Heifer consuming pellet from GF system.

Description of animals selected for grazing trial

Heifers were bred by artificial insemination in mid-May followed by natural service to a clean-up bull. A pregnancy exam was conducted in late July and again between September and October on all heifers; two KIN and four LAC heifers were later found to be open in 2015, and one KIN and three LAC heifers in 2016.

As a consequence of imposing the aforementioned selection criteria, in 2015, the 10 lowest (−0.56 ± 0.26 kg DMI d^{-1}) and 10 highest (+0.54 ± 0.27 kg DMI d^{-1}) RFI KIN heifers with average Day 0 BW of 457.9 ± 33.4 kg and 463.0 ± 14.1 kg, and backfat of 3.3 ± 1.3 mm and 3.4 ± 1.0 mm, respectively were selected from the RFI_{fat} test group at 465 ± 15 days of age. Similarly, the 10 lowest (−0.36 ± 0.11 kg DMI d^{-1}) and 10 highest (+0.49 ± 0.22 kg DMI d^{-1}) RFI LAC heifers with average Day 0 BW of 500.0 ± 32.3 kg and 483.3 ± 28.4 kg, and backfat of 8.3 ± 2.2 mm and 6.8 ± 1.9 mm were selected at 513 ± 22 days of age.

Similarly, in 2016, the 10 lowest (−0.71 ± 0.30 kg DMI d^{-1}) and 10 highest (+0.75 ± 0.36 kg DMI d^{-1}) RFI KIN heifers with an average Day 0 BW of 434.3 ± 35.2 kg and 432.9 ± 23.6 kg, and backfat of 4.5 ± 0.5 mm and 4.8 ± 1.1 mm were selected from the RFI_{fat} test group at 463 ± 10 days of age. The 10 lowest (−0.48 ± 0.26 kg DMI day^{-1}) and 10 highest (+0.38 ± 0.34 kg DMI day^{-1}) RFI LAC heifers with an average Day 0 BW of 501.4 ± 37.8 kg and 510.6 ± 18.6 kg, and backfat of 9.1 ± 2.0 mm and 9.7 ± 2.1 mm were also selected at 508 ± 11 days of age.

4.3.3 Forage and pellet sample collection

Each year, an *n*-alkane labelled pellet (Table 4.2) consisting of ground barley grain, ground wheat grain, canola meal, corn distillers grain, canola oil, finely grated beeswax (Tegart Apiaries Ltd. Fairview, AB, Canada) and dotriacontane (C32; Minakem, Beuvry La Foret, France) was extruded at the Crop Diversification Centre (Brooks, AB, Canada). Beeswax was removed from the pellet in 2016 to improve palatability and intake. Barley was chosen as the primary ingredient to ensure that the meadow bromegrass contributed the greatest proportion of the C31 *n*-alkane profile in the diet. Pellet samples were collected during the extrusion process for subsequent *n*-alkane analysis to ensure a uniform *n*-alkane profile was achieved throughout the batch mix. Post extrusion, pellets were coated with canola oil to aid in pellet integrity.

Table 4. 2 Ingredient composition of *n*-alkane pellet delivered during the trial period in each year.

	2015	2016
Ingredient composition (% as-fed)		
Finely ground barley grain	55	55.7
Finely ground wheat grain	20	20
Canola meal	16	16
Corn DDGS	5.3	5.3
Canola oil (post-extrusion)	2.5	2.5
Finely grated beeswax	0.7	-
Dotriacontane (C32)	0.04	0.04

4.3.4 Animal management: Pasture intake period

Two groups of twenty heifers from each KIN and LAC in each of two years were assigned a paddock equipped with GF and provided with ad libitum water and beef calving premix mineral (Masterfeeds, Red Deer, AB (2015); Hi Pro Feeds (2016)). The grazing periods began July 28, 2015 and July 27, 2016 for KIN heifers, and August 11, 2015 and August 4, 2016 for LAC heifers (Figure 4.2). During the trial period, animals had access to ad libitum meadow bromegrass forage (Table 4.3) at LAC allocated daily based on 5% of average group BW. Five-metre wide lengths were created using single-strand portable electric fencing within a paddock, excluding space on either extremity of the paddock where forage yield was less uniform. These lengths were further subdivided into daily grazing strips, with the size of the area allocated adjusted daily to accommodate differences in yield as determined by sonar in 2015 (from 0.0943 to 0.1137 ha) and 2016 (from 0.0840 to 0.0900 ha). As heifers were moved daily, waterers and mineral tubs were also moved to each new section.

Heifers grazed for an 18-d period which consisted of a 5- to 8-d adaptation period (Day −8 or −5 to −1), followed by 13 d of once-daily dosing with an *n*-alkane pellet (Day 0 to 12) and a 5-d fecal collection period (Day 8 to 12). From Day 0 to 7, heifers were grazed on back-fenced sections (fenced to exclude previously-grazed area) of the paddock and from Day 8 to 12 were

moved to another paddock set up with single-strand portable electric fencing to enable animals to access only a confined area of pasture.

Table 4. 3 Summary of forage nutrient composition of meadow bromegrass pastures located at LRDC, available to KIN and LAC heifers in 2015 and 2016.

	2015		2016	
	KIN	LAC	KIN	LAC
DM, %	32.97	28.0	37.6	36.4
NE_m, MJ/kg DM	5.61	5.61	5.52	5.52
CP, % DM	19.06	15.2	13.31	11.27
NDF, % DM	45.53	47.1	53.41	56.12

4.3.5 Supplement delivery

To administer *n*-alkane-marked pellets, heifers were moved to a handling facility once daily from Day 0 to 12 at 0815 h and were offered 1000 ± 0.5 g $head^{-1}$ of *n*-alkane pellets in individual pens. Orts were collected and weighed daily to be later analyzed for DM.

Daily GF pellet intake was measured through automated monitoring of the number of cup drops delivered to each animal, identified via its RFID tag. Cup drop samples were collected daily to ensure delivery of consistent pellet weight. In 2015, KIN heifers received *n*-alkane pellets (Table 4.4) from GF in an attempt to use this automated system as the sole delivery strategy to dose the C32 marker. However, this approach resulted in highly variable pellet intake, ranging from 0 to 0.79 kg DM d^{-1} (mean 0.19 kg DM d^{-1}) within the first three days (Day 0 to 2) of the trial. Therefore, a hand dosing protocol was implemented beginning on Day 3. In 2016, KIN heifers received soybean meal-based Calf-Manna® multi-species performance supplement (MannaPro Corporation, St. Louis, MO) from GF. In both years, LAC heifers received DG bull pellets (Masterfeeds, Inc. Red Deer, AB, Canada) containing barley, beef vitamin-trace mineral premix, calcium carbonate, corn distillers grain screenings, sodium chloride, wheat/wheat middlings and zinc chelate.

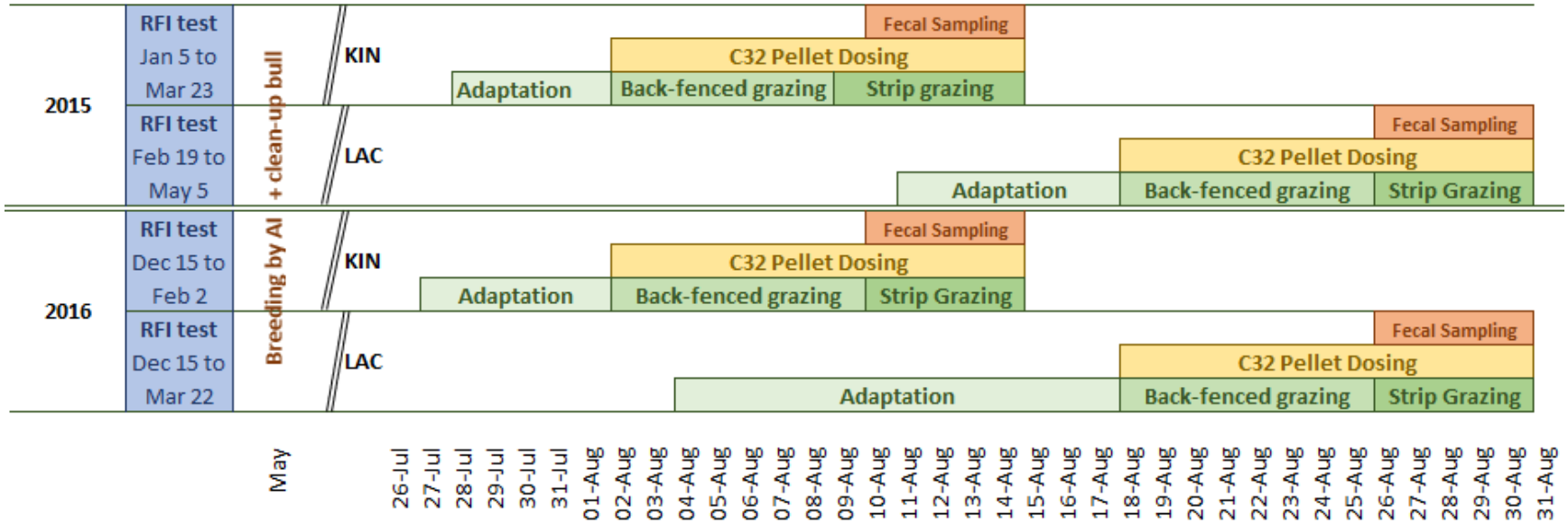

Figure 4. 2 Timeline of events throughout trial period for KIN and LAC heifers in 2015 and 2016.

Table 4. 4 Strategy for delivery and nutrient composition of pellets dispensed from GreenFeed (GF) system and hand-dosed (HD) to individual heifers.

	2015		2016		
	C32 pellet	**Bull DG**	**C32 pellet**	**Bull DG**	**Calf-Manna®**
Delivery method					
KIN heifers	HD/GF	-	HD	-	GF
LAC heifers	HD	GF	HD	GF	-
n	5	2	6	4	4
Nutrient composition (% DM)					
% DM	90.64	88.60	84.49	90.97	91.65
CP	17.64	17.80	19.40	16.30	27.08
ADF	13.08	8.05	9.88	10.28	6.98
Ca	0.23	2.22	0.29	1.78	1.09
P	0.53	0.52	0.57	0.74	0.84
Mg	0.24	0.22	0.34	0.31	0.28
K	0.63	0.67	0.70	0.92	1.60

4.3.6 Sample collection

Four days prior to grazing, six 0.125 m^2 quadrats were clipped from each pasture and analyzed for quality, percent DM and DM yield (DMY). Pluck samples were also collected from each allocated area. From Day 0 to 7, six quadrat samples were collected from each strip prior to grazing. One pluck sample was also collected in the early afternoon from each area being grazed. On Days 8 to 12, six quadrat samples were collected from the strip prior to grazing. Six pluck samples per strip were also collected each afternoon for quality and *n*-alkane analysis, and percent DM determination. Within 24 h following grazing, a 0.125 m^2 residue quadrat was clipped from each strip (daily allocation) to estimate disappearance.

Fecal grab samples were collected once daily on Day 0 and twice daily at 0815 h and 1515 h from Day 8 to 12 and stored in a cooler on ice until placed in a freezer at − 20°C for later processing.

From Day 8 to 12, one pellet sample of each type (Table 4.4) was collected to be later analyzed for DM and *n*-alkane composition. Subsamples were randomly collected between two and four times during each trial for subsequent quality analysis to ensure consistency throughout the trial.

4.3.7 Sample processing and analysis

Sample processing

Pellet, ort and fecal samples were dried at 60°C for three days in a forced-air oven and ground in a Wiley grinder through a 1 mm screen. Forage subsamples were dried at 80°C for three days to calculate percent DM. Subsamples for quality and *n*-alkane analysis were dried for a minimum of five days in a forced-air oven at 55°C and further separated into subsamples for either quality or *n*-alkane analysis, and ground to 2 mm or 1 mm, respectively.

Forage quality analysis

Forage samples were scanned for quality by NIRS (model 6500, Foss NIRSystems, Eden Prairie, MN). Raw spectral data were recorded as Log 1/R from 400 to 2498 nm at intervals of 2 nm and were transformed using the standard normal variant procedure. A calibration dataset of 238 samples was selected from a larger sample set (n=1050) for NIRS calibration using WinISI^{TM} 4 software (Infrasoft International, Silver Spring, MD). These samples were chosen by the equation in order to capture variability seen in CP, ADF and NDF. Calibrations were assessed using the coefficient of determination for the prediction (R^2) and the standard error of calibration (SEC). Calibrations were validated using cross-validation procedure and assessed by reference to the standard error of cross validation (SECV) and variance accounted for during validation (1−VR). Summary statistics are presented below (Table 4.5) for the calibration used.

Table 4. 5 Summary of NIRS calibration statistics for forage quality analysis.

Nutrient	n	Mean	SD	Min.	Max.	SEC	R^2	SECV	1-VR
CP	238	10.34	2.53	2.75	17.93	0.41	0.97	0.48	0.96
ADF	238	34.69	2.90	25.99	43.39	1.38	0.78	1.48	0.74
NDF	238	54.07	3.46	43.68	64.46	1.48	0.82	1.74	0.75

Pellet quality analysis

Pellet samples were analyzed for dry matter (DM), crude protein (CP), acid detergent fibre (ADF), calcium, phosphorus, magnesium and potassium. The chemical compositions of each pellet are presented in Table 3.4 as determined by Cumberland Valley Analytical Services (CVAS; Hagerstown, MD). In brief, DM was determined by drying at 135°C to a constant weight as per Association of Official Analytical Chemists (AOAC) official procedures (AOAC 2000, Official Method 930.15). Crude protein (CP) was calculated as 6.25 x N (AOAC 1990, Official Method 973.03). Acid detergent fibre (ADF) and mineral contents were determined by AOAC (2010) official procedures 973.18 and 985.01, respectively, with modifications as described online (CVAS, 2017).

n-alkane analysis

Fecal samples, plucked pasture samples, and *n*-alkane pellet samples were analyzed for *n*-alkanes as described by Moshtaghi-Nia and Wittenberg (2002), with the following modifications. Briefly, a known amount of dried and ground sample was digested overnight at 90°C in an Isotemp oven (Fisher Scientific, Ottawa, ON) with ethanolic potassium hydroxide and an internal standard containing C34. After cooling, 8 ml of heptane and 8 ml of warm distilled water were added. Samples were vortexed before warming in a water bath set to between 50°C and 60°C for at least five minutes to allow the phases to separate. Following the addition of three, 5-ml aliquots of heptane, the extract was evaporated to dryness by placing the vials in a water bath maintained between 70°C and 90°C, using compressed air to hasten the process. After dissolving

the alkanes with heptane in a water bath, the solution was filtered through a silica gel column for a total of five times, rinsing the vial each time with heptane. The filtrate was then evaporated to dryness using the same method as above, and samples were prepared for the gas chromatograph (GC) by adding *n*-undecane to the vials, warming and rinsing with the solution, and transferring to GC vials. An in-house standard with a well-characterized *n*-alkane profile was extracted in duplicate and analyzed between every 44^{th} sample to ensure consistency. In addition, a blank sample was analyzed with the same frequency to ensure samples were free from contamination. The reconstituted samples were analyzed using a Varian Model 3900 gas chromatograph, set to split injection mode at 300°C. The GC was calibrated using an external standard containing all *n*-alkanes between C24 and C36. *n*-alkane profiles are summarized in Tables 3.6 and 3.7 for 2015 and 2016, respectively.

Table 4. 6 Mean (± SD) n-alkane composition (mg kg^{-1} DM) of meadow bromegrass and pellets offered via hand-dosing (*n*-alkane C32-labelled pellet) and GreenFeed (GF; DG Bull or Calf-Manna® pellet) in 2015.

Alkane chain length	Feedstuff		
	Meadow bromegrass	C32-labelled pellet	GF pellet
		KIN 2015	
n	*87*	*10*	*0*
C_{24}	4.21 (0.22)	1.48 (0.25)	
C_{25}	6.95 (0.14)	42.57 (0.15)	
C_{26}	4.07 (0.26)	5.32 (0.15)	
C_{27}	46.30 (0.60)	184.81 (0.34)	
C_{28}	2.46 (0.08)	4.56 (0.04)	
C_{29}	77.15 (1.35)	113.96 (0.21)	
C_{30}	2.37 (0.04)	3.73 (0.04)	
C_{31}	96.12 (1.80)	85.24 (0.26)	
C_{32}	1.41 (0.03)	257.12 (0.45)	
C_{33}	47.70 (1.26)	12.88 (0.04)	
C_{34}	426.27 (1.22)	438.42 (1.00)	
C_{35}	3.65 (0.11)	3.01 (0.10)	
C_{36}	0.53 (0.10)	0.09 (0.09)	
		LAC 2015	
n	*97*	*2*	*8*
C_{24}	3.26 (0.14)	2.58 (1.66)	1.14 (0.11)
C_{25}	6.83 (0.14)	42.25 (0.60)	2.7 (0.05)
C_{26}	3.13 (0.14)	5.44 (0.33)	0.57 (0.08)
C_{27}	47.36 (0.59)	182.83 (1.35)	3.67 (0.07)
C_{28}	2.55 (0.12)	4.46 (0.04)	0.53 (0.16)
C_{29}	87.82 (1.41)	111.98 (086)	11.25 (0.06)
C_{30}	3.40 (0.18)	3.63 (0.12)	0.60 (0.09)
C_{31}	121.46 (1.85)	83.66 (0.66)	10.16 (0.09)
C_{32}	1.88 (0.05)	251.85 (1.77)	0.18 (0.12)
C_{33}	45.90 (0.77)	12.45 (0.07)	0.75 (0.12)
C_{34}	436.84 (1.11)	438.64 (0.68)	434.51 (0.87)
C_{35}	3.09 (0.06)	3.29 (0.49)	0 0
C_{36}	2.60 (0.06)	0 0	0 0

Table 4. 7 Mean (± SD) *n*-alkane composition (mg kg^{-1} DM) of meadow bromegrass and pellets offered via hand-dosing (*n*-alkane C32-labelled pellet) and GreenFeed (GF; DG Bull or Calf-Manna® pellet) in 2016.

Alkane chain length	Feedstuff		
	Meadow bromegrass	C32-labelled pellet	GF pellet
		KIN 2016	
n	*98*	*14*	*23*
C_{24}	1.43 (0.08)	1.16 (0.03)	1.23 (0.10)
C_{25}	6.09 (0.16)	2.62 (0.07)	0.81 (0.20)
C_{26}	1.90 (0.10)	0.43 (0.10)	0.50 (0.09)
C_{27}	48.46 (0.84)	3.08 (0.27)	2.33 (0.31)
C_{28}	2.44 (0.13)	0.56 (0.18)	0.22 (0.13)
C_{29}	81.22 (1.42)	11.96 (0.21)	8.11 (0.28)
C_{30}	3.39 (0.06)	1.62 (0.03)	0.81 (0.06)
C_{31}	117.04 (2.00)	9.06 (0.25)	12.82 (0.30)
C_{32}	1.85 (0.04)	369.68 (7.11)	1.06 (0.26)
C_{33}	41.08 (0.71)	2.57 (0.08)	1.53 (0.04)
C_{34}	491.80 (1.01)	499.10 (0.66)	500.42 (1.16)
C_{35}	2.85 (0.07)	0 0	0 0
C_{36}	4.57 (0.12)	3.86 (0.12)	4.09 (0.09)
		LAC 2016	
n	*97*	*16*	*21*
C_{24}	0.95 (0.02)	1.50 (0.16)	0.92 (0.03)
C_{25}	5.68 (0.14)	2.59 (0.04)	6.33 (0.05)
C_{26}	1.68 (0.10)	1.14 (0.13)	0.77 (0.06)
C_{27}	49.36 (4.17)	2.70 (0.03)	6.44 (0.05)
C_{28}	3.15 (0.15)	0.91 (0.10)	1.27 (0.11)
C_{29}	84.82 (1.07)	9.77 (0.91)	12.36 (0.09)
C_{30}	4.10 (0.06)	1.75 (0.08)	1.05 (0.06)
C_{31}	135.50 (2.33)	8.45 (0.12)	16.06 (0.12)
C_{32}	2.14 (0.04)	361.77 (5.38)	0.68 (0.12)
C_{33}	40.47 (0.66)	2.40 (0.04)	2.60 (0.02)
C_{34}	488.25 (1.08)	491.86 (1.06)	498.29 (1.13)
C_{35}	2.50 (0.06)	0 0	0 0
C_{36}	6.81 (0.11)	6.14 (0.19)	5.03 (0.16)

fNIRS analysis

Fecal samples were pooled by animal such that each composite sample consisted of equal amounts of up to 10, but at minimum six, dried and ground fecal samples collected in the morning and afternoon from Day 8 to 12.

Samples were treated as described in Johnson et al. (2017). Briefly, before scanning, the fecal composite samples were dried in a forced-air oven at 60°C for a minimum of 4 h to eliminate any recaptured moisture and then placed in a desiccator for 1 h to cool to ambient temperature. Samples were then packed into quartz-lens sample cups and stored in a desiccator until NIRS scanning. Samples were scanned using a Foss NIRS 6500 scanning monochromator at the Grazingland Animal Nutrition Laboratory (Temple, TX). Reflectance energy (log 1/R) was measured and recorded at 2 mm intervals from 400 to 2498 nm, and stored using Infrasoft International software, version 1.5 (Win ISI Port, Matilda, PA). A spectral library was created from fecal spectra compiled from six LRDC studies (n = 123) in which pregnant heifers grazed meadow bromegrass pasture: i) two studies conducted at LRDC in 2012 and 2013 as described in Manafiazer et al. (2015) and ii) the four studies described herein with KIN and LAC heifers in 2015 and 2016.

Prior to calibration, fecal spectra were subjected to a standard multiplicative scatter correction (MSC) to correct for mean and standardization at each wavelength, as well as a second derivative transformation, with a gap and smooth software setting of four. Modified partial least squares (MPLS) regression approach was then used to develop calibration equations for DMI and C31 and C32 concentration using fNIRS spectra as the independent variable. A total of 256 wavelengths were used for calibration development, and two outlier elimination passes were used to identify and eliminate outliers based on a Mahalonobis distance (GH) ≥ 8 and a critical 'T' statistic ≥ 2.5 (Showers et al., 2006).

Cross- and test-set methods were used to evaluate the accuracy of prediction equations. Cross-validation was accomplished as described by Williams (2005), using the same samples for validation as were used for calibration development. Calibration performance was evaluated using the coefficient of determination for calibration (R^2_c) and standard error of calibration (SEC), which defines how well calibration samples fit the reference data. Predictive accuracy of the calibration equations was evaluated using the coefficient of determination for cross validation (R^2_{cv}), standard error of cross-validation (SECV), and the coefficient of determination for test-set validation (R^2_v). Estimates of DMI were either derived directly from fNIRS analysis (DMI_{fNIRS}) or indirectly via estimation of C31 and C32 concentration in feces ($DMI_{fNIRSC31C32}$), using the traditional *n*-alkane method to determine forage and pellet *n*-alkane profiles and the Moshtaghi-Nia and Wittenberg (2002) equation to estimate DMI.

4.3.8 Trait derivations and intake calculations

Efficiency measures under drylot conditions

Residual feed intake, unadjusted (RFI) and adjusted for off-test backfat (BF) thickness (RFI_{fat}) were calculated as previously described by Basarab et al. (2003). Body weight at the mid-point of the test (MIDWT) and average daily gain (ADG, regressed over duration of test; kg d^{-1}) for each heifer were calculated using a linear regression of the animal's observed BW against day on test. Duration of test varied, where KIN 2015 = 57 days, LAC 2015 = 52 days. KIN 2016 = 63 days and LAC 2016 = 64 days. Residual feed intake was calculated as the difference between actual standardized DMI (DMSI) and expected feed intake (EFI). Average daily feed intake for each heifer, as recorded from the GrowSafe ® Feed Intake system, was multiplied by the feed dry matter percentage to obtain daily dry matter intake (DMI), then multiplied by the metabolizable energy (ME) content of the ration and divided by 10 to standardize DMI to an

energy density of 10 MJ ME kg^{-1} DM (DMSI). Standardizing DMSI made the results comparable to previously-reported research findings (Arthur et al. 2001; Basarab et al. 2003; Nkrumah et al. 2006). Thereafter, DMSI was divided by the number of days on test to give average standardized daily DMI (SDMI; kg DM d^{-1}). For each animal within a contemporary group, SDMI was then regressed on ADG and metabolic MIDWT ($kg^{0.75}$) to estimate EFI using PROC GLM (SAS Institute, Inc. 2009) and the following model:

$$\text{Model 1: } Y_i = \beta_0 + \beta_1 ADG_i + \beta_2 MIDWT_i^{0.75} + e_i,$$

where Y_i is the SDMI for animal *i*, β_0 is the regression intercept, β_1 is the partial regression coefficient of SDMI on ADG, β_2 is the partial regression coefficient of SDMI on metabolic MIDWT, and e_i is the random error term. A second model was developed to adjust RFI for backfat thickness (mm) in an effort to remove effects of body fatness and sexual maturity on feed intake:

$$\text{Model 2: } Y_{ij} = \beta_0 + \beta_1 ADG_i + \beta_2 MIDWT_j^{0.75} + \beta_3 BFend_k + e_{ijk},$$

where β_3 is the partial regression coefficient of SDMI on off-test ultrasound backfat thickness. RFI and RFI_{fat} were then computed for each animal as the deviation of SDMI from the expected feed intake (EFI).

Forage intake

Forage DMI was estimated on an individual animal basis using data from Day 0 to 12 via the methods described below.

1) Paired *n*-alkane methodology: $DMI_{Alkane}(kg\ DM\ d^{-1}) = \frac{\left(\frac{F_i}{F_j}\right) x\ D_j\ x\ ((IS\ x\ S_j) - (IS\ x\ S_i))}{H_i - \left(\frac{F_i}{F_j}\right) x\ H_j}$

Where $F_{i,j}$ = fecal C31, C32 concentrations (mg kg^{-1} DM), D_j = dose rate of C32 (mg kg^{-1}), IS = intake of dosed pellet (kg DM d^{-1}), $S_{i,j}$ = pellet C31, C32 concentrations (mg kg^{-1} DM) and $H_{i,j}$ = forage C31, C32 concentrations (mg kg^{-1} DM) (Moshtaghi-Nia and Wittenberg, 2002).

The *n*-alkane profile of pellets delivered via GreenFeed and hand dosing were considered in intake calculations

This formula was also used to calculate $DMI_{fNIRSC31C32}$, with fecal C31 and C32 concentrations ($F_{i,j}$) determined via fNIRS.

2) NRC Equation 10-1 (NRC 2016):

$$Forage\ NE_m\ intake,\ Mcal\ d^{-1} = BW^{0.75} * (0.2435 * NE_m - 0.0466 * NE_m^2 - 0.0869)$$

$$DMI_{NRC},\ kg\ DM\ d^{-1} = forage\ NE_m\ intake\ (Mcal\ d^{-1})/NE_m$$

where BW = average body weight (kg) over the feeding period and NE_m = standing forage net energy for maintenance (Mcal kg^{-1} DM).

3) Minson and McDonald (1987):

$$DMI_{Minson},\ kg\ DM\ d^{-1} = (1.185 + 0.00454BW - 0.0000026BW^2 + 0.315ADG)^2$$

where BW = average BW over the feeding period (kg) and ADG = average daily gain (kg d^{-1}).

4) Mertens et al. (1987):

$$DMI_{Mertens},\ kg\ DM\ d^{-1} = ((120/NDF)/100) * BW$$

where BW = average BW over the feeding period (kg) and NDF = forage neutral detergent fibre (% DM).

Intake estimates for DMI_{NRC}, DMI_{Minson} and $DMI_{Mertens}$ were averaged over Days 8 to 12 of the trial period as BW, NE_m, and ADG were assumed to be constant over this period. Similarly, $DMI_{fNIRSC31C32}$ was estimated over the 5-d period as fNIRS analysis was conducted on 5-d fecal composites. However, DMI_{Alkane} was estimated on a daily basis from Day 8 to 12 as *n*-alkane analysis was conducted on fecal, forage and pellet samples collected daily.

4.3.6 Statistical analysis

Dependent variables included RFI_{fat} and $DMI_{fNIRSC31C32}$, $DMI_{NRC,}$ DMI_{Minson} and $DMI_{Mertens}$, collectively referred to as DMI_{method}. As RFI_{fat} can be more difficult to measure compared to DMI_{Alkane} and DMI_{fNIRS}, these dependent variables are either alternative approaches

($DMI_{fNIRSC31C32}$) to estimating DMI using DMI_{Alkane} or DMI_{fNIRS}, or are reference values (DMI_{NRC}, DMI_{Minson} and $DMI_{Mertens}$). These variables were compared to independent variables DMI_x (either DMI_{Alkane} or DMI_{fNIRS}) using PROC MIXED of the SAS University Edition software (version 3.71, SAS Institute Inc. 2017, Cary, NC). The regression model also included DMI_x, as well as Year (2015 or 2016) and Herd (KIN or LAC) as class variables, and interactions between each of these. The main effects (Year, Herd, and Year×Herd) tested for differences in intercept, while interactions with DMI_x (Year×DMI_x, Herd×DMI_x, and Year×Herd×DMI_x) tested whether slope was affected by Year and/or Herd. The ESTIMATE procedure was used to estimate intercept. Each of these models tested whether the regression of RFI_{fat} or DMI_{method} on DMI_x had the same slope and intercept in the various Years and Herds, and thus whether the relationship was consistent under various conditions. The models listed here show the factors and interactions present in the MIXED procedure, as further described in Tables 4.10 and 4.11. The DMI variables, whether dependent or independent, were continuous. Any dependent variable with DMI implies that there is a regression value associated with it. All dependent variables in the model were fixed effects.

1) $DMI_{method} = DMI_{Alkane}$ Year Herd Year×Herd Year×DMI_{Alkane} Herd×DMI_{Alkane} Year×Herd×DMI_{Alkane}
2) $DMI_{method} = DMI_{fNIRS}$ Year Herd Year×Herd Year×DMI_{fNIRS} Herd×DMI_{fNIRS} Year×Herd×DMI_{fNIRS}

The PROC CORR procedure (version 3.71, SAS Institute Inc. 2017, Cary, NC) was used to examine correlations between RFI_{fat} and DMI for each method. The CORR procedure was run

for both individual trials (KIN 2015, LAC 2015, KIN 2016, LAC 2016; n = 20 for each trial), as well as all animals as a single group (n = 80).

In order to assess whether High- and Low-RFI_{fat} heifers could be distinguished using any of the DMI estimation methods, PROC MIXED was used to compare High- and Low-RFI heifers, for each DMI estimation method in each Herd-Year (i.e. contemporary group), with High and Low as class variables and the RFI_{fat} or DMI_{method} as a continuous variable. CONTRAST and ESTIMATE statements were used to compare LS means, with differences among treatment means tested using a Tukey test at $\alpha = 0.05$ level of significance.

4.4 RESULTS AND DISCUSSION

4.4.1 Calibration and validation of fNIRS equations

Prediction of DMI_{fNIRS}

Calibration statistics (Table 4.8) obtained for DMI_{fNIRS} ranged from 0.23 (SEC) and 0.90 (R^2_c) for LAC 2016 to 0.77 (SEC) and 0.41 (R^2_c) for LAC 2015. Calibration accuracies for prediction equations were outside the range of the acceptable criteria of $R^2 > 0.80$ and SEC < 2.0×SEL described by Westerhaus (1989) and Li et al. (2007), where SEL is the standard error of the laboratory reference method, and the reference method in this case is DMI_{Alkane}. The cross-validation accuracies (SECV and R^2_{cv}) ranged from 0.72 (SEC) and 0.07 (R^2_{cv}) for LAC 2016 to 1.08 (SEC) and 0.16 (R^2_{cv}) for LAC 2015.

Calibration and cross-validation summary statistics for the combined trial fNIRS equation for DMI are presented in Table 4.9. These calibrations were developed using the samples from six meadow bromegrass grazing trials (n=123) conducted between 2012 and 2016. The range in calibration and validation accuracies across the individual trial equations was likely due to insufficient population sizes (n = 16 to 20), as accuracies were improved when individual trial

data sets were compiled. The calibration (R^2_c) and validation (R^2_{cv}) accuracies for this equation were 0.66 and 0.78, respectively. The R^2_{cv} reported here is greater than values reported by Boval et al. (0.52; 2004); Valiente et al. (0.45; 2004), Keli et al. (0.20; 2007) and Johnson (0.73; 2014) where fecal composite samples from cattle or sheep were used to predict average intake. The SEC and R^2_{cv} for this study are outside the range recommended by Westerhaus (1989) and Li et al. ($R^2 > 0.80$ and SEC < 2.0×SEL; 2007), suggesting that these equations are unsuitable for DMI prediction.

Table 4. 8 Summary statistics for calibration and cross-validation of fNIRS prediction equations for *n*-alkane estimated DMI (DMI_{Alkane}, kg DM d^{-1}) and fecal *n*-alkane C31 and C32 concentration using 5-d composite fecal samples from pregnant heifers from KIN and LAC in four trials conducted at LAC in 2015 and 2016.

						Calibration[2]		Cross-validation	
Year	Herd	N	Range	Mean (SD)	Outliers[1]	SEC	R^2_c	SECV	R^2_{cv}
DMI_{Alkane}									
2015	KIN	16	8.5 - 13.9	11.23 (0.89)		0.69	0.41	0.99	0.16
2015	LAC	20	7.9 - 13.9	10.90 (1.00)		0.77	0.41	1.08	0.16
2016	KIN	20	8.0 - 13.7	10.83 (0.94)		0.68	0.48	1.05	0.07
2016	LAC	20	8.3 - 12.7	10.46 (0.73)		0.23	0.90	0.72	0.07
Fecal C31 n-alkane concentration, mg/kg									
2015	KIN	16	288.3 - 400.3	344.30 (18.67)		10.68	0.67	17.00	0.27
2015	LAC	19	331.0 - 410.5	370.75 (13.26)	1	2.86	0.95	12.39	0.23
2016	KIN	20	252.7 - 363.0	307.83 (18.39)		10.58	0.67	14.37	0.43
2016	LAC	19	274.8 - 394.2	334.53 (19.90)	1	14.13	0.50	19.33	0.10
Fecal C32 n-alkane concentration, mg/kg									
2015	KIN	16	63.2 - 197.9	130.53 (22.45)		14.78	0.57	34.21	0.25
2015	LAC	19	65.7 - 113.8	89.73 (8.02)	1	1.33	0.97	6.69	0.34
2016	KIN	20	70.2 - 151.8	110.98 (13.60)		9.83	0.48	13.33	0.16
2016	LAC	20	83.2 - 157.3	120.25 (12.36)		8.18	0.56	12.26	0.06

[1]Outliers were identified as having a "GH" statistic > 0.80 or a "T" statistic > 2.5 and were not included in the calibration equation.
[2]Calibration included 100% of the samples in the dataset.
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 4. 9 Summary statistics for calibration and cross-validation of fNIRS prediction equations for *n*-alkane estimated DMI (DMI_{Alkane}, kg DM d^{-1}) and fecal *n*-alkane C31 and C32 concentration (mg kg^{-1}) using 5-day composite fecal samples from pregnant heifers from six LRDC trials conducted from 2012 to 2016.

					Calibration[2]		Cross-validation	
Trial	N	Range	Mean (SD)	Outliers[1]	SEC	R^2_c	SECV	R^2_{cv}
DMI_{Alkane}, kg d^{-1}								
Bromegrass calibration	123	6.82-12.1	9.82 (1.54)	4	0.81	0.66	0.73	0.78
Fecal C31 n-alkane concentration, mg kg^{-1}								
Bromegrass calibration	123	272.3-461.4	369.0 (46.5)	1	15. 2	0.89	21.1	0.79
Fecal C32 n-alkane concentration, mg kg^{-1}								
Bromegrass calibration	123	71.9-170.2	117.1 (18.6)	5	10.7	0.60	12.2	0.48

[1]Outliers were identified as having a "GH" statistic > 0.80 or a "T" statistic > 2.5 and were not included in the calibration equation.

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

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

Prediction of fecal n-alkane concentration using fNIRS

Calibration statistics obtained for C31 ranged from 2.86 (SEC) and 0.95 (R^2_c) for LAC 2015 to 14.13 (SEC) and 0.50 (R^2_c) for LAC 2016 (Table 4.8). Values for C32 ranged from 1.33 (SEC) and 0.97 (R^2_c) for LAC 2015 to 14.78 (SEC) and 0.57 (R^2_c) for KIN 2015 (Table 4.8). Calibration accuracies for prediction equations were outside the range of the acceptable criteria of $R^2 > 0.80$ and SEC < 2.0×SEL described by Westerhaus (1989) and Li et al. (2007), with the exception of LAC 2015 C31 and C32. The cross-validation accuracies for C31 ranged from 12.39 (SECV) and 0.23 (R^2_{cv}) for LAC 2015 to 19.33 (SECV) and 0.10 (R^2_{cv}) for LAC 2016. Cross-validation accuracies for C32 ranged from 6.69 (SECV) and 0.34 (R^2_{cv}) for LAC 2015 to 34.21 (SECV) and 0.25 (R^2_{cv}) for KIN 2015. The large range in calibration and validation accuracies across the individual trial equations observed for DMI_{fNIRS} described above were also apparent with C31 and C32 and were likely due to insufficient population sizes (n = 16 to 20), as accuracies were improved for both C31 (SECV = 21.1, $R^2_{cv} = 0.79$) and C32 (SECV = 12.2, $R^2_{cv} = 0.48$) when individual trial data sets were compiled (n = 123; Table 4.9). Furthermore, it has been suggested (Boval et al. 2004) that calibration datasets for NIRS equation development should include samples representing all factors that contribute to spectral diversity such as plant species, location, soil type, management, season and year. Logically, the same rationale would apply to fNIRS calibration datasets. However, the fecal samples in this study represented only a single forage species grazed at one location and season, by animals of similar breed composition and physiological status and under similar management. Therefore, the low SEC and SEC-V and moderately high R^2 for DMI_{Alkane} (Tables 4.8 and 4.9) may be within reasonable expectations for this type of sample set but the predictive ability of these equations when applied to other datasets would likely be poor. Thus, while it may take several additional years and resources to develop a prediction equation which is suitable for estimation of DMI or *n*-alkane concentration across

diverse trials, in the meantime this approach may allow for accurate estimates to be obtained via fNIRS for meadow bromegrass studies.

Calibration and cross-validation summary statistics obtained for C31 and C32 using the combined trial fNIRS equation are presented in Table 4.9. As with the calibrations for $DMI_{fNIRS,}$ cross-validation was used to evaluate performance of the calibration developed with all samples in the data set (n = 123). The calibration accuracies for this equation were 15.2 (SEC) and 0.89 (R^2_c) for C31, and 10.7 (SEC) and 0.60 (R^2_c) for C32. The SEC and R^2_c for both C31 and C32 are outside the range recommended by Westerhaus (1989) and Li et al. (2007; $R^2 > 0.80$ and SEC < 2.0×SEL), however C31 predictions were closer to the acceptable criteria compared to estimates of C32 based on the observed SEC and R^2_c values. It has been speculated by Ru et al. (2002) and Garnsworthy and Unal (2004) that some naturally-occurring *n*-alkanes (including C31) represent more than pure hydrocarbons, which may explain the improved accuracy of fNIRS predictions for C31, whose spectra may be influenced by structural interactions between the *n*-alkane and other chemical or structural entities such as cellulose, lignin, and tannins. Alternatively, improved accuracy could also be due to the greater range and higher concentration of C31 in these studies compared to C32 in our dataset as Ru et al. (2002) also reported that *n*-alkanes present in deer feces (C24 to C36 ranging in concentration from 5.67 to 716.58 ppm) in higher concentrations were more highly predictable, as indicated by improved validation statistics, than those found in lower quantities. Cross-validation is intended to provide an assessment of the predictive performance of samples outside the current calibration dataset. However, in this case, as in Johnson (2014), it was likely limited by the narrow diversity of the sample set. Therefore, the current dataset is not large enough to confirm use of fNIRS to predict DMI, as it lacks the statistical strength to predict independent datasets. Larger data sets are

required to increase robustness of the fNIRS calibration and improve its ability to validate prediction equations. While the results from this study were comparable to previously reported data, the calibrations developed for the prediction of DMI were unable to provide an acceptable predictive equation based on the recommendations by Westerhaus (1989) and Li et al. (2007) as R^2_v values were less than 0.80 and SEC values were greater than 2 × SEL. However, these recommendations may not be entirely suitable to evaluate fNIRS equations for the prediction of DMI as they were established for single element nutritive parameters such as CP. Variations in DMI however can be influenced by many factors, including those that are unobservable in fecal samples such as rumen size or feeding behaviour (Fanchone et al. 2007). Johnson (2014) has suggested that for this reason, fNIRS equations may never be able to predict DMI with the same accuracy as equations developed for the prediction of diet quality, such as CP or NDF.

Overall, the capacity of fNIRS equations to predict individual-animal DMI is limited, as the equations developed in this study reported calibration and validation R^2 values less than 0.90 (with the exception of LAC 2015 data). These equations are limited in robustness, as the calibration contains fewer than 2000 samples (Johnson 2014). However, calibrations may be improved in the future using statistical software that will allow for trial effects to be blocked, resulting in a greater proportion of the variation associated with individual animal feed intake.

The results from the discriminant analysis illustrating the variation in fecal spectra between trials are presented in Figure 4.3. These trials are well-suited for discriminant analysis as they were similar with regards to breed composition, stage of production, age, diet, location and season across years (Johnson et al. 2015). The discriminant analysis (Figure 4.3) revealed that variations in the fecal spectra are unrelated to these aforementioned factors, with trial effects representing the largest variation in fecal spectra. The variation across trials is evident by the

clustering of individual trials, depicted in Figure 4.3, which is common in fNIRS spectra. However, the observed variation between trials was less distinct than in a study by Johnson et al. (2015) which may be attributable to differences in breed of cattle in the current study compared to previous work in which all studies were conducted using a single breed.

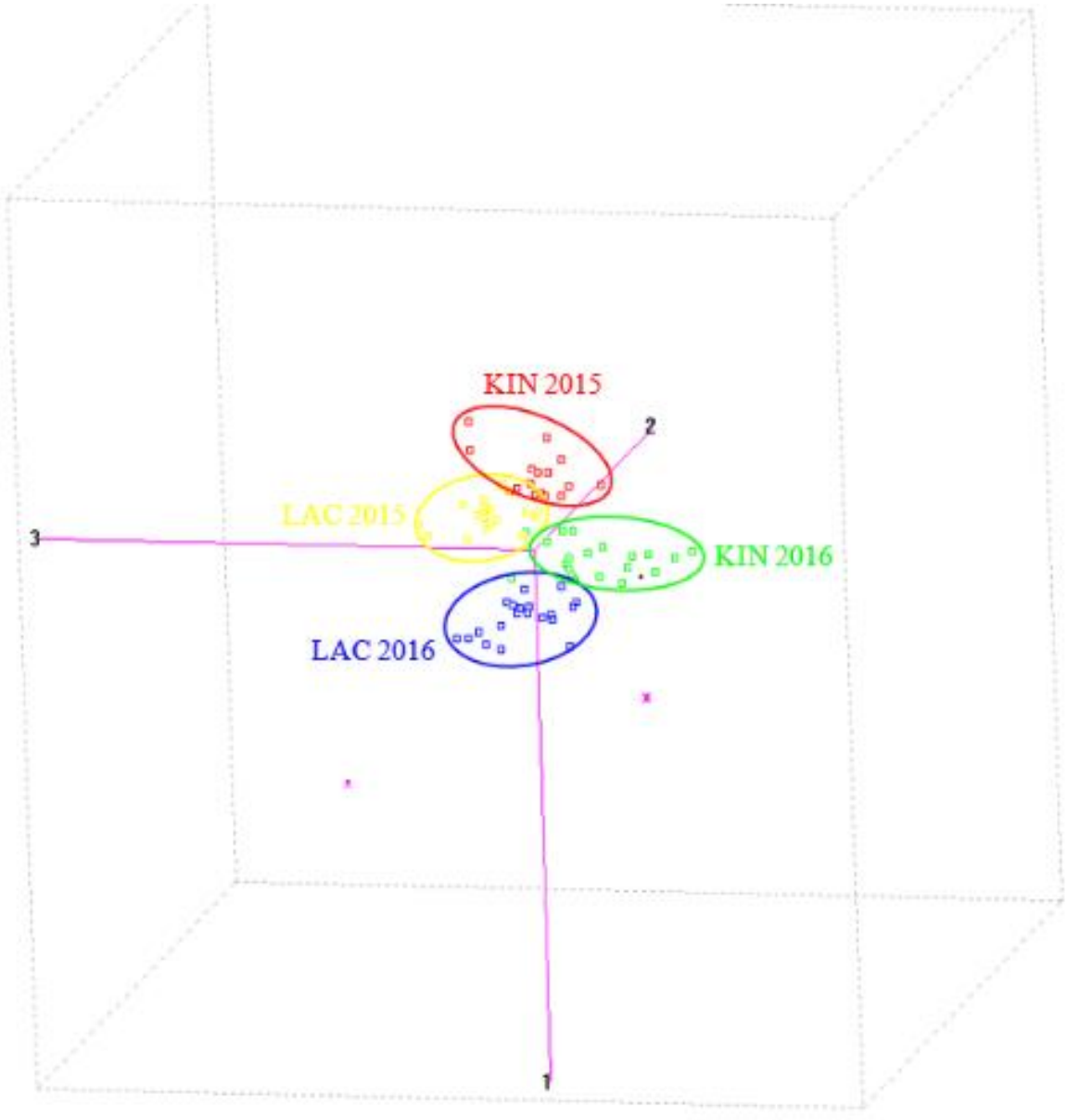

Figure 4. 3 Three-dimensional discriminant analysis of fecal NIRS spectra demonstrating trial effects where each point represents the 5-d fecal composite from an individual animal and each color represents a Herd-Year.

4.4.2 DMI_{Alkane} and DMI_{fNIRS} regression models

Significance of relationships between dependent variables RFI_{fat}, $DMI_{fNIRSC31C32}$, DMI_{NRC}, DMI_{Minson} or $DMI_{Mertens}$ and continuous variables DMI_{Alkane} or DMI_{fNIRS} are depicted in Table 4.10 and Table 4.11, respectively. Final fitted models and summary statistics for each model are presented in Table 4.12.

Although DMI_{Alkane} was significant for RFI_{fat} ($P = 0.0063$; Table 4.10), suggesting a potential for forage DMI estimates using the *n*-alkane method to predict RFI_{fat}, the R^2 (0.0919) and RSD (0.5856) were low (Table 4.12). This suggests that the model is neither precise nor accurate. Neither year, herd nor any interactions between these variables were significant for the model between DMI_{Alkane} and RFI_{fat}. Year and Herd factors encompassed animal variables such as breed, age, BW and ADG, as well as environmental variables such as weather and forage quality during the grazing period. They also included differences in the diet offered during the RFI test period between herds, which was potentially the most influential factor in this model. As suggested by Durunna et al. (2014), animals should be evaluated for efficiency across diets and seasons due to differences in maintenance requirements, fat deposition, conceptus growth, lactation, activity and thermoregulation.

For both the DMI_{Alkane} (Table 4.10) and DMI_{fNIRS} (Table 4.11) regression models, Year was significant ($P < 0.0001$) for DMI_{Minson} and $DMI_{Mertens}$, a reflection of BW, ADG or forage quality. Herd was significant for all DMI methods ($DMI_{fNIRSC31C32}$ $P < 0.05$; DMI_{NRC}, DMI_{Minson} and $DMI_{Mertens}$ $P < 0.0001$), but not for RFI_{fat}. Heifers from each herd differed in mean BW, BF and age, with LAC heifers in both years being heavier and older on average compared to KIN heifers. Although KIN heifers were more divergent in RFI_{fat} ranking, mean RFI_{fat} was similar between herds. Year*Herd was significant for DMI_{Minson} ($P < 0.0001$ for DMI_{Alkane} model;

P = 0.0002 for DMI_{fNIRS} model). As the Minson and MacDonald (1987) equation predicts DMI_{Minson} based on BW and ADG, differences in BW between herds and years (KIN 2016 < KIN 2015 < LAC 2015 < LAC 2016) could explain the significant effect of Year and Year*Herd for the DMI_{Minson} models. The Mertens et al. (1987) equation predicts $DMI_{Mertens}$ based on NDF and BW, and the significant effect of Year was likely due to the 9 – 11 % (DM basis) greater NDF in forage grazed in 2016 than in 2015. There were no significant interactions between Year*DMI, Herd*DMI, or Herd*Year*DMI for any of the dependent variables.

While DMI_{Alkane} was able to predict $DMI_{fNIRSC31C32}$ (P = 0.0163) and DMI_{NRC} (P = 0.0287), there was no significant relationship between DMI_{Alkane} and DMI_{Minson} or $DMI_{Mertens}$, nor was the relationship between DMI_{fNIRS} and RFI_{fat} or any DMI estimation method significant, with the exception of DMI_{Minson} (P = 0.0354).

The final fitted model for DMI_{Alkane} (Table 4.12) and RFI_{fat} includes an intercept and linear regression on DMI_{Alkane} with a weak (R^2 = 0.0919) but significant (P = 0.0063; Table 4.10; Figure 4.4) positive relationship between DMI_{Alkane} and RFI_{fat} Similarly, there was a weak but significant positive relationship between DMI_{Alkane} and $DMI_{fNIRSC31C32}$ (R^2 = 0.1437, P = 0.0163; Figure 4.5), with a higher average intake for KIN heifers (intercept = 6.0715) compared to LAC heifers (intercept = 5.4212). Conversely, with DMI_{NRC} as the dependent variable in this model, LAC heifers had a higher average intake (intercept = 10.2737) than KIN heifers (intercept = 11.2077), and a stronger positive relationship (R^2 = 0.4334, P = 0.0287; Figure 4.6). While there was no relationship between DMI_{Alkane} and DMI_{Minson}, LAC heifer DMI_{Minson} estimates were similar between years, and higher than KIN in both years; while KIN DMI_{Minson} estimates were higher in 2016 than in 2015 (Table 4.12). Similarly, there was no relationship between DMI_{Alkane} and $DMI_{Mertens}$, however a significant effect of year and herd was noted.

Table 4. 10 Significance table[1] for *n*-alkane-estimated DMI (DMI_{Alkane}) multiple regression models[2].

y	DMI_{Alkane}	Year	Herd	Year*Herd	Year* DMI_{Alkane}	Herd* DMI_{Alkane}	Herd*Year* DMI_{Alkane}
RFI_{fat}	**	NS	NS	NS	NS	NS	NS
	0.0063	0.5392	0.5060	0.2447	0.0615	0.3021	0.7155
$DMI_{fNIRSC31C32}$	*	NS	*	NS	NS	NS	NS
	0.0163	0.6730	0.0444	0.7630	0.9348	0.5792	0.7437
DMI_{NRC}	*	NS	***	NS	NS	NS	NS
	0.0287	0.3998	<0.0001	0.2783	0.7544	0.9268	0.5517
DMI_{Minson}	NS	***	***	***	NS	NS	NS
	0.0612	<0.0001	<0.0001	<0.0001	0.9959	0.4792	0.9505
$DMI_{Mertens}$	NS	***	***	NS	NS	NS	NS
	0.2054	<0.0001	<0.0001	0.7247	0.6817	0.7940	0.2352

[1]* *P < 0.05* ** *P < 0.01* *** *P < 0.0001*

[2]Year =2015 or 2016; Herd=KIN or LAC; RFI_{fat} = residual feed intake adjusted for backfat thickness; $DMI_{fNIRSC31C32}$ = dry matter intake as estimated using fecal C31 and C32 values derived from fNIRS analyses; DMI_{NRC} = dry matter intake as estimated using the NRC equation; DMI_{Minson} = dry matter intake as estimated using the Minson equation; $DMI_{Mertens}$ = dry matter intake as estimated using the Mertens equation.

Table 4. 11 Significance table[1] for fNIRS-estimated DMI (DMI_{fNIRS}) multiple regression models[2].

y	DMI_{fNIRS}	Year	Herd	Year*Herd	Year* DMI_{fNIRS}	Herd* DMI_{fNIRS}	Herd*Year* DMI_{fNIRS}
RFI_{fat}	NS	NS	NS	NS	NS	NS	NS
	0.2009	0.5543	0.7491	0.8202	0.9468	0.2393	0.3595
$DMI_{fNIRSC31C32}$	NS	NS	*	NS	NS	NS	NS
	0.6297	0.8231	0.0130	0.8469	0.1569	0.0911	0.7059
DMI_{NRC}	NS	NS	***	NS	NS	NS	NS
	0.2712	0.3366	<0.0001	0.0798	0.5527	0.2337	0.8712
DMI_{Minson}	*	***	***	**	NS	NS	NS
	0.0354	<0.0001	<0.0001	0.0002	0.6766	0.5456	0.7743
$DMI_{Mertens}$	NS	***	***	NS	NS	NS	NS
	0.1585	<0.0001	<0.0001	0.8111	0.6649	0.1254	0.7406

[1]* *P < 0.05* ** *P < 0.01* *** *P < 0.0001*

[2]Year=2015 or 2016; Herd=KIN or LAC; RFI_{fat} = residual feed intake adjusted for backfat thickness; $DMI_{fNIRSC31C32}$ = dry matter intake as estimated using fecal C31 and C32 values derived from fNIRS analyses; DMI_{NRC} = dry matter intake as estimated using the NRC equation; DMI_{Minson} = dry matter intake as estimated using the Minson equation; $DMI_{Mertens}$ = dry matter intake as estimated using the Mertens equation.

Dry matter intake estimates for LAC heifers $DMI_{Mertens}$ were higher than KIN for both years, and 2015 estimates were higher than 2016 for both herds (Table 4.12). Overall, R^2 values were no greater than 0.70, and therefore the potential to use DMI_{Alkane} to predict RFI_{fat} or DMI as estimated using indirect fNIRS, NRC, Minson or Mertens equations is limited. This is particularly evident when animals are divergent in efficiency ranking because these prediction equations do not account for physiological differences between animals. Furthermore, high RSD values ($0.53 < RSD < 1.39$) and lack of slope (Table 4.12) indicate that DMI_{Alkane} is estimated using different parameters than any of the other DMI methods, with a poor relationship between methods.

The final fitted model for DMI_{fNIRS} (Table 4.13) and RFI_{fat} is an intercept alone as there were no significant main effects or interactions. This is contrary to findings by Johnson (2014), where fNIRS was used to identify significant ($P = 0.04$) differences in DMI across low- and high-RFI herds, albeit with a narrower range of divergence compared to observed DMI (fNIRS 123.2 to 126.8 $g/BW^{0.75}$ versus observed 115.4 to 133.3 $g/BW^{0.75}$). However, Johnson (2014) found the ability of fNIRS to directly predict RFI was limited without an accurate prediction equation ($R^2_c = 0.15$, $R^2_{cv} = 0.07$). The fitted models for DMI_{fNIRS} and $DMI_{fNIRSC31C32}$ (Figure 4.7) and DMI_{fNIRS} and DMI_{NRC} (Figure 4.8) in this study had significant herd effects only: KIN heifer $DMI_{fNIRSC31C32}$ estimates were higher than LAC, and more variable as evident by the larger range of DMI estimates (Table 4.14), whereas LAC heifer DMI_{NRC} estimates were higher than KIN. The fitted model for DMI_{fNIRS} and DMI_{Minson} had separate intercepts and linear regressions for each year and herd as Year, Herd, and Year*Herd were significant (Figure 4.9). LAC heifer DMI_{Minson} estimates were higher than KIN in both years, with higher

values in 2016 than in 2015 for both herds. The $DMI_{Mertens}$ model had significant Year and Herd effects, as indicated in Table 4.13. LAC heifers had higher $DMI_{Mertens}$ estimates than KIN in both years, and 2015 estimates were higher than 2016 estimates for both herds (Table 4.13). As with the DMI_{Alkane} models, R^2 values were no greater than 0.70 for the DMI_{fNIRS} models, and therefore the potential to use DMI_{fNIRS} to predict RFI_{fat} or DMI as estimated using indirect fNIRS, NRC, Minson or Mertens equations is limited. As with the DMI_{Alkane} models, this is particularly evident when animals are divergent in efficiency ranking because these prediction equations do not account for physiological differences between animals. Furthermore, high RSD values ($0.54 < RSD < 1.43$) and lack of slope (Table 4.12) indicate that DMI_{fNIRS} is estimated differently than any of the other DMI methods, with a poor relationship between methods.

Table 4. 12 Final fitted models (coefficients ± SE) and summary statistics for MIXED multiple regression model comparisons of DMI_{Alkane}[1] with RFI_{fat} and DMI estimates[2] via indirect fNIRS, NRC, Minson and Mertens methods.

y	Final Fitted Models	R-Square	Root MSE (RSD)
RFI_{fat}	$y = -2.2501$ (± 0.8050) + 0.2150(± 0.0765)×DMI_{Alkane}	0.0919	0.5856
$DMI_{fNIRSC31C32}$	KIN: $y=6.0715$ (± 1.9951) + 0.4564(± 0.1858)×DMI_{Alkane} LAC: $y=5.4212$ (± 1.9281) + 0.4564(± 0.1858)×DMI_{Alkane}	0.1437	1.3904
DMI_{NRC}	KIN: $y=10.2737$ (± 0.7658) + 0.1590(± 0.0713)×DMI_{Alkane} LAC: $y=11.2077$ (± 0.7401) + 0.1590(± 0.0713)×DMI_{Alkane}	0.4334	0.5337
DMI_{Minson}	2015 KIN: $y=8.3136$ (± 0.1635) 2015 LAC: $y=10.0528$ (± 0.1635) 2016 KIN: $y=9.9637$ (± 0.1635) 2016 LAC: $y=10.0262$ (± 0.1635)	0.5169	0.7311
$DMI_{Mertens}$	2015 KIN: $y=11.3795$ (± 0.1329) 2015 LAC: $y=12.4994$ (± 0.1329) 2016 KIN: $y=9.7210$ (± 0.1329) 2016 LAC: $y=10.8408$ (± 0.1329)	0.6884	0.6862

[1]DMI_{Alkane} = dry matter intake estimated using the *n*-alkane technique
[2]RFI_{fat} = residual feed intake adjusted for backfat thickness; $DMI_{fNIRSC31C32}$ = dry matter intake estimated indirectly using fecal C31 and C32 values derived from fNIRS analysis; DMI_{NRC} = dry matter intake as estimated using the NRC equation; DMI_{Minson} = dry matter intake as estimated using the Minson equation; $DMI_{Mertens}$ = dry matter intake as estimated using the Mertens equation.

Table 4. 13 Final fitted models (coefficients ± SE) and summary statistics for MIXED multiple regression model comparisons of DMI_{fNIRS}[1] with RFI_{fat} and DMI estimates[2] via indirect fNIRS, NRC, Minson and Mertens methods.

y	Final Fitted Models	R-Square	Root MSE (RSD)
RFI_{fat}	*y=0.0047* (± 0.0683)	0.0000	0.6106
$DMI_{fNIRSC31C32}$	KIN: *y=10.9416* (± 0.2268) LAC: *y=10.1257* (± 0.2268)	0.0766	1.4345
DMI_{NRC}	KIN: *y=11.9706* (± 0.0865) LAC: *y=12.8470* (± 0.0865)	0.3968	0.5470
DMI_{Minson}	2015 KIN: *y=5.4646* (± 1.3900) + *0.2821*(± 0.1315)×DMI_{fNIRS} 2015 LAC: *y=6.9886* (± 1.4371) + *0.2821*(± 0.1315)×DMI_{fNIRS} 2016 KIN: *y=6.8870* (± 1.4430) + *0.2821*(± 0.1315)×DMI_{fNIRS} 2016 LAC: *y=7.0722* (± 1.3861) + *0.2821*(± 0.1315)×DMI_{fNIRS}	0.4920	0.7074
$DMI_{Mertens}$	2015 KIN: *y=11.3795* (± 0.1329) 2015 LAC: *y=12.4994* (± 0.1329) 2016 KIN: *y=9.7210* (± 0.1329) 2016 LAC: *y=10.8408* (± 0.1329)	0.6883	0.6861

[1]DMI_{fNIRS} = dry matter intake estimated using the fecal near infrared reflectance spectroscopy.
[2]RFI_{fat} = residual feed intake adjusted for backfat thickness; $DMI_{fNIRSC31C32}$ = dry matter intake estimated indirectly using fecal C31 and C32 values derived from fNIRS analysis; DMI_{NRC} = dry matter intake as estimated using the NRC equation; DMI_{Minson} = dry matter intake as estimated using the Minson equation; $DMI_{Mertens}$ = dry matter intake as estimated using the Mertens equation.

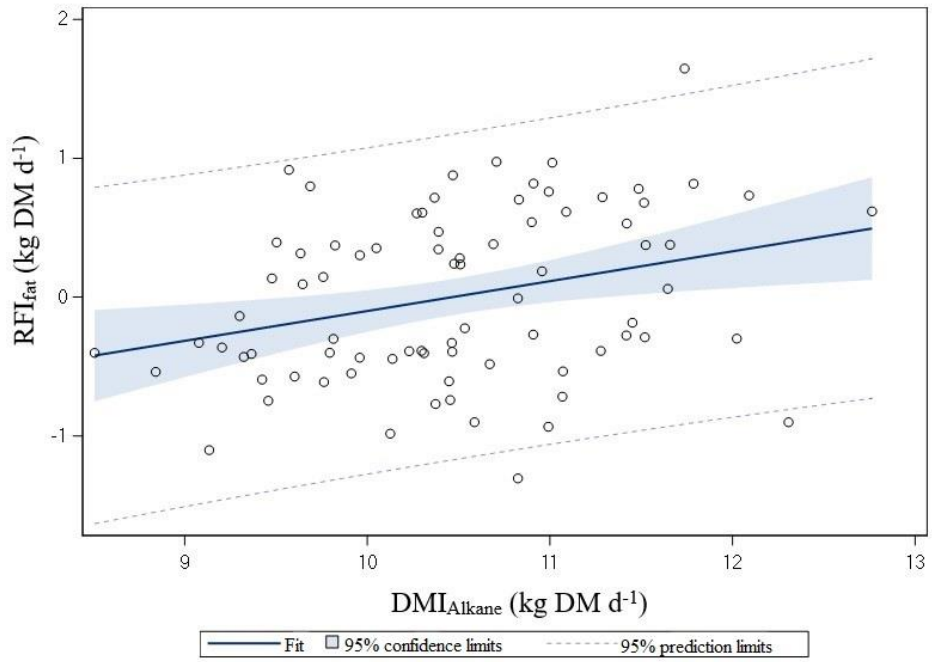

Figure 4. 4 Fit plot for RFI_{fat} depicting the final fitted MIXED multiple regression model for the comparison of DMI_{Alkane} with RFIfat.

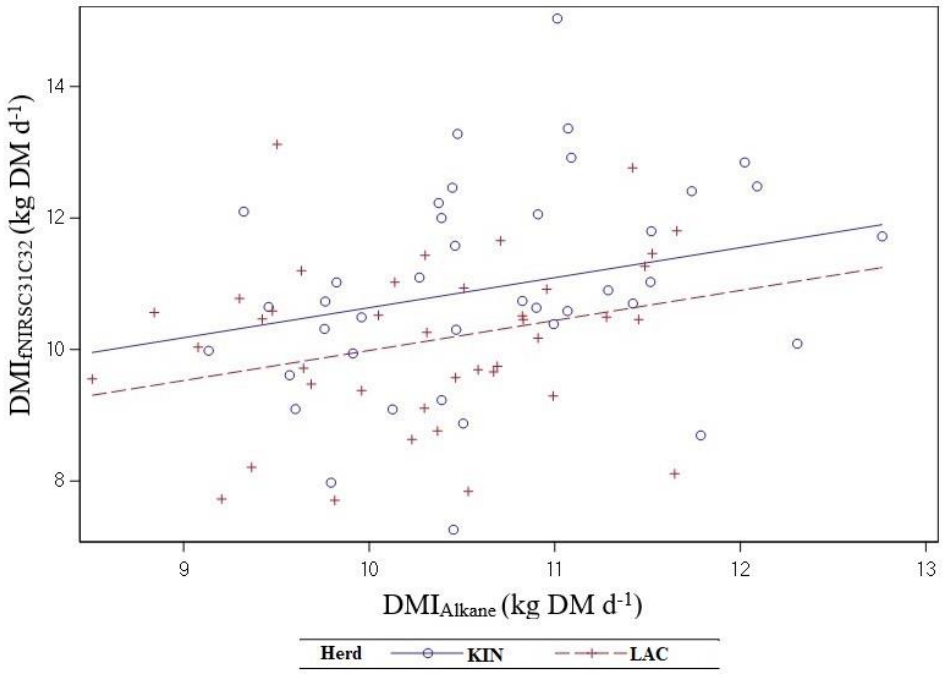

Figure 4. 5 Analysis of covariance of $DMI_{fNIRSC31C32}$ depicting the final fitted MIXED multiple regression model for the comparison of DMI_{Alkane} with DMI estimates using the indirect fNIRS method ($DMI_{fNIRSC31C32}$).

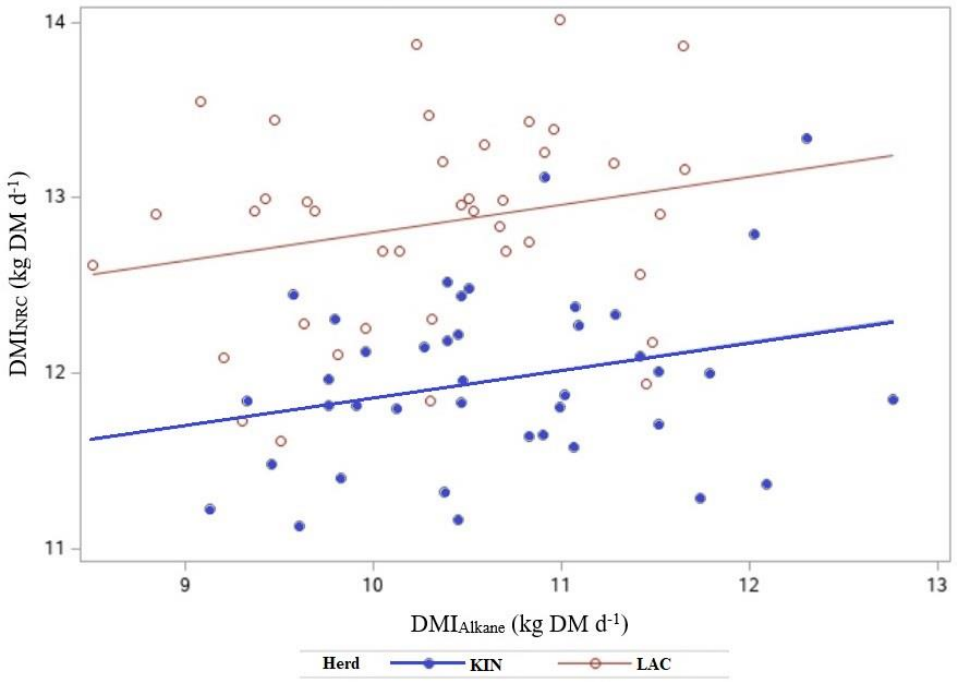

Figure 4. 6 Analysis of covariance for DMI_{NRC} depicting the final fitted MIXED multiple regression model for the comparison of DMI_{Alkane} with DMI estimates using the NRC equation (DMI_{NRC}).

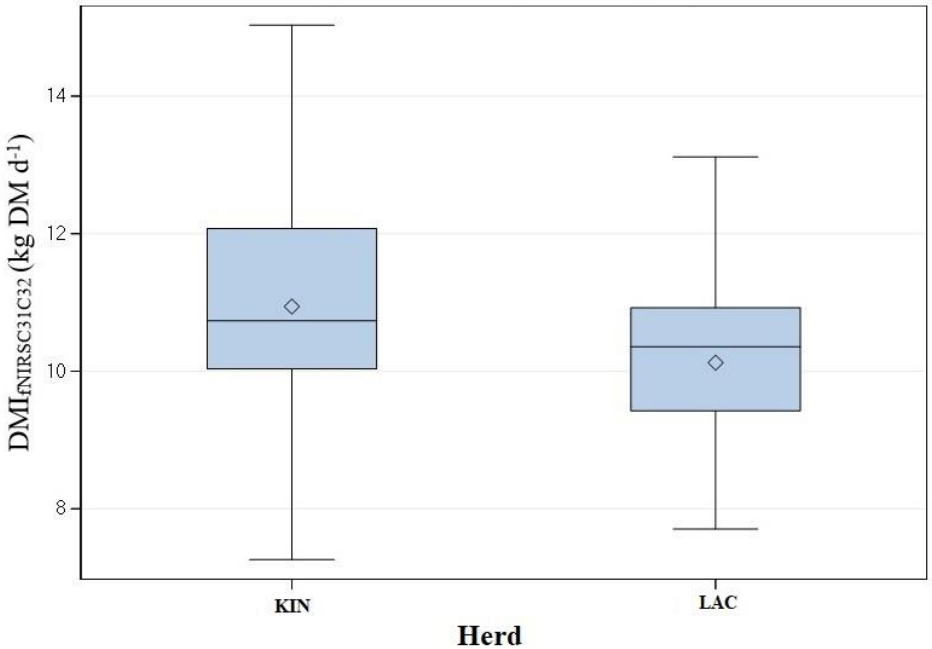

Figure 4. 7 Distribution of $DMI_{fNIRSC31C32}$ depicting the final fitted MIXED multiple regression model for the comparison of DMI_{fNIRS} with DMI estimates using the indirect fNIRS method ($DMI_{fNIRSC31C32}$).

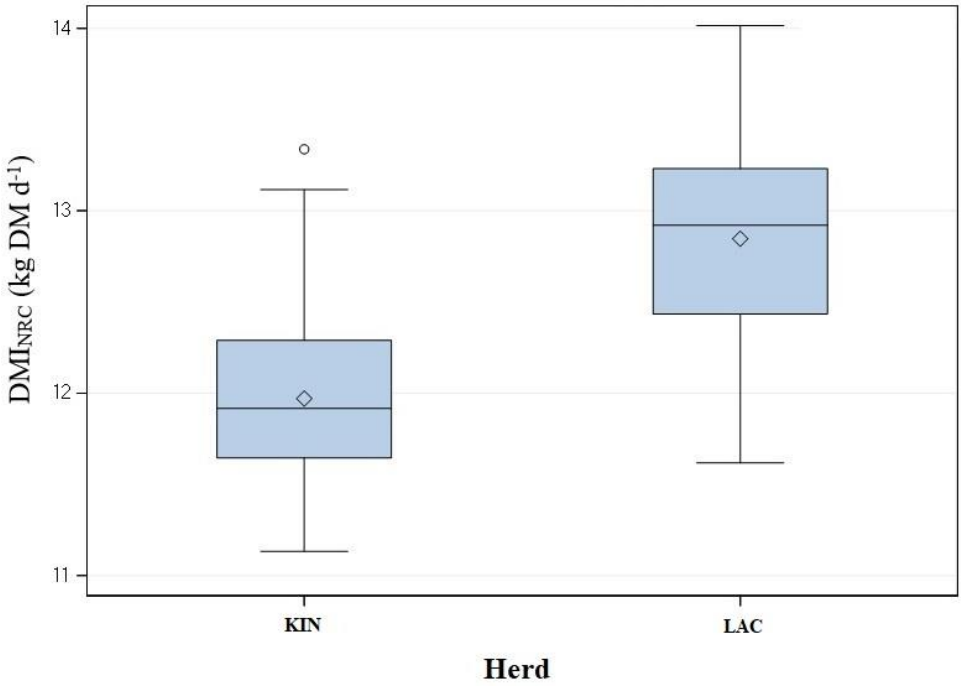

Figure 4. 8 Distribution of DMI_{NRC} depicting the final fitted MIXED multiple regression model for the comparison of DMI_{fNIRS} with DMI estimates using the NRC equation (DMI_{NRC}).

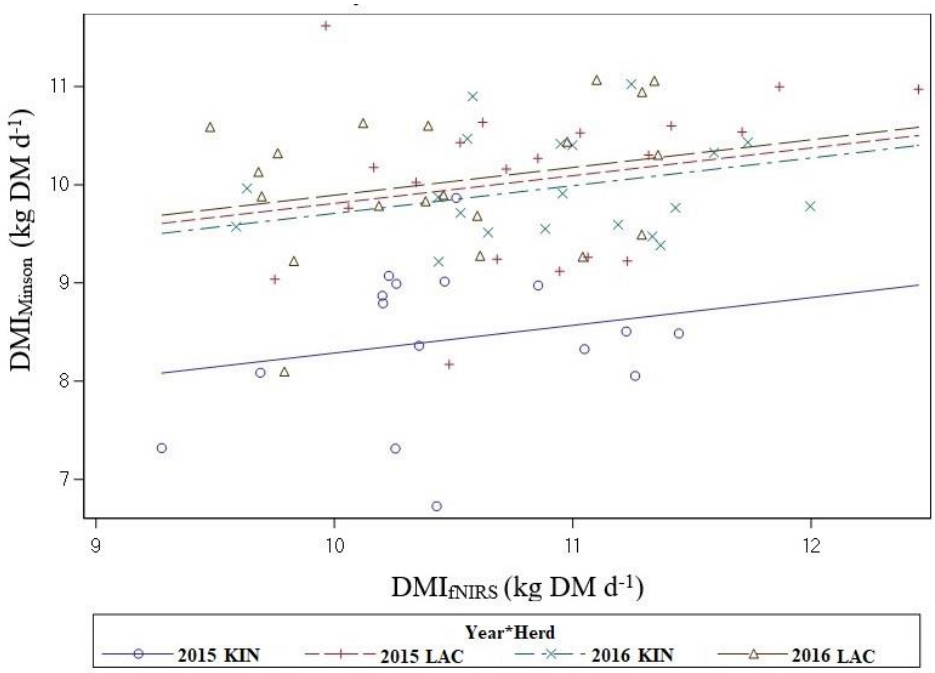

Figure 4. 9 Analysis of covariance for DMI_{Minson} depicting the final fitted MIXED multiple regression model for the comparison of DMI_{fNIRS} with DMI estimates using the Minson equation (DMI_{Minson}).

4.4.3 Correlation between RFI_{fat} and DMI methods

Five-day average individual animal forage DMI and RFI_{fat} are summarized by Herd and Year in Tables 4.14 and 4.15. All mean DMI estimates were within biological reason based on likely intake between 1.5% and 3% BW. As indicated in Table 4.14, RFI_{fat} for KIN 2015 heifers ranged from −1.10 to 0.97 kg DM d^{-1} and was correlated to DMI_{Alkane} ($r = 0.64$, $P = 0.0024$; Table 4.15) but was not significantly correlated to other DMI methods. In addition, DMI_{Alkane} was also correlated to DMI_{fNIRS} ($r = 0.51$, $P = 0.0462$), but had no correlation with equation-based estimates of DMI (NRC, Minson, Mertens). Further, DMI_{fNIRS} was correlated to DMI_{NRC} ($r = 0.52$, $P = 0.0395$) however $DMI_{fNIRSC31C32}$ was not correlated to any other DMI estimates. DMI_{NRC} was correlated to DMI_{Minson} ($r = 0.51$, $P = 0.0208$) and $DMI_{Mertens}$ ($r = 0.86$, $P < 0.0001$), which is logical given that NRC and Mertens equations each use BW and some measure of forage quality, while the Minson equation includes BW and ADG. With grazing steers, Undi et al. (2008) also found that DMI estimates using the NRC and Minson methods were correlated ($r = 0.30$, $P = 0.001$), though the correlations in the current study were stronger ($0.51 < r < 0.92$) but with significance varying from $P < 0.05$ to $P < 0.0001$. DMI_{Minson} was correlated to $DMI_{Mertens}$ ($r = 0.57$, $P = 0.0090$). Overall, DMI_{NRC} predicted a significantly higher DMI compared to $DMI_{Mertens}$, $DMI_{fNIRSC31C32}$, DMI_{Alkane}, DMI_{fNIRS}, while DMI_{Minson} was significantly lower than other intake methods' predicted DMI (Table 4.14). The smallest range in intake estimates (11.13 to 13.12 kg DM d^{-1}; Table 4.14) was observed for DMI_{NRC}, suggesting that it may not be useful for detecting differences in intake when intake is affected by animal factors other than BW or net energy (NE_m) requirements. This is consistent with results reported by Undi et al. (2008): these authors also found that the NRC equation provided the least variable results between individual animals compared to estimates using the Minson and *n*-alkane

techniques to estimate DMI. Similarly, Smit et al. (2005) found that using a net energy equation to estimate intake of grazing lactating dairy cows provided less variable results than the *n*-alkane technique. This is logical based on the variables used to predict DMI_{NRC}.

As indicated in Table 4.14, RFI_{fat} for LAC 2015 heifers ranged from −0.54 to 0.82 kg DM d^{-1} (range 1.36) and was correlated to DMI_{Alkane} ($r = 0.61$, $p = 0.0041$; Table 4.15) and $DMI_{fNIRSC31C32}$ ($r = 0.57$, $p = 0.0087$). DMI_{Alkane} and DMI_{fNIRS} were not correlated to any other DMI prediction methods. $DMI_{fNIRSC31C32}$ was correlated to DMI_{NRC} ($r = - 0.45$, $p = 0.0448$) and $DMI_{Mertens}$ ($r = 0.47$, $p = 0.0376$). As with both KIN heifer groups, DMI_{NRC} was correlated to DMI_{Minson} ($r = 0.71$, $p = 0.0004$) and $DMI_{Mertens}$ ($r = 0.95$, $p < 0.0001$), and DMI_{Minson} was correlated to $DMI_{Mertens}$ ($r = 0.47$, $p < 0.0351$). Once again, DMI_{NRC} provided the highest estimate of DMI, followed by $DMI_{Mertens}$, $DMI_{fNIRS,}$ $DMI_{fNIRSC31C32}$, DMI_{Alkane}, DMI_{Minson}. DMI_{NRC} and $DMI_{Mertens}$ were significantly higher than other methods, and DMI_{NRC} once again had the smallest range and coefficient of variation (%CV).

As indicated in Table 4.14, RFI_{fat} for KIN 2016 heifers ranged from −1.31 to 1.65kg DM d^{-1} (range 2. 95), however it was not significantly correlated with any DMI method. Significant correlations were observed between DMI_{Alkane} and DMI_{fNIRS} ($r = 0.50$, $p = 0.0236$; Table 4.15) and $DMI_{fNIRSC31C32}$ ($r = 0.47$, $p = 0.0352$), but DMI_{fNIRS} and $DMI_{fNIRSC31C32}$ were not correlated to any other DMI methods. As with for KIN 2015, DMI_{NRC} was correlated to DMI_{Minson} ($r = 0.92$, $P < 0.0001$) and $DMI_{Mertens}$ ($r = 0.99$, $P < 0.0001$), and DMI_{Minson} was correlated to $DMI_{Mertens}$ ($r = 0.86$, $p < 0.0001$). As described in Table 4.14, DMI_{NRC} was significantly higher than other estimates, followed by $DMI_{fNIRSC31C32}$, DMI_{fNIRS}, DMI_{Alkane}, DMI_{Minson} and $DMI_{Mertens}$.

As indicated in Table 4.14, RFI_{fat} for LAC 2016 heifers ranged from −0.93 to 0.98 kg DM d^{-1}, however it was not significantly correlated to any DMI prediction method

estimates (Table 4.16). As was observed with the KIN heifers in both years, but not the 2015 LAC heifers, DMI_{Alkane} for 2016 LAC heifers was correlated to DMI_{fNIRS} (r = 0.44, P = 0.0498). DMI_{fNIRS} was negatively correlated to $DMI_{fNIRSC31C32}$ (r = - 0.48, P = 0.0311), but $DMI_{fNIRSC31C32}$ was not significantly correlated to any other DMI prediction method estimates. As with all previous groups, DMI_{NRC} was correlated to DMI_{Minson} (r = 0.53, P = 0.0147) and $DMI_{Mertens}$ (r = 0.91, P < 0.0001), however DMI_{Minson} and $DMI_{Mertens}$ were not correlated in this case. Once again, DMI_{NRC} provided a significantly higher average estimate of DMI compared to other methods, followed by $DMI_{Mertens}$, $DMI_{fNIRS,}$ DMI_{Alkane}, DMI_{Minson} and $DMI_{fNIRSC31C32}$ (Table 4.14).

When comparing all trials as a pooled group (n = 80; Table 4.14), RFI_{fat} ranged from −1.30 to 1.65 kg DM d^{-1} (range 2.95) and was correlated to DMI_{Alkane} (r = 0.30, P = 0.0063; Table 4.16) and $DMI_{fNIRSC31C32}$ (r = 0.25, P = 0.0230). DMI_{Alkane} was also correlated to DMI_{fNIRS} (r = 0.32, P = 0.0054) and $DMI_{fNIRSC31C32}$ (r = 0.31, P = 0.0049). DMI_{fNIRS} was correlated to DMI_{Minson} (r = 0.28, P = 0.0140), and $DMI_{fNIRSC31C32}$ was correlated to DMI_{NRC} (r = - 0.32, P = 0.0034) and DMI_{Minson} (r = -0.27, P = 0.0167). As with each individual group, DMI_{NRC} was correlated to DMI_{Minson} (r = 0.61, P < 0.0001) and $DMI_{Mertens}$ (r = 0.62, P < 0.0001), however DMI_{Minson} and $DMI_{Mertens}$ were not significantly correlated when *n* was pooled. Following a similar trend as with individual groups, DMI_{NRC} provided a significantly higher estimate of DMI than other methods, followed by $DMI_{Mertens}$, $DMI_{fNIRS,}$ $DMI_{fNIRSC31C32}$, DMI_{Alkane} and DMI_{Minson}, which was significantly lower than estimates via other methods. $DMI_{fNIRSC31C32}$ had the greatest range and %CV (> 10%) for all groups and as a pooled group, while DMI_{NRC} varied the least.

For both herds in each year, and when summarizing the four trials as a pooled group (n = 80), DMI_{NRC} was greater than DMI_{Alkane}, which was greater than DMI_{Minson} (Table 4.14). Conversely,

in a three-year grazing study where *n*-alkane controlled release capsules were used to dose steers, Undi et al. (2008) reported that the Minson equation provided higher DMI estimates than the NRC equation ($P < 0.05$), with the *n*-alkane technique yielding intermediate estimates of DMI. Differences between the current study and trials described by Undi et al. (2008) may be due to physiological differences between animals. Undi et al. (2008) studied intake in steers which may have differed in growth rate compared to the heifers examined herein. Further, the Minson equation was developed for use in steers (Minson and MacDonald 1987). Although NRC (2016) does suggest it may be used in heifers, the data presented here suggests that this requires further study.

DMI_{Alkane} was statistically lower ($P < 0.0001$) than DMI_{NRC} for both herds in both years regardless of RFI ranking, which is contrary to over-estimation reported by Smit et al. (2005). In a study of grazing lactating dairy cows by Smit et al. (2005) the net energy method used resulted in estimated intake of 0.4 to 2.9 kg DM d^{-1} lower than the *n*-alkane method. Further, the difference between methods was more distinct using C32:C31 pairings compared to C32:C33, with a greater magnitude of difference in year 2 of their study compared to year 1. It is possible that physiological status and diet type may affect fecal recovery rates, providing variable intake estimates across trials (Olivàn et al. 2007; Elwert et al. 2008). Furthermore, the carrier material used, frequency of dosing and fecal sampling schedules may also influence fecal excretion patterns of dosed *n*-alkanes (Bezabih et al. 2012). In both 2015 heifer herds, RFI_{fat} was correlated with DMI_{Alkane} ($r > 0.60$, $P < 0.01$; Table 4.15), however this trend was not repeated in 2016, when both herds of heifers had non-significant correlations ($r < 0.2$, $P > 0.5$; Table 4.16). Despite the lack of correlation in 2016, 2015 RFI_{fat} had a higher degree of correlation and significance compared to results published by Hafla et al. (2013), who found that postweaning

RFI was moderately correlated (P = 0.01; r = 0.38) with forage intake of pregnant Bonsmara females measured in GrowSafe feed bunks. It is possible that differences in forage quality (lower CP and higher NDF in 2016 than 2015) may impact the relationship between intake and RFI and therefore, along with other factors, may explain the variation in correlation and significance between trials or locations.

Table 4. 14 Summary of RFI_{fat} and five-day individual animal forage DMI (kg d^{-1}) estimates using six different techniques for KIN and LAC heifers in 2015 and 2016, and as one pooled group.

Intake Technique	n	Mean[1]	SD	%CV	Min.	Max.
			KIN 2015 Heifers			
RFI_{fat}	20	-0.01	0.62	-	-1.10	0.97
DMI_{Alkane}	20	10.63b	0.90	8.50	9.13	12.76
DMI_{fNIRS}	16	10.48b	0.58	5.49	9.28	11.45
$DMI_{fNIRSC31C32}$	20	10.94b	1.94	17.73	7.26	15.03
DMI_{NRC}	20	11.98a	0.46	3.84	11.13	13.12
DMI_{Minson}	20	8.31c	0.80	9.64	6.73	9.87
$DMI_{Mertens}$	20	11.36b	0.68	6.00	10.36	12.96
			LAC 2015 Heifers			
RFI_{fat}	20	0.06	0.47	-	-0.54	0.82
DMI_{Alkane}	20	10.15b	0.89	8.80	8.51	11.66
DMI_{fNIRS}	20	10.86b	0.68	6.26	9.75	12.45
$DMI_{fNIRSC31C32}$	20	10.17b	1.42	13.96	7.73	13.12
DMI_{NRC}	20	12.71a	0.59	4.63	11.62	13.87
DMI_{Minson}	20	10.05b	0.83	8.25	8.17	11.62
$DMI_{Mertens}$	20	12.52a	0.78	6.27	11.08	13.87
			KIN 2016 Heifers			
RFI_{fat}	20	0.02	0.82	-	-1.31	1.65
DMI_{Alkane}	20	10.71b	0.86	8.05	9.46	12.31
DMI_{fNIRS}	20	10.90b	0.63	5.75	9.59	12.00
$DMI_{fNIRSC31C32}$	20	10.94b	1.16	10.60	9.09	12.92
DMI_{NRC}	20	11.96a	0.55	4.63	11.17	13.34
DMI_{Minson}	20	9.96b	0.51	5.12	9.22	11.02
$DMI_{Mertens}$	20	9.74b	0.66	6.74	8.85	11.41
			LAC 2016 Heifers			
RFI_{fat}	20	-0.05	0.53	-	-0.93	0.98
DMI_{Alkane}	20	10.46b	0.73	7.00	9.08	11.65
DMI_{fNIRS}	20	10.47b	0.64	6.08	9.48	11.36
$DMI_{fNIRSC31C32}$	20	10.08b	1.15	11.41	7.71	12.76
DMI_{NRC}	20	12.99a	0.57	4.38	11.94	14.01
DMI_{Minson}	20	10.13b	0.74	7.38	8.10	11.07
$DMI_{Mertens}$	20	10.82b	0.63	5.81	9.34	11.87
			All Trials			
RFI_{fat}	80	0.00	0.61	-	-1.30	1.65
DMI_{Alkane}	80	10.49b	0.86	8.21	8.51	12.76
DMI_{fNIRS}	76	10.69b	0.65	6.12	9.28	12.45
$DMI_{fNIRSC31C32}$	80	10.53b	1.48	14.08	7.26	15.03
DMI_{NRC}	80	12.41a	0.70	5.64	11.13	14.01
DMI_{Minson}	80	9.59c	1.03	10.76	6.73	11.62
$DMI_{Mertens}$	80	11.11b	1.21	10.92	8.85	13.87

[1]Unequal means were observed using PROC MIXED LSMEANS residual panel. Letters indicate Adj $P < 0.0001$ using Tukey's test.

Table 4. 15 Correlation[1] between RFI_{fat} and five-day individual animal forage DMI (kg DM d^{-1}) estimates for KIN and LAC heifers using six different techniques in 2015.

Intake Technique	**RFI_{fat}**	**DMI_{Alkane}**	**DMI_{fNIRS}**	**$DMI_{fNIRSC31C32}$**	**DMI_{NRC}**	**DMI_{Minson}**	**$DMI_{Mertens}$**
				KIN 2015 Heifers			
RFI_{fat}	-	0.64**	0.17NS	0.25NS	0.22NS	0.05NS	0.21NS
DMI_{Alkane}		-	0.51*	0.24NS	0.27NS	0.12NS	0.11NS
DMI_{fNIRS}			-	0.28NS	0.52*	0.21NS	0.49NS
$DMI_{fNIRSC31C32}$				-	0.01NS	-0.13NS	0.11NS
DMI_{NRC}					-	0.51*	0.86***
DMI_{Minson}						-	0.57**
$DMI_{Mertens}$							-
				LAC 2015 Heifers			
RFI_{fat}	-	0.61**	0.20NS	0.57**	-0.25NS	-0.00NS	-0.32NS
DMI_{Alkane}		-	0.12NS	0.26NS	0.30NS	0.8NS	0.26NS
DMI_{fNIRS}			-	-0.07NS	0.14NS	0.30NS	0.05NS
$DMI_{fNIRSC31C32}$				-	-0.45*	-0.24NS	-0.47*
DMI_{NRC}					-	0.71**	0.95***
DMI_{Minson}						-	0.47*
$DMI_{Mertens}$							-

[1] ** $P < 0.05$ ** $P < 0.01$ *** $P < 0.0001$*

Table 4. 16 Correlation[1] between RFI_{fat} and five-day individual animal forage DMI (kg DM d^{-1}) estimates for KIN and LAC heifers using six different techniques in 2016, and as one pooled group (four groups over two years).

Intake Technique	**RFI_{fat}**	**DMI_{Alkane}**	**DMI_{fNIRS}**	**$DMI_{fNIRSC31C32}$**	**DMI_{NRC}**	**DMI_{Minson}**	**$DMI_{Mertens}$**
				KIN 2016 Heifers			
RFI_{fat}	-	0.15NS	0.29NS	0.25NS	-0.00NS	0.10NS	-0.04NS
DMI_{Alkane}		-	0.50*	0.47*	0.26NS	0.22NS	0.27NS
DMI_{fNIRS}			-	-0.07NS	0.25NS	0.13NS	0.28NS
$DMI_{fNIRSC31C32}$				-	-0.27NS	-0.32NS	-0.24NS
DMI_{NRC}					-	0.92***	0.99***
DMI_{Minson}						-	0.86***
$DMI_{Mertens}$							-
				LAC 2016 Heifers			
RFI_{fat}	-	-0.12NS	-0.12NS	0.10NS	-0.16NS	0.13NS	-0.20NS
DMI_{Alkane}		-	0.44*	0.18NS	0.07NS	0.25NS	-0.12NS
DMI_{fNIRS}			-	-0.48*	0.00NS	0.30NS	-0.01NS
$DMI_{fNIRSC31C32}$				-	-0.14NS	-0.28NS	-0.11NS
DMI_{NRC}					-	0.53*	0.91***
DMI_{Minson}						-	0.38NS
$DMI_{Mertens}$							-
				All Trials			
RFI_{fat}	-	0.30**	0.15NS	0.25*	-0.04NS	0.05NS	-0.02NS
DMI_{Alkane}		-	0.32**	0.31**	0.05NS	0.07NS	-0.10NS
DMI_{fNIRS}			-	-0.04NS	0.08NS	0.28*	0.07NS
$DMI_{fNIRSC31C32}$				-	-0.32**	-0.27*	-0.20NS
DMI_{NRC}					-	0.61***	0.62***
DMI_{Minson}						-	0.15NS
$DMI_{Mertens}$							-

[1] Adjusted P using Tukey's test ** $P < 0.05$* *** $P < 0.01$* **** $P < 0.0001$*

4.4.4 Differences in DMI between RFI_{fat} groups

As shown in Table 4.15, although differences in RFI_{fat} between high- and low-RFI_{fat} animals were highly significant ($P < 0.0001$) for all groups, DMI_{Alkane} was only significantly different ($P < 0.05$) between high- and low-RFI_{fat} groups in 2015, although the rationale for this is not clear. The lack of statistical significance in 2016 is contrary to results from Johnson (2014), who observed in consecutive RFI and feed intake test periods that both the fNIRS ($P = 0.03$) and *n*-alkane ($P = 0.04$) methods predicted DMI differences (g/kg $BW^{0.75}$) between low- and high-RFI pregnant Bonsmara females fed a 70:30 diet of sorghum:alfalfa hay.

Figure 4.10 illustrates differences in DMI_{Alkane} between high- and low-RFI_{fat} animals by day of trial and as a 5-d mean. Significant differences ($P < 0.05$, Table 4.17) were noted for DMI_{fNIRS} and $DMI_{fNIRSC31C32}$ between high- and low-RFI_{fat} groups only in LAC 2015 heifers. No significant differences existed between high- and low-RFI groups for DMI estimates using any of the three equation-based methods in either herd or year. As there was no reference measure of individual animal DMI in this study, it is difficult to speculate whether the inconsistent relationship between RFI_{fat} and DMI_{Alkane} is attributable to shortcomings of the *n*-alkane technique, or to re-ranking of animals between the RFI ranking and pasture intake periods. Durunna et al. (2011) found that 51% of 190 replacement heifers studied over three years had a different RFI class, suggesting that re-ranking does occur despite having the same diet and similar environmental conditions, and may be due to differences in maturity of animals. Further, Manafiazer et al. (2015) found that RFI_{fat} measured in 171 crossbred heifers on drylot was significantly ($P = 0.04$) positively correlated ($r_p = 0.30$) with DMI estimates using the *n*-alkane technique on pasture approximately two months later.

Table 4. 17 Least squares means (± SEM) for RFI_{fat} and dry matter intake (DMI; kg DM d^{-1}) estimates by six methods for heifers in each herd and year of trial (n = 20[1]).

Intake estimation method[2]	High-RFI	Low-RFI	Adj. P[3]
		KIN 2015	
RFI_{fat}	0.54 ± 0.09	-0.56 ± 0.08	<0.0001
DMI_{Alkane}	11.04 ± 0.27	10.21 ± 0.25	0.0369
DMI_{fNIRS}	10.56 ± 0.19	10.38 ± 0.23	0.5718
$DMI_{fNIRSC31C32}$	11.25 ± 0.60	10.63 ± 0.64	0.4942
DMI_{NRC}	12.02 ± 0.08	11.94 ± 0.20	0.7061
DMI_{Minson}	8.32 ± 0.28	8.31 ± 0.28	0.9877
$DMI_{Mertens}$	11.42 ± 0.17	11.29 ± 0.26	0.6856
		LAC 2015	
RFI_{fat}	0.48 ± 0.07	-0.37 ± 0.04	<0.0001
DMI_{Alkane}	10.62 ± 0.26	9.69 ± 0.23	0.0153
DMI_{fNIRS}	11.16 ± 0.22	10.56 ± 0.17	0.0472
$DMI_{fNIRSC31C32}$	11.03 ± 0.33	9.30 ± 0.38	0.0029
DMI_{NRC}	12.60 ± 0.18	12.82 ± 0.19	0.4139
DMI_{Minson}	10.12 ± 0.23	9.98 ± 0.30	0.7327
$DMI_{Mertens}$	12.30 ± 0.23	12.73 ± 0.26	0.2341
		KIN 2016	
RFI_{fat}	0.75 ± 0.11	-0.71 ± 0.10	<0.0001
DMI_{Alkane}	10.79 ± 0.26	10.64 ± 0.30	0.7152
DMI_{fNIRS}	11.06 ± 0.13	10.75 ± 0.25	0.2933
$DMI_{fNIRSC31C32}$	11.01 ± 0.39	10.87 ± 0.36	0.7898
DMI_{NRC}	11.98 ± 0.15	11.94 ± 0.20	0.8784
DMI_{Minson}	10.07 ± 0.17	9.86 ± 0.16	0.3832
$DMI_{Mertens}$	11.42 ± 0.17	11.29 ± 0.26	0.6856
		LAC 2016	
RFI_{fat}	0.38 ± 0.11	-0.48 ± 0.08	<0.0001
DMI_{Alkane}	10.42 ± 0.21	10.50 ± 0.27	0.8054
DMI_{fNIRS}	10.47 ± 0.19	10.47 ± 0.22	0.9876
$DMI_{fNIRSC31C32}$	10.08 ± 0.35	10.08 ± 0.40	0.9978
DMI_{NRC}	13.09 ± 0.15	12.88 ± 0.21	0.4273
DMI_{Minson}	10.32 ± 0.16	9.73 ± 0.27	0.0758
$DMI_{Mertens}$	10.92 ± 0.13	10.72 ± 0.26	0.5007

[1]For KIN 2015 DMI_{fNIRS} only, n = 16.

[2] RFI_{fat} = residual feed intake adjusted for backfat thickness; DMI_{Alkane}, DMI_{fNIRS}, $DMI_{fNIRSC31C32}$, DMI_{NRC}, DMI_{Minson}, $DMI_{Mertens}$ are dry matter intake estimated using n-alkane, direct fNIRS, indirect fNIRS using fecal C31 and C32, and the NRC, Minson and Mertens equations, respectively.

[3] Adjusted P using Tukey's test

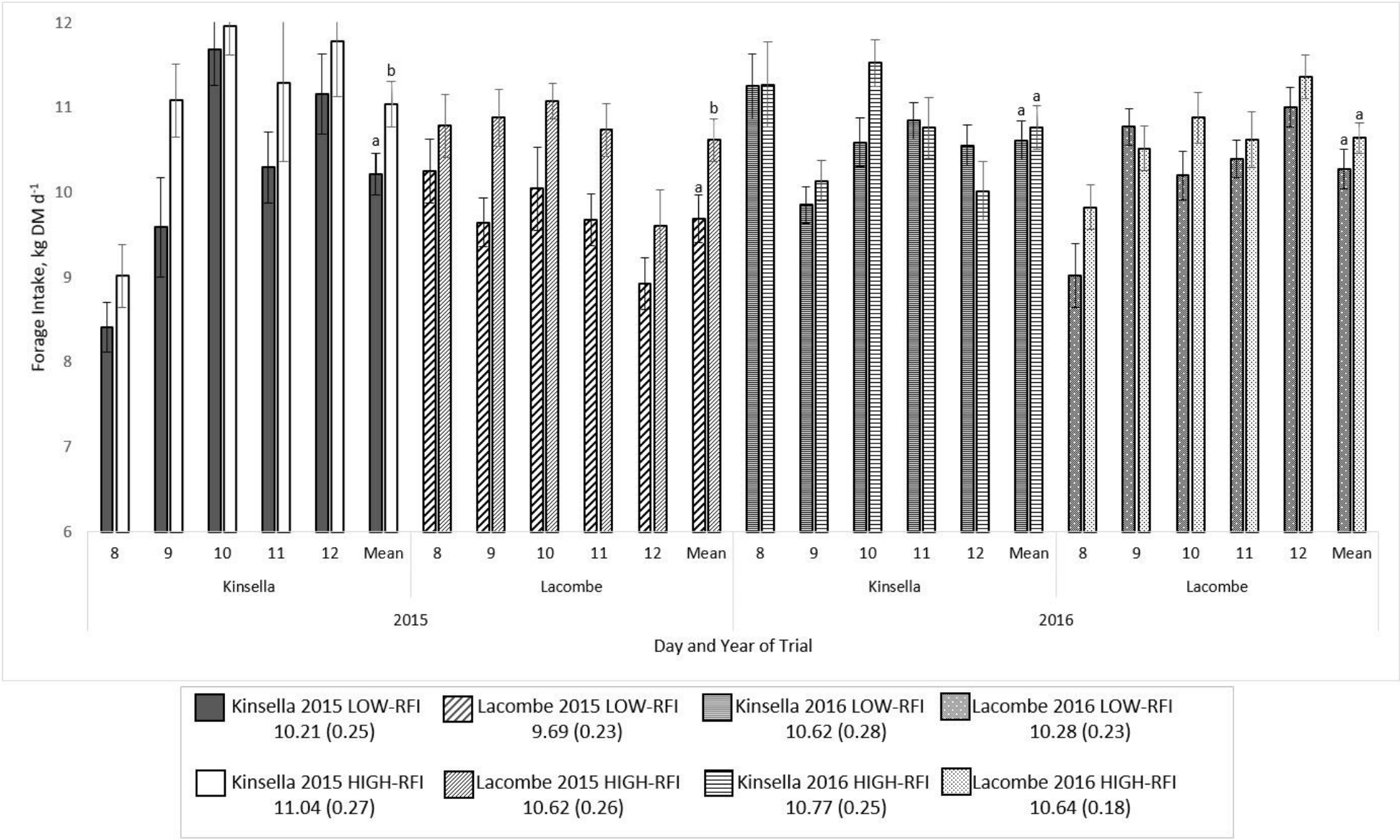

Figure 4. 10 Least squares means (SEM) of daily grazed forage intake (kg DM d^{-1}) by day of trial, and as a five-day mean for low- and high-RFI_{fat} heifers (n = 20) by herd and year of trial as estimated using the n-alkane method. Different letters indicate significant differences ($P < 0.05$) between mean DMI_{Alkane} of low- and high-RFI_{fat} groups.

4.5 CONCLUSION

Results from this study suggest that fNIRS profiling cannot be used to predict individual-animal forage DMI or C31 and C32 *n*-alkane concentration. Neither direct nor indirect ($DMI_{fNIRSC31C32}$) fNIRS methods were found to be suitable for the prediction of individual-animal DMI on pasture when using DMI_{Alkane} as the reference DMI value. The calibration equations were limited in robustness, likely due to insufficient sample size and thus further studies are required to increase the calibration dataset in order to fully evaluate the potential of this technique.

For each herd in both years, the NRC equation provided the least variable estimates of intake and over-estimated DMI compared to the *n*-alkane, fNIRS and $fNIRS_{C31C32}$ techniques. The $fNIRS_{C31C32}$ method provided the most variable estimates of DMI. Congruent with this study's hypothesis, results indicated that DMI as measured by direct and indirect fNIRS and *n*-alkane methods differ in their predictions of individual-animal pasture intake compared to traditional prediction equations. However, neither direct nor indirect fNIRS methods were significantly correlated with previously-measured RFI_{fat} except in the LAC 2015 herd. While DMI_{Alkane} was correlated to RFI_{fat} rankings in 2015, this was not repeated in 2016. None of the equation-based DMI prediction methods provided estimates which differed significantly between RFI_{fat} groups, indicating that these methods are unable to account for biological differences between animals.

Based on the results of this study, it can be concluded that the *n*-alkane technique provides the most reasonable estimates of individual-animal DMI given its ability to provide statistically significant differences between high- and low-RFI_{fat} heifers. However, further research may be warranted as findings from this study were not repeated in both years.

5. MANUSCRIPT II

Evaluation of swath-grazed *Triticale hexaploide* for intake determination using the *n*-alkane method

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5.1 ABSTRACT

Extended grazing strategies, including swath grazing, are gaining in popularity in Canadian beef production. As such, it would be advantageous to have a better understanding of individual animal intake in extended grazing environments in order to characterize nutrient use efficiency. While the *n*-alkane technique has been employed in many confinement and some pasture grazing environments, few studies have examined this method for its ability to estimate individual animal intake in extended grazing. The objectives of this study were to assess the suitability of swath-grazed triticale for *n*-alkane intake studies, by examining i) its *n*-alkane profile, ii) its morphology (through the measure of plant part weights) and iii) residual starch content to estimate uniformity of grazing. This was done over two years at Lacombe Research and Development Centre (LRDC) with one group of 20 three-year-old Angus and Angus-cross cows grazing daily-allocated triticale swath in each year (Trial 1 and Trial 2). Forage samples were collected at the onset of the trial, representing forage on offer pre-grazing (AM), as well as at 7 hr into grazing (PM). In Trial 2, plant part samples were also collected prior to grazing. These were analyzed via NIRS for quality, as well as by *n*-alkane method for C31 and C32 profile.

In Trial 1, starch ($P < 0.0001$) and C31 concentration ($P = 0.0002$) were significantly lower in PM compared to AM samples, suggesting cows were selectively grazing. In Trial 2, weight of plant parts [separated into either two (head, leaf/stem) or three (head, leaf and stem) plant part fractions] as well as C31 and C32 concentrations in plant parts differed ($P < 0.0001$). The results of this study suggest that the extreme differences between triticale plant parts, coupled with preferential grazing of heads over leaf and stem material and the unsuitable grazing

conditions caused by unpredictable winter weather, resulted in inconsistent consumption of *n*-alkanes.

5.2 INTRODUCTION

Over the last decade, Canadian beef producers have continued to adopt extended grazing strategies such as bale, stockpiled, and swath grazing to decrease feed production and overwintering costs (Baron et al. 2014, 2016; Sheppard et al. 2015). It has been estimated that cost savings of up to 60% may be realized when swath grazing crops such as triticale, barley or corn, compared to confined feeding in a feedlot or feeding site (Baron et al. 2014). Secondary benefits include improved soil fertility, reduced labour, reduced land use and reduced carbon footprint of beef production compared to traditional overwintering methods (AAFC 2017).

Accurate measurement of individual animal intake in extended grazing environments is necessary to characterize nutrient use efficiency and to enable producers to include this trait in breeding decisions. The *n*-alkane technique (Moshtaghi-Nia and Wittenberg 2002), which is based on the use of naturally-occurring and synthetic waxes of adjacent chain length as markers, has been used to estimate intake in many studies in both summer grazing (Manafiazer et al. 2015) and confinement feeding (Johnson 2014). However, this method has not been used to determine intake in winter grazing systems including swath grazing. Accurate measurement of intake can only be achieved if the following conditions regarding the marker are met: reaches steady state in the rumen; is non-toxic; chemically discrete and inert, with no effect on digestion; is completely recovered in feces; and has physical characteristics, such as gut passage rate and density, that are similar to the associated content of the digestive tract (Kotb and Luckey, 1972).

Spring triticale has been successfully swath grazed from an animal performance perspective (Baron et al. 2014). Although differences in quality and digestibility between plant

parts (head, or leaf and stem; Baron et al. 2015) have been demonstrated, the *n*-alkane profiles have not been examined. Therefore, it is warranted to examine triticale plant parts, as has been done for various other plant species in previous studies (Tulloch 1973; Dove et al. 1996; Dove and Mayes, 1996; Boadi et al., 2002). Large differences in the *n*-alkane profiles of triticale plant parts, coupled with selective grazing of these plant parts will negate use of this technique for measure of individual animal intake. Dove et al. (1992, 1999) has suggested it is possible to use the *n*-alkane technique to estimate total intake as well as intake of distinct plant parts using statistical software to compute the combination of consumed plant parts which best matches fecal *n*-alkane profiles. Therefore, it may be possible to use the *n*-alkane technique to estimate individual animal intake of swathed triticale forage despite potential differences in plant part *n*-alkane profile.

The purpose of this manuscript is to examine the *n*-alkane profile and morphology of triticale, as well as selective grazing as measured by residual grazing material to assess the potential use of the *n*-alkane technique to measure intake in swath grazed cattle.

5.3 MATERIALS AND METHODS

5.3.1 Test pasture preparation

As depicted in Figure 5.2, Bunker spring triticale (*Triticale hexaploide)* was seeded into orthic black chernozem soil at the Agriculture and Agri-Food Canada Lacombe Research and Development Centre (LRDC; Lacombe, Alberta, Canada) in June 2015 at a rate of 150 kg ha^{-1} using a broadcast seeder. An overall field dry matter yield (DMY) was determined prior to swathing by collecting nine, 100-cm lengths of a row in the standing crop from representative locations within the paddock. The paddock was swathed using a 7.62 m swather on September 17, 2015 (Trial 1) and September 20, 2016 (Trial 2) at the soft dough stage.

5.3.2 Animal selection criteria and management

All procedures involving animals were reviewed and approved by the University of Alberta Animal Care and Use Committee (Livestock) and LRDC Animal Care Committee. All animals were cared for in accordance with the guidelines of the Canadian Council on Animal Care (CCAC).

From October 5 to 18, 2015, 38 crossbred Aberdeen Angus-Hereford and Charolais-Red Angus cows (Trial 1) were housed in a drylot pen with free choice access to water, mineral, and shelter, as well as to barley silage delivered in a bunk. In addition, a GreenFeed (GF; C-Lock, Inc., Rapid City, USA) station which offered DG bull pellet – plain (barley, beef vitamin-trace mineral premix, calcium carbonate, corn distillers grain screenings, sodium chloride, wheat/wheat middlings and zinc chelate; Masterfeeds, Inc. Red Deer, AB, Canada) was placed in the pen to adapt the cows to the unit. On October 19, 2015, the selected cows were moved to the triticale swaths where barley silage and GF were also available, though with the addition of panels to allow only one cow near the GF hood. The barley silage was removed over a 5-d period, and on October 23, 2015 the bull DG pellets were replaced with C32 pellets. Similarly, in preparation for Trial 2, 24 cows on swath were adapted to GF with DG Bull pellets beginning on October 17, 2016. Cows were given free choice access to water, mineral, barley silage and triticale swath, with barley silage removed over a 5-d period.

Description of grazing animals selected for trial

Following a pregnancy check in October 2015 (Trial 1), 20 cows with an average Day 0 BW of 656.4 ± 49.8 kg, and backfat of 11.7 ± 4.3 mm began the trial period at 1301 ± 13 days of age. Due to temperament, only 18 cows completed the trial period.

Similarly, in October 2016 (Trial 2), 20 cows were selected for the grazing portion of the study. However, due to wet grazing conditions resulting in a lack of available forage, only 12 cows completed the trial period at 1297 ± 18 days of age with an average Day 0 BW of 645.4 ± 25.4 kg, and backfat of 8.0 ± 2.8 mm.

5.3.3 n-alkane pellet preparation

As described in Manuscript 1, *n*-alkane labelled pellets (Table 5.1) consisting of ground barley grain, ground wheat, canola meal, corn distillers grain, finely grated beeswax (Tegart Apiaries Ltd. Fairview, AB, Canada) and dotriacontane (C32, which served as an external *n*-alkane marker; Minakem, Beuvry La Foret, France) were extruded at the Crop Diversification Centre (Brooks, AB, Canada) for both Trials 1 and 2. For Trial 2, beeswax was removed from the diet to improve palatability and intake. Post extrusion, pellets were coated with canola oil to aid in pellet integrity. In both years, samples of extruded pellets were collected at multiple times throughout the process for subsequent *n*-alkane analysis to ensure a uniform *n*-alkane profile was achieved throughout the batch mix.

Table 5. 1 Ingredient composition of n-alkane pellet delivered during trial period for Trial 1 and Trial 2.

	Trial 1	Trial 2
Ingredient composition (%)		
Barley	55	55.7
Wheat	20	20
Canola meal	16	16
Corn DDGS	5.3	5.3
Canola oil (post-extrusion)	2.5	2.5
Beeswax	0.7	
Dotriacontane (C32)	0.04	0.04

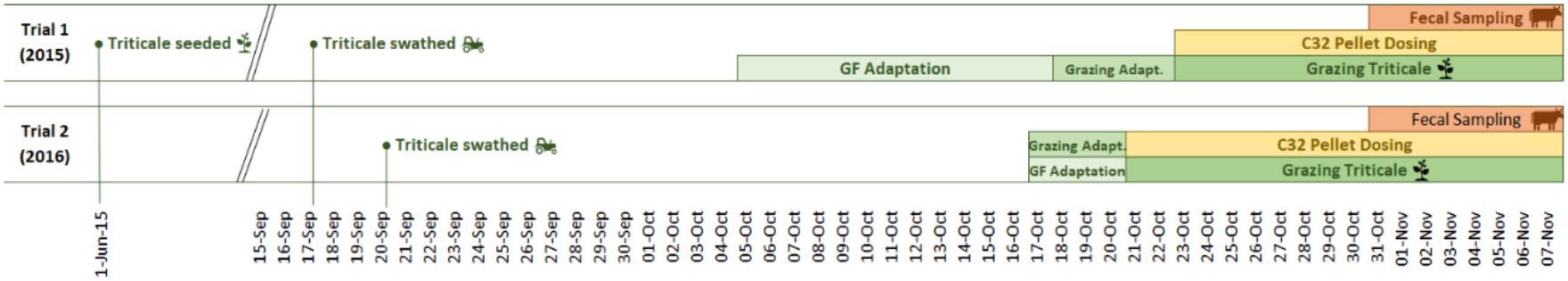

Figure 5. 1 Timeline of events leading up to and during Trials 1 and 2.

5.3.4 Animal management: Swath intake period

Cows ($n_{Trial\ 1}$ = 19, $n_{Trial\ 2}$ = 12) were assigned to a paddock equipped with GreenFeed systems and provided *ad libitum* access to water and mineral. Mean monthly temperatures were slightly above (1.1 to 2.2°C in 2015; - 2.9 to 5.1°C in 2016) the long-term (1971 to 2000) average of 4.4 and - 4.4°C, respectively in October and November. Total monthly precipitation from October to November in each year was 22.0 and 51.1 mm, respectively compared to a long-term average of 35.6 mm (Environment Canada).

Grazing began on October 19, 2015 and October 17, 2016 at LRDC for Trials 1 and 2, respectively. Cows grazed swathed triticale forage (Table 5.2) for approximately 22 d which consisted of a 4- to 6-d adaption period, (Day −4 to −1), 13 d of once-daily dosing with an *n*-alkane pellet (Day 0 to 12) beginning October 23 (Day 0) in each year and a 5 to 8-d fecal collection period (Day 8 to 12 or 15).

More specifically, from Day 0 to 7, cows grazed a larger section of paddock ("grazing adaptation"; Figures 5.1 and 5.2) and from Day 8 to 12, cows were moved to a paddock with single-strand portable electric fencing, with access to swaths allocated into daily strips based on an estimated forage DM utilization rate of 1.8% of average group BW. Each grazing strip measured 7 m wide and 20 m long (140 m^2) within the paddock in Trial 1, and between 6.4 and 6.7 m wide and 24 m long (153 and 161 m^2) in Trial 2. The area allocated was reduced on Day 13 to maintain consistent grazing density with reduced animal numbers, as cows were split into two smaller groups in strips on a second swath, for other research objectives not discussed here. Cows were moved every morning following *n*-alkane dosing; waterers and mineral tubs were moved concurrently to each new section. Forage allocation was restricted in order to encourage

homogeneous grazing by preventing animals from selectively grazing plant parts, as well as to reduce waste via trampling.

Table 5. 2 Nutrient composition of swathed triticale prior to being grazed in Trial 1 and Trial 2.		
Nutrient composition, % DM	Trial 1	Trial 2
DM, %	74.5	50.4
CP	7.0	8.0
NDF	58.3	56.4
ADF	40.1	38.6
Starch	6.9	5.8

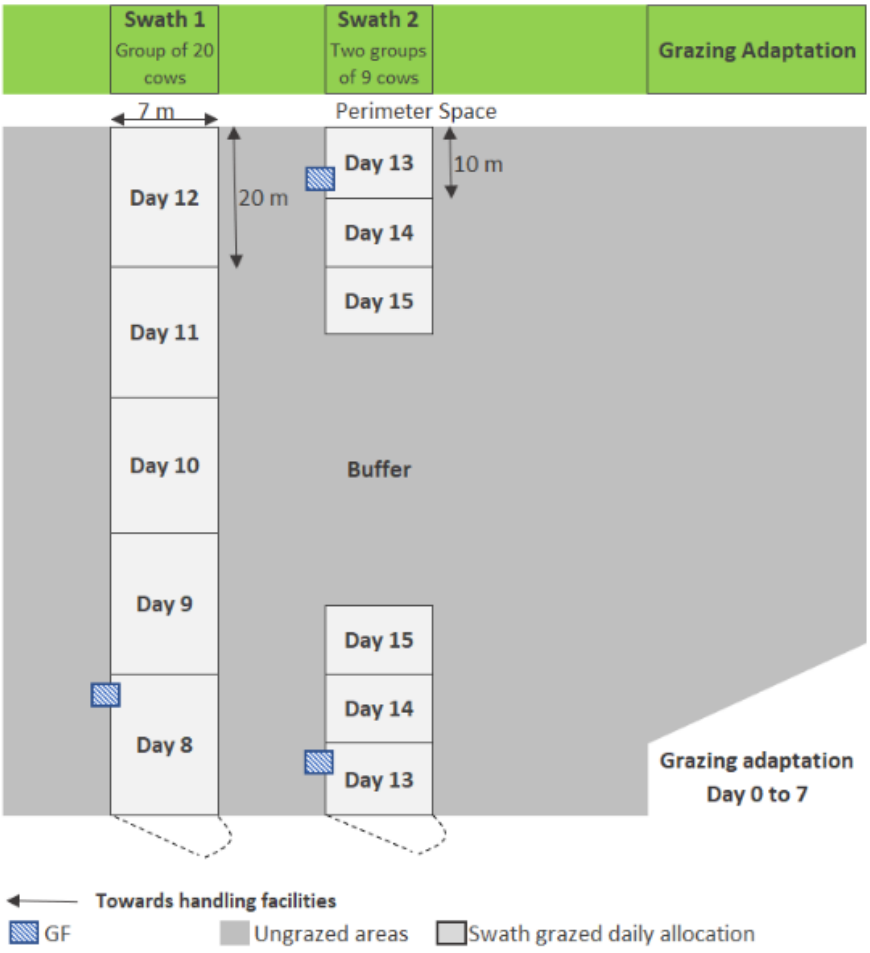

Figure 5. 2 Diagram of paddock layout outlining animal movement in Trial 1, with strips set up the length of two swaths. In Trial 2, the layout was similar though oriented from West to East rather than South to North. Cows were moved once- (beginning Day 0) or twice-daily (Day 8 onward) to handling facilities for C32 pellet dosing and/or fecal collection.

Supplement delivery

To administer the *n*-alkane-marked pellets, cows were moved to a handling facility once daily from Day 0 to 15 at 0815 h and were offered 1000 ± 0.5 g head^{-1} of *n*-alkane pellets in individual pens. Orts were collected and weighed daily to be later analyzed for DM.

Daily GF pellet intake was measured through automated monitoring of the number of cup drops delivered to each animal, identified by an RFID tag. Cup drop samples (n = 10) were collected daily to ensure delivery of consistent pellet weight. In Trial 1, cows received *n*-alkane pellets from GF in an attempt to use this automated system to dose the C32 marker. However, this approach resulted in highly variable pellet intake, ranging from 0 to 1.28 kg DM d^{-1} (mean 0.35 kg DM d^{-1}) within the first four days (Day 0 to 3) of the trial. Therefore, a hand dosing protocol was implemented beginning on Day 4 of Trial 1. In Trial 2, DG bull pellets were used in the GF system (Table 5.3).

Table 5. 3 Nutrient composition and strategy for delivery of pellets dispensed from GreenFeed system and hand-dosed to individual animals.

	Trial 1 C32 pellet	**Trial 2 C32 pellet**	**Trial 2 DG Bull pellet**
Delivery method			
GreenFeed system	✓		✓
Hand dosing	✓	✓	
Chemical composition (% DM)			
% DM	96.20	95.71	95.84
CP	17.84	19.42	16.43
ADF	13.01	9.86	10.50
Ca	0.23	0.28	1.53
P	0.52	0.57	0.72
Mg	0.24	0.33	0.30
K	0.63	0.70	0.90

Fecal sample collection

Fecal grab samples were collected once at 0815 h on Day 0 to serve as a baseline *n*-alkane fecal profile, and twice daily at 0815 h and 1515 h from Day 8 to 15. Fecal samples from each animal were stored in a cooler on ice until placed in a freezer at −20°C for later processing.

5.3.5 Forage and pellet sample collection

Prior to grazing (AM; Time 0 hr), one grab sample was collected from each strip, measuring either 20 m or 10 m long, to be allocated on Days 8 through 12 or 13 through 15, respectively (Figure 5.2). In Trial 1, one grab sample was collected from the daily grazing allocation at 1500 h daily (PM), at Time 7 hr post entry. It was noted through visual observation that the cows had consumed most triticale heads by this time. In both Trial 1 and 2, a visual estimate of percent utilization at both 7 and 24 hr was recorded. However, in Trial 2 two separate PM samples were collected due to poor weather and grazing conditions. The first sampling method (A) used required collection of forage from a clean, ungrazed section of swath, and then heads were removed to mimic the selective grazing strategy observed in Trial 1. The second sampling method (B) was used to collect forage daily from each strip at 1500 h and rinsed of most mud and manure contamination prior to further processing. This sample represented the actual forage accessible at 7 hr post-entry.

In addition, three 0.125 m^2 quadrat samples from each strip were taken at 24 hr post-entry to estimate residual forage biomass and percent disappearance. Each sample was weighted based on location within the strip, as the greatest mass located in the centre of the swath, with less residue towards the outer edges of the swath.

In Trial 2, prior to grazing, a sample of 10 culms (entire stem, head and leaves) was collected from the bottom, middle and top of the swathed forage, and from the length of each

strip to be grazed within a 24-hr period, as previously described. These grab samples were separated into plant parts by removing the heads at the base of the florets using scissors, and weighing and drying separately from leaves and stems, processed for later *n*-alkane analysis to assess differences in *n*-alkane profile between heads, leaves and stems.

From Day 8 to 15, a pellet sample of each type (when applicable; Bull DG and/or C32-marked pellet, Table 5.3) was collected daily to be later analyzed for DM and *n*-alkane composition. Subsamples were randomly collected throughout each trial for later quality analysis.

5.3.6 Sample processing and analysis

Sample processing

Pellet, ort and fecal samples were dried at 60°C for three days in a forced-air oven and ground through a 1 mm screen in a Wiley grinder. Forage subsamples for DM were dried in a Blue M oven (Thermal Product Solutions, New Columbia, PA) at 80°C for three days. Forage subsamples for quality and *n*-alkane analysis were dried for a minimum of five days in a forced-air oven at 55°C and ground to 2 mm or 1 mm, for subsequent *n*-alkane or quality analysis, respectively.

Forage quality analysis

Forage samples were scanned for quality by NIRS (model 6500, Foss NIRSystems, Silver Spring, MD). Raw spectral data were recorded as Log 1/R from 400 to 2498 nm at intervals of 2 nm and were transformed using the standard normal variant procedure. A calibration dataset of between 92 and 96 (117 for starch) samples was selected from a larger sample set (n=1050) for NIRS calibration using WinISI^{TM} 4 software (Infrasoft International, Silver Spring, MD). Calibrations were assessed using the coefficient of determination for the prediction (R^2) and the standard error of calibration (SEC). Calibrations were validated using cross-validation procedure

and assessed by reference to the standard error of cross validation (SECV) and variance accounted for during validation (1-VR). Summary statistics are presented in Table 5.4 for the calibration used.

Table 5. 4 Summary of NIRS calibration statistics.

Constituent	n	Mean	SD	Min.	Max.	SEC	R^2	SECV	1-VR
CP	92	12.5	4.40	0.0	25.7	0.75	0.97	1.04	0.944
ADF	95	34.7	5.45	18.5	51.1	1.27	0.95	1.63	0.910
NDF	95	50.6	7.16	29.1	72.0	1.92	0.93	2.33	0.893
Starch	117	8.8	6.66	0.0	28.8	2.06	0.91	2.43	0.87

Pellet quality analysis

Pellet samples were analyzed for dry matter (DM), crude protein (CP), acid detergent fibre (ADF), calcium, phosphorus, magnesium and potassium by Cumberland Valley Analytical Services (CVAS; Hagerstown, MD), as described in Table 5.3. In brief, DM was determined by drying at 135°C to a constant weight as per Association of Official Analytical Chemists (AOAC) official procedures (AOAC 2000, Official Method 930.15). Crude protein was calculated as 6.25 x N (AOAC 1990, Official Method 973.03). Acid detergent fibre and mineral content were determined by AOAC (2010) official procedures 973.18 and 985.01, respectively, with modifications as described online (CVAS, 2017).

n-alkane analysis

As described in Manuscript 1, fecal samples, triticale forage samples, and *n*-alkane pellet samples were analyzed for *n*-alkanes as described by Moshtaghi-Nia and Wittenberg (2002), with some modifications. Briefly, a known amount of dried and ground sample was digested overnight at 90°C in an Isotemp oven (Fisher Scientific, Ottawa, ON) with ethanolic potassium hydroxide and an internal standard containing C34. After cooling, 8 ml of heptane and 8 ml of warm distilled water were added. Samples were vortexed before warming in a water bath set to between 50°C and 60°C for at least five minutes to allow the phases to separate. Following the

addition of three, 5-ml aliquots of heptane, the extract was evaporated to dryness by placing the vials in a water bath maintained between 70°C and 90°C, using compressed air to hasten the evaporation process. After dissolving the alkanes with heptane in a water bath, the solution was filtered through a silica gel column five times, rinsing the vial each time with heptane. The filtrate was then evaporated to dryness using the same method as above, and samples were prepared by adding *n*-undecane to the vials, warming and rinsing with the solution, and transferring to GC vials. Samples were then analysed using a Varian Model 3600 gas chromatograph (GC) (Varian, Inc. Walnut Creek, CA) using split injection mode at 300°C. An in-house standard with a well-characterized *n*-alkane profile was extracted in duplicate with every run to ensure consistency, as well as a blank to ensure sample integrity. The GC was calibrated using an external standard containing analytical standards of all *n*-alkane chain lengths between C24 and C36 (Sigma-Aldrich).

While all chain lengths between C24 and C36 were analyzed, statistical comparisons focused on C31 and C32 as this is a common pairing when using the *n*-alkane method to estimate DMI, where the odd-chain *n*-alkane, C31, is most abundant in the forage, and the even-chain *n*-alkane, C32, is comparatively low in forage and therefore dosed using a synthetic source (Olivàn et al. 2007).

5.3.5 Statistical analysis

Paired t-tests were used to compare AM (0-hr.) and PM (7-hr.) mean *n*-alkane C31 and C32 concentrations, as well as starch concentration (Trials 1 and 2). *n*-alkane C31 and C32 concentrations (mg kg^{-1}), content (mg in sample) and plant part dry weights (g) of head vs. leaf and stem (Trial 2 only) were also compared using paired t-tests.

To test for normality, the null hypothesis was stated that the distribution of the residuals was not different from a normal distribution for *n*-alkane profiles and plant part dry weights, head vs. leaf vs. stem *n*-alkane profiles. Failing to reject the null hypothesis, *n*-alkane profiles and plant part dry weights, head vs. leaf vs. stem *n*-alkane profiles were then compared using a randomized complete block design (proc MIXED: SAS University Edition version 3.71. SAS Institute, Inc. 2017) with sample as a random block effect. A power analysis to ensure Power ≥ 0.8 was run to ensure an appropriate level of significance was used, given the low *n*. Differences among treatment means were tested using a Tukey test at $\alpha = 0.05$ level of significance. The same procedures were also used to assess differences in plant weights.

5.4 RESULTS AND DISCUSSION

5.4.1 Quality and *n*-alkane profile of swath-grazed triticale

In Trial 1, CP and starch in triticale samples collected at 0-(AM) and 7-hr (PM) into grazing decreased from 7.0 % to 4.9 % ($P < 0.0001$) and from 6.2 % to 0.1 % ($P < 0.0001$), respectively in AM compared to PM samples, as depicted in Table 4.5. In addition, NDF and ADF increased from 58.6 % in AM samples to 62.7 % ($P < 0.0001$) in PM samples and 40.3 % in AM samples to 45.4 % in PM samples ($P < 0.0001$), respectively. This decline in quality suggests that cows preferentially grazed plant parts containing starch and protein, selectively grazing the heads before the leaves and stalkier stem material. To the author's knowledge, no published literature exists that characterizes selective grazing of plant parts in a swath, however numerous studies have demonstrated that cattle are able to selectively graze between plant species in standing pasture (Jamieson and Hodgson 1979; Stephens and Krebs 1986; Cuchillo-Hilario et al. 2017). Despite differences in sampling protocol in Trial 2 similar trends were apparent; however, the magnitude of difference was less (Table 5.5), as described in detail

below. Challenges were apparent for both sample collection methods A and B used for Trial 2 PM sample collection. Method A does not reflect actual grazing behaviour in Trial 2 and method B results were affected by mud and manure that remained following rinsing. As a consequence of this contamination, results from preliminary quality analysis in PM samples collected using method B indicated that mud and manure removal by rinsing was not effective (5.95% greater ash content in B compared to A samples) and yielded biased results (global H > 3.0 for all quality constituents), and therefore no further analysis was completed. While starch content was similar in AM samples in both years (6.2 mg kg^{-1} in 2015 and 6.0 mg kg^{-1}in 2016) and decreased ($P < 0.0001$) in PM samples in both years, the magnitude of decrease was greater in Trial 1 ($Diff_{Trial\ 1} = 6.1$, $Diff_{Trial\ 2A} = 2.3$). This may have been due to excess moisture, causing mould and degradation in the forage, and thus influencing the NIRS spectra. Further, NIRS calibrations for starch are difficult to develop and accuracy may be limited at low starch levels. Crude protein (CP) decreased only slightly from 8.3 % in AM to 7.4 % ($P < 0.05$) in Trial 2 PM samples, and changes in NDF and ADF were not significantly different between AM and PM samples.

In addition to differences in starch content of AM and PM samples, significant differences in the C31 concentration were also apparent between AM and PM samples ($Diff_{Trial\ 1}$ = 24.5 mg kg^{-1} ($P = 0.0002$), $Diff_{Trial\ 2A}$ = 28.0 mg kg^{-1} ($P < 0.0001$), $Diff_{Trial\ 2B}$ = 11.7 mg kg^{-1} ($P = 0.0168$); Table 5.5). The concentration of C31 was higher in AM compared to PM samples in both trials and for samples collected using methods A ($P < 0.0001$) and B ($P = 0.0168$) in Trial 2. Although AM samples had mean C31 concentrations of 66.5 mg kg^{-1} and 60.0 mg kg^{-1} in Trials 1 and 2 respectively, the PM samples from both trials and both Trial 2 collection methods have average C31 concentrations below 50 mg kg^{-1}. With

C31 concentrations in PM samples below this recommended minimum concentration for forages intended for *n*-alkane intake studies (Casson et al. 1990; Laredo et al. 1991; Boadi et al. 2002), it is possible that estimates of DMI using the *n*-alkane method would be biased.

Similar to trends observed with C31, C32 concentrations were higher in AM compared to PM samples in both trials using both Trial 2 PM sample collection methods ($Diff_{Trial\ 1}$ = 0.2 mg kg^{-1} (P = 0.4221), $Diff_{Trial\ 2A}$ = 0.5 mg kg^{-1} (P = 0.0056) and $Diff_{Trial\ 2B}$ = 0.7 mg kg^{-1} (P = 0.0001); Table 5.5). Further, C31/C32 ratios were significantly different between AM and PM samples in Trial 1 (Diff. 14.8, P < 0.0001), and while still significant, to a lesser degree in Trial 2 using PM sample collection methods A (Diff. 9.9, P = 0.0099) and B (Diff. −16.8, P < 0.0001). Observed differences between *n*-alkane concentrations in AM and PM samples, along with observed differences in nutrient profile of residual material provide further evidence that cows were selectively grazing heads. Therefore, it may be speculated that cows had grazed all heads available to them within the first seven hours of grazing in Trial 1 based on the quality and *n*-alkane analyses of the PM sample collected daily at 15h00. In Trial 2, while the trend in nutritive and *n*-alkane profiles still existed, smaller differences between the two sample collection periods may be associated with changes in PM sampling protocol necessitated by adverse weather resulting in use of a simulated grazing scenario.

Differences in *n*-alkanes concentrations in AM samples between the two trials were not surprising given that wax production in the plant cuticle, including *n*-alkanes which are found within, can be affected by soil moisture (Baker 1980), plant maturity (Laredo et al. 1991) as well as differences in climatic, environmental and other soil conditions (Boadi et al. 2002). To a lesser extent, these factors may also explain the day-to-day variation between samples collected within

the same trial as growing conditions may have varied slightly throughout the field. In addition, the presence of visible mould, which appeared to differ in severity throughout the swath may account for some of the variability in *n*-alkane profile between samples within Trial 2. Moulds will consume carbon atoms in organic material, therefore their presence may have led to a decrease in *n*-alkane concentration in triticale samples. Although previous publications have not addressed the effect of mould on the *n*-alkane profile of forages, this would be a worthwhile area of research to better understand the effect on the *n*-alkane profile of swath-grazed forages, which could be susceptible to moulding if harvested and exposed to elevated temperature and moisture prior to the extended grazing season (AARD 2008).

Variability in the PM sample *n*-alkane profile and selective grazing of plant parts which differ in their *n*-alkane profile in Trial 1 suggest it is necessary to differentiate forage material offered from waste material by characterizing their respective *n*-alkane profiles. Weighting of the initial proportions and remaining residue of each plant part and their respective *n*-alkane profiles may allow for a more accurate representation of the *n*-alkane concentration consumed and thus more accurate estimation of DMI using the *n*-alkane method when swath grazing. Unfortunately, characterization of residue material was not possible under the wet grazing conditions in Trial 2.

Table 5. 5 Summary of quality data and C31 and C32 n-alkane concentration of triticale swath samples by trial and time[1] of collection.

	Trial	n	AM				PM				Diff[2]	SE	P-value
			Mean	Min.	Max.	CV	Mean	Min.	Max.	CV			
DM (%)	1	5	74.6	72.8	76.3	2.1	72.1	64.3	79.3	6.3	1.0	2.2	0.6644
	2A[3]	7	55.6	43.6	73.5	17.8	57.8	48.2	64.7	12.1	−2.2	2.4	0.3899
CP (% DM)	1	17	7.0	5.8	7.7	7.6	4.9	3.8	6.0	10.7	0.8	0.2	<0.0001
	2A	12	8.3	6.1	9.8	11.0	7.4	6.6	9.2	11.4	0.9	0.4	0.0264
NDF (% DM)	1	17	58.6	51.3	61.7	4.9	62.7	61.5	63.9	1.1	-4.1	0.7	<0.0001
	2A	12	56.5	54.3	60.9	3.1	55.8	53.6	58.1	2.6	0.7	0.7	0.2890
ADF (% DM)	1	17	40.3	34.4	42.7	5.5	45.4	44.3	46.9	1.4	−5.0	0.6	<0.0001
	2A	12	39.3	37.6	43.1	3.6	38.6	37.1	40.5	2.6	0.7	0.4	0.0758
Starch (% DM)	1	17	6.2	2.4	16.6	60.4	0.1	0.0	1.0	287.9	6.1	0.9	<0.0001
	2A	12	6.0	3.8	7.9	19.9	3.7	2.6	5.3	24.8	2.3	0.3	<0.0001
C31 (mg kg^{-1})	1	17	66.5	35.2	100.4	27.4	42.0	31.1	49.9	11.6	24.5	5.0	0.0002
	2A	12	60.0	45.0	72.7	14.4	31.5	16.6	43.5	28.0	28.4	2.9	<0.0001
	2B[4]	12					48.3	36.2	66.5	20.5	11.7	4.1	0.0168
C32 (mg kg^{-1})	1	17	1.6	0.8	2.9	37.8	1.5	0.0	2.4	36.5	0.2	0.2	0.4221
	2A	12	1.6	1.0	2.5	28.2	1.1	0.0	2.4	52.8	0.5	0.1	0.0056
	2B	12					0.9	0.6	1.1	19.4	0.7	0.1	0.0001
C31/C32	1	17	42.9	28.0	56.7	18.7	28.9	20.3	42.1	21.1	14.8	2.0	<0.0001
	2A	12	38.6	28.1	50.0	17.5	28.0	16.8	45.1	32.2	9.9	3.1	0.0099
	2B	12					55.4	48.0	63.9	10.9	−16.8	2.7	<0.0001

[1]AM represents a sample collected at approximately 08h00, or Time 0 hr, before grazing; PM represents a sample collected at approximately 15h00, or Time 7 hr, 7 hours following entry to the paddock (see footnotes 3 and 4 for exceptions to the PM sample protocol in Trial 2).
[2]Paired t-test used to compare AM to PM means. Difference of (AM – PM) shown.
[3]PM sample collection method A: culms collected from clean, ungrazed section of swath with heads removed. Represents presumed selective grazing had all forage material been accessible.
[4]PM sample collection method B: culms collected from grazed section of swath and rinsed of most mud and manure. Represents actual forage on offer at time of collection.

5.4.2 C31 and C32 *n*-alkane profile and morphology of swath-grazed triticale plant parts

As indicated in Table 5.6, C31 and C32 concentrations differed in the plant parts sampled, with higher C31 and C32 concentrations ($P < 0.0001$) in the head (90.6 mg kg^{-1} and 6.4 mg kg^{-1}, respectively) than in the leaf/stem (47.7 mg kg^{-1} and 1.8 mg kg^{-1}, respectively). As previously discussed, the concentration of C31 was higher in AM than PM samples; the elevated C31 concentration in head compared to leaf/stem samples then further supports the hypothesis that heads were being selectively grazed. Leaf/stem samples also had an average C31 concentration slightly below 50 mg kg^{-1}, which is the recommended minimum concentration in forages used for *n*-alkane intake studies (Casson et al. 1990; Laredo et al. 1991; Boadi et al. 2002). However, C31 content (mg) was lower ($P = 0.0057$) in head samples compared to leaf/stem samples (Diff. −0.4 mg), with similar C32 content between plant part samples (Diff. 0.0 mg, $P = 0.0421$). Leaf/stem samples had a higher coefficient of variation (CV) for both C31 and C32 concentration and content compared to head samples, though variability of C31 content was greater than 10% for both plant parts sampled. Based on sample weight, leaf/stem (31.0 g) made up the largest portion (72 %) of the plant compared to the head (12.2 g), which explains why the significance was less when examining *n*-alkane content rather than concentration in each plant fraction.

Further separation of plant parts into head, leaf and stem, provided additional evidence that there are significant differences in *n*-alkane concentrations between plant parts (Table 5.7). The concentration of C31 was higher ($P < 0.0001$) in leaves (169.8 mg kg^{-1}) than in heads (109.4 mg kg^{-1}), and in heads compared to stems (28.5 mg kg^{-1}). The concentration of C32 was higher ($P < 0.0001$) in heads (7.0 mg kg^{-1}) than in leaves (3.0 mg kg^{-1}), and in leaves compared to stems (0.9 mg kg^{-1}). When comparing C31 and C32 content of plant parts (Table 5.7), only head

n-alkane content (C31 = 1.7 mg, C32 = 0.1 mg) was higher ($P < 0.0001$) than leaf (C31 = 0.8 mg, C32 = 0.0 mg) and stem (C31 = 1.0 mg, C32 = 0.0 mg) samples. The ratio of C31/C32 was highest in leaves (57.1), compared to stem (30.5) and head (15.6) which were also both different ($P < 0.0001$). To the author's knowledge, *n*-alkane content has not been explored in plant parts of any species. Further, although multiple studies have characterized the *n*-alkane concentrations within plant parts, few have been conducted in Canadian-grown forage species including triticale. Dove et al. (1996) examined *Lolium perenne* and *Phalaris aquatica* grown under controlled conditions in Australia. With the exception of C33, they observed highest *n*-alkane concentrations (mg kg^{-1} OM) in the inflorescence, followed by the leaf and other plant parts (sheath, base and stem) when harvested weekly between 40 and 142 days post seeding. However, Dove and Mayes (1991) postulated that it would theoretically be possible to use differences in *n*-alkane profiles of plant parts to estimate the selection of plant parts in the same way that species selection is estimated. This theory assumes that differences between plant parts within a species are consistent, and that forage sampled is representative of that consumed. Similarly, Smith et al. (2001) studied common grass species in Africa and also concluded that plant parts from the same species have significantly different *n*-alkane profiles. Evidence of differences in the hydrocarbon profile of plants has been reported by Tulloch (1973) who studied both spring wheat and durum wheat in Saskatoon, SK at various stages of growth. While Tulloch did not analyze samples specifically for their *n*-alkane profile, he found that both the concentration and composition of hydrocarbons varied between plant parts. Consequently, one can conclude that, *n*-alkanes making up a portion of the hydrocarbon composition of plants, *n*-alkane concentration would vary between plant parts of these wheat species under Canadian growing conditions as well. Although most previous studies have found the highest concentration of *n*-alkanes in the head, Laredo et

al. (1991) studied eight species of forage in Australia and noted that the *n*-alkane concentration in leaves was influenced by the phenological stage of the plant, morphological composition, age and season. These observations are relevant to the Western Canadian production environment in which swath-grazed forages are grazed at a later stage of maturity, age and season than forages previously described in the literature. These factors could account for the observed increase in C31 concentrations of swathed triticale leaves compared to heads which is contrary to previously published literature findings.

Trial 2 triticale plant part samples had significantly different weights, with the largest proportion of the mass of the plant in the stem (62.9 %), followed by heads (28.4 %), then leaves (8.8 %). These differences in weight are comparable to values reported by Baron et al. (2015), who studied different cultivars of triticale at the same location as the current study. Further, Bilgili et al. (2009) who studied 33 lines of triticale in Mediterranean conditions reported that stems made up 60.9 % of total DM, heads 26.8 % and leaves 12.3 %, which is similar to the results in the present study. These authors also reported that leaves were more digestible (660 g kg^{-1}) compared to heads (622 g kg^{-1}) and stems (520 g kg^{-1}), which could potentially play a role in rumen retention time and alter the pattern of fecal *n*-alkane excretion. Therefore, the relationship between plant part *n*-alkane profile, digestibility and rumen retention time requires further investigation to understand potential impact on DMI estimates using the *n*-alkane technique.

When designing grazing trials for forage species with plant parts that differ in *n*-alkane profile and can be selectively grazed, estimates of DMI may vary dramatically due to the *n*-alkane profile consumed based on selectivity or grazing behaviour. A study of mature dairy cows ranked for RFI as calves (6 to 8 months of age) by Gregorini et al. (2015) found that low-RFI

(efficient) cattle strip-grazing *ad libitum* perennial ryegrass tended to graze most when fresh pasture was allocated each morning, compared to high-RFI cattle who were less efficient grazers and spread their grazing throughout the day. Furthermore Basarab et al. (2013), in a summary of multiple RFI studies reported that low-RFI_{fat} cattle have lower feeding event duration and frequency compared to high-RFI_{fat} animals, with distinct diurnal patterns of feeding behaviour between RFI_{fat} groups in confinement. Based on results from these previous studies, and with differences in quality and *n*-alkane profile between AM (pre-grazing) and PM (mid-grazing) samples presented here, it may be speculated that cattle divergent in RFI_{fat} would consume different proportions of plant parts. As low-RFI (efficient) cattle tend to graze most in the early morning, when most heads are available to be grazed, they might consume a different *n*-alkane profile than high-RFI (inefficient) cattle that tend to graze more consistently throughout the entire day. However, previous studies have not distinguished grazing selectivity between RFI groups and therefore further investigation is required in swath grazing systems with RFI-divergent animals.

Visual observation suggested that there was variation in plant part morphology of swathed triticale (Figure 5.3), however it is difficult to speculate on the effect of these differences as our samples were composed of plant parts from 10 culms. Under both plant part separation protocols, there were significant differences between both heads and leaf/stems (Table 5.6), and heads, leaves and stems (Table 5.7) in terms of both C31 and C32 concentration and content, and weight, which may be an indicator of morphology.

Figure 5. 3 Photo demonstrating visual variation in head morphology of swathed triticale forage.

Table 5. 6 C31 and C32 n-alkane concentration (mg kg^{-1}) and content[1] (mg), and dry weight of triticale swath plant part (head, leaf/stem) samples[2] collected in Trial 2.

	Head				Leaf/Stem				Diff[4]	SE
	Mean[3]	Min.	Max.	CV	Mean	Min.	Max.	CV		
C31 (mg kg^{-1})	90.6a	69.6	100.8	11.8	47.7b	32.5	58.1	16.7	42.9	4.7
C32 (mg kg^{-1})	6.4a	5.3	7.2	9.4	1.8b	0.0	2.7	45.2	4.6	0.3
C31 (mg)	1.1b	0.6	1.6	31.1	1.5a	0.9	2.2	31.5	−0.4	0.1
C32 (mg)	0.1a	0.0	0.1	28.2	0.1b	0.0	0.1	48.8	0.0	0.0
C31/C32	14.2b	12.6	16.6	9.4	25.4a	17.9	40.6	33.9	−11.4	2.6
Weight (g)	12.2b	7.8	16.6	23.4	31.0a	20.3	40.5	22.7	−18.8	1.4

[1]Indicates mg of C_n present in entire sample, made up of 10 culms. Considers weight of sample collected.
[2]*n* = 10 samples, each comprised of 10 culms.
[3]Letters indicate Adj $P < 0.05$
[4]Paired t-test used to compare head to leaf/stem means. Difference of (head – leaf/stem) shown.

Table 5. 7 C31 and C32 n-alkane concentration (mg kg^{-1}) and content[1] (mg), and dry weight of triticale swath plant part samples[2,3] (head, leaf, stem) collected for Trial 2.

	Head				Leaf				Stem				SE
	Mean	**Min.**	**Max.**	**CV**	**Mean**	**Min.**	**Max.**	**CV**	**Mean**	**Min.**	**Max.**	**CV**	
C31 (mg kg^{-1})	109.4b	98.1	131.2	10.6	169.8a	154.8	181.1	5.9	28.5c	24.3	34.2	13.6	3.7
C32 (mg kg^{-1})	7.0a	6.1	8.5	12.0	3.0b	2.6	3.3	10.4	0.9c	0.9	1.1	6.6	0.2
C31 (mg)	1.7a	1.4	2.5	23.0	0.8b	0.7	1.2	19.9	1.0b	0.8	1.2	13.5	0.1
C32 (mg)	0.0a	0.0	0.2	29.6	0.0b	0.0	0.0	15.1	0.0b	0.0	0.0	10.5	0.0
C31/C32	15.6c	14.1	16.7	5.8	57.1a	52.3	61.9	6.6	30.5b	23.0	37.5	16.5	1.5
Weight (g)	15.9b	11.9	24.0	27.0	4.9c	3.9	6.6	19.1	35.2a	29.2	39.5	11.1	1.4

[1]Indicates mg of C_n present in entire sample, made up of 10 culms. Considers weight of sample collected.
[2]*n* = 6 samples, each comprised of 10 culms.
[3]Comparisons made using randomized complete block design, where each block corresponds to a single sample made up of 10 culms.
[4]Unequal variance was observed among plant parts for all variables. Letters indicate Adj $P < 0.001$

5.8 CONCLUSIONS

In Trial 1, AM samples were significant higher in CP and starch, as well as significant lower in NDF and ADF compared to PM triticale samples. Furthermore, C31 concentrations decreased significantly between AM and PM samples, with a mean PM C31 concentration below 50 mg kg^{-1}. These factors in Trial 1 were suggestive of selective grazing behaviour within the first seven hours of grazing, and therefore sampling of plant parts was included in the protocol for Trial 2.

Trial 2 was conducted in extremely wet grazing conditions and trampling in muddy conditions required further changes to the forage sampling protocol with PM samples collected only to mimic grazing based on indicators from Trial 1. For this reason, while significant decreases in CP, starch, C31 and C32 concentration, and C31/C32 ratio were similar to AM and PM samples in Trial 2, this data could not be used as evidence that selective grazing occurred.

As theorized based on the published *n*-alkane profiles of other plant species, differences were found in the C31 profile between plant parts of swath-grazed triticale. This was true whether plants were separated into two or three plant part fractions.

Hypothetically, although plant parts of many grazed forage species differ in their *n*-alkane profile, it may be possible to estimate individual-animal DMI by weighting the respective contribution of each plant part to the forage grazed assuming that each animal consumes a similar, consistent proportion of each plant part on a daily basis and that this residual material can be accurately measured. This may be achieved through careful grazing allocation with most grass species, however it was found in this study that the extreme differences between triticale plant parts, which may be applicable to most cereal crops, coupled with preferential grazing of heads over leaf and stem material and the unsuitable grazing conditions caused by unpredictable

winter weather, resulted in inconsistent consumption of *n*-alkanes. By definition, marker-based intake strategies require consistent delivery of the marker.

Further studies involving *n*-alkane estimation of DMI and cereal crops in the extended grazing season should be conducted across multiple years, as results in this study were highly variable between trials. Furthermore, sampling of residual forage material may aid in better characterizing the *n*-alkane profile of forage consumed and thus in estimating DMI. Alkane and quality analysis of plant part samples, as well as quantification of the proportion of each plant part present at each sampling time, may also aid in developing a strategy for DMI estimation using the *n*-alkane technique under similar conditions. In addition, given the differences between *n*-alkane profile of plant parts, and the occurrence of selective grazing which may alter the *n*-alkane profile on offer throughout the period of grazing allocation (usually 24 h), separate grazing areas might be warranted when studying groups of animals divergent in RFI ranking as diurnal variation in grazing behaviour might influence the profile of plant parts, and thus *n*-alkanes, consumed.

6. GENERAL DISCUSSION

Accurate measurement of individual animal intake, including on pasture, is essential for improved feed efficiency in the beef sector, and particularly for improved economic and environmental sustainability. An improved understanding of individual animal intake will improve producers' ability to meet nutrient requirements in grazing cattle and may provide an opportunity to select animals based on efficiency traits, such as RFI. As indicated, it may be useful as a tool to decrease feed costs and improve production efficiency via reduced feed intake as well as manure and methane production without increasing mature cow size, as it is independent of body weight and average daily gain (Basarab et al. 2002, 2011, 2013). However, most research to date has measured RFI in confinement using automated feeding stations over a minimum 63-d test period (Wang et al. 2014), which can be costly when RFI testing many breeding prospects. Furthermore, differences in maturity or diet type (Durunna et al. 2011) may lead to re-ranking between parities and thus animals may need to be evaluated for efficiency across diets and seasons (Durunna et al. 2014). To address gaps in the current literature, the potential to estimate individual-animal intake in two classes of animals (heifers, cows) was explored over two grazing periods in two non-confinement systems: i) pasture and ii) swath grazing. Intake was estimated in heifers from two sites with divergent RFI rankings, grazing meadowbrome grass. In addition, the potential to measure individual-animal DMI using the *n*-alkane technique was examined in cows with divergent RFI when grazing swathed triticale; the first effort to measure individual animal intake in a cereal-based extended grazing system.

The current work: i) compared the *n*-alkane method, fNIRS and traditional NRC equations as methods of predicting intake of beef heifers on pasture; ii) compared the *n*-alkane method and fNIRS to traditional prediction equations (NRC, Mertens, Minson) for predicting beef cattle intake in non-confinement environments; iii) correlated estimates of intake on pasture

(estimated using *n*-alkane, fNIRS) to previously-measured RFI rankings; and iv) examined the *n*-alkane profile, residual starch content and morphology of swath-grazed triticale in order to assess its suitability for *n*-alkane intake studies.

6.1 RFI and methods of estimating intake on pasture

Manuscript I supports the hypothesis that intake on pasture as measured by direct and indirect fNIRS and *n*-alkane methods differ in their predictions of individual-animal pasture intake compared to traditional prediction equations. This can be explained by previous research by Arthur et al. (2001), who concluded that RFI accounts for between-animal variation in feed intake unexplained by differences in BW and ADG. Therefore, traditional equation-based DMI estimation techniques are not suitable for individual animals when other factors, such as feed efficiency, are variable. However contrary to our hypothesis, fNIRS and *n*-alkane methods were not found to be suitable for predicting individual animal intake on pasture when using previously-measured RFI rankings as a reference. The *n*-alkane method was correlated to RFI rankings in 2015, but not in 2016, perhaps related to differences in forage quality between years, and therefore this relationship requires further investigation. The relationship between DMI_{Alkane} and RFI_{fat} in 2015, is similar to findings by Manafiazer et al. (2015) and Johnson (2014). Manafiazer et al. (2015) found a positive correlation ($r_p = 0.30$) between RFI_{fat} measured in crossbred beef heifers when in a drylot and grazing tame pasture using the *n*-alkane technique to estimate intake approximately two months after the initial RFI ranking. Similarly, Johnson (2014) observed that both the fNIRS ($P = 0.03$) and *n*-alkane ($P = 0.04$) methods predicted DMI differences (g/kg $BW^{0.75}$) between low- and high-RFI pregnant females. In the current study and in that described by Manafiazer et al. (2015), heifers may have exhibited different "feeding behaviours" to acquire feedstuffs at different times throughout the day among RFI groups and

pasture intake periods, having to acquire feed via a GrowSafe bunk or grazing, respectively. However, in Johnson (2014) heifers were fed using Calan gate feeders during both the RFI and intake test periods, which were conducted consecutively. A challenge with using RFI rankings to validate intake estimates is that these measurement periods are distinct in time and place, with different feedstuffs available in different environments and thus may result in re-ranking of animals. This challenge is echoed by Durunna et al. (2014), who reported that due to differences in maintenance requirements, fat deposition, conceptus growth, lactation, activity and thermoregulation, animals should be evaluated for efficiency across diets and seasons. Furthermore, there is currently no accepted gold standard which can be used to validate individual animal intake estimates in non-confinement. It would therefore be logical that re-ranking may have occurred in the current study between the time of RFI ranking and intake estimation on pasture.

In Manuscript II, congruent with our hypothesis, significant differences in the *n*-alkane profile between plant parts of swath-grazed triticale were found. In addition, significant differences were found between nutrient content of AM (pre-grazing) and PM (after 7 h of grazing) triticale samples suggesting that animals were selectively grazing heads followed by remaining plant parts. Further, using plant part weights as indicators of morphological variation, there was large (> 20% CV) variability in the weight of head and leaf/stem samples. These observations collectively indicate that the use of triticale, and perhaps other cereal-grain crops, as a forage in *n*-alkane intake studies may be limited. Therefore, while it was hypothesized that despite differences in *n*-alkane profile between plant parts, grazing management to encourage uniform consumption of plant parts would allow for prediction of intake using the *n*-alkane method, this may not be the case. In addition to variability in plant part morphology, many

challenges related to winter grazing of swathed material may limit the use of the *n*-alkane method for prediction of individual animal intake in these settings. In Trial 2, these challenges included restricted intake due to the inability of animals to access all forage and the contamination of forage via trampling into the soil in wet conditions.

6.2 Implications of research for the industry

Despite the extensive research conducted on RFI to demonstrate its worth as a selection criterion for feed efficiency (Arthur and Herd 2008; Basarab et al. 2013; Nkrumah et al. 2014), and acceptance of RFI as a selection criteria by several purebred associations, the cost, time and equipment required for RFI testing of animals under the current protocol (Wang et al. 2006) have perhaps contributed to slow adoption within the beef industry. In addition, the skills used by animals to acquire feed (grazing vs bunk feeding) as well as the ability to utilize various sources of nutrients (forage- vs. concentrate-based diets) may differ between feed and animal types. For these reasons, the ability to test animals for RFI, or some trait related to this (such as intake), in non-confinement in a more rapid or cost-effective manner may be the next step to making RFI more widely used as a selection criterion by beef producers. Furthermore, this selection tool must be applicable to beef cattle management, environmental conditions and diets commonly used in Canadian agriculture, including extended grazing systems.

This research addresses the concept of using either the *n*-alkane method or fNIRS technology (directly or indirectly, to estimate fecal C31 and C32) as indicators of animal efficiency over a short period of time (5 d). The direct estimation of DMI via fNIRS is desirable as it requires only fecal collection and allows for rapid, non-destructive and cost-effective analysis. However, a robust calibration data set is required. While the *n*-alkane method is more labor intensive, including dosing of animals and collection of fecal, forage and pellet samples, as

well as more costly and time-consuming wet chemistry, it appears this method provides the most accurate estimation of DMI, and thus indication of feed efficiency, as demonstrated in the 2015 pasture intake trial. Similarly, a robust calibration data set is required when using fNIRS to estimate fecal C31 and C32 concentration. However, this method still requires wet chemistry on forage and pellet samples using the traditional *n*-alkane method. None of the equation-based DMI estimation methods were correlated to RFI rankings, and therefore although these methods may still be useful in some instances, for example to estimate feed requirements for a group of animals, they should not be used to evaluate individual animal efficiency. This of course is evident in the definition of RFI, as it is not influenced by BW or ADG (Basarab et al. 2011); factors which are used in the estimation of DMI via equation-based methods. These same shortcomings would likely be true of intake estimation in swath grazing systems as well, however there are further challenges when grazing a cereal crop.

6.3 Future research

It is evident from this research that further work using both the *n*-alkane and fNIRS methods are required to better estimate intake in non-confinement environments. This includes use of larger groups of animals grazing various types of forages. These animals could also be followed through to extended grazing, and with repeated measures on the same group of animals, a more complete picture of this relationship may become evident. Although previous research has shown some re-ranking of animals over time, the value in this approach would be in having a larger, more diverse calibration data set for fNIRS. However, the cost of such a study may be prohibitive without also examining other congruent research objectives. Studies of longer duration may also provide additional information regarding the impact of extreme heat (during pasture grazing) or cold (during extended grazing) environmental conditions, as these

temperature swings may influence intake measurements in non-confinement and thus the apparent relationship between intake and RFI ranking. In addition, a behaviour component to the intake studies as it has been demonstrated that low- and high-RFI animals differ in grazing behaviour (Basarab et al. 2013); perhaps dominance (or subordinance) is also related to RFI or DMI, which may be particularly evident under daily-allocated extended grazing management. While these factors may influence outcomes of RFI and pasture intake studies, they have not been examined to date.

In addition, sensitivity of the DMI estimation methods used should be considered in future research. Several factors contribute to the sensitivity of *n*-alkane and fNIRS analyses including alkane recovery rates and accuracy of *n*-alkane extraction, dosed pellet intake measurement (subject to human error when collecting orts), and fNIRS spectra calibration. These factors should be considered when determining statistical power with respect to sensitivity. Further, benchmarks for evaluation criteria such as R^2 and SEC should be re-examined to account for these factors.

Further investigation is also warranted to determine whether DMI estimation of individual animals grazing cereal grain crops is possible using either of the *n*-alkane or fNIRS techniques. This may require preliminary research feeding cereal crops in confinement, with intake also measured via GrowSafe or a similar system, to better understand the effect of plant part selection on the resulting *n*-alkane profile of feces. However, this method would require a careful approach as plant part selection may differ when grazing compared to bunk feeding. Alternatively, grazing management may be altered to increase grazing pressure sufficiently to obtain a consistent residue on a daily basis. Although this might be achieved through twice-daily

forage allocation, forage availability may be reduced due to trampling with such high grazing pressure.

Although many studies have been conducted using the GreenFeed System (Zimmerman and Zimmerman 2016), a novel aspect of both the pasture and swath trials was to use this system to dose animals with the *n*-alkane marked pellet. While unsuccessful in both pasture and swath settings, use of this system may be more effective with an extended adaptation period, following recommendations as described by Hristov et al. (2015), or delivery of highly palatable feedstuffs. Improving familiarity with the system or incentive to visit the GreenFeed or other automated feeding station may encourage consumption of the marked pellet and eliminate the repeated handling required when C32 is dosed manually on an individual animal-basis. While this strategy would still require handling for collection of fecal samples, grazing disruption would be minimized, allowing for more accurate estimate of intake on pasture and collection of other efficiency data, such as methane emissions on pasture.

7. GENERAL CONCLUSIONS

Intake on pasture as measured by direct and indirect fNIRS and *n*-alkane methods differed in their predictions of individual-animal pasture intake compared to traditional prediction equations. Of the DMI estimation methods studied in Manuscript I, it can be concluded that traditional equation-based DMI estimation techniques are not suitable for individual animals when other factors, such as feed efficiency, are variable. However, the *n*-alkane method may be suitable as evidenced by the correlation ($p < 0.05$) between DMI_{Alkane} and RFI_{fat} in 2015. This provides further evidence to support literature previously published by Manafiazer et al. (2015) and Johnson (2014). The fNIRS and $fNIRS_{C31C32}$ methods require further investigation, including more robust calibration equations, as these were not found to be suitable for predicting individual animal intake on pasture when using previously-measured RFI rankings as a reference with the exception of the LAC 2015 heifers.

Data presented in Manuscript II indicated that significant differences exist in the *n*-alkane profile between plant parts of swath-grazed triticale. As this is the first known attempt to characterize the *n*-alkane profile of triticale plant parts, this may be useful in future Canadian extended grazing studies using any cereal crop. In addition, selective grazing occurred as evidenced by the significant differences between nutrient content of AM and PM triticale samples. Further, using plant part weights as indicators of morphological variation, there was large (> 20% CV) variability in the weight of head and leaf/stem samples. These observations collectively indicate that the use of triticale as a forage in *n*-alkane intake studies may be limited. It was hypothesized that despite differences in *n*-alkane profile between plant parts, grazing management to encourage uniform consumption of plant parts would allow for prediction of intake using the *n*-alkane method. However, sufficient grazing pressure to ensure consistent

residual biomass and therefore plant part consumption may be difficult to achieve. In addition to variability in plant part morphology, inclement weather including excess precipitation created additional challenges when estimating residual biomass, restricted swath intake due to the inability of animals to access all forage material under snow or mud, and caused contamination of forage via trampling. Therefore, these challenges associated with winter grazing of swathed material may limit the use of the *n*-alkane method for prediction of individual animal intake.

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