8-2012

Evaluation of Internal Markers for Predicting Digestibility and Fecal Output by Cattle Fed Bermudagrass Hays of Varying Quality

Juvenal Kanani
University of Arkansas, Fayetteville

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EVALUATION OF INTERNAL MARKERS FOR PREDICTING DIGESTIBILITY AND FECAL OUTPUT BY CATTLE FED BERMUDAGRASS HAYS OF VARYING QUALITY

A dissertation submitted in partial fulfillment
of the requirements for the degree of
Doctor of Philosophy in Animal Science

By

Juvenal Kanani
National University of Rwanda
Bachelor of Science in Animal Production, 2000
Texas A&M University-Kingsville
Master of Science in Animal Science, 2003

August 2012
University of Arkansas

ABSTRACT

The potential of in situ rumen undegradable dry matter (RUDM), indigestible neutral-detergent fiber (INDF), indigestible acid-detergent fiber (IADF), acid-detergent insoluble ash (ADIA), alkaline-peroxide lignin (APL), and acid-detergent lignin (ADL) to predict digestibility (DMD) and fecal output (FO) by cattle fed bermudagrass [*Cynodon dactylon* (L.) Pers.] hay-diets categorized by their low (L), medium low (ML), medium high (MH), or high (H) CP concentrations (79, 111, 131, and 164 g/kg DM, respectively) was evaluated. The second objective was to evaluate the effects of time (0600, 1200, 1800, and 2400 h) of fecal sampling on the prediction of FO and DMD. A replicated 4 × 4 Latin-Square with one period missing was employed where diets were offered in three 15-d periods to provide 2 replicates per diet per period (n = 24). Actual DMI, FO, and DMD were determined based on hay offered, orts, and feces excreted. Hay, orts, and feces were analyzed for RUDM, INDF, IADF, ADL, APL, and ADIA concentrations. Fecal recoveries of internal markers were expressed as the ratio of the quantity of marker excreted per unit of marker consumed. Estimate of FO and DMD were calculated by the marker ratio technique.

All in situ markers and ADL recoveries differed from 1. Estimates of DMD were underestimated while FO estimates were overestimated for all in situ markers. Recovery of APL tended to differ from 1, but ADIA recovery was not different from 1. Estimates of FO and DMD derived using APL and ADIA were not different from TC. Time of sampling affected the concentration of $IADF_a$ while ADIA and APL concentrations in fecal samples were not different. Estimates of FO and DMD by all fecal sampling times and their different combinations were not different from actual FO and DMD. Therefore APL and ADIA have the potential to predict FO and DMD of bermudagrass of various qualities fed to cattle and fecal sampling time may not be an issue when using internal markers.

This dissertation is approved for recommendation

to the Graduate Council.

Dissertation Director:

_____

Dr. Kenneth P. Coffey

Dissertation Committee:

_____

Dr. Dirk Philipp

_____

Dr. Elizabeth B. Kegley

_____

Dr. Charles P. West

_____

Dr. Shane Gadberry

_____

Dr. John Jennings

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ACKNOWLEDGMENTS

First and foremost, I wish to express my deep appreciation and thanks to Dr. Dirk Philipp and Dr. Kenneth P. Coffey for their invaluable help, advice, patience, and support during my four-year period at the University of Arkansas, Fayetteville.

My deep gratitude and thanks are also extended to Dr. Elizabeth B. Kegley, Dr. Charles P. West, Dr. Shane Gadberry, and Dr. John Jennings for being part of my research committee and their important suggestions for improving the quality of this dissertation research.

I would like to extend my thanks to all members of the University of Arkansas community, in particular all faculty and staff in the Department of Animal Science for their team work sprit, conducing environment, encouragement, and for their commitment to the student success and achievement.

My sincere thanks are extended to Robert Rein, Ashley Young, Doug Galloway, and Karen Anschutz for their technical and physical help during the experiment and laboratory analysis.

Finally, I express my sincere thanks to the Government of Rwanda, the University of Arkansas, and the Robert McNamara Fellowship Program of the World Bank for their invaluable financial support. It would be impossible to come to the conclusion of my studies without their support.

DEDICATION

To my parents with love,

My family with all my love,

My wife Justine Elise Mivumbi,

My daughter Fiona Isingizwe Kanani, and

My son Brice Kanani

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LIST OF ABBREVIATIONS

Item	Term
ADF	acid-detergent fiber
ADIA	acid-detergent insoluble ash
$ADIA_{1234}$	acid-detergent insoluble ash of four samplings per day
$ADIA_c$	acid-detergent insoluble ash in consumed forage
$ADIA_f$	acid-detergent insoluble ash in feces
$ADIA_{TC}$	acid-detergent insoluble ash from total collection
ADL	acid-detergent lignin determined by 72 % sulfuric acid
ADL_C	acid-detergent lignin in consumed forage
ADL_f	acid-detergent lignin in feces
AHP	alkaline hydrogen peroxide solution (pH = 11.5)
AIA	acid-insoluble ash
APL	alkaline-peroxide lignin
APL_{1234}	alkaline-peroxide lignin of four samplings per day
APL_c	alkaline-peroxide lignin in consumed forage
APL_f	alkaline-peroxide lignin in feces
APL_{TC}	alkaline-peroxide lignin from total fecal collection
BW	body weight
CF	correction factor
CP	crude protein
CRD	controlled-release device
CV	coefficient of variation

DM	dry matter
DM loss	dry matter loss
DMD	dry matter digestibility
DMI	dry matter intake
FO	fecal output
GIT	gastrointestinal tract
H	high crude protein diet (CP = 164 g/kg DM)
HEM	hemicelluloses
IACUC	institutional animal care and use committee
IADF	indigestible acid-detergent fiber
$IADF_a$	adjusted indigestible acid-detergent fiber
$IADF_{a1234}$	adjusted indigestible acid-detergent fiber of four samplings per day
$IADF_{ac}$	adjusted indigestible acid-detergent fiber in consumed forages
$IADF_{fa}$	adjusted indigestible acid-detergent fiber in feces
$IADF_{aTC}$	adjusted indigestible acid-detergent fiber from total collection
$IADF_c$	indigestible acid-detergent fiber in consumed forage
$IADF_f$	indigestible acid-detergent fiber in feces
INDF	indigestible neutral-detergent fiber
$INDF_a$	adjusted indigestible neutral-detergent fiber
$INDF_{ac}$	adjusted indigestible neutral-detergent fiber in consumed forage
$INDF_{fa}$	adjusted indigestible neutral-detergent fiber in feces
$INDF_c$	indigestible neutral-detergent fiber in consumed forage
$INDF_f$	indigestible neutral-detergent fiber in feces

IVIADF	in vitro indigestible acid-detergent fiber
IVINDF	in vitro indigestible neutral-detergent fiber
K_P	rate of passage
L	low crude protein diet (CP = 79 g/kg DM)
M	marker
M_{fc}	marker concentration in feces
M_{fd}	marker concentration in feed consumed
M_{ftime}	marker concentration in feces at a particular sampling time
MH	medium high crude protein diet (CP = 131 g/kg DM)
ML	medium low crude protein diet (CP = 111 g/kg DM)
M_{of}	marker concentration in forage offered
M_{or}	marker concentration in ort
NDF	neutral detergent fiber
N_{fc}	concentration of a particular nutrient in feces
N_{fd}	concentration of a particular nutrient in feed
OMD	organic matter digestibility
pDNDF	potentially degradable neutral-detergent fiber
Q_{of}	quantity of feed offered
Q_{or}	quantity of ort
R	coefficient of determination
r	correlation coefficient (linear relationship)
RUDM	rumen undegradable dry matter
$RUDM_a$	adjusted rumen undegradable dry matter

$RUDM_{ac}$	adjusted rumen undegradable dry matter in consumed forage
$RUDM_{fa}$	adjusted rumen undegradable dry matter in feces
$RUDM_{c}$	rumen undegradable dry matter in consumed forage
$RUDM_{f}$	rumen undegradable dry matter in feces
SEM	standard error of the means
SW	sample weight
T	time of grab sampling
TA	total ash
TC	total collection
TDN	total digestible nutrient
TMR	total mixed ration
FDA	United States Food and Drug Administration
WREC	watershed research and education center

Key words: Bermudagrass, internal markers, digestibility, fecal output, sampling time, cattle

Chapter I

Introduction

Bermudagrass [*Cynodon dactylon* (L.) Pers.] is a warm-season perennial grass that is widely grown in the southeastern US. This grass is adapted to a wide range of soil types, is drought tolerant, and persists under high grazing pressure (Burton and Hanna, 1995). In Arkansas, bermudagrass constitutes the backbone of beef farms and is either grazed or used as hay. Approximately 809,400 ha of bermudagrass exist in the state (UACES, 2006).

In addition, the abundance of non-commercial fertilizer sources, largely from poultry litter, has improved soil fertility to an extent that bermudagrass hay now often exceeds crude protein (CP) concentrations of 160 g/kg. This led to hay with CP concentrations exceeding those of samples used to develop the bermudagrass energy equation currently used in Arkansas. Also, data compilation of the last 20 years from different laboratory analyses report a large range of bermudagrass CP contents with an average of 132 g/kg with a normal range (± 1 SD) of 95 to 170 g/kg of CP (Gadberry and Gunsaulis, 2010). Furthermore, Coblentz et al. (2001) and Gadberry et al. (2005) have reported an overestimation of bermudagrass energy based on predicted TDN obtained using the current Arkansas energy equation and the digestible organic matter (DMO).

One of the most effective ways to estimate energy value of the feed is to conduct an in vivo digestion study and determine organic matter digestibility (OMD), which is theoretically equal to TDN (Lofgreen, 1953). However, in vivo techniques to determine dry matter (DM) intake (DMI), fecal output (FO), and DM digestibility (DMD) are labor-intensive, expensive, and require large amounts of test forage (Weiss, 1994; Ordakowski et al., 2001, Coleman et al.,

2003). Alternatively, indirect methods using external and internal markers can be used (Penning and Johnson, 1983 a & b; Cochran et al., 1986; Cochran et al., 1987; Pond et al., 1987; Owens and Hanson, 1992). Internal markers present advantages of being an integral part of the forage or feed consumed by the animal, and can be fed with minimal effects on the normal animal's feeding behavior (Ferret et al., 1999). However, fecal recovery of an internal marker for any novel feedstuff must be validated before its use (Titgemeyer, 1997) because of varying results observed when a particular marker is applied across a wide range of forages (Sunvold and Cochran, 1991). Therefore, the global objective of this dissertation research was to evaluate the potential of different internal markers to predict FO from and digestibility of bermudagrass hay of varying quality fed to cattle, and to determine the fecal sampling frequencies that can provide adequate estimates of daily fecal excretion.

Chapter II

Literature review

Intake, digestibility, and energy value of bermudagrass

Generally, DMI and DMD of warm season-grasses such as bermudagrass are lower than those of cool-season grasses (Minson, 1990). Consequently, energy and CP supply are the most limiting factors for the performance of cattle consuming warm-season grasses (Minson, 1990). Energy deficiencies occur most often in forage-fed animals due to limited digestible energy intake, especially with high-fiber and low-energy forages where physical fill limits intake (Mertens, 1994). Prolonged periods of energy deficiency result in slow growth, weight loss, delayed puberty, decreased fertility, and reduced milk or fiber production (Pond et al., 1995). Knowledge of forage intake and digestibility is important to determine if daily nutrient requirements are being met and to decide whether a warm-season grass-based diet requires supplementation. Traditionally, DMI and DMD are determined by conducting in vivo digestion trials where total collections of feed, orts, and feces are performed (Cochran and Galyean, 1994). However, the in vivo method requires total fecal collection (TC) which is laborious and often unfeasible for testing a wide range of samples with a large number of animals. Alternatively, external and internal markers can be applied to estimate DMI, DMD, and FO of the feedstuff by ruminants.

Use of markers to estimate intake, digestibility, and fecal output

There are two types of markers: external markers which are substances added to the diet at a known rate per day or at known concentration in the diet, and internal markers which are

inherent constituents of feedstuffs offered to the animal (Cochran et al., 1987). The criteria that characterize an ideal marker were summarized by Owens and Hanson (1992). Such a marker should not be absorbed, affect or be affected by the gastro-intestinal tract (GIT) of the animal or its microbial population. Additionally, markers should be intimately associated with the material they mark and should exhibit the same flow through the GIT, and be specific and sensitive to the method of analysis (Nelson et al., 1990; Lippke, 2002). All marker calculations are based on the same principle that the amount of marker excreted equals the amount of marker consumed, because they are considered indigestible and the degree of concentration of marker is proportional to the degree of digestion (disappearance) of feed. Markers can be used to estimate DMD according to the following relationship (Burns et al., 1994):

$$\text{DMD (\%)} = 100\text{-}[100 \times (M_{fd} / M_{fc})] \quad [1]$$

where M_{fd} is the marker concentration in feed, and M_{fc} is the marker concentration in feces.

It is also possible to use an external marker or internal marker to determine FO. Fecal output can then be calculated for either external or internal markers using the following formulas (Cochran and Galyean, 1994):

$$\text{FO (kg/d)} = \text{marker dose (mg/d)} / M_{fc}\,\text{(mg/kg)} \quad [2]$$

$$\text{FO (kg/d)} = \text{DMI (g/d)} \times M_{fd}\,\text{(g/kg)} / M_{fc}\,\text{(g/kg)} \quad [3]$$

Estimates of FO and DMD can then be combined to predict DMI as follows:

$$\text{Intake (DMI, kg/d)} = \text{FO}/1\text{-}(\text{DMD} / 100) \quad [4]$$

If intake is unknown, the digestion coefficient for different nutrients in the feed can be measured as follows (Cochran and Galyean, 1994):

$$\text{Digestibility (\%)} = 100\text{-}100\ (\%M_{fd} / \%M_{fc}) \times (\%\ N_{fc} / \%N_{fd}) \quad [5]$$

where N_{fc} is the concentration of a particular nutrient in the feces, and N_{fd} is the concentration of a particular nutrient in the feed.

External markers

External markers are indigestible substances added to the diet at a known rate (Van Soest, 1994). They may be administered orally, infused into the rumen through fistula, or given by controlled-release devices (Marais, 2000; Lippke, 2002). In an attempt to overcome the difficulty and expense in conducting conventional in vivo digestion trials, the use of inert markers to predict the digestibility of feeds and to estimate digesta flow and FO has received attention (Undersander et al., 1987; Owens and Hanson, 1992). Each external marker has its own particular benefits and limitations. A discussion of these individual markers is therefore warranted.

Chromic oxide (Cr_2O_3)

This compound (or similarly chromium sesquioxide) has been the most extensively used external marker to estimate intake and digestibility in confined and grazing animals during the past 50 years (Lippke, 2002), before the discovery of rare earth elements and the utilization of elements such as titanium dioxide (TiO_2). Chromic oxide is orally administered to animals as gelatin capsules or mixed with the ration.

The primary disadvantage of chromic oxide is that it moves through the digestive tract of the animal independently of undigested particles of the diet, and consequently fecal concentrations of Cr_2O_3 exhibit diurnal variation (Lippke, 2002). In an attempt to solve that problem, several doses per day have been proposed by different authors (Brandyberry et al.,

1991; Luginbuhl et al., 1994) by inserting chromic oxide into the rumen through a cannula, but increased dosing up to six times a day is considered impractical. Another solution to chromium daily variation in feces has been the development of controlled-release devices (CRD) for continuous release of the marker into the gut. The CRD reduced diurnal variation of Cr_2O_3 considerably; however, the release rate appeared to be diet-dependent which requires prior validation with a small number of animals before the trial. Luginbuhl et al. (1994) achieved a constant fecal excretion of chromium after 8 d dosing with a controlled release bolus containing chromic oxide. However, Hatfield et al. (1991) reported that both the continuous release bolus and dosing twice a day overestimated the actual FO in sheep fed alfalfa (*Medicago sativa* L.). Santos and Petit (1996), however, reported that grab samples taken once a day provided reliable estimates of FO ($R = 0.96$, $P < 0.05$) with a slow-release bolus of chromic oxide. With this protocol, an adaptation of at least 10 d was required before samples could be taken when using chromic oxide as an external marker. Additionally, chromic oxide analysis requires calibration with fecal samples from animals free of chromium ingestion and on the same diet as that one used in the experiment (Holt, 1993). Titgemeyer et al. (2001) reported that chromic oxide recovery deviated from 1 in several experiments, while Myers et al. (2006) raised concerns about carcinogenic properties of Cr_2O_3 and potential human health hazards due to Cr_2O_3 inhalation.

Titanium oxide (TiO_2)

This compound was proposed as an alternative to Cr_2O_3 and presents less negative health properties than Cr_2O_3 (Myers et al., 2004). In addition, the United States Food and Drug Administration (FDA) recommends the use of TiO_2 while the use of Cr_2O_3 is not approved as a dietary additive in the United States (Titgemeyer et al., 2001). Studies comparing Cr_2O_3 and

TiO_2 in pigs (Jagger et al., 1992), cattle (Titgemeyer et al., 2001), and sheep (Myers et al., 2006) revealed that TiO_2 can be an appropriate alternative to Cr_2O_3. Furthermore, Glindemann et al. (2009) reported an overall TiO_2 recovery of 1.04 in sheep (ranging from 0.96 to 1.09), but during stall feeding, TiO_2 had different recoveries ($P < 0.001$; between 0.99 and 1.08) due to different diets (unsupplemented hay diet vs. hay supplemented with concentrate). Intake and TiO_2 excretion reached equilibrium after 5 d of TiO_2 administration. The administration of TiO_2 twice per day reduced the variability in fecal TiO_2 concentration and increased the accuracy of FO prediction than dosing once a day or fecal sampling at different time periods.

Ytterbium (Yb)

Like other rare earth elements (Er, Dy, and Y), Yb can be added to feed to increase its total concentration in the diet and to facilitate analysis. Ytterbium oxide was proposed as an alternative to Cr_2O_3 in animal nutrition studies and presents satisfactory biological properties with no major health problem or carcinogenicity (Delagarde et al., 2010). Brandyberry et al. (1991) reported that continuous release of ytterbium acetate and ytterbium chloride yielded the same estimates as chromium oxide for fecal flow. Delagarde et al. (2010) reported that ytterbium oxide had the same accuracy as chromic oxide for estimating daily FO variations in cows fed a total mixed ration (TMR) at variable feeding levels. Also, rare earth elements (La, Yb, and Tb) applied to a particular feed can be flow markers for undigested particles from the marked feed (Ellis et al., 2002).

Internal markers

Internal markers are plant constituents that are neither digested nor absorbed by the animal. These markers help to estimate intake and digestibility of a given feed by animals with

minimal disturbances in feeding behavior (Ferret et al., 1999).The use of internal marker assumes that the content of indigestible feed material (marker) gradually increases while the ingested feeds pass through the GIT due to the removal of digestible feed components by digestion and absorption processes (Sampaio et al., 2011b). Current indigestible feed components that have been tested as internal markers (Undersander et al., 1987) can be categorized into the following groups: 1) in situ or in vitro markers, rumen undegradable dry matter (RUDM), indigestible NDF (INDF), and indigestible ADF (IADF); 2) lignin-based markers, acid-detergent lignin (ADL); permanganate lignin, acetyl bromide-soluble lignin, and alkaline peroxide lignin (APL); 3) ash-based markers, acid-insoluble ash (AIA) and acid-detergent insoluble ash (ADIA); and 4) n-alkanes.

Rumen undegradable DM

The RUDM is obtained by incubation of feed or feces samples in the rumen (in situ) or incubated with rumen fluids (in vitro) for extended periods of time to allow the rumen microbes access all potentially digestible material. The remaining portion, after washing and drying, is the RUDM. Furthermore, the indigestible DM residue can be sequentially refluxed in neutral-detergent solution and acid-detergent solution to obtain INDF and IADF, respectively. Huhtanen et al. (1994) and Detmann et al. (2001) recommended the use of RUDM as an internal marker because of low analytical cost compared to INDF and IADF. However, error from in situ procedure has been associated with contamination from microbial debris, feed, and rumen contents (Huhtanen et al., 1994; Casali et al., 2009), and the removal of these contaminants on the in situ residue requires detergent solution (Van Soest, 1994). Sample contamination during in situ evaluation of RUDM has been found to be variable among different bags used and replicate

samples (Casali et al., 2009; Sampaio et al., 2011b). This pattern can cause inconsistencies in marker recovery, which indicates that caution should be observed when using RUDM as an internal marker.

Graham and Aman (1984) reported that in vitro and in situ methods produced similar kinetics of ruminal degradation for barley straw constituents (*Hordeum vulgare*). On the other hand, Varel and Kreikemeier (1995) reported, based on a study comparing in vitro with in situ methods, that lag time was 3.5 h less, rate of disappearance was 0.03/h faster, and extent of digestion was 6.0% greater for in situ than for the in vitro method for determining NDF digestion kinetics of alfalfa or bromegrass in cattle. Low concentration of microorganisms in the in vitro inocula may increase lag time, slow the rate of digestion, and lower the extent of digestion compared with the in situ method.

Indigestible ADF and NDF

Indigestible NDF and ADF have been proposed by Lippke et al. (1986) and Judkins et al. (1990) to overcome the problem of low concentrations and variable recovery of lignin and AIA contamination in consumed forages. Indigestible ADF was the best predictor of organic matter digestibility (OMD) of several forages in sheep (Penning and Johnson, 1983b) compared to in vitro technique. Indigestible ADF was also used with success in another experiment with sheep and steers (Nelson et al., 1990). Indigestible ADF and NDF provided acceptable estimates of digestibility of alfalfa cubes, tall wheatgrass *(Agropyron elongatum),* and soybean meal diets (Cochran et al., 1986). However, further investigations have been recommended by these authors for the applicability of IADF and INDF as markers for use in cattle consuming a diverse range of diets or fresh, immature forage. Berchielli et al. (2005) concluded that INDF and IADF can be

used as predictors of FO and digesta flow in cows by using in situ techniques. In a study using IADF, Vanzant et al. (2002) reported that acceptable fecal marker recovery was obtained from cattle consuming tall fescue hay (*Festuca arundinacea* Schreb.) by using bulk in vitro incubation with either Ankom #1020 or Ankom #F57 (ANKOM Technology Corp., Fairport, NY, USA) polyester bags. Indigestible NDF could be a useful marker if measured using standardized in vitro and in situ methods and if recovery is satisfactory (Lund et al., 2007); and has the advantage of being degraded at a predictable rate (Ellis et al., 1999). Using in vitro incubation (144 h) with either an acid/pepsin pretreatment or control of feed, ort, and feces, Sunvold and Cochran (1991) reported a fecal recovery of IADF-based markers in the range of 0.70 to 0.80 in steers limit-fed various grasses, leading to an underestimation of OMD.

Acid-detergent lignin (ADL)

Lignin has been considered to be indigestible and recoverable in feces (Ellis et al. 1946; Forbes and Garricus, 1948; Elam and Davis, 1961) for many years because no enzyme for lignin degradation appears to exist in ruminants. As a part of the fiber fraction, forage lignin increases in concentration as plants mature. Also, as an end product of routine fiber analyses, some authors have considered ADL as a potential internal marker (Waldo et al., 1972; Van Soest, 1982), while others have reported inconsistencies in lignin recovery (Fahey and Jung, 1983; Cochran et al., 1986). According to Van Soest (1987), acceptable results can be obtained for ADL as an internal marker when its concentration is at least 60 g/kg of DM. Lignin may not be an adequate internal marker because of potential degradability or complex formation with carbohydrates during its transit in the GI tract of ruminants (Jasra and Johnson, 2000). Incomplete lignin recovery resulted in underestimation of digestibility when ADL was used as an internal marker (Merchen,

1993). In addition, variable positive and negative digestion coefficients were obtained using sheep and goat rumen liquor on forage samples (forbs, shrubs, and grass) of three phenological stages (Jasra and Johnson, 2000). Positive ADL recoveries have been reported by Fahey et al. (1979) and Fahey and Jung (1983), and are attributable to the formation of an artifact during the gastrointestinal transit of consumed forage. Furthermore, Neilson and Richards (1978) reported that nearly 50% of the lignin in forage may conjugate with carbohydrates and form a complex that will be measured in feces as lignin. Another issue for lignin is that its lower concentration in immature forage and the variability in lignin content in different plants make analysis difficult with drastic variability across the range of particular forages due to maturity. Finally, Muntifering (1982) reported that lignin [permanganate ($KMnO_4$) lignin], ADL, and acetyl bromide-soluble lignin appeared to have low and variable recovery regardless of method of determination.

Alkaline-peroxide lignin (APL)

Alkaline peroxide lignin constitutes a core portion of lignin more indigestible (Marais, 2000). Treatment of crop residues with alkaline hydrogen peroxide improved digestibility due to the removal of up to half of the lignin (Lewis et al., 1988; Bhargava et al., 1989; Amjed et al., 1992). Alkaline hydrogen peroxide (AHP) incubation in the ADL procedures, particularly when incorporated before the acid-detergent extraction, improved the recovery rate of lignin in feces (Cochran et al. (1988). Fecal recoveries of APL averaged close to 1 (0.978 and 0.959) in two experiments with sheep and cows fed mature prairie grass hay (Momont et al., 1994), but were more variable (0.989, 1.060, and 0.925) in steers limit-fed (17.5 g/kg BW) alfalfa, bromegrass [*Bromus inermis* Leyss.], and prairie hay (Sunvold and Cochran, 1991).

Acid-insoluble ash (AIA)

Acid insoluble ash (AIA) is obtained by drying and ashing samples in a muffle furnace followed by boiling the ashed samples in 2N HCl for 5 min, filtering, and rinsing to neutral pH, and finally drying and re-ashing the remaining residue (Van Keulen and Young, 1977). The DMD estimates by AIA ratio were similar to those measured by total fecal collection (Van Keulen and Young, 1977).The mean recovery rates in feces estimated by AIA using concentrated HCl, 2N HCl and 4N HCl procedures were 0.97 ± 0.067, 0.97 ± 0.061, and 1.03 ± 0.071, and were not statistically different from 1. In a study comparing AIA and permanganate lignin as potential internal markers to predict digestibility of cattle diets, the average recovery of permanganate lignin were 0.52 ± 0.018 and 0.59 ± 0.018, compared with an average recovery of AIA of 1.02 ± 0.048 and 0.99 ± 0.030 for early cutting and late cutting dates of mixed grass hays (Thonney et al., 1979); consequently, the permanganate lignin ratio underestimated the digestibility while predicted values of DMD by AIA were similar to the TC values. However, different results were obtained when AIA was used to estimate OMD of alfalfa fed to wether sheep (Penning and Johnson, 1983a). Diets containing less than 7.5 g/kg of AIA may yield biased results when used to estimate digestibility (Thonney et al., 1985). Furthermore, AIA as internal marker should be used with caution because fecal recovery rate can be affected by soil contamination of ingested feed (Sunvold and Cochran, 1991).

Acid-detergent insoluble ash (ADIA)

The acid-detergent insoluble ash (ADIA) is a preferred method, shorter, and less expensive to analyze (Van Soest, 1994). Acid-detergent insoluble ash is obtained by ashing the remaining DM after acid-detergent extraction in a muffle furnace at 500^{o}C for 8 h. The ADIA

procedure has been recommended for use as the most reliable internal marker technique (Undersander et al., 1987; Van Soest, 1994; Bodine et al., 2002). In a study comparing ADIA with TC, Bodine et al. (2002) found similar estimates of DMD of alfalfa, bermudagrass, and unsupplemented prairie hay diets fed to steers. In addition, Stafford et al. (1996) reported excellent recovery rates of ADIA (average of 1.02) by cattle fed low-quality, tallgrass-prairie hay with different supplements. The ADIA recovery was not impacted by the type of supplement and their level. However, due to its relation with inorganic matter, ADIA is susceptible to soil contamination during the feeding process (Appeddu and Bodine, 2002). Soil ingestion by grazing animals can account for up to 11.5 % of total intake (Mayland et al., 1977) and feces can be contaminated during sample collection, processing and storage, which may result in over-estimated digestibility.

*Plant alkane*s

Alkanes are components of the plant-cuticular wax and are relatively indigestible in the ruminant digestive tract. They are saturated straight-chain hydrocarbons with a chain length of 21-35 carbons (Dove and Mayes, 1996). They are found in most forage species (Russell et al. 2000), and the n-alkanes with odd-numbered carbons predominate (90%).

The use of n-alkanes as an internal marker has been proposed by Mayes et al. (1986) and is based on the same principle of analyzing n-alkanes in feed consumed and feces to estimate DMD. Fecal recovery increased with increased chain length, and tritriacontane ($C_{33}H_{68}$) is commonly used to predict digestibility (Mayes et al., 1986). According to Laredo et al. (1991), concentrations of some long chain n-alkanes such as tritriacontane and pentatriacontane ($C_{35}H_{72}$) are very low in some tropical forage species. Casson et al. (1990) recommended that odd-chain

n-alkanes concentration should be at least 50 mg/kg for accurate prediction of DMD to avoid lack of column sensitivity which can allow the detection of lower n-alkanes concentration. Even and odd-chained alkanes are determined by capillary gas chromatography (Marais, 2000) and the concentration is computed according to the below formula (Russell et al., 2000):

$$\text{mg of n-alkanes/kg sample} = (P_{alkane} \times 0.6 \text{ mg} \times 100)/(P_{is} \times SW \times DM) \quad [6]$$

where P_{alkane} is the peak area of alkane; 0.6 mg represent 0.6 ml of a standard solution containing 1.0 mg of dotria-contane per ml of n-hexane; P_{is} is the peak area of internal standard; SW is the sample weight; and DM, is the dry matter of the sample.

Several studies have reported incomplete recovery of n-alkanes in feces, which suggests that long chain n-alkanes disappear during gastrointestinal passage (Mayes et al., 1988). To overcome the problem of low recovery of n-alkanes in feces, Mayes et al. (1986) proposed dosing an animal with an external marker (even-chain alkanes) for estimation of FO while closely related odd-chain alkanes can be used to estimate digestibility. The combination of external and internal marker allows the prediction of DMI, because recoveries of the two markers would cancel out when performing intake calculations (Dove and Mayes, 1991). In a study comparing n-alkanes and IADF as internal markers to predict digestibility, Russell et al. (2000) reported that neither marker was completely recoverable in feces and was not consistent across forage species tested, although the recovery of n-alkanes was greater in general than IADF. Both markers underestimated ($P < 0.05$) the actual digestibility values of the forages tested.

Summary

Different internal markers have been developed and tested to estimate DMI, DMD, FO, rate of passage (K_p), rate of digestion (K_d), and energy content of feeds (TDN). Until now, no marker has presented 100% quantitative recovery across wide varieties of diets. Therefore, it is imperative that researchers validate or define recovery of internal markers for the diets they are studying before calculating digestibility, FO, and DMI. A large number of samples can be evaluated by external and internal markers to estimate nutritive value and save labor. However, variability in these estimations can be largely due to differences in forage species, stage of maturity, and marker types. One of the concerns with internal markers is that most components used are available in small quantities in forages, potentially magnifying errors in analytical procedures. In addition, some internal markers, such as silica, acid insoluble ash (AIA), and acid detergent insoluble ash (ADIA) can easily be contaminated by soil present on the forage or fecal sample or if animals consume soil intentionally. Finally, the direct method of fecal collection is always the most accurate whenever feasible.

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Chapter III

Evaluation of in situ internal markers for predicting digestibility and fecal output in cattle fed bermudagrass hays of varied nutrient composition

Abstract

The potential of in situ rumen undegradable dry matter (RUDM), indigestible neutral-detergent fiber (INDF), and indigestible acid-detergent fiber (IADF) for predicting digestibility (DMD) and fecal output (FO) by cattle offered bermudagrass [*Cynodon dactylon* (L.) Pers.] hay of varying qualities was evaluated. Eight ruminally cannulated cows (594 ± 100.3 kg) were allocated randomly to 4 bermudagrass hay diets categorized by their low (L), medium low (ML), medium high (MH), and high (H) crude protein (CP) concentrations (79, 111, 131, and 164 g CP/kg DM, respectively). Diets were offered in 3 periods to provide 2 replicates per diet each period (n = 24). Cows were housed in individual pens and offered their respective hay at a total of 20 g DM/kg of BW in equal feedings at 0800 and 1600 h for a 10-d adaptation period followed by a 5-d total fecal collection (TC) in each period. Duplicate samples of each of the hay, ort, and fecal samples from each period were incubated in Dacron bags for 144 h in the rumen of 2 cows for each of the digestion periods, followed by a sequential analysis of NDF and ADF. Recovery of RUDM, INDF, and IADF and their respective adjusted values ($RUDM_a$, $INDF_a$, and $IADF_a$, respectively) were expressed as the ratio of the quantity of marker excreted in the feces per unit of marker consumed. Data for in vivo DMI, DMD, FO and the chemical composition of the diets were analyzed as a replicated 4 × 4 Latin-Square design with one period missing using PROC GLM of SAS. Effects of cow, diet, and period were included in the model. Data for recovery, estimates of DMD, and FO were also analyzed using PROC GLM of SAS,

where diet, method, and diet by method interaction were included in the model. Diet affected DMI ($P = 0.01$) but did not affect FO ($P = 0.12$) and apparent DMD ($P = 0.18$). All fecal recovery rates differed by marker ($P < 0.01$) and diet, but not by the diet × marker interaction ($P = 0.99$). Fecal output estimates were affected ($P < 0.01$) by diet and marker while DMD was affected by the diet × marker interaction ($P = 0.019$). Indigestible NDF, ADF, and RUDM determined by in situ incubation appeared to be inadequate internal markers because of incomplete recovery and potential variability in DMD prediction across diets.
Key words: Bermudagrass, digestibility, in situ internal markers, cattle.

1. Introduction

In forage-based ruminant feeding, knowledge of the nutritive value of the basal diet is crucial to decide whether supplements are needed to meet the animal's energy and other nutrient requirements. One way of estimating energy values of feed is to conduct an in vivo digestion study and to determine organic matter digestibility (OMD), which is theoretically equal to total digestible nutrients (TDN) or digestible energy (DE; Lofgreen, 1956). However, the in vivo method requires total fecal collection, which is laborious, and in addition unfeasible to test a wide range of samples with a large number of animals (Undersander et al., 1987). Alternatively, indirect measurements using external and internal markers can be used to estimate digestibility of consumed feeds, especially forages. The use of reliable internal markers offers more advantages than external markers as long as they are fully recoverable and thus indigestible. When this assumption is not fulfilled, an adjustment for incomplete recovery can be applied (Owens and Hanson, 1992; Cochran and Galyean, 1994).

Several studies have evaluated internal markers with in vitro disappearance techniques to compare the results with in vivo responses (Undersander et al., 1987). However, few researchers have evaluated indigestible feed components using the in situ disappearance technique. Comparing in vitro and in situ procedures for RUDM, INDF, and IADF determination, Huhtanen et al. (1994) recommended the use of the in situ procedure. However, the in situ procedure has produced variable results (Judkins et al., 1990) while promising results were obtained by Fondevila et al. (1995) and Ferret et al. (1999). In addition, the variability of internal markers in predicting digestibility and FO across different types of forages (Sunvold and Cochran, 1991) requires a validation of marker recovery on a specified diet before its application in research. Therefore, our objective was to evaluate the potential of in situ RUDM, INDF, and IADF as internal markers in predicting apparent FO and DMD of bermudagrass hay of varying qualities by cattle.

2. Materials and Methods

2.1. Location, treatments, and experimental design for in vivo digestion

The study was conducted at the University of Arkansas Division of Agriculture Watershed Research and Education Center (WREC) located in Fayetteville, AR. Eight ruminally cannulated cows (n = 8, BW = 594 ± 100.3 kg) were stratified by weight and allocated to 1 of 2 blocks containing 4 cows each. Each block of 4 cows was assigned to a replicated 4 × 4 Latin-Square experimental design with one period missing. Four diet treatments of bermudagrass hay (Table 3.1) were duplicated in the 2 squares. The 4 bermudagrass hays varied in nutritional quality and were designated as follows based on their CP concentrations: low (L, CP = 79 g/kg DM); medium low (ML, CP = 111 g/kg DM); medium high (MH, CP = 131 g/kg DM); and high

(H, CP = 164 g/kg DM).The combination of 8 cows used for 3 periods resulted in 24 total in vivo observations, or 6 observations per hay treatment. Each period consisted of a 10-d adaptation period followed by 5-d of total fecal collection.

Cows were housed individually in 3.0 × 4.3 m pens with solid concrete floors covered with rubber mats. Cows were allowed to move freely within their respective pens. Each pen was fitted with plastic sheets on the rails between pens to avoid inadvertent cross-contamination of feces across pens. Cows were moved from their pens and allowed to graze for 14 d between each period to exercise and reduce the carryover effects of the previous hay treatment. The protocol used in this research was approved by the Institutional Animal Care and Use Committee of the University of Arkansas (IACUC approved protocol #10016).

2.2. Hay acquisition

Bermudagrass hay used in this study was harvested at 3 different locations: The University of Arkansas Livestock and Forestry Research and Extension Station near Batesville, AR (3 bales), WREC (5 bales), and the University of Arkansas Southeast Research and Extension Center in Monticello, AR (4 bales) to represent a wide range in quality and maturity. The bales were large round bales weighing between 364 to 500 kg with average bale dimensions of 1.2 × 1.5 m. Core samples from each bale (n = 3) were taken with Star Quality Samplers (Edmond, AB, Canada) at the round side in different directions in each bale to a depth of 0.46 m. The core samples were analyzed for CP, and then the bales were grouped based on CP concentration, irrespective of location, into 1 of the 4 groups described previously. One bale from each treatment (total of 12) was fed to 2 cows during each period. A total of 12 large round bales were used for the 45 d feeding of the 3 periods.

2.3. Feeding and sample collection

A total of 20 g/kg BW was offered as long hay in equal amounts at 0800 and 1600. This feeding level was chosen to minimize refusal. Water was provided for ad libitum consumption via rubber water tanks and each cow received 114 g of a commercial cattle mineral supplement[1] (Purina Wind and Rain® All Season 7.5 Complete) per day. Feed sampling began on d 9, orts on d 10, and feces on d 11. Samples of each hay offered were taken at each feeding sequence, placed in paper bags, weighed immediately, and dried in a forced-air oven at 50°C until no further weight loss was detected. Orts (refusals) were collected each morning before feeding (0700 h), weighed, and a representative sample was placed in paper bags. Samples were then weighed and dried in a forced-air oven at 50° C until no further weight loss was detected. Total feces from each cow were collected throughout the day beginning at 0800 on day 11 by scraping them directly from the pen rubber mats. Feces were stored temporarily in plastic-lined trash cans. At 0800 each day, total feces per cow were weighed, mixed in a commercial concrete mixer (Mixer Model 043206 Type A, Monarch Industries Inc., Canada), and a representative fecal sample (approximately 300 g of fresh feces) from the individual total daily fecal excretion was taken and placed on paper or aluminum plates, and dried in a forced-air oven at 50° C for determination of total FO and subsequent analysis of chemical composition and marker concentrations.

[1] Contained 135-160 g/kg Ca, 75 g/kg P, 182.5-217.5 g/kg salt, 5 g/kg Mg, 10 g/kg K, 3600 µg/kg Zn, 2115 µg/kg Mn, 1100 µg/kg Cu, 50 µg/kg Co, 115 µg/kg I, 27 µg/kg Se, 660,000 IU/kg Vitamin A, 66000 IU/kg Vitamin D, and 660 IU/kg Vitamin E

Fecal grab samples were taken directly from the rectum of each cow at 0600, 1200, 1800, and 2400 daily during the 5-d total collection period for a subsequent study. Total FO of each cow was corrected to include the dry weight of the 20 fecal grab samples taken per period.

2.4. In situ analysis

After drying samples of hay, orts, and feces to a constant weight for dry matter (DM) determination, samples of each period were composited by diet treatments for hay offered, and by cow for orts and feces. Then, samples of hay, orts, and feces were ground to pass a 2-mm screen of a Wiley mill (Thomas Scientific, Swedesboro, NJ). Duplicate Dacron bags (10 × 20-cm; 53 ± 10-*u*m pore size; ANKOM Technology Corp., Fairport, NY, USA) were filled with 5 g of each ground forage, ort, or fecal sample and closed with rubber bands. In total, there were 24 samples of hay offered, 48 samples of feces, and 36 samples of orts (3 cows in period 2, and 3 cows in period 3 did not have orts).

Six ruminally-cannulated cows were used for the in situ evaluation. During the incubation, cows were offered a total of 20 g DM /kg of BW of a bermudagrass hay-based diet (17.5 g/kg BW of hay, 2.5 g/kg BW of concentrate mix) in equal meals at 0800 and 1600 h and had ad libitum access to water. The composition of the diet fed during the in situ trial is summarized in Table 3.2.

Individual bags of hay, ort, and fecal samples were placed in 36 × 50-cm mesh bags and inserted into the ventral rumen immediately prior to feeding on d 10 of the study. Samples from each period were inserted into the rumen of 2 cows in order to provide replication of the rumen environment for each period. After 144 h of incubation, the Dacron bags were removed from the rumen and were subjected to a hand washing (rinsing) with cold-water until the water was clear (approximately 10 times) to prevent any loss of sample due to washing machine use. All rinsed

bags were dried to a constant weight at 50^{o} C and allowed to equilibrate to ambient temperature prior to weighing.

2.5. Dry matter loss analysis and adjustment of concentrations of markers

The lack of uniformity of particle size of forage, ort, and feces may result in variable and incomplete in situ INDF and IADF recovery rates (Lippke et al., 1986; Lund et al., 2007). However, once the marker is not completely recoverable in feces, an adjustment for incomplete recovery can be made (Owens and Hanson, 1992; Cochran and Galyean, 1994). In this study, after initial evaluation of RUDM, INDF, and IADF, recovery rates were adjusted based on the proportion of each marker that washed out of the sample bags that were not incubated in the rumen, but were subjected to washing procedures similar to those used for the bags incubated in the rumen. The correction (adjustment) factor (CF) was calculated as the ratio of DM remaining after washing to the initial sample weight on a DM basis. The initial DM incubated for in situ RUDM, INDF, and IADF evaluation was then multiplied by CF to obtain the initial DM corrected for differential DM loss of forage, ort, and feces.

2.6. Chemical analysis of forages, orts, feces and internal markers

Forage samples were analyzed for DM, total ash (TA), and total N by AOAC (2001) procedures 2001.12 and 2001.11, respectively. Organic matter was calculated as the weight lost from combustion of DM. Neutral-detergent fiber, ADF, and ADL in forage, ort, and feces were analyzed sequentially by the methods of Van Soest et al. (1991) and the batch procedure outlined by ANKOM Technology Corp. (Fairport, NY, USA). Sodium sulfite or heat-stable α-amylase was not added to the neutral-detergent solution. The same method was used to analyze INDF and

IADF on the residual DM from the in situ procedure by placing 0.5 ± 0.01 g in filter bags and analyzing these sequentially for NDF and ADF. Hemicelluloses were estimated from the values obtained in sequential analyses of NDF and ADF and was calculated as the difference between NDF and ADF.

2.7. Recovery rate, digestibility, and fecal output calculation

The concentration of marker in consumed forage (M_{fd}) was expressed as follows:

$$M_{fd} = [(M_{of} \times Q_{of})-(M_{or} \times Q_{or})] / DMI \quad [1]$$

where M_{of} is the concentration of marker in hay offered; Q_{of} is the amount of hay offered; M_{or} is the concentration of marker in orts; Q_{or} is the amount of orts refused (Q_{or}), and DMI is the actual DMI.

The recovery of RUDM, adjusted RUDM ($RUDM_a$), INDF, adjusted INDF ($INDF_a$), IADF, and adjusted IADF ($IADF_a$) were expressed as the ratio of the quantity of marker excreted in the feces per unit of marker consumed according to the following relationship:

$$R \text{ (recovery)} = (M_{fc} \times FO) / (M_{fd} \times DMI) \text{ or}$$

$$R = (M_{fc} \times FO) / [(M_{of} \times Q_{of}) - (M_{or} \times Q_{or})] \quad [2]$$

where FO is the fecal DM excreted; M_{fd} is the marker concentration in consumed feed; M_{fc} is the marker concentration in feces.

Apparent dry matter digestibility (DMD) was calculated by the following formula:

$$DMD = 1000 \times (DMI-FO) / DMI \quad [3]$$

The estimate of dry matter digestibility (DMD) using internal markers was given by the following expression:

$$DMD = 1000 \times (1- M_{fd} / M_{fc}) \quad [4]$$

Estimate of FO using internal markers was given by the following expression:

$$FO = DMI \times M_{fd} / M_{fc} \quad [5]$$

2.8. Statistical analysis

Data for intake, digestibility, chemical composition and DM loss from the in situ bags due to washing were analyzed as a replicated 4 × 4 Latin-Square design with one period missing using PROC GLM of SAS (SAS Int. Inc., Cary, NC, USA, 2009). Effects of cow, diet and period were included in the model. Cow was considered as the experimental unit for the diet effects and differences were considered significant at $P < 0.05$. Data of internal marker recovery and estimates of apparent DMD and FO were analyzed using PROC GLM of SAS, where diet, marker, and diet by marker interaction were included in the model and significant differences were noted at $P < 0.05$. Treatment means were reported as least squares means and were estimated and separated by the LSMEANS and PDIFF options in SAS when the overall treatment effect was significant ($P < 0.05$). When diet × marker interaction was not significant, the comparisons of the LSMEANS among themselves and with the means of observed values of FO and DMD were conducted using the ESTIMATE statement in GLM which calculated the difference of each pair of means and tested if it differed from zero. The F-protected t-test was used to determine if the marker ratio estimates differed from 1.

3. Results

3.1. Intake, digestibility, and fecal output

Data for DMI, FO, and apparent DMD for the different bermudagrass hay qualities are presented in Table 3.3. Forage DMI was affected by diet ($P = 0.01$), while FO ($P = 0.12$) and

apparent DMD ($P = 0.18$) were not affected by diet. Forage DMI was greater on MH and H than the L diet.

3.2. Internal marker concentration

The marker concentrations in consumed feeds and feces before and after adjustment are presented in Table 3.4. The concentrations of all internal markers in forage consumed were affected by type of diet ($P < 0.01$), and generally decreased as forage CP increased. Diet tended ($P = 0.05$) to affect the concentrations of RUDM in the feces but did not affect ($P \geq 0.14$) the fecal concentrations of the other internal markers.

3.3. Recovery of internal markers

The recovery rates of each marker before and after adjustment are presented in Table 3.5 and were affected by marker ($P < 0.01$) and diet ($P < 0.01$) but not by the diet × marker interaction ($P = 0.99$). Adjusting marker concentrations for the amount of marker loss due to washing resulted in an improvement ($P < 0.05$) in marker recovery. However, recoveries of all markers differed from 1 (Table 3.6; $P < 0.01$).

Results of 0-h (A fraction analysis) are presented in Tables 3.7 and 3.8. Dry matter loss and CF were affected by sample type ($P < 0.01$) and diet quality ($P < 0.01$), but not the diet quality × sample type interaction ($P \geq 0.62$). Dry matter loss was greater ($P < 0.05$) for feces than for hay or orts and was lower ($P < 0.05$) for L hay than for the other qualities of hay. The resulting CF was a reflection of DM loss.

3.4. Estimates of FO and apparent DMD

Estimates of FO (Table 3.9) were affected by type of marker ($P < 0.01$) and diet ($P < 0.01$), but not by the diet × marker interaction ($P = 0.90$). In general, FO was overestimated ($P < 0.01$) because of incomplete recovery of these in situ markers in feces. However, there was an improvement ($P < 0.001$) in prediction of actual FO when the markers were adjusted for washing losses. Adjusted IADF was the closest in predicting FO (4207 vs. 4588 g/d; CV = 8.7 %).

Estimates of DMD (Table 3.10) were impacted by diet ($P < 0.01$), marker ($P < 0.01$), and the diet × marker interaction ($P = 0.019$). Adjusted IADF accurately predicted the DMD of ML, MH, and H hays, but failed to predict the DMD of L bermudagrass hay. Also, $RUDM_a$ and $INDF_a$ accurately predicted DMD on MH and H diets but not on L and ML diets.

4. Discussion

4.1. Effect of diets on DMI, DMD, and FO

Our diet treatments were categorized by their L, ML, MH, and H CP concentration in hay and were offered at fixed feed intake (20 g/kg DM). In this study, DMI was affected by diet, which was unexpected because bermudagrass hay was offered at restricted intake, indicating that ad libitum intake was less than 20 g/kg on most of these hays. There were no differences in forage DMD among treatments. Cows on lower CP diets consumed less feed (263, 118, 42, and 72 g of orts/kg DM offered, respectively for L, ML, MH, and H CP content diets) and consequently the low DMI may have lowered the rate of passage (k_p; Thonney et al., 1985) of consumed feed, which in turn mitigated the expected difference in DMD. Also, the NDF in high quality hay was not in agreement with CP concentration. However, a numerical increase in DMD was observed when CP content increased.

4.2. Marker concentration and recovery

The fecal recovery rates of unadjusted and adjusted RUDM were incomplete. However, RUDM measured with 7 d in situ incubation (nylon bags 125 × 100-mm, 50 µm pore size) was the best internal marker with the average recovery rate of 0.992 in ryegrass and 1.000 in alfalfa fed alone (Ferret et al.,1999). Also, Sampaio et al. (2011b) obtained a RUDM recovery rate of 0.990 with cattle consuming different diets. In comparison to our results, differences may be due to different diets and bags used (non-woven textiles bags, 100 g/m^2; 4 × 5 cm) along with an incubation period of 264 h instead of 144 h as in our study. Furthermore, an average recovery rate for RUDM (96-h incubation) in sheep on cereal straw-based diets of 1.080 was reported by Fondevila et al. (1995), who cautioned the use of RUDM as an internal marker when used on different diets in different feeding conditions. Although RUDM can be used as a lower cost internal marker compared to INDF and IADF (Huhtanen et al., 1994; Detmann et al., 2001), sample contamination during in situ evaluation of RUDM is one of the shortcomings of this procedure, and can cause greater variability of results (Valente et al., 2011). Contamination can differentially affect the RUDM residues due to differences in bags and feed utilized and is not uniform among replicates (Casali et al., 2009). Therefore, caution should be observed when using RUDM as an internal marker.

The fecal recovery of unadjusted and adjusted INDF was also incomplete in this study. Incomplete fecal recovery (0.86) was observed from alfalfa fed to lambs by Undersander et al. (1987). Large ranges (0.830 to 1.11 and 0.781 to 0.997) of fecal recovery rates of INDF, either measured by in situ (6 d incubation) or in vitro, were observed on bermudagrass from different varieties (Lippke et al., 1986). However, there was less variation, but incomplete (0.868) fecal recovery due to cutting age (maturity). In addition, the in vitro INDF (IVNDF) fecal recoveries

were 1.012, 0.432, 0.966, and 0.915, respectively for alfalfa cubes, tall fescue, tall wheatgrass and soybean meal (SBM), and prairie hay (Cochran et al., 1986). In contrast to our results, an average recovery rate for INDF (96-h incubation) of 0.964 in sheep on cereal straw-based diets was achieved by Fondevila et al. (1995) while Sampaio et al. (2011b) obtained a fecal recovery of 0.989 from cattle fed various diets with an in situ incubation of 264 h using non-woven textiles bags (100 g/m^2; 4 × 5 cm).

The adjusted IADF recovery was the best among the in situ markers evaluated, even though their recoveries differed from 1. The recovery of IADF was consistently least among the markers evaluated (ADL, APL, AIA, IADF) in a study by (Sunvold and Cochran, 1991). In their study, the recovery of IADF-based markers fell in the range of 0.70 and 0.80 (0.803, 0.801, and 0.702 for alfalfa (*Medicago sativa* L.), bromegrass (*Bromus inermis* Leyss.), and prairie hay, respectively). Different recovery rates of IADF were observed on ryegrass fed alone with hays of varying quality (0.881, 0.741, and 1.050; high, medium high and low quality, respectively) and on alfalfa (0.937) in ewes (Ferret et al., 1999). Complete IADF recovery was achieved with alfalfa fed to lambs (1.01; Undersander et al., 1987), and with cattle fed various diets (1.02, Sampaio et al., 2011b) using a 264 h incubation and bags with different pore size (non-woven textiles bags; 100 g/m^2; 4 × 5 cm).

As mentioned previously, one of the main shortcomings of in situ markers is sample contamination during the in situ procedure. The main sources of contamination are microbial contamination, substrates (feed), and bag characteristics (Vanzant et al., 1998), and in addition, contamination is not homogenous for all replicate samples incubated (Casali et al., 2009; Sampaio et al., 2011a&b). The contamination issue is more problematic for RUDM than for

other in situ markers because neutral- and acid-detergent solutions remove many contaminants, and in particular, microbes.

The second limitation to the use of in situ techniques is the initial particle loss often described as the soluble fraction of DM (Huhtanen and Sveinbjörnsson, 2006), which differs between forage and feces (Lippke et al., 1986). Differences in particle size between hay, ort, and feces lead to incomplete recovery of in situ internal markers (RUDM, INDF and IADF; Lippke et al., 1986; Huhtanen et al., 1994; Lund et al., 2007). During the in situ process, sample material that disappears from the Dacron bags is considered as being digested while a fraction of the sample may leave the bag actually due to small particle size. These differences in particle size between feeds and feces after grinding to 2-mm screen could be responsible for the variable and generally less than complete fecal recovery of the in situ internal markers observed. Furthermore, using bags of different porosity (nylon, 50 µm; F57 (ANKOM), and non-woven textile (NWT-100 g/m^2) may yield varying results of marker recovery (Valente et al., 2011). Thus, selecting the appropriate bag type is of utmost importance. To overcome the problem of difference in DM loss, marker recovery rate should be calculated using an INDF: NDF ratio instead of INDF: DM ratio (Huhtanen et al., 1994) if it is assumed that particles leaving the nylon bags come from only potentially degradable NDF (pdNDF) in feed and in feces. In this study, after adjusting for 0-h DM loss, there was an improvement in recovery rates on all 3 internal markers, but still the recovery rates were incomplete and different from 1. Another source of error may be associated with loss of particles that is higher for feces than feed during NDF or ADF analysis (Lund et al., 2007) and Udén (2006). Average particle loss of NDF (g/kg NDF) was 40 and 120, respectively for forage and feces (Lund et al., 2007).

4.3. Marker effects on prediction of FO and DMD

Fecal output estimates from markers overestimated actual values of FO obtained from the TC trial. Similar overestimation of FO by in situ indigestible fiber components, measured after incubation of 144 d, was reported by Soares et al. (2011) for buffalo fed elephant grass (*Pennisetum purpureum*). Estimated and actual FO were in perfect agreement for RUDM, INDF, and IADF measured from 264 h in situ incubation in cows consuming different diets (Sampaio et al., 2011b). However, IADF measured by in situ incubation (144 h) predicted FO (1.83 vs. 1.73 kg/d, respectively for TC and IADF) on Tifton-85 [*Cynodon dactylon* (L.) Pers. x C. transvaalensis Burtt Davy] hay fed to cattle (340 kg BW) while INDF overestimated fecal output (1.83 vs. 2.32 kg/d; Berchielli et al., 2005).

Dry matter digestibility was variable among diet treatments and was underestimated by all in situ markers. Similarly, IADF yielded estimates of forage OMD that differed ($P < 0.05$) from that of TC for alfalfa, bromegrass, and prairie hay diets (Sunvold and Cochran, 1991). Thus, further investigations were warranted for the applicability of IADF and INDF as markers for cattle consuming diverse ranges of diets or fresh, immature forage. In addition, Judkins et al. (1990) and Sunvold and Cochran (1991) came to the conclusion that indigestible fiber fractions underestimated the DMD of forages. The OMD estimation of grass-hay diets (bromegrass, and prairie hay) and alfalfa were consistently less ($P < 0.05$) than those derived from TC. Furthermore, Arthington and Brown (2005) found that IADF measured by in vitro technique underestimated bermudagrass OMD compared with TC (502 vs. 538 g/kg DM, respectively).

In contrast to our results, some studies have reported promising results when using in situ or in vitro markers. According to Ferret et al. (1999), RUDM was the best predictor of DMD with the prediction equation (0.132 + 0.80x, n = 26, R = 0.91), explaining 83% of the variation in

DMD. The IADF was the best marker in predicting digestibility of several forages in sheep (Penning and Johnson, 1983). In addition, ash-free IADF determined by 48-h ruminal fluid incubation, 24-h pepsin-HCl hydrolysis, and then a 96-h in vitro incubation appeared to be a suitable marker to estimate digestibility by forage fed or grazing cattle (Nelson et al., 1990). The estimate of OMD was 631 vs. 646 g/kg DM from TC. Indigestible ADF and NDF provided acceptable estimates of digestibility with alfalfa cubes (*Medicago sativa* L.) and tall wheatgrass [*Agropyron elongatum* (Host) P. Beauv.] plus soybean meal diet although a variable relationship between in vivo DMD and DMD estimates by these markers was observed (Cochran et al., 1986).

In addition to the problem of incomplete fecal recovery in the present study, the fact that there was a diet × marker interaction becomes a hindrance to the use of these in situ markers on varied qualities of bermudagrass. Therefore, one in situ-based marker may not be able to predict the DMD digestibility of bermudagrass hay across a wide range of protein concentrations.

In summary, for in situ indigestible fiber fractions to have the potential to be used as internal markers, several conditions or assumptions must be met. These conditions and assumptions include adequate incubation period, accounting for particle loss during in situ and fiber analysis, grinding samples with a proper diameter screen and using proper nylon bags with acceptable pore sizes.

5. Implications

Based on the results of this study, RUDM, INDF and IADF and their corresponding adjusted markers ($RUDM_a$, $INDF_a$ and $IADF_a$), determined by in situ incubation, are not adequate internal markers for varying qualities of bermudagrass hays fed to cattle because of low

and variable marker recovery. Consequently, none of the in situ internal markers accurately predicted apparent digestibility and fecal output. However, an adequate adjustment based on DM loss occurring during in situ process and fiber analysis due to differences in particle sizes among forage, ort, and feces may provide acceptable fecal recovery for fecal output and digestibility prediction.

Acknowledgements

This research was supported by the University of Arkansas, Division of Agriculture.

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Table 3.1
Chemical composition (g/kg dry matter, DM) of bermudagrass hay fed during an in vivo experiment for estimating marker recovery based on different crude protein (CP) levels.

	Treatments[a]					
Item[b]	L	ML	MH	H	SEM[c]	*P*-value
DM	885	872	867	875	10.6	0.754
OM	946[d]	913[e]	912[e]	919[e]	8.2	0.038
TA	57	82	83	76	6.0	0.052
CP	79[f]	111[e]	131[e]	164[d]	6.4	<0.001
NDF	768	712	690	740	19.1	0.085
ADF	428[d]	348[e]	332[e]	370[de]	19.4	0.035
HEM	340	364	358	370	9.1	0.191
ADL	45[d]	33[ef]	31[f]	41[de]	2.9	0.029

[a]L, low CP hay (CP = 79 g/kg DM); ML, medium low CP hay (CP = 111 g/kg DM); MH, medium high CP hay (CP = 131 g/kg DM); and H, high CP hay (CP = 164 g/kg DM).
[b]DM, dry matter; OM, organic matter; TA, total ash, CP, crude protein , NDF, neutral-detergent fiber, ADF, acid detergent fiber; HEM, hemicellulose; ADL, acid-detergent lignin.
[c]SEM, standard error of the mean.
[def]Means with different superscripts in the same row differ at $P < 0.05$.

Table 3.2

Chemical composition (g/kg DM) of the diet fed during the in situ trial to estimate marker recovery from bermudagrass hays with differing concentrations of crude protein.

Components	Chemical composition[a]					
	DM	TA	OM	CP	NDF	ADF
Bermudagrass hay	875	79	921	108	673	287
Concentrate[b]	920	99	901	210	218	57

[a]DM, dry matter; TA, total ash, OM, organic matter; CP, crude protein, NDF, neutral-detergent fiber, and ADF, acid-detergent fiber

[b]Concentrate contained (on as-fed basis): cracked corn (372 g/kg), wheat shorts (200 g/kg), soybean meal (347 g/kg), molasses (40 g/kg), limestone (3 g/kg), TM salt[1] (33 g/kg), and vitamin A, D, and E premix[2] (2 g/kg), and vitamin E premix (3 g/kg) and was offered at 2.5 g/kg BW.

[1]TM salt contained 135-160 g/kg Ca, 75 g/kg P, 182.5-217.5 g/k salt, 5 g/kg Mg, 10 g/kg K, 3,600 µg/kg Zn, 2,115 µg/kg Mn, 1,100 µg/kg Cu, 50 µg/kg Co, 115 µg/kg I, 27 µg/kg Se, 660, 000 IU/kg Vitamin A, 66000 IU/kg Vitamin D, and 660 IU/kg Vitamin E.

[2]Vitamin A, D, and E premix contained 88,000,000 IU Vitamin A/kg, 1,760,000 IU Vitamin D/kg, and 1,100 IU Vitamin E/kg.

Table 3.3

Dry matter intake (DMI), fecal output (FO), and dry matter digestibility (DMD) of bermudagrass hay with differing concentrations of crude protein (CP) fed to cattle for estimating internal marker recovery based on total collection (TC).

	Treatments[a]					
Item	L	ML	MH	H	SEM[b]	*P*-value
DMI (g/d)	7736[d]	9015[cd]	10205[c]	9780[c]	423.7	0.01
FO (g/d, on DM basis)	3755	4081	4719	4275	254.3	0.12
DMD (g/kg DM)	511	544	535	567	16.4	0.18

[a]L, low CP hay (CP = 79 g/kg DM); ML, medium low CP hay (CP = 111 g/kg DM); MH, medium high CP hay (CP=131 g/kg DM); and H, high CP hay (CP = 164 g/kg DM).

[b]SEM, standard error of the mean.

[cd]Means with different superscripts in the same row differ at $P < 0.05$.

Table 3.4

Concentration (g/kg dry matter, DM) of various internal markers in consumed bermudagrass hays with differing concentrations of crude protein and associated feces as determined by total collection and in situ procedures (144 h incubation) before and after correction for DM loss.

	Treatments[a]					
Item[b]	L	ML	MH	H	SEM[c]	*P*-value
Uncorrected[1]						
$RUDM_c$	401[d]	349[e]	329[e]	324[e]	9.4	0.01
$RUDM_f$	575	591	595	619	12.4	0.17
$INDF_c$	320[d]	267[e]	252[e]	245[e]	9.0	0.01
$INDF_f$	444	442	450	469	9.8	0.30
$IADF_c$	180[d]	133[e]	127[e]	125[e]	6.6	0.01
$IADF_f$	250	234	233	251	6.4	0.14
Corrected[2]						
$RUDM_a$	471[d]	439[e]	417[e]	409[f]	8.4	<0.001
$RUDM_{fa}$	788[e]	870[d]	852[de]	871[d]	21.5	0.05
$INDF_a$	375[d]	336[e]	319[ef]	310[f]	7.9	<0.001
$INDF_{fa}$	609	652	644	661	15.7	0.15
$IADF_a$	211[d]	167[e]	161[e]	158[e]	5.9	<0.001
$IADF_{fa}$	343	345	334	354	8.7	0.49

[a]L, low CP hay(CP = 79 g/kg DM); ML, medium low CP hay (CP = 111 g/kg DM); MH, medium high CP hay (CP=131 g/kg DM); and H, high CP hay (CP = 164 g/kg DM).

[b]$RUDM_c$, rumen undegradable dry matter concentration in the consumed diet; $RUDM_f$, rumen undegradable dry matter concentration in feces; $INDF_c$, indigestible neutral-detergent fiber concentration in the consumed diet; $INDF_f$, indigestible neutral-detergent fiber concentration in feces; $IADF_c$, indigestible acid-detergent fiber concentration in the consumed diet; and $IADF_f$, indigestible acid-detergent fiber concentration in feces; $RUDM_a$, adjusted rumen undegradable dry matter concentration in the consumed diet; $RUDM_{fa}$, adjusted rumen undegradable dry matter concentration in feces; $INDF_a$, adjusted indigestible neutral-detergent fiber concentration in the consumed diet; $INDF_{fa}$, adjusted indigestible-neutral detergent fiber concentration in feces; $IADF_a$, adjusted indigestible acid-detergent fiber concentration in the consumed diet; and $IADF_{fa}$, adjusted indigestible acid-detergent fiber concentration in feces.

[c]SEM, standard error of the mean.

[de]Means with the same superscripts within row differ at $P < 0.05$.

[1]Marker in hay and feces before correcting for differences in dry matter loss.

[2]Marker in hay and feces obtained after correcting for difference in dry matter loss.

Table 3.5

Recovery (g/kg) of internal markers in feces from cattle fed bermudagrass hays varying in crude protein concentrations. Values are given for markers pre- and post-correction for particle loss during the analytical procedures.

	Treatments[a]						*P*-value [d]		
Item[b]	L	ML	MH	H	Average	SEM[c]	D	M	D × M
RUDM	0.709	0.777	0.845	0.824	0.789[f]	0.0136	<0.01	<0.01	0.99
$RUDM_a$	0.824	0.904	0.951	0.926	0.901[e]				
INDF	0.696	0.771	0.844	0.829	0.785[f]				
$INDF_a$	0.801	0.890	0.941	0.924	0.889[e]				
IADF	0.713	0.840	0.870	0.885	0.827[f]				
$IADF_a$	0.811	0.957	0.968	0.960	0.924[e]				
Average	0.759[i]	0.856[h]	0.903[g]	0.891[g]					

[a]L, low CP hay (CP = 79 g/kg DM); ML, medium low CP hay (CP = 111 g/kg DM); MH, medium high CP hay (CP = 131 g/kg DM); and H, high CP hay (CP = 164 g/kg DM).

[b]RUDM, rumen undegradable dry matter; $RUDM_a$, adjusted rumen undegradable dry matter; INDF, indigestible neutral-detergent fiber; $INDF_a$, adjusted indigestible neutral-detergent fiber; IADF, indigestible acid-detergent fiber; $IADF_a$, adjusted indigestible acid-detergent fiber.

[c]SEM, standard error of the mean.

[d]D, diet; M, marker; and D × M, diet by marker interaction.

[ef]Means with different superscripts within column differ at $P < 0.05$.

[ghi]Means with different superscripts row differ at $P < 0.05$.

Table 3.6

Recoveries of corrected and uncorrected internal markers and their corresponding confidence intervals (95%). Fecal recovery of a particular marker is considered complete if its confidence interval includes the theoretical value (1) of TC.

		Confidence interval			
Parameter[a]	Fecal recovery (g/kg)	Low limit	Upper limit	SEM[b]	*P*-value[c]
RUDM	0.789	0.750	0.828	0.0187	<0.001
$RUDM_a$	0.901	0.863	0.939	0.0184	<0.001
INDF	0.785	0.745	0.825	0.0193	<0.001
$INDF_a$	0.889	0.850	0.928	0.0189	<0.001
IADF	0.827	0.782	0.872	0.0219	<0.001
$IADF_a$	0.924	0.878	0.970	0.0220	0.0022

[a]RUDM, rumen undegradable dry matter; $RUDM_a$, adjusted rumen undegradable dry matter; INDF, indigestible neutral-detergent fiber; $INDF_a$, adjusted indigestible neutral-detergent fiber; IADF, indigestible acid-detergent fiber; $IADF_a$, adjusted indigestible acid-detergent fiber.

[b]SEM, standard error of the mean.

[c]Probability that the fecal recovery mean of a particular marker differ from 1.

Table 3.7

Average dry matter loss (DM loss, g/kg DM) and resulting correction factor (CF) for forage, ort, and fecal samples hand-washed prior in situ incubation. Values are averages from all diet treatments.

	Particle types[a]				
Item[b]	Forage	Ort	Feces	SEM[c]	*P*-value
DM loss	192[e]	175[e]	294[d]	6.0	<0.01
CF	0.808[d]	0.822[d]	0.706[e]	0.0060	<0.01

[a]Forage, ort, and fecal samples ground to 2-mm screen put in Dacron bags (Dacron bags, 10 cm by 20 cm; 53 ±10-*u*m pore size) and hand-washed.

[b]DM loss, dry matter loss (g/kg DM) at 0-h incubation; CF, correction factor.

[c]SEM, standard error of the mean.

[de]Means with different superscript within a row differ at $P < 0.05$.

Table 3.8

Average dry matter loss (DM loss, g/kg DM) and resulting correction factor (CF) for different treatment samples hand-washed prior in situ incubation. Values are averages for each diet treatment.

	Treatment[a]					
Item[b]	L	ML	MH	H	SEM[c]	*P*-value
DM loss	188[e]	237[d]	227[d]	230[d]	7.0	<0.01
CF	0.812[d]	0.762[e]	0.770[e]	0.770[e]	0.0070	<0.01

[a]L, low CP hay (CP = 79 g/kg DM); ML, medium low CP hay (CP = 111 g/kg DM); MH, medium high CP hay (CP = 131 g/kg DM); and H, high CP hay (CP = 164 g/kg DM).

[b]DM loss, dry matter loss (g/kg DM) at 0-h incubation for each diet type; CF, correction factor, (the ratio of remaining DM after washing over initial sample weight).

[c]SEM, standard error of the mean.

[de]Means with different superscripts within a row differ at $P < 0.05$.

Table 3.9

Fecal output (g/d) estimates derived from different internal markers including both unadjusted and adjusted values. Means comparisons were made between all markers and TC.

	Treatments[a]						*P*-value[d]		
Item[b]	L	ML	MH	H	Average	SEM[c]	D	M	D ×M
TC	3663	4055	4764	4347	4207[g]	105.7	<0.01	<0.01	0.904
RUDM	5305	5302	5643	5241	5373[e]				
$RUDM_a$	4552	4556	5010	4691	4702[f]				
INDF	5461	5405	5682	5245	5448[e]				
INDFa	4687	4648	5047	4695	4769[f]				
IADF	5375	5025	5520	5053	5243[e]				
$IADF_a$	4605	4319	4902	4526	4588[f]				
Average[1]	4807[i]	4759[i]	5223[h]	4828[i]					

[a]L, low CP hay (CP = 79 g/kg DM); ML, medium low CP hay (CP = 111 g/kg DM); MH, medium high CP hay (CP = 131 g/kg DM); and H, high CP hay (CP = 164 g/kg DM).

[b]RUDM, rumen undegradable dry matter; $RUDM_a$, adjusted rumen undegradable dry matter; INDF, indigestible neutral-detergent fiber; $INDF_a$, adjusted indigestible neutral-detergent fiber; IADF, indigestible acid-detergent fiber; $IADF_a$, adjusted indigestible acid-detergent fiber.

[c]SEM, standard error of the mean.

[d]D, diet; M, marker; and D × M, diet by marker interaction.

[efg]Means with different superscripts within a column differ at $P < 0.05$.

[hi]Means with different superscripts within a row differ at $P < 0.05$.

[1]Averages for treatments across the different markers.

Table 3.10

Least square estimates of digestibility (DMD, g/kg DM) derived from the diet × marker interaction, presenting values based on both unadjusted and adjusted values. Mean comparisons were made between all markers and TC.

	Treatment means (g/kg DM)[a]					
Item[b]	L	ML	MH	H	SEM[c]	Effect[d]
TC	519^{eH}	537^{eH}	532^{eH}	568^{eH}	20.3	D × M
RUDM	287^{fJ}	411^{eJ}	448^{eI}	474^{eI}		
$RUDM_a$	398^{fI}	489^{eI}	511^{eH}	533^{eH}		
INDF	267^{gJ}	400^{fJ}	443^{efI}	473^{eI}		
$INDF_a$	381^{fI}	480^{eI}	506^{eH}	532^{eH}		
IADF	272^{gJ}	440^{fJ}	457^{efI}	494^{eI}		
$IADF_a$	386^{fI}	514^{eHI}	519^{eH}	550^{eH}		

[a]L, low CP hay (CP = 79 g/kg DM); ML, medium low CP hay (CP = 111 g/kg DM); MH, medium high CP hay (CP = 131 g/kg DM); and H, high CP hay (CP = 164 g/kg DM).
[b]RUDM, rumen undegradable dry matter; $RUDM_a$, adjusted rumen undegradable dry matter; INDF, indigestible neutral-detergent fiber; $INDF_a$, adjusted indigestible neutral-detergent fiber; IADF, indigestible acid-detergent fiber; $IADF_a$, adjusted indigestible acid-detergent fiber.
[c]SEM, standard error of the means.
[d] D, Diet ($P < 0.001$); M, Marker ($P < 0.001$); D × M, diet by marker interaction ($P = 0.019$).
[efg]Means with different superscripts within row differ at $P < 0.05$.
[HIJ]Means with different superscripts within column differ at $P < 0.05$.

Chapter IV

Using acid-detergent lignin, alkaline-peroxide lignin and acid-detergent insoluble ash to predict fecal output and digestibility by cattle offered bermudagrass hays of varying nutrient composition

Abstract

The potential of acid-detergent insoluble ash (ADIA), alkaline-peroxide lignin (APL), and acid-detergent lignin (ADL) to predict fecal output (FO) and dry matter (DM) digestibility (DMD) by cattle offered bermudagrass [*Cynodon dactylon* (L.) Pers.] hay of different qualities was evaluated. Eight ruminally cannulated cows (594 ± 100.3 kg) were allocated randomly to 4 bermudagrass hay diets categorized by their low (L), medium low (ML), medium high (MH), or high (H) crude protein (CP) concentrations (79, 111, 131, and 164 g CP/kg DM, respectively). Diets were offered in 3 periods to provide 2 replicates per diet per period (n = 24). Cows were offered hay individually at a total of 20 g/kg of BW in equal feedings at 0800 and 1600 h for a 10-d adaptation followed by a 5-d total fecal collection (TC) each period. Hay, ort, and feces from each period were analyzed for ADL, APL, and ADIA concentrations. Actual DM intake (DMI), DMD, and FO were determined based on hay offered, ort, and feces excreted. Recovery of APL, ADL, and ADIA were expressed as the ratio of the quantity of marker excreted per unit of marker consumed. Data for ADL, APL, and ADIA recovery and marker-based estimates of FO and DMD were analyzed as a replicated 4 × 4 Latin-Square with one period missing using PROC GLM of SAS, where the effects of diet, marker, and the diet by marker interaction were included in the model. Average ADL recovery differed from 1 ($P < 0.01$), and that of APL tended to differ ($P = 0.081$) from 1, but ADIA recovery was not different from 1 ($P = 0.204$).

Estimates of FO and DMD derived using APL and ADIA were not different ($P \geq 0.28$) from TC while those using ADL differed ($P < 0.05$) from that of TC. In addition, there was no diet by marker interaction ($P \geq 0.224$) for both FO and DMD. Therefore ADIA and APL are potential internal markers to predict FO and DMD of bermudagrass of varying nutrient composition fed to cattle.

Key words: Bermudagrass, digestibility, alkaline-peroxide lignin, acid-detergent insoluble ash, cattle

1. Introduction

Diet formulation with accurate energy and nutrient digestibility values requires reliable methods of obtaining these values (Sales et al., 2004). Traditionally, dry matter digestibility (DMD) and that of corresponding nutrients are determined by the in vivo total fecal collection (TC) procedure. Although considered the most accurate, this procedure is labor intensive, time consuming, and quasi unfeasible to evaluate a wide range of feed samples requiring a large number of animals. In an attempt to overcome this problem, indirect methods using internal markers have been proposed (Penning and Johnson, 1983 a&b; Cochran et al., 1986; Cochran et al., 1987; Pond et al., 1987; Owens and Hanson, 1992). The use of internal markers requires the determination of the concentration of the marker and any other nutrient in representative samples of diet consumed and feces excreted. In addition, the use of internal marker ratio to estimate DMD is possible under the assumption that the marker is completely recoverable in feces.

Although lignin has been considered to be indigestible and recoverable in feces (Ellis et al., 1946; Forbes and Garrigus, 1948; Waldo et al., 1972) for many years, recent studies indicate that lignin may not be an adequate internal marker because of potential degradability or

formation of insoluble carbohydrate complex during its transit in the gastrointestinal tract (GIT) of ruminants (Cochran et al. 1986; Jasra and Johnson, 2000). According to Van Soest (1987), successful results can be obtained for ADL as an internal marker when its concentration is at least 60 g/kg of the DM. The addition of alkaline hydrogen peroxide (AHP) solution before ADF analysis appeared to improve the recoveries of lignin from plants and feces (Cochran et al., 1988). Digestibility estimates using APL in their trial were similar to those of total collection estimates when sheep were fed either immature or dormant grasses. In later digestion trials using lambs, APL gave variable digestibility estimates, even though lignin recovery was estimated to be near 100% (Momont et al., 1994). On the other hand, acid-detergent insoluble ash (ADIA) has been presented as a t reliable internal marker (Van soest, 1994), but is susceptible to soil contamination during the feeding process (Appedu and Bodine, 2002). In the previously-conducted experiment described in Chapter 3, none of the in situ-based internal markers presented a satisfactory fecal recovery to estimate fecal output (FO) and DMD. Therefore, the objective of this study was to evaluate the potential of ADL, APL and ADIA to be used as internal markers to determine FO and DMD of bermudagrass hay of various qualities by cattle.

2. Materials and Methods

A total collection experiment was conducted for 3 periods using 8 cows offered bermudagrass hay of varied crude protein (CP) concentrations (Chapter 3, Table 3.1). Location, experimental design, treatments, feeding and sample collection were described in detail in Chapter 3.

2.1. Chemical analysis of ADL, APL, and ADIA in forage, orts and feces

ADL procedure: Forage, ort, and fecal samples collected during the in vivo experiment were ground to pass a 1-mm screen Wiley mill (Arthur H. Thomas Scientific, Philadelphia, PA, USA) and sequentially analyzed for NDF, ADF and ADL using neutral-detergent solution, acid-detergent solution, and 72% sulfuric acid, respectively, according to the batch procedures outlined by ANKOM Technology Corp. (Fairport, NY, USA) and Van Soest et al. (1991). Samples were run in duplicate and when the coefficient of variation (CV) was greater than 5%, samples were rerun again until the CV was equal to or less than 5%.

APL Procedure: To overcome the problem of inconsistencies in lignin recovery, the ADL procedure was modified to include an alkaline-hydrogen peroxide (AHP) pretreatment of samples before the acid-detergent analysis (Cochran et al., 1988). Alkaline-peroxide lignin was isolated by pre-treating forage, ort, and fecal samples in AHP solution (1% H_2O_2 + NaOH) with pH adjusted to 11.5. The new procedure is an updated combination of procedures for fiber analysis (Van Soest et al., 1991; Cochran et al., 1988; and Sunvold and Cochran, 1991). One half-gram (0.5 ± 0.01 g) of each sample of forage, ort, and feces was put directly into filter bags (ANKOM Technology Corp. #F57, Fairport, NY, USA) instead of incubating samples in filter tubes. The bags were sealed, and samples were spread uniformly inside the filter bags. Filter bags (n = 24) were placed into a 2000 mL beaker and AHP solution was added at a rate of 50 mL AHP solution per bag. The bags were incubated for 24 h with agitation. After 24 h, bags were rinsed with hot distilled water (100^{o}C) until the pH became neutral (pH = 7). The filter bags were soaked in acetone for 3-5 min. After soaking, the filter bags were spread out on a plate and placed under a ventilation hood for at least 30 min to evaporate the acetone before drying the filter bags in oven at 100^{o}C for 8 h. Samples were cooled in desiccators for 20 min prior to weighing and recording the filter bag and sample residue. The weight obtained minus the initial

bag weight constituted the AHP residue. The AHP residue was analyzed sequentially for ADF and ADL content using acid-detergent solution and 72% sulfuric acid according to the batch procedures outlined by ANKOM Technology Corp. (Fairport, NY, USA) and Van Soest et al. (1991). The ADL residue was ashed in a muffle furnace at 500^{o}C for 8 h, and the mass of ash from the ADL residue was subtracted from the mass of the ADL residue. The residue was then divided by the original sample weight to obtain ash-free APL. Samples were run in duplicate and where the CV between replicates was greater than 5%, samples were rerun again until the CV was equal or less than 5%. In addition, samples were incubated 24 h instead of 48 h as it was suggested by Sunvold and Cochran (1991) because the difference in AHP residue was not significantly different to justify the long incubation based on preliminary samples we analyzed.

Procedure for ADIA: Approximately 0.5 ± 0.01g of forage, ort, and fecal samples were put in filter bags (ANKOM Corp. #F57) and analyzed for ADF according to Van Soest et al. (1991). The ADF residue was then burned in a muffle furnace at 500^{o}C for 8 h. The ADIA concentrations were calculated as the residual ash after ashing divided by the initial sample weight.

2.2. Marker recovery calculation, digestibility and fecal output estimation

The concentration of marker in consumed forage was calculated using the formula [1] in Chapter 3. The recovery rates of ADL, APL and ADIA, which are the ratios of the quantity of marker excreted in the feces per unit of marker consumed, were calculated using formula [2] of Chapter 3. The estimated DMD by internal marker was given by one minus the ratio of marker concentration in feed divided by marker concentration in feces according to the formula [4] of

Chapter 3. Estimates of FO were expressed as the ratio of the unit of marker consumed per unit of marker excreted multiplied by the actual DMI according to the formula [5] of Chapter 3.

2.3. Statistical Analysis

Data for chemical composition (Table 3.1), DMI, FO, and apparent DMD (Table 3.3) of the diet treatments were analyzed in Chapter 3. Data for marker recovery (ADL, APL, and ADIA) and estimates of FO and DMD were analyzed using PROC GLM of SAS (SAS Int. Inc., Cary, NC, USA, 2009), where diet, marker, and diet × marker interaction were included in the model. Results are reported as the least-squares means (LSMEANS). When significant differences were detected ($P < 0.05$), means were separated using the LSMEANS, PDIFF option in SAS (SAS Institute). Also, the correlation (PROC CORR) function was used to determine the best predictors of FO and DMD. When the diet × marker interaction was not significant, the comparisons of the LSMEANS among themselves and with the means of observed values of FO and DMD were made by the ESTIMATE statement in PROC GLM. This calculates the difference of each pair of means and tests if it is different from zero. A t-test was run to determined if the marker recovery rates were different from 1.

3. Results

Actual in vivo data for DMI, DMD, and FO were reported and discussed in Chapter 3 (Table 3.3) and were used to calculate marker recoveries and accuracy.

3.1. Internal marker concentration

Hay and fecal marker concentrations are presented in Table 4.1. Concentrations of ADL differed by diet ($P < 0.001$) and were greater for L and H diets and lower for ML and MH diets. The ADL concentration in feces tended to be impacted by diet ($P = 0.10$). The APL in hay and feces was not affected ($P \geq 0.121$) by diet. Diet tended ($P < 0.09$) to affect the ADIA concentration in hay consumed and affected ($P < 0.001$) the ADIA content in feces. Fecal ADIA concentrations did not appear to be related to forage CP concentrations, as the greatest ($P < 0.05$) concentrations of ADIA were from cows offered the ML and MH treatments.

3.2. Recovery of internal markers

Results for marker recovery are presented in Tables 4.2 and 4.3. In general, diet treatments did not alter ($P = 0.51$) the recovery of ADL, APL, and ADIA, but recovery differences were observed among markers ($P < 0.004$). In addition, the diet × marker interaction tended to affect marker recovery ($P < 0.062$). Recovery of ADL differed ($P < 0.05$) from that of ADIA and APL, and that of ADIA and APL did not differ ($P \geq 0.05$) from each other. The overall average ADL recovery differed from 1 (Table 4.3; $P < 0.001$) while that of ADIA was not different from 1 ($P = 0.204$) and that of APL tended to differ from 1 ($P = 0.081$).

3.3. Estimates of FO and apparent DMD

Estimates of FO differed by marker ($P = 0.011$, Table 4.4) and diet ($P < 0.01$), but the diet × marker interaction did not affect estimates of FO ($P = 0.497$). Fecal output estimates by APL and ADIA were not different from each other ($P = 0.74$, Table 4.5) and not different ($P \geq 0.39$) from that of TC, while that of ADL differed ($P = 0.002$) from that of TC and underestimated FO. Estimates of DMD were affected by marker ($P = 0.002$, Table 4.6) and diet

(P = 0.002), but not the diet × marker interaction (P = 0.224). The DMD estimates of ADIA and APL were not different (P = 0.54, Table 4.7) from each other and not different ($P \geq 0.28$) from that of TC values, while ADL overestimated ($P < 0.001$) DMD. In general, estimates of ADL were different from all other estimates and overestimated the apparent DMD by over 10% while underestimating FO by over 13%. Furthermore, the correlation coefficients between actual DMD values vs. estimated indicated that ADL and APL had low and similar coefficients of correlation (r = 0.45 and 0.43) while ADIA had a high correlation coefficient (r = 0.72). For FO, the correlation coefficients between actual and estimated values were 0.76, 0.85 and 0.88, respectively for ADL, APL, and ADIA.

4. Discussion

4.1. Diet effect on marker concentration

The ADL concentrations obtained from the forages used in this study varied between 32 to 43 g/kg DM. Similar to our results, Bass et al. (2012) reported an average ADL concentration of 38 g/kg DM in bermudagrass hay baled at normal moisture concentration after 42 d storage in a study conducted in Northwest Arkansas.

The average APL concentration in feeds and feces excreted in this study was 24.4 and 56.0 g/kg DM; respectively. A fecal APL concentration of 49 ± 2.4 g/kg was reported by Momont et al. (1994) for cows fed prairie hay. Furthermore, the APL concentrations in that study did not show any significant variability (P = 0.94) over sampling time, and daily fecal excretion of APL was not affected by DMI (P = 0.52). Slightly lower APL concentrations were obtained by Sunvold and Cochran (1991). The APL concentrations (g/kg) in forage and feces were 18 and 46 for bromegrass, and 19 and 45 for prairie hay. Greater values for APL concentrations were

obtained by Cochran et al. (1988). The APL concentrations determined by incubation of forage samples in AHP solution before acid-detergent extraction were 39 and 55 g/kg in immature and dormant grass, respectively. The high APL concentration in that study may have resulted from the analytical method used (incubation of samples in filter tubes and use of Whatman paper for filtration). As expected, APL concentrations were less than ADL concentrations due to the removal of core and non-core lignin fractions (Amjed et al., 1992) when forage samples were incubated in AHP before ADF extraction. It is estimated that up to 50% of the lignin in roughage may be removed with AHP treatment (Lewis et al., 1988; Bhargava et al., 1989).

Average concentrations of ADIA in feed and feces for this study were 26 and 58 g/kg DM. Fecal ADIA concentration of 59 g/kg DM was reported from lambs fed alfalfa (Undersander et al.,1987), or prairie hay (57.5 g/kg DM; Stafford et al. 1996), and steers fed tall grass prairie hay (52.5 g/kg DM; Olson et al., 2008), while lower fecal ADIA concentrations (46 g/kg DM) were found from steers fed alfalfa (Stafford et al., 1996) and from dairy cattle diets (Porter, 1987).

4.2. Marker effect on recovery

The closest fecal recovery rates to 1 were obtained with ADIA (1.029) and APL (1.061) while that of ADL was greater than 1. Furthermore, ADL and APL recoveries were more variable than the ADIA. Steers fed alfalfa cubes had incomplete ADL recovery rate (0.519), while steers consuming tall wheatgrass plus soybean meal (SBM) had a positive recovery (1.164, Cochran et al., 1986). Fecal recoveries of ADL were 0.920, 1.065 and 1.145 in steers fed alfalfa, bromegrass and prairie hay, respectively (Sunvold and Cochran, 1991). Incomplete ADL fecal recoveries (0.776 and 0.938) were obtained from lambs fed prairie hay and lucerne hay,

respectively (Krysl et al., 1988). However, Ferret et al. (1999) achieved ADL fecal recovery close to 1 in ryegrass diets. The positive and incomplete recovery of ADL is attributable to the formation of an artifact during the transit of ingested forage in the GIT of a ruminant (Muntifering, 1982; Fahey and Jung, 1983).The biodegradation of lignin during its transit in the GIT may also occur (Jasra and Johnson, 2000), and is due to the formation of a soluble lignin-carbohydrate complex in the rumen environment (Fahey et al. 1979; Merchen, 1993). Furthermore, nearly 50% of the lignin in forage may conjugate with carbohydrates and form a complex that will be measured in feces as lignin (Neilson and Richards, 1978). On the other hand, Elam and Davis (1961) reported that up to 12.9% of lignin in consumed feed was digested. Incomplete fecal recovery of lignin as an internal marker may be associated with its low concentration in immature forages and the variability in lignin content in different plant species. According to Van Soest (1987), ADL should only be used as an internal marker when its concentration is at least 60 g/kg of the DM. While some authors consider lignin an inadequate internal marker, other argues that lignin can be used with certain types of diets.

The overall APL fecal recovery was 1.06. Closer fecal APL recovery (0.98 ± 0.025) with a range from 0.824 to 1.180 was obtained in lambs fed prairie hay (Momont et al., 1994); supplementation with SBM, urea and sulfur, or urea and methionine did not affect APL recovery (P = 0.47). Also, Cochran et al. (1988) found a mean fecal APL recovery of 0.976 using steers fed dormant bluestem grass(*Andropogon gerardii* Vitman), and noted that the addition of AHP improved the recovery of lignin from plants and feces, and the AHP incubation in ADL procedure should be incorporated before the acid-detergent extraction. In a subsequent study (Sunvold and Cochran, 1991), average fecal APL recovery rates were 0.892, 1.064, and 0.925 from steers fed alfalfa, bromegrass, and prairie hay, respectively. Excellent APL fecal recovery

of 1.00 was achieved in sheep fed ad libitum fescue hay, although actual and predicted digestibility values differed (Judkins et al., 1990). However, incomplete APL fecal recovery (0.788) was observed on cows fed finger millet (*Eleusine coracona*) straw with supplements (Renuka et al., 2003).

In this study, the ADIA fecal recovery was 1.03. Similar fecal recovery (0.993) was reported by Bodine et al. (2002) on steers fed alfalfa, bermudagrass and prairie hay without supplements. The fecal recovery rate of ADIA was also close to 1(1.052 ± 0.0248) from lambs fed alfalfa (Undersander et al., 1987), and from steers fed forage-based diets with different levels of supplements (Stafford et al., 1996). Supplementation did not have an effect on ADIA recovery. However, ADIA recovery of 0.937 was reported in cattle consuming supplemented finger millet straw (Renuka et al., 2003). Although over-recovery may occur due to soil contamination, ADIA had the potential to perform as an internal marker due to rapid analysis, low cost, and low analytical error compared to ADL or APL (Van Soest, 1994).

4.3. Marker effect on prediction of DMD

The results of the study showed that ADIA and APL are potential internal markers that can predict FO and DMD of bermudagrass hay with a wide range of CP concentrations, while ADL underestimated FO and overestimated the DMD. Generally, the ability of an internal marker to estimate FO and DMD reflects its fecal recovery. Underestimation and overestimation of DMD by ADL was reported on steers fed various diets (Cochran et al., 1986). In another study, ADL digestion coefficients differed from those of TC (Cochran et al., 1988). According to Miraglia et al. (1999), apparent DMD cannot be estimated by ADL because of incomplete recovery and subsequent underestimation of digestibility. Underestimation of DMD was also

reported on lambs fed prairie and lucerne hay due to incomplete lignin recovery (Krysl et al., 1988; Merchen, 1993).

In this study, APL produced estimates of FO and DMD similar to those of TC. Similar results were reported on steers fed bluestem-range grass when AHP incubation was performed before acid-detergent extraction (Cochran et al., 1988). Estimates of digestibility by APL were also similar to TC values when sheep were fed either immature or dormant grasses (Momont et al., 1994). However, in a later digestion trial using lambs, APL exhibited variable digestibility estimates even though lignin recovery was estimated to be near 1. In addition, Sunvold and Cochran (1991) observed that APL ratio performed similarly to ADL ratio in estimating forage OMD. Both predicted the actual OMD of bromegrass but failed to predict the actual OMD of alfalfa diets. Estimates of DMD were underestimated in cattle consuming finger millet with supplement due to the incomplete fecal recovery observed (Renuka et al., 2003).

Among the markers evaluated, ADIA was the best in predicting FO and DMD. The ADIA was the most accurate internal marker in predicting in vivo DMD of alfalfa fed to lambs (Undersander et al., 1987). The mean estimates of DMD and OMD by ADIA were 604 and 650 g/kg DM and were similar to 595 and 643 g/kg DM from in vivo DMD, which resulted in the highest correlation and least mean differences between predicted DMD and actual DMD values (Undersander et al., 1987). Values of digestibility derived from ADIA were similar to TC values on 3 different diets fed to dairy cattle (Porter, 1987). However, ADIA underestimated DMD by 26.9% with steers fed prairie hay supplemented with corn and soybean meal. No differences were found with steers fed alfalfa, bermudagrass and prairies without supplements (Bodine et al., 2002).

5. Implications

Estimates for digestibility and FO using ADL were different from all other estimates and overestimated the apparent digestibility (597 vs. 539 g/kg) while underestimating FO (3655 vs. 4207 g/d). However, ADIA and APL are potential internal markers for predicting FO and DMD by cattle fed bermudagrass hay of varying quality while ADL should be used with caution. Estimates of APL presented more variability and correlated less with TC values than those of ADIA.

Acknowledgements

This study was supported by University of Arkansas, Division of Agriculture.

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Table 4.1
Concentration (g/kg dry matter, DM) of internal markers in consumed bermudagrass hays of varying crude protein concentrations and associated feces.

	Diet treatments[a]					
Item[b]	L	ML	MH	H	SEM[c]	*P*-value
ADL_c	42.8[d]	32.4[e]	32.2[e]	37.6[d]	1.60	0.001
ADL_f	93.3	84.8	86.6	94.7	3.02	0.100
APL_c	26.4	24.4	22.4	24.4	1.12	0.121
APL_f	59.5	52.8	52.4	59.4	3.20	0.257
$ADIA_c$	25.4	31.9	26.9	20.0	1.28	0.09
$ADIA_f$	51.4[e]	65.1[d]	60.3[d]	53.5[e]	1.73	0.001

[a]L, low crude protein hay (CP = 79 g/kg DM); ML, medium low crude protein hay (CP = 111 g/kg DM); MH, medium high crude protein hay (CP = 131 g/kg DM); and H, high crude protein hay (CP = 164 g/kg DM).

[b]ADL_c, acid-detergent lignin in the forage; ADL_f, acid-detergent lignin in feces; APL_c, alkaline-peroxide lignin in the forage; APL_f, alkaline-peroxide lignin in feces; $ADIA_c$, acid-detergent insoluble ash in the forage; $ADIA_f$, acid-detergent insoluble ash in feces.

[c]SEM, standard error of the mean.

[def]Means with different superscripts within a row differ at $P < 0.05$.

Table 4.2

Recovery (g/kg) of internal markers relative to the value 1 (for 100% recovery) in feces for each bermudagrass hay treatment.

	Treatments[a]						*P*-value[d]		
Item[b]	L	ML	MH	H	Average	SEM[c]	D	M	D × M
ADL	1.09	1.20	1.29	1.07	1.16[f]	0.028	0.51	0.004	0.062
APL	1.09	1.02	1.06	1.07	1.06[e]	0.028			
ADIA	1.00	0.96	1.02	1.13	1.03[e]	0.028			
Average[1]	1.06	1.06	1.12	1.09					

[a]L, low crude protein hay (CP = 79 g/kg DM); ML, medium low crude protein hay (CP = 111 g/kg DM); MH, medium high crude protein hay (CP = 131 g/kg DM); and H, high crude protein hay (CP = 164 g/kg DM).

[b]ADL, acid-detergent lignin; APL, alkaline-peroxide lignin; ADIA, acid-detergent insoluble ash.

[c]SEM, standard error of the mean.

[d]D, diet effect, M, marker effect, and D × M, diet by marker interaction.

[ef]Means with different superscripts in the same column differ at $P < 0.05$.

[1]Average per treatment across different markers.

Table 4.3

Internal marker recoveries and their corresponding confidence intervals. Fecal recovery of a particular marker is complete if its confidence interval (95%) contains the theoretical value (1) of TC.

		Confidence interval			
Parameter[a]	Fecal recovery (g/kg)	Low limit	Upper limit	SEM[b]	*P*-value[c]
ADL	1.163	1.090	1.235	0.172	<0.001
APL	1.061	0.992	1.131	0.165	0.081
ADIA	1.029	0.983	1.075	0.023	0.204

[a]ADL, acid-detergent lignin; APL, alkaline-peroxide lignin; ADIA, acid-detergent insoluble ash.

[b]SEM, standard error of the mean.

[c]Probability that the fecal recovery mean of a particular marker is not different from 1.

Table 4.4

Estimates of fecal output (FO, g/d) using different internal markers compared with values derived from total collection (TC).

	Treatments[a]						*P*-value[d]		
Item[b]	L	ML	MH	H	Average	SEM[c]	D	M	D × M
TC	3788	4090	4734	4218	4207[e]	122.2	<0.01	0.011	0.497
ADL	3369	3472	3739	4040	3655[f]				
APL	3510	4088	4593	4046	4059[e]				
ADIA	3712	4289	4656	3806	4116[e]				
Average	3595[i]	3985[h]	4430[g]	4028[h]					

[a]L, low crude protein hay (CP = 79 g/kg DM); ML, medium low crude protein hay (CP = 111 g/kg DM); MH, medium high crude protein hay (CP = 131 g/kg DM); and H, high crude protein hay (CP = 164 g/kg DM).

[b]ADL, acid-detergent lignin; APL, alkaline peroxide lignin; ADIA, acid-detergent insoluble ash.

[c]SEM, standard error of the mean.

[d]D, diet effect; M, marker effect; D × M, diet by marker interaction.

[ef]Means with different superscripts in the same column differ at $P < 0.05$.

[ghi]Means with different superscripts in the same row differ at $P < 0.05$.

Table 4.5

Comparison of different internal markers for predicting fecal output (FO). Estimates are the difference among marker values and between marker values and those values from total fecal collection (TC).

Parameter[a]	Estimate (g/d)	SED[b]	*P*-value
ADIA vs. TC	-91	173.0	0.60
ADL vs. TC	-552	173.0	0.002
APL vs. TC	-148	173.0	0.39
ADIA vs. ADL	461	173.0	0.009
ADIA vs. APL	57	173.0	0.74
ADL vs. APL	-404	173.0	0.02

[a]Comparison of estimates of fecal output (g/d) by different markers (ADL, acid-detergent. lignin, APL, alkaline-peroxide lignin, ADIA, acid-detergent insoluble ash with total collection (TC) or among themselves.

[b]SED, standard error of the difference of the means.

Table 4.6

Estimates of dry matter digestibility (DMD, g/kg DM) from different internal markers compared with total collection (TC).

	Treatments[a]						*P*-value[d]		
Item[b]	L	ML	MH	H	Average	SEM[c]	D	M	D × M
TC	506	543	534	572	539[f]	11.1	0.002	0.002	0.224
ADL	547	613	635	592	597[e]	11.1			
APL	543	539	552	590	556[f]	11.1			
ADIA	507	520	543	617	547[f]	11.1			
Average	526[i]	554[hi]	566[gh]	593[g]					

[a]L, low crude protein hay (CP = 79 g/kg DM); ML, medium low crude protein hay (CP = 111 g/kg DM); MH, medium high crude protein hay (CP = 131 g/kg DM); and H, high crude protein hay (CP = 164 g/kg DM).

[b]ADL, acid-detergent lignin; APL, alkaline-peroxide lignin; ADIA, acid-detergent insoluble ash.

[c]SEM, standard error of the mean.

[d]D, diet effect, M, marker effect, and D × M, diet by marker interaction.

[ef]Means with different superscripts in the same column differ at $P < 0.05$.

[ghi]Means with different superscripts in the same row differ at $P < 0.05$.

Table 4.7

Comparison of different internal markers for predicting apparent dry matter digestibility (DMD). Estimates are the difference each pair of marker values, and between marker values and those values from total fecal collection (TC)

Parameter[a]	Estimate (g/kg)	SED[b]	*P*-value
ADL vs. TC	57.8	15.75	<0.001
APL vs. TC	17.0	15.75	0.28
ADIA vs. TC	8.0	15.75	0.64
ADL vs. APL	40.8	15.75	0.012
ADL vs. ADIA	-50.0	15.75	0.002
ADIA vs. APL	-9.1	15.75	0.54

[a]Comparison of estimates of digestibility by different internal markers (ADL, acid-detergent lignin, APL, alkaline-peroxide lignin, ADIA, acid-detergent insoluble ash with total fecal collection (TC) or among themselves.

[b]SED, standard error of the difference of the means.

Chapter V

Diurnal variation in fecal concentrations of indigestible-acid detergent fiber, acid-detergent insoluble ash, and alkaline-peroxide lignin from cattle fed bermudagrass hays of varying nutrient content

Abstract

The effect of time of fecal sampling on the accuracy of adjusted indigestible acid-detergent fiber ($IADF_a$), acid-detergent insoluble ash (ADIA), and alkaline-peroxide lignin (APL) for the prediction of fecal output (FO) in cattle was evaluated. Eight ruminally cannulated cows (594 ± 100.3 kg) were allocated randomly to 4 bermudagrass [*Cynodon dactylon* [L.] Pers.] hay diets having a wide range of crude protein concentrations (79-164 g/kg DM) with 2 replicates per diet for 3 periods (n = 24). Cows were offered their respective hay individually at a total of 20 g/kg of BW in equal feedings at 0800 and 1600 h for a 10-d adaptation period followed by a 5-d total fecal collection (TC) period in 3.0 × 4.3-m pens fitted with rubber mats. Fecal grab samples were taken each day during the fecal collection period at 0600, 1200, 1800, and 2400 h either directly from the rectum or from fresh feces, and were composited by cow and time across the 5 d of total fecal collection. Duplicate samples of each hay, ort, and fecal sample were incubated for 144 h in the rumen of 2 cows for each period (n = 6 cows), followed by a sequential analysis of neutral-detergent fiber and acid-detergent fiber (ADF) to obtain $IADF_a$. Additionally, forage, ort, and fecal samples were analyzed for concentrations of APL and ADIA. Time of sampling affected ($P < 0.05$) the fecal concentrations of, and estimates of DMD from IADF, but not those of ADIA and APL ($P \geq 0.16$), and did not affect ($P \geq 0.14$) estimates of FO using either marker. Estimates of FO and DMD by in vivo TC or markers from different

sampling times and all different combinations of sampling time were not different ($P \geq 0.29$) across internal markers. Therefore, there is little variation in concentrations of ADIA and APL in daily fecal excretion giving researchers greater flexibility in their fecal grab sampling schedules to be used in the prediction of FO and DMD.

Key words: Digestibility, fecal sampling time, internal markers, bermudagrass, cattle

1. Introduction

Due to the expense and difficulty involved in testing a large number of forages using in vivo techniques for measuring DMI, FO, and DMD in ruminant animals, indirect methods using external and internal markers can be applied (Penning and Johnson a & b, 1983; Cochran et al., 1986; Cochran et al., 1987; Pond et al., 1987; Owens and Hanson, 1992). Internal markers, which are inherent constituents of feed that are neither digested nor absorbed by the animal (Cochran et al., 1987), are the best options for estimating DMI, FO, and DMD. These markers are expected to have a flow through the gastrointestinal tract similar to that of the digesta they mark (Owens and Hanson, 1992; Sampaio et al., 2011a).

The experiment described in Chapters 3 and 4 determined that APL, ADIA, and adjusted IADF were the most suitable internal markers to predict DMD and FO by cattle fed bermudagrass hays with a range of CP concentrations. Several studies have reported diurnal variation in fecal concentration of external markers (Titgemeyer, 1997), but few studies (Momont et al., 1994; Sampaio et al., 2011a) have evaluated diurnal fecal concentration patterns of internal markers. Bias in estimating fecal excretion can have two sources; firstly, failure of markers to be totally recoverable in feces (long term bias), and secondly, failure or inconsistencies in obtaining a representative sample of the total feces excreted (Sampaio et al., 2011a&b). Diurnal fecal

variation can be overcome by collecting enough samples throughout the day to provide a composite sample in which the marker concentration is close to the concentration of the entire day (Titgemeyer, 1997). To alleviate the tedious work of total fecal collection for estimating apparent DMD of cattle feeds, information is needed on the variation of internal markers during a 24-h period to determine whether or not sampling time affects marker recovery. Therefore, the objective of this study was to evaluate the effect of time of fecal sampling on the accuracy of $IADF_a$, ADIA, and APL in predicting FO and DMD in cattle fed bermudagrass hays with a range of CP concentrations.

2. Materials and Methods

The site of the study, the experimental layout, and diet treatments were described in Chapter 3. Values of DMI, DMD, and FO based on TC were also described in Chapter 3. All other procedures used in this part of the study were approved by the Institutional Animal Care and Use Committee of the University of Arkansas (IACUC approved protocol #10016).

2.1. Fecal grab sample collection and preparation for in situ analysis

Fecal grab samples (approximately 300 g for each sample) were taken 4 times daily (0600, 1200, 1800, and 2400 h) directly from the rectum of each cow or from freshly excreted feces and were oven-dried at 50^{o}C. Dried fecal grab samples were composited by cow and time of sampling within period, then ground to pass a 2-mm screen of a Wiley mill (Thomas Scientific, Swedesboro, NJ). Dacron bags (10 × 20 cm; 53 ± 10-µm pore size; ANKOM Technology Corp., Fairport, NY, USA) were filled with 5 g of ground feces and closed with

rubber bands. Duplicate bags (n = 24 × 4 × 2 = 192) were prepared for each fecal sample representing each cow and sampling time within each period.

2.2. In situ experiment for analyzing IADF

A total of 6 cows (585 ± 37.8 kg) were used for in situ marker determination, with samples from each period in the digestion study assigned to 2 of the 6 cows. Duplicate fecal grab samples along with hay and ort samples were incubated for 144 h (6 d), and the remaining DM over initial sample weight was RUDM. The INDF and IADF were analyzed by extracting the residue in NDF and ADF solution (Cochran et al., 1986). A complete description of the in situ diets and procedures can be found in Chapter 3.

2.3. Chemical analysis of $IADF_a$, APL, and ADIA in fecal grab samples

Residual DM from the in situ incubation was analyzed sequentially for NDF and ADF by the method of Van Soest et al. (1991) and the batch procedure of ANKOM Technology Corp. (Fairport, NY, USA) to determine indigestible ADF. Adjusted IADF ($IADF_a$) was obtained by dividing the IADF concentration by the corresponding correction factor (CF) obtained as described in Chapter 3.

Hay, ort, and fecal grab samples were ground to pass 1-mm screen Willey mill and analyzed for ADIA (Van Soest et al., 1991) using the ANKOM procedure (ANKOM Technology Corp., Fairport, NY, USA), for which 0.5 ± 0.01 g of sample was analyzed for ADF, and the remaining ADF residue was ashed in a muffle furnace (Thermolyne Sybron, Thermolyne Corporation, Dubuque, IA, USA) at 500^{o}C for 8 h. Alkaline-peroxide lignin analysis was performed by the modified procedure of Cochran et al. (1988) and Sunvold and Cochran (1991),

for which 0.5 ± 0.01 g of sample were placed in filter bags (ANKOM Corp., #F57) instead of using filter tubes, then incubated in alkaline-hydroxide peroxide (AHP, pH = 11.5) solution for 24 h, and rinsed to neutral pH with hot distilled water after incubation instead of filtration using Whatman filter paper. The AHP residue was then sequentially analyzed for ADF and ADL to obtain APL concentrations in fecal grab samples.

2.4. Calculation of DMD and FO using $IADF_a$, ADIA, and APL from fecal grab samples

The concentrations of $IADF_a$ in consumed forage were reported in Chapter 3 (Table 3.4), and those of APL and ADIA were reported in Chapter 4 (Table 4.1). Apparent in vivo FO was determined directly, and DMD was calculated using formula [3] of Chapter 3. The estimated DMD using the fecal grab samples taken at different times was calculated by the following formula:

$$DMD = 100 \times (1 - M_{fd} / M_{ftime}) \quad [1]$$

where M_{fd} is the marker concentration in consumed feed; M_{ftime} is the marker concentration in each fecal grab sample at a particular sampling time.

Estimates of FO by fecal grab samples taken at different times were calculated according to the following expression:

$$FO = DMI \times M_{fd} / M_{ftime} \quad [2]$$

As we had 4 sampling times, the resulting single sample times and all possible 2-, 3-, and 4-way combinations of the 4 sampling times resulted in 15 different combinations of sampling time means to compare to in vivo total collection data (TC). These values were compared to determine diurnal variation in marker concentration as well as to determine how close the concentrations of markers in the grab samples were to those obtained by TC, and to determine

which time or combination of times of sampling could provide the closest prediction of FO and DMD to those from TC.

2.5. Statistical analysis

Data for marker concentrations in grab samples, and FO and DMD estimates derived from the marker concentration at different sampling times and their different combinations (15) were analyzed as a replicated 4 × 4 Latin-Square design with one period missing using PROC GLM of SAS (SAS Inst. Inc., Cary, NC, USA, 2009). Effects of period, cow, diet, marker, sampling time, and the 2- and 3-way interactions among diet, marker, and sampling time were included in the model and significance was noted at $P < 0.05$. In cases where no marker × time or diet × marker × time interaction was detected, each individual marker was analyzed separately to determine if there was any potential diet × time interaction within each individual marker. The model included diet, time and a diet × time interaction term.

3. Results

The analysis of the entire data set (period = 3; diet = 4, cow within diet = 2, time with all sampling time combinations = 15, marker = 3; n = 1080) where diet, marker, and time were included in the model revealed that diet, marker, and the interaction diet × marker affected ($P < 0.001$) the estimates of FO and DMD, but time of sampling had no effect ($P \geq 0.96$) on the prediction of FO and DMD. In addition, the interactions of marker × time, diet × time, and diet × marker × time of sampling were not significant (data not shown; $P \geq 0.99$). Therefore, it was concluded that the three markers behave similarly regarding their prediction of FO and DMD.

Thus, the following results are related to the analysis of data for each individual marker for which diet, time, and diet × time interaction were included in the model.

3.1. Marker concentration in feces by sampling time

The chemical composition of the diet treatments and values of DMI, DMD, and FO derived from TC have been presented and discussed in Chapter 3. Concentrations of internal markers in feces and effects of time of grab-sampling are displayed in Table 5.1. There was no diet × time of sampling interaction ($P \geq 0.60$) for all 3 markers. Fecal concentrations of $IADF_a$ were affected by sampling time ($P < 0.01$) and diet ($P = 0.01$). Concentration of $IADF_a$ in fecal grab samples taken at 0600, 1200, and 1800h were greater ($P < 0.05$) than those derived from TC. The concentrations of ADIA and APL were not affected by sampling time ($P = 0.45$ and $P = 0.22$, respectively), but diet affected ($P < 0.01$) fecal ADIA and APL concentrations.

3.2. Fecal output estimation and digestibility by sampling time

Estimates of FO and DMD by different fecal grab sampling times (1, 2, 3, and 4) are also presented in Table 5.1. Diet ($P < 0.01$), time ($P < 0.03$), and the diet × time ($P < 0.02$) interaction affected predictions of DMD using $IADF_a$. Time of sampling ($P \geq 0.16$) and diet × time ($P \geq 0.86$) had no effect on the prediction of DMD by ADIA and APL.

Estimates of FO derived using the mean marker concentrations across the 4 fecal grab samplings per day for the 3 internal markers ($IADF_{a1234}$, $ADIA_{1234}$, APL_{1234}) differed among markers ($P = 0.03$, Table 5.2) but estimates of FO from all 3 markers were not different from the FO value obtained by TC procedure. Also, estimates of DMD determined from a combination of the 4 fecal grab samplings per day differed ($P = 0.002$) by internal markers but only the DMD

estimated by APL differed ($P < 0.05$) from that of TC. Diet affected ($P < 0.01$) DMD and FO ($P < 0.001$) estimates, and the diet × marker interaction affected DMD ($P = 0.003$) but not FO ($P = 0.16$) estimates. Alkaline peroxide lignin (APL_{1234}) overestimated (575 vs. 509), and $IADF_{a1234}$ underestimated (399 vs. 509) the DMD of low quality bermudagrass (Table 5.3). The DMD estimates derived from the mean of the 4 sampling times from the different markers were not different ($P > 0.05$) from those from TC within the ML, MH, and H bermudagrass hays.

Estimates of FO and DMD (Table 5.4) by $IADF_a$, ADIA, and APL using samples from different fecal sampling times (1, 2, 3, 4) and their different 2-, 3-, and 4-way combinations were not different from in vivo values ($P \geq 0.60$ and $P \geq 0.29$; respectively). Diet had an effect ($P < 0.01$) on the prediction of FO and DMD for all internal markers while time ($P > 0.29$) and diet × time did not impact ($P \geq 0.82$) FO and DMD prediction.

4. Discussion

4.1. Effects of diet and sampling time on marker concentration

There was little variation in concentration of ADIA and APL within a 24 h sampling period, whereas, the concentration of $IADF_a$ presented some variability. Other researchers have reported concerning diurnal variation of internal markers. Fecal lignin concentrations were relatively uniform within day and not impacted by a sampling schedule of 3-h intervals for 48 h (Elam and Davis, 1961), and daily variation in lignin (72 % sulfuric acid) content of feces from sheep on a diet of timothy [*Phleum pratense* L.] hay was also very small (Ellis et al., 1946). Furthermore, no interaction between diet and time was detected in their study.

No differences in fecal concentrations of IADF and INDF were observed among samples taken 4 times daily (0130, 0730, 1330, 1930 h) when compared with the IADF and INDF

concentrations provided by a representative sample from TC (Sampaio et al., 2011b). Also, uniformity of fecal excretion patterns of indigestible dry matter, INDF, and IADF in a digestion trial with cattle fed different diets such as elephant grass (*Pennisetum purpureum* Schumach.) silage, corn (*Zea mays* L.) silage, and signal grass (*Brachiaria decumbens* Stapf) hay, led to recommendations that 4 fecal samplings that are evenly distributed during the day can help to obtain FO estimations free of bias (Sampaio et al., 2011a). Also, fecal IADF content from grazing sheep varied little across 5 d within a period (Nelson et al., 1990), further supporting that variation in fecal concentrations of IADF may have little fluctuation.

In this study, fecal APL concentrations showed very small diurnal fluctuations across sampling times. Sampling time had no effect on APL concentrations in feces, resulting in no diurnal variation in APL excretion in previous work (Momont et al., 1994). A lack of diurnal or day-to-day variation was also reported on acid-insoluble ash (Van Keulen and Young, 1977; Thonney et al., 1985) and ADIA (Porter, 1987) concentrations in feces.

Comparing external (chromium and titanium dioxide) and internal markers (RUDM, INDF, and IADF), Sampaio et al. (2011a) noted that external markers presented a higher oscillation range (between 23.0 and 21.2%) than internal markers (6.6, 5.8, and 8.5%), meaning that fecal concentrations of internal markers from samples gathered throughout the day are closer to the average fecal concentrations than those of external markers. These same authors reported an oscillation rate of 8.5% for IADF; while in this study, the oscillation rate was 6.1% for $IADF_a$, 5.0% for ADIA, and 5.7% for APL. The oscillation rate, which is calculated as the difference between the maximum fecal concentration of a marker (C_{max}) and the minimum (C_{min}) divided by the overall mean fecal marker concentration ($A_{o,}$ Sampaio et al., 2011a), provides information on the variability of the marker around the mean fecal concentration. Ideal markers should flow

similarly to and be physically associated with the digesta they mark (Owens and Hanson, 1992). Internal markers, which are natural components of feeds, are expected to flow similarly with the digesta through the gastrointestinal tract of the animal (Sampaio et al., 2011a&b); which may explain why there was little variation of fecal content of the internal markers studied. Furthermore, some variations observed in marker concentrations in feces may have been caused by differences in diet and the feeding frequencies (Vanzant et al., 1998), and also by the natural event of transit and degradation of consumed feed, although continuous in the rumen, there is a time when ruminant animal may be processing greater amounts of feed (Sampaio et al, 2011a&b), and this may explain why there was some variability in fecal concentrations.

4.2. Estimates of FO and DMD

In this study, all sampling times (4 times with a 6-h interval) and their different combinations produced similar results that were not different from TC, thus, fecal sampling time had little effect on the prediction of FO and DMD. No differences between actual and predicted values of DMD, FO, DMI using fecal grab samples and representative samples from total fecal collection were reported in previous work (Momont et al., 1994), which supports the findings from this study. Porter (1987) reported that 2 fecal grab samplings per day for 14 d can provide acceptable estimates of DMD on individual cows with 95% confidence.

5. Implications

The results of this study revealed that time of sampling affected the concentration of $IADF_a$ but did not alter the ADIA and APL concentrations in fecal grab samples across sampling times or from that in TC. Consequently, estimates of DMD by a representative sample from TC

and that from all grab sampling times and their different combinations were not different from actual DMD regardless of which internal marker was used. Similarly, FO estimated by in vivo, samples from TC, or samples from different sampling times, and all different combinations of sampling times were not different across internal markers. Therefore, there was little variation in concentrations of ADIA and APL in daily fecal excretion and multiple daily fecal samplings may not be necessary to obtain a representative sample of cow fecal excretion.

Acknowledgements

This research was supported by University of Arkansas, Division of Agriculture.

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Table 5.1
Mean fecal concentrations (g/kg dry matter, DM), and estimates of fecal output (FO, g/d), and dry matter digestibility (DMD, g/kg DM) using adjusted indigestible acid-detergent fiber ($IADF_a$), acid-insoluble ash (ADIA), and alkaline-peroxide lignin (APL) from feces sampled at different times compared with actual fecal concentrations, FO, and DMD values from total collection (TC).

	Time of sampling[a]						*P*-value[e]		
Marker[b]	1	2	3	4	TC[c]	SEM[d]	D	T	D × T
Fecal concentrations (g/kg DM)									
$IADF_a$	363[f]	372[f]	361[fg]	350[gh]	345[h]	4.1	0.01	<0.01	0.96
ADIA	59	58	61	58	58	1.5	<0.01	0.45	0.60
APL	55	59	58	58	56	1.2	<0.01	0.22	0.92
FO (g/d)									
$IADF_a$	4366	4242	4363	4526	4207	94.0	<0.01	0.14	0.35
ADIA	4036	4069	3928	4071	4207	125.4	<0.01	0.64	0.78
APL	4105	3903	3907	3922	4207	135.8	<0.01	0.38	0.99
DMD (g/kg DM)									
$IADF_a$	516[fg]	530[f]	518[fg]	500[g]	539[f]	9.1	<0.01	0.03	0.02
ADIA	557	554	573	551	539	9.6	<0.01	0.16	0.86
APL	550	576	571	574	539	13.5	0.30	0.20	0.98

[a]Different sampling times (1 = 0600, 2 = 1200, 3 = 1800, and 4 = 2400 h).
[b]$IADF_a$, adjusted indigestible acid-detergent fiber; ADIA, acid-detergent insoluble ash; and APL, alkaline-peroxide lignin.
[c]TC, total fecal collection.
[d]SEM, standard error of the mean.
[e]D, diet; T, sampling time; D × T, diet by sampling time interaction.
[fgh]Means with different superscripts in the same row differ, $P < 0.05$.

Table 5.2
Comparison of in vivo dry matter digestibility (DMD, g/kg DM) and fecal output (FO, g/d) with estimates obtained by different internal markers using the mean of 4 fecal grab samples per day.

	Marker[a]					*P*-value[e]		
Item[b]	$IADF_{1234}$	$ADIA_{1234}$	APL_{1234}	TC[c]	SEM[d]	D	M	D × M
FO (g/d)	4370[f]	3990[g]	3930[g]	4207[fg]	111.4	<0.001	0.03	0.16
DMD (g/kg)	517[g]	561[fg]	571[f]	539[g]	10.5	<0.001	0.002	0.003

[a]$IADF_a$, adjusted indigestible acid-detergent fiber; ADIA, acid-detergent insoluble ash; APL, alkaline-peroxide lignin. Each value represents the mean from four grab samples per day (0600, 1200, 1800, and 2400).
[b]FO, fecal output; DMD, dry matter digestibility.
[c]TC, total fecal collection.
[d]SEM, standard error of the mean.
[e]D, diet effect; M, marker effect; D × M, diet by marker interaction.
[fg]Means with different superscripts in the same row differ at $P < 0.05$.

Table 5.3
Dry matter digestibility (DMD, g/kg DM) of bermudagrass hay diets varying in crude protein concentrations estimated using total collection or the mean concentration of different internal markers across 4 fecal grab samples daily.

Method[b]	Treatments[a]				SEM[c]	Effect
	L	ML	MH	H		
TC	509[e]	543[de]	535[de]	570[de]	21.4	D × M
$ADIA_{1234}$	545[de]	531[de]	563[de]	607[d]		
APL_{1234}	575[d]	557[de]	559[de]	591[d]		
$IADF_{a1234}$	399[f]	546[de]	547[de]	574[d]		

[a]L, low crude protein (CP = 79 g/kg DM); ML, medium low crude protein (CP = 111 g/kg DM); MH, medium high crude protein (CP = 131 g/kg DM); H, high quality diet (CP = 164 g/kg DM).
[b]TC, total collection, $IADF_{a1234}$, adjusted indigestible acid-detergent fiber using 4 sampling times ; $ADIA_{1234}$, acid-detergent insoluble ash using 4 sampling times; and APL_{1234}, alkaline-peroxide lignin using 4 sampling times. Each marker value represents the mean from four grab samples per day (0600, 1200, 1800, and 2400).
[c]SEM, standard error of the mean.
[def]Means with different superscripts within row and column differ at $P < 0.05$.

Table 5.4

Comparison of the actual in vivo estimates of fecal output (FO, g/d) and dry matter (DM) digestibility (DMD,g/kg DM) and their corresponding estimates determined using adjusted indigestible acid-detergent fiber ($IADF_a$), acid-detergent insoluble ash (ADIA), and alkaline-peroxide lignin (APL) using samples from different sampling times and their combinations.[a]

		Time of sampling[b]																	*P*-value[f]		
Item[c]	Marker	1	2	3	4	12	13	14	23	24	34	123	124	134	234	1234	TC[d]	SEM[e]	D	T	D × T
FO																					
	$IADF_a$	4366	4242	4363	4526	4300	4361	4439	4299	4439	4440	4318	4369	4412	4370	4367	4207	85.5	<0.01	0.60	0.99
	ADIA	4036	4069	3928	4073	4039	3945	4046	3952	4057	3962	3967	4043	3975	3979	3986	4207	100.0	<0.01	0.94	0.99
	APL	4105	3903	3907	3922	3987	3992	3995	3896	3888	3895	3954	3888	3955	3887	3934	4207	125.5	<0.01	0.94	0.99
DMD																					
	$IADF_a$	516	530	518	500	523	517	508	524	508	509	522	516	512	517	517	539	8.6	<0.01	0.29	0.82
	ADIA	557	554	573	551	557	565	555	565	555	563	564	556	562	562	561	539	9.2	<0.01	0.83	0.99
	APL	550	576	571	574	565	554	564	575	578	575	568	578	568	576	571	539	12.7	0.003	0.72	0.99

[a]There was no diet × time interaction for DMD and FO ($P > 0.82$) on all markers.
[b]1, sampled at 0600; 2, sampled at 1200; 3, sampled at 1800, 4, sampled at 2400, and their different combinations of sampling times.
[c]FO, fecal output; DM, dry matter digestibility.
[d]TC, total collection.
[e]SEM, standard error of the means.
[f]D, diet; T, time effect; D × T, diet by time interaction.

Chapter VI

Conclusion

The objective of this study was to evaluate the potential of different internal markers in predicting the nutritive value of bermudagrass hay of varying quality fed to cattle with the long-term goal to improve the accuracy of currently used bermudagrass TDN equation for Arkansas. An additional objective was to determine the fecal sampling frequencies that can provide an adequate estimate of daily fecal excretion. The results of this investigation showed varying results in marker recovery, in particular for the in situ indigestible components of feed materials. This is mainly due to differential loss of particles among hay offered, ort, and feces during the in situ procedure. The results of this study revealed that incomplete recovery of the in situ markers can be improved by appropriate adjustments of marker recovery.

Incomplete and positive recoveries were also noted for acid-detergent lignin, alkaline-peroxide lignin, and acid-detergent insoluble ash, with greater variability for acid-detergent lignin and alkaline-peroxide lignin. However, the overall recovery rates for alkaline-peroxide lignin and acid-detergent insoluble ash were the closest to 1, and derived fecal output and dry matter digestibility using those markers were similar to the actual values. Furthermore, this study found that time of fecal grab sampling within a 24-h period had little effect on fecal concentrations of alkaline-peroxide lignin and acid-detergent insoluble ash. The predicted fecal output and dry matter digestibility were not different from the actual in vivo values regardless of time of sampling or their combination, which suggests that researchers have considerable flexibility in developing a multiple daily sampling schedule to predict fecal output and dry matter digestibility in cattle consuming bermudagrass hay of varying crude protein concentrations.

With the two potential internal markers identified (ADIA and APL), their estimates of DMD or OMD can be used to update the current TDN equation without conducting total collection. This can be achieved by sampling forage offered and feces at any particular time of the day and analyze the concentration of the internal marker in feed and feces and then apply the marker ratio formula to calculate the digestibility of the bermudagrass hay diets. But, before applying the selected internal marker, an attempt was made to assess the relationship between actual DMD and estimated TDN using the current bermudagrass TDN equation for Arkansas.

The data consisted of 24 in vivo DMD observations of the four diet treatments (L, ML, MH, H) fed during the 3 periods, the chemical compositions (CP, NDF, ADF) of the diet treatments, and estimated TDN using the current bermudagrass TDN equation for Arkansas [111.8 + 0.95 CP - 0.70 NDF - 0.36 ADF]. The relationship between observed DMD and TDN estimated using the Arkansas TDN equation for bermudagrass is presented in the Figure 6.1 and in Table 6.1. There was a positive relationship between DMD from TC and calculated TDN ($Y = 0.84x + 133.7$; $R^2 = 0.337$). Also, the simple correlation coefficient between DMD and TDN estimates was positive and significant ($r = 0.58$, $P = 0.002$). The current bermudagrass TDN equation accurately predicted the energy content of L diet (Table 6.2; 501 vs. 511 g/kg DM), but overestimated the energy content of ML, MH, and H diets. Overestimation of bermudagrass energy by the current TDN energy equation was also reported by Gadberry et al. (2005), and bias increased as hay CP increased up to the MH level. The decline in bias from MH to H diet was mainly due to the relatively high NDF observed on that hay, which decreased the calculated TDN. It appears from these results that the current TDN equation for bermudagrass predicted accurately the energy of low quality hay, but not that of higher quality hay. The overestimate of

energy content by the current TDN equation of ML, MH, and H diets may be associated with the narrow range of bermudagrass hay quality used to develop the current TDN expression.

References

Gadberry, M.S., Troxel, T.R., Jennings, J.A., Davis, G.V., 2005. Influence of crude protein content of bermudagrass hay on in vitro organic matter digestibility and the Arkansas TDN equation. Un. Ark. Coop. Ext. Service. AAES Research Series 535.

Figure 6.1
Relationship between observed dry matter digestibility (DMD, g/kg DM) and predicted total digestible nutrient (TDN, g/kg DM) estimates using the Arkansas TDN equation for bermudagrass

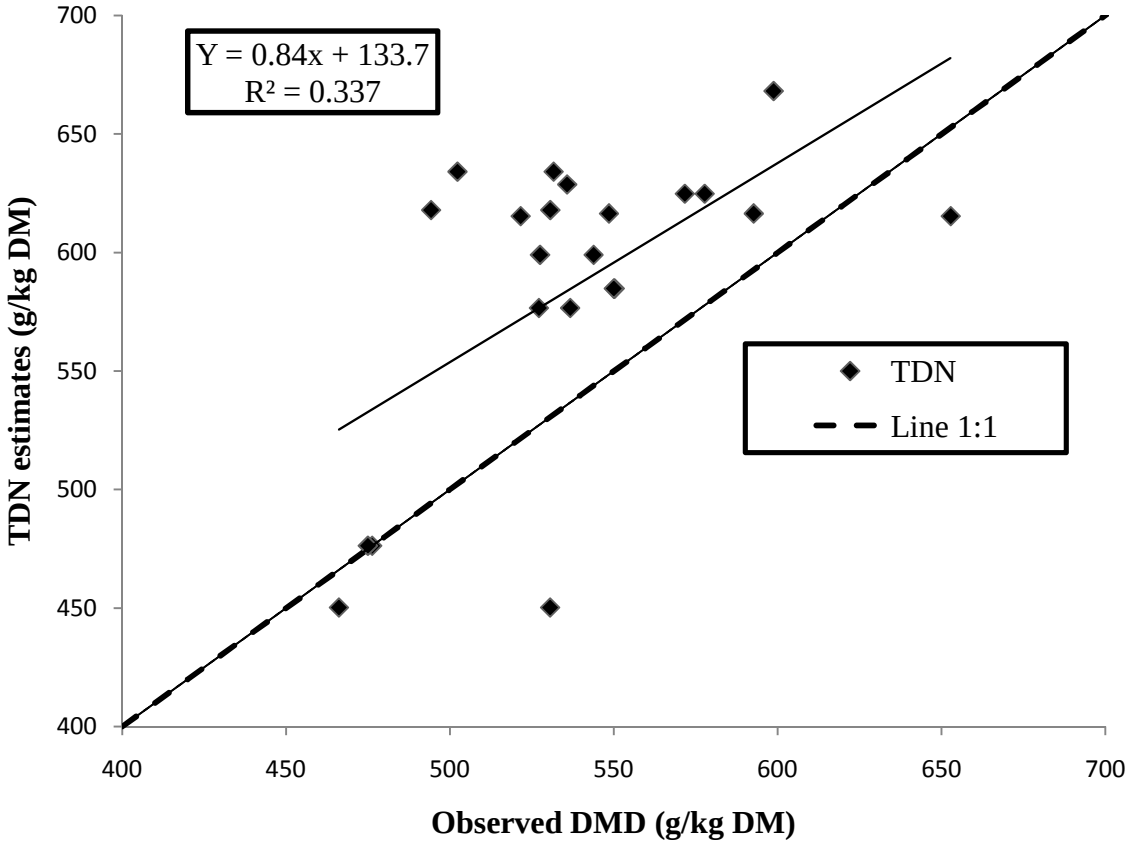

Table 6.1

Correlation coefficient among different variables and their corresponding *P*-values

Item[a]	DMD	CP	NDF	ADF
CP	0.50[b]			
	0.013[c]			
NDF	-0.46	-0.28		
	0.024	0.19		
ADF	-0.47	-0.48	0.93	
	0.02	0.017	<0.001	
TDN	0.58	0.75	-0.84	-0.93
	0.002	<0.001	<0.001	<0.001

[a]CP, crude protein; NDF, neutral-detergent fiber; ADF, acid-detergent fiber; TDN, total digestible nutrient calculated using the Arkansas TDN equation for bermudagrass.

[b]Correlation coefficient.

[c]*P*-value, probability that the correlation coefficient is significant.

Table 6.2

Estimates of energy of varying qualities bermudagrass hays using the Arkansas total digestible nutrient (TDN) equation

	Diet treatments[a]					
Item[b]	L	ML	MH	H	SEM[c]	*P*-value
CP g/kg	79[f]	111[e]	131[e]	164[d]	6.4	<0.001
NDF, g/kg	768	712	690	740	19.1	0.085
ADF, g/kg	428[d]	348[e]	332[e]	370[de]	19.4	0.035
TDN, g/kg	501[f]	600[e]	640[d]	623[de]	16.4	<0.01
DMD, g/kg	511	544	535	567	13.4	0.180
Bias TDN vs. DMD, g/kg	-10	56	105	56		

[a]L, low CP; ML, medium low CP; MH, medium high CP; H, high CP diet.

[b]CP, crude protein; NDF, neutral-detergent fiber; ADF, acid-detergent fiber; TDN, total digestible nutrient estimated using current bermudagrass TDN equation [111.8 + 0.95CP - 0.7 NDF - 0.36 ADF]; DMD, dry matter digestibility; Bias, difference between TDN calculated and the actual values of DMD.

[c]SEM, standard error of the mean.

[def]Means with different superscripts within a row differ at $P < 0.05$.