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VALIDATING LABORATORY TECHNIQUES THAT INFLUENCE ESTIMATES OF INTAKE AND PERFORMANCE IN BEEF CATTLE

Hannah C. Hamilton
University of Nebraska-Lincoln, hhamilton6@unl.edu

VALIDATING LABORATORY TECHNIQUES THAT INFLUENCE ESTIMATES OF INTAKE AND PERFORMANCE IN BEEF CATTLE

by

Hannah C. Hamilton

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VALIDATING LABORATORY TECHNIQUES THAT INFLUENCE ESTIMATES OF INTAKE AND PERFORMANCE IN BEEF CATTLE

Hannah C. Hamilton, M.S.

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Advisor: James C. MacDonald

Two experiments were conducted to evaluate effects of inoculum source on *in vitro* and *in situ* digestion procedures performed on grass hay and corn residue samples. Steers were fed 70% brome or 70% corn residue. Inoculum from each steer was used to perform *in vitro* procedures to determine IVDMD, organic matter digestibility (OMD), and neutral detergent fiber (NDF) digestibility and for *in situ* procedures to determine NDF digestibility. There were no interactions for inoculum source and IVDMD, OMD, or NDF digestibility.

Three cattle digestion studies were used to evaluate the relationship between TDN and digested OM (DIGOM). Total tract collection and OM analysis of feed and feces were used to determine digested OM. Gross energy of feed and feces was determined using bomb calorimetry and used to calculate TDN. The difference between TDN and DIGOM was least (3.58 percentage units) for traditional corn diets. However, the difference between TDN and DIGOM was greater (9.96 percentage units) for diets containing wet distillers grains.

n-Alkanes and long-chain alcohols were used as markers to delineate the parts of the corn plant and, separately, 8 western rangeland grasses and legumes. The corn plant parts were easily delineated with over 98% of the variation between variables described

within a 2-dimensional plane with visible separation. The PCA for the 8 species of the western rangeland had less distinctive separation with only 90.5 or 93.2% of the differences described 2-dimensionally, depending on the growth stage.

Plant waxes were utilized to predict dietary intake of 26 heifers that were individually fed a ration of 70% corn silage and 30% alfalfa with a daily dose of an internal marker. Predicted values of intake overestimated actual intakes, but improved if the diet was assumed to be a total mixed ration. A sensitivity test was conducted to examine the effects of incomplete dose consumption. Predictions were improved when accounting for losses in the amount of internal marker eaten, which likely occurred in practice.

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Introduction

Within the beef and dairy industry, it has become increasingly important to evaluate feed intake and the factors that affect an animal's intake. Factors that influence intake, and ultimately feed efficiency, include factors such as animal variation due to size, age, breed, production level, environmental factors, and growth hormones (NRC, 2000). For example, when fed the same forage, a steer that weighs 1,200 pounds will consume more each day when compared to a lighter steer that only weighs 900 pounds due to differences in rumen volume. Furthermore, if a cow is lactating it will consume more feed than a cow that is not lactating because of the difference in energy demands (Stricklin et al., 1976). Other factors such as selectivity and nutritional value of available forage can also impact total intake. Knowing the energy content of a feed source can allow for a better prediction of how much an animal can eat. For example, as forage quality increases, shown by an increase in TDN content, the amount that an animal can eat also increases. Differences in quality affect digestibility and passage rate. This can also be seen with an increase in leaf to stem ratio. Lower quality forage contains more stem which includes more cell wall contents which is not as easily digested. There is also an increase in lignin which cannot be digested by rumen microbes (Van Soest et al. 1978).

Feed intake can be a powerful tool to predict animal performance. Having a better grasp on intake allows for better management practices and increase efficiency and profitability. According to Arthur et al. (2001) 55 to 75% of the total costs associated with beef cattle production are feed costs. Additionally, a 5% improvement in feed

efficiency could have four times the economic impact than that of a 5% increase in average daily gain (Basarab et al., 2002). With this economic advantage, research has been aimed at improving efficiency of feed and forage use with the focus primarily placed on reducing input costs. This review will focus on behaviors of grazing animals and how different factors affect intake.

Chapter I: Review of Literature

Animal Selectivity

Grazing is considered the act of searching for forage, selecting forages, and then biting or eating the selected forage. Ruminants spend a significant amount of time, 4-14 h in a 24 h period, sampling and looking for high quality forages that meet energetic and nutritional needs (Stricklin et al. 1976). They are able to selectively eat or bite off certain parts of a plant, thus consuming more digestible and energetically efficient choices.

Unlike some other ruminants, cattle have a relatively inflexible upper lip leading to a decrease in selectivity when compared to other ruminants such as sheep or goats. However, this can be counteracted by taking smaller bites, spending more time eating, and restricting which forages they consume (Lyons and Machen, 2012). Selection follows a preferential list beginning with the newest, freshest, green growth. If that is not available, they will then begin to move to older green forage, then green stems, dry leaves, and ultimately resort to dry stems (Lyons and Machen, 2012). Forage availability can have a large impact on a ruminant's selectivity.

Wallice de Vries and Daleboudt (1994) looked at grazing behaviors in an environment that was sparse relative to energy density in a two year study with 13 observation periods that utilized both an Agrostis/Festuca and Lolium grassland. Cattle were observed for patch selection where bite counts were taken for different vegetative structures (short, tall, and mature grass). Samples from the selected patches were analyzed for organic matter digestibility to measure energy content. Steers selected the

short and tall patches over the mature patches despite the relatively low intake rate of digestible organic matter in the short patches. They found that digestible organic matter intake rate gave the poorest prediction of selection. However, matching for digestibility gave the best explanation of selection. Wallice de Vries and Daleboudt (1994) determined that selectivity appeared to be controlled by the cost of searching for and discriminating between different forage types.

Ginane and D'Hour (2003) offered heifers the choice between a tall abundant reproductive sward (RS), and a short (8cm) or tall (14cm) vegetative sward (VS). They wanted to assess the trade-off in preference between RS and VS as diet quality and accessibility of the alternative decreased. There were six heifers per treatment placed in experimental conditions allowing for different dietary choices for 10-day periods. Feeding choices were recorded for 2 days per period from dawn to dusk. Fecal samples were collected the last 5 days of the period to estimate total intake and diet quality from nitrogen and chromic oxide contents in feces. As expected, the authors observed preference increase for VS as RS matured for both short and tall treatments. Decreased preference for VS depended on RS maturity. Even though potential DM and digestible organic matter intake rates remained higher on RS, they expressed strong preference for the VS. Increased grazing time and biting rate allowed heifers to maintain both total intake and diet digestibility (Ginane and D'Hour, 2003).

Forage availability accounts for the amount of certain parts of the plant material that a grazing animal will choose to eat. When there is ample amount of high quality

forage, less time is spent grazing. In contrast, when there is a sparse amount of low quality forage, more time is taken for grazing. This can also be affected by stocking rate. Increasing stocking rate cause cattle to graze longer and have less available forage per animal (Stricklin et al., 1976). Knowing the amount and quality of forage available becomes crucial when managing a pasture system.

Estimation of Intake

Ruminant animals consume a diet that differs not only in plant species, but also in plant parts, which all possess different nutrient content. To accurately estimate nutrient intake, both diet composition and an estimate of total intake must be quantified. Estimating intake can be difficult, so the relationship between intake, digestibility of the whole diet, and fecal output is used to make predictions. This ratio takes into account that intake equals the amount of fecal output, divided by one minus the digestibility of the whole diet (Dove and Mayes, 2005). However, attempting total fecal collection in a grazing setting can be a large amount of work and possibly disturb the natural grazing patterns of the animals. Thus, estimating fecal output using an external marker can be more successful.

Often there are large errors associated with determining intake and diet composition. Indoor testing while feeding a known amount, often provides validation. Many external markers, such as chromium oxide, have been assessed previously, but none have been found to be ideal (Kotb and Luckey, 1972).

One previous method uses quantification of microscopic plant material fragments collected from esophageal-fistulated animals, stomach contents, digesta or feces (Coates et al., 1987). These samples are then used to describe identifiable fragments within the digesta and feces coming from each plant species. However, this technique has some limitations. When samples are collected, they are generally collected over just a few minutes. But, the animals are often grazing for days or weeks. The samples taken may not be accurately representing the entire grazing period of that animal. Repeated collections at different times give a more representative sample. Mayes and Dove (2000) suggest that there may be an inherent difference with a fistulated animal that has been surgically altered, thus creating different choices while grazing. Samples might also differ from material actually ingested as it is altered by chewing, saliva, or quick bypass past the site of collection. There have been advancements in this method with an introduction of a remote control device produced by Raats and Clarke (1996). This allows for collections to occur throughout the day without disturbing normal grazing behavior (Raats and Clarke, 1996). Even with frequent sampling, the sample itself can be troublesome. When coming from a large sample, often times fragments cannot be identified as coming from a certain plant species which hinders the ability to accurately estimate quantitative diet composition. With that said, this method can still be useful for identifying the presence or absence of a certain plant species or plant part in the diet.

Corn Residue Grazing

Understanding the utility of corn residue is important. More than 90 million acres of land are planted to corn with the largest portion of the crop grown in the central

states like Iowa, Illinois, Indiana, Nebraska, and Ohio (Capehart, 2015). There has been a large shift from grazing land being converted to acres of corn. This has left a shortage of grass but opened a large opportunity to utilize another type of forage. Corn residue grazing can offer a sustainable, efficient grazing system. Grazing residue also allows farmer's to utilize increased flexibility for fall and winter pasture, which helps reduce overall feed costs. One acre of corn residue is enough to sustain a 1,000 pound cow or equivalent animal for 1.5 to 2 months (Samples and McCutcheon, 2002). To touch on previous topics, corn residue grazing will be looked at as it pertains to changes in availability, nutritive value or quality, and the selection of different parts of the corn plant.

Residue Plant Parts. Almost 40 percent of the total corn plant, in weight, is left as residue after harvest. This equates to a yield of 6,000 pounds of residue if we assume 120 bushels of corn. This doesn't include the more than 150 pounds of corn per acre missed during harvest (Samples and McCutcheon, 2012). Corn residue can be broken down into the respective parts of the plant. Understanding the different nutritive values that each part possesses is important. Because the profiles of grazing fields are changing constantly, cattle are continually changing their preferences based on availability. Traditionally, corn residue has been viewed as a low quality forage source due to its relatively low digestibility. Parts of the corn residue have different digestibility values, with stalk and cob being the least digestible, but composing the largest portion (60.11%) of the plant (Watson et al., 2015). When compared on a dry matter basis there is significantly more stalk out in the field. Stalk makes up over 50% of the residue on a dry

matter basis. Both leaf and cob compose about 20% of residue, with a combined 40% on a dry matter basis. Husk comes in last with only 10% of the residue on a dry matter basis (Samples and McCutcheon, 2012). Others have reported a large range in quality of the different parts with leaf and husk having the highest IVDMD and ranging in NDF content from 90% NDF in the cob to 70% NDF in the husk (Fernandez-Rivera and Klopfenstein, 1989; Gutierrez-Ornelas and Klopfenstein, 1991; McGee et al., 2013).

This range in digestibility highly influences the forage quality of residue based on the percentage of these parts available for the animal to consume. An improvement in feed efficiency was found when corn residue was harvested using a John Deer 569 round baler with the Hillco single pass round bale system, compared to conventional harvesting methods (Updike et al., 2015). Further, feed efficiency was improved by harvesting 2 rows of stalks plus tailings using the Cornrower system compared to raked and baled stalks (King et al., 2016). This improvement in feed efficiency is attributed to a higher ratio of leaf and husk to cob and stalk in the residue.

Nutritive Value of Corn Residue Traditionally corn residue has been viewed as a low quality forage source due to its low digestibility. Residue is not considered ideal for growing animals or maintaining milk producing females year round without some form of supplement (Watson et al., 2015). Total digestible nutrient value was found to be 65.85%, with a crude protein level of 6.5%. Corn residue has a neutral detergent fiber (NDF) content of 65%, calcium content of 0.62%, and a phosphorus content of 0.09%, all on a dry matter basis (NRC, 1996). Parts of the corn residue have different *in vitro* dry

matter digestibility values (IVDMD), with stalk and cob being the least digestible (51 and 60% respectively; Watson et al., 2015). The leaf and husk are the most digestible parts with 48.3, and 60.9% respectively. Crude protein values follow a similar pattern with leaf at 5.9%, husk at 4.1%, cob at 2.5% and stalk with 4.8% crude protein (Watson et al., 2015).

Gutierrez-Omelas et al. (1991) demonstrated that more than ninety percent of grain left in the field disappeared, despite the time of the grazing season. Other plant parts had different disappearing rates. After only 36 days of grazing, 88.4% of the grain was gone. However, only 33.4% of leaf blades and 39.7% of the husks disappeared after day 36. It can be assumed that cattle are selecting the more digestible plant parts first and then moving on to the less digestible parts when availability becomes more limited.

The Development of Plant Wax as Markers

Plants contain a complex mixture of aliphatic lipid compounds on their external surface. These compounds form a cuticular or epicuticular wax that is seen to greatly differ among plant species and even among plant parts (Dove and Mayes, 2005). The highest concentrations are generally located or measured in the leaf and flower partitions of the plant (Dove and Mayes, 1991). Many components make up this wax including *n*-alkanes, monoesters, primary alcohols, long-chain fatty acids, secondary alcohols, ketones, and β-diketones, all with differing concentration levels. Most research is focused on the use of *n*-alkanes to predict grazing intake. The relative ease of analysis and

inertness are a few reasons *n*-alkanes are used more than other compounds within the wax to predict intake (Dove and Mayes, 2005).

Oro et al. (1965) found a high correlation between the pattern of *n*-alkanes extracted from cattle feces and the pattern of *n*-alkanes from the diet consumed. They could definitively distinguish cattle selecting the leaf fraction of their diet when they looked at the corresponding leaf alkane patterns (Oro, 1965). Shortly after, Body and Hansen (1978), compared concentrations of other cuticular wax components in perennial ryegrass and sheep feces. Grace and Body (1981) demonstrated that the cuticular long-chain fatty acids could be recovered from feces and quantified. This study confirmed the use of plant wax components as indigestible internal markers.

From this work Mayes and Lamb (1984) began evaluating *n*-alkanes as a possible marker to estimate digestibility. These long chain hydrocarbons are found in vascular plants with chain lengths ranging from 21 to 37 carbon atoms. Over 90% of *n*-alkanes possess an odd numbered chain. The most dominant chains in pasture species are C29, C31, and C33 (Dove and Mayes, 2005). However, Mayes et al. (1986) found that the recovery of these compounds in feces was not complete. To combat this, it was proposed that animals could be dosed with a synthetic, even-chain *n*-alkane as external markers for the estimation of fecal output. Thus, the plant alkane is working as an internal marker to provide an estimate of digestibility, while the dosed *n*-alkane acts as the external marker to relate fecal output.

Using n-alkanes to Estimate Intake

To estimation intake using plant-waxes the plant odd-chain *n*-alkane (*i*) and dosed even *n*-alkane (*j*), intake (*I*), plant *n*-alkane concentrations (H_i and H_j), fecal concentrations (F_i and F_j), fecal recoveries (R_i and R_j), and the dose rate of alkane (*j*) must be taken into account. This is used in the following equation (Dov and Mayes, 1991):

$$\frac{Dose\ ratej}{\left(\frac{Fj \times Ri}{Fi \times Rj}\right) \times (Hi - plant\ contentj)}$$

Evaluating the ratio between fecal *n*-alkane concentrations and not their actual values is important. In contrast to other methods with separate internal and external markers to estimate digestibility and fecal output separately, an unbiased estimate of intake can be obtained if the fecal recoveries and the dosed alkanes are equal (Dove and Mayes, 2005). Less important, is if plant contains the dosed alkane as the concentration is accounted for in the denominator of the prediction equation (Dove and Mayes, 2005).

Using these ratios it is possible to use adjacent longer chain alkanes from fecal recovery to predict intake (Dove et al., 1996). For example, intake can be predicted accurately for C31 or C33 alkane by dosing C32 alkane, as they are very similar. It is assumed that recoveries are the same for plant and dosed alkanes and therefore is not accounted for when predicting intake (Dove et al., 1996).

Alkanes vs. Other Intake Techniques. Alkanes have the opportunity to allow for individual intake estimates when compared with other techniques because it

accommodates the digestibility of the diet in individual animals (Dove and Mayes, 2005). Along with an even-chain dose to allow for intake estimation, a dosed external marker, with a 95% fecal recovery, can also be used for the estimation of fecal output and whole-diet digestibility (Dove and Mayes, 2005). Alkane estimation is also effective in estimating diet composition and can even delineate individual plant species and plant parts. Less error and bias is achieved because the plant, fecal, and external marker alkane concentrations can then be determined at the same time by the same analytical procedure (Dove and Mayes, 2005).

Fiber Content

For a long period of time crude fiber (CF) analysis was a standard to determine the amount of fiber within a forage source (Preston, 2014). However, this method is a concern because of the test's inability to account for digested carbohydrates in feeds. This can be attributed to the variable amounts of an indigestible fraction called lignin, which is removed during the crude fiber procedure. So, acid detergent fiber (ADF) and NDF were developed to help improve this analytical procedure. Feed digestibility is related with ADF, and NDF is related partially to voluntary intake and availability of net energy (Preston, 2014). Both of these measurements more closely relate to predicted animal performance and tend to have more impact than CF. A forage based diet is often fed to beef cattle. Because forage, grass or residue, plays a major role in most cattle diets, knowing the energy value of forages is critical when predicting performance. *In vitro* and in situ procedures are used to estimate *in vivo* values as they are more cost and time efficient. These two procedures are traditionally carried out using an inoculum retrieved

from a donor on a 30% concentrate diet to evaluate feed (Vanzant et al., 1998). However, dietary components can influence the ruminal microbial population and potentially affect the rate or extent of digestion (Varvikko and Lindberg, 1985; Nocek and Russel, 1988).

Because of the importance of determining *in vivo* digestibility of grazed forages, the use of a calibration forage sample set could be useful in adjusting *in vitro* digestibility estimates of forage samples to *in vivo* digestibility (Giesert, 2007; Walker, 2014). McLeod and Minson (2006) suggested that *in vitro* data should be adjusted with a standard set of feeds with known *in vivo* digestibility to accurately represent *in vivo* digestibility of those feed samples. Because of the nature of run variability, Stalker et al. (2013) proposed regression equations that can be used to adjust the data to *in vivo* digestibility values, which allows for comparisons among separate *in vitro* runs.

When working with both grass and residue samples it is essential to know whether multiple donor diets are necessary to get accurate digestibility estimates. An interaction of forage type and inoculum source may indicate a need to obtain rumen fluid from donors fed the forage being tested. Soder (2005) saw differences in IVDMD values when evaluating different inoculums with a total mixed ration and a pasture diet.

Microbial Effects on Fiber Digestion. A ruminant animal is able to digest fiber due to the vast populations of bacteria, protozoa, and fungi species living symbiotically within the rumen. These microorganisms are able to degrade and ferment carbohydrates in plant cells, which in turn provide volatile fatty acids and protein for the host animal. Ruminal fermentation has a large impact on the metabolic processes of the animal and the

functions of the microorganisms that live within. The environment of the rumen is well adapted for the maintenance of a diverse and large microbial population. The rumen has a relatively constant supply of substrate and water. The temperature is constant and the pH is kept slightly acidic with the capacity of saliva to buffer (Masson and Phillipson, 1951). There is a constant removal of VFAs via passage into the lower digestive tract, and absorption through the rumen wall.

To assess the comparative influences of microbial groups (bacteria, protozoa, and fungi) on the overall process of plant cell wall digestion in the rumen, representatives of these groups were selected by physical and chemical treatments of whole rumen fluid and used to construct an artificial rumen ecosystem (Lee et al., 2000). It was shown that cellulolysis of orchardgrass cell wall differed between organisms at different states of incubation periods. Cellulolysis was greatest ($P < 0.05$) during the early stages of incubation by bacterial populations. However, during the late stages of incubation cellulolysis was greatest by the fungal populations. The protozoa by themselves did not degrade the cell wall material. The overall process of cell wall digestion can be attributed first to fungal populations, followed by bacteria, and lastly protozoa. Fungi have the ability to penetrate deeply into plant tissues and utilize cell wall components of the plant material where bacteria are not able. Lee et al. (2000) suggested that fungal activity could potentially be sufficient to account for all of the observed degradation. However, Cheng et al. (1991) suggested bacteria are responsible for the majority of the feed digestion in the rumen based purely on the numerical predominance and metabolic diversity the bacterial populations have to offer. The primary fibrolytic bacteria in the rumen are

Fibrobacter succinogenes, Ruminococcus flavefaciens and Ruminococcus albus (Cheng et al., 1991). These bacteria have the potential to colonize and degrade fiber due to the array of different species and carbohydrases that are available in the rumen ecosystem (Hungate, 1966). The interaction between bacteria and fiber depends on the microbial type and the plant tissue type (Akin and Rigsby, 1985). Akin and Rigsby (1985) suggested that tissues which are more easily digested, like mesophyll, are degraded by the surrounding bacteria that are not physically attached to the particle. They concluded that this indicates that extracellular enzymes are at work when looking at the degradation of these tissues (Akin and Rigsby, 1985). However, some of the major fiber digesters actually require attachment to the more resistant tissues (Akin and Rigsby, 1985).These tissues that require attachment are pitted, split, or cracked open by the bacteria and either degraded or partially degraded depending on the type of plant and its maturity.

In contrast with the substantial information on predominant rumen fibrolytic bacteria, the role of rumen protozoa on fiber digestion is still controversial. This might be due to the difficulty in the ability to cultivate protozoa routinely in the absence of growing bacteria. Results of biochemical, cultural and microscopic studies show that the contribution of protozoa depends on the complex interactions between the protozoa, bacteria, and diet composition (Jouany, 1996). Dijkstra and Tamminga (1995) evaluated the role of protozoa and bacteria in fiber digestion using a mathematical model of microbial metabolism in the rumen. The model provides a base for which knowledge on protozoal-bacterial interrelationships can be formulated to represent the main factors of protozoal and bacterial metabolism. This model considered uptake of bacteria by

protozoa, selective retention of protozoa in the rumen and microbial substrate preferences.

Large protozoa populations are generally found with diets consisting of equal amounts of roughages and concentrates. In contrast, feeding high levels of high-grain diets reduced protozoa numbers or even eliminated protozoa (Jouany, 1996). The ability of the protozoa to utilize protein, both dietary and microbial, influenced its own biomass in the rumen, in response to changes in dietary nitrogen levels. Predicted protozoal organic matter was slightly reduced when dietary nitrogen levels increased. This led to an increase in fibrolytic bacterial organic matter. In conclusion they showed that, in general, increases in intake level reduced protozoal contribution. Substitution of roughages by concentrates increased the protozoal contribution of NDF degradation in the rumen (Dijkstra and Tamminga, 1995).

Anaerobic fungi degrade un-lignified tissues in plants (Akin, 1989). However, they are more efficient in colonizing and weakening cellulose tissue that has been lignified (Akin and Rigsby, 1985).The fungi are able to degrade the entire sclerenchyma more extensively and readily when compared to bacteria. Rumen fungi produce high levels of cellulases and hemicellulases and are particularly proficient in producing xylanases (Wubah et al., 1993). This allows the fungi to cause splits and fractures in the xylem and lignified vascular tissues (Akin, 1989). In certain cases it is possible for the fungi to penetrate the cuticle. With these fiber-degrading characteristics, rumen fungi allow for modifying the physical barriers to degradation.

Plant Effects on Fiber Digestion

Plant composition is a determining factor when looking at fiber digestibility. The chemical and physical nature of forages can create a barrier that inhibits bacteria from accessing and digesting the available nutrients. Mowat et al. (1969) suggested that as a plant ages, there is a change in plant composition. During the early stage of plant growth, the cell must be able to grow in size. At this stage in development the cell wall is considered the primary wall. The primary wall is capable of elongating because there are no polymers within the cell that are cross-linked (Moore and Hatfield, 1994). The middle lamella is the area between two adjacent cells. The middle lamella is composed mostly of pectin which is highly digestible. It has been observed that legumes contain large amounts of pectin compared to grasses which have relatively low concentrations. The primary cell wall also consists of many polysaccharides. Some of these include cellulose, β-glucans, heteroglucans, heteroxylans and glucuronarabinoxylans (Moore and Hatfield, 1994).

When the plant begins to mature, lignification begins and forms a secondary wall. Cellulose is the major polysaccharide seen in the secondary wall. Lignin starts to be deposited in the middle lamella and the primary wall (Terashima et al., 1993). Lignin is covalently bound to cell wall polysaccharides creating cross-linkages (Ralph et al., 1995). Lignin is one of the key elements that limits cell wall digestibility. Van Soest and Moore (1965) suggested that there was a similar relationship between lignin and cell wall digestibility between grasses and legumes. This was also observed in certain grasses with silica instead of lignin. To determine the difference in lignin content and digestibility

between species and plant parts, Mowat et al. (1969) conducted a study that analyzed fifty six forage samples with varying composition. They showed that there was variability within cell wall constituents with a range of lignin from 3.7 to 19.1%, in cellulose from 12.2 to 39.3%, and in total silica from 0.3 to 9.8%. They also looked at the varying chemical components within different parts of the plant. For example they found that leaves within grasses and legumes had a lower content of all chemical constituents, excluding silica, when compared with stems. As the plant matured, all of the chemical components increased (Mowat et al., 1969).

Along with lignin and silica, phenolic compounds (cinnamic acid, ferulic acid, and vanillin) are thought to inhibit digestion of forages within the rumen (Jung and Allen, 1995). Phenolic acids are toxic to rumen bacteria and protozoa. Vincent and Jung (1986) conducted a study that evaluated the influence of phenolic compounds on forage digestibility and the toxic effects they may have on the rumen organisms. They found that Vanillin may interfere with the attachment of *B. succinogens* to cellulose. This could be due to the phenolic compounds affecting growth habits, septum formations, and cell divisions, resulting in an increased amount of free floating bacteria.

The cuticle of the plant is another factor that should be considered when looking at plant composition. The cuticle is a protective layer on the epidermis that prevents the entry of microbes into the plant tissue to further degrade the other tissues (Monson et al., 1972). Akin and Rigsby (1985) observed no degradation of the cuticle by ruminal microbes in either warm or cool season grasses. Instead, the cuticle remained intact and

allowed binding between the residues of vascular bundles, sclerenchyma, and other non-degraded tissues (Akin and Rigsby, 1985). However, to overcome this, the cuticle can be cracked to allow microbial populations to penetrate into the leaves. Hanna et al. (1978) demonstrated this when they studied various lines of pearl millet and found that the cracking of the cuticle occurred under stress and accounted in part for the improved digestibility in certain varieties of these forages.

Plant composition, anatomy, and structural features are important in forage intake when considering the amount of particle reduction needed by mastication (Ellis, 1978). The physical bulk of less digestible forages and the capacity within the ruminants' gastrointestinal tract are limiting factors when considering forage intake by ruminants (Ellis, 1978). Plant anatomy and structural features that change the shape and physical presence can be important in forage intake. The way these plants are reduced in particle size by mastication is also a very important role within fiber digestion (Akin, 1989). Grass stems could potentially be a major contributor to reduce feed quality due to the extensive lignification throughout the stem which negatively affects intake (Akin, 1989). Legumes may prove to have an advantage over grasses as they require less mastication to reduce particle size (Akin, 1989).

Estimation of intake continues to be a measurement of interest. An estimate of how much an animal will consume is critical when evaluating supplements, rations, stocking rate, or predicting animal performance. Dry matter intake (DMI) is impacted by

many different things including animal weight, condition, stage of production, environmental conditions, forage quality, and amount or type of supplement provided.

Cattle require a daily amount of nutrients including protein, water, vitamins and minerals. The concentration of these nutrients varies in different food sources, therefore dictates how much the animal needs to consume to meet its requirements.

Forage fed cattle are limited by the capacity of the digestive tract. It is rare to have forage digestibility greater than 70% of DM. When you approach this high quality of forage the animal is no longer regulating feed intake by the capacity of its digestive tract. Forage intake is highly correlated with forage quality. The greater the rate of digestion and passage from higher quality forage, the greater the DMI when compared to forage that is lower in digestibility. Feed intake then becomes a physiological or chemostatic mechanism for controlling ad libitum intake when diets are high in digestible energy (Lalman, 2003).

When evaluating a high forage diet, which is “energetically dilute” and less digestible, regulation of intake relates to physical fill (Waldo, 1986). Cell wall concentration of forage diets can be considered one of the best chemical predictors of intake. Gastrointestinal tract fill is also important because it can cause a volume limitation that physically limits intake. The conversion of forage to an animal product can primarily be contributed to dry matter intake, digestibility, and the efficiency of converting digested energy to metabolizable energy and metabolizable energy to net energy in the animal product (Waldo, 1986).

Animal Effects on Fiber Digestion. When cattle ruminate they are regurgitating a bolus of incompletely chewed or digested feed. In order for the microbes to have a greater surface area to attach and digest fiber rapidly and efficiently, plant material must be ground down to a smaller particle size. Welch and Smith (1970) investigated how forages of different chemical composition affect rumination time in cattle. A wide range of forages with different compositions was used over three experiments. The study began with a two day period of fasting where no long forages were fed. This was followed by a single long forage test feed and a re-feeding at day 5. They saw that during the fast, rumination time decreased rapidly, declining to almost zero. Following the test meal, rumination time increased quickly. In experiment one, the meal made of straw produced the greatest total rumination time of 578 minutes following the meal. The high-quality orchardgrass showed a rumination time of 369 minutes. After the first fed meal, rumination returned to almost zero before the next re-feeding. Immediately after re-feeding, a spike in rumination was observed. Again, within 24 hours after feeding, rumination returned to normal. Experiment two had similar results to experiment one, but alfalfa meal pellets were offered during the fasting periods at 2.27 kg per head per day in Exp. 2. The rumination times produced by the single meals of straw were compared to those rumination times produced by good quality mixed second-cutting hay. The straw test meal produced 539 minutes of rumination and the second-cutting hay resulted in 387 minutes ($P = 0.05$). Experiment three was similar to Exp. 1, except that the test meals were either early-cut orchardgrass or a mature orchardgrass. Feeding the late-cut, mature orchardgrass resulted in more rumination time when compared with the rumination time

produced by the early-cut material. Cell wall component in the forages was correlated, with a slope of 0.94, with the amount of rumination time produced by the test meal. Reducing particle sized allowed passage through the gastrointestinal tract, which was important in maintaining intake. As cell wall percentage increased, rumination time per gram of dry matter increased. However, rumination time per unit of cell wall remained the same. Poorer quality forage required more rumination time for particle size reduction. Longer rumination time is needed per gram of dry matter ingested when the forage quality is reduced (Welch and Smith, 1970).

Fiber digestion in ruminants requires investigation into several aspects related to the degradation of the fiber particle. Bacterial concentration can be a major factor in the level of degradation to the fiber particles. More research is needed in each specific microorganism species within the rumen. For example, much debate on whether protozoa have a significant effect on fiber digestion is still continuing today. Although cultures of these protozoa are often difficult to achieve alone, it would be extremely beneficial to find a method that was efficient enough to see their effects within the rumen without the assistance or interference with rumen bacteria and fungi. Observing these interactions may prove, in fact, not be possible as the rumen interconnections between microorganisms play a significant role in digestion. The role of fungi is still cloudy as well, with a basic knowledge that they somehow contribute to fiber digestion. More study to further develop their roles within the rumen would also be beneficial.

All aspects of digestion need to be considered when evaluating fiber digestion. All of these factors allow the ruminant animal to digest these particles. Research needs to continue to investigate as this niche is a substantial part of ruminant nutrition. Further development within this research could allow for an increase in ruminant performance, which becomes extremely useful, both educationally and economically.

Energy

Energy is the potential to do work, which can be measured and defined using a set of standard conditions and therefore can be considered absolute. In other disciplines with electrical, mechanical, and chemical backgrounds it is common for energy to be expressed in joules. However, joules can be converted to ergs, watt-seconds, or calories. Nutritionists have moved toward using purified benzoic acid to standardize combustion calorimeters. This is because benzoic acid has been measured electrically and computed in terms of joules/g mole. The calorie has been measured using the amount of energy it takes to raise one gram of water from 16.5^{o}C to 17.5 ^{o}C at atmospheric pressure, which is equal to 4.184 joules (NRC, 2000).

Gross energy (GE), or heat of combustion, is the energy that is released when an organic substance is burned or oxidized and turned completely into water and carbon dioxide. Thus, GE is related to the substance's chemical composition. However, it does not allow us to understand the availability of that energy for an animal to utilize. This makes GE an easy measurement to make, but gives little value in assessing a particular diet or feed ingredient as an energy source for an animal.

After measuring GE, it can then be used in combination with the subtraction of the energy loss measured in feces to calculate digestible energy (DE). This is measured relatively easy and can reveal information about diet digestibility. However, DE does not fully account for some other energy losses associated with the metabolism and digestion of food. The DE overestimates the value of feedstuffs like hay or straw, which are high in fiber, relative to grain which is highly digestible and low in fiber.

Many studies have been done in finishing and growing diets to show the increased performance response with the use of distillers grains (DGS; Farlin, 1981; DeHaan et al, 1982; Firkins et al., 1985; Larson et al., 1993; Fanning et al., 1999; Trenkle, 1997a; Trenkle, 1997b; Vander Pol et al., 2005). In all instances, the energy supplied by DGS was consistently greater than corn, with 30-40% inclusion of DGS having an average of 24% greater feeding value than that of corn (Klopfenstein et al, 2007).

The Nebraska Corn Board and the University of Nebraska developed a review that showed digestible and metabolizable energy value for corn and DGS using beef cattle. They found that metabolizable energy values were less compared to corn. Additionally, they noticed significant variation for DGS energy values between studies (Nebraska Corn Board, 2005). Stein et al. (2005) reported an ME range for DDGS of 3,058 to 3,738 kcal/kg, and an average of 3,378 kcal/kg. These reported values were significantly greater than previous values reported on distillers grains (NRC, 1988).

Hastad et al. (2004) showed results that indicated DE and ME values (3,800 and 3, 642 kcal/ kg, respectively) estimated from metabolism trials were 6 to 15% greater

than data determined directly from growth performance. These differences in values were attributed to the differences in feed intake between the metabolism and growth studies.

A review of previous work using finishing or growing diets with no byproducts shows a positive correlation between DE and digested OM. The average DE (Mcal/kg) was 3.38 while the average digested OM was 81.2%. This relationship showed a 0.03 slope with a 0.74 intercept and an r^2 value of 0.36, a relatively poor relationship (Zinn, 1989; Zinn, 1990; Zinn and Plasencia, 1993; Zinn, 1994; Zinn et al., 1995; Calderon-Cortes, 1996; Zinn et al., 2000; Carrasco, 2013). However, little work has been done to relate digested OM to TDN in diets containing distillers grains.

Total Digestible Nutrients

Total Digestible Nutrients is related to DE, which allows prediction of animal performance (Rasby and Martin, 2016). By multiplying the %TDN content by 2, DE can be calculated. Additionally, TDN can be converted to DE energy using 1 kg of TDN equal to 4.4 Mcal of DE (Swift, 1957; NRC, 2000). Organic matter digestibility (OMD) is related to TDN. However, the relationship is not established for diets containing byproducts. Traditionally, TDN is based on proximate analysis, which is no longer commonly used. These analyses were also based on diets containing primarily corn, fat, and alfalfa but none containing byproducts. A study done by Olson et al. (2014) explored empirical predictions for DE on tallgrass prairie hay. This study found that GE fell in a narrow range, while intake, NDF, intake of digestible OM, OM digestion, and DE varied widely among the grass samples. It was found that GE content of the grass was a poor indicator of DE (r^2 = 0.39, slope=1.5). Conversely, the prediction of DE from intake of

digestible OM was highly accurate (r^2 = 0.91, slope = 0.061). Organic matter digestion (%) was also highly correlated with DE (r^2 = 0.93, slope = 0.04). In concurrence with Olsen, others have explored the relationship with OM digestion and the estimation of DE (Minson, 1982; Moir, 1961; Rittenhouse et al., 1971).

Often times, forage quality can be interpreted using TDN. An equation calculating TDN uses proximate analysis to sum up digestible crude protein (DCP), digestible crude fiber (DCF), digestible nitrogen-free extract (DNFE), and 2.25 times (due to crude fat being 2.25 times the energy density of carbohydrates) digestible ether extract (DEE; Rasby and Martin, 2016). Calculated TDN values tend to under predict the feeding value of concentrates relative to forage (Rasby and Martin, 2016). This is most likely due to the nature of error originating from proximate analysis assumptions. For example, DEE does not take into consideration that some lipid are low in energy or not digestible such as waxes, pigments, fat-soluble vitamins, and sterols. The DCP fraction is determined by Kjeldahl analysis, which does not account for nitrates or any fiber bound or heat damaged proteins. Kjeldahl also assumes all nitrogen in the food comes from true protein. However, Jones (1931) showed that all amino acids, and in turn proteins, are not all of equal value. Additionally the DCF fraction assumes all fiber to have the same energy content. Van Soest and Robertson (1977) showed that 82% of feed lignin was recovered in the DCF fraction for grasses compared to 21% of feed cellulose. Lastly, the DNFE fraction is not directly measured, but calculated by difference and causes any error to be compounded. Because of these limitations many have moved to modern analytical procedures.

Conclusions

Further research into what animals choose to graze will help answer the very broad and important question of intake. There are techniques such as *n*-alkane markers that can help us try to predict choices. By creating plant profiles using *n*-alkanes, improvement can be made in determining the composition of a particular diet an animal chooses to eat. As an outcome better inferences can be made about the quality and amounts of plants that are being chosen.

Improving lab techniques to assess these different types of forages is also extremely important. It is essential to have accurate estimates of feedstuff values such as energy or NDF content. Having reliable estimates allows for a more accurate model to base inferences about performance and other characteristics of a particular diet or ingredient. The inclusion of lab standards to adjust values to known *in vivo*

values is critical when assessing feedstuff with *in vitro* and *in situ* procedures.

Understanding the relationship between different measures of energy will be instrumental in estimating the value of a feedstuff. Relating measurable energy inputs and outputs to traditional TDN values may prove to be problematic with the inclusion of byproducts in cattle diets. Therefore, investigating these relationships to understand the more complex association between measures such as digested organic matter and TDN will remain essential.

With the improvement of these techniques movement toward precision and accuracy of predicting intake increases. Predicting DMI is not an exact science. However, understanding the factors that can affect DMI can improve the usefulness of predictions. Advancing techniques to accurately predict intake is something that is going to be key when making prediction about animal performance.

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Chapter II: Effects of Inoculum Source on *in vitro* and *in situ* Digestion Procedures Performed on Corn Residue Samples

H. C. Hamilton[*], J. L. Gramkow[*], J. C. MacDonald[*], G. E. Erickson*, T. J. Klopfenstein[*]

[*]Department of Animal Science, University of Nebraska-Lincoln Lincoln, NE 68583

Abstract

In vitro and *in situ* procedures are traditionally carried out using inoculum retrieved from a donor on a 30% concentrate diet. Diet of the donor (inoculum source) may impact digestibility estimates. Therefore, two studies were conducted to assess effects of donor diets on *in vitro* organic matter digestibility (IVOMD), and NDF digestibility. In Exp.1, four ruminally cannulated crossbred steers (725 kg BW) were fed a mixed diet of 70% bromegrass hay and 30% dry distillers grains plus solubles (DDGS) or a corn residue diet with 70% corn stalks and 30% Sweet Bran. Exp. 2 used 6 ruminally cannulated crossbred steers (286 kg BW) and also compared inoculum source from steers fed 70% bromegrass hay or corn residue, but both diets contained 30% Sweet Bran. Five grass and 9 residue samples (forage type) were incubated in each inoculum from each individual steer to test for an interaction of inoculum source and forage type. Exp. 1 contained 3 runs (n = 6) and Exp. 2 was a crossover design with two periods and two runs per period. Steer inoculum source within run was the experimental unit (n = 12). In Exp. 1 and Exp. 2 no 3-way interaction (inoculum x forage x time) was observed for NDF digestibility, *in vitro* or *in situ* ($P > 0.85$) incubated at 24 or 48 h. There were no interactions for Exp. 1 and Exp. 2 on NDF digestibility for incubation time (24 or 48 h) by forage type ($P = 0.79$; $P = 0.19$) or inoculum source by forage type ($P = 0.99$; $P = 0.34$). Exp. 1 demonstrated a tendency for an interaction for inoculum source by incubation time ($P = 0.11$) where forage samples inoculated with rumen fluid from steers fed corn residue had greater NDF digestibility at 48 h ($P = 0.03$), but not at 24 h ($P = 0.90$). Similarly, Exp. 2 showed an interaction for inoculum source by incubation time (*P*

<0.01) where NDF digestibility was greatest at 72 h for forages incubated in inoculum from steers fed a residue diet. The NDF digestibility was consistently greater for all forage types when the inoculum was from steers fed a residue diet. There was no effect of inoculum source on IVDMD (P = 0.25) or IVOMD (P = 0.41). Maintaining two sets of donors for *in vitro* or *in situ* procedures involving corn residue is not necessary. However, donor diet affects NDF digestibility estimates of residue samples. Therefore, when assessing energy values using *in situ* or *in vitro* techniques, a set of standards with established *in vivo* digestibility values should be used for adjustment.

Key words: corn residue, *in situ*, *in vitro*, neutral detergent fiber digestibility

Introduction

Increased corn production has increased the availability of corn residue to be used as a feed source for cattle. Traditionally, corn residue has been viewed as a low quality forage source due to its relatively low digestibility. Parts of the corn residue have different digestibility values, with stalk and cob being the least digestible, but composing the largest portion (60.11%) of the plant (Watson, 2015). Others have reported a large range in quality of the different parts with leaf and husk having the highest IVDMD and varying NDF content (Fernandez-Rivera and Klopfenstein, 1989; Gutierrez-Ornelas and Klopfenstein, 1991; McGee et al., 2013).

While there is a large shift to grazing and feeding corn residue, grazing traditional grasses still remains important. Forage makes up 80% of the feed inputs into most beef production systems (Klopfenstein, 2001). Because forage plays a major role in cattle diets, knowing the energy value of forages is critical when estimating animal performance. *In vitro* and *in situ* procedures are used to estimate *in vivo* values as they are more cost and time efficient than *in vivo* digestibility. These two procedures are traditionally carried out using an inoculum retrieved from a donor on a 30% concentrate diet to evaluate feed (Vanzant, 1998). However, dietary components can influence the ruminal microbial population and potentially affect the rate or extent of digestion (Varvikko and Lindberg, 1985; Nocek, 1988).

Because of the importance of determining *in vivo* digestibility of grazed forages the use of a calibration forage sample set could be useful in adjusting *in vitro* digestibility estimates of forage samples to *in vivo* digestibility (Giesert, 2007; Walker, 2014).

McLeod and Minson (2006) suggested that *in vitro* data should be adjusted with a standard set of feeds with known *in vivo* digestibility to accurately represent *in vivo* digestibility of those feed samples. Because of the nature of run variability, Stalker (2013) proposed regression equations that can be used to adjust the data to *in vivo* digestibility values, which allows for comparisons among separate *in vitro* runs.

When working with both grass and residue samples knowing whether multiple donor diets are necessary to get accurate digestibility estimates is essential. An interaction of forage type and inoculum source may indicate a need to obtain rumen fluid from donors fed the same forage being tested. Therefore, the objective of this study was to evaluate the effects of different donor diets on *in vitro* and *in situ* digestibility estimates (IVDMD, IVOMD, and NDF digestibility) to determine if two sets of donor steers would need to be routinely maintained for these procedures.

Materials and Methods

Two *in vitro* and *in situ* digestibility experiments were conducted at the University of Nebraska. Animal use procedures were reviewed and approved by the University of Nebraska Institutional Animal Care and Use Committee.

Experiment 1

Four ruminally cannulated crossbred steers (275 kg BW) were utilized to compare two forage diets to provide inoculum to incubate corn residue and grass samples *in vitro* and *in situ*. The first was a mixed diet consisting of 70% bromegrass hay and 30% dry distillers grains plus solubes (DDGS) and the second was a high corn residue diet with 70% conventionally baled stalks and 30% Sweet Bran. Each diet was fed to two steers.

Both diets contained a supplement with 0.14% salt, 0.86% trace mineral, and 0.09% vitamin ADE.

Corn residue samples were selected to represent variation in the proportion of stem, cob, leaf, and husk which included 2-row, 4-row, 6-row, 8-row, conventional bale, leaf, husk, stalk and cob. Selected samples differed in overall quality (Table 2.1). A New Holland Cornrower Corn Head was used to obtain bales with 2, 4, 6, and 8 rows as described by Updike (2015). The Cornrower head uses an attachment to cut the stems and blow them into a windrow between the wheels of the combine. The straw spreader is disengaged, allowing for the exiting residue to fall onto the windrow of stalks. The number of rows being cut at once can be adjusted from 2 to 8. The residue exiting the combine includes all of the cobs, a majority of the husks, some leaves, and some of the upper 1 / 3 portion of the stems. The 8 row bale includes all of the stalk material, thus, may be equivalent to conventionally baled stalks. An improvement in feed efficiency was found when corn residue was harvested using a John Deer 569 round baler with the Hillco single pass round bale system, compared to conventional harvesting methods (Updike et al., 2015). Further, feed efficiency was improved by harvesting 2 rows of stalks plus tailings using the Cornrower system compared to raked and baled stalks (King et. al., 2016). This improvement in feed efficiency is attributed to a higher ratio of leaf and husk to cob and stalk in the residue. Conventionally baled stalks from another single field were also used.

Cob, stalk, husk and leaf were taken from a 40 ha irrigated corn field located at the Agriculture Research and Development Center located near Mead, NE (McGee,

2013). Ears and leaf blade were removed on site prior to transport to prevent loss. Stalks were cut at the top of the crown roots and bundled. Leaves and stalks were stored to air dry in an open air barn. Ears were husked and separated. Samples were bagged and left open inside a climate controlled building to allow the plant parts to dry. Stalk, cob, and leaf samples were all chopped using the Ohio Mill, and further ground through a Wiley Mill using 1mm and 2mm screens (McGee, 2013). Samples were then composited by plant part.

Five chopped hays, as described by Giesert (2007), with known *in vivo* NDF digestibility values were used as non-corn residue samples to compare effects of inoculum with other forage types. The hays were immature smooth bromegrass (good brome hay), mature smooth bromegrass (poor brome hay), low quality brome or immature meadow hay (meadow hay), mature brome hay used in an individual barn feeding system (mature brome hay), and prairie grass hay (prairie hay).The prairie hay consisted of a mixture of warm and cool season grass species (Table 2.1).

All grass and residue samples were ground through Wiley Mill using a 1 mm screen for *in vitro* and 2mm screen for *in situ* (Thomas Scientific, Swedesboro, NJ). The Tilley and Terry (1963) *in vitro* method was followed with the following modifications. Inoculum for *in vitro* NDF digestibility was obtained by collecting whole rumen contents from each steer, with two steers per treatment, strained through 4 layers of cheesecloth. Rumen fluid from each steer was prepared separately so that steer within run was the experiment unit. Each of the strained ruminal fluid samples were then mixed with McDougall's buffer (1:1 ratio; Weiss 1994) containing 1 g urea / L. Residue and non-

residue samples of 0.5 g were weighed into a 100 mL tube where 50 mL of one of the four inoculum, was added to each tube. All samples were tested with each inoculum from each individual steer, to determine the effects of each diet for each sample. Test tubes were placed in a water bath at 39°C and incubated for 24 or 48 hours. Fermentation was ended by removing tubes from the water bath and placing them in the freezer immediately. The runs were performed at one week intervals, beginning one week after donor steers were offered their respective diets. Tubes were later thawed in a 39°C for 10 minutes and evaluated for NDF content to estimate NDF digestibility. Tubes were poured into a 600 mL beaker and rinsed with NDF solution added up to 150 mL total volume. The solution was brought to a boil on a hot plate and allowed to reflux for one hour. The beaker content was then filtered through Whatman 541 filter paper, rinsed with distilled water, and dried in a 100° C oven for 6 h (Van Soest, 1991). This process was repeated in 3 runs, where steer within run was the experimental unit (n = 6). Three *in vitro* tubes per experimental unit were averaged for digestibility estimates.

The NDF digestibility of the corn residue samples was also determined utilizing *in situ* rumen incubation. Residue samples were weighed (1.25 g) into small (5 x 10 cm) nylon bags (Ankom Technology Corp., Macedon, NY). Bags were sealed three times with an Ankom heat sealer (Ankom Technology Corp. Macedon, NY). Three bags of 9 samples were placed in the rumen of each of the four steers, with two steers per treatment and 81 bags per steer separated into 3 time points (n = 4). This entire process was repeated with 2 runs performed at 48 hour intervals to complete Exp. 1. Individual bags were placed in mesh zipper bags fitted with weights and incubated for 28 h, 36 h, and 48

h. After incubation, bags were washed in a washing machine using a 1 min agitation and 2 min spin cycle, and repeated 5 times (Haugen et al., 2006). They were then rinsed with distilled water and stored in the freezer. Determination of NDF on the remaining residue was done using the Ankom Fiber Analyzer (Ankom Technology Corp. Macedon, NY).

All data were analyzed using the MIXED procedures of SAS (SAS Inst., Inc., Cary, N.C.). The effects of run, inoculum source, incubation time, and forage type were included in the model. Diet by time and diet by time by sample interactions were also tested. Differences were considered significant at $P < 0.05$.

Experiment 2

Six ruminally canulated crossbred steers (286 kg BW) were fed either a mixed diet consisting of 70% brome grass hay and 30% Sweet Bran or a high corn residue diet with 70% conventionally baled stalks ground through a 3 inch screen and 30% Sweet Bran. Both diets contained a supplement with 0.14% salt, 0.86% trace mineral, and 0.09% vitamin ADE. Experiment 2 consisted of two periods in a crossover design with two runs per period. Periods were 4 weeks long with a 2 week adaptation and a 2 week collection. One *in vitro* run and one *in situ* run were done each week of collection consistent with suggested procedures outlined by Vanzant et al. (1998). Ten samples were analyzed.

Residue samples were the same as Exp.1 for 2-row, 8-row and conventional bale samples. Additional samples of husk and husklage were also used. Husks were obtained from Hoegemeyer Seed. Husks were sifted through a 3 foot by 5 foot metal screen by hand to remove any remaining corn. The husklage was produced with the use of a John

Deere 569 round baler that was modified with the Hillco single pass round bale system as described by Updike (2016). Similar to Exp. 1, grass samples consisted of good brome, poor brome, prairie hay, meadow hay, and mature brome. These samples were chosen because they have been evaluated *in vivo.*

All grass and residue samples were ground through a CT 193 Cyclotec™ Sample Mill (Foss, Hillerød, Denmark) using a 2-mm screen for *in vitro* and a Wiley Mill using a 2-mm screen for *in situ*. Samples were tested for IVDMD and *in vitro* organic matter digestibility (IVOMD) using the *in vitro* methods described in Exp. 1. However for Exp. 2, fermentation was ended by adding 5 mL of 20% hydrochloric acid and 3 mL of 5% pepsin. Tubes were then incubated for an additional 24 hand then frozen immediately. Tube contents were filtered through Whatman 541 filter paper, rinsed with distilled water, and dried in a 100 °C oven for six hours to determine IVDMD. Filters were then placed in ceramic crucibles and allowed to ash for 6 h at 600 ºC in a muffle furnace to determine IVOMD. This process was repeated in 2 runs for each period, and steer inoculum source within run was the experimental unit (n = 12). Three *in vitro* tubes per experimental unit were averaged for digestibility estimates.

The NDF digestibility of samples was also determined utilizing *in situ* rumen incubation. Residue samples were weighed (1.25 g) into small (5 x 10 cm) nylon bags (Ankom Technology Corp., Macedon, NY). Bags were sealed three times with an Ankom heat sealer (Ankom Technology Corp. Macedon, NY). Three bags of each sample were placed in the rumen of each of the 6 steers, with 3 steers per treatment and 120 bags per steer separated into 4 time points. Individual bags were placed in mesh zipper bags fitted

with weights and incubated for 36 h, 48 h, 60 h, and 72 h. After the incubation period, bags were pulled from the animal and placed in a washing machine where they were agitated with water in a washing machine for 1 min and spun for 1 min for 5 cycles. They were then rinsed with distilled water and stored in the freezer. The Ankom Fiber Analyzer (Ankom Technology Corp. Macedon, NY) was used to analyze NDF of the remaining residue. This process was repeated in 2 runs a week apart for each period.

All data were analyzed using the MIXED procedures of SAS (SAS Inst., Inc., Cary, N.C.). This experiment used a crossover design with 2 periods and 2 runs per period. The experimental unit was steer within run. The effects of run, inoculum source, incubation time, and forage type were included in the model. Inoculum source by incubation time and inoculum source by incubation time by forage type interactions were also tested. Differences were considered significant at $P < 0.05$.

Results

Experiment 1: In vitro

No 3-way interaction was observed for incubation time by forage type by inoculum source ($P = 0.99$) for Exp. 1 (Table 2.2). There were no interactions for forage type by inoculum source ($P = 0.99$; Table 2.3). There was a tendency for an interaction for inoculum source by incubation time ($P = 0.11$) where inoculum source significantly ($P = 0.03$) affected NDF digestibility at 48 h, but not at 24 h (Table 2.4). Inoculum source was significantly different with samples having a greater NDF digestibility when incubated in inoculum from a steer fed a residue diet when compared to that of a brome

diet (Table 2.5). There was an effect of run ($P < 0.01$), and an effect of time ($P < 0.01$) illustrating that runs are variable (data not shown). Run 1, 2, and 3 had average NDF digestibility values of 41.1%, 48.3%, and 42.7% across both inoculum sources and both forage types.

Experiment 1: In situ

No 3-way interaction was observed for incubation time by forage type by inoculum source ($P = 0.99$; Table 2.6). There was a main effect ($P < 0.01$) of inoculum source for *in situ* NDF digestibility where samples incubated in an animal consuming a residue diet had greater NDF digestibility than samples incubated in an animal consuming a grass diet. There was a significant interaction ($P = 0.01$) between incubation time and inoculum source where NDF digestibility increased over time, with the greatest NDF digestibility at 48 h ($P = 0.03$) for samples incubated in steers fed a residue diet (Table 2.7), but NDF digestibility was not different at 24 h ($P = 0.90$). There was an interaction of incubation time and inoculum source ($P < 0.01$) where, at 28 and 48 hours, inoculum source impacted NDF digestibility ($P < 0.02$). However, there was no effect of inoculum source at 36 hours ($P = 0.45$). There was no interaction ($P = 0.96$) for inoculum source and forage type (Table 2.8). Average NDF digestibility was greater for residue samples when the donor was fed a high corn residue diet ($P = 0.01$; Table 2.9). There was an effect of run ($P < 0.01$; data not shown) demonstrating variability between runs, similar to *in vitro* results.

Experiment 2: In vitro

No interaction was observed for inoculum source and forage type for IVDMD (P = 0.99; Table 2.10). There was no interaction between inoculum source and forage type for IVOMD (P = 0.98; Table 2.11). There was no effect of inoculum source for IVDMD (P = 0.41) or for IVOMD (P = 0.25; Table 2.12).

Experiment 2: In situ

There was no 3-way interaction observed for forage type by incubation time by inoculum source (P = 0.85; Table 2.13). There was no interaction for inoculum source by forage type (P = 0.19; Table 2.14). There was an interaction for inoculum source by incubation time (P = 0.01; Table 2.15). Digestibility of NDF was greatest at 36 h for both forage types incubated in an inoculum source obtained from steers consuming a residue diet (P = 0.03). There was no significant difference for NDF digestibility at 48 h (P = 0.13). However, at 60 and 72 h NDF digestibility was greatest for both forage types incubated in an inoculum source obtained from steers consuming a residue diet (P < 0.01). There was a main effect for incubation time (P < 0.01) where NDF digestibility increased over time and was greatest at 72 h (Table 2.16). There was also a main effect for inoculum source where NDF digestibility was greatest for both forage types when incubated in an inoculum obtained from steers consuming a residue based diet (P < 0.01; Table 2.17).

Discussion

In Exp. 2, Sweet Bran at 30% inclusion level was included across experiment to assure there was no effect of DDGS in Exp. 1. As anticipated, the husk and leaf had the

greatest average NDF digestibility for Exp. 1, with *in situ* values of 49.7% and 51.3%, and *in vitro* values of 52.5% and 44.5%, respectively. Exp. 2 showed a similar trend with *in situ* NDF digestibility being greatest for husk at 68.1%. Compared to the husk and leaf, the stalk and cob had lesser average NDF digestibility *in situ* of 27.1% and 29.3%, respectively, in Exp. 1. Similarly in Exp. 1 stalk and cob had lesser average NDF digestibility *in vitro* of 39.8% and 45.5%, respectively than the leaf and husk. Exp. 2 followed the same trend with lower quality residue samples, such as husklage and conventionally baled residue, having lower NDF digestibility *in situ* at 49.8% and 52.6%, respectively. Higher quality husk and leaf compared to the stalk and cob has been reported in the literature (Fernandez-Rivera and Klopfenstein, 1989; Gutierrez-Ornelas and Klopfenstein, 1991; McGee et al., 2013).

There was no interaction in Exp. 2 for IVDMD and IVOMD with inoculum source suggesting that DMD or OMD is not affected by a diet change. However, Soder (2005) saw differences in IVDMD values when evaluating different inoculums with a total mixed ration and a pasture diet. Others have supported these results by showing that source of inoculum did have a significant effect on IVDMD (Bezeau, 1965; Cherney, 1993; Holden, 2000). However, others are in disagreement, reporting similar IVDMD regardless of donor diet (Quicke et al., 1959; Marinucci et al., 1992).

The digestibility of NDF is dependent on the amount of time spent inside the rumen (NRC, 2000). As expected, NDF digestibility increased over time for all procedures showing an increased disappearance with increased time incubated. Run was significant for all procedures which suggests variation between runs. McLeod and

Minson (2006) proposed accurate predictions were possible with standardized procedures and samples of known *in vivo* digestibilities included in each run. As suggested by Stalker (2013), a regression equation derived from standards with known *in vivo* digestibility should be included in each *in vitro* run to be used to adjust IVDMD values to *in vivo* values. Peterson (2006) suggested adjusting IVOMD values to in vivo values to increase precision of animal performance. This is also in agreement with Giesert (2007) who developed a set of forage standards to estimate *in vivo* digestibility values. Additionally, according to Weiss (1994) samples with unknown *in vivo* digestibility values can be evaluated *in vitro* and adjusted using regression equations developed from *in vivo* digestibility values.

This study shows that the diet of the donor animal does affect NDF digestibility estimates of corn residue samples. However there was no interaction for sample and diet type. Greater NDF digestibility estimates for both *in vitro* and *in situ* procedures for both sample types were observed when incubated in an inoculum from a steer consuming a residue diet compared to the brome diet. Continually maintaining different donor animals on different diets to perform these procedures is not necessary; one set of animals on a 30% concentrate diet is sufficient.

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Table 2.1 Varying nutritive quality of forage and grass samples[1].

Sample	CP	NDF
2 Row	6.1	83
4 Row	6.0	90.8
8 Row	7.8	78.9
Cob	7.8	90.1
Conventional	7.8	73.8
Stalk	4.3	79.5
Husk	5.7	84.0
Leaf	3.9	72.9
Mature Brome Hay	8.2	71.4
Meadow Hay	7.6	60.0
Poor Brome Hay	7.5	69.6
Prairie Hay	7.9	68.3
Good Brome	9.3	66.7

[1] 2 Row: Harvested with a Holland Cornrower Corn Head with 2 rows engaged; 4 Row: Harvested with a Holland Cornrower Corn Head with 4 rows engaged; 8 Row: Harvested with a Holland Cornrower Corn Head with 8 rows engaged; Husk, leaf, stalk, cob: were harvested and separated by hand; Conventional: corn residue was raked and baled

Table 2.2 Three way interaction of inoculum source, incubation time, and forage type on *in vitro* NDF digestibility[1] (%) of different forages for Experiment 1.

	24 h			48 h		
Sample	Brome[2]	Residue	*P* - Value[3]	Brome	Residue	*P* - Value
2 Row	41.2	40.4	0.86	57.9	57.6	0.95
4 Row	37.9	35.6	0.60	48.6	51.6	0.50
6 Row	33.8	33.1	0.87	47.3	48.7	0.74
8 Row	36.3	35.7	0.88	47.8	49.2	0.75
Cob	34.7	36.2	0.73	43.5	44.7	0.78
Conventional	31.3	32.8	0.74	41.6	44.8	0.45
Husk	44.0	44.8	0.85	60.0	61.1	0.81
Stalk	36.9	41.4	0.30	48.1	51.7	0.40
Leaf	40.3	38.8	0.75	49.9	53.2	0.45
Good Brome Hay	46.2	48.6	0.58	63.1	65.9	0.52
Meadow Hay	47.9	41.3	0.13	53.9	58.7	0.27
Poor Brome Hay	32.2	34.0	0.68	41.8	45.3	0.43
Prairie Hay	27.5	26.8	0.87	38.3	39.6	0.76
Mature Brome Hay	37.6	36.2	0.74	47.4	51.7	0.32

[1]Forage type x incubation time x inoculum source; $P = 0.99$, SEM = 2.1

[2] Brome diet consists of 70% bromegrass hay and 30% DDGS; Residue diet consists of 70% corn residue and 30% Sweet Bran

[3]NDF digestibility averaged across run

Table 2.3 Interaction of inoculum source and forage type on *in vitro* NDF digestibility[1] (%) for Experiment 1.

	Inoculum source[2]		
Sample	Brome	Residue	*P* - value[3]
2 Row	49.5	49.0	0.86
4 Row	43.2	43.6	0.92
6 Row	40.6	40.9	0.91
8 Row	42.1	42.5	0.91
Cob	39.0	40.5	0.66
Conventional	36.4	38.8	0.77
Husk	52.0	52.9	0.76
Stalk	42.5	46.5	0.19
Leaf	45.1	46.0	0.77
Good Brome Hay	54.6	57.3	0.39
Meadow Hay	50.9	50.0	0.77
Prairie Hay	37.0	39.6	0.40
Prairie Hay	32.9	33.2	0.92
Mature Brome hay	42.5	43.9	0.64

[1]Forage type x inoculum source interaction; $P = 0.99$, SEM = 2.3

[2] Brome diet consists of 70% bromegrass hay and 30% DDGS; Residue diet consists of 70% corn residue and 30% Sweet Bran

[3]NDF digestibility averaged across run

Table 2.4 Interaction of inoculum source and incubation time on *in vitro* NDF digestibility[1] (%) for Experiment 1.

	Inoculum source[2]		
Time (h)	Brome	Residue	*P* - value[3]
24	37.7	37.5	0.90
48	49.2	51.7	0.03

[1]Inoculum source x incubation time interaction; $P = 0.11$, SEM = 1.2

[2] Brome diet consists of 70% bromegrass hay and 30% DDGS; Residue diet consists of 70% corn residue and 30% Sweet Bran

[3]NDF digestibility averaged across all forage samples

Table 2.5 Main effect of inoculum source on *in vitro* NDF digestibility (%) for Experiment 1.

	Inoculum source[2]			
	Brome	Residue	SEM	*P* - value
NDF Digestibility[1]	43.5	44.6	0.58	0.16

[1]NDF digestibility averaged across all forage samples

[2] Brome diet consists of 70% bromegrass hay and 30% DDGS; Residue diet consists of 70% corn residue and 30% Sweet Bran

Table 2.6 Three way interaction of inoculum source, incubation time, and forage type on *in situ* NDF digestibility[1] (%) of different forages for Experiment 1.

	28 h			36 h			48 h		
Sample	Brome[2]	Residue	*P* - Value[3]	Brome	Residue	*P* - Value	Brome	Residue	*P* - Value
2 Row	31.0	39.2	0.16	46.7	47.9	0.84	54.4	59.8	0.35
4 Row	28.7	33.7	0.38	42.6	42.6	0.99	49.7	50.7	0.86
6 Row	28.1	32.0	0.49	40.0	39.9	0.87	47.7	49.2	0.57
8 Row	19.2	26.3	0.21	35.5	39.2	0.52	42.0	46.3	0.45
Cob	19.6	23.0	0.55	25.7	30.5	0.40	30.1	33.7	0.54
Conventional	29.8	33.6	0.51	39.8	38.6	0.78	43.0	42.4	0.91
Husk	36.4	46.4	0.08	48.0	46.1	0.73	59.4	62.1	0.64
Leaf	43.0	46.9	0.49	53.4	52.5	0.98	54.3	57.5	0.80
Stalk	15.7	21.7	0.29	28.7	31.0	0.67	37.6	40.9	0.56

[1]Forage type x incubation time x inoculum source; $P = 0.99$; SEM = 2.0; LSD = 0.04

[2] Brome diet consists of 70% bromegrass hay and 30% DDGS; Residue diet consists of 70% corn residue and 30% Sweet Bran

[3]NDF digestibility averaged across run

Table 2.7 Interaction of inoculum source and incubation time on *in situ* NDF digestibility[1] (%) for Experiment 1.

	Inoculum source[2]			
Time (h)	Brome	Residue	SEM	*P* - value[3]
28	27.9	33.7	2.1	<0.01
36	40.0	40.9	2.1	0.45
48	46.5	49.2	2.1	0.02

[1]Inoculum source x incubation time interaction; $P = 0.01$

[2] Brome diet consists of 70% bromegrass hay and 30% DDGS; Residue diet consists of 70% corn residue and 30% Sweet Bran

[3]NDF digestibility averaged across all forage samples

Table 2.8 Interaction of inoculum source and forage type on *in situ* NDF digestibility[1] (%) for Experiment 1.

	Inoculum source[2]		
Sample[3]	Brome	Residue	*P* - value
2 Row	44.1	49.0	0.23
4 Row	40.4	42.4	0.44
6 Row	38.6	40.5	0.65
8 Row	32.2	37.3	0.14
Cob	25.1	29.1	0.40
Conventional	37.7	38.2	0.87
Husk	48.0	51.5	0.61
Leaf	50.2	52.3	0.78
Stalk	27.3	31.2	0.25

[1]Forage type x inoculum source interaction, $P = 0.96$, SEM =3.8

[2] Brome diet consists of 70% bromegrass hay and 30% DDGS; Residue diet consists of 70% corn residue and 30% Sweet Bran

[3]NDF digestibility averaged across run

Table 2.9 Main effect of inoculum source on *in situ* NDF digestibility (%) for Experiment 1.

	Inoculum source[2]			
	Brome	Residue	SEM	*P* - value
NDF Digestibility[1]	40.0	43.5	1.7	0.01

[2]Brome diet consists of 70% bromegrass hay and 30% DDGS; Residue diet consists of 70% corn residue and 30% Sweet Bran

[1]NDF digestibility averaged across all forage samples

Table 2.10 Interaction of inoculum source and forage type on *in vitro* DMD[1] for Experiment 2.

	Inoculum source[2]		
Sample[3]	Brome	Residue	*P* - value
2 Row	49.9	51.5	0.62
8 Row	41.9	44.2	0.50
Conventional	45.5	48.4	0.39
Husk	61.7	60.7	0.76
Husklage	34.9	38.9	0.23
Good Brome Hay	57.8	58.8	0.75
Mature Brome Hay	49.2	49.6	0.90
Meadow Hay	55.5	56.0	0.88
Poor Brome Hay	51.6	51.5	0.98
Prairie Hay	48.9	49.3	0.89

[1]Inoculum source x forage type; $P = 0.99$, SEM = 2.4

[2] Brome diet consists of 70% bromegrass hay and 30% Sweet Ban; Residue diet consists of 70% corn residue and 30% Sweet Bran

[3]DMD averaged across run

Table 2.11 Interaction of inoculum source and forage type on *in vitro* OMD[1] for Experiment 2.

	Inoculum source[2]		
Sample[3]	Brome	Residue	*P* - value
2 Row	52.3	53.2	0.79
8 Row	43.9	46.1	0.50
Conventional	47.5	50.4	0.39
Husk	62.8	61.2	0.65
Husklage	35.8	40.1	0.21
Good Brome Hay	60.3	60.2	0.98
Mature Brome Hay	49.9	50.4	0.89
Meadow Hay	59.0	59.4	0.92
Poor Brome Hay	52.6	51.9	0.83
Prairie Hay	50.8	50.8	0.99

[1]Diet x sample; $P = 0.98$, SEM = 2.4

[2] Brome diet consists of 70% bromegrass hay and 30% Sweet Ban; Residue diet consists of 70% corn residue and 30% Sweet Bran

[3]OMD averaged across run

Table 2.12 Main effect of inoculum source on *in vitro* estimates[1] for Experiment 2.

	Inoculum source[2]			
	Brome	Residue	SEM	*P* - value
IVDMD, %DM	49.7	50.9	0.79	0.41
IVOMD, %DM	51.5	52.4	0.74	0.25

[2] Brome diet consists of 70% bromegrass hay and 30% Sweet Ban; Residue diet consists of 70% corn residue and 30% Sweet Bran

[1]Averaged across run

Table 2.13 Three way interaction of inoculum source, forage type, and incubation time on *in situ* NDF digestibility[1] (%) of different forages for Experiment 2.

	36 h			48 h			60 h			72 h		
Sample	Brome[2]	Residue	*P*-value[3]	Brome	Residue	*P*-value	Brome	Residue	*P*-value	Brome	Residue	*P*-value
2 Row	48.4	49.4	0.65	52.3	55.5	0.18	57.4	60.8	0.16	63.3	69.4	0.01
8 Row	46.0	47.8	0.46	51.2	51.9	0.79	53.7	58.1	0.07	58.9	66.3	<0.01
Conventional	44.9	45.3	0.87	49.8	50.7	0.71	51.3	57.6	0.01	56.4	64.5	<0.01
Husk	55.2	65.7	<0.01	62.4	64.2	0.44	65.7	73.1	<0.01	75.2	83.5	<0.01
Husklage	39.6	39.7	0.98	48.4	47.4	0.69	50.2	53.6	0.16	53.9	65.9	<0.01
Good Brome Hay	47.9	49.0	0.65	50.8	52.8	0.41	54.9	60.6	0.02	60.4	65.5	0.03
Mature Brome Hay	46.2	46.5	0.92	50.3	50.4	0.99	52.4	56.5	0.08	55.4	62.1	0.01
Meadow Hay	53.9	55.1	0.63	55.9	57.7	0.46	61.4	63.6	0.35	63.5	69.5	0.01
Poor Brome Hay	43.0	43.9	0.71	47.6	47.9	0.88	48.5	55.7	<0.01	55.5	61.8	0.01
Prairie Hay	46.6	46.2	0.85	46.9	48.5	0.51	51.9	55.6	0.12	56.2	61.0	0.05

[1]Forage type x incubation time x inoculum source; SEM = 2.0; $P = 0.85$

[2] Brome diet consists of 70% bromegrass hay and 30% Sweet Ban; Residue diet consists of 70% corn residue and 30% Sweet Bran

[3]NDF digestibility averaged across run

Table 2.14 Interaction of inoculum source and forage type on *in situ* NDF digestibility[1] (%) for Experiment 2.

	Inoculum source[2]		
Sample[3]	Brome	Residue	*P* - value
2 Row	55.3	58.8	<0.01
8 Row	52.5	56.0	<0.01
Conventional	50.6	54.5	<0.01
Husk	64.6	71.6	<0.01
Husklage	48.0	51.7	<0.01
Good Brome Hay	53.5	57.0	<0.01
Mature Brome Hay	51.1	53.9	0.02
Meadow Hay	58.7	61.5	0.02
Poor Brome Hay	48.7	52.3	<0.01
Prairie Hay	50.4	52.8	<0.01

[1]Inoculum source x forage type *P* = 0.19, SEM = 1.2
[2] Brome diet consists of 70% bromegrass hay and 30% Sweet Ban; Residue diet consists of 70% corn residue and 30% Sweet Bran
[3]DMD averaged across run

Table 2.15 Interaction of inoculum source and incubation time on *in situ* NDF digestibility[1] (%) for Experiment 2.

	Inoculum source[2]		
Time (h)	Brome	Residue	*P* – value[3]
36	47.2	48.9	0.03
48	51.6	52.7	0.13
60	54.7	59.5	<0.01
72	59.9	66.9	<0.01

[1]Inoculum source x incubation time interaction; $P = 0.01$, SEM = 1.0

[2] Brome diet consists of 70% bromegrass hay and 30% Sweet Ban; Residue diet consists of 70% corn residue and 30% Sweet Bran

[3]NDF digestibility averaged across all forage samples

Table 2.16 Main effect of incubation time on *in situ* NDF digestibility[1] (%) for Experiment 2.

	Time (h)					
	36	48	60	72	SEM	*P* - value
NDF Digestibility	48	52.1	57.1	63.4	0.54	<0.01

[1]NDF digestibility averaged across all forage samples

Table 2.17 Main effect of inoculum source on *in situ* NDF digestibility[1] (%) for Experiment 2.

	Inoculum source[2]			
	Brome	Residue	SEM	*P* - value
NDF Digestibility	53.3	57.0	0.38	<0.01

[2]Brome diet consists of 70% bromegrass hay and 30% Sweet Ban; Residue diet consists of 70% corn residue and 30% Sweet Bran

[1]NDF digestibility averaged across all forage samples

Chapter III: Relationship between Dietary TDN and Digested OM in Beef Cattle Growing and Finishing Diets

H. C. Hamilton[*], J. L. Gramkow[*], J. C. MacDonald[*], G.E. Erickson*, T. J. Klopfenstein[*], A. K. Watson[*]

[*]Department of Animal Science, University of Nebraska-Lincoln Lincoln, NE 68583

Abstract

The relationship between organic matter digestibility (OMD) and TDN is unestablished for diets containing byproducts. Three cattle digestion studies were used to evaluate the relationship between TDN and digested OM (DIGOM). Total tract collection and OM analysis of feed and feces determined OMD, which was multiplied by dietary OM content to determine digested OM (% of DM). Gross energy of feed and feces was determined by bomb calorimetry. Dietary DE was converted to TDN using 4.4 Mcal DE / kg TDN. Exp. 1 utilized 45% HMC, 40% Sweet Bran, 10% corn silage diets and 5% supplement (DM basis); Exp. 2 used diets containing 18% modified DGS (DM basis) and increasing amounts of a corn stover pellet, all containing 18% DGS, replacing dry rolled corn (DRC). Exp. 3 compared 80% DRC-based diets with corn oil or tallow to diets with 25.5% distillers solubles, or 56% wet DGS. Regression was used to relate DIGOM to TDN. The initial model included experiment, animal within experiment, and treatment within experiment. A significant treatment within experiment effect ($P < 0.01$) resulted in independent regression models for each experiment being fitted. Exp. 1 and 2 showed no treatment effect and no interaction between treatment and DIGOM. In Exp. 3 there was no treatment effect ($P = 0.14$). Results from Exp.1 indicate DIGOM was 3.58 percentage units (ppt) less than TDN content. In Exp. 2, DIGOM was 11.1 ppt less than TDN content. In Exp. 3, DIGOM in the corn diet was 3.96 ppt less than TDN. For the tallow and corn oil diet, DIGOM was 0.34 ppt less and 0.37 ppt greater than TDN, respectively. In the solubles and wet DGS diets, DIGOM was less than TDN by, 5.88 ppt and 9.96 ppt, respectively. The increase in ppt was consistent with an increase in gross energy (GE)

across diets within experiment. These results suggest DIGOM is consistent relative to TDN of traditional, corn based diets. In finishing diets containing DGS additional DE supplied by DGS is not accounted for when evaluating only DIGOM. Measuring DE content of diets used in digestion trials is important when trying to estimate feeding values.

Keywords: Bomb Calorimetry, Digestible Energy, digested OM, TDN

Introduction

Energy is the potential to do work, which can be measured and defined using a set of standard conditions and therefore can be considered absolute.

Gross energy (GE), or heat of combustion, is the energy that is released when an organic substance is or oxidized to water and carbon dioxide, and is impacted by GE chemical composition, but does not allow us to understand the availability of that energy for an animal to utilize. Measuring GE is easy, but gives little value in assessing a particular diet or feed ingredient as an energy source for an animal. Digestible energy (DE) is the difference of GE in feed and feces. Values of DE overestimate the value of feedstuffs like hay or straw, which are high in fiber, relative to grain which is highly digestible and low in fiber.

Total digestible nutrients (TDN) is directly related to DE. Thus, TDN can be converted to DE energy using 1 lb of TDN equal to 2 Mcal of DE. Previously, TDN was based on proximate analysis, which is no longer commonly used. These analyses were also based on diets containing primarily corn, fat, and alfalfa but none containing byproducts. Organic matter digestibility (OMD) is related to TDN and is commonly measured in digestion studies. However, the relationship between OMD and TDN is unestablished for diets containing byproducts. When the amount of wet distillers grains plus solubles (WDGS) is increased in a diet there is an increase in feed efficiency but a decrease in OMD (Ham et al., 1994). Bomb calorimetry can directly determine total energy content of the feed and feces. The objective of this study was to compare digested

organic matter (DIGOM), determined by previous digestibility trials, and calculated TDN values using bomb calorimetry.

Materials and Methods

This study utilized three previously conducted digestion trials which used total tract collection and OM analysis of feed and feces to determine OMD. Organic matter digestibility values were multiplied by dietary OM content to determine digested organic matter (DIGOM, % DM). Dietary DE was calculated from heat of combustion found using a bomb calorimeter (Parr Instrument Company, Moline, IL) as described in Appendix 3.1. Dietary DE was converted to TDN using 4.4 Mcal DE / kg TDN. A summary of mathematic equations is provided in Appendix 3.2. The TDN and DIGOM were compared by regressing the TDN on the DIGOM using the GLM Procedure of SAS.

Digestion data were analyzed using the Mixed Procedures of SAS with treatment as a fixed effect and steer within period as experimental unit.

Experiment 1

A digestion study was completed by Harding et al. (2015) utilizing 4 ruminally cannulated steers in a switchback design with three, 21-d periods. All steers were fed a basal diet consisting of 40% Sweet Bran®, 45% HMC, 10% corn silage, and 5% supplement (DM basis; Table 3.1). Steers were assigned randomly to one of two treatments, with treatments consisting of the basal diet treated with the enzyme (ENZ) or the basal diet without the enzyme treatment (CON). Fecal and diet samples collected during the trial were freeze-dried, ground through a Wiley mill with a 1-mm screen

(Thomas Scientific, Swedesboro, NJ) and composited by steer within period. Fecal samples were analyzed for titanium dioxide concentration to estimate DM excretion. Fecal and diet samples were analyzed for DM and OM to estimate total tract digestibility.

Experiment 2

A digestion study completed by Gramkow et al. (2016) used 6 steers in a 4 × 6 Youden square. The negative control (NEGCON) contained 60% untreated corn stover, 18% MDGS, 18% distillers solubles and 4% supplement (DM basis; Table 3.2). The positive control (POSCON) consisted of 60% CaO treated corn stover, 18% MDGS, 18% distillers solubles, and 4% supplement. The third treatment (CONV) was a pellet containing the same proportions of CaO treated corn stover, solubles, MDGS and supplement. The corn stover for all treatments was harvested from the same field. The corn residue that was left was raked into windrows and baled with a conventional square baler. Treatment four (MOG) was also a pellet containing the same proportions of CaO treated corn stover, solubles, DDG, and supplement. The corn stover for this treatment was harvested using a single pass round baler pulled behind the combine (John Deere; Hillco Technologies Inc.). Fecal and diet samples collected during the trial were freeze-dried, ground through a Wiley mill with a 1-mm screen and composited by steer within period. Fecal samples were analyzed for titanium dioxide concentration to estimate DM excretion. Fecal and diet samples were analyzed for DM and OM to estimate total tract digestibility.

Experiment 3

A digestion study completed by Bremer (2010) utilized 5 ruminally cannulated steers were in a completely randomized, five-twenty-one day-period Latin square designed study. Diets compared 80% DRC-based diets with one of two supplemental fat sources (tallow or corn oil) to diets with 25.5% distillers solubles, or 56% wet DGS (Table 3.3). Fecal and diet samples collected during the trial were freeze-dried, ground through a Wiley mill with a 1-mm screen, and composited by steer within period. Fecal samples were analyzed for titanium dioxide concentration to estimate DM excretion. Fecal and diet samples were analyzed for DM and OM to estimate total tract digestibility.

Calculations

Energy intake was calculated:

$$\left(\frac{Hc\left(\frac{cal}{g}\right) \times \%\ inclulsion\ of\ ingredient}{1{,}000{,}000\ \left(\frac{cal}{Mcal}\right) 453.59\ \left(\frac{g}{lbs}\right)}\right) \times DMI\ (lbs)$$

where *Hc* is the heat of combustion measured from the sample burning in the bomb calorimeter, *% inclusion of the ingredient* is percent inclusion in the diet, and DMI is dry matter intake of the animal in pounds. The denominator is the conversion from calories to megacalories and grams to pounds.

Fecal Energy was calculated:

$$\left(\frac{Hc\left(\frac{cal}{g}\right)}{1{,}000{,}000\ \left(\frac{cal}{Mcal}\right) 453.59\ \left(\frac{g}{lbs}\right)}\right) \times Fecal\ Output\ (DM, lbs)$$

where *Hc* is the heat of combustion measured from the sample burning in the bomb calorimeter, *Fecal Output* is the pounds of feces excreted by the animal on a dry matter

basis. The denominator is the conversion from calories to megacalories and grams to pounds.

DE was calculated by subtracting fecal energy from energy intake. Total digestible nutrients was calculated:

$$\left(\frac{DE}{\frac{4.4\ Mcal\ DE}{2.2\ lbs\ TDN}} \right) \times 100$$

where *DE* is the difference between energy intake and fecal energy and DMI is dry matter intake of the animal in pounds. The first denominator is the conversion from calories to megacalories, grams to pounds and DE to TDN using the assumption of 4.4 Mcal DE / kg TDN.

Regression

Regression was used to relate digestible OM to TDN. The initial model included experiment, animal within experiment, and treatment within experiment. An isopleth with a slope of 1 originating from zero is indicated with a dotted line to show relative differences of slope. A two tailed t-test was used to statistically test if the slopes were different from 1. Individual points were used to represent animal within period for each experiment. The treatment average was used as the observation for regression models that compared GE and the difference between DIGOM and TDN.

Results

Intercepts for a unified regression model was not significant ($P = 0.32$). A significant treatment within experiment effect ($P < 0.01$) resulted in independent regression models for each experiment.

Experiment 1

Treatments for Exp. 1 were significantly different ($P < 0.01$) for DIGOM relative to TDN (Table 3.4). However, Exp.1 showed no treatment effect for DIGOM. Therefore, a single slope with a linear relationship was used. The linear regression line gave a slope of 0.97, a y-intercept of 6.19, and an r^2 value of 0.89 (Figure 3.2). The two treatments had an average of 76.9% digested OM as a percent of DM. The average DE was found to be 1.61 Mcal/lb. From DE, TDN was calculated as 80.5% TDN. Results indicate digested OM was 3.58 percentage units (ppt) less than TDN content (Table 3.5)

Experiment 2

Treatments for Exp. 2 were significantly different ($P < 0.01$) for DIGOM relative to TDN (Table 3.4). However, Exp. 2 showed no treatment effect for DIGOM. Therefore, a single slope with a linear relationship was used. The linear regression line gave a slope of 1.10, a y-intercept of 4.58, and an r^2 value of 0.85 (Figure 3.3). The four treatments had an average of 65.3% digested OM as a percent of DM. The average DE was found to be 1.53 Mcal/lb. TDN was calculated with an average of 76.5% TDN. Digested OM was 11.1 ppt less than TDN content (Table 3.5).

Experiment 3

In Exp. 3, there was a tendency for a treatment effect ($P > 0.14$). Therefore treatments were evaluated using separate regression lines and treatments remained separate for further analysis (Corn, CornOil, Tallow, Solubles, WDGS). The corn treatment had a slope of 1.11, a y-intercept of 12.5, and an r^2 value of 0.99 (Figure 3.4). The corn diet had 79.18% digested OM as a percent of DM. The DE was found to be 1.50 Mcal / lb. The TDN was calculated with an average of 75.2% TDN. Digested OM was 3.96 ppt more than TDN content (Table 3.5). The tallow treatment had a slope of 0.99, a y-intercept of 1.09, and an r^2 value of 0.99 (Figure 3.4). The tallow diet had 79.3% digested OM as a percent of DM. The DE was found to be 1.59 Mcal/lb. The average TDN was 79.7% for Exp. 3. Digested OM was 0.34 ppt less than TDN content (Table 3.5). The corn oil treatment had a slope of 1.18, a y-intercept of 13.5, and an r^2 value of 0.99 (Figure 3.4). The corn oil diet had 76.2% digested OM as a percent of DM. The DE was found to be 1.52 Mcal/lb. TDN was calculated with an average of 75.9% TDN. Digested OM was 0.37 ppt more than TDN content (Table 3.5). The solubles treatment had a slope of 1.25, a y-intercept of 13.9, and an r^2 value of 0.99 (Figure 3.4). The solubles diet had 80.7% digested OM as a percent of DM. The DE was found to be 1.73 Mcal/lb. TDN was calculated with an average of 86.6% TDN. digested OM was 5.88 ppt less than TDN content (Table 3.5). The WDGS treatment had a slope of 1.25, a y-intercept of 13.8, and an r^2 value of 0.99 (Figure 3.4). The WDGS diet had 73.8% digested OM as a percent of DM. The DE was found to be 1.67 Mcal / lb. TDN was calculated with an average of 83.7% TDN. Digested OM was 9.95 ppt less than TDN content (Table 3.5).

Differences between TDN and digested OM were plotted relative to GE of the diet (cal / g, % DM), for all experiments, the slope was 0.025, a y-intercept of 105, and an r^2 value of 0.70 (Figure 3.5). This figure shows that with increasing amount of GE in the diet there is also an observed increase in the difference between TDN and digested OM. If Exp. 3 is viewed independently, the slope of the line is 0.02, with a y-intercept of 97.8, and an r^2 value of 0.93 (Figure 3.6). This figure shows that the corn diet had a negative difference (-4.0) between TDN and digested OM, meaning digested OM was 4 ppt greater than TDN. The corn diet also had the least GE in the diet. On the other end of the spectrum, the WDGS diet had a difference of 10.0 with TDN being 10 ppt greater than digested OM. Similarly, WDGS diet had the highest GE of all treatments.

There were no significant differences for OM intake (kg) across all treatments ($P = 0.88$). There were no significant differences in energy intake (Mcal) across all treatments ($P = 0.28$). However, OM excreted (kg) was significantly different ($P < 0.01$), with WDGS and 18% MDGS treatments having the greatest energy intake compared to other treatments. There were significant differences ($P < 0.01$) in energy excreted (Mcal) with WDGS and 18% MDGS having the greatest energy excreted, Corn, Corn Oil, Tallow, and 45% HMC being intermediate, and solubles having the least energy excreted. The ratio for consumed energy relative to consumed OM was different across treatments ($P < 0.01$), with WDGS and 18% MDGS having the greatest ratio, solubles the next greatest ratio, followed by 45% HMC. Corn oil and tallow had the fourth greatest ratio and Corn had the lowest ratio. The ratio for excreted OM relative to excreted energy was significantly different ($P < 0.01$), with solubles and WDGS having the greatest ratio,

corn, corn oil, tallow, and 45% HMC being intermediate, and 18% MDGS having the lowest ratio (Table 3.6).

Discussion

Traditionally, TDN was based on proximate analysis, which is no longer commonly used because of concerns with cumulative error. These analyses were also based on diets containing primarily corn, fat, and alfalfa but none containing byproducts. Stein et al. (2005) reported an ME range for DDGS of 3,058 to 3,738 kcal/kg with an average of 3,378 kcal/kg. These reported values were significantly greater than previous values reported on distillers grains (NRC, 1989). Olson et al. (2014) explored empirical predictions for DE on tallgrass prairie hay. This study found that GE fell in a narrow range, while intake, NDF, intake of digestible OM, OM digestion, and DE varied widely among the grass samples. Results indicated that GE content of the grass was a poor indicator of DE (r^2 = 0.39, slope=1.5). Conversely, the prediction of DE from intake of digestible OM was highly accurate (r^2 = 0.91, slope = 0.061). Organic matter digestion (%) was also highly correlated with DE (r^2 = 0.93, slope = 0.04). In concurrence with Olsen, others have explored the relationship with OM digestion and the estimation of DE (Moir, 1961; Rittenhouse et al., 1971; Minson, 1982).

Digested OM was plotted relative to TDN to explore the relationship between the two. In agreement with the previous studies done by Zinn et al. (1989, 1990, 1993, 1994, 1995, 2000), digested OM is consistent relative to TDN content of traditional corn based diets. These results are also similar to those of (Calderon-Cortes and Zinn, 1996;

Plascencia et al., 2011; Carrasco et al., 2013). Peter et al. (2000) assessed the effects of corn milling coproducts and observed that the difference between TDN and DIGOM for DDGS was only -0.3 ppt. However, OMD appeared relatively low to other reported values. The values of GE and DIGOM were highly correlated, which was consistent with previous studies (Olsen, 2014).

Results from Exp. 2 and 3 with diets containing DGS showed there was an additional supply of DE, which was not accounted for when using only digested OM. Additional DE is likely due to the protein and fat content of DGS, which supplies additional energy relative to OM content. All treatments consumed the same amount of OM but varied in energy intake. This is more apparent when expressed as a ratio with energy intake. The 18% MDGS treatment had the greatest ratio for energy intake relative to OM intake. Conversely, 18% MDGS had the smallest ratio for energy excreted relative to OM excreted. This suggests that there is more energy being consumed but never realized in the feces. These results are consistent with results suggesting fecal energy is the largest and most variable loss of intake energy, and consequently DE (Brown, 1966).

A large proportion of variation in TDN due to the fiber component has been observed for by-products (Owens et al., 2010). The fiber content of DGS could reduce energy supplied, but would remain in feces as OM, which is why greater OM was excreted from treatments containing DGS. This effect has been previously suggested, where crude fiber is the component of the diet that mainly affects digestibility (Cole, 1974). Cole (1974) also evaluated several studies that have shown decreased dietary

crude fiber levels increase the digestibility of organic matter. As suggested by Galyean et al. (2016), dietary factors such as NDF, ether extract (EE), and starch will likely bias energy predictions. It is suggested that inclusion of these factors in the model could improve precision compared with a single-variable regression approach (Galyean et al., 2016).

According to Owens et al. 2010, the impact of fat on digestible OM estimates is relatively small for most feeds. However, some feeds that have high lipid content will provide a considerable proportion of their DE from fat (Owens et al., 2010).

Values for DE on a range of feed ingredients, including DGS, are limited (Cole, 1974). Historically, TDN values were used to estimate DE. From this association, empirical relationships became acceptable in the absence of actual DE values. A relationship of 4.4 Mcal DE per 1kg TDN was used in this study. Other suggested conversion factors are 4.37, 4.50, 4.47, and 4.42 (Crampton, 1957; Maynard, 1953; Robinson, 1965; Zivkovic, 1963). Altering the conversion factor would ultimately lead to different absolute values of the calculated TDN in which affects the relationship of TDN to DIGOM. Variation among these values mainly comes from differences in chemical analysis and digestibility coefficients (Cole, 1974).

The difference between TDN and digested OM is much greater for diets containing DGS. When the percent difference between TDN and digested OM is expressed in terms of GE within an individual experiment, the relationship becomes

uniform across diets. Therefore, it is essential to measure digestible energy content of diets in digestion trials.

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Table 3.1 Diet fed to finishing steers in a digestion trial (Harding, 2015).

Ingredient, % DM	CON[1]	ENZ[2]
HMC[3]	45	45
Sweet Bran	40	40
Corn Silage	10	10
Supplement	5	5
Enzyme	-	+

[1] Basal diet without the enzyme treatment
[2] Basal diet treated with the enzyme
[3]HMC: High moisture corn

Table 3.2 Diet fed to growing steers in a digestion trail (Gramkow, 2016).

Ingredient, % DM	NEGCON[1]	POSCON[2]	Pellet C[3]	Pellet S[4]
MDGS[5]	18	18	18	18
Solubles	18	18	18	18
Corn Stalks	60	-	-	
CaO Trt Corn	-	60	60	60
Pellet C	-	-	100*	-
Pellet S	-	-	-	100*
Supplement	4	4	4	4

[1] Negative control containing untreated corn stover
[2] Positive control containing CaO treated corn stover
[3] Pellet containing CaO treated corn stover harvested by being raked into a windrow and baled with a conventional square baler
[4]Pellet containing CaO treated corn stover harvested using a single pass round baler pulled behind the combine
* Both pellet treatments were included at 100% of the diet (DM basis) as a complete pelleted feed. Other inclusions are shown to indicate identical proportions of ingredients for all treatments.
[5] MDGS: Modified distillers grains

Table 3.3 Diet fed to steers in a digestibility experiment evaluating dietary fat sources (Bremer, 2010).

Ingredient, % DM	Corn	Corn Oil	Tallow	Solubles	WDGS
DRC[1]	80	82.7	82.7	62	31.5
Grass Hay	7.5	7.5	7.5	7.5	7.5
Supplement	5	5	5	5	5
Molasses	7.5	-	-	-	-
Corn Oil	-	4.8	-	-	-
Tallow	-	-	4.8	-	-
Solubles	-	-	-	25.5	-
WDGS[2]	-	-	-	-	56

[1] DRC: Dry rolled corn

[2] WDGS: Wet distillers grains

Table 3.4 Relationship between DIGOM *x* and TDN *y* for all experiments 1, 2 and 3.

	r-squared	Treatment[1]	*P* – value[2]
Experiment 1			
$y = 0.923 (\pm 0.14)\ x + 9.27 (\pm 11.1)$	0.913	CON	0.61
$y = 1.13 (\pm 0.19)\ x - 6.08 (\pm 14.5)$	0.897	ENZ	0.52
$y = 0.967 (\pm 0.11)\ x + 6.16 (\pm 8.20)$	0.892	EXP 1	0.76
Experiment 2			
$y = 1.08 (\pm 0.19)\ x + 6.61 (\pm 12.2)$	0.818	NEGCON	0.70
$y = 1.23 (\pm 0.33)\ x - 4.11 (\pm 21.1)$	0.878	POSCON	0.52
$y = 1.25 (\pm 0.17)\ x - 6.37 (\pm 11.4)$	0.856	Pellet C	0.22
$y = 1.22 (\pm 0.19)\ x - 3.07 (\pm 13.4)$	0.659	Pellet S	0.23
$y = 1.10 (\pm 0.08)\ x + 4.59 (\pm 5.14)$	0.852	EXP 2	0.21
Experiment 3			
$y = 1.11 (\pm 0.05)\ x - 12.5 (\pm 4.12)$	0.993	Corn	0.11
$y = 1.10 (\pm 0.07)\ x - 7.70 (\pm 5.44)$	0.987	Corn Oil	0.25
$y = 1.24 (\pm 0.08)\ x - 13.88 (\pm 6.72)$	0.987	Solubles	0.04
$y = 0.990 (\pm 0.04)\ x + 1.09 (\pm 3.44)$	0.994	Tallow	0.84
$y = 1.15 (\pm 0.05)\ x - 0.887 (\pm 3.49)$	0.995	WDGS	0.04
$y = 0.898\ (\pm 0.19)\ x + 10.32 (\pm 14.8)$	0.495	EXP 3	0.59

[1] CON: Basal diet without the enzyme treatment; ENZ: Basil diet treated with the enzyme; EXP 1: combined treatments; NEGCON: Negative control containing untreated corn stover; POSCON: Positive control containing CaO treated corn stover; Pellet C: Pellet containing CaO treated corn stover harvested by being raked into a windrow and baled with a conventional square baler; Pellet S: Pellet containing CaO treated corn stover harvested using a single pass round baler pulled behind the combine; EXP 2: combined treatments; Corn: 80% DRC; Corn Oil: 82.7% DRC, 4.8% Corn Oil; Solubles: 62% DRC, 25.5 Solubles; Tallow: 62.7 DRC, 4.8% Tallow; WDGS: 31.5 DRC, 56% WDGS; EXP 3: combined treatments.

[2]Two tailed t-test for the hypothesis that the slope is equal to 1

Table 3.5 Average TDN and digested OM for treatments for experiments 1-3.

	Corn[3]	CornOil[3]	Tallow[3]	45% HMC[4]	Solubles[3]	WDGS[3]	18% MDGS[5]
TDN[1], % of DM	75.2	75.9	79.7	80.5	86.6	83.7	76.4
Digested OM[1], % of DM	79.2	76.2	79.3	76.9	80.7	73.8	65.3
Difference[2]	-4.0	-0.4	0.3	3.6	5.9	10.0	11.1

[1] Treatment average across animal and period
[2] Percentage unit difference between TDN and Digested OM
[3] Treatments from Exp. 3
[4] Treatments from Exp. 1
[5] Treatments from Exp. 2

Table 3.6 Difference in diet and fecal energy relative to OM content for all experiment treatments.

	Treatment								
	Corn	45% HMC[1]	Corn Oil	Tallow	Solubles	WDGS	18% MDGS[2]	SEM	*P* - Value
Consumed									
OM, lb	10.7	9.53	9.34	10.0	9.34	10.0	9.93	0.84	0.88
GE, Mcal	46.4	44.9	42.7	45.6	45.1	51.0	50.9	4.23	0.28
Excreted									
OM, lb	1.92^{bc}	1.80^{bc}	2.01^{bc}	1.87^{bc}	1.35^{c}	2.32^{ab}	2.71^{a}	0.30	<0.01
GE, Mcal	9.61^{bc}	9.17^{bc}	10.5^{bc}	9.58^{bc}	7.30^{c}	12.4^{ab}	13.5^{a}	1.49	<0.01
Ratio, GE/lb OM[3]									
Consumed	4.32^{e}	4.72^{c}	4.56^{d}	4.56^{d}	4.83^{b}	5.07^{a}	5.11^{a}	0.025	<0.01
Excreted	5.00^{bc}	5.11^{bc}	5.25^{ab}	5.18^{abc}	5.40^{a}	5.36^{a}	5.00^{c}	0.087	<0.01

[1]Treatment average for Exp. 1

[2] Treatment average for Exp.2

[3] Consumed: Consumed GE (Mcal) was divided by consumed OM (lb). Excreted: Excreted GE (Mcal) was divided by consumed OM (lb).

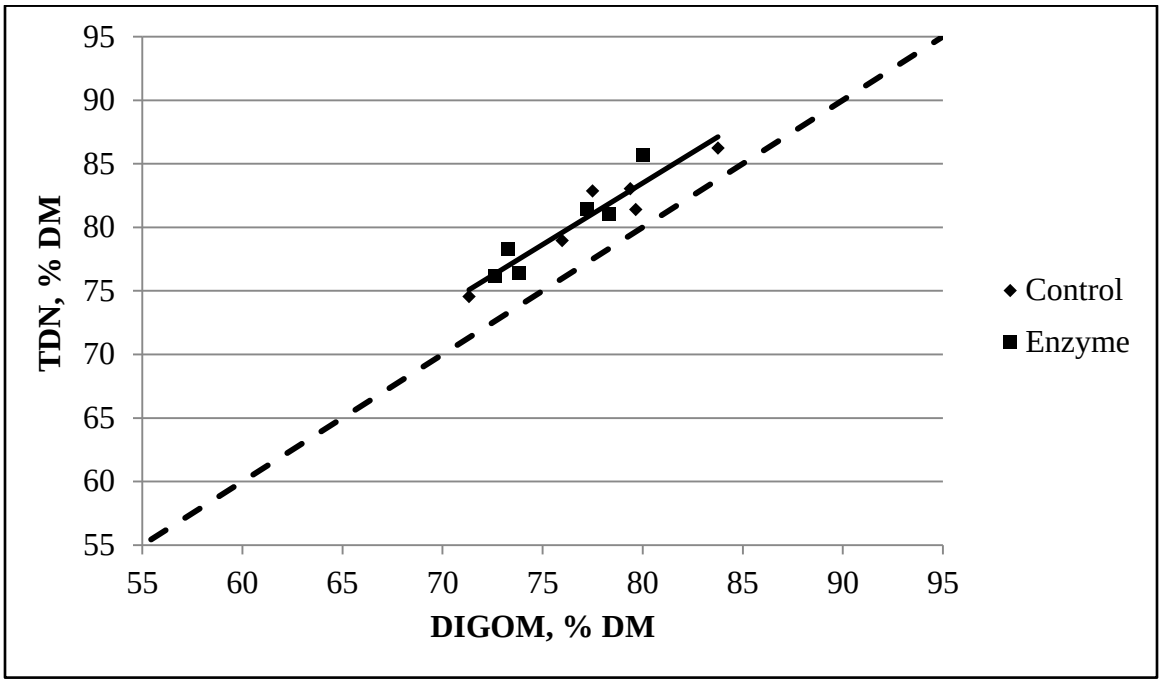

Figure 3.2 TDN vs DIGOM in 2 finishing diets w/o DGS (Exp. 1). Control (diamonds) and enzyme (squares) data are shown in the graph where individual data points indicate animal as the experimental unit. The regression equation for the data was TDN = [0.967 (±0.106) × DIGOM] + 6.16 (±8.20) % (r^2 =0.892).

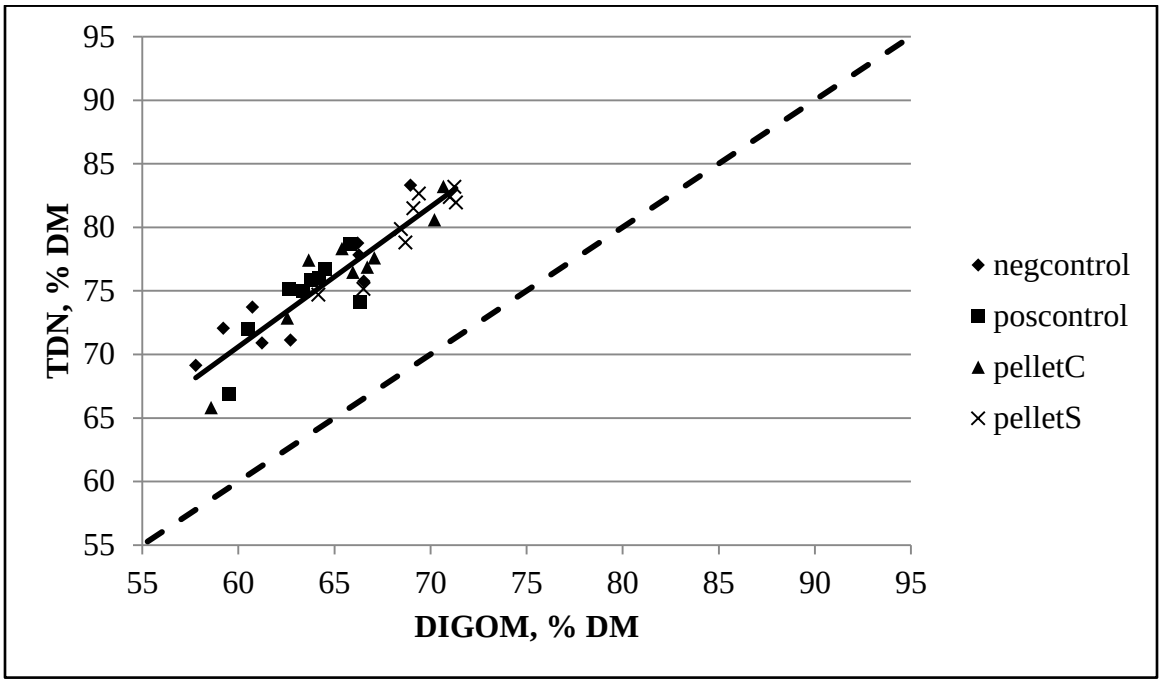

Figure 3.3 TDN vs DIGOM of growing diets with DGS. Negcontrol (diamond), poscontrol (square), pellet C (triangle), and pellet S (exes) data are shown in the graph where individual data points indicate animal as the experimental unit. The regression equation for the data was TDN = [1.10 (±0.0786) × DIGOM] + 4.59 (±5.14) % (r^2 =0.852).

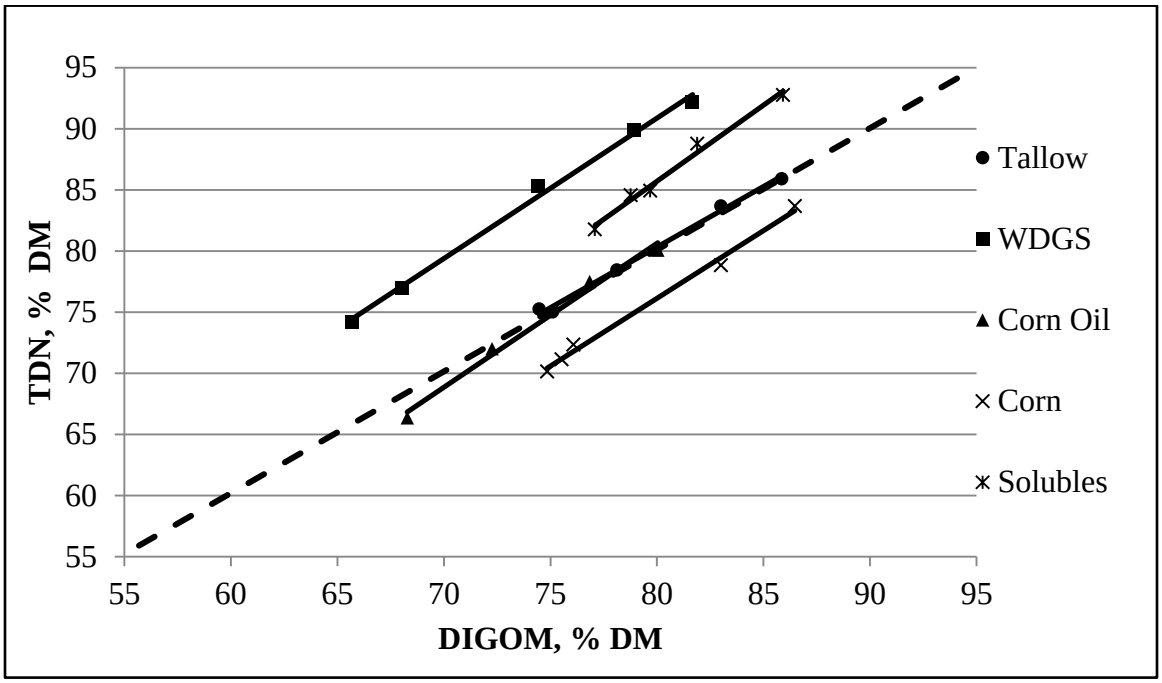

Figure 3.4 TDN vs DIGOM in 5 finishing diets. Tallow (circles), WDGS (squares), Corn Oil (triangles), Corn (exes), and Solubles (asterisks) data are shown in the graph where individual data points indicate animal as the experimental unit. The regression equation for the Tallow treatment was TDN = [0.990 (±0.0433) × DIGOM] + 1.09(±3.44) % (r^2 =0.994). The regression equation for the WDGS treatment was TDN = [1.15 (±0.0471) × DIGOM] -0.887(±3.49) % (r^2 =0.995). The regression equation for the Corn Oil treatment was TDN = [1.10 (±0.0712) × DIGOM] -7.70(±5.44) % (r^2 =0.987). The regression equation for the Corn treatment was TDN = [1.11 (±0.0519) × DIGOM] -12.5(±4.12) % (r^2 =0.993). The regression equation for the Solubles treatment was TDN = [1.24 (±0.0833) × DIGOM] -13.9(±6.72) % (r^2 =0.987).

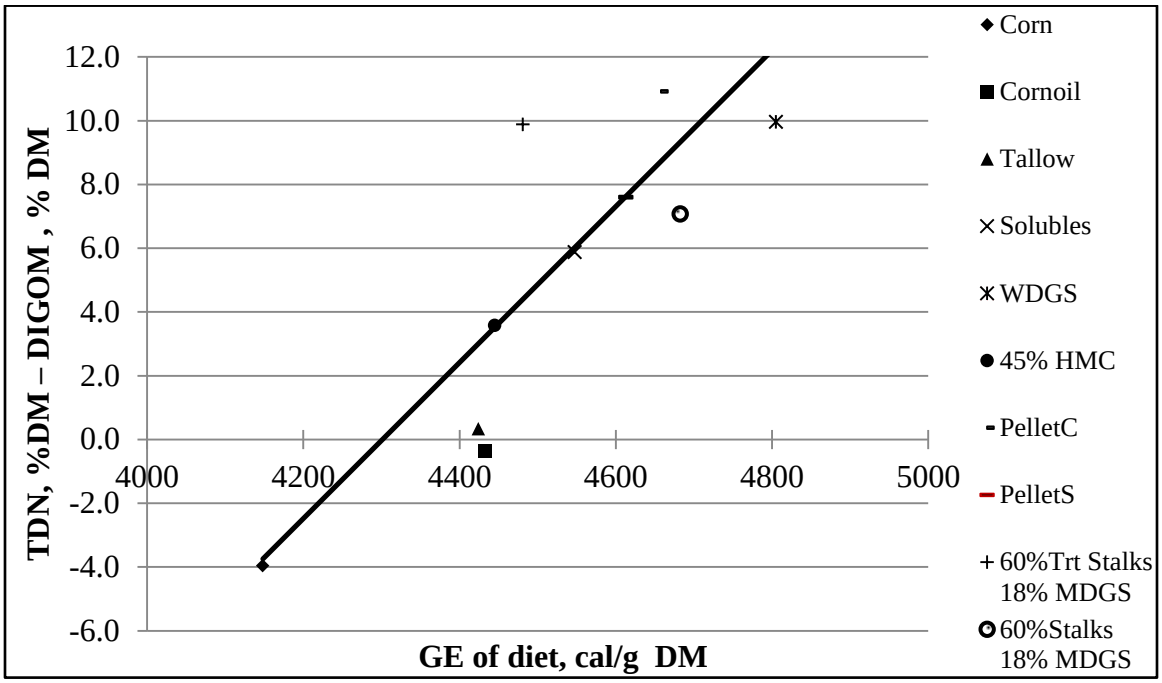

Figure 3.5 GE vs TDN-DIGOM in all diets. 45% HMC (circles), Corn Oil (squares), Tallow (triangles), Solubles (exes), WDGS (asterisks), Corn (diamonds), Pellet C (small hyphens), Pellet S (large hyphens), 60% treated stalks (crosses), and 60% stalks (rings) data are shown in the graph where individual data points indicate treatment average as the experimental unit. The regression equation for the all experiments combined was GE = [26.5 (±5.81) × TDN-DIGOM] + 4360 (±45.5) cal/g (r^2 =0.698).

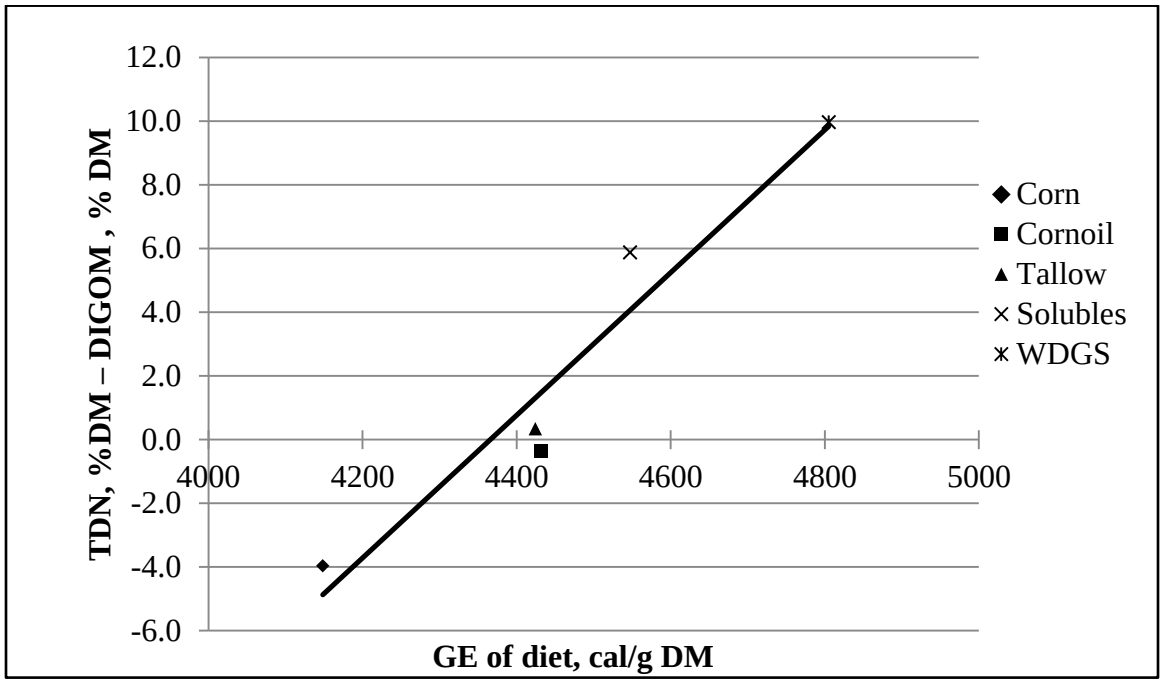

Figure 3.6 GE vs TDN-DIGOM in five finishing diets. Corn Oil (squares), Tallow (triangles), Solubles (exes), WDGS (asterisks), and Corn (diamonds) data are shown in the graph where individual data points indicate treatment average as the experimental unit. The regression equation for the all treatments combined was GE = [41.5 (±6.60) × TDN-DIGOM] + 4373 (±36.1) cal/g (r^2 =0.930).

Chapter IV: Evaluation of the Utility of Plant-waxes to Predict Forage Intake in Grazing Cattle

H. C. Hamilton[*], J. D. Volesky†, R. M. Lewis*

[*]Department of Animal Science, University of Nebraska-Lincoln Lincoln, NE 68583

† Department of Agronomy and Horticulture, West Central Research and Extension Center, North Platte, NE 68588

Abstract

Although key to the efficiency of a cattle operation, feed intake is challenging to evaluate in a grazing setting. Plant-waxes are a complex mixture of lipids found on the surface of plants. When sufficiently unique among plants, the composition of diets can be determined from the pattern of these compounds in the forages ingested. Therefore, even within forage-based systems, plant-wax markers may be used to estimate dietary choices and feed intake. *n*-Alkanes and long-chain alcohols were used as markers to attempt to delineate the parts of the corn plant and, separately, 8 western rangeland grasses and legumes at 2 stages of growth (peak vegetative and maturity). These markers were transformed into a set of linearly uncorrelated variables using principal component analysis (PCA). Based on PCA over 98% of the variation among different parts of the corn plant could be described within a 2-dimensional plane, with clear separation of those plant-parts. This technique therefore could be useful in a monoculture, such as a corn residue field, to determine the plant parts predominating in the diet. The PCA for the 8 species of the western rangeland had less distinctive separation with only 90.5 or 93.2% of the differences described 2-dimensionally, depending on the growth stage. Delineating plants in a complex sward was more difficult, particularly among like species. The use of more markers may help to more clearly distinguish plants within western rangelands. Alternatively, grouping plants into relevant categories, such as C3 and C4 grasses, may be sufficient to predict forage intakes.

Key words: feed intake, long-chain alcohols, *n*-alkanes, plant-wax markers, prediction

Introduction

Within the beef and dairy industry, determining the factors that affect animal intakes is important. One approach for doing so is based on amount and composition of plant-wax markers. Plants contain a complex mixture of aliphatic lipid compounds on their external surface that are essentially inert within the digestive system of cattle. Of particular interest are the *n*-alkanes (**ALK**; saturated straight-chain hydrocarbons) and long-chain alcohols (**LCOH**). The concentrations of these compounds can differ greatly among plant species, and even among plant parts, providing a marker profile or signature of a plant. When these profiles are distinctive enough, the composition of cattle diets can be determined from the pattern of these compounds in the forages ingested. The number of plants that can be delineated depends on the number and profiles of ALK and LCOH measured in the individual plants or plant-parts. It has been suggested by Bungalho (2004) and Ali (2005) that there are large between-species differences for ALK and LCOH concentrations making plant profiles markedly different. However, as the complexity of a sward increases, such as within a mixed grassland, the number of markers needed to distinguish plants increases. The objective of this study was to assess the ability to delineate the plant composition of corn residue and of a diverse western rangeland using ALK and LCOH as plant markers.

Materials and Methods

Corn Plant Collection and Preparation

Cob, stalk, husk and leaf samples were taken from 238 endrow plants from a 40 ha irrigated corn field located at the Eastern Nebraksa Research and Development Center located near Mead, NE. Ears and leaf blade were removed on site prior to transport to prevent loss. Stalks were cut at the top of the crown roots and bundled. Leaves and stalks were stored to air dry in an open air barn. Ears were husked and separated. Samples were bagged and left open inside a climate controlled building to allow the plant parts to dry. Stalk, cob, and leaf samples were all chopped using an "Ohio" Ensilage Cutter (The Silver Mfg Co., Salem, OH) and ground further through a Wiley Mill (Thomas Scientific, Swedesboro, NJ) using 1-mm screen (McGee, 2013). Samples were then composited plant part.

Western Rangeland Collection and Preparation

Forage samples were collected at the West Central Research and Extension Center (WCREC) in North Platte, Nebraska. Collection sites were primarily native mixed-grass rangeland within the rolling plains and breaks of Major Land Resource Area 73. Ecological sites included loamy upland, loamy lowland and loess breaks (Table 4.1). The forages were 3 cool-season (C3) grasses (cheatgrass (*Bromus tectorum*); needle-and-thread (*Hesperostipa comata*); western wheatgrass (*Pascopyrum smithii*)), 3 warm-season (C4) grasses (blue grama (*Bouteloua gracilis*); little bluestem (*Schizachyrium scoparium*); sideoats grama (*Bouteloua curtipendula*)), and 2 legumes (leadplant (*Amorpha canescens*); sweet clover (*Melilotus officinalis*)). Forage samples were collected at peak vegetative and mature states between late-April and late-August 2015. Peak vegetative stage of growth was defined as just before stem elongation for the

grasses and before flowering for the legumes. At the mature stage, grasses were fully headed and beginning seed ripening. Legumes were past flowering and in seed development.

Robust plants were clipped at ground level, bagged in a Ziploc bag, and shipped overnight in Styrofoam coolers with icepacks. All samples were prepared in the Ruminant Nutrition Laboratory at the University of Nebraska-Lincoln. Upon arrival samples were visually divided in half. One half of the sample was placed in a 60 ºC forced air oven for 48 h to determine dry matter. The other half of the sample was separated by hand into leaf and stem. Depending on the stage at which the plant was collected the reproductive portion was additionally divided. All separated fractions were placed in a 60 ºC forced air oven for 48 h to determine dry matter. After drying all samples were removed from the oven and ground through a Wiley Mill using a 1-mm screen (Thomas Scientific, Swedesboro, NJ).

Analysis of n-alkanes

Extractions were performed in duplicate (Mayes and Dove, 2006). Ground samples of 0.200-0.204 g were weighed into 16 mm X 100 mm borosilicate glass culture tubes fitted with screw caps lined with PTFE inserts. Docosane (C_{22}) and tetratriacontane (C_{34}) were added by weight at a concentration of 0.3 mg / g to serve as internal alkane standards. An internal alcohol standard, *n*-heptacosanol (1-C_{27}-ol) at a concentration of 1.5 mg / g was added by weight. Two mL of 1 M ethanolic KOH solution (97% ethanol: 3% deionized water, v / v) was added to each tube. Tubes were capped securely to ensure a

complete seal and heated overnight (16 h) at 90 ºC in a dry-block heater (Techne DB-3, Techne Ltd., Duxford, Cambridge, UK).

After heating samples were allowed to cool to 50-60 ºC. Aliquots of 2 mL of *n*-heptane and 0.6 mL of distilled deionized water were added to each tube. Tubes were mixed using a Vortex Maxi Mix I for 5 s (Thermo Fisher Scientific, Waltham, MA). The top (non-aqueous) layer was aspirated from the tube using a polyethylene transfer pipette and transferred to a 4 mL glass vial. A second 2 mL administration of heptane was added to the tubes, mixed, and aspirated into the same 4 mL vial. Vials were then placed in a dry-block heater under a Techne Sample Concentrator with a gentle flow of air to dry vials (Bibby Scientific Limited, Staffordshire, OSA, UK).

Vials were reconstituted with 0.3 mL of heptane and rolled to ensure all sample was dissolved from the sides of the glass. The sample was aspirated with a polyethylene transfer pipette and transferred to an ISOLUTE Single fritted reservoir, or column (3 mL 20 µm PE, Biotage LLC, Charlotte NC), containing a 1 mL bed volume of silica-gel (70-230 mesh, Fisher Scientific, Fairlawn, NJ). The sample was first eluted, during solid phase extraction, with 2 administrations of 1.5 mL of heptane to collect hydrocarbons. Secondly, crude alcohol fractions were eluted by a following 2 administrations of 1.5 mL of heptane/ethyl-acetate (80:20 v / v). Hydrocarbon and crude alcohol fractions were then placed in a dry-block heater under a Techne Sample Concentrator with a gentle flow of air to dry vials. Using the crude alcohol vials, the LCOH fractions were separated from the sterol/stanol fractions by a second solid phase extraction and heptane elution. A volume of 100 µL of warmed saturated urea in ethanol was added to a 1 mL column,

double fritted, (Biotage LLC, Charlotte NC) and sealed with a luer mat (SPEware, Baldwin Park, CA). The crude alcohol vials were reconstituted with 200 µL of heptane. An aliquot of 50 µL was transferred to the column. Columns were placed in a 70 °C oven and the oven was immediately turned off. After 20 min in the oven, columns were removed from the oven and placed in a fume hood to evaporate overnight. The next day, sterol/stanol fractions were eluted first by adding 0.5 mL to each column, a total of 3 times. The columns were then purged with 60 psi airflow for 5 min and rinsed with 4 rounds of 0.5 mL of distilled water. The LCOH fractions were then derivatized by heating at 60 ºC on a dry block heater, overnight, in 200 µL of a pyridine/acetic anhydride solution (5:1 v / v). *n*-Alkane elutes and LCOH fractions were evaporated to dryness, and then re-dissolved in 200 µl of *n*-dodecane for chromatographic analysis.

Quantification of ALK and LCOH was carried out by gas chromatography (GC), using an Agilent 7820A GC (Agilent Technologies, Inc., Santa Clara, CA). *n*-Alkane and LCOH extracts were injected (0.5 µl) via a 7650A Series auto-sampler through a splitless liner into a bonded-phase, non-polar column (Agilent J&W DB-column, 30-meter, 0.530 mm internal diameter and 0.5 µm film thickness). Helium functioned as the carrier gas at a constant flow of 4 mL / min. Temperature programming was: 280 °C for the injector; 340 °C for the detector; and, 170 °C for 4 min for the column oven followed by a first ramp of 30 °C / min to 215 °C with a 1 min hold, and then a second ramp of 6 °C / min to 300 °C with a 20 min hold. Samples of an ALK and LCOH standard solution mixture (C_{21} to C_{36}; $C_{20}OH$ to $C_{30}OH$, Sigma Aldrich, St. Louis, MO) were included in the GC analyses to determine peak identification and standard response factors. Chromatograph

data were analyzed using Agilent ChemStation software (Rev. B.04.02 SP1). Peak areas were determined with auto-integration and manual review of chromatograms. *n*-Alkane and LCOH concentrations were calculated relative to known amounts of the internal standards (C_{22}, C_{34}, and $C_{27}OH$), according to the equations outlined by Mayes and Dove (2006).

Potential differences between the concentration of individual ALK and LCOH within plant or corn plant part were tested using the using the MIXED procedure in SAS 9.3 (SAS Inst., Inc., Cary, N.C.). The model fitted was:

$$y_{ijk} = \mu + P_i + E_{(i)j} + \varepsilon_{ijk}$$

where y_{ijk} is the ALK or LCOH concentration in plant or plant part (i = 1, …, 8, for the 8 plants or i = 1, …, 5, for the 5 corn plant parts) for extraction $E_{(i)j}$ (j = 1 or 2, for the 2 extractions for each plant) with μ the overall mean concentration. Plant was fitted as a fixed effect. Random effects were extraction nested within operator ($E_{(i)j}$) and the residual (ε_{ijk}).

Potential differences between the concentration of individual ALK and LCOH were tested using the using the MIXED procedure in SAS 9.3 (SAS Inst., Inc., Cary, N.C.). The model fitted was:

$$y_{ijk} = \mu + P_i + S_j + (PS)_{ij} + \varepsilon_{ijk}$$

where y_{ijk} is the ALK or LCOH concentration in plant (i = 1, …, 8, for the 8 plants) for stage S_j (j = 1 or 2, for the 2 growth stages) with μ the overall mean concentration. Plant,

stage, and their interaction [$(PS)_{ij}$], was fitted as fixed effects. The random effect was the residual (ε_{ijk}).

Statistical analyses were conducted using GenStat for Windows 17th Edition to create biplots based on principal component analysis (**PCA**) of the ALK and LCOH concentrations of the relevant plants or plant-parts. For the corn plant parts, concentrations of 4 ALK (C_{27}, C_{29}, C_{31} and C_{33}) and 3 LCOH concentrations ($C_{26}OH$, $C_{28}OH$ and $C_{30}OH$) were used. For the plant species in the western rangelands, an additional ALK (C_{35}) and 2 additional LCOH were used ($C_{24}OH$ and $C_{32}OH$) as markers given the greater complexity of plant mixture. The concentrations of $C_{24}OH$ and $C_{32}OH$ were estimated from nearby standard response factors. Because the concentrations of ALK and LCOH differed appreciably among the plant species, the concentrations were normalized to a unit scale within ALK and within LCOH by dividing individual concentrations by their respective sum.

Results

Corn Plant

The ALK and LCOH concentration of the 5 corn plant parts are provided in Table 4.2. There was large variation in the plant-wax content of the plant parts. The concentration of C_{27} was relatively low in all parts. The C_{26}-OH compound was predominant in the husk compared to all other parts ($P < 0.01$). The leaf of the plant had consistently greater concentrations of all compounds, excluding $C_{26}OH$ ($P < 0.01$). Grain, stalks and cobs seemed to have the least overall concentrations of all compounds. Based on the PCA, 79.9% of the variation between plant parts was described by the first

principal component (**PC**; x-axis; Fig.4.1). An additional 18.1% was defined by the second PC (y-axis), with effectively all variation (98%) among plant parts being explained by just these first 2 PC.

Western Rangeland

The ALK and LCOH concentration of the 8 plant species found in western rangelands at their peak vegetative and mature states are provided in Tables 4.3 and 4.4, respectively. There was large variation in the plant-wax content of plants within and across growth stages (Fig. 4.2; Fig. 4.3). Leadplant and sweet clover contained greater concentrations of C_{29} during both vegetative and mature states ($P < 0.01$). Blue grama had greater concentrations of C_{33} when compared to other plants ($P < 0.01$). All plants had low concentrations of C_{35}.

Concentrations of LCOH, when present, were considerably greater than ALK concentrations. The concentration of C_{28}OH was greatest in cheatgrass and needle-and-thread at maturity ($P < 0.01$). The compound C_{32}OH only appeared at extremely high concentrations in vegetative and mature warm-season grasses (blue grama, little bluestem and sideoats grama).

The PCA for vegetative plants showed that 55.3% of the variation between plant parts was described on the first PC (x-axis; Fig. 4.4). An additional 35.2% was defined on the second PC (y-axis) for a total of 90.5% of variation being defined by the first 2 components. For mature plants, 65.8% of the variation was described by the first PC, followed by 27.4% on the second PC, totaling 93.2% of the variation being defined along the first 2 axes (Fig. 4.5).

When evaluated by plant-part, there were appreciable differences between plant species ($P < 0.01$) and part by growth stage ($P < 0.01$). Blue grama, little bluestem and sideoats grama, all C4 grasses, were the only forages that contained $C_{32}OH$ in the leaf portion in both vegetative and mature states (Fig. 4.6; Fig. 4.7).

At maturity, the reproductive part of the C4 grasses contributed to the greater ($P < 0.01$) concentrations of $C_{32}OH$ found in the whole plants (Table 4.4; Fig. 4.8). In sweet clover and leadplant, C_{29} concentration was predominant in their reproductive portion compared to other plants. Alternatively, C_{27} was greatest for little bluestem and western wheatgrass ($P < 0.01$). For the long-chain alcohols, $C_{28}OH$ was predominant in needle-and-thread, cheatgrass and leadplant compared to other plants. However, sweet clover and western wheatgrass had greater concentrations of $C_{26}OH$ in their reproductive portions ($P < 0.01$).

The stem portion followed similar trends as the leaf and reproductive portions where blue grama, little bluestem, and sideoats grama were the only forages that contained $C_{32}OH$ at both stages of maturity (Fig. 4.9, 4.10). Sweet clover had very high concentrations of C_{29} at both the mature and vegetative states. Little bluestem and blue grama had greater amounts of C_{31} at both stages than the other plants, with western wheat grass having more in the vegetative state. Similar to the reproductive portion, the stem had high levels of $C_{28}OH$ for cheatgrass and needle-and-thread in the mature state, but all forages had high levels of $C_{28}OH$ in the stems in the vegetative state.

Discussion

To distinguish plants based on their plant-wax profiles, the patterns of these markers must be sufficiently different. As seen previously by López López (2015), large variations between plant species in their LCOH profiles made their inclusion as additional markers useful for greater delineation among plant species. However, the addition of LCOH as markers has not always improved diet composition predictions (Vargas Jurado, 2015). In the current study, corn and western range forages did appreciably differ in their LCOH profiles, justifying their inclusion as plant markers.

Using PCA, plant parts of corn and some plant species of western range could be discriminated based on their ALK and LCOH concentrations. The distribution of the 8 forage species in a 2-dimensional space clearly shows the ability to discriminate legumes from grasses. It has been suggested that ALK and LCOH may discriminate better between components (Dove and Mayes, 2005). The greater concentrations of C_{29}, C_{24}OH and C_{26}OH in legumes resulted in their clustering. Greater concentrations of C_{32}OH made C4 grasses stand out, particularly blue grama that also had greater concentrations of C_{33}. Stronger separation of the grasses was captured by the second PC, but they still could not be clearly differentiated. Cheatgrass, western wheatgrass, little bluestem, and sideoats grama clustered together and were not separable based on their ALK and LCOH profiles alone.

The parts of the corn plant were clearly more discernable. Leaf had greater concentrations of C_{28}OH and C_{30}OH making its cluster very distinct. Husk and cob clusters were also distinct from the other plant-parts with greater concentrations of C_{29};

however, husk and cob were themselves distinct due to their different C_{31} and $C_{26}OH$ concentrations. Stalk and grain were the most closely related, with greater concentrations of C_{27}. However, stalks contained more C_{33} allowing it to appear separate from grain. Using ALK and LCOH concentrations, the corn plants parts could be clearly delineated.

Plant-wax markers in this context could be utilized to improve management strategies for residue grazing. Predicting dietary choices may give insight into how an animals choices change over time. With an animal grazing corn residue, the animal first chooses the more digestible parts of the corn plant and moves to less digestible portions once the other portions have become less available. These choices could be more closely determined with the use of plant-waxes. Because we are able to tell the different portions of the corn plant apart, it is likely these differences would continue to be evident in the feces. Knowing diet composition could allow producers to make informed decisions about when to move cattle based on changes in their dietary choices.

Available forage is always changing, so it is important when using plant-wax markers to collect a sample of the forages on offer. Large variation in ALK and LCOH concentrations between growth stages of different plant species makes creating a universal database impractical. This means that forage samples need to be representative of the forage the animal is going to be able to consume in the given time of fecal sampling. Depending on the degree of change between sampling, more frequent sampling may be required in order to adequately represent the available forage.

In this study, specie-specific profiles of the plant-wax markers were not unequivocally distinct leading to possible difficulty distinguishing among plants

comprising a complex western rangeland. To overcome this difficulty, as is suggested by Dove and Mayes (2005), decreasing the number of dietary components by grouping species in the diet on a logical basis could improve matters. In a western rangeland it may be more useful to group forages together such as C3 grasses, C4 grasses, and legumes (Kelman, 2003). It then would become easier to delineate those groups from one another vs. individual plant species.

Alternatively, or perhaps even in conjunction with grouping like forages, increasing the number of 'discriminators' could be used to help tell forages apart (Dove and Mayes 2005). This could be achieved by increasing the number of ALK and LCOH markers being used, which would likely improve the ability to discriminate more plants. Likewise, using other plant-wax markers like alkenes or long-chain fatty acids in combination with ALK and LCOH could improve the distinctiveness of individual plant profiles (Kelman, 2003; Ali, 2005; Ferreira, 2010).

In conclusion, plant-waxes appear useful for assessing dietary choices in cattle grazing a monoculture like corn residue. Such information may benefit management decisions, including deciding when animals should be moved to alternative grazing areas. However, to delineate choices in a complex sward such as western rangelands, more plant markers will be needed to more clearly distinguish plant species.

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Table 4.1 Collection data

Date Collected	Plant Name[1]	Phenology[2]	GPS Coordinates	Elevation	Soil Description
4/22/15	CG	veg	41°05’17” 100°45’56”	2831	Cozad silt loam 1-3% slope
5/4/15	CG	mat	41°05’17” 100°45’56”	2832	Cozad silt loam 1-3% slope
5/12/15	NT	veg	41°04’45” 100°4’09”	2933	Coly silt loam 17-30% slope
5/12/15	WW	veg	41°04’45” 100°4’09”	2933	Coly silt loam 17-30% slope
5/29/15	SC	veg	41°03’33” 100°45’44”	3025	Valent sand, rolling
6/9/15	NT	mat	41°04’45” 100°4’09”	2933	Coly silt loam 17-30% slope
6/9/15	LB	veg	41°04’42” 100°46’09”	2952	Coly silt loam 17-30% slope
6/15/15	BG	veg	41°04’42” 100°46’09”	2952	Coly silt loam 17-30% slope
6/15/15	SG	veg	41°04’42” 100°46’09”	2952	Coly silt loam 17-30% slope
6/16/15	LP	veg	40°58’24” 100°45’38”	3045	Valent sand, rolling
7/1/15	WW	mat	41°04’45” 100°4’09”	2933	Coly silt loam 17-30% slope
7/1/15	SC	mat	41°03’33” 100°45’44”	3025	Valent sand, rolling
7/21/15	LP	mat	40°58’24” 100°45’38”	3045	Valent sand, rolling
7/31/15	BG	mat	41°04’42” 100°46’09”	2952	Coly silt loam 17-30% slope
7/31/15	SG	mat	41°04’42” 100°46’09”	2952	Coly silt loam 17-30% slope
8/19/15	LB	mat	41°04’42” 100°46’09”	2952	Coly silt loam 17-30% slope

[1] 1The forage species were legumes: Sweet Clover (SC) and Leadplant (LP); C3 grasses: Cheatgrass (CG), Needle-and-Thread (NT), and Western Wheatgrass (WW); C4 grasses: Blue Grama (BG), Little Bluestem (LB), and Sideoats grama (SG)
[2]veg: vegetative; mat: mature

Table 4.2 Mean (mg kg^{-1}) *n*-alkane and long-chain alcohol concentrations (mg kg^{-1} DM) for corn plant parts.

	Plant part						
	Cob	Husk	Leaf	Stalk	Grain	SEM	*P* – value[1]
n-alkanes							
C_{27}	4.60^{b}	4.25^{b}	9.29^{a}	2.59^{d}	2.99^{cd}	0.75	< 0.01
C_{29}	11.1^{c}	17.1^{b}	30.1^{a}	3.21^{de}	2.27^{e}	0.83	< 0.01
C_{31}	7.41^{c}	19.5^{b}	56.5^{a}	3.65^{de}	2.29^{e}	0.85	< 0.01
C_{33}	5.42^{c}	9.80^{b}	45.6^{a}	4.77^{c}	3.31^{d}	0.58	< 0.01
Long-chain alcohols							
C_{26}OH	19.7^{c}	70.3^{a}	54.9^{b}	20.1^{c}	18.3^{c}	0.93	< 0.01
C_{28}OH	3.23^{c}	25.7^{b}	57.2^{a}	5.10^{c}	2.24^{dc}	1.0	< 0.01
C_{30}OH	3.87^{d}	30.9^{b}	74.3^{a}	5.52^{c}	4.34^{dc}	0.63	< 0.01

[abcd] From the F-test, means with differing superscripts are different (P < 0.05)

Table 4.3 Mean (mg kg^{-1}) n-alkane and long-chain alcohol concentrations for 8 forage species[1] at peak vegetative stages of growth.

	C3 grasses			C4 grasses			Legumes			
	CG	NT	WW	BG	LB	SG	LP	SC	SEM	*P* - value
n-alkane										
C_{27}	47.3^{b}	30.7^{d}	9.60^{h}	13.8^{g}	18.2^{f}	29.1^{e}	93.36^{a}	38.8^{c}	0.25	< 0.01
C_{29}	57.7^{d}	84.9^{c}	31.1^{f}	49.0^{e}	28.0^{f}	28.6^{f}	143.8^{b}	268.3^{a}	2.07	< 0.01
C_{31}	39.6^{f}	89.8^{b}	59.1^{c}	179.4^{a}	50.5^{e}	21.2^{g}	38.3^{f}	53.0^{d}	0.7	< 0.01
C_{33}	39.9^{b}	28.5^{c}	25.5^{d}	121.2^{a}	8.37^{g}	15.7^{f}	5.48^{h}	22.2^{e}	0.29	< 0.01
C_{35}	3.19^{d}	29.8^{a}	1.42^{e}	21.1^{b}	1.24^{e}	6.98^{c}	0.443^{f}	1.42^{e}	0.14	< 0.01
Long-chain alcohol										
$C_{24}OH$	351.2^{a}	0.00^{c}	0.00^{c}	0.00^{c}	0.00^{c}	0.00^{c}	193.0^{b}	188.6^{b}	6.52	< 0.01
$C_{26}OH$	74.0^{c}	84.8^{c}	40.9^{d}	0.00^{e}	44.2^{d}	77.7^{c}	658.2^{b}	2464^{a}	5.88	< 0.01
$C_{28}OH$	1309^{c}	4126^{a}	560.1^{d}	727.2^{d}	337.0^{e}	1269^{c}	2856^{b}	159.8^{e}	64	< 0.01
$C_{30}OH$	58.5^{f}	180.3^{cd}	29.5^{g}	141.2^{e}	196.2^{c}	157.7^{de}	1077^{a}	522.6^{b}	6.09	< 0.01
$C_{32}OH$	0.00^{d}	0.00^{d}	0.00^{d}	2703^{b}	5871^{a}	900^{c}	0.00^{d}	0.00^{d}	101.5	< 0.01

[abcd] From the F-test, means with differing superscripts are different (P < 0.05)

[1]The forage species were legumes: Sweet Clover (SC) and Leadplant (LP); C3 grasses: Cheatgrass (CG), Needle and Thread (NT), and Western Wheatgrass (WW); C4 grasses: Blue Grama (BG), Little Bluestem (LB), and Sideoats grama (SG)

Table 4.4 Mean (mg kg-1) n-alkane and long-chain alcohol concentrations for 8 forage species[1] at mature stages of growth.

	C3 grasses			C4 grasses			Legumes			
	CG	NT	WW	BG	LB	SG	LP	SC	SEM	*P* - value
n-alkane										
C_{27}	42.2^{cd}	30.7^{e}	50.9^{ab}	12.4^{f}	57.7^{a}	35.5d^{e}	48.9^{bc}	37.0^{de}	2.27	< 0.01
C_{29}	94.1^{d}	113.7^{c}	34.5^{f}	148.7^{b}	58.8^{e}	35.6^{f}	181.5^{a}	51.3^{e}	3.35	< 0.01
C_{31}	152.4^{c}	92.1^{d}	56.6^{e}	46.9^{e}	44.8^{e}	45.5^{e}	273.4^{b}	440.0^{a}	0.96	< 0.01
C_{33}	22.3^{bc}	22.4^{b}	6.26^{f}	75.1^{a}	18.9^{de}	19.4^{cd}	16.5^{e}	7.53^{f}	0.13	< 0.01
C_{35}	1.66^{e}	15.5^{b}	0.79^{g}	18.8^{a}	4.44^{c}	3.73^{d}	0.00^{h}	1.25^{f}	1.63	< 0.01
Long-chain alcohol										
C_{24}OH	77.9^{c}	0.00^{f}	0.00^{f}	28.6^{e}	68.9^{d}	26.1^{e}	234.8^{a}	219.1^{b}	27.6	< 0.01
C_{26}OH	144.4^{c}	271.0^{b}	28.7^{d}	0.00^{d}	140.5^{c}	324.5^{b}	757.4^{a}	689.7^{a}	35.14	< 0.01
C_{28}OH	5066^{b}	5934^{a}	39.3^{e}	111.9^{e}	134.7^{e}	1624^{d}	2057^{c}	108.1^{e}	7.65	< 0.01
C_{30}OH	72.0^{e}	168.6^{c}	21.6^{f}	125.4^{d}	120.4^{d}	262.3^{b}	537.6^{a}	116.1^{d}	6.15	< 0.01
C_{32}OH	0.00^{d}	35.5^{d}	0.00^{d}	11253^{b}	13200^{a}	1244^{c}	94.9^{d}	0.00^{d}	112.2	< 0.01

^{abcd} From the F-test, means with differing superscripts are different (P < 0.05)

[1]The forage species were legumes: Sweet Clover (SC) and Leadplant (LP); C3 grasses: Cheatgrass (CG), Needle-and-Thread (NT), and Western Wheatgrass (WW); C4 grasses: Blue Grama (BG), Little Bluestem (LB), and Sideoats grama (SG)

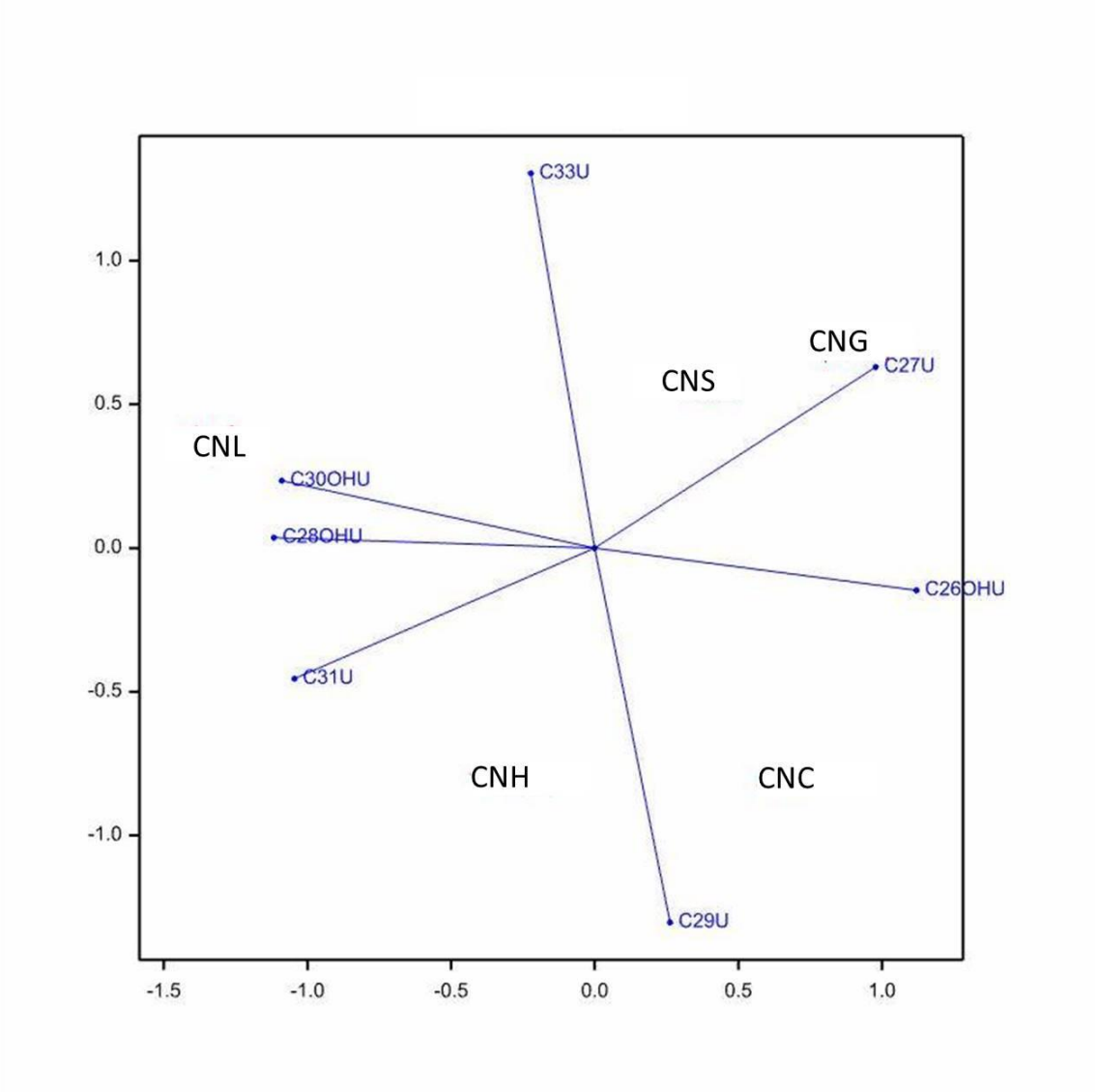

Figure 4.1 Biplot showing the 5 corn plant parts in a 2-dimensional space derived from principal component analysis. Based on concentrations of 4 *n*-alkanes (C27U, C29U, C31U and C33U) and 3 long-chain alcohols (C26OHU, C28OHU and C30OHU) once normalized to a unit scale. The corn plant parts were cob (CNC), leaf (CNL), husk (CNH), stalk (CNS) and grain (CNG).

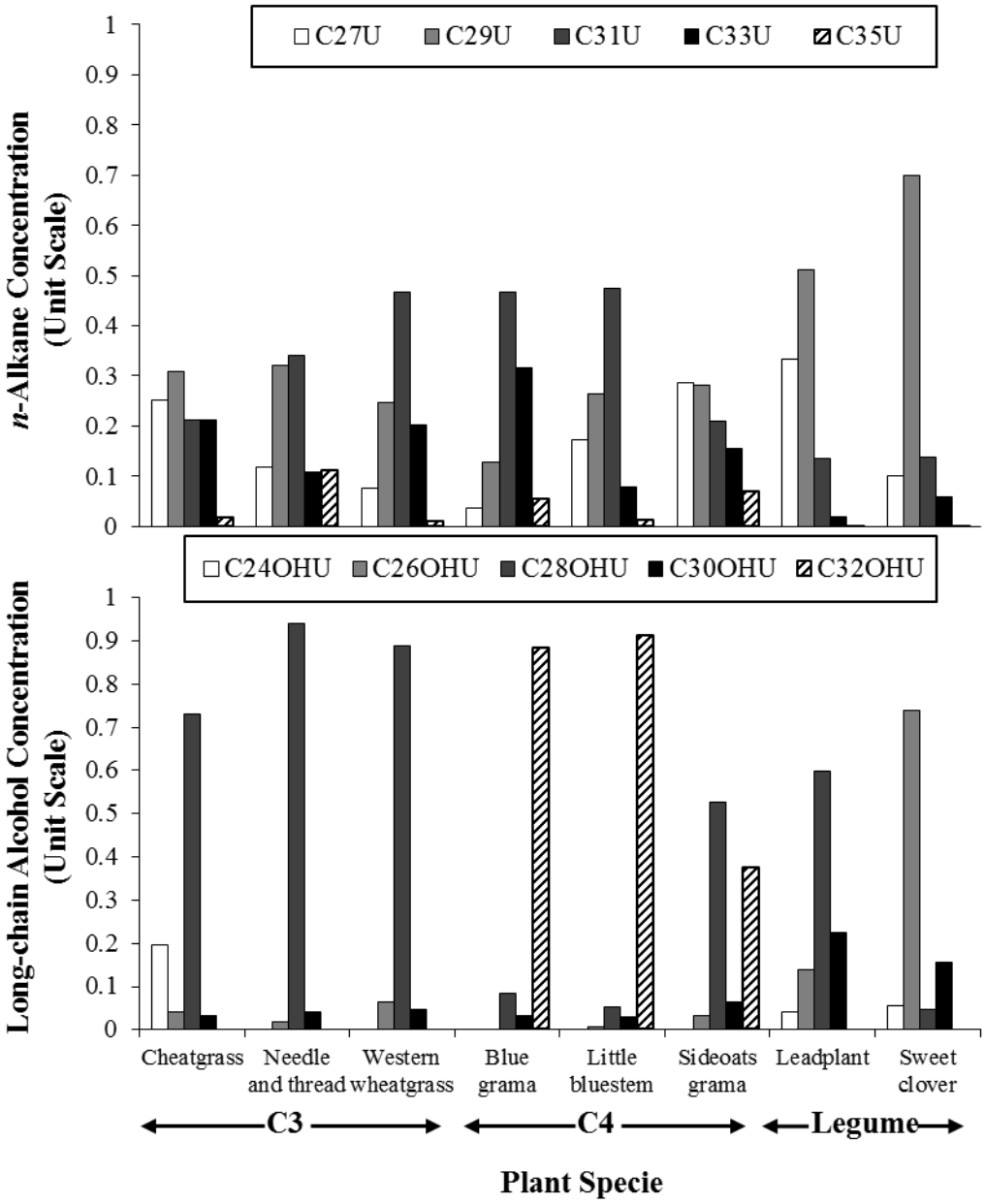

Figure 4.2 Mean *n*-alkane (C27U, C29U, C31U, C33U and C35U) and long-chain alcohol (C24OHU, C26OHU, C28OHU, C30OHU and C32OHU) concentrations, normalized to a unit scale, in the entire plant for 8 forage species at peak vegetative state.

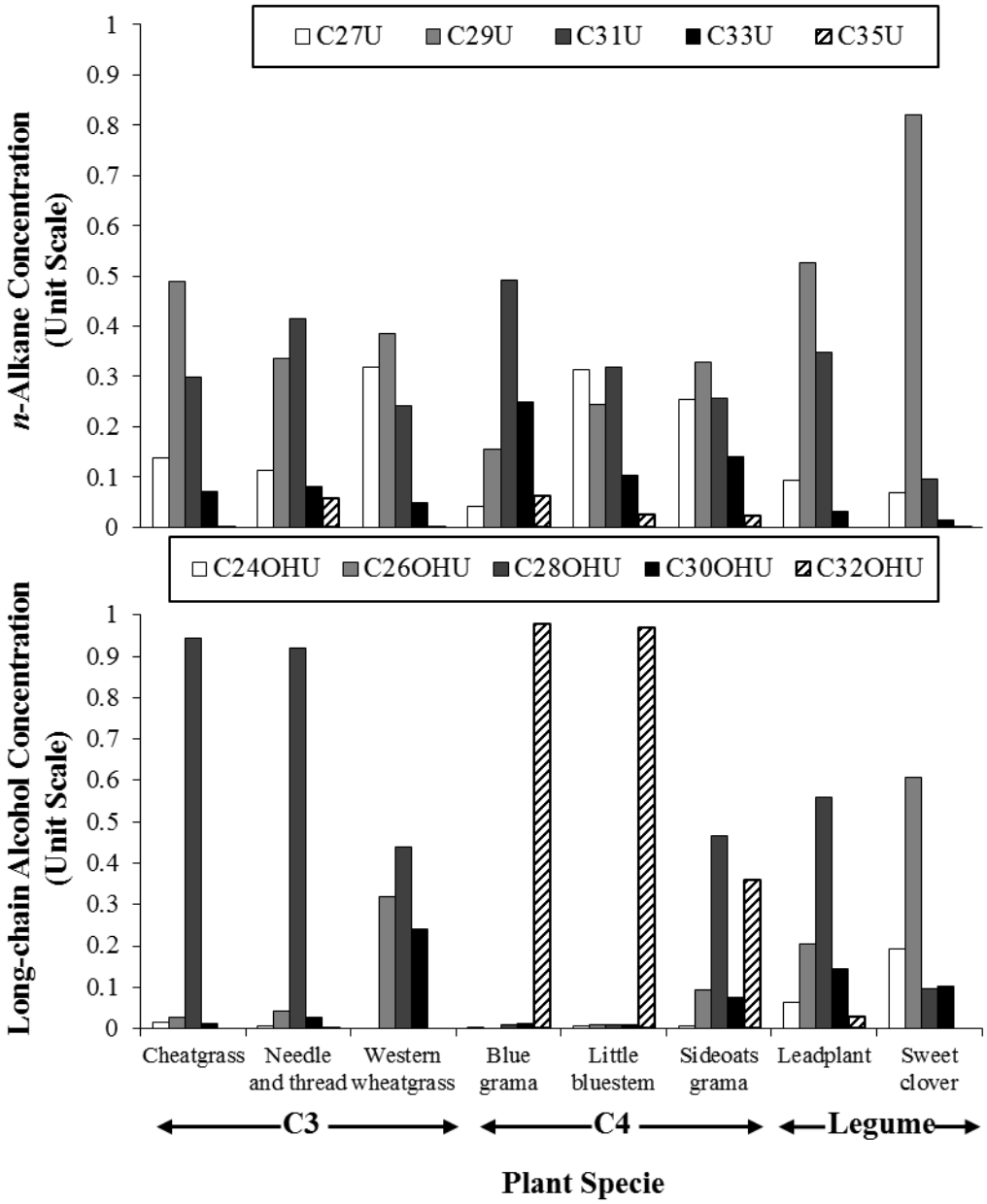

Figure 4.3 Mean *n*-alkane (C27U, C29U, C31U, C33U and C35U) and long-chain alcohol (C24OHU, C26OHU, C28OHU, C30OHU and C32OHU) concentrations, normalized to a unit scale, in the entire plant for 8 forage species at mature state.

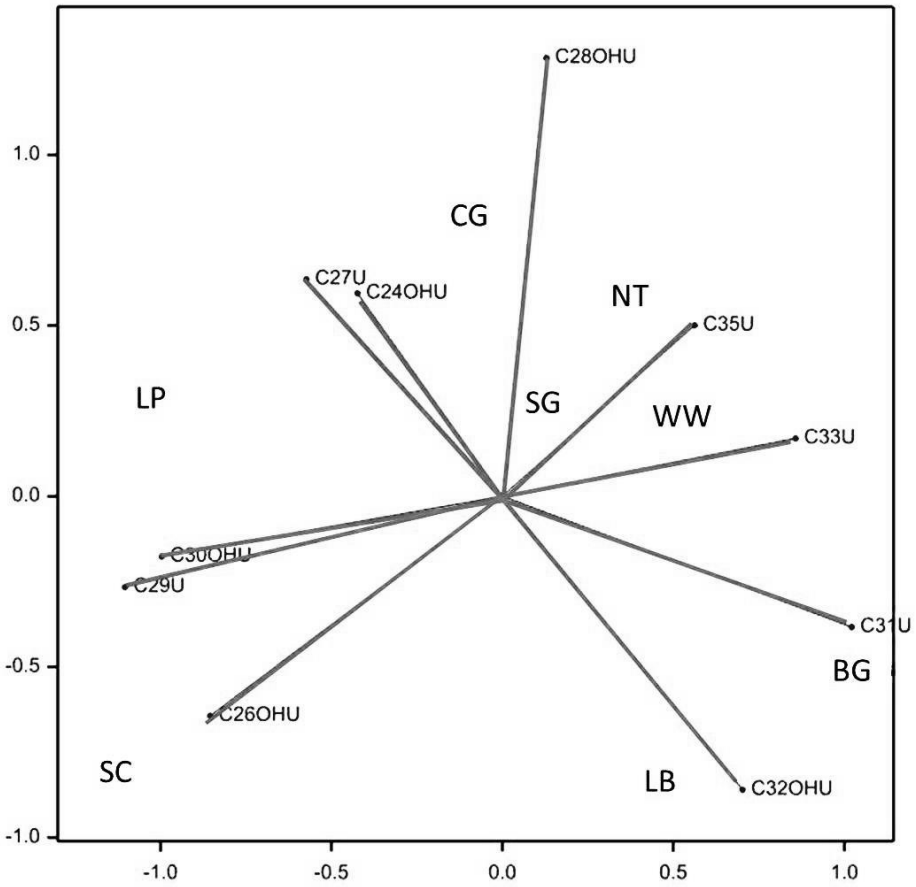

Figure 4.4 Biplot showing the 8 forage species at their peak vegetative state in a 2-dimensional space. Derived from principal component analyses based on concentrations of 5 *n*-alkanes (C27U, C29U, C31U, C33U and C35U) and 5 long-chain alcohols (C24OHU, C26OHU, C28OHU, C30OHU and C32OHU) once normalized to a unit scale. The forage species were C3 grasses [Cheatgrass (CG), Needle-and-Thread (NT), Western Wheatgrass (WW)], C4 grasses [Blue Grama (BG), Little Bluestem (LB), Sideoats grama (SG)], and legumes [(Sweet Clover (SC), Leadplant (LP)].

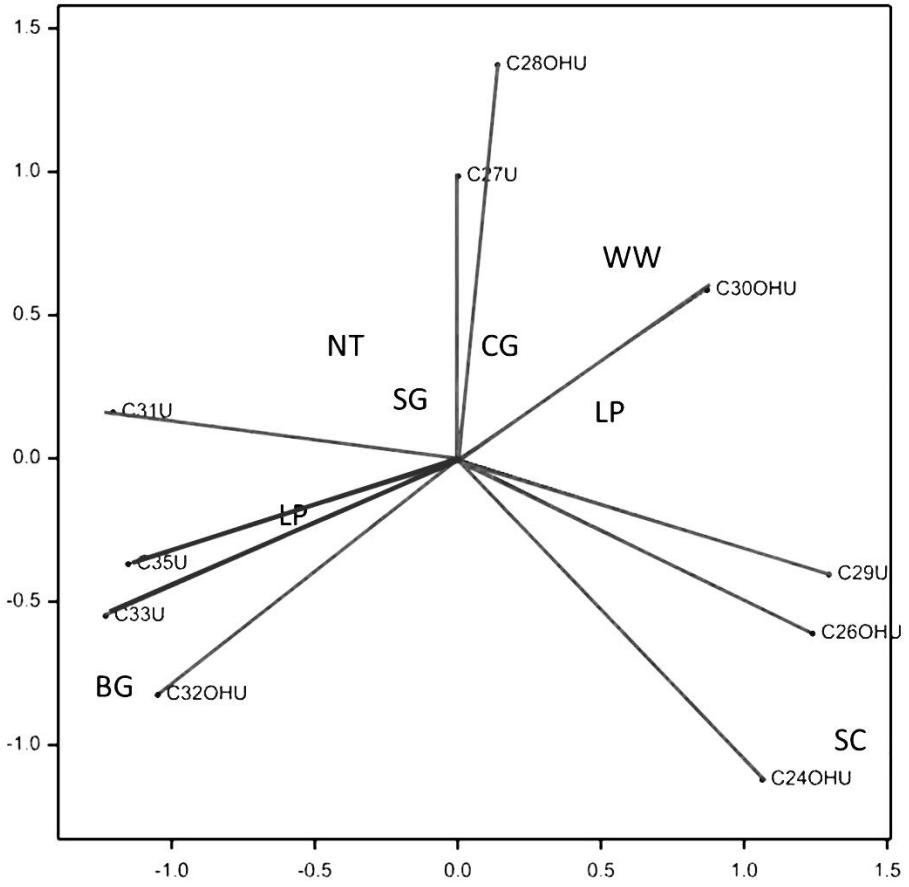

Figure 4.5 Biplot showing the 8 forage species at mature state in a 2-dimensional space. Derived from principal component analyses based on concentrations of 5 *n*-alkanes (C27U, C29U, C31U, C33U and C35U) and 5 long-chain alcohols (C24OHU, C26OHU, C28OHU, C30OHU and C32OHU) once normalized to a unit scale. The forage species were C3 grasses [Cheatgrass (CG), Needle-and-thread (NT), Western Wheatgrass (WW)], C4 grasses [Blue Grama (BG), Little Bluestem (LB), Sideoats grama (SG)], and legumes [(Sweet Clover (SC), Leadplant (LP)].

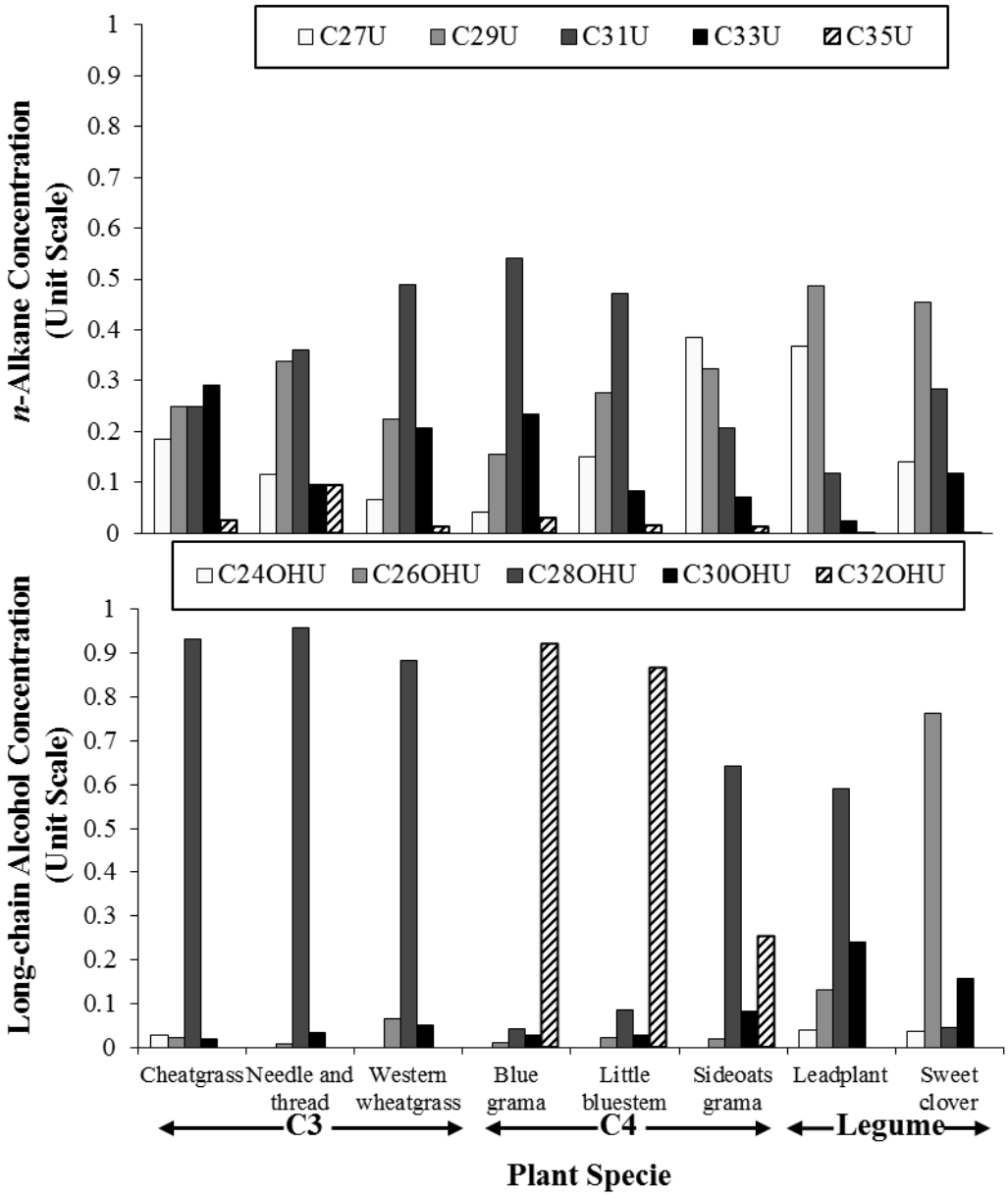

Figure 4.6 Mean *n*-alkane (C27U, C29U, C31U, C33U and C35U) and long-chain alcohol (C24OHU, C26OHU, C28OHU, C30OHU and C32OHU) concentrations, normalized to a unit scale, in the leaf portion for 8 forage species at peak vegetative state.

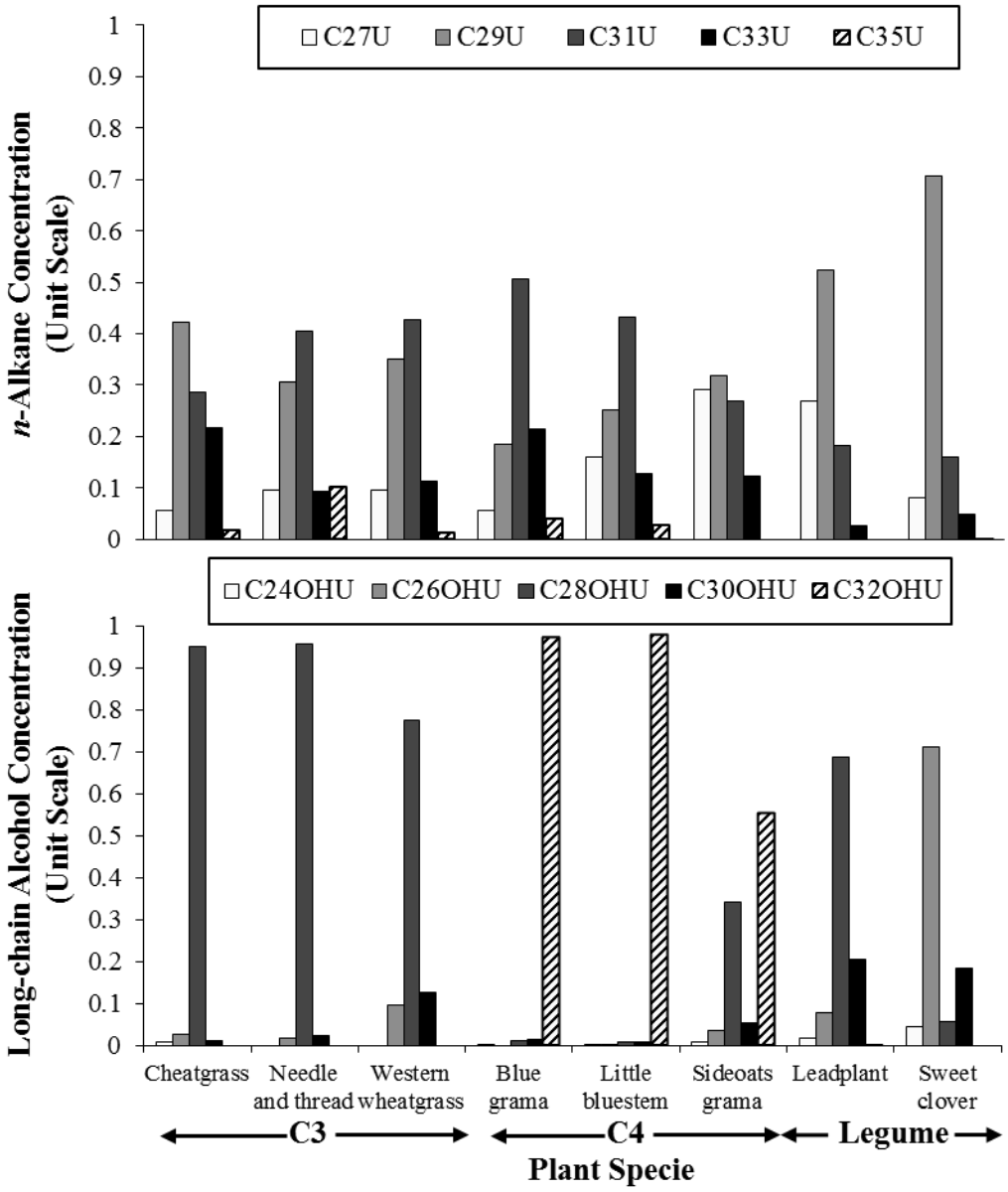

Figure 4.7 Mean *n*-alkane (C27U, C29U, C31U, C33U and C35U) and long-chain alcohol (C24OHU, C26OHU, C28OHU, C30OHU and C32OHU) concentrations, normalized to a unit scale, in the leaf portion for 8 forage species at mature state.

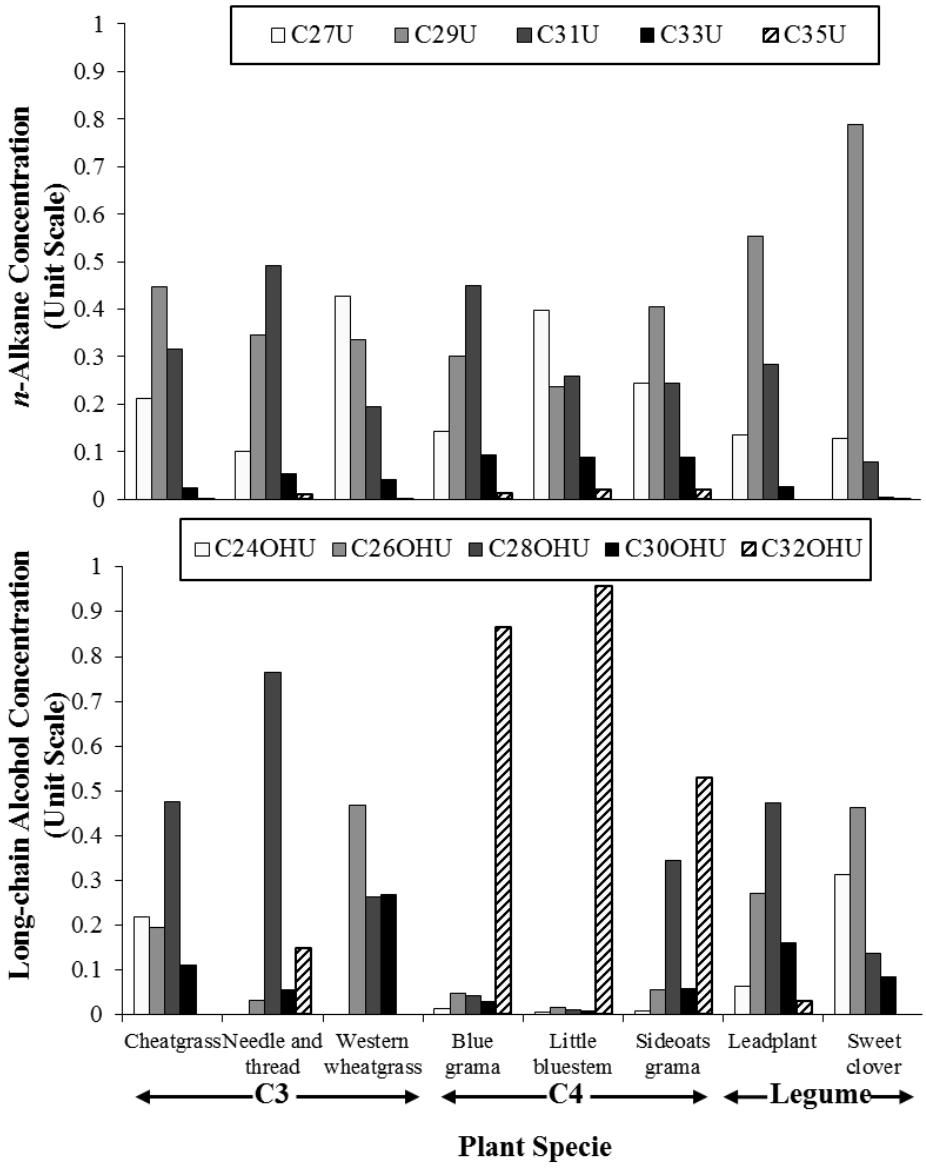

Figure 4.8 Mean *n*-alkane (C27U, C29U, C31U, C33U and C35U) and long-chain alcohol (C24OHU, C26OHU, C28OHU, C30OHU and C32OHU) concentrations, normalized to a unit scale, in the reproductive (flower/seed) portion for 8 forage species at mature state.

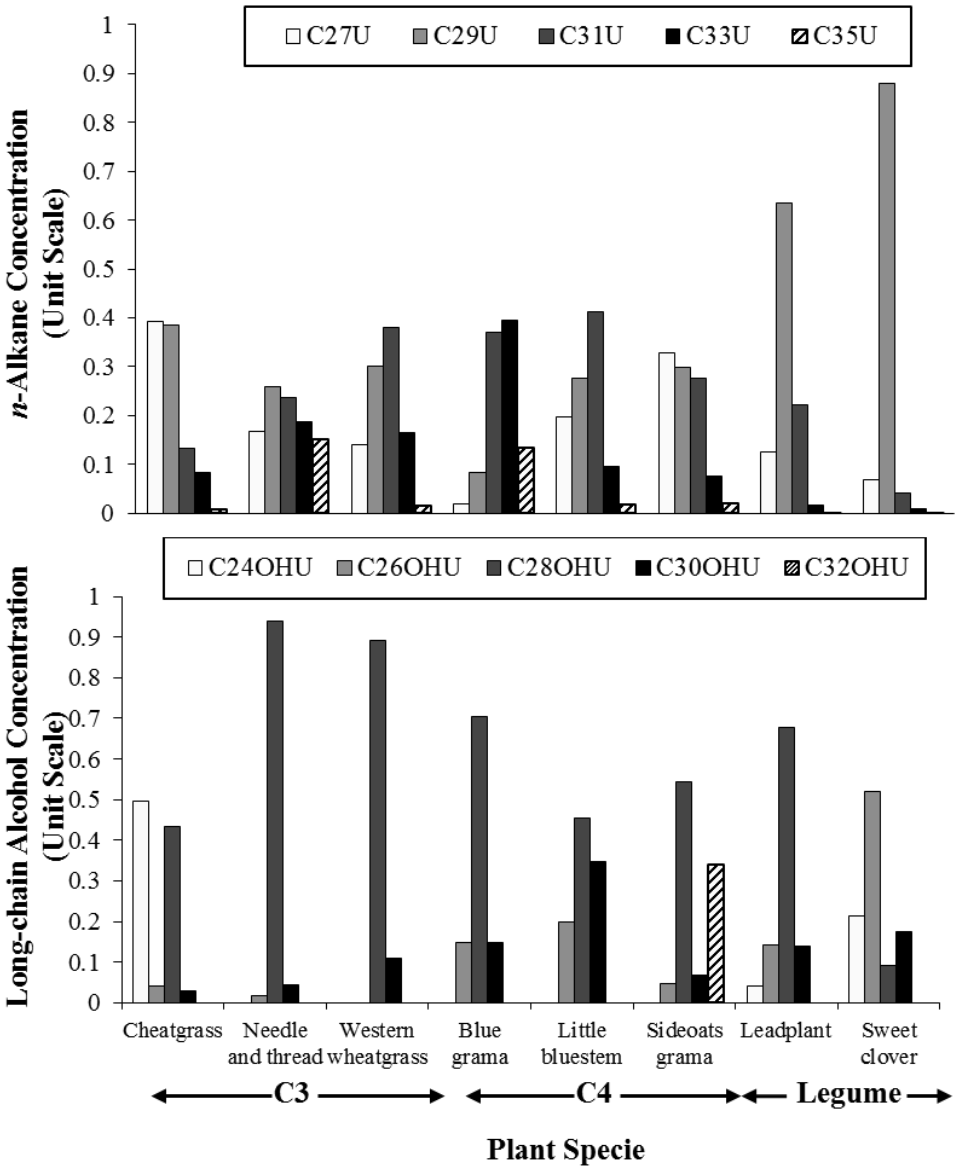

Figure 4.9 Mean *n*-alkane (C27U, C29U, C31U, C33U and C35U) and long-chain alcohol (C24OHU, C26OHU, C28OHU, C30OHU and C32OHU) concentrations, normalized to a unit scale, in the stem portion for 8 forage species at peak vegetative state.

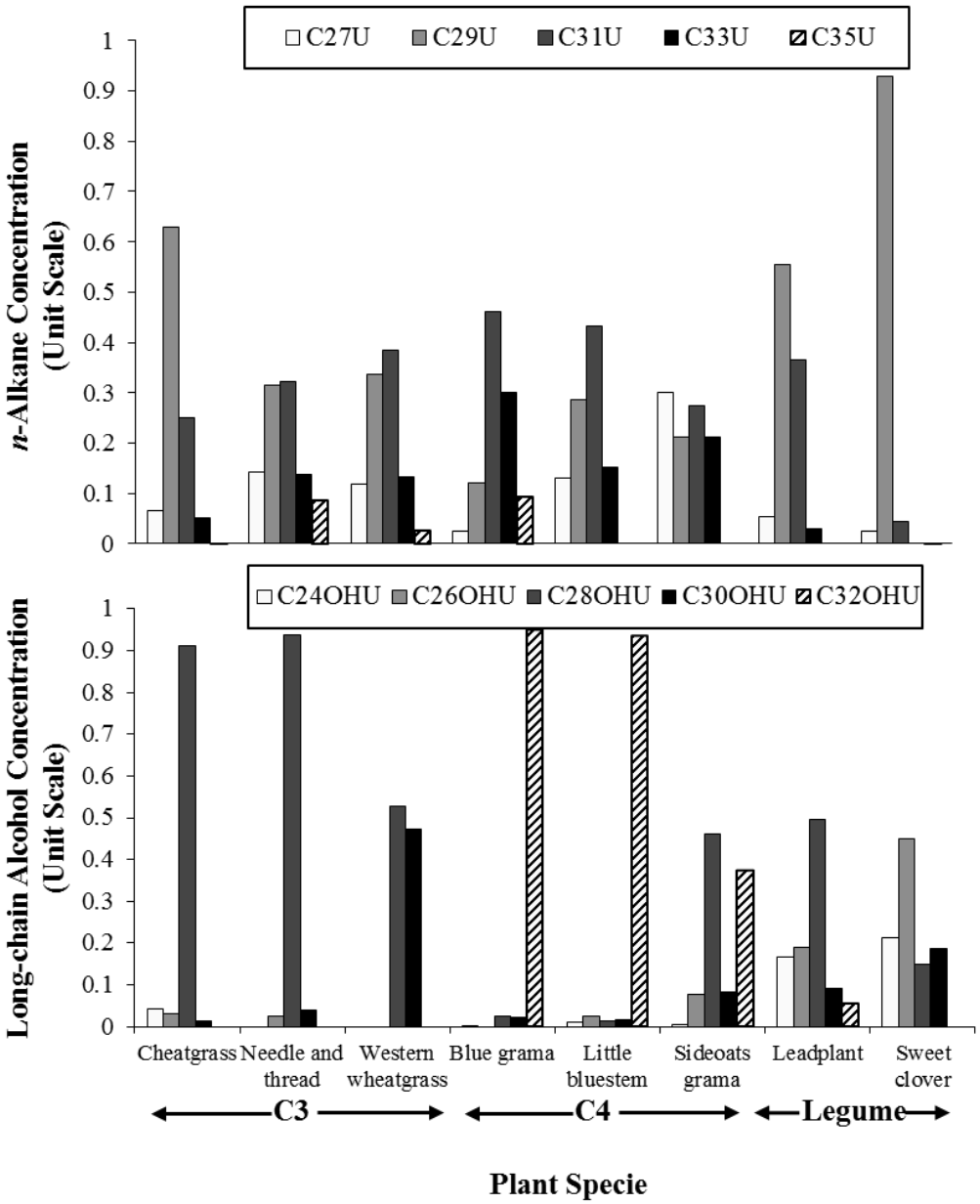

Figure 4.10 Mean *n*-alkane (C27U, C29U, C31U, C33U and C35U) and long-chain alcohol (C24OHU, C26OHU, C28OHU, C30OHU and C32OHU) concentrations, normalized to a unit scale, in the stem portion for 8 forage species at mature state.

Chapter V: Evaluation of Plant-waxes to Predict Intake in Individually Fed Cattle

H. C. Hamilton[*], H. Freetly †, R. M. Lewis*

[*]Department of Animal Science, University of Nebraska-Lincoln Lincoln, NE 68583

† Roman L. Hruska U. S. Meat Animal Research Center, USDA-ARS, Clay Center, NE 68933

Abstract

Determining feed intake of livestock in grazing settings can be difficult. A technique utilizing plant waxes, a complex mixture of lipids found on the surface of plants, could be influential to achieve that aim. To test the reliability of this methodology, *n*-alkanes were utilized in an indoor feeding study to predict intake. Twenty-six heifers (438.9 ± 10.1 kg BW) were individually fed a ration of 70% corn silage and 30% alfalfa, along with a daily dose of an *n*-alkane marker (C_{32}). Fecal samples were collected the last 5 d of feeding along with individual intakes. Feed and fecal samples were tested for marker concentrations. The relative concentrations of C_{33} or C_{31} in the herbages and fecal samples to that of the dosed C_{32} were used to predict intake. Predicted intakes were then regressed on measured intakes. Predictions based on explicitly modelling the 2 components of the diet offered were used as the benchmark. In that case, predicted intakes tended to be higher than actual intakes (slope 1.35 ± 0.21; r^2 0.623). However, if the diet was assumed to be a total mixed ration (TMR), predictions were more reliable with the slope of the line numerically closer to unity (slope 1.07 ± 0.17; r^2 0.623) but not statistically different ($P = 0.44$) than those obtained from the benchmark scenario. A sensitivity test was conducted to examine the effects of incomplete consumption of the C_{32} dose by the heifers by assuming either 97.5% or 95% was eaten. Predictions were improved, with slopes even closer to unity. To account for the relatively wide range in measured intakes, the regression of predicted on actual intakes was repeated with both expressed as natural logarithms. Although transformation improved predictions when considering the feed offered as a 2-component mixture, such was not the case when considering the feed offered as a TMR. At the accuracy obtained, predictions of intake

could be reliably made within 2 to 3 kg of their observed values. However, finer distinctions in intake may even be more difficult to achieve in an applied setting.

Key Words: feed intake, *n*-alkanes, plant-wax markers, prediction

Introduction

The landscape and climate of the west central U.S. provide a large opportunity for range and crop land to be utilized for grazing cattle. The nutritional value of these available forages is highly variable with nutrient quality and quantity often being the limiting factor in livestock production. Determining the dietary choices and intakes of livestock in these settings can be difficult. One approach for making these predictions is based on plant-wax markers. Plants contain a complex mixture of aliphatic lipid compounds on their external surface that are primarily inert within the digestive system of cattle. These waxes include the *n*-alkanes (ALK; saturated straight-chain hydrocarbons) and long-chain alcohols (LCOH). The concentrations of these compounds can differ greatly among plant species, and even among plant parts, providing a marker profile or signature of a plant (Mayes et al., 1986). Predicting intake using this method is based on the relative rather than absolute concentrations of fecal ALK. This is because fecal recoveries of adjacent longer-chain ALK have been shown to be very similar (Mayes et al., 1986; Dove and Mayes 1996). Therefore, intakes can be accurately predicted using the ratio of a dosed ALK (C_{32}) with that of two adjacent ALK (C_{31} or C_{33}) commonly found in herbages (Dove and Mayes 1996). To account for some differences in the recoveries of C_{31} and C_{33}, final intakes are often predicted by averaging predictions derived using both C_{31}:C_{32} and C_{33}:C_{32} ratios. The objective of this study was to test the utility of using plant wax markers to predict feed intakes in cattle under controlled (pen) conditions.

Materials and Methods

This study was conducted at the Roman L. Hruska U. S. Meat Animal Research Center (USMARC), Clay Center, NE. Animals were raised in accordance with the Guide for the Care and Use of Animals in Agricultural Research and Teaching (FASS, 2010), and their care was approved by both the USMARC and University of Nebraska-Lincoln (UNL) Animal Care and Use Committees. Laboratory analysis was conducted in the Ruminant Nutrition Lab at UNL.

Experimental layout

Twenty-four spring born commercial MARC II heifers were used in this study with an average body weight of 452.6 ± 35.2 kg. Animals were assigned randomly to 2 pens in a drylot. Each pen had an automatic water trough and was equipped with a Broadbent Feeding System (American Calan, Northwood, NH) with 12 doors.

At the start of the study, heifers were allowed a 20 d period to adjust to the facilities and feed. Doors remained unlocked, with heifers having access to any feed bunk. During this period, the heifers' preferences for particular doors for feeding were noted; when assignments to feeding bunks were made, those preferences were considered. Following the adjustment period (defined as d 1), heifers were fitted with a sensor key corresponding with a single feed door. The doors were locked restricting a heifer's access to her assigned gate. Heifers were allowed a further 14 d to fully acclimate to the feeding system. Data used in the analyses were collected for 10 d thereafter. In total, the study lasted 44 d.

Throughout the study, the heifers were carefully monitored (visually observed at least twice daily) to confirm their unrestricted access to feed and water.

Feeding strategy

Heifers were offered *ad libitum* access to a total mixed ration (TMR) comprised of 69.8% corn silage, 30% ground alfalfa hay, and 0.2% salt, on a dry matter basis, throughout the study. Chemical composition of the dietary components is provided in Table 5.1. The TMR was added to the feed bunk at 8 a.m. daily, with refusals removed at least weekly.

Starting on d 8, a supplement of 0.23 kg of Country Lane 12% Sweet All Stock Feed (Orscheln Farm and Home, LLC, Moberly MO) was offered prior to feeding to acclimate the heifers to a dosing regimen. From d 15 to 25, two internal ALK markers – C_{32} (Dotriacontane) and C_{36} (Hexatriacontane; Minakem, SAS, France) – were added to the supplement, each at 625 mg/d (approximately 1.3 mg/kg BW per d). The calculated daily dose of ALK was melted (at 85 ^{0}C) onto 20 g of pre-weighed ground soy bean hulls (approximately 4 mm). The soybean hulls with ALK and supplement were mixed together and stored in gallon Ziploc bags until use. Animals were observed for complete ingestion of the dose, which was poured into a rubber feeding tub and placed inside each individual bunk. If the animal did not consume the entire dose before feeding, the dose was emptied out on top of their daily TMR.

Between d 8 and d 21, weekly intakes were recorded. Daily intakes were collected during the final 5 d of the study (d 22 to 26).

Body weights and fecal sampling

Animals were weighed starting at 8:00 a.m. (without fasting) on day 1, 2 and 22 to 26. At the start of each weighing, the accuracy of the weigh scale was validated with "true" weights.

Fecal samples were collected on d 22 to 26. Following weighing, animals were restricted in a squeeze chute. A rectal fecal sample was collected using a new glove and sleeve for each animal. Fecal samples were placed into an aluminum pan fitted with a lid for transport. Samples were stored on ice and transported back to the lab for analysis. Once at the UNL lab, fecal samples were placed in a forced-air drying oven at 60 ^{0}C until dried. Dried samples were ground through a Wiley mill using a 1-mm screen (Thomas Scientific, Swedesboro, NJ)

Analysis of n-alkanes

Feed, fecal, and supplement samples were analyzed for concentrations of ALK and LCOH. Extractions were performed in duplicate (Mayes and Dove, 2006). Ground feed samples of 0.200-0.204 g (0.100 to 0.104 g for feces) were weighed into 16 mm X 100 mm borosilicate glass culture tubes fitted with screw caps lined with PTFE inserts. Docosane (C_{22}) and tetratriacontane (C_{34}) were added by weight at a concentration of 0.3 mg / g to serve as internal ALK standards. An internal LCOH standard, *n*-heptacosanol (1-C_{27}-ol) at a concentration of 1.5 mg / g was added by weight. Two (1.5 for feces) milliliters of 1 M ethanolic KOH solution (97% ethanol: 3% deionized water, v / v) was added to each tube. Tubes were capped securely to ensure a complete seal and heated overnight (16 h) at 90 ºC in a dry-block heater (Techne DB-3,Techne Ltd., Duxford, Cambridge, UK).

After heating, samples were allowed to cool to 50 to 60 ºC. Aliquots of 2 (1.5 for feces) mL of n-heptane and 0.6 (0.5 for feces) mL of distilled deionized water were added to each tube. Tubes were mixed using a Vortex Maxi Mix I for 5 s (Thermo Fisher Scientific, Waltham, MA). The top (non-aqueous) layer was aspirated from the tube using a polyethylene transfer pipette and transferred to a 4 mL glass vial. A second 2 (1.5 for feces) mL administration of heptane was added to the tubes, mixed, and aspirated into the same 4 mL vial. Vials were then placed in a dry-block heater under a Techne Sample Concentrator with a gentle flow of air to dry vials (Bibby Scientific Limited, Staffordshire, OSA, UK).

Vials were reconstituted with 0.3 mL of heptane and rolled to ensure all sample was dissolved from the sides of the glass. The sample was aspirated with a polyethylene transfer pipette and transferring to an ISOLUTE Single fritted reservoir, or column (3 mL 20 µm PE, Biotage LLC, Charlotte NC), containing a 1 mL bed volume of silica-gel (70-230 mesh, Fisher Scientific, Fairlawn, NJ). The sample was first eluted, during solid phase extraction, with two administrations of 1.5 mL of heptane to collect hydrocarbons. Secondly, crude alcohol fractions were eluted by a following 2 administrations of 1.5 mL of heptane/ethyl-acetate (80:20 v / v). Hydrocarbon and crude alcohol fractions were then placed in a dry-block heater under a Techne Sample Concentrator with a gentle flow of air to dry vials. Using the crude alcohol vials, the LCOH fractions were separated from the sterol/stanol fractions by a second solid phase extraction and heptane elution. A volume of 100 µL of warmed saturated urea in ethanol was added to a 1 mL column, double fritted (Biotage LLC, Charlotte NC), and sealed with a luer mat (SPEware, Baldwin Park, CA). The crude alcohol vials were reconstituted with 200 µL of heptane.

An aliquot of 50 µL was transferred to the column. Columns were placed in a 70 °C oven and the oven was immediately turned off. After 20 min, oven columns were removed from the oven and placed in a fume hood to evaporate overnight. The next day, sterol/stanol fractions were eluted first by adding 0.5 mL to each column, a total of 3 times. The columns were then purged with 60 psi airflow for 5 min and rinsed with 4 rounds of 0.5 mL of distilled water. The LCOH fractions were then derivatized by heating at 60 ºC on a dry block heater, overnight, in 200 µL of a pyridine/acetic anhydride solution (5:1 v / v). *n*-Alkane elutes and LCOH fractions were evaporated to dryness, and then re-dissolved in 200 µl of *n*-dodecane for chromatographic analysis.

Quantification of ALK and LCOH was carried out by gas chromatography (GC), using an Agilent 7820A GC (Agilent Technologies, Inc., Santa Clara, CA). *n*-Alkane and LCOH extracts were injected (0.5 µl) via a 7650A Series auto-sampler through a splitless liner into a bonded-phase, non-polar column (Agilent J&W DB-column, 30-meter, 0.530 mm internal diameter and 0.5 µm film thickness). Helium functioned as the carrier gas at a constant flow of 4 mL / min. Temperature programming was: 280 °C for the injector; 340 °C for the detector; and, 170 °C for 4 min for the column oven followed by a first ramp of 30 °C / min to 215 °C with a 1 min hold, and then a second ramp of 6 °C / min to 300 °C with a 20 min hold. Samples of an ALK and LCOH standard solution mixture (C_{21} to C_{36}; $C_{20}OH$ to $C_{30}OH$, SigmaAldrich, St. Louis, MO) were included in the GC analyses to determine peak identification and standard response factors. Chromatograph data were analyzed using Agilent ChemStation software (Rev. B.04.02 SP1). Peak areas were determined with auto-integration and manual review of chromatograms. The ALK and LCOH concentrations were calculated relative to known amounts of the internal

standards (C_{22}, C_{34}, and $C_{27}OH$), according to the equations outlined by Mayes and Dove (2006). Prediction of animal intakes was conducted using the equation from Mayes and Dove (2005), where intake is calculated directly from herbage and fecal ALK concentrations, and the dose rate of ALK.

Statistical analyses

Statistical analyses were conducted using GenStat for Windows 17th Edition to create biplots based on principal component analysis (PCA). The ALK (C_{27}, C_{29}, C_{31} and C_{33}) and LCOH ($C_{26}OH$, $C_{28}OH$ and $C_{30}OH$) concentrations of the relevant diet components were used in the analysis.

Additional statistical analyses were conducted using SAS 9.3 (SAS Institute Inc., Cary, NC). For each animal, daily feed intakes were averaged for the last 5 d of the experiment. The predicted and observed mean daily intakes of all 26 heifers were compared by regressing the predicted on the observed intakes using the GLM Procedure of SAS. The reliabilities of the prediction was assessed by testing the hypotheses that the slope was not different from unity and that the intercept was not different from zero.

As the benchmark for comparison, intakes were predicted when explicitly modelling the 2-components of the diet offered, a mixture of 70% corn silage (CS) and 30% alfalfa (ALF). The hypothesis that the slope equaled one was then tested using a 2-tailed t-test, where the test statistic was derived from the difference between the estimated slope and 1, and its associated standard error. The change in slope and intercept for other scenarios was tested as a marginal difference (effect) relative to this benchmark scenario.

The scenarios tested involved 2 facets. Intakes predicted when considering the diet as a single feed (TMR) were compared with considering the diet as a 2-component mixture. The sensitivity of those predictions to incomplete ingestion of the dosed marker were further tested by assuming that 100%, 97.5% or 95% of the target dose intake were consumed. Including the benchmark, 6 scenarios were assessed.

Because of the relatively wide range in measured food intake (mean 9.2 (SD 0.9) kg / d), the regression analyses were repeated with both predicted and observed intakes expressed as natural logarithms. With the log-transformation, the residual SD describes the proportional rather than absolute error of a single observation. Six scenarios were again evaluated, with predictions based on the 2-component mixture used as the benchmark scenario.

Results

The ALK and LCOH concentration of the TMR, its components, and the supplement are provided in Table 5.2. Principal component analysis was conducted using the 3 feedstuffs (TMR, CS and ALF) and 4 ALK and 3 LCOH. Nearly all variation was defined by the first principal component (PC; 99.97%) with a slight amount of additional variation (0.02%) defined by the second PC (Figure 5.1). There was strong separation of CS and ALF, with TMR in the middle. However all of the plant waxes clustered with the ALF, suggesting there was very little information being offered by the plant wax contents of the CS. Therefore, delineating CS and ALF as separate components of a mixed diet will be difficult.

In Fig. 5.2, the regression of predicted on observed intake is shown for the 26 heifers when modelling the diet offered based on its composition of 70% CS and 30% ALF. The slope and intercept were 1.35 (± 0.21) and 1.85 (± 1.98), respectively, with r^2 0.62. The slope differed from 1 (P = 0.07), although the intercept did not differ from 0 (P = 0.30). On average, the observed intakes were over-predicted. When the diet was instead evaluated as a TMR, predictions improved (Fig. 5.3; slope 1.07 (± 0.17); intercept -1.21 (± 1.57); r^2 0.623) although the slope and intercept did not differ from those obtained for the 2-component mixture (benchmark; P = 0.79).

The sensitivity of the predictions to losses in the amount of internal marker actually eaten – 97.5% and 95% of the target dose intake – were also evaluated. Slopes and intercepts from the fit of the regressions are provided in Table 5.3, and plotted in Fig. 5.4. Although the slopes numerically closer to 1, when considering the feed as either a 2-component mix of CS and ALF or as a single TMR, the predictions of feed intakes were similar to those when heifers were presumed to consume the entire daily dose (P = 0.30).

Given the relatively wide range in the observed intakes, the regression of the natural logarithm of observed on the natural logarithm of the predicted feed intake was also fitted. As shown in Fig 5.5, when considering the feed as a 2-component mixture, the log-transformed predicted and observed feed intakes more closely aligned (slope 1.19 (± 0.19); intercept -0.30 (± 0.42); r^2 0.625). The intercept of the regression did not differ from zero (P = 0.29) and the slope did not differ from unity (P = 0.30). When predictions were based on the regression of the log of observed on predicted feed intake as a TMR, the alignment worsened numerically (Fig. 5.6; slope 1.16 (± 0.18); intercept -0.41 (±

0.41); r^2 0.625). Still, the slopes and intercepts did not differ from those for the benchmark scenario ($P = 0.84$).

Similar to the non-transformed data, when accounting for possible losses in the amount of internal marker actually eaten, the predictions of feed intakes were similar to those when heifers were presumed to consume the entire daily dose ($P = 0.84$; Fig. 5.7). Such was the case when considering the feed as either a 2-component mix of CS and ALF or as a single TMR.

Discussion

Indoor validation studies have shown that the plant-wax procedure provides reliable estimates of measured intake in sheep (Mayes et al., 1986; Vulich et al. 1991; Dove and Olivan, 1998; Sibbald et al., 2000; Dove et al. 2002; Lewis et al. 2003; Valiente et al., 2003). In this study in cattle, predicted intakes were, on average, greater than observed intakes. From the sensitivity analyses, the extent of that difference may in part reflect that the complete dose of the internal marker was not ingested by the animals. This could be due to the initial weighing of the dose, losses when transferring the dose from storage bags to the feed bunk, or waste during feeding. Due to its low concentrations of C_{31} and C_{33}, CS offered relatively little discerning information with regards to the plant-wax profile of the mixed diet. Therefore, it may not be an ideal feedstuff for validating predictions using this method. Perhaps because of such variability in the mixing and feeding of dietary components, considering the feedstuff as a TMR was beneficial to predictions. This makes it imperative in further studies to consider and account for these possible errors.

In conclusion, the plant-wax technique could be reliably used in, at least, a controlled setting to predict individual animal intakes. At the accuracy obtained, predictions of intake could be reliably made within 2 to 3 kg of their observed values. However, finer demarcations in intake may be difficult to achieve in an applied setting. Based on the results of this indoor study, it becomes a reasonable assumption that this technique could be used to predict intake in a grazing setting given that the differences in feed intake of interest are sufficiently large.

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Table 5.1 Chemical composition of the forages and feed used

Foodstuff	OM, % DM	NDF, % DM	CP, % DM	Fat, % DM
Main Diet				
TMR[1]	93.18	50.11	12.53	2.40
Corn silage	95.26	31.84	9.42	2.96
Alfalfa	89.03	55.70	24.79	1.95
Supplement				
Whole supplement[2]	90.08	61.50	14.19	4.92
Sweet feed	87.70	38.22	16.76	3.14
Soybean hulls	93.02	74.04	11.05	0.89

[1] TMR: Total mixed ration is composed of 70% corn silage and 30% alfalfa

[2] Whole supplement contained 1.1 kg of sweet feed, 20 g of soybean hulls, 625 mg of C32, and 625 mg C36

Table 5.2 Mean (SE) alkane concentrations (mg kg^{-1}) for the TMR, its components, and the supplement.

	Alkanes				Long-chain alcohols		
Foodstuff	C_{27}	C_{29}	C_{31}	C_{33}	$C_{26}OH$	$C_{28}OH$	$C_{30}OH$
Main Diet							
TMR[1]	6.38	28.0	80.2	10.9	41.6	88.8	683.9
	(5.30)	(7.51)	(3.19)	(3.93)	(5.38)	(3.02)	(2.45)
Corn Silage	1.90	5.45	8.92	5.76	13.6	16.9	39.2
	(2.11)	(1.3)	(2.00)	(3.02)	(1.90)	(2.35)	(0.520)
Alfalfa	11.1	61.5	210.0	16.8	85.6	171.4	2274.6
	(4.12)	(1.59)	(3.86)	(3.22)	(9.81)	(0.793)	(4.64)
Supplement							
Whole supplement[2]	2.98	5.60	11.4	8.14	34.4	109.7	55.7
	(5.04)	(4.93)	(3.43)	(1.03)	(7.69)	(9.22)	(1.95)
Soybean Hulls	0.761	2.72	13.5	3.47	0	11.3	54.7
	(4.96)	(5.15)	(6.80)	(5.73)	(0)	(3.47)	(5.3)
Sweet Feed	6.15	10.6	7.76	4.36	38.4	85.2	47.7
	(0.39)	(0.95)	(2.04)	(1.41)	(2.51)	(5.65)	(0.70)

[1] TMR: Total mixed ration is composed of 70% corn silage and 30% alfalfa
[2] Whole supplement contained 1.1 kg of sweet feed and 20 g of soybean hulls

Table 5.3 Relationship between predicted intake and measured intake.

	r-square	Diet[1]	Dose[2]
y = 1.35 (± 0.21) x - 1.85 (±1.98)	0.623	CS/ALF	100
y = 1.31 (± 0.21) x - 1.81 (± 1.93)	0.623	CS/ALF	97.5
y = 1.28 (± 0.20) x - 1.76 (± 1.88)	0.623	CS/ALF	95
y = 1.07 (± 0.21) x - 1.21 (± 1.98)	0.623	TMR	100
y = 1.04 (± 0.17) x - 1.18 (± 1.53)	0.623	TMR	97.5
y = 1.02 (± 0.16) x - 1.15 (± 1.49)	0.623	TMR	95.0
y = 1.19 (± 0.19) x - 0.30 (± 0.42)	0.625	ln (CS/ALF)	100
y = 1.19 (± 0.19) x - 0.32 (± 0.42)	0.625	ln (CS/ALF)	97.5
y = 1.19 (± 0.19) x – 0.35 (± 0.42)	0.625	ln (CS/ALF)	95
y = 1.16 (± 0.18) x - 0.41 (± 0.41)	0.625	ln (TMR)	100
y = 1.16 (± 0.18) x - 0.44 (± 0.41)	0.625	ln (TMR)	97.5
y = 1.16 (± 0.18) x - 0.47 (± 0.41)	0.625	ln (TMR)	95.0

[1] CS/ALF: Assumption of 70% corn silage and 30% corn silage; TMR: Assumption of total mixed ration; ln(x): using log transformation data for both predicted and actual intake

[2] 100: assumes animal consumed 100% of a 625 mg /d dose; 97.5%: assumes animal consumed 97.5% of a 625 mg /d dose; 95%: assumes animal consumed 95% of a 625 mg /d dose

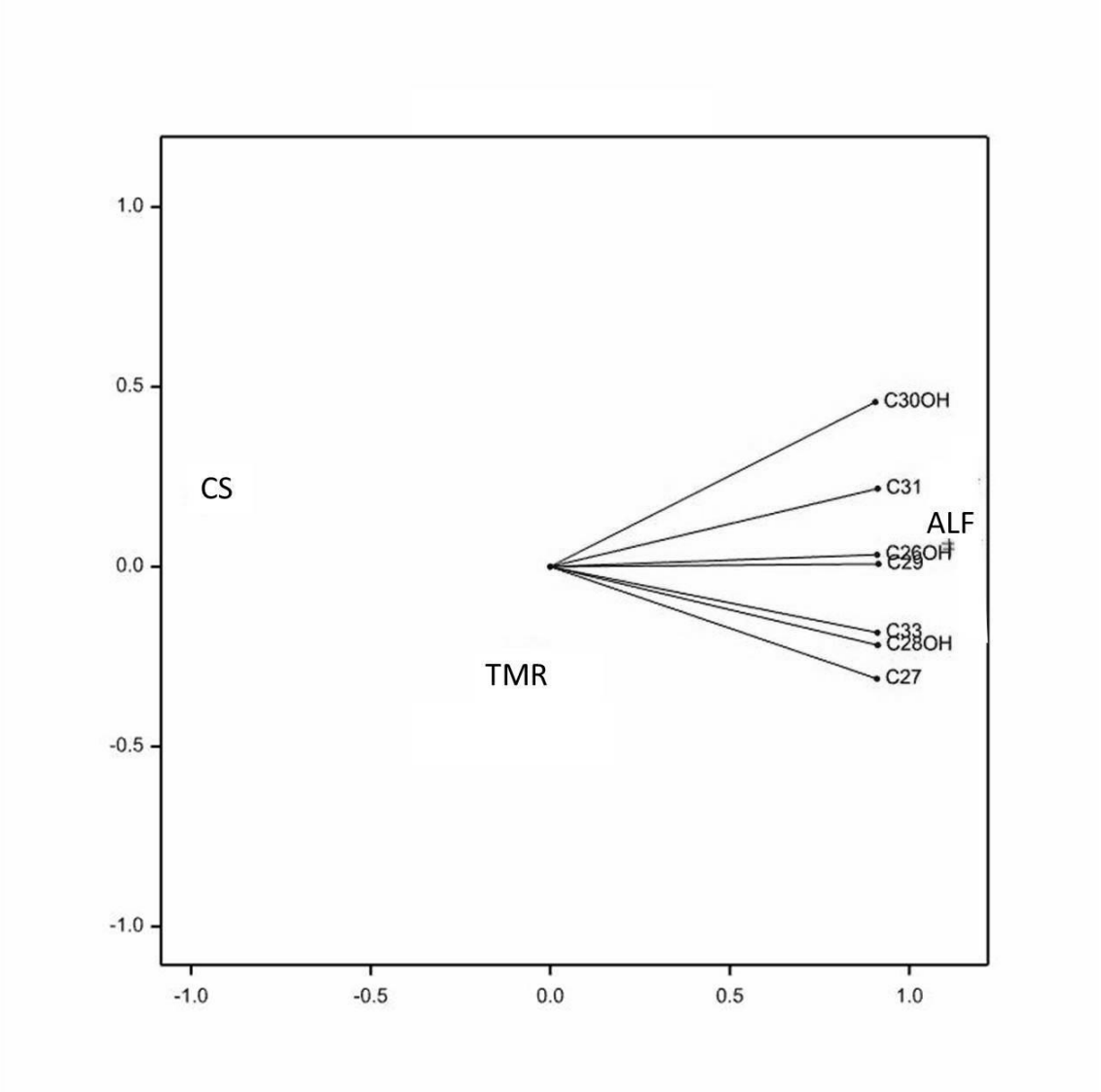

Figure 5.1 Biplot showing the 2 dietary components and a total mixed ration in a 2-dimensional space. Plot derived from principal component analysis based on concentrations of 4 *n*-alkanes (C27, C29, C31 and C33) and 3 long-chain alcohols (C26OH, C28OH and C30OH). The components were alfalfa (ALF), corn silage (CS) and the total mixed ration (TMR).

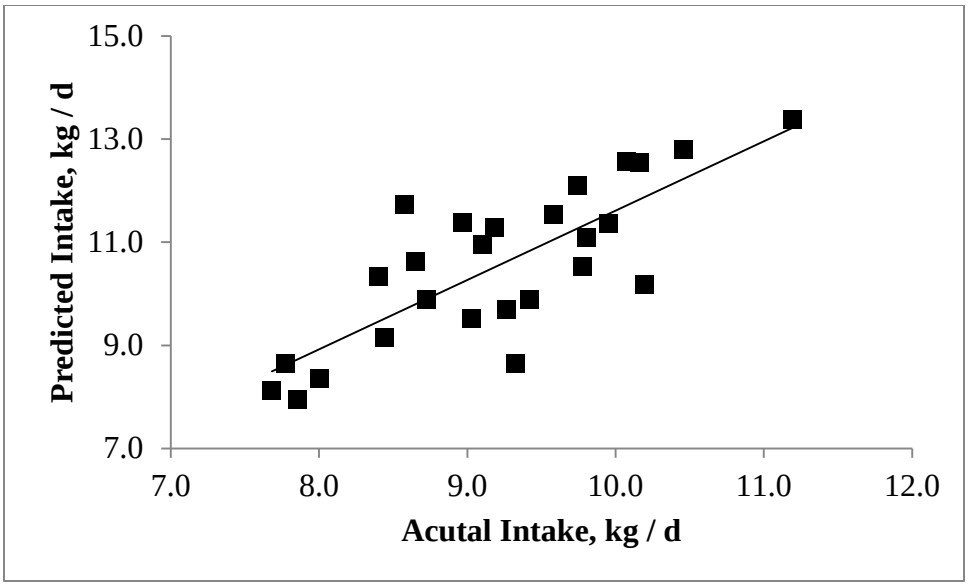

Figure 5.2 Predicted vs. observed dry matter intake (kg/d) of 26 heifers consuming a mixed diet in a drylot. Predictions were based on explicitly modelling the 2 components of the diet, approximately 70% corn silage and 30% alfalfa. The solid line shows the fit of the regression (slope 1.35 (± 0.21); intercept - 1.85 (± 1.98); r^2 0.623).

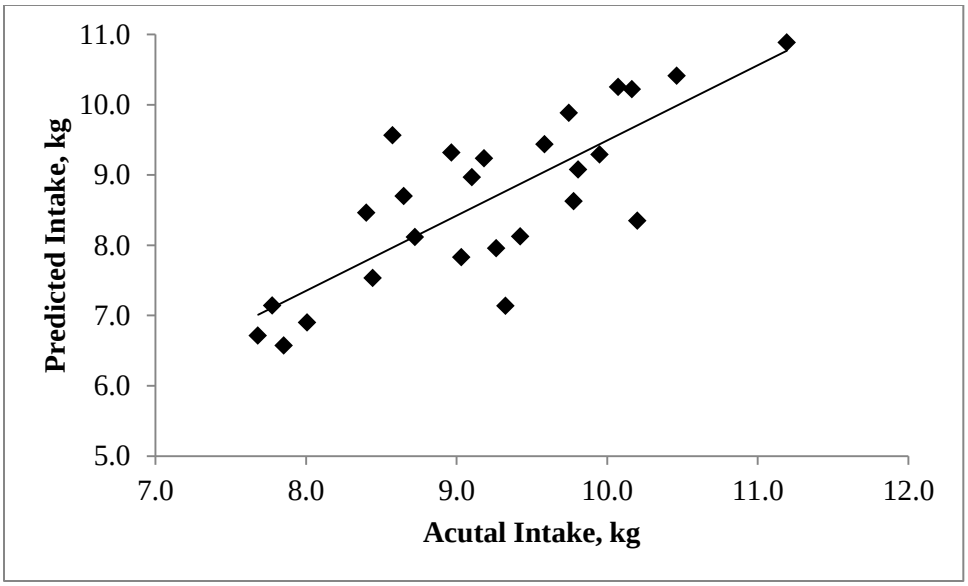

Figure 5.3 Predicted vs. observed dry matter intake (kg/d) of 26 heifers consuming a mixed diet in a drylot. Predictions were based on considering the diet offered as a total mixed ration. The solid line shows the fit of the regression (slope 1.07 (± 0.17); intercept - 1.21 (±1.57); r^2 0.623).

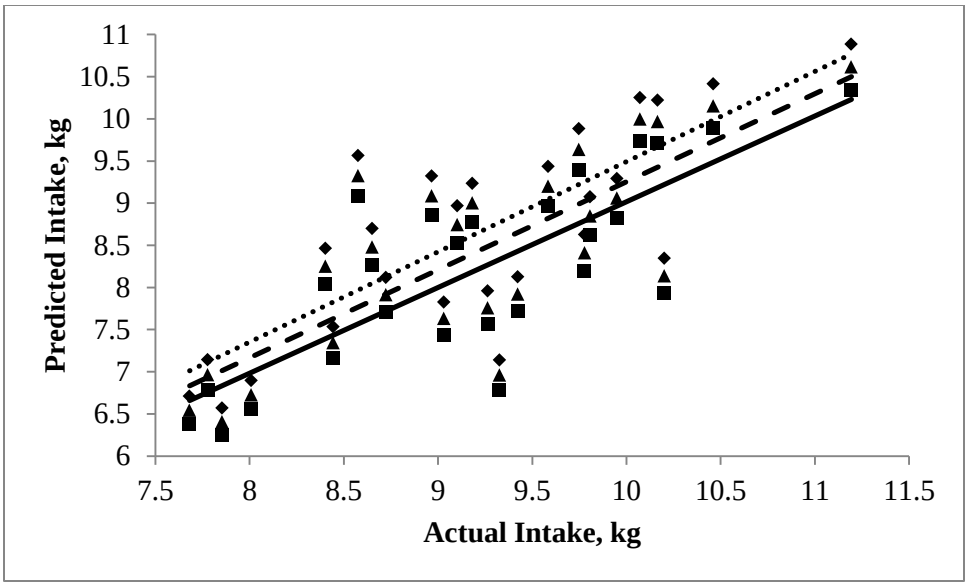

Figure 5.4 Predicted vs. observed dry matter intake (kg/d) of 26 heifers consuming a mixed diet in a drylot. Predictions were based on considering the diet offered as a total mixed ration and 100% dose consumption (diamonds), 97.5% dose consumption (triangles), and 95% dose consumption (squares). The dotted line shows the fit of the regression for 100% consumption (slope 1.07 (± 0.21); intercept – 1.21 (±1.98); r^2 0.623). The dashed line shows the fit of the regression for 97.5% consumption (slope 1.04 (± 0.17); intercept – 1.18 (±1.53); r^2 0.623). The solid line shows the fit of the regression for 95% consumption (slope 1.01 (± 0.16); intercept – 1.15 (±1.49); r^2 0.623).

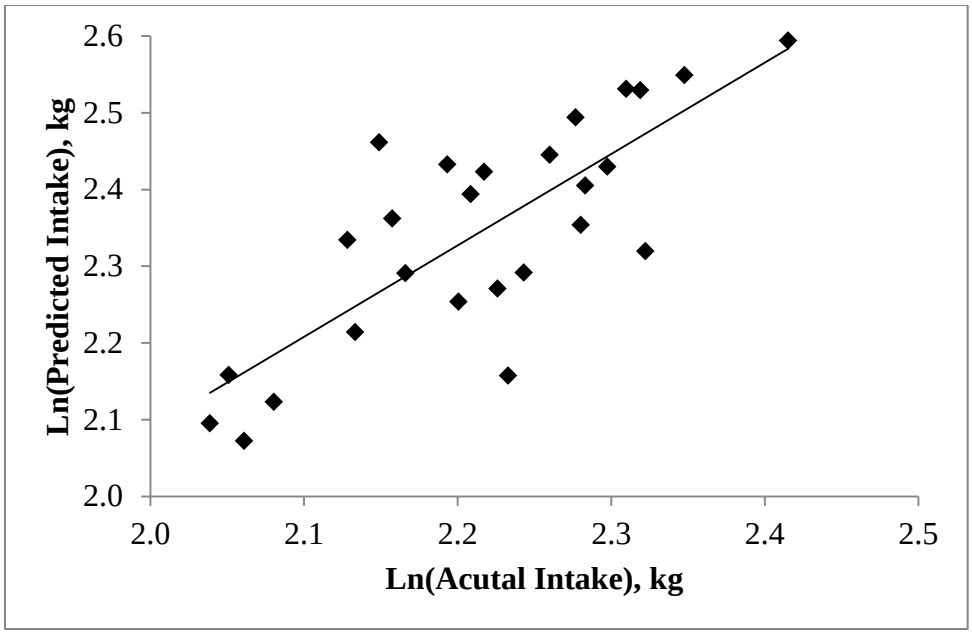

Figure 5.5 Natural log of predicted vs. natural log of observed intake (kg/d) of 26 heifers consuming a mixed diet in a drylot. Predictions were based on explicitly modeling the 2 components of the diet, approximately 70% corn silage and 30% alfalfa. The solid line shows the fit of the regression (slope 1.19 (± 0.19); intercept - 0.30 (± 0.42); r^2 0.623).

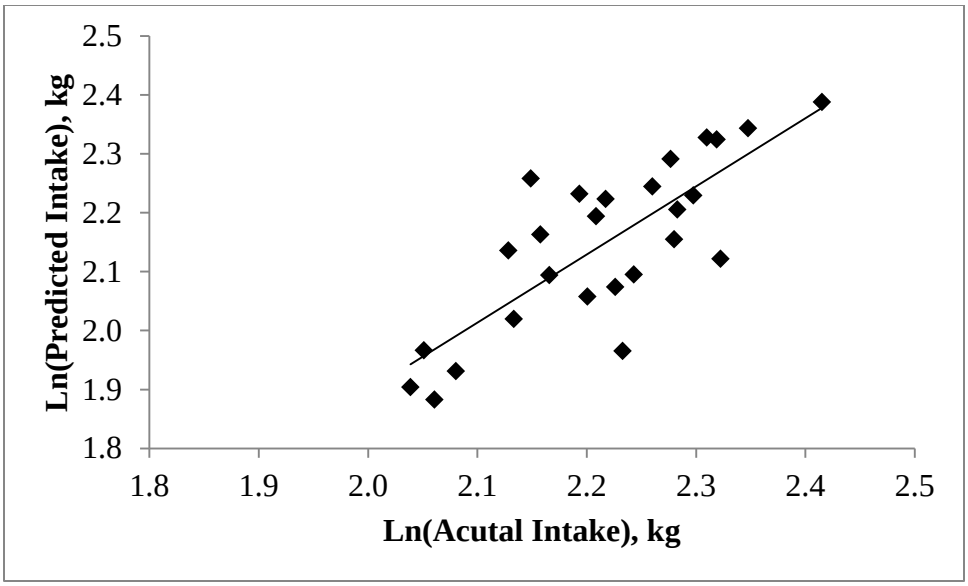

Figure 5.6 Natural log of predicted vs. natural log of observed dry matter intake (kg/d) of 26 heifers consuming a mixed diet in a drylot. Predictions were based on considering the diet offered as a total mixed ration. The solid line shows the fit of the regression (slope 1.16 (± 0.18); intercept – 0.414 (± 0.41); r^2 0.625).

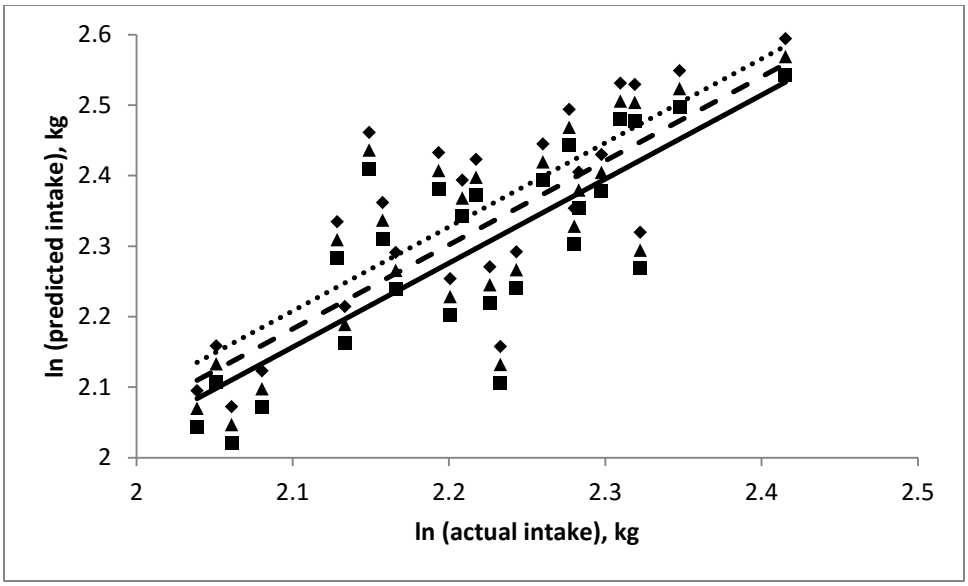

Figure 5.7 Natural log of predicted vs. natural log of observed dry matter intake (kg/d) of 26 heifers consuming a mixed diet in a drylot. Predictions were based on explicitly modelling the 2 components of the diet, approximately 70% corn silage and 30% alfalfa and 100% dose consumption (diamonds), 97.5% dose consumption (triangles), and 95% dose consumption (squares). The dotted line shows the fit of the regression for 100% consumption (slope 1.19 (± 0.19); intercept – 0.30 (±0.42); r^2 0.625). The dashed line shows the fit of the regression for 97.5% consumption (slope 1.19 (± 0.19); intercept – 0.32 (±0.42); r^2 0.625). The solid line shows the fit of the regression for 95% consumption (slope 1.19 (± 0.19); intercept – 0.35 (±0.42); r^2 0.625).

Appendix 3.1: A description of bomb calorimeter standardization

A Parr 1281 Bomb Calorimeter (Parr Instrument Company, Moline, Illinois) was used to analyze samples for energy content. The instrument used 99.5% pure oxygen set to 450 psi on the outlet gauge. Nitrogen gas used was water and oil free, set to 80 psi on the outlet gauge. Precut fuses (part # 845DD2) were used to ignite samples. The machine was standardized using 10 benzoic acid samples. Only samples ranging between 803 EE and 813 EE were accepted during calibration. After 10 benzoic standard samples, the relative standard deviation was checked. A satisfactory calibration had a relative standard deviation less than 0.15. After calibration, all benzoic acid standards should read at 6318 ± 18 cal. If at any time a benzoic acid standard did not read in this range, the machine was serviced. Standardizing the machine was performed every 500 samples or after replacing O-rings. A benzoic acid standard was analyzed every day before analyzing any other samples to assure the machine was functioning properly. Samples ranging between 0.400 - 0.404g were weighed into combustion capsules. Because of the nature of the samples a pellet could not be formed and was weighed loosely into the capsule. All samples then had 0.2000-0.2999g of mineral oil added with a glass dropper. The capsule was allowed to sit overnight to allow full dispersion of the mineral oil to assure a thorough and complete burn of entire sample. The sample was then burned in the machine. The recorded temperature rise was then used to calculate heat of combustion (Hc): $\frac{WT-e1-e2-e3-(Hcs)(Ms)}{m}$; where (*W)* is the energy equivalent of the calorimeter, (*T)* is the observed temperature rise, *(e1)* is the heat produced by burning nitrogen in the air, (*e2)* is the heat produced by formation of sulfiric acid, (*e3)* is the heat produced by the

heating wire and cotton thread, (*m)* is the mass of the sample, (*Hcs)* is the heat of combustion for the spiked material, and (*ms)* is the mass of the spiking material.