ESTIMATION OF APPARENT DIGESTIBILITY OF SIX FORAGES USING TWO DIFFERENT DIGESTIBILITY MARKERS

By

CHUNALA ALEXICO NJOMBWA

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To my family

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LIST OF ABBREVIATIONS

ADF	Acid – detergent fiber
ADL	Acid – detergent lignin
AIA	Acid insoluble ash
APL	Acid peroxide lignin
CIAT	International centre for tropical agriculture
CP	Crude protein
D	Day
DM	Dry matter
GIT	Gastro – intestinal tract
H	hour
NAA	Neutron activation analysis
NDF	Neutral – detergent fiber
OM	Organic matter
OMD	Organic matter digestibility
VFA	Volatile fatty acids

Abstract of Thesis Presented to the Graduate School
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By

Chunala Alexico Njombwa

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The objectives of the two experiments were to compare apparent total tract digestibility of nutrients of summer and winter annual forages; and to compare the efficacy of TiO_2 and Cr_2O_3 as digestibility markers for fresh forages fed ad libitum, and to determine effects of performing 2× vs. 3× per day fecal sample collection to measure digestibility. Ryegrass, ryegrass + oat, and ryegrass + triticale, Mulato II, millet and sorghum were fed to 12 Angus and Angus-crossbred heifers. Heifers were dosed at 1200 h with 10 g of Cr_2O_3 and 10 g of TiO_2 via gelatin capsules. Feed and fecal samples were collected within 5 days. In experiment 1, no effect of forage, marker, sampling schedule, or marker x sampling schedule interaction was found for the digestibility variables measured ($P > 0.05$). In experiment 2, sampling effect, sampling protocol x marker and forage x marker were observed ($P < 0.05$) on digestibility of forages. Both Cr_2O_3 and TiO_2 may be used indistinctively to estimate digestibility of winter forages while only TiO_2 may be used for summer forages. Increasing sampling frequency to 3x a day may yield more desirable results when Cr_2O_3 is used for fresh summer forages. In addition, a survey was conducted to determine status of commercial beef production in Malawi. Lack of proper strategic breeding and animal performance

monitoring, inadequate nutritional management and insufficient farm mechanization in majority beef farms indicate that beef production is at infant stage in Malawi. Research and dissemination of technologies to farmers should be intensified to enhance beef production.

CHAPTER 1
INTRODUCTION

One of the most fundamental measurements used to determine the nutritive value of a feed is digestibility. Digestibility of a feed determines the amount of nutrients that are actually absorbed by an animal and, therefore, the availability of these nutrients for maintenance, growth, reproduction, and production of other desirable products such as meat and milk (Ibrahim and Olaloku, 2000). Feedstuffs of high digestibility are often associated with high nutritive values and accelerated animal performance. Consequently, feedstuffs of low digestibility are associated with low nutritive quality and may not provide sufficient nutrients for successful animal performance (Kham et al., 2003). Therefore, knowledge of the digestibility of feed should be assessed in livestock production systems to ensure that livestock producers optimize efficiency of production within their operation. This would allow livestock producers the opportunity to choose, produce, and provide feeds of high quality that offer more nutrients for animal growth and production, resulting in higher yields.

Classically, several techniques have been used to estimate digestibility. Among various techniques in use, markers have been widely accepted applications to estimate digestibility and organic matter intake. Markers are indicator substances which are inert (non-digestible) in the gastrointestinal tract (GIT) of an animal. Characteristics of a good marker are: 1) they are strictly non-absorbable; 2) they do not affect or are affected by the GIT or microbial population; 3) they are physically similar to or closely associated with feed material; and 4) methods of estimation in digesta samples must be specific, sensitive, and not interfere with other analyses (Kham et al., 2003).

Although markers are widely used to estimate feed digestibility, erratic results have been reported when administered across a wide range of feedstuffs. It has been reported that some markers yield accurate estimates of digestibility of specific feeds while overestimating or underestimating digestibility of other feeds (Sunvold and Cochran, 1991). Thus, there may be an inconsistency in feed evaluation which may jeopardize the process of evaluating and allocating the feed to meet maintenance and achieve desired growth and performance of the animal. This also makes it difficult to determine the most efficient feeding strategies to maximize animal productivity and economic returns. Therefore, research aimed at evaluating accuracy of markers in estimating digestibility of various feeds is of extreme importance. This ensures more precise and accurate estimates of digestibility which can enhance production of high valued feeds and the allocation of feeds to appropriate groups of animals; thereby, enhancing animal performance and returns.

Chromic oxide (Cr_2O_3) has been the most commonly used digestibility marker. However, there are reports that Cr_2O_3 is associated with health risks such as carcinogenic effects (Myers et al., 2004). As a consequence, titanium dioxide (TiO_2) has been explored as a potential alternative digestibility marker with no reported health risks that may be legally added to feeds as color additive at amounts that do not exceed 1% of finished product (Titgemeyer et al., 2001). Several studies have indicated the feasibility of using TiO_2 as a viable total-tract digestibility marker in rats, chicken, pigs, dairy cows (Myers et al., 2004), and beef cattle (Titgemeyer et al., 2001). However, little research has been completed to test its efficacy in estimating digestibility of some forms of feedstuffs and in various feeding conditions.

Currently, there is no available data to assess the viability of using TiO_2 as a digestibility marker in beef cattle fed fresh forages on an ad libitum basis, or to quantify the effect of collecting fecal samples 2× or 3× per day. Therefore, the efficacy of TiO_2 as a marker for these conditions is unknown, limiting its application as a digestibility marker in experiments.

Therefore, two studies were conducted with objectives to: 1) compare total tract digestibility of nutrients for three cool-season and three warm-season forages and 2) to determine the efficacy of two digestibility markers titanium dioxide (TiO_2) and chromic oxide (Cr_2O_3) sampled 2× or 3× per day on estimating digestibility. Ryegrass (*Lolium multiflorum* Lam.), ryegrass combined with oat (*Avena sativa*), and ryegrass combined with triticale (*Triticosecale rimpau*) were the cool-season forages evaluated, whereas sorghum (*Sorghum bicolor*), pearl millet (*Pennisetum glaucum*), and Mulato II (*Brachiaria* hybrid) were the warm-season grasses evaluated.

CHAPTER 2
LITERATURE REVIEW

Animal productivity and profitability of livestock enterprises are directly linked to nutrition which is mostly determined by amount of feed consumed, nutritive quality, and digestibility of the diet consumed by livestock (Marais, 2000). Digestibility provides an estimate of the quantity of consumed feed, or specific components of the feed available for an animal to digest and absorb. These components are used for growth, maintenance, reproduction, and production of meat or milk of livestock species. However, digestibility is useful only if it is accurately estimated (Morais et al., 2010). Therefore, accurate knowledge of the digestibility of feedstuffs is essential for the establishment of effective feeding strategies to optimize the profitability of livestock production systems.

Forage Quality and Adaptation

Forages form a larger proportion of ruminant diets; however, producers should be aware of the existence of variations in seasonal response of forages if they are to maximize forage production and utilization. Some forages are well adapted and more productive during the cool periods of the year (i.e., winter), whereas others are more productive during the warm periods of the year (i.e., summer), hence the terms cool-season and warm-season forages.

Cool Season Grasses

Annual ryegrass (*Lolium multiflorum*) is one of the most widely grown cool-season grass in southeastern United States (US) covering greater than one million hectares, annually. Ryegrass is considered the best quality winter forage for the southeastern US due to its high dry matter (DM) digestibility (typically > 65%), excellent animal

performance, low seed costs, seed availability, and adaptation to a wide variety of environments. Ryegrass also has a crude protein (CP) content that exceeds the requirements for most classes of livestock (Blount et al., 2009; Blount et al., 2010). Annual ryegrass can be seeded alone but a more common production practice is to seed it in mixtures with other cool-season forages such as oats and triticale.

Triticale (*Triticosecale rimpau*) is a hybrid between wheat (*Triticum*) and rye (*Secale*). It take its name from the first five letters of Triticum and last four letters of Secale. Triticale is well adapted to the southern parts of US and peninsular Florida. It has the forage quality of wheat and the excellent disease resistance of rye. Dry matter digestibility of triticale ranges from 60% to 79% while CP content ranges from 11% to 22%, depending on stage of maturity (Keuren and Underwood, 1990). Triticale is best utilized as ensiled haylage or silage because it does not respond well to intense grazing; however, when used in grazing systems, it is important to consider blending it with ryegrass to promote a longer growing season (Blount et al., 2010).

Oat (*Avena sativa*) is a palatable grass with a DM digestibility range of 56% to 77% and CP content ranging from 11% to 20% depending on the stage of maturity (Keuren and Underwood, 1990). Peak season of forage production for ryegrass is later than that of oat, rye or triticale. Therefore, when grown in a mixture of oat with rye or triticale provides a faster growing component of the blend, earlier grazing, and as the oat declines in late winter, ryegrass production peaks. The result is an extension of cool-season forage production with high quality ryegrass forage (Blount et al., 2009: Blount et al., 2010).

Warm Season Grasses

Warm-season grasses are the dominant forage crops used for livestock production in tropical regions of the world. The majority of these warm-season grasses have seasonal growth, with most of forage production occurring during the spring, summer, and early fall months (Vendramini et al., 2010). Sorghum (*Sorghum bicolor*), pearl millet (*Pennisetum glaucum*), and Mulato II (*Brachiaria* hybrid) are among the most widely used warm-season grasses in the US. As with other warm season grasses, these grasses are characterized by high DM content, and relatively low CP and DM digestibility compared to cool season grasses. Banks (1998) reported DM yield of ≈11 tons per ha, ≈12% CP and DM digestibility of ≈67% for sorghum. Pearl millet has slightly greater feeding value as compared with sorghum, with DM yield of ≈12 ton per ha, CP content of ≈18% and DM digestibility ≈69%. Both sorghum and pearl millet may be used as fresh fodder, pasture, silage, or hay (Lang, 2001). However, stage of maturity at harvest, soil fertility, and management impact the quality of sorghum and pearl millet.

Although sorghum is widely used as forage, there are concerns about the potential toxic effects to animals. Leaves of sorghum plants may be toxic as a result of high concentrations of hydrogen cyanide (prussic acid), especially in young dark-blue colored regrowth after experiencing drought conditions. Unlike sorghum, pearl millet does not produce prussic acid (Banks, 1998; Cook et al., 2005). However, there have been incidences of nitrate poisoning from pearl millet. Nitrate poisoning results from the ingestion of forage containing high concentrations of nitrates (NO_3; Lang, 2001). Normally, safe levels of nitrates in forages vary depending on the physiological state of

the cattle. Nitrate nitrogen (NO_3) concentrations > 0.88% are considered unsafe for pregnant females whereas for non-pregnant females, NO_3 concentrations > 1.76% are considered toxic. In addition, forages with NO_3 concentrations < 1.76% may be limit-fed to non-pregnant animals at specific inclusion percentages. For example, NO_3 concentrations between 1.54 and 1.76% in feedstuffs should be supplied at the rate of 25% of total DM of the ration whereas concentrations of 0.88 to 1.54% and 0.66 to 0.88% may be limit-fed at inclusion rates of 35 to 40% and 50%, respectively. As for pregnant females, limited inclusion rates of 50% must be observed when concentrations of NO_3 are between 0.44 and 0.66% (Lang, 2001).

Accumulation of NO_3 occurs in plants that are fertilized with N at high rates, but grow at slow rates. Slow growth usually occurs because of insufficient soil moisture, but may also be the result of heavy cloud cover, shading, cool temperatures, or frost. Controlling access to toxic forage and dilution with other feeds are methods that may be used to control toxicity (Hannaway and Larson, 2004). In addition, application of N fertilizer in pearl millet should remain below 250 kg per ha in order to reduce concentrations of NO_3 (Banks, 2002). Furthermore, Banks (2002) indicated that application of N fertilizer for Pearl Millet should be applied at 70% of the application rates used for corn fertilization with the first 50% applied at planting and the remaining 50% after first cutting, to avoid risk of toxicity.

As technology in agriculture advances, forage breeding programs have been established to create better quality forages that can enhance livestock production to feed the growing human population. One of the products of cross breeding and selection is the development of the cultivar Mulato II (*Brachiaria hybrido*). Mulato II is a

Brachiaria hybrid developed and released in 2004 by the Tropical Forage Programme, International Centre for Tropical Agriculture (CIAT), Cali, Colombia. Mulato II is a cross between *Brachiaria ruziziensis*, *Brachiaria brizantha* and *Brachiaria decumbens*. Mulato II is adapted to many soil types ranging from sand to clay (pH range of 5.5–6.0) and is superior in tolerating drought (up to 6 months), burning, and pests (such as spittle bugs; Vendramini et al., 2011; Argel et al., 2007). In addition, it also demonstrates high plant vigor and fast recovery after grazing (Argel et al., 2007). Argel et al. (2007) also reported Mulato II to have DM yields of ≈3 ton/ha/cutting, CP of ≈11.4%, and DM digestibility of ≈66% during periods of rain. However, CP (8.4%) and IVDMD (61%) declined during the dry season. Generally, Mulato II has a range of 11 to 16% for CP and 55 to 60% for TDN (Vendramini et al., 2011). Mulato II does not tolerate water logging but has demonstrated good compatibility with other forages, particularly legumes.

In most regions of Sub Saharan Africa and Malawi (located 13^0 55´S and 33^0 42´E) in particular, napier grass (*Pennisetum purpureum*) is commonly used in livestock production systems. Unlike other grasses used in other parts of the world, napier grass is favored because it is a multipurpose grass. Apart from being used as livestock feed, napier grass may also be planted along contours during cultivation to reduce soil erosion. Therefore, napier grass is a good fit in the Malawi agricultural production system, which is characterized by high land pressure and competition for land between human food production and livestock feed production. napier grass produces DM ranging from 2 to10 ton/ha when unfertilized, 10 to 30 ton/ha/yr and may increase to 85 ton/ha/yr when fertilized. These production rates appear to be significantly greater than

those reported for mulato II, sorghum, and millet (Cook et al., 2005). However, napier grass requires deep well-drained loam soils with pH ranging from 4.5 to 8.2 and requires 150 to 300 kg/ha/yr of N to achieve high nutrient and DM production. These requirements increase the cost of production of napier grass.

Unlike the temperate grasses such as annual ryegrass, oat, and triticale, CP content in Napier grass drops more rapidly with maturity. At 6 wk regrowth, 10% CP of Napier grass was reported, whereas at 10 wk of regrowth, the CP content decreased to 7.6% (Cook et al., 2005). This is attributed to efficiency of N utilization of Carbon 4 (C4) grasses as stage of maturity increases. Napier grass belongs to a group of C4 grasses. The C4 grasses are efficient in utilization of N, thus there is a rapid decrease in N content with advanced maturity resulting in reduced CP. In general, CP content in leaves ranges from 9.5 to 19% whereas DM digestibility ranges from 68 to 74%. Napier grass is regarded as palatable, with high quality, and tolerant to drought conditions (Cook et al., 2005), but quality is affected by soil fertility, harvesting frequencies, and management.

Variation of Forage Digestibility

Forage plants form a great proportion of diets of ruminant animals. Just like other plants, forage plant cells have walls which form the structural framework that provides mechanical support for the plant, involved in water balance, ion exchange and also enclose and hold together all organelles (Moore and Jung, 2001). The cell walls constitute cellulose, hemicellulose, and lignin. During primary growth, cells experience an increase in size (Jung and Allen, 1995; Moore and Jung, 2001). However, after increasing in size and elongation, secondary growth is initiated in which cells undergo extensive thickening of walls (Jung and Allen, 1995). During this development,

cellulose, and hemicellulose composition of cell wall increases (Buxton and Redfearn, 1997). In addition to this, there is increased deposition of phenolic acids and lignin starting from outside the cell wall progressing to the inside part of the cell wall (Jung and Allen, 1995). This is done to enhance the structure and strength of the cell wall, facilitate water transportation, create a major line of defense against pathogens, insects and other herbivores and also to impede degradation of cell wall polysaccharides (Hatfield and Vermervis, 2001). When ruminants consume forages, digestion of a great proportion of these forages is aided by bacteria and fungi that exist in the rumen. The rumen microbes release enzymes which hydrolyze cellulose and hemicellulose to produce volatile fatty acids (VFA) such as propionate (substrate for gluconeogenesis), acetate (precursor for milk fat and a source of energy for muscles) and butyrate (source of energy for rumen cells). These VFAs are absorbed in the rumen and used in metabolic processes that support life and performance of ruminants.

The rate and efficiency at which microbes digest the cell wall components is what determines digestibility of forages (Jung and Allen, 1995). However, microbial efficiency in digestion of the cell wall components is affected by plant development. At maturity, the plant cell walls accumulate high cellulose and hemicellulose, both of which are encrusted with lignin. The cellulose and hemicellulose are compacted together and this reduces surface area for microbial attachment, thereby increasing the duration of hydrolysis and reducing forage digestibility. In addition to this, lignin provides a physical barrier and also shields microbial enzymes from accessing cellulose and hemicellulose. Through phenolic acids, lignin develops linkages with cell wall polysaccharides and these linkages change the orientation of cellulose, reducing the opportunity for

hydrolysis of cellulose to occur. The reduced rate and efficiency of rumen microbes in digestion of the cell wall result in a reduction in overall digestibility of forages (Jung and Allen, 1995).

Many studies (McMillan et al., 2006; Firdous and Gilan, 1999; Cochran et al., 1986; Sunvold et al., 1991) have reported variations in total tract digestibility of different forages. In general, these differences are attributed to variation in levels of accumulation of cell materials which is affected by nature of the forage, stage of maturity, species, and proportion of different components of the forage (Buxton et al., 1997; Firdous and Gilan, 1999).

Class of the forage

Forages can be classified into Carbon 3 (C3) and Carbon 4 (C4) plants depending on the way in which they assimilate carbon dioxide into their system. The first products of photosynthesis in C3 plants are compounds with three carbon atoms, whereas in C4 plants the first compounds have four carbon atoms (Ehleringer et al., 1997). Many studies have reported differences in digestibility between C3 and C4 plants. In most situations, C3 plants have greater digestibility than C4 plants due to differences in structural composition. In general, C4 plants contain a greater proportion of vascular bundles and also have tightly packed mesophyll cells, compared to C3 plants that have a larger proportion of leaf mesophyll cells which are loosely packed (with large intercellular spaces), which provides enough surface area for microbial attachment in the rumen thereby increasing the rate of digestion (McMillan et al., 2006; Akin, 1986; Buxton and Redfearn, 1997). The higher proportion of vascular bundles in C4 plants increases the proportion of forage requiring microbial digestion, reducing the rate and efficiency of digestion because microbes require additional time to digest forage. Tightly

packed mesophyll cells in C4 forages reduces surface area for microbial attachment in the rumen, decreasing the rate of digestion (McMillan et al., 2006). Temperate grasses and legumes are generally regarded as C3 plants, whereas tropical grasses are regarded as C4 plants (Akin, 1986; Sollenberger, 2011). Therefore, these differences between C3 and C4 describe why temperate or winter grasses, such as ryegrass, oat, and triticale have greater digestibility than tropical or warm season grasses such as sorghum, millet, and Mulato II.

In addition, variations in digestibility have been reported between legumes and grasses. In general, legumes are more digestible than grasses, since legumes tend to have less fiber and greater CP content (Buxton and Redfearn, 1997). This provides readily available nitrogen for protein synthesis by rumen microbes thereby enhancing degradation of legumes in the rumen. Whereas grasses have low CP content and increased levels of cellulose and hemicellulose, which requires a longer duration for microbes to digest, hence decreasing digestibility (Sollenberger, 2011).

Forage maturity

Within the same species of forage, digestibility varies at different stages of maturity. Under typical conditions, young forages have greater digestibility than mature forages (McMillan et al., 2006). Ammar et al. (2010) reported a decline in digestibility of *Avena sativa* (oat), *Trifolium alexandrium*, and *Vicia sativa* forages as stages of maturity increased. As the forages mature, the compaction and quantity of cell wall contents, such as cellulose and hemicellulose increase. Accumulation of these components reduces digestibility of the forage because rumen microbes require additional time to digest these structural carbohydrates to become available for animal use. In addition, as the forage plant cell develops, phenolic acids and lignin are deposited in the maturing

cell wall in specific structural conformations, and in a strict developmental sequence to enhance the strength of the cell wall (Buxton and Redfearn, 1997).

Lignin is the key element that limits cell-wall digestibility. The phenolic acids facilitate linkages between lignin and cell wall polysaccharides resulting into changes in orientation of these polysaccharides thereby physically shielding them from enzymatic hydrolysis (Jung and Allen., 1995). As the cell wall matures, lignin composition changes from guaiacyl-type to syringyl-type lignin. Unlike p-hydroxyphenol and guaiacyl-type lignin, the syringyl-type lignin protects a greater proportion of cell wall from digestion because it is more linear in structure and extends further into the secondary wall of the cell, thereby linking with more polysaccharides and reducing cell wall digestibility (Jung and Allen., 1995). Therefore, increased concentrations and composition of lignin reduces the percentage of the digestible portion of the forage resulting in reduced digestibility of the forages as stage of maturity increases.

Within the vertical orientation of forage

Moving up the plant, there is variation in digestibility. Studies have demonstrated that digestibility is increased towards the upper portion of the plant compared to the lower portions of the plant. Normally, lower portions of the plant have a low leaf to stem ratio (Ball et. al., 2001). Stem material of all forages has vascular tissues which have more sclerenchyma cells. The sclerenchyma cells are greater in cell-wall concentration than cell content due to extensive secondary thickening and they also contain high concentrations of lignin, reducing digestibility. In contrast, forage leaves tend to have more mesophyll cells that undergo little secondary wall thickening and deposit virtually no lignin, thus fewer materials is present to prohibit digestion resulting in increased digestibility (Jung and Allen, 1995).

Methods of Estimating Digestibility

Several methods of estimating digestibility have been developed for experimental use. Digestibility estimations have been developed for both in vitro and in vivo experimental methods.

In Vitro Methods

In vitro methods involve assimilating conditions of the rumen in a laboratory and estimate the breakdown of forage or feed. Forage or feed is combined with rumen fluid and allowed to undergo anaerobic fermentation for 24 h (to represent similar conditions in the rumen (Kham et al., 2003). After incubation for 24 h, the mixture is exposed to hydrochloric acid and pepsin for 48 h, to represent similar conditions to the ruminant abomasum (Kham et al., 2003). Although this method is less costly, less labor intensive, and can provide good estimates of feed digestibility, the major challenge has been to create similar conditions to the rumen and abomasum (Judkins et al., 1990). Characteristics such as gastric motility, interaction of microflora in the rumen environment, physiological changes in the animal that may affect digestibility, and interactions between feed constituents in the rumen environment have been difficult to duplicate. In some cases, these conditions have resulted in digestibility values that may not be achieved with in vivo methods (Judkins et al., 1990; Cochran et al., 1986).

In Vivo Methods

Unlike the in vitro method, in vivo methods of estimating digestibility are performed in the gastrointestinal tract. This method involves measuring feed intake and fecal output, which are used for calculating feed digestibility. Three methods for in vivo estimates of digestibility have been developed: 1) total fecal collection, 2) in sacco, and 3) indigestible markers.

Total fecal collection

The total fecal collection method involves collection and weights of all feed intake and fecal material, estimating the difference between the two. This method is an excellent estimate of digestibility; however, this technique also is labor intensive, expensive, and frequently impractical for larger animals, because of the number of times animals need to be handled (Rymer, 2000).

In sacco technique

The in sacco technique, feed samples are placed in nylon bags and are mechanically suspended in the rumen of ruminally fistulated animals for a specific period of time to allow microbial digestion to take place. The microbes (especially protozoa) and digestive agents found in the rumen enter the bags through the pores and digest the feed; therefore, the ideal pore size should range from 40 to 60 μm (Vanzant et al., 1998). Larger pore sizes are discouraged because they result in loss of feed from the bag, whereas smaller pore sizes frequently become blocked, restricting circulation across the bag resulting in reduced rate of degradation of the feed (Vanzant et al., 1998). This technique helps to estimate lag extent (delay in digestion attributed to time required for wetting of feed and attachment of bacteria to the feed) and rate of DM and nutrient disappearance (Udén and Van Soest, 1984). However, the in sacco technique fails to mimic some processes of digestion, such as mastication, rumination, and passage which affect digestion of feed in the rumen (Ørskov et al., 1980; Vanzant et al., 1998).

Marker techniques

Marker techniques have been widely applied in animal nutrition experiments. They are used to estimate digestibility of DM and nutrients, determine ruminal passage of digesta and fluids, and also DM intake of grazing ruminants (Cochran et al., 1986; Marais, 2000). The marker techniques involve the use of markers which serve as indicator substances which are inert in the GIT (Rymer, 2000). Concentrations of markers in the feed and in the feces are determined and these concentrations are used to calculate estimated digestibility of the feed.

Concepts of Marker Techniques

Categories and Analysis of Digestibility Markers

There are two categories of digestibility markers; internal markers and external markers. Internal markers are indigestible materials occurring naturally in forages or feeds such as silica, Acid Insoluble Ash (AIA), and lignin. These digestibility markers form an integral part of feedstuffs (Marais, 2000). In contrast, external markers are materials that are not part of the normal diet and are added to the diet. Common external markers are metal oxides such as chromic oxide, rare earth metals such as dysprosium chloride, and chromium mordanted fiber (Kham et al., 2002). Compared to internal digestibility markers, these external digestibility markers are expensive.

Several analytical methods for external digestibility markers have been developed (Short et al., 1996; Titgemeyer et al., 2001; Myers et al., 2004; Fenton and Fenton, 1979; Hill and Anderson, 1958; Czarnocki et al., 1961). However, the common trait among these methods is the use of light absorbance properties of some digestibility markers. Reactions of some external digestibility markers such as TiO_2, Fe_2O_3, Cr_2O_3, Ytterbium oxide and Dysprosium chloride with reagents, result into a solution changing

color. When TiO_2 reacts with H_2O_2 the end result is production of an orange or yellow color (Titgemeyer et al., 2001). The color produced by the external marker has a distinct absorbance at specific wavelengths of light and this property is used to quantify concentrations of the marker in fecal samples. Of the inert digestibility markers, research has more recently focused on TiO_2 (Short et al., 1996; Titgemeyer et al., 2001; and Myers et al., 2004). In separate studies, Myers et al. (2004) used light wavelength of 406 nm whereas Titgemeyer et al. (2001) used 410 nm to determine absorbance and concentration of TiO_2 in fecal samples.

The six-step procedure for calculating TiO_2 concentrations in feed and feces is as follows: 1) preparation of duplicate 0.5 g samples into 250-mL macro-Kjeldahl digestion tubes including a baseline sample of feces (or duodenal, ileal digesta,or forage) devoid of TiO_2 for background correction (Myer et al., 2004); 2) addition of a reaction catalyst containing 3.5g K_2SO_4 and 0.4g $CuSO_4$ to each vial; 3) addition of 13 mL of concentrated H_2SO_4 to each vial and digest samples at 420°C for 2 h; 4) remove heat and allow cooling for a minimum of 30 min; 5) addition of 10 mL 30% H_2O_2 to each vial and allow cooling for 30 minutes; and 6) allow total liquid weight to increase to 100 g with distilled water and filtering through Whatman No. 541 filter paper to remove precipitate and finally, followed by absorbance measurement at 410 nm. The spectrophotometer is calibrated with working standards, prepared by adding 0, 2, 4, 6, 8, and 10 mg of TiO_2 to blank tubes.

The analysis of Cr_2O_3 usually requires an initial oxidation of the organic matter by dry (Fenton and Fenton, 1979) or wet (Hill and Anderson, 1958; Czarnocki et al., 1961) ashing, followed with a more severe oxidation of Cr_2O_3 into the predominantly soluble

dichromate form using combinations of either sulfuric acid, nitric acid, perchloric acid, hydrogen peroxide or sodium molybdate. The concentration of dichromate ion may be determined spectrophotometrically at 440 nm wavelength or by atomic absorption spectroscopy. These procedures generally require sample sizes of 0.5 to 1.0 g (Suzuki and Early, 1991). Some rare earth external digestibility markers like Lanthanum oxide and Samarium may be analyzed by Neutron Activation Analysis (NAA). This involves bombarding the sample with neutrons, causing elements (markers) to form radioactive isotopes. The radioactive emissions and radioactive decay paths for each element are known. Using this information, it is possible to study the spectra of emissions of radioactive samples, and determine the concentration of the elements within it. This analytical procedure does not destroy the sample (Glascock, 2004). Internal digestibility markers such as lignin, NDF and ADF can be analyzed using the cell wall analytical procedures by Van Soest (Van Soest et al., 1991; Goering and Van Soest, 1970). These techniques involve boiling samples in detergent solution and this removes all other portions of the cell except the targeted internal marker (Goering and Van Soest, 1970). The concentration of the internal marker AIA can be determined by drying and ashing samples in 2 M hydrochloric acid for 5 minutes. The ash content is then determined gravimetrically after filtering, washing the hydrolysate to remove the acid and reashing (Van Keulen and Young, 1977). After calculating the concentration of the marker in the feed and feces, digestibility is estimated.

Characteristics of Good Digestibility Markers

A good digestibility marker cannot be digestible or absorbable in the GIT (Marais, 2000) and should pass through the GIT unaffected. Publications have established that internal digestibility markers are more susceptible to digestion and absorption compared

to external markers, because internal markers are natural components of the feedstuffs. When estimating digestibility of feedstuffs using markers, the concentration of marker in the feed and fecal material are critical parameters. When the marker is digested or absorbed in the GIT, there is reduced recovery and concentrations in the feces resulting in overestimation of digestibility (Owens et al., 1992; Rymer, 2000; Judkins et al., 1990). Therefore, an excellent marker must be indigestible and non-absorbable to ensure full recovery in the fecal material, thereby providing an accurate estimation of the digestibility of the feed.

In addition, an excellent marker must not alter the function of the GIT (Sunvold and Cochran, 1991). A ruminant GIT is a complex system involving complex relationships among microbes, the endocrine system, and enzymes which are coordinated under specified ranges of pH and temperature (Kham et al., 2003). Interference of this environment will result in malfunctioning of the GIT, reducing its ability to digest feeds. Some external markers, such as rare earth metals, are believed to influence gut fill, thereby reducing DM intake. Therefore, markers that affect the normal function of the GIT should be avoided by scientists to ensure accurate estimation of digestibility.

A good marker must be physically similar to or closely associate with feed material, which ensures even distribution of the marker in the feed and fecal material (Marais, 2000). This reduces chances of inaccurate estimation of the digestibility of the feed due to varying concentrations of the marker in fecal material. An ideal digestibility marker must have a specific method of analysis and the method must not interfere with other analyses (Marais, 2000). A marker that fulfills all of these conditions is considered

ideal for use in digestibility experiments because they are likely to have a high recovery rate, enhancing accurate estimation of digestibility.

In general, most external markers (metal oxides and rare earths) have been found superior in estimating digestibility compared to internal digestibility markers. In most cases, they may also be applied across a range of feeds and feedstuffs in different feeding conditions but provide estimations of digestibility and fecal output not significantly different from the total fecal collection method. Average estimates of daily fecal output obtained from analyzing both Cobalt and Ytterbium concentrations (2.39 and 2.59 kg/d, respectively) did not differ from total collection value (2.48 kg/d) (Brandyberry et al., 1991). In addition, Myers et al. (2004) observed a reduction in the diurnal effect on the excretion pattern of both TiO_2 and Cr_2O_3 in sheep. However, use of TiO_2 as a marker underestimated (from 1.6 to 4.3 %) digestibility compared to total fecal collection (Titgemeyer et al., 2001). In contrast, TiO_2 was equally effective as Cr_2O_3 in estimating rate of passage of digesta in sheep (Myers et al., 2004).

Among external digestibility markers, some are less effective than others. Pond et al. (1985) indicated the possibility of reduced recovery in feces and overestimation of digestibility by Fe_2O_3, which was attributed to Fe_2O_3 being heavy and not mixing well with digesta. In addition, rare earth metals are more expensive than some metal oxides. Furthermore, detection of some of these rare earth elements requires specialized analysis such as neutron activation which is expensive (Prigge et al., 1981). Therefore, Cr_2O_3 has been the most favored marker in digestibility trials because it is cheap, rarely found in feedstuffs, easy to analyze, and in most cases, it provides similar digestion coefficients as total fecal collection (Mroz et al., 1996; Brisson, 1956). More recently,

TiO_2 has become a useful marker which is easy to analyze, has no reported cases of carcinogenic effects, and can be legally added to feeds as a color additive (Titgemeyer et. al., 2001).

For internal digestibility markers, Sunvold and Cochran (1991) recommended acid detergent lignin (ADL), acid peroxide lignin (APL), and AIA as sound markers for estimating organic matter digestibility (OMD) of grass hay diets. Results indicated OMD estimates derived by ADL ratio, APL ratio and AIA ratio (63.5%, 62.0% and 63.5% respectively) for brome grass hay, which was similar to total fecal collection (61.2%) measurement. Likewise, OMD for prairie hay by ADL, APL, and AIA ratio (60.1%, 48.2% and 54.9% respectively) did not differ from total fecal collection (54.4%) measurements (Sunvold and Cochran, 1991). However, these markers yielded OMD estimates of alfalfa that differed from those derived by total fecal collection, which was attributed to relatively low concentrations of these marker (less than 6% in Alfalfa) and possibility of contamination, especially with AIA (Sunvold and Cochran, 1991).

High variations in digestibility estimates by AIA and silica have been associated to contamination of feedstuffs with dirt (Van Dyne and Lofgreen, 1964; Van Keulen and Young, 1977). Furthermore, Fahey and Jung (1983) explained the possibility of losing lignin during analysis as a result of destruction by reagents, pseudo digestion, true digestion, and metabolization in the GIT. Therefore, the application of internal digestibility markers is limited since there is currently no single marker that is effective in estimating digestibility of all feeds and feedstuffs. Therefore, it is prudent to utilize markers that have been identified as accurate to assess digestibility to avoid erratic results (Judkins et. al., 1990).

Utilization of Digestibility Markers

External digestibility markers may be administered orally by mixing with the diet before feeding or dosed using gelatin capsules. Digestibility markers may also be placed directly into the rumen in fistulated animals. Several studies have reported variations in digestibility or feed intake estimation with different methods of administering markers (Brandberry et al., 1991; Prigge et al., 1981; Langland et al., 1963; Brisson et al., 1957). Directly incorporating the marker with the feed may resulted in estimations of digestibility which could not be accurate and this in other cases, could be attributed to the fact that animals may not consume all of the feed provided to them, and it is difficult to account for the marker remaining in the orts, hence erratic estimation of digestibility (Brandyberry et al., 1991). Direct placement of marker in the rumen is mostly recommended when the objective is to estimate the passage rate of the feed (Myers et al., 2005).

After completion of an adaptation period, fecal samples should be collected, composited, and concentration of the marker in both feces and feed measured. Fecal sampling frequency depends on the frequency of external marker administration and the objective of the experiment. More frequent marker administration (6× per day) may require less frequent fecal sample collection, whereas less frequent marker administration (1× or 2× per day) may require more frequent fecal sampling (Brisson et al., 1957; Prigge et al., 1981). No significant differences were reported in fecal output obtained by Co and Yb_2O_3 (2.39 and 2.60 kg/d, respectively) using fecal samples collected in the morning or afternoon only compared to estimates (2.48 kg/d) by total fecal collection (Brandyberry et al.,1991).

In addition, 4× and 8× per day fecal sampling were used to determine interval required for marker equilibrium and to evaluate diurnal effects on marker recovery, respectively (Brandyberry et al., 1991). Fecal outputs in beef cows were evaluated using $YbCl_3$ as digestibility marker (Prigge et al., 1981). Fecal output estimates of 2.74 and 3.00 kg/d from samples collected 1× per d at 0800 h and 1600 h, respectively, did not differ from the 2.87 kg/d obtained from 2× per d sampling at 0800 and 1600 and these did not further differ from 2.83 kg/d fecal output obtained by total collection (Prigge et al., 1981). In addition, it was determined that samples should be taken at 4 h intervals for 48 hours to estimate diurnal variation of Cr_2O_3 and $YbCl_3$ excretion (Prigge et al., 1981). Prior to that (Brisson, 1956), it was determined that fecal samples should be collected 4× per day to successfully establish excretion patterns of Cr_2O_3 in cattle dosed 1×, 2×, or 6× per day. Again, Brisson et al. (1957) recommended 1× per day fecal sampling to estimate fecal output when cows were dosed 6× per day at equal intervals. In addition, Myers et al. (2005) indicated the possibility of reduced frequency of sampling in sheep dosed 2× without markedly affecting mean concentrations of Cr_2O_3 and TiO_2. Although variations in frequency of fecal sample collection exist, it is important that fecal samples are collected in such a way that represents multiple time periods to overcome diurnal variations associated with external marker excretion (Myers et al., 2005; Prigge et al., 1981; Brisson et al., 1957; Hardison et al., 1955; Langland et al., 1963).

Once concentrations of marker in feed and fecal samples are determined, digestibility of forage or feed is calculated using the formula: DM digestibility = 100 – (100 * % marker in feed / % marker in feces).

Advantages of Marker Techniques

Unlike total fecal collection techniques (where all feces are collected and weighed), with marker techniques only small samples of feces are utilized, which reduces collection of all feces (Rymer, 2000). In certain situations, particularly with cattle, total fecal collection is especially challenging. Thus, markers provide an alternative by using fecal samples from the rectum and using the marker to estimate total fecal output.

Challenges of Using Digestibility Markers

Although markers are widely used to estimate feed intake, passage rate, and digestibility, speculation still remains regarding the accuracy of specific markers used in estimating these responses. Erratic results have been reported when markers were administered across a wide range of feeds under different feeding conditions (Sunvold et al., 1991). These variations have been attributed to a range of factors such as metabolism and absorption of markers in the GIT, diurnal effects on marker recovery, uneven distribution of markers in the feces, sampling errors, and analytical errors which affect measurement of marker concentrations in feed and feces (Rymer, 2000). In addition, the mechanism of administration of external markers, also affects recovery, as a result of residual markers that are not accounted for when the marker is incorporated into the diet (Brandyberry et al., 1991). These factors negatively affect recovery and concentration of markers in feces, thereby influencing variations in estimates of DM digestibility, passage rate, and DM intake in nutrition experiments (Marais, 2000). Therefore, it is important to note that markers are selected for specific diets, feeding conditions, and that these selected markers should be validated before use in different

feeds and feeding conditions in order to achieve accurate estimates of digestibility (Judkins et al., 1990).

Chromic oxide and Titanium dioxide

Both Cr_2O_3 and TiO_2 are metal oxides. Chromic oxide is the most commonly used digestibility marker, and it's utilization was first reported in 1918. As a digestibility marker, Cr_2O_3 was used in 72% of studies published by the Journal of Animal Science between 1986 and 1995 (Titgemeyer, 1997). In addition, chromic oxide also is the most extensively studied external digestibility marker and has been found to be effective in ruminants, monogastrics, birds and in multiple feedstuffs such as grass hay, legume hay, and concentrates (Hill and Anderson, 1958; Brandberry et. al., 1991; and Titgemeyer et. al., 2001). Additional studies (Hardison et al., 1955; Brisson et al., 1957; Langland et al., 1963; and Prigge et al., 1981; Fenton and Fenton, 1979; Czarnock et. al., 1961; Suzuki et. al., 1991) have developed analytical procedures, evaluated and derived techniques based on dosing, recommended fecal collection frequencies on different diets and feeding conditions when Cr_2O_3 is used in digestibility experiments. However, although Cr_2O_3 has been the most frequently used marker, there are reports associating it with health risks (specifically those that may be carcinogenic; Myers et al., 2004).

In contrast, TiO_2 has been recently explored as a potential alternative digestibility marker, with less health risks, that may be legally added to feeds (Titgemeyer et al., 2001). Several studies have indicated the feasibility of using TiO_2 as a viable total–tract digestibility marker in rats, chicken, pigs, dairy cows (Myers et al., 2004) and beef steers (Titgemeyer et al., 2001). However little has been done to test its efficacy in estimating digestibility in a variety of forms of feed or feeding conditions. Currently, there is no

available data to explain the viability of using TiO_2 as a digestibility marker in beef animals fed fresh forages on an ad libitum basis. In addition, no data is available to either quantify the effect of differing sampling frequencies. Therefore, additional data is necessary to further evaluate the use of TiO_2 to broaden its application as a digestibility marker in digestibility experiments.

CHAPTER 3
ESTIMATION OF APPARENT DIGESTIBILITY OF SIX FORAGES USING TWO DIFFERENT DIGESTIBILITY MARKERS

Materials and Methods

Two experiments were carried out with the objectives of comparing summer and winter annual forages in terms of apparent total tract digestibility of nutrients. Secondary objectives were to compare the efficacy of titanium dioxide (TiO_2) and chromic oxide (Cr_2O_3) as digestibility markers for fresh forages fed on an ad libitum basis, and to determine the effects of performing a 2× vs. 3× per day fecal sample collection protocol to measure digestibility.

Annual Winter Forages (Experiment 1)

The study was conducted at the University of Florida Feed Efficiency Facility (FEF) in Marianna, FL. Ryegrass (*Lolium multiflorum* Lam cultivar Prine), blend of Ryegrass (*Lolium multiflorum* Lam cultivar Prine) and Oat (*Avena sativa* cultivar Horizon 201), and the blend of ryegrass (*Lolium multiflorum* Lam cultivar Prine) and triticale (*Triticosecale rimpau* cultivar Trical 342) were the forages used in this study. In November 2010 these forages were sown on prepared seedbeds in 0.7 ha paddocks at the following rates: 35 kg ha^{-1} of ryegrass; 67 kg ha^{-1} of oat combined with ryegrass sown at the rate of 30 kg ha^{-1}; and triticale was sown at the rate of 274 kg ha^{-1} in combination with ryegrass at 17 kg ha^{-1}. All pastures were fertilized twice by the Altha, FL Farmers Coop. The first fertilization was done 28 d after planting at the rate of 57 kg ha^{-1} of N (NH_4NO_3) and 22 kg ha^{-1} of S while the second was done after another 28 d from the first fertilization at the rate of 57 kg ha^{-1} of N (NH_4NO_3) and 11 kg ha^{-1} of S. All grasses were cut fresh using a chopper at 15 cm stubble height. Forages were cut every morning starting at d 98 from planting and continued until the end of the study.

Herbage mass of each forage treatment was estimated at the beginning of the experiment. Ryegrass, Oat + Ryegrass, and Triticale + Ryegrass treatments had herbage mass estimated at 2,258 kg ha^{-1}, 3,463 kg ha^{-1} and 5,198 kg ha^{-1} respectively (Table 3-1). The botanical composition of each treatment was described in order to estimate DM contribution of each forage to the total herbage mass of the treatment (Table 3-1). For Ryegrass treatment, 100% of the total herbage mass was from Ryegrass alone. Ryegrass contributed 91.9%, whereas Oat and weeds contributed 4.1% each to the total herbage mass of Oat + Ryegrass treatment. Triticale made 61.1% while Ryegrass was 38.9% of the total herbage mass of Triticale + Ryegrass treatment. Furthermore, Oat + Ryegrass, and Triticale + Ryegrass treatments had DM contents of 16.34% and 16.48% respectively whereas the Ryegrass treatment had 15.81% DM. The CP content for Ryegrass treatment was 17.73%, NDF and ADF estimates were 41.67% and 22.67% respectively. For Oat + Ryegrass treatment, CP was at 18.27%, while NDF was 44.46% and ADF was 24.13%. As for Triticale + Ryegrass treatment, the CP, NDF and ADF were estimated at 16.32%, 49.56%, and 27.56% respectively (Table 3-1).

Annual Summer Forages (Experiment 2)

In experiment 2, three summer forages, Mulato II (hybrid *Brachiaria*), “Tifleaf 3” Pearl Millet (*Pennisetum glaucum*), and “Hay Day” Sorghum sudan (*Sorghum bicolor*) were separately planted in three pens (0.7 ha each) in June 2011. Mulato II was planted on June 2, 2011 on a prepared seedbed at a seeding rate of 1 kg ha^{-1}. On June 27, 2011, “Tifleaf 3” Pearl Millet and “Hay Day” Sorghum sudan were planted in the remaining two pens on a prepared seedbeds at a seeding rates of 33 kg ha^{-1} both. All grasses were fertilized by Altha, FL Farmers Coop. with 57 kg ha^{-1} of N (NH_4NO_3) and

11 kg ha^{-1} of S on d 44 for Mulato II, whereas for Pearl Millet and Sorghum sudan was done on d 19 from planting. These grasses were cut fresh every morning using a chopper at 15 cm stable height starting from d 91 after planting and this continued until the end of the study.

All three summer forages were characterized in terms of botanical composition, yield and nutrient content on DM basis (Table 3-2). The herbage masses for Mulato II, Pearl Millet and Sorghum sudan were 3,132 kg DM ha^{-1}, 1,519 kg DM $ha^{-1}$and 1,644 kg DM ha^{-1} respectively. Millet contributed 100% to the total herbage mass of Pearl Millet treatment, while Sorghum sudan made 91.5% of total herbage mass of Sorghum sudan treatment with the remaining 3.5% being a contribution from weeds. Mulato II had a significant proportion of weeds, such that Mulato II contributed 51.8% to the total herbage mass of the Mulato II treatment while the remaining 48.2% was a contribution from weeds. Furthermore, DM compositions for Mulato II, Pearl Millet and Sorghum sudan were 32.55%, 22.30% and 20.73% respectively. In addition, Pearl Millet had the highest CP (23.18%) followed by Sorghum sudan (18.29%) and lastly, Mulato II at 17.83%. The NDF for Sorghum sudan was 54.99% followed by Mulato II at 54.33% and Pearl Millet at 51.74%. The ADF estimates indicate that Mullato II had lowest value 27.28% while Pearl Millet and Sorghum sudan had 30.93% and 30.78% respectively (Table 3-2).

Animals and Management (Experiment 1)

Angus and Angus-crossbred heifers (n=12) were used in this study. On d 0, heifers were randomly assigned to one of the 3 forage treatments and allowed to graze for 28 d. These heifers were weighed on d 29 (364 ± 52 kg of BW), stratified by weight, and then randomly assigned to pens (2 heifers per pen) in the University of Florida Feed

Efficiency Facility (FEF) located in Marianna, FL. From d 29 to 44 heifers were offered daily fresh cuts of the same forage treatments they were grazing and were also provided with fresh water both on ad libitum basis throughout the study period. Individual intake was monitored in the FEF using a GrowSafe system (GrowSafe Systems Ltd., Alberta, Canada). Every heifer became the experimental unit once entering the FEF. Again, from d 29 to d 44, heifers were bolus-dosed with 2 gelatin capsules: one containing 10 g of TiO_2 and the other containing 10 g of Cr_2O_3 using a balling gun. These gelatin capsules were fed once per day at 1200 h. Feed samples were collected from d 39 to d 43 and fecal samples were collected by rectal grabs from d 40 to d 44 of the study allowing 12 d stabilization of the digestibility markers and also for heifers to adapt to the marker and feeding in the FEF.

Animals and Management (Experiment 2)

A total of 12 Angus and Angus-crossbred heifers were selected for enrollment in the study and were moved into the Feed Efficiency Facility and penned in groups of 6 per pen. Heifers were offered water and bahia grass hay on ad libitum basis for 1 wk to adapt them to eating from the FEF bunks before commencement of the study. On d 0, Heifers were weighed (194 ± 11 kg of BW), stratified by weight, and randomly assigned to pens (2 heifers per pen) in the FEF. Heifers were fed grass fresh cuttings from their respective treatments and were also provided with fresh water both on ad libitum basis throughout the study period. Individual intake was monitored in the FEF using a GrowSafe system (GrowSafe Systems Ltd., Alberta, Canada). Every heifer became the experimental unit once entering the FEF, as feed intake was recorded individually. Beginning on d 7, heifers were bolus-dosed with 2 gelatin capsules: one containing 10 g of TiO_2 and the other containing 10 g of Cr_2O_3 using a balling gun. These gelatin

capsules were fed only once per d at 1200 h from d 7 until d 19. Feed samples were collected from d 14 to d 18 and fecal samples were collected by rectal grabs from d 15 to d 19 of the study. This was to allow 8 days of marker stabilization and adaptation of animals to the feed.

Sample Collection and Preparation (Experiment 1 and 2)

Feed samples for daily DM determination were collected in paper bags 2× daily at 0800 h and 1600 h, and the average DM value of the two samples was used to determine daily DM intake. Feed samples were dried at 100°C for 24 h in order to determine DM. Feed samples for nutritive value analyses were collected once daily after feeding and were stored in plastic bags and frozen at -20°C for posterior freeze-drying to avoid loss of nutrients through continued activities of enzymes in the forages soon after cutting. These samples were then freeze dried at -50^{o}C in order to reduce loss of volatile elements in the forage through heat destruction. Thereafter, the samples were ground through a 2-mm screen before analysis. Forage samples from the cutting pastures were collected from 3 areas of 0.25 m^2 within each pasture to determine the botanical composition of the forages. These samples were stored in paper bags and transported to the lab. All 3 samples per pasture were combined into 1 sample and weighed. For experiment 1, while still fresh, each forage sample was sorted into the different species present in each pasture as follows: RG treatment: sorted into ryegrass and “other species” (weeds, etc.). T+RG (Triticale and Ryegrass) treatment: sorted into Triticale, Ryegrass, and “other species”. O+RG (Oat and Rygrass) treatment: sort into Oat, Ryegrass, and “other species”. Likewise, for experiment 2, fresh forage samples were sorted into different species present in each pasture as follows: Mulato and other species for Mulato II treatment, Pearl millet and other species for Pearl Millet treatment,

and finally, Sorghum sudan and other species for Sorghum sudan treatment. After sorting, each subsample was weighed fresh and then dried at 100°C for 24 h to determine percentage DM contribution of each species to the total herbage mass expressed as kg of DM ha^{-1}.

Fecal samples were collected three times a d at 0800 h, 1200 h and 1600 h by grabbing from the rectum. After every collection, fecal samples were stored in plastic bags and frozen at -20°C for posterior freeze-drying at -50^{o}C and subsequent grinding through a 2 mm screen. Fecal samples for the 5 collection days were composited within heifer. Two separate pools of composited samples were created to test the effect of adding a noon fecal collection: one containing equal amounts of 0800 h, 1200 h, and 1600 h fecal samples while the other part containing equal amounts of 0800 h and 1600 h fecal samples. These two pools of composited samples were analyzed separately.

Sample Analysis (Experiment 1 and 2)

TiO_2 analysis

In both experiment 1 and 2, TiO_2 was analyzed following the procedure of Myers et al. (2004). A 1.0 g sample of dried feces was weighed into weight papers in duplicate and each sample wrapped in a weight paper was placed into 250 mL macro-Kjeldahl digestion tubes. In each run, a blank sample was included (fecal sample devoid of Titanium dioxide and treated as the rest of the samples) to account for any absorbance due to the components of the fecal matter. Again, included in each run, were standards prepared by adding 0, 2, 4, 6, 8, and 10 mg of TiO_2 each one placed in digestion tubes without fecal samples. As with other samples, weight paper was included in each of the 0 standards. These standards were used to develop a calibration curve. One CT-37 FisherTab tablet (containing 3.5g K_2SO_4 + 0.4g $CuSO_4$) was added to each digestion

tube as a reaction catalyst and subsequently, 3 mL of concentrated sulfuric acid was added to each digestion tube. With the manifold on top of the tubes, samples were digested for 2 h at 420^{o}C. After turning off the digestor, cooling was allowed for at least 30 min. then, 10 mL of 30% H_2O_2 was added to each digestion tube and further cooling allowed for another 30 min. Later, total liquid weight of each tube was brought to 100 g using distilled water. Thereafter, the liquid was filtered through Fisherbrand P8 Grade filter paper to remove any precipitate. A 96-well plate map was created and from each sample 200 µL was transferred into the plate in duplicate wells using a pipette. At least two wells of the plate were left unfilled to be used as a correction for empty well absorbance. The absorbance was read at 405 nm wavelength using a Bechman DU – 500 Spectrophotometer.

Cr_2O_3 analysis

For Cr_2O_3 concentration in feces, approximately 0.5 g ± 0.05 g of ground samples were dried at 105°C for 24 h to determine DM, after which the samples were ashed at 550°C for 3 h to determine OM. The method of Williams et al. (1962) was used to digest Cr_2O_3 in the samples. Briefly, 3 mL of acid manganese sulfate and 4 mL of potassium bromate were added to the ashed samples and heated in a hot plate for approximately 7 minutes after which, 12.5 mL of calcium chloride were added and samples were brought to volume in a 100-mL volumetric flask. After digestion, Cr_2O_3 concentration was determined by atomic absorption spectrophotometry (358 nm with an air-plus-acetylene flame; AA-6300; Shimadzu Corp., Kyoto, Japan).

Analysis of NDF, ADF, and DM

Determination of NDF in samples was conducted using an Ankom 200 Fiber Analyzer (Ankom Technology, Fairport, NY) according to procedures of Van Soest et al.

(1991; as modified by Ankom Technology). Approximately 0.5 g ± 0.05 g of the ground composite fecal sample for each animal and each diet were placed into individual F57 filter bags (Ankom Technology) and heat sealed. During the NDF procedure, heat stable α-amylase (Ankom Technology) and sodium sulfite were added to both feed and fecal samples (dried at 55°C for 2 d).

Crude protein

To determine CP (N × 6.25) concentrations in feed and feces, approximately 0.250 g ± 0.005 g of ground feed or fecal samples were placed into a crucible for total N analysis by rapid combustion using a macro elemental N analyzer (Vario Max CN, Elementar Americas Inc., Mt. Laurel, NJ) following official method 992.15 (AOAC, 1995).

Statistical Analysis (experiment 1 and 2)

Both experiment 1 and 2 were split-split plot design in which the whole plot tested the forage treatment effect, the split plot tested the fecal collection schedule (2× vs. 3×) and the split-split plot tested the marker effect (Cr_2O_3 vs. TiO_2) using heifer as the experimental unit. The data was analyzed using the MIXED procedure of SAS. All values reported are least square means and significance was declared if $P < 0.05$. The model used to analyze the results was:

$$Yijk = \mu + Ti + Bj + (TB)ij + Ck + (TC)ij + (BC)jk + (TBC)ijk + Eijk$$

Where;

Yijk = digestibility of forage

μ = general mean

Ti = forage effect (i = 1, 2, and 3)

Bj = time of collection effect (j = 2× and 3×)

(TB)ij = forage × time of collection

Ck = marker effect (k = TiO_2 and Cr_2O_3)

(TC)ij = forage × marker effect

(BC)jk = time of collection × marker effect

(TBC)ijk = forage × time of collection × marker

Eijk = experimental error.

Table 3-1. Composition, yield and analyzed nutrient content (DM basis) of the winter annual pastures used in Exp. 1.

Item	Treatment[a] Ryegrass[b]	Oat + Ryegrass[c]	Triticale + Ryegrass[d]
Herbage mass at the beginning of digestibility phase, kg DM ha^{-1}	2,258	3,463	5,198
Botanical composition, % of total herbage mass DM[e]			
Triticale	-	-	61.1
Ryegrass	100	91.9	38.9
Oat	-	4.1	-
Weeds	0	4.1	0
Analyzed composition, % of DM			
DM	15.81	16.34	16.48
CP	17.73	18.27	16.32
NDF	41.67	44.46	49.56
ADF	22.67	24.13	27.56

[a]Three 0.7 ha pastures were planted on a prepared seedbed for each treatment. Pastures were planted on November of 2010 and fertilized 28 d after planting with 57 kg ha^{-1} of N (NH_4NO_3) and 22 kg ha^{-1} of S. A second fertilization with 57 kg ha^{-1} of N (NH_4NO_3) and 11 kg ha^{-1} of S took place 56 d after planting.
[b]Seeding rate = 35 kg ha^{-1} of *Lolium multiflorum* Lam cv. Prine.
[c]Seeding rate = 67 kg ha^{-1} of oat (*Avena sativa* cv. Horizon 201) plus 30 kg ha^{-1} of ryegrass (*Lolium multiflorum* Lam cv. Prine).
[d]Seeding rate = 274 kg ha^{-1} of triticale (*Triticosecale rimpau* cv. Trical 324) plus 17 kg ha^{-1} of ryegrass (*Lolium multiflorum* Lam cv. Prine).
[e]Average of three 0.25 m^2 samples taken from each 0.7 ha pasture.

Table 3-2. Composition, yield and analyzed nutrient content (DM basis) of the summer annual pastures used in Exp. 2.

Item	Treatment[a] Mulato II[b]	Millet[c]	Sorghum[d]
Herbage mass at the beginning of digestibility phase, kg DM ha^{-1}	3,132	1,519	1,644
Botanical composition, % of total herbage mass DM[e]			
Mulato *Brachiaria*	51.8	-	-
Sorghum Sudan	-	-	96.5
Millet	-	100	-
Weeds	48.2	0	3.5
Analyzed composition, % of DM			
DM	32.55	22.30	20.73
CP	17.83	23.18	18.29
NDF	54.33	51.74	54.99
ADF	27.28	30.93	30.78

[a]Three 0.7 Ha pastures were planted on a prepared seedbed for each treatment. Pastures were planted on June 2, 2011 (Mulato) and on June 27, 2011 (Sorghum Sudan and Millet). All pastures were fertilized on July 15, 2011 with 57 kg ha^{-1} of N (NH_4NO_3) and 11 kg ha^{-1} of S.
[b]Seeding rate = 1 kg ha^{-1} of *Brachiaria hybrid* Mulato II.
[c]Seeding rate = 33 kg ha^{-1} of "Tifleaf 3" Pearl Millet (*Pennisetum glaucum*).
[d]Seeding rate = 33 kg ha^{-1} of "Hay Day" Sorghum Sudan (*Sorghum bicolor*).
[e]Average of three 0.25 m^2 samples taken from each 0.7 ha pasture.

CHAPTER 4
RESULTS AND DISCUSSION

Results

Experiment 1

Daily nutrient intake by the heifers during the study period was estimated. No forage treatment effect was observed ($P > 0.05$) on DM, OM, CP NDF and ADF intake (Table 4-1). Intakes for DM, OM, CP, NDF and ADF across the forages averaged 4.54 kg/d, 4.05 kg/d, 0.79 kg/d, 2.05 kg/d and 1.0 kg/d, respectively. No significant forage treatment effect was observed ($P > 0.05$) on the total tract digestibility of winter forages (Table 4-1). Across forage treatments, average apparent nutrient digestibility were 59%, 61%, 60%, 49%, and 35% for DM, OM, CP NDF and ADF respectively. Furthermore, neither fecal sample collection protocol (2× vs. 3× per day) nor markers affected apparent total tract digestibility ($P > 0.05$) of DM, OM, CP NDF and ADF from the three winter forages (Table 4-1). In addition, no interactions were found ($P > 0.05$) between sample collection protocol and marker for apparent nutrients digestibility of DM, OM, CP, NDF, and ADF across all winter forages used in this study (Table 4-1).

It is worth noting that within TiO_2 as a marker, there were no significant differences ($P > 0.05$) in apparent total tract digestibility of nutrients across sampling schedules (Table 4-2). Averaged across 2× and 3× sampling schedules, the apparent total tract nutrient digestibility measured using TiO_2, were 62.1%, 64.2%, 62.7%, 52.1%, and 37.8% for DM, OM, CP NDF and ADF respectively. Similarly to TiO_2, no significant effects ($P > 0.05$) were observed in apparent total tract digestibility of nutrients across 2× and 3× sampling schedules when Cr_2O_3 was used as a marker (Table 4-2). The averages across sampling schedules were 56.3% for DM, 58.6% for OM, 57.5% for CP,

46.2% for NDF, and 31.4% for ADF. In addition, no interactions were observed (P > 0.05) between the markers and forages (Table 4-2).

Experiment 2

During the study period, daily intake of DM, OM, CP, NDF and ADF was not affected (P > 0.05) by forage treatment (Table 4-3). Across the forages, intake of DM, OM, CP, NDF and ADF was averaged 3.6 kg/d, 2.98 kg/d, 0.7 kg/d, 1.93 kg/d and 0.87 kg/d respectively. Similarly to nutrient intake, apparent total tract digestibility by heifers was not affected (P > 0.05) by forage treatment. The values of apparent total tract digestibility across the forage treatments averaged 56.2%, 65.9%, 68.4%, 49.7% and 32.7% for DM, OM, CP, NDF and ADF respectively. There was no effect of marker (P > 0.05) on apparent total tract digestibility of DM, OM, CP NDF and ADF from the three summer forages. Furthermore, there was no interaction (P > 0.05) between sample collection protocol and marker for apparent digestibility of ADF. However, unlike in experiment 1, interactions were found (P < 0.05) between sample collection protocol and marker for apparent nutrients digestibility of DM, OM, CP and NDF across all summer forages used in this study (Table 4-3).

Under a 2× sampling protocol, apparent total tract digestibility of DM, OM, CP and NDF measured using Cr_2O_3 were significantly decreased (P < 0.05) compared with those obtained using TiO_2 (Table 4-4). Total tract apparent digestibility of DM, OM, CP and NDF were 7.7%, 5.7%, 5.4% and 8.8% lower with Cr_2O_3 than when using TiO_2 under a 2× sampling protocol, respectively. Similarly, using Cr_2O_3 under a 2× sampling protocol yielded digestibility values of DM, OM, CP and NDF that were decreased (P < 0.05) compared with estimates obtained with either Cr_2O_3 or TiO_2 under a 3× sampling protocol. However, no differences (P > 0.05) were observed in total tract nutrient

digestibility of summer forages when a 3× fecal sampling protocol was implemented (Table 4-4).

However, ADF digestibility calculated with reference to Cr_2O_3 under a 2× sampling protocol did not differ ($P > 0.05$) from ADF digestibility measured with TiO_2 under a 2× sampling protocol and Cr_2O_3 under 3× sampling protocol. Under 2× sampling protocol, ADF digestibility estimated by Cr_2O_3 (23.8%) was 16.3% less than the values estimated with reference to TiO_2 (39.9%) under 3× sampling protocol. Apparent total tract digestibility of DM, OM, CP, NDF and ADF measured using TiO_2 under 2× sampling protocol were not different ($P > 0.05$) from those measured with Cr_2O_3 or TiO_2 under 3× sampling protocol Table 4-4).

There was a marker effect ($P < 0.05$) on apparent total tract digestibility of DM, OM, CP and NDF across sampling schedules (Table 4-5). Averaged across 2× and 3× sampling schedules, the apparent total tract nutrient digestibility measured using TiO_2, were 58.3%, 67.6%, 69.8%, and 52.0% for DM, OM, CP and NDF respectively. Whereas using Cr_2O_3, apparent total tract digestibility of DM, OM, CP and NDF across 2× and 3× sampling protocol were 53.7%, 64.3%, 66.6%, and 46% respectively. However, no marker effect ($P > 0.05$) was found on apparent digestibility of ADF. In addition, an interaction was observed between marker and forage ($P > 0.05$) on the apparent total tract digestibility of all nutrients measured (Table 4-5). When using TiO_2, no differences were observed in nutrient digestibility across forages (Table 4-6). However, when using Cr_2O_3, the total tract digestibility of DM and NDF were decreased ($P < 0.05$) in Pearl millet compared with Mulato II (Table 4-6).

Discussion

Nutrient Digestibility of Forages

The lack of differences in nutrient digestibility in forages may result from the relatively high quality of the forages tested. Mean DM apparent total tract digestibility of winter grasses (combinations of Oat + Ryegrass, and Triticale + Ryegrass) obtained in this study were within the ranges reported by Keuren and Underwood (1990) of the grasses separately. Dry Matter digestibility ranges of 60% to 79% and 56% to 77% for Triticale and Oat respectively were reported by Keuren and Underwood (1990). Similarly, mean DM digestibility for Mulato II and Sorghum sudan reported in this study fell within the ranges of previous reports(Vendramini et al., 2011; Lang, 2001) with DM digestibility for Mulato II ranging from 55 to 60%, while a range of 55 to 70% for DM digestibility of Sorghum sudan. However, DM digestibility for Ryegrass and Millet were slightly lower than the ranges by other reports (Blount et al., 2009; Keuren and Underwood, 1990) and this may be a factor of differences in forage management and increased rate of passage in the GIT due to low DM content since harvesting in this study was done at an early stage of growth.

Effectiveness of TiO_2 and Cr_2O_3 as Digestibility Markers

The effectiveness of TiO_2 in estimating digestibility of winter forages was similar to that of Cr_2O_3 in our study. Furthermore, collecting fecal samples by rectal grab 2× a day (at 0800 h and 1600 h) was as sufficient as obtaining samples 3× a day (0800 h, 1200 h and 1600 h) in estimating digestibility of winter grasses. Contrary to our findings, Titgemeyer et al. (2001) reported that total tract DM digestibility measured using TiO_2 was underestimated ($P < 0.01$) by 1.1 to 5.5 percentage units while total tract DM digestibility calculated using Cr_2O_3 was overestimated ($P < 0.01$) by 2.0 percentage

units in study 2 except in study 3 where estimates calculated using Cr_2O_3 were not different from (P = 0.31) those obtained by total fecal collection. These observations were collected from 2 steers limit fed corn-based diets in study 2 and 8 steers fed corn-based diets on ad libitum basis in study 3. This contrast may be as a result of difference in the diets and method of administering the marker. Digestibility markers behave differently when used across different diets (Sunvold and Cochran, 1991; Judkins et al., 1990). In our experiments, forage diets were used whereas Titgemeyer et al. (2001) used corn based diets and this may have attributed to Cr_2O_3 and TiO_2 behaving differently. In addition, in our experiment, the markers were packed in gelatin capsules and administered orally using a balling gun and this ensured that the entire marker was taken by the animal. In contrast, markers were mixed with feed or dietary supplements in feeders in the study by Titgemeyer et al. (2001) and this may have resulted in some proportions of the markers not being consumed by steers thereby affecting marker recovery and digestibility calculations.

Unlike in the winter forage study, the presence of an interaction between sampling protocol and markers in summer forage study indicates differences in the effectiveness of Cr_2O_3 and TiO_2 to estimate digestibility of forages with varying characteristics. As indicated by Judkins et al. (1990) different digestibility markers behave differently when used across different diets, this may be applicable in our study as total tract digestibility estimates of summer forages calculated with reference to Cr_2O_3 were affected by sampling frequency while those calculated with reference to TiO_2 were not affected. This may have to do with issues concerning marker and digesta association. Similar studies (Titgemeyer, 1997; Owen and Hardson, 1992; Preggie et al., 1981) have

described Cr_2O_3 as a marker that does not mix completely with digesta in the GIT. Chromic oxide often is criticized because it does not associate specifically with either the particulate or fluid phase (Titgemeyer, 1997). Again, it was reviewed by Titgemeyer (1997) that Cr_2O_3 does not seem to mix completely with ruminal contents, particularly when supplied in gelatin boluses and that collection of representative fecal sample can alleviate the effect. Currently, it has not been clearly established as to how specific feed types affect the behavior of the Cr_2O_3. However, Titgemeyer (1997) suggested that the variable fecal recoveries and diurnal pattern of excretion for Cr_2O_3 presumably are a result of temporal sequestration of the Cr_2O_3 in the rumen that results from poor mixing with digesta. All summer forages in this study had DM almost 2× that of individual winter forage which means their retention time in the rumen may be higher than that of winter forages and this may have allowed more time for temporal sequestration of Cr_2O_3 in the rumen hence differences in marker concentration in the fecal sample thereby affecting digestibility values calculated with reference to Cr_2O_3 when fecal samples were collected 2× a day (at 0800 h and 1600 h).

The variation between total tract digestibility of summer forages calculated with reference to Cr_2O_3 and those calculated with reference to TiO2 under 2x sampling, may be as a result of differences in the rate of recovery, excretion pattern and ability of marker to mix with digesta when used in different forages. Many reports (Titgemeyer, 1997; and Priggie et al., 1981) have indicated diurnal variations in the flow of Cr_2O_3 and these variations have been reported to be more pronounced when Cr_2O_3 is dosed once. Although not much has been reported on the behavior of TiO_2 in comparison with Cr_2O_3, Myers et al. (2005) reported consistently higher mean concentrations ($P < 0.05$) of TiO_2

in fecal samples than Cr_2O_3 at every sampling time in all three experiments. These observations were obtained from eight ewes fed 100% forage diet (brome hay), 50% forage diet and 25% forage diets in experiments 1, 2, and 3 respectively. Markers were dosed intraruminally twice a day (0600 and 1800 h) and samples were collected at 6 h interval for six days in all experiments. Again, Myer et al. (2005) observed a more erratic pattern of the flow of TiO_2 excretion and further described this as a reflection of the normal pattern of digesta flow and thus providing a more accurate representation of marker excretion pattern than Cr_2O_3. This implies that TiO_2 associated well with the digesta than Cr_2O_3 in diets used and that TiO_2 recovery was good compared to Cr_2O_3. In our study, collecting fecal samples 2× a day (0800 h and 1600 h) may have been insufficient to accurately estimate total tract digestibility of the summer forages using Cr_2O_3. However, increasing the sampling frequency to 3× a day may have reduced effects of diurnal variations and recovery and incomplete mixing with digesta thereby resulting in collection of representative fecal samples hence total tract digestibility of nutrient calculated with reference to Cr_2O_3 did not differ from those calculated with reference to TiO_2 when fecal samples were collected 2× a day (0800 h and 1600 h) and 3× a day (0800 h, 1200 h and 1600 h).

When using TiO_2, no differences were observed in nutrient digestibility across forages. However, when using Cr_2O_3 the total tract digestibility of DM and NDF were decreased in Pearl millet compared with Mulato II. Although it is hard to find a factor influencing this variation, but differences in DM and NDF content between Pearl millet (22.30%) and Mulato II (32.55%) may have increased the rate of passage for Pearl

millet thereby affecting Cr_2O_3 recovery and subsequent estimates of DM and NDF digestibility.

Conclusion

Titanium dioxide and chromic oxide are indigestible markers that can be used to calculate total tract digestibility of fresh winter and summer forages fed to ruminants. Collecting fecal samples at 0800 and 1600 h was sufficient for measuring digestibility in winter forages, eliminating the need for a 1200 h fecal sampling. Both Cr_2O_3 and TiO_2 may be used indistinctively to estimate digestibility of winter forages while only TiO_2 may be used to estimate total tract digestibility for summer forages if fecal samples are collected 2× a day at 0800 h and 1600 h. Caution should be observed when using Cr_2O_3 in estimating total tract digestibility of fresh summer forages as 2× sampling frequency (0800 h and 1600 h) may result in underestimations of nutrient digestibility. Increasing sampling frequency to 3× a day (0800 h, 1200 h and 1600 h) may yield more desirable results when Cr_2O_3 is used as a digestibility marker for fresh summer forages. However standard errors of the mean reported in this study, especially for digestibility of fiber fractions may be of concern. The use of digestibility markers that associate more intimately with the forage (e.g. internal markers) and comparing our results with total fecal collection method should be tested in future studies.

Table 4-1. Nutrient intake and digestibility by heifers fed winter forages, using Cr2O3 or TiO2 as indigestible marker and under two fecal sampling protocols in Exp. 1.

	Treatment[a]				*P*-value			
Item	Ryegrass	Oat + Ryegrass[b]	Triticale + Ryegrass[c]	SEM[d]	Forage treatment	Sampling protocol[e]	Marker[f]	Sampling × Marker[g]
Intake[h], kg/d								
DM	4.34	4.88	4.4	0.72	0.85	-	-	-
OM	3.87	4.31	3.97	0.64	0.88	-	-	-
CP	0.77	0.89	0.71	0.12	0.6	-	-	-
NDF	1.81	2.17	2.18	0.33	0.68	-	-	-
ADF	0.88	1.04	1.1	0.17	0.64	-	-	-
Digestibility, %								
DM	50.3	62	65.4	5.9	0.21	0.31	0.1	0.87
OM	51.8	64.8	67.5	5.8	0.18	0.25	0.1	0.91
CP	55.8	58.2	66.1	5	0.36	0.57	0.13	0.85
NDF	30.8	57.3	59.4	8.7	0.08	0.33	0.14	0.96
ADF	13.9	42.9	46.8	9.7	0.07	0.14	0.18	0.62

[a]Winter forages were cut fresh every day from 0.7 ha pastures at 0800 h and offered ad libitum.
[b]Combination of Oat and Ryegrass.
[c]Combination of Triticale and Ryegrass.
[d]Pooled standard error of treatment means, n = 4 heifers/treatment.
[e]Effect of fecal sample collection protocol: 2 samples per day (0800 and 1600 h) vs.3 samples/d (0800, 1200 and 1600 h).
[f]Effect of indigestible marker used to calculate apparent total tract digestibility: 10 g/d of each Cr_2O_3 and TiO_2 were dosed once daily at 1200 h in two separate gelatin capsules.
[g]Sampling protocol × indigestible marker interaction.
[h]Intake during the 5-d digestibility measurement period of the experiment.

Table 4-2. Apparent total tract digestibility of nutrients measured using Cr2O3 or TiO2 as indigestible markers in heifers fed three winter forages in Exp. 1.

	Marker[a]			*P*-value	
Item	Cr_2O_3	TiO_2	SEM[b]	Marker effect	Marker × forage[c]
Digestibility, %					
DM	56.3	62.1	3.82	0.13	0.82
OM	58.6	64.2	3.75	0.13	0.81
CP	57.5	62.7	3.38	0.16	0.89
NDF	46.2	52.1	5.44	0.18	0.85
ADF	31.4	37.8	6.07	0.21	0.77

[a]Heifers were dosed once daily with 10 g/d of both Cr_2O_3 and TiO_2 once daily at 1200 h in two separate gelatin capsules.
[b]Pooled standard error of treatment means, n = 4 heifers/treatment.
[c]Effect of interaction between forage consumed (ryegrass, oat + ryegrass, or triticale + ryegrass) and marker used.

Table 4-3. Nutrient intake and digestibility by heifers fed summer forages, using Cr2O3 or TiO2 as indigestible marker and under two fecal sampling protocols in Exp. 2.

	Treatment[a]				*P*-value			
Item	Mulato II	Millet	Sorghum	SEM[b]	Forage treatment.	Sampling protocol[c]	Marker[d]	Sampling × Marker[e]
Intake[f], kg/d								
DM	3.79	3.55	3.46	0.66	0.94	-	-	-
OM	3.12	2.96	2.86	0.56	0.95	-	-	-
CP	0.72	0.73	0.65	0.10	0.85	-	-	-
NDF	2.05	1.88	1.87	0.39	0.93	-	-	-
ADF	0.92	0.84	0.84	0.16	0.92	-	-	-
Digestibility, %								
DM	57.0	51.6	60.1	5.39	0.55	0.05	0.07	0.02
OM	64.1	63.6	70.1	4.32	0.47	0.04	0.09	0.02
CP	67.8	65.9	71.4	4.09	0.63	0.05	0.08	0.03
NDF	50.7	44.5	53.9	6.40	0.58	0.06	0.07	0.02
ADF	30.8	27.6	39.6	8.92	0.63	0.08	0.17	0.20

[a]Summer forages were cut fresh every day from 0.7 ha pastures at 0800 h and offered ad libitum.
[b]Pooled standard error of treatment means, n = 4 heifers/treatment.
[c]Effect of fecal sample collection protocol: 2 samples per day (0800 and 1600 h) vs. 3 samples/d (0800, 1200 and 1600 h).
[d]Effect of indigestible marker used to calculate apparent total tract digestibility: 10 g/d of each Cr_2O_3 and TiO_2 were dosed once daily at 1200 h in two separate gelatin capsules.
[e]Sampling protocol × indigestible marker interaction.
[f]Intake during the 5-d digestibility measurement period of the experiment.

Table 4-4. Interaction between indigestible marker used and fecal collection protocol on digestibility of nutrients in heifers fed three summer forages in Exp. 2.

	2 samples/d[k]		3 samples/d[l]		
Item	Cr_2O_3	TiO_2	Cr_2O_3	TiO_2	SEM[m]
Digestibility, %					
DM	48.2[a]	55.9[b]	59.2[b]	61.6[b]	3.9
OM	60.2[a]	65.9[b]	68.4[b]	70.0[b]	3.03
CP	62.6[a]	68.0[b]	70.6[b]	72.2[b]	2.89
NDF	40.5[a]	49.3[b]	53.0[b]	55.9[b]	4.41
ADF	23.8[a]	30.6[ab]	36.3[ab]	39.9[b]	6.15

[a,b]Within a row, means without a common superscript differ ($P < 0.05$).
[k]Fecal samples collected by rectal grab at 0800 and 1600 h.
[l]Fecal samples collected by rectal grab at 0800, 1200, and 1600 h.
[m]Pooled standard error of treatment means, n = 4 heifers/treatment.

Table 4-5. Apparent total tract digestibility of nutrients measured using Cr2O3 or TiO2 as indigestible markers in heifers fed three summer forages in Exp. 2.

	Marker[a]			*P*-value	
Item	Cr_2O_3	TiO_2	SEM[b]	Marker effect	Marker × forage[c]
Digestibility, %					
DM	53.7	58.3	3.25	0.02	0.005
OM	64.3	67.6	2.61	0.03	0.006
CP	66.6	69.8	2.47	0.03	0.006
NDF	46.8	52	3.82	0.02	0.005
ADF	30.1	34.9	5.29	0.06	0.004

[a]Heifers were dosed once daily with 10 g/d of both Cr_2O_3 and TiO_2 once daily at 1200 h in two separate gelatin capsules.
[b]Pooled standard error of treatment means, n = 4 heifers/treatment.
[c]Effect of interaction between forage consumed (Mulato *Brachiaria*, Sorghum Sudan, or Millet) and marker used.

Table 4-6. Interaction between type of forage consumed and indigestible marker used on digestibility of nutrients in heifers fed three summer forages in Exp. 2.

	Mulato II[k]		Millet[l]		Sorghum[m]		
Item	Cr_2O_3	TiO_2	Cr_2O_3	TiO_2	Cr_2O_3	TiO_2	SEM[n]
Digestibility[o], %							
DM	59.0[b]	53.5[ab]	46.0[a]	57.2[ab]	56.1[ab]	64.1[b]	4.47
OM	65.9[ab]	63.9[ab]	59.3[a]	67.9[ab]	67.8[ab]	73.6[b]	3.43
CP	69.3[ab]	67.8[ab]	61.9[a]	69.8[ab]	68.6[ab]	74.3[b]	3.29
NDF	53.1[b]	49.9[ab]	38.1[a]	50.9[ab]	49.2[ab]	58.5[b]	5.24
ADF	34.8[ab]	30.5[ab]	20.7[a]	34.6[ab]	34.7[ab]	44.5[b]	6.93

[a,b]Within a row, means without a common superscript differ ($P < 0.05$).
[k]*Brachiaria hybrid* Mulato II planted in three 0.7-ha pastures at a seeding rate of 1 kg ha^{-1}.
[l]"Tifleaf 3" Pearl Millet (*Pennisetum glaucum*) planted in three 0.7-ha pastures at a seeding rate of 33 kg ha^{-1}.
[m]"Hay Day" Sorghum Sudan (*Sorghum bicolor*) planted in three 0.7-ha pastures at a seeding rate of 33 kg ha^{-1}.
[n]Pooled standard error of treatment means, n = 4 heifers/treatment.
[o]To measure digestibility, 10 g/d of each Cr_2O_3 and TiO_2 were dosed once daily at 1200 h in two separate gelatin capsules to use and indigestible marker and fecal samples collected by rectal grab at 0800, 1200, and 1600 h during 5 consecutive days.

CHAPTER 5
COMMERCIAL BEEF PRODUCTION IN MALAWI

Introductory Remarks

Malawi, located in sub Saharan Africa, has a huge potential for beef production. This is attributed to extensive availability of grasses during rainy season and abundant crop residues (groundnuts haulms, maize stover) which can be used as feed for beef cattle. Currently, the beef cattle population is approximately 1,060,000 (Department of Animal Health and Livestock Development, 2011). Most of these cattle are kept by small scale farmers in herds of ≤ 7 animals under traditional systems that only depend on grazing on communal range lands without any supplementation (Chintsanya et al., 2004). Apart from generating income, small scale farmers use beef cattle as source of power for farming activities, paying dowries (wedding gifts) and as a symbol of wealth in the society (Chintsanya et al., 2004).

In 1957, the Malawi government and development partners started promoting stall feeding programs with the aim of improving beef cattle productivity and quality (Spurling et al., 1972). This led to commencement of what is known as commercial beef production. Commercial beef production is a high input and business oriented production system characterized by intensive feeding of beef cattle up to the time they are ready for sale or slaughter (Chintsanya et al., 2004). Therefore, commercial beef producing farmers regard beef cattle raring as a business from which they can generate income on a regular basis. Currently, there is no clear documentation about general management (breeds, breeding methods, feeding, housing, parasites, and diseases management) of beef cattle on these farms hence limited chances of improving productivity and profitability of beef enterprise. Therefore, our objectives were to

conduct a survey to ascertain 1) breeds and breeding methods, 2) nutritional management, 3) parasites and diseases management, and 4) to determine animal housing, farm equipment and animal handling facilities available in commercial beef cattle farms of Malawi. This information is important for determining research needs that can lead to improvement of beef production in Malawi.

This survey took place on nine commercial beef producing farms of Malawi and this represented 100% of recorded commercial beef farms. During the interviews, farm managers were asked to provide information on animal reproduction, statistics, nutrition management, animal health and farm mechanization. In order to capture this information, both closed and open ended questions were used and in the survey and responses for each question are presented as percentages in Tables 5-1, 5-2, 5-3, 5-4, 5-5 and 5-6.

Results and Discussion

Farm Ownership and Objectives

Out of 9 commercial beef farms in Malawi, 78% are privately owned while 22% belong to the government. The majority (67%) of the farms are exclusively for producing finished beef animals ready for slaughtering while 22%, especially government owned beef farms, breed stock for stall feeding and also act as site for indigenous gene conservation. Only 11% of the farms purchase poor grade small animals, and then improve them through intensive feeding before selling these animals to other farmers for finishing (Table 5-1). However, there is no reference point to determine when the animals are ready for selling to other farmers.

Breeds, Breeding Methods and Animal Performance

These farms have Zebu, Brahman, and crosses between Zebu and Brahman. However, 44% of the farms have Zebu and Brahman, while 33% have Zebu, Brahman, and crosses between Zebu and Brahman, 11% have crosses between Zebu and Brahman, whereas another one farm (11) keep only the Zebu (Table 5-1). In most of these farms (67%) the population of animals is below 1,000 whereas only 33% of the farms have cattle population greater than 1,000. This is attributed to the size of business capital which limits number of animals a farm can have as a breeding or starter stock. Only 44% of the farms have a breeding season and this is usually from January to March so that calving takes place somewhere around October at the start of the rains to ensure good pastures for calves. The other 56% do not observe a breeding season because they mostly buy animals from other farmers and keep them for upgrading before selling. While within the 56%, others have limited knowledge on the importance of having a strategic breeding season. In addition, natural service (bull) is the method used in all farms that practice cattle breeding (Table 5-2). This is because, natural service is considered cheap, easy to apply, more effective and efficient. Other alternative methods of breeding like artificial insemination (AI) have been mentioned and described by these commercial farmers as expensive (as it needs expertise, facilities for storing semen) and therefore, not preferred.

The average pregnancy rates on most farms (67%) ranges between 70 – 80% with one farm (11%) recording as high as 81-90%. It is worth noting that 11% of the visited farms do not observe pregnancy rates as they don’t realize the importance of doing so. Again, none of the visited farms observe and record average birth weight, these farmers also do not know the potential birth weights of the breeds of animals they are keeping

when kept under optimal management (Table 5-2). Furthermore, many farms (56%) involved in breeding beef cattle, registered calf mortality rate of <10% for the whole of 2011, whereas the remainder registered no death at all. Again, majority of farmers (89%) do not observe or record weaning weights of their stock and also do not know the potential weaning weight of the breeds they are keeping (Table 5-3). This Lack of animal performance record keeping may limit improvement in management as farmers do not realize whether applied management methods are enhancing animal performance to its potential thus may result in low productivity and profitability of the enterprise. Finally, average mature weight of the animals ranged from 300 – 500 kg on 78% of farms that sell finished beef animals to abattoirs. While at one farm, mature weights were not recorded. Furthermore, it was a challenge to obtain number of animals sold per annum, because on some farms, this information is regarded as a business secret and is not shared to anyone. However, from the six farms which provided the information, the range of animals sold per year was between 300 and 1000 (Table 5-3).

Nutritional Management

On majority of the farms (56%), animals graze on the rangeland and thereafter offered supplement in form of concentrates and grasses. About half of the remaining farms (22%), animals are fed in stalls all the time (zero grazing), whereas on the other farms (22%), animals simply graze on rangeland without any supplementation (Table 5-4). Furthermore, out of the farms that practice zero grazing and supplementation, a majority (78%) do not grow any forage for their animals. They simply buy from the local businesses who source the grass from the rangelands at the end of each rainy season. This grass is preserved in form of hay before selling when feed is scarce more especially during the dry period of the year. A few commercial beef producing farms

(22%) grow forages like Rhodes grass, Napier grass and maize, and all these farms, cultivated forages only occupy less than 5% of the total land area with Rhodes grass occupying large hectarage because of its relative low cost of production and high production (Table 5-4). Forage production is good during the rainy season (December – April) and is poor during dry season (May – November). This is due to the fact that forage production is only based on rain as a source of water. Most of these forages are preserved in form of hay, with a few in form of silage because hay is easier and cheaper to make as it does not require special facilities (like pit, plastics for wrapping) than does silage (Table 5-5). In addition, majority (78%) of the visited farms do supplementation of some form. The most common supplement is maize bran because it is purchased at a low price and in abundant supply. However, other farms provide their animals with molasses (by product after making sugar from sugarcanes) as supplements (Table 5-5).

Parasites and Diseases Management

The most common parasite reported is the Tick on 56% of visited farms, followed by worms (33%) and tsetse fly (11%). Ticks and tsetseflies are controlled by spraying chemicals whereas worms are controlled by oral administration of drugs (dewormers). Foot and Mouth Disease (FMD) was reported common at 34% of the visited commercial farms (especially those located in the southern part of Malawi), while 22% of the farms reported East Cost Fever (ECF) as a problem (especially in farms located in the central part of Malawi). Other diseases like Heart water was reported at 22% of the visited farms while another 22% of farms indicated pneumonia and birth complications as among the common health problems encountered. It is interesting to note that 22% of the visited farms did not express any problem with these diseases. Heart water, FMD, ECF, pneumonia are being controlled by a combination of drugs (vaccinations) and

husbandry practices such as isolation and hygiene in the pens (Table 5-6). Farmers wait for veterinary assistance in cases when complications occur during birth.

Animal Housing and Handling Facility

All the visited farms have open pens for housing their animals. At least 89% of the visited farms have cluches for handling animals. While only 11% have no animal handling facility and this limits inspection on animal health and performance (Table 5-6).

Conclusion and Recommendation

The beef industry in Malawi is not well developed. This is evident by lack of proper strategic breeding and animal performance monitoring, inadequate nutritional management and insufficient farm mechanization. However, there is great potential for improvement due to availability of well adapted breed of cattle (Zebu and Brahman) to Malawi, abundant feed availability (green grasses in wet season and crop residues in dry season) which can improve animal performance if properly conditioned, and also availability of enthusiastic farmers who can adopt new technologies upon receiving adequate training in beef cattle management. In view of this, it is imperative to develop standards of beef cattle management through studies in breed performance under Malawi conditions. More focus should be on reproductive performance, growth performance, disease management and characterization of feed quality and quantity across the nation to enhance proper diet formulation. Thereafter, the developed standards should be effectively disseminated to commercial beef producers for implementation. This approach can uplift beef production thereby increasing its contribution to Malawi's economy.

Table 5-1. Farm ownership, objectives and cattle breeds presented in percentages of the nine visited beef producing farms.

Item	Gov[a].	Private	Beef production	Breeding & gene conservation	Upgrading animals	Zebu only[b]	Zebu & Brahman	Zebu × Brahman[c]	All breeds[d]
Ownership	22	78	-	-	-	-	-	-	-
Objective	-	-	67	22	11	-	-	-	-
Cattle breeds	-	-	-	-	-	11.1	44.4	11.1	33.3

[a]Government
[b]Malawi zebu cattle breed
[c]Cross between Malawi zebu and Brahman
[d]Farms keeping Malawi zebu, Brahman and also crosses between Malawi zebu and Brahman.

Table 5-2. Cattle population, breeding season, breeding methods, pregnancy rate and birth weight expressed as percentage of 9 beef producing farms.

Item	<1000	>1000	Yes	No	Jan – Mar[a]	NM[b]	Not applicable	70-80 %	81-90 %	Not observed	Not recorded	Not known
Cattle population	67	33	-	-	-	-	-	-	-	-	-	-
Have breeding season	-	-	44	56	-	-	-	-	-	-	-	-
Breeding season	-	-	-	-	100	-	-	-	-	-	-	-
Breeding method	-	-	-	-	-	89	11	-	-	-	-	-
Conception rate	-	-	-	-	-	-	11	67	11	11	-	-
Birth weight (BW)	-	-	-	-	-	-	-	-	-	-	100	-
Breed potential BW	-	-	-	-	-	-	-	-	-	-	-	100

[a]Between months of January and March
[b]Natural mating

Table 5-3. Mortality rates, weaning and mature weight and number of animals sold per annum expressed as percentage of 9 visited beef producing farms of Malawi.

Item	0%	</= 10%	Not applicable	Not observed	Not known	300-500	<500	>500	Not disclosed
Mortality rate	33	56	11	-	-	-	-	-	-
Weaning weight –ww[a]	-	-	11	89	-	-	-	-	-
Breed potential WW	-	-	-	-	100	-	-	-	-
Mature weight (kg)	-	-	11	11	-	78	-	-	-
Animals sold per annum	-	-	-	-	-	-	33	33	34

[a] ww = weaning weight

Table 5-4. Animal feeding and feed production expressed as percentage of 9 visited beef producing farms of Malawi.

Item	Stall feeding only	Grazing only	G &S[a]	None	Rhodes, Napier & Corn	< 5%	Rhodes grass	Corn/ maize
Feeding practice	22	22	56	-	-	-	-	-
Cultivated forages	-	-	-	78	22	-	-	-
Land allocated to pastures	-	-	-	-	-	100	-	-
Pasture occupying largest land	-	-	-	-	-	-	100	-
Most productive forage	-	-	-	-	-	-	50	50

[a] Grazing and Stall feeding

Table 5-5. Forage preservation and feed supplementation practices expressed as percentage of 9 beef producing farms of Malawi.

Item	Yes	No	Hay only	Silage only	Hay & silage	Not applicable	Corn bran	Cheap
Forage preservation	78	22	-	-	-	-	-	-
Form of forage preservation	-	-	56	0	22	22	-	-
Supplementation	78	22	-	-	-	-	-	-
Common supplements	-	-	-	-	-	-	100	-
Why corn bran common supplement	-	-	-	-	-	-	-	100

Table 5-6. Animal housing, disease and parasite management expressed as percentage of 9 beef producing farms of Malawi.

Item	Open pen	Clutch	None	Ticks	Worms	Tsetsefly	Drugs & good hygiene	FMD[a]	HW[b]	Pneumonia & BC[c]	ECF[d]
Animal housing	100	-	-	-	-	-	-	-	-	-	-
Animal handling facility	-	89	11	-	-	-	-	-	-	-	-
Common pests / parasites	-	-	-	56	33	11	-	-	-	-	-
Pests / parasite control	-	-	-	-	-	-	100	-	-	-	-
Common diseases	-	-	-	-	-	-	-	34	22	22	22
Disease control	-	-	-	-	-	-	100		-	-	-

[a] Foot and Mouth Disease
[b] Heart Water diseases
[c] Pneumonia and Birth Complication
[d] East Cost Fever diseases

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BIOGRAPHICAL SKETCH

Chunala Alexico Njombwa was born in Dowa, Malawi. There he grew up on a small family farm, growing tobacco, peanuts and corn. Chunala attended primary and secondary education at Mondwe and Madisi in Dowa District respectively. Thereafter, he graduated from Mzuzu University with a bachelor's degree in the field of forestry in 2007. Upon graduation, he got a teaching job at a government secondary school in Malawi. After that he joined Bio – Energy Resources Limited, where he worked as a Senior Planting Technician in 2008 before joining the Department of Agricultural Research Services in 2009. In 2010, Chunala moved to Florida, US to pursue his master's degree in animal sciences at University of Florida with financial support from United States Agency for International Development (USAID) under the supervision of Dr. Cliff Lamb. His major focus has been on forage evaluation and nutrition of beef cattle.