FECAL NEAR-INFRARED REFLECTANCE SPECTROSCOPY CALIBRATIONS FOR PREDICTING DIET QUALITY AND INTAKE OF DONKEYS

A Dissertation

by

NEGUSSE FESSEHAYE KIDANE

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

DOCTOR OF PHILOSOPHY

December 2005

Major Subject: Rangeland Ecology and Management

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Approved by:

Chair of Committee, Jerry W. Stuth
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ABSTRACT

Fecal Near-Infrared Reflectance Spectroscopy Calibrations for Predicting

Diet Quality and Intake of Donkeys. (December 2005)

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Chair of Advisory Committee: Dr. Jerry W. Stuth

The objective of these studies was to develop near-infrared reflectance spectroscopy calibration equations from diet-fecal pair datasets to predict the diet quality and intake of donkeys. One hundred-forty diet-fecal pair samples were generated from two independent in vivo feeding trials conducted in the United States (N = 100) and Africa (N = 40). At each site, ten female donkeys were fed mixed diets blended from 25 forage and crop residues. The modified partial least square model (MPLS) was used to develop calibration equations for crude protein (CP), digestible organic matter (DOM), dry matter digestibility (DDM) and organic matter digestibility (OMD), for the US, Africa and US/Africa combined datasets, and dry matter (DM) and organic matter (OM) intake calibrations from the US datasets.

Crude protein (CP) equations were developed with standard error of calibration (SEC) < 1.0 and coefficient of determination (R^2) > 0.90, (SEL = 0.5). The US, US/Africa and Africa CP equations had SEC value of 0.77, 0.97 and 0.88 with corresponding R^2 of 0.97, 0.95 and 0.88, respectively. Validation of the US CP equation resulted in a standard error of prediction (SEP) of 1.79 with corresponding coefficient of correlation (r^2) of 0.82 and slope of 0.84 indicating high accuracy of prediction.

In vivo derived DOM equations were also developed for the US, Africa and US/Africa datasets with SEC values of 2.58, 4.91 and 3.52, and R^2 of 0.60, 0.81 and 0.84, respectively. In addition, the SEC and R^2 values were 3.25 and 0.72 for US OMD, 3.28 and 0.79 for US DDM, and 4.2 and 0.85 for US/Africa OMD, and 4.3 and 0.87 for US/Africa DDM equation, respectively.

Calibration equations for predicting DMI and OMI have resulted in SEC values of 3.45 and 3.21 (g/kgw$^{0.75}$) and R^2 values of 0.89 and 0.84, respectively. The present study explored the relationship between DMI and diet quality attributes. Crude protein and digestible organic matter to crude protein ration (DOM/CP) with r^2 values of 0.60 and 0.39, respectively, have shown good correlations with intake.

The present studies have confirmed the potential for the fecal NIRS profiling for predicting CP, DOM, DDM, OMD, DMI and OMI of donkeys. Both calibration and validation results have indicated that the present donkey equations were comparable to previously developed equations for ruminants; they have the capability for accurate prediction of diet quality and intake, and can be a useful tool for monitoring the nutritional well-being of donkeys with acceptable accuracy. Research works to further expand the present calibration equations with additional diet-fecal samples particularly from Africa that did not meet the required accuracy level is recommended.

DEDICATION

I dedicate this dissertation to my late Grandaunt, W/r Haimanot Gubsa, for the love and care she showed me during my childhood. She inspired me to excel academically. To my beloved wife Seniat Seghid who has always been at my side during the entire study period for her love and care. Last but not least, to my loving son Abner Negusse for his patience during the long hours of my absence from home when he desperately needs me.

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CHAPTER I

INTRODUCTION

Free grazing livestock production has been a dominant animal agriculture supporting the daily life of millions of people in many countries. Both equines and bovines are sources of food such as meat and milk, draught power for traction and transportation, organic fertilizer to maintain soil fertility, and cash income from sale of livestock and livestock products. In same system livestock (equine and bovine) also create an employment, store wealth, and provide a variety of social and cultural services.

The primary purpose of equines is draught power for transporting people and goods, and for plowing field and threshing crops. Ramaswamy (1998) estimated that over two billion people in developing countries use both equine and bovine draught animals for agricultural operations and small-scale transportation. According to the author the use of draught animals saves 20 billion tons of petroleum, which is worth 10 billion dollars per year.

Donkeys (*Equus asinus)* are among the early-domesticated equines that have been around as long as mankind (Saul et al. 1997). Today, there are more than 40 million donkeys distributed throughout the world (Fernando and Starkey 1998; Blench 1999; FAO 2003). In Africa, the donkey population is estimated to be 13 million (Starkey and Starkey 2001) of which about 45% (6 million) are found in East Africa (FAO 2003). The use of donkeys has extensively been for transport (riding, packing) and traction (cultivation) although in some areas they are used for meat and milk production (Blench, 1999; Fernando and Starkey 1998). In Africa, donkeys provide cheap, renewable and ecologically sustainable draught power for agricultural and

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This dissertation follows the style and format of *Rangeland Ecology and Management.*

household activities (Aganga and Tsopito 1998). They are important for transporting small-scale agricultural inputs from distribution centers to farms, and agricultural products (grains, vegetables) and fuel wood from fields to homesteads and local markets. As traction animal donkeys in association with oxen, provide the energy source for plowing of land, threshing crops and weeding fields. These services by donkey spare people in developing countries some of the hardest labor, and increase crop production, food supply and household income (Pearson and Vall 1998). Moreover, with the increasing livestock population and livestock pressure on the grazing lands (Starkey and Starkey 2001) and the increasing price of working oxen (Aganga and Tsopito 1998) farmers in many developing countries are not able to continue maintaining draught oxen for work purpose. Consequently, the use of donkey for traction is becoming a preferred choice for many farmers.

Despite these great contributions to society, donkeys have been almost totally neglected both by researchers and developers (Ghebreab et al. 1999; Pearson et al. 1999; Starkey and Starkey 2001) and little is known about the management and nutrition aspects of donkey production. Nutrition is the most pervasive constraint to donkey production in developing countries (Buvanendran 1989; Pearson et al. 1999). In many countries feeding of donkeys is almost entirely based on grazing on communally owned grasslands (Ghebreab 1999) where forage resources are inadequate both in quality and quantity. During the dry seasons where range forage production is inadequate to support donkeys, in order to reduce nutritional stress farmers practice some nutritional intervention by supplementing animals with crop residues and household food wastes. However, improvement on the performance of donkeys even through supplementation was of marginal value.

Like other grazing animals, optimum levels of performance of donkeys in terms of draught power may be achieved when the animals get all necessary nutrients both quantitatively and

qualitatively and when these resources are adequately managed. There is a general consensus that for successful nutritional management of free grazing animals the following three primary factors must be known: primarily the amount of forage available for the animal and subsequent intake (Coleman 1989), secondly the diet quality/composition of forage selected by the animal (Mayes and Dove 2000) and finally the nutritional status of the animal (Stuth et al. 1991; Stuth 1998; Stuth et al 1999).

Monitoring the nutritional status of free-grazing animals is becoming an increasingly important aspect of range management. However, livestock producers have had limited ability to assess the nutritional status of free-ranging herbivores in a manner sufficiently quantitative for precise nutritional management (Stuth et al. 1999). One of the basic problems confronting the livestock producers is lack of a simple and affordable technique for estimating the nutrient composition of diets of free-grazing animals (Mofareh et al. 1997; Walker et al. 200l). These constraints further limit the ability of livestock producers to adjust animal number and kind (stocking rate) in response to the available forage quality (Holechek 1998).

Many variants of nutritional profiling approaches including direct animal observations, analysis of either esophageal fistula extrusa or forage samples derived from hand plucked, and analysis of fecal materials have been used for determining diet quality. In reference to the key indicators of the nutritional quality, currently used conventional methods are wrought with problems that deem them incompatible (Ossiya 1999b). In particular, the complexity of the techniques, the cost and labor requirements are excessive for advisory purpose (Bruno-Soares et al.1998). The advantages and limitations of the application of the various techniques under free-grazing condition have been discussed in detail next chapter and elsewhere (Van Soest 1982; 1994, Holechek et al.1982; Mayes and Doves 2000).

As an alternative approach, pastoralists have been using traditional methods including visual appraisal of forage (Niamir-Fuller 1998) and body condition score of animals (Stuth et al.1999; Pearson and Quassat 2000). The use of these methods to assess the nutritional status of animals, especially under free grazing situation has several weaknesses. Primarily, visual assessment of forage is subjective and cannot be reliably used to mimic the selectivity of free-ranging animals Van Soest 1994). Secondly, body condition score indicates past nutrition (Lyons 1990; Stuth et al. 1999) or may reflect factors such as long distance movements and parasite load, which is not directly related to the nutritive value of forage.

Until 1990 neither conventional nor traditional methods had provided a complete solution to address the problem in the nutritional management of animals under free grazing situations. Consequently, during the last decade several attempts have been made in developing other objective monitoring methodologies capable of determining at least the current nutrient supply compared to animal's demand. Considerable progress has been made in developing useful analytical tools based on fecal and forage analyses. Particularly, with the advent of near infrared reflectance spectroscopy (NIRS) fecal profiling techniques, analysis of feces has been of over-riding importance in determining diet quality (e.g. crude protein and digestibility) and as an important driving variable modeling the complexity of dry matter intake. As a result several fecal-NIRS calibration equations have been developed for various animal species including, cattle (Lyons 1990; Lyons and Stuth 1992; Lyons et al. 1993; Coates 1998, 1999; Ossiya 1999a; Gibbs et al. 2002; Awuma 2003), goats (Leite and Stuth 1995; Awuma 2003), sheep (Ossiya 1999a; Awuma 2003; Li et al. 2004), white tailed deer (Gallagher 1990), and Rocky Mountain elk (Keating 2005). In addition, albeit few research works have been carried out to assess the potential for NIRS with non-ruminants, results have demonstrated successful use of the technique.

Once suitable NIRS calibration equations have been developed, fecal-NIRS methods are routinely used for characterizing the diet quality in grazing animals with acceptable accuracy. These techniques have become a prime choice for many rangeland managers and received wide legitimacy as a diagnostic and monitoring tool worldwide. The methods aid animal producers in making sound interpretation of the nutritional status of the animals in terms of diet quality (Stuth et al. 1999) and assist them in making judicious use of available resource (forage, feed supplements).

Through much of the development of the fecal NIRS technique, the focus has been overwhelmingly on ruminants and little, if any, attention have been given to draught equines. The apparent increased use of donkeys for work in areas such as Africa has led to a demand for accurate information on donkey's nutritional status. Until recent time, however, no research has been done to examine the potential for NIRS fecal profiling to characterize the nutrient quality of free grazing donkeys. On the other hand, limited studies conducted by Lyons and Stuth (1992) have demonstrated that application of fecal NIRS calibrations developed for one species cannot be used to predict diet quality consumed by another species. As this study shows, there is a fundamental difference in biochemical composition of fecal samples from different species of animals rooted in the inherent physiological and behavioral variation of the animals. Equines are different from ruminant both physiologically and behaviorally, and therefore NIRS calibrations developed for cattle, sheep or goats may not be reliable for predicting the diet quality of donkeys. Hence, there is a need for development of fecal NIRS profiling equation specifically for donkeys. This study therefore seeks to address the following question "can fecal-NIRS profiling predict the nutritional status of free grazing donkeys in particular and equines in general?"

Hypothesis

The null hypothesis of the study is that analysis of fecal materials via near-infrared reflectance spectroscopy (NIRS) cannot predict the diet quality of free grazing donkeys, and cannot be used as monitoring tool to assess the nutritional status of free grazing donkeys with acceptable accuracy, both in Africa and US.

Objectives of the Study

The overall objective of the study was to explore the potential for near-infrared reflectance spectroscopy (NIRS) for predicting the diet quality including the chemical composition and functional properties of forage consumed by donkeys. The specific objectives were:

1. To develop fecal-NIRS calibration equations for predicting the crude protein and digestible organic matter of diets for donkeys.
2. To determine the effect of physiological state of donkeys on the performance of NIRS calibration equations in predicting diet quality.
3. To determine the relationship between diet quality and dry matter intake of donkeys fed mixed diets.
4. To determine the potential of fecal-NIRS calibration equation for predicting the feed intake of donkeys.

CHAPTER II

LITERATURE REVIEW

The Economic Importance of Donkeys

Draught animals, both equine and bovine, provide an essential power resource in developing countries worldwide (Pearson and Smith 1992; Pritchard et al. 2005). Particularly, the potential of donkeys is noted in the growing body of literature. The total world population of draught animals is estimated at 400 million (Panin and Ellis-Jones 1994) of which more than 10% are donkeys. Over 95% of the donkeys are found in developing countries (Pritchard et al. 2005) and East Africa region (Eritrea, Ethiopia, Kenya, Tanzania and Uganda) alone accounts for more than 5.6 million donkeys.

Donkeys have played and still play, an important role in meeting the power requirement of farming systems in many parts of the world, particularly sub-Saharan Africa (Pearson and Vall 1998, Panin and Ellis-Jones 1994). The great majority of working donkeys (probably over 95%) are used specifically for transportation (goods, grains, fuelwood, water, people) and traction (tillage, weeding, threshing) (Pearson and Quassat 2000; Gebre-Wold et al. 1999; Aganga et al. 2000; Starkey and Starkey 2001). Recent information regarding the contribution of donkey power to the economies of the developing countries has shown that donkeys contribute a considerable portion of the power requirement for crop production and transportation. According to Ramaswamy (1998) who conducted the economic analysis, the work performed annually by donkeys and other draught animals would require 20 million tons of petroleum, valued at US$10 billion, if it were performed by motorized vehicles. Therefore, animal power is relatively cheap, locally available and has low cost of maintenance. The use of this power for agriculture generally enables farmers in sub-Saharan Africa to increase agricultural production and improve

the quality of life. For this it has been argued, convincingly, that donkeys should be seen at least as an adjunct to other livestock (oxen or cows).

Challenges in Using Donkeys for Draught Power

Despite the socio-economic contribution to undeveloped societies and their future potential (Wilson 1981) donkeys are often neglected in the allocation of resources, especially feed (Gebreab et al. 1999; Pritchard et al. 2005). Efficient use of working animals depends on an understanding of the capabilities of the animals for work, their husbandry requirements and the factors, which influence their performance (Pearson and Vall 1998). In any livestock enterprises one of the most important factors determining animal productivity and profitability is optimum level of feeding (Maxwell and Milne 1995; Aganga et al. 2000; Tamminga and Chen 2000; Devendra and Sevilla 2002). According to Pearson and Vall (1998) and other scientist working in Africa, feeding animals is most challenging in grazing areas, particularly in the dry lands of the sub-Saharan Africa where availability of forage, quantitatively and qualitatively, is uncertain. For instance, Starkey and Starkey (2001) reported that in the pastoral and low-input mixed farming systems of sub-Saharan Africa grazing donkeys suffer from either permanent or seasonal nutritional stress. The principal factors limiting donkeys' performance are low protein content of forages and low energy intake due to the high fiber content of forages (Ghebreab et al. 1999; Gebre-Wold et al. 1999; Pearson and Ouassat 2000).

In many countries of the sub-Saharan Africa, donkey owners are well aware of the impact of diet quality on the performance of their animals (Mofareh et al. 1997) and have recognized the importance of supplementary feeding strategies. At times, particularly during the long, dry season and after heavy work, some farmers feed their donkeys with crop residues including sorghum/maize stover, dried stalks, leaves, wheat/maize bran, and teff straw (Aganga and

Tsopito 1998; Gebre-Wold et al. 1999). Nevertheless, improvements in work performance are not always realized in the animal following supplementary feeding (Pearson and Vall 1998). The major reason is that farmers are often limited to practice these strategies effectively and on time mainly due to lack of reliable information on the quality and quantity of the grazing animal's diet at the time of feeding (Mofareh et al. 1997). This limitation in turn is attributed to lack of a rapid method of determining diet quality of free ranging herbivores (Lyons and Stuth 1992) and the inability of the donkey owners to monitor the nutritional status of the their donkeys objectively.

Conventional and Traditional Techniques for Assessing the Diet Quality and Nutritional Status of Free Grazing Animals

Determination of forage or diet quality is a key factor in management decision concerning the nutritional well being of animals (Roberts et al. 2004). Equally important is information about the amount of feed consumed (intake) by the animal (Mayes and Dove 2000).

A variety of direct and indirect techniques have been developed to assess the forage quality and nutritional well being of free grazing animals. These include bulk sampling by clipping, hand plucking of material an animal might consume, and use of cages or quadrats which exclude grazing animals (Van Soest, 1994; Holecheck et al 1982, 1998). However, no single type of measurement gives reliable quantitative description of the nutritional relationship between forage and animals. The advantages and limitations of the various techniques have been extensively reviewed in Van Soest (1982, 1994) and Holecheck et al. (1982, 1998).

Van Soest (1994) ascribed that a more direct approach to measuring forage quality is to assay the plant material that is actually consumed by the animal via direct feeding or esophageal fistulation. Several chemical analytical techniques are currently employed to determine digestibility and crude protein concentration forage samples. For example, the digestibility of

forages can be determined in at least three different ways namely, in vivo (Stern et al. 1997, Tamminga and Chen 2000), in virto (Uden and Van Soest 1982, Van Soest 1994; Mayes and Dove 2000), and in situ (Ørskov and Ryle 1990; Ørskov 2000) and marker (Marais 2000) methods. The protein concentration of forages also has been determined by wet chemistry procedures such as the Kjeldahl method (Bekers et al. 1996).

Many researchers in the field of range animal nutrition (Coleman et al. 1989; Lyons and Stuth 1992; Stuth 1998; Ossiya 1999a) have noted that currently employed scientific methods have limitations for use in extensive rangelands with complex vegetation. For instance, in vivo determination of digestibility is the de facto standard (Coleman and Moore 2003) but because of the obvious limitations of expense, time and labor it is not practical to use as a routine analysis (Van Soest 1994; Stern et al. 1997). Additionally, in vivo measurement of nutrients digestion is subjected to inherent animal variation (Stern et al. 1997) and requires surgically cannulated animals (Van Soest 1994; Mayes and Dove 2000). The in vitro technique described by Tilley and Terry (1963) has also been the most commonly used method for predicting digestibility of forages. The method also has been criticized for two major drawbacks: (1) use of ruminally-cannulated animals (Stern et al. 1997; Mayes and Dove 2000) that involves pain and stress to the animal (2) samples obtained by either esophageal fistula or by rumen emptying do not exactly reflect that of the forage eaten because samples are contaminated with salivary mineral and organic compounds (Van Soest 1994), and (3) extrusa samples are collected over a period of few minutes, where as the test animals may be grazing or browsing for days (Mayes and Dove).

The well known kjeldahl method for the determination of forage nitrogen provides precise and accurate measurement but Bekers et al. (1996) noted that the method is rather complex, time consuming and expensive. A detailed description of the advantage and limitations of the major analytical techniques for measuring forage quality have been presented in (Van Soest 1982,

1994; Lyons 1990; Stern et al. 1997; Ossiya 1999a; Mayes and Dove 2000; Awuma 2003) and therefore no need for further elaboration here. Even so, from a practical standpoint the common limitations which preclude the use of the above conventional techniques under free condition can be summarized in two categories: first the techniques require high cost, intensive labor, skilled man power and more time, and second they are limited to pure scientific applications rather than management or monitoring tool for free grazing animals.

Traditionally, in many countries donkey owners have been using visual assessment of body condition score and body weight as a routine method to assess the nutritional status of their animals (Pearson and Quassat 2000). Body condition is used as a rough guide to adjust the amount of feed given. Yet there are disadvantages in relying on body measurements alone. The limitations include (1) body condition reflects past nutritional (Lyons 1990), (2) non-nutrition related changes in body condition and body weight can occur when the animals are sick, burdened with parasite, heat stressed or engaged in heavy work conditions (Pearson and Quassat 2000) and (3) body condition measurement is a subjective judgment (Ossiya 1999b). Pearson and Quassat (2000) also argued that successive measures of body weight does not accurately reflect changes in nutritional status of the animal because it can be affected by several factors including consistency in weighing technique, handling stress, gut fill, pregnancy and water intake at each weighing interval.

Importance of Diet Quality Information in Free Grazing Animal Nutrition Management

Terms describing nutritional attributes of forages have often been confused, with forage quality and forage nutritive value used interchangeably (Coleman et al. 1999). Forage quality/nutritive value has been defined in various ways. Nutritive value is conventionally

classified into three general components: digestibility, feed consumption, and energetic efficiency (Van Soest 1994). According to Stuth et al. (1999) forage quality implies digestibility, crude protein (CP), secondary compounds and mineral contents. Both crude protein and digestibility are indicators of the nutritional value of the forages and they play a fundamental role in determining the nutritional status of animals. In this dissertation therefore, the term forage/diet quality rather than nutritive value would be used to describe the quality of both forage and crop residue origin diets. Several authors (Stuth et al. 1991, Stuth 1998; Ossiya 1999b; Ndikumana et al. 2000; Awuma 2003) have noted that in extensive livestock production system improvement in animal nutrition may be virtually impossible without first addressing the issue of predicting forage productivity and the nutritional value of forage. Measuring or estimating the chemical composition and functional properties (digestibility and intake) of the diets of free grazing animals, however, is limited mainly by the selective grazing behavior of the animals (Boval et al. 2004) and other associated conditions. Regarding the former, Lamoot et al. (2004) pointed out that under free grazing situation diet composition depends on the forage habitat type, varies among seasons and changes over years. Additionally, according to Van Soest (1994) forage quality varies tremendously with age of the plant and the portion of the plant being consumed.

To successfully mitigate the nutritional stress of free grazing animals by seasonal or annual herd mobility, which is a common practice in pastoral livestock production systems, livestock producers (agro-pastoralists and pastoralists) have to be provided with reliable information on forage quality (Ndikumana et al. 2000). Likewise, a sound and strategic intervention with supplementary feeding, which is common practice in mixed crop livestock production systems, requires an estimate of how much protein and energy the animal has consumed from grazing pasture (Nsahlai et al. 2000) as well as crop residues. Ndikumana et al. (2000) noted that any

forage quality assessment technique should be able to generate reliable indicators, which are focused on the needs of the end users (agro-pastoralists and pastoralists). The indicators then should enable livestock producers to make economically viable decisions regarding feed inputs and management as well as grazing management.

In response to the needs of livestock producers, there have been attempts by several authors to devise a useful technique that applies for free grazing animals. Particularly, scientists at Texas A&M University have been actively engaged in devising an analytical technique. Since early 1990's Prof Jerry Stuth and his coworkers have been productive in supplying suitable methodology for monitoring the nutritional quality of forages through fecal profiling using near infrared reflectance spectroscopy (NIRS). The technique relies on the relationship between chemical properties as determined by defined reference methods and absorption of light at different wavelengths in the near infrared region, measured by reflectance (Cozzolino and Moron 2004). To date, fecal NIRS technique has received a wide legitimacy worldwide with NIRS fecal profiling laboratories being established in Argentina, Australia, Ethiopia, India, Kenya, Tanzania and Uganda (Stuth, personal communications).

Near Infrared Reflectance Spectroscopy and Its Application

Near-infrared reflectance spectroscopy (NIRS) technology is widely used for a vast number of applications, including in feed industries (Roberts et al. 2004), textile industries (Montalvo and Von Hoven 2004), medical and pharmaceutical advances (Schulz 2004) and agriculture (Shenk and Westerhaus 1993; Shenk et al. 2001). In agriculture, the technique has become an important tool for the routine quantitative determination of organic constituents (Nahm 1992) and it is routinely used for nutritional analysis of feedstuff and forages (Roberts et al. 2004). The most wide spread use of NIRS has been for the determination of protein, moisture, starch, lipids

and cellulose of grains, and forages (hay and silage) (Skoog et al. 1998; van Kempen et al. 1996; Foley et al. 1998; Reeves and Van Kessel 2000; Roberts et al. 2004) samples obtained from either food/feed mills, feedlots, or monoculture pastures.

Moreover, recent studies have demonstrated successful use of the NIRS to predict forage quality for livestock obtained via esophageal extrusa (Lyons and Stuth 1992; Leite and Stuth 1995; Volesky and Coleman 1996), hand clipped forage or stall-fed mixed diets (Awuma 2003; Li et al. 2004; Keating 2005). The theory of the near infrared spectroscopy and mathematical basis of calibration have been thoroughly explained by several authors (Hrushka 1987; Murray and Williams 1987; Workman and Shenk 2004). Therefore, the next section of the chapter will briefly review the basic principles of NIRS technique and its application in free grazing animal management.

Basic Principles of Near Infrared Reflectance Spectroscopy

Spectroscopy literally means looking at light and is based on the analysis of the interaction of electromagnetic radiation with matter (Deaville and Flinn 2000). The near-infrared (NIR) region of the spectrum extends from the upper wavelength end of the visible region at about 770nm to 2500-nm (13,000 to 40000 cm^{-1}) (Skoog et al. 1998). Interestingly, most analytical use of NIRS is between 1100 and 2500-nm (Deaville and Flinn 2000). This region is dominated by overtone and combination bands of fundamental vibrations occurring in the mid-infrared (Pasikatan et al. 2001). The frequency intervals are considered to be the region of the spectrum in which the reflected light intensity best correlates with the concentration of analyzed chemical species (Nikolich et al. 2001).

Near-infrared reflectance spectroscopy basically uses the principle that molecular bonds absorb specific frequencies of light to obtain information about the number and type of organic bonds present in a compound (van Kempen et al. 1996). Using the light in the infrared region (*Ir spectroscopy*) the vibrational modes in irradiated molecules are then activated and provide primary information on the stretching and bending of organic bonds primarily of C-H, O-H, and N-H bonds (Norris et al. 1976, Foley et al. 1998; and Stuth et al. 2003). The stretching and bending of organic bonds produces interactions between the radiation and a biological material, and yields an abundance of chemical information about the material in question (Shenk and et al. 2001).

The ranges of the main absorption bands vary widely depending on the type of the samples. For instance, to water they are at 1940- and 1450-nm, of aliphatic C-H bonds at 2310-, 1725-, 1400- and 1210-nm, of O-H bonds around 2100- and 1600-nm and N-H bonds at 2180 and 2055-nm (Harvey 2000). Forage materials are found to exhibit identifiable C-H, N-H, and O-H absorption bands in the 1400-2500–nm region (Workman and Shenk 2004).

Using instruments such as the scanning monochromator (Nahm 1992) a broad array of information about the chemical composition of the organic or biological material is extracted (Pasikatan et al. 2001). This spectral information is regressed against the values of reference data obtained from wet chemistry to create calibration equations, which are then used to predict the nutrient or chemical composition of unknown or independent samples (Clark et al. 1995; Shenk et al. 1992).

Calibration Procedures

According to Westerhaus (1992), calibration is the process of estimating the mathematical relationship between spectra and analytical measurements made on a group of samples. These

calibrations are based on the statistical analysis, termed "chemometrics" of the relationship between mathematically transformed spectra and the frequency of chemical bonds in an organic matrix, termed "reference values (Landua et al. 2005). The use of near-infrared reflectance spectroscopy is therefore to obtain spectra from which quantitative or qualitative information for an analyte of interest can be extracted (CapMunday et al. 2004). Near -infrared reflectance spectroscopy instruments determine the concentration of component by measuring log (1/R) values, which then be related to the amount of the component as determined by standard laboratory method (Hruschka 1987). Quantitative analysis by NIRS is based on the Beer-Lambert Law (Crooks 1978). Beer's law states that log (1/R) is proportional to the concentration of the chemical bond absorbing the NIRS energy (Westerhaus 1992). In its simplest form, the Beer-Lambert law indicates that the more molecules of a certain type present in the sample, the more energy will be absorbed at wavelengths specific to those molecules (Williams 1987). As a result the degree of absorbance can be used to determine the concentration of those molecules present in the samples (Williams 1987). The mathematical derivation of the law has extensively been described by several authors elsewhere (Crooks 1978, Birth and Hecht 1987, Williams 1987).

Application of near-infrared reflectance spectroscopy is a secondary method in that the instrument first has to be calibrated with samples analyzed by the conventional methods (Neumeiter et al.1997). The standard procedure is to prepare a number of calibrations or training samples, determine the analyte concentration using a reference method and measure the spectral response (Faber and Kowalski 1997). Once a database of sample spectra and analyte values has been obtained, it is necessary to relate the spectral information to the analyte information, that is, to calibrate (Faber and Kowalski 1997; Westerhaus et al. 2004). In this regard the near infrared reflectance spectroscopy calibration involves regression of the spectral response (independent

variable) against the verified reference (dependent variable) obtained by conventional laboratory procedures (van Kempen et al. 1996). The independent variables are the mathematical combination of log (1/R) values at various wavelengths (Hruschka 1987). Following the above stated Beer's law, the mathematical relationship between log (1/R) and the analytical measurements is assumed to be linear (Westerhaus 1992).

The general essence of calibration is to ensure that the range of spectral variation found in the whole population is represented in the samples selected for analysis for calibration development (Foley 1998). For calibration various statistical models including, Principal Component Regression (Fearn 1992), Multiple Linear Regression (Ruano-Romos et al. 1999; Reeves 2000) and Partial Least Square Regression (Faber and Kowalski 1997; Nikolich et al. 2001) can be used. Each statistical model allows exploration of relationships of reflectance/absorption values of diverse sets of chemical bonds in the dried/wet sample and wet chemistry values of samples (Westerhaus et al. 2004).

Since spectra are influenced both by chemical (absorption by chemical bonds) and physical (e.g., scatter, pathlength, surface reflectance) effects, before any calibration is made a derivation of a mathematical relationship must be calculated between reference values and the spectral data (Smith et al. 2001; Westerhaus et al. 2004). According to Williams (1987) the most successful mathematical treatments of spectra to date are the first or second derivative of the log (1/R) and smoothed log (1/R). These mathematical pretreatments/ transformations through derivation procedures are undertaken in the form of (ψ, χ, γ, ρ,).

Where: ψ order of derivative (first and second derivative) of log (1/R)

χ the gap in data points over which the derivative is calculated

γ number of data point over which first smoothing is applied

ρ number of data points over which the second smoothing is applied

Derivatives are an approach to addressing two of the basic problems with near-infrared spectra: overlapping peaks and large baseline variations (Hruschka 1987). First derivative eliminates a common baseline offset, and a second derivative removes a tilted baseline shift (Westerhaus et al. (2004) and separates overlapping bands (Hruschka 1987) whereas smoothing is a means of reducing instrument/sample noise (Williams 1987). In addition, standard normal variate (SNV) correction and detrending are used to remove the major effects of light scattering from the spectra (Duckworth 2004). SNV correction is applied to correct for the effect of multiplicative interferences of scatter and particle size and detrending usually follows to attempt to remove the additional variation in baseline shift and curvelinearity (Hruschka 1987; Duckworth 2004).

Selecting Prediction Equations

The usual end product of calibration is a prediction equation that converts the spectra data for one sample into a prediction constituent (Fearn 1992). Since each math treatment or transformation results in a separate equation, the performance of each equation is determined by considering various statistical tests (Shenk and Westerhaus 1992; Cozzolino and Moron 2004). In most studies, the quality of NIRS calibration is evaluated in terms of linearity and accuracy (Landau et al. 2005). Westerhaus et al. (2004) listed around 17 different statistical parameters useful for determining the quality of NIRS calibration models, however, which terms are used often depends on the type of software used. Stuth et al. (1998) recommended that standard error of calibration (SEC), coefficient of determination (R^2), wavelength coefficient magnitude, F-statistics and biological interpretation of wavelength as most commonly used parameters. Although all these statistical parameters are useful in determining both the feasibility of an NIRS method as well as an estimation of calibration model quality (Westerhaus et al.2004) several

authors (Lyons and Stuth 1992; Leite and Stuth 1995; Ruano-Romos et al. 1999; Cozzolino and Labandera 2002) suggested that the best calibration equations should be characterized by a large coefficient of determination (R^2), a low standard error of calibration (SEC), and a high F values for each selected wavelength. Both SEC and R^2 values indicate how well the equation will perform within the same population.

The coefficient of determination (R^2) is a measure of linearity and indicates the portion of the variance (mean deviation square) of the reference values, which is explained by the NIRS analysis. The statistical formula used to compute R^2 is give by the following formula:

$$R^2 = \frac{\Sigma_i \text{ (NIRS analysis}_i\text{-1/n } \Sigma_i \text{ reference value}_i\text{)}}{\Sigma_i \text{ (reference value}_i\text{– 1/n } \Sigma_i \text{ reference value}_i\text{)}} \qquad \text{[Eq.2.1]}$$

with i = 1 … n samples

The standard error of calibration (SEC) is calculated as the standard deviation of all NIRS analysis values from the reference values of the calibration samples and given by

$$\text{SEC} = \sqrt{\Sigma \frac{\text{(NIRS analysis value}_i\text{- reference value}_i\text{)}}{\text{n-1}}} \qquad \text{[Eq.2.2]}$$

with i = 1 …n samples and p wavelength or factor in calibration

The interpretative value of the SEC during calibration is dependent among other things on the standard error of laboratory (SEL) and the scatter of the features in the population. Hruschka (1987) proposed that good SEC values are one to two times the SEL and it will never be smaller than the SEL (over fitting). Although these parameters have been extensively used by several researchers as major criteria for selection, recently, Cozzolino and Moron (2004) and Agnew et al. (2004) argued that in judging the quality of a calibration equation, the standard error of cross validation (SECV) is better parameter compared to SEC because the former avoids over fitting.

According to the authors the SECV represents the variability in the difference between predicted and reference values when the equation is applied sequentially to subset of data from the calibration data set. Particularly, when developing calibration equation using multivariate regression models such as Principal Component Regression (PCR), Partial Least Square (PLS) and Modified Partial Least Square (MPLS), cross validation is carried out to identify and prevent an over-fitting, which may be expressed in a repeat rise of the SECV (Cowe et al. 1992). Cross validation builds models by leaving out a portion of the calibration samples (depends on the population size) for which the concentrations are estimated. This step is repeated until all the calibration samples have been left out once and prediction error is calculated from the difference between the predicted and the known reference values as follows:

$$SECV = \sqrt{\Sigma i \frac{(NIRS_i - \text{reference value}_i)^2}{n-1}} \quad \text{[Eq.2.3]}$$

with i = 1 …n samples

Eventually, after having obtained the prediction error all calibration samples are used to build up the final model. Thus the SECV represents a true estimate of how the calibration will perform when predicting unknown samples (Agnew et al. 2004). However, Marten and Naes (1987), and Naes et al. (2002) pointed out that the SECV procedure might give over-optimistic results, in particular if data are replicated, but justified in situations with calibration samples that are randomly selected from a natural population.

Another important criterion used in selecting best calibration equation is examination of the biological significance of the dominant wavelengths in the equation in relation to the constituent of interest. Generally, dominant wavelengths are selected based on first the magnitude of the regression coefficient of wavelength and second on the F value (Ossiya 1999a; Awuma 2003). Once wavelengths have been selected then they should be interpreted for their biological meaning in relation to the constituent of interest.

Validation of Developed Calibration Equations

In addition to the cross validation (SECV), once a calibration equation has been established it should be validated for its performance using external (independent) data that were not part of the calibration set. In this validation procedure, how well a calibration model performs is evaluated by examining various validation statistics, including standard error of prediction (or performance, SEP), correlation coefficient (r), bias (means deviation) and slope (Cozzolino and Moron 2004). The standard error of prediction (SEP) is the standard deviation of all NIRS values from the reference values for the validation sample sets (Williams 1987) and is calculated as:

$$SEP = \sqrt{\frac{\Sigma_i (\text{NIRS-analysis value}_i - \text{reference value}_i)^2}{n-1}} \quad \text{[Eq.2.4]}$$

with i = 1…n samples

Recently, Landau et al (2005) argued that the SEP is superior to the SECV because the former encompasses bias i.e. the mean difference between the predicted and actual values in a validation data set, and corrects the predicted value accordingly. However, since it is expensive and time consuming to produce independent data for validation, most workers (Landau et al. 2005) recommend the use of SECV as a major criterion of validation.

Indirect Prediction of Diet Quality of Free Grazing Animals from NIRS Fecal Sample Scanning

Unlike stall-feeding, in free grazing system predicting nutritional quality of forages consumed by free grazing animals is still challenging. One prominent reason is that samples of forage are not always easy to obtain, especially when animals have free choice at pasture (Stuth 1999; Coleman et al. 1999). As alternative technique fecal indices have been used to predict the

nutritional quality of forages harvested by grazing animals (Lyons 1990; Mayes and Dove 2002). The underlying assumption is that feces are the product of eroding and synthesizing digestive processes, consisting of residue of undigested feed and plant tissue and component of microbial and animal origin (Van Soestt 1982; Lyons and Stuth 1992; Van Soestt, 1994; Leite and Stuth 1995, Tolleson et al. 2000; Mayes and Dove 2002). Several authors (Lyons and Stuth 1992; Coleman et al. 1995; Mayes and Dove 2002) have suggested that feces should contain information about the amount and characteristics of the diet ingested by the animal and can be used for predicting the constituent of the diet. In contrast, Van Soest (1994) has argued that fecal composition will tell nothing about the amount and quality of the more digestible components that are not presented in the feces.

Despite the above disparity, research literatures have indicated that near infrared spectroscopy can be a viable tool to extract usable information about forage quality from the spectral characteristics of animal feces. In early 1990s' the work by Lyons and Stuth (1992) first proved that identification of key organic chemical bonds in feces through NIRS could successfully predict dietary constituents of forages ingested by free grazing cattle. This concept resulted in the development of NIRS equations from scanning fecal sample and predicting the diet crude protein (CP) and digestible organic matter (DOM) of free ranging cattle (Lyons and Stuth 1992). Since the advent of this fecal-NIRS technique several researchers (Lyons 1990; Lyons and Stuth 1992; Leite and Stuth 1995; Ossiya 1999a; Keating 2005; Awuma 2003; Li et al. 2004) in the Grazignland Animal Nutrition Laboratory (GANLAB) at Texas A&M University, have developed elegant NIRS calibration equations which could be used as a routine method of evaluating the values of diets ingested by both ruminants and non-ruminant herbivores.

Application of Fecal-NIRS for Predicting the Diet Quality of Free Grazing Ruminants

The validity and usefulness of NIRS fecal profiling to characterize the diet quality of free grazing ruminants has been demonstrated both in domestic and wild ruminants under different agro-ecological zones. Most previous studies have concentrated on testing the potential of the technology to predict the chemical composition (crude protein concentration, CP), functional properties (digestibility of forages) (Lovett et al. 2004; Roberts et al. 2004) and animal response specifically dry matter intake (Agnew et al. 2004; Boval et al. 2004) of animals. Therefore, the following review section will deal with prior accomplished research works on fecal NIRS for predicting mainly crude protein, digestible organic matter and dry matter intake of different species of animals.

Predicting Crude Protein and Digestible Organic Matter Concentration

The first use of NIR spectroscopy for predicting forage quality from fecal samples was demonstrated in the US (Brooks et al. 1984; Coleman and Stuth 1989; Lyons and Stuth 1992). Brooks et al. (1984) were the first researchers that developed calibration equation analyzing forage quality for elk from fecal material. Lyons and Stuth (1992) developed NIRS calibration equations to predict the CP and DOM content of forages consumed by cattle. Their experiments were conducted in two different locations College Station and La Copita, in Texas, including sub tropical savannas and deciduous hardwood woodlands. In this experiment, the reference diet samples were collected from esophageal-fistulated steers and sample digestibility was determined by in vitro procedures. Lyons and Stuth (1992) reported a standard error of calibration (SEC) for College Station DOM and CP of 1.66 and 0.89, respectively. The coefficients of determination values (R^2) for the same site were, 0.80 and 0.92 for DOM and CP, respectively. For La Copita DOM equation Lyons and Stuth (1992) found SEC=1.75 and

R^2=0.69, CP equation SEC= 0.88 and R^2= 0.88. In a study performed to validate the 1992 equation Lyons and Stuth (1995) found high correlation coefficient for crude protein CP (R^2 =0.98) and digestible organic matter DOM (R^2 =0.87) indicating a strong relationships between conventional chemistry values of the diet samples collected from esophageal-fistulated steers and NIRS prediction from fecal samples collected from intact mature cows.

In the US, Leite and Stuth (1995) demonstrated the use of NIRS fecal profiling to determine the crude protein (CP) and digestible organic matter (DOM) of forage consumed by goats grazing in sub-tropical deciduous woodlands in Texas. In this study, diet samples were collected from esophageally fistulated goats for in vitro digestion, whereas fecal samples were obtained from intact goats. The authors reported SEC values for CP and DOM equations 1.12 and 2.02, respectively and the R^2 equals to 0.94 and 0.93 for CP and DOM, respectively. Validation trials performed in Post Oak Woodland and subtropical thornshrub of Texas obtained R^2 values of 0.94 and 0.93, respectively for CP and DOM equations indicating that both selected equations could be used for predicting the nutritional status of goats. More recently, Landau et al. (2004) also used fecal NIRS to monitor the diet of Mediterranean goats in Israel. To generate diet-fecal pair data a stall fed experiment was conducted with mixed diets consisted of hay, concentrates or combination of browsed species. Landau et al. (2004) established calibration equations to predict crude protein and *in vitro* dry matter digestibility and they reported coefficient of determination (R^2) and standard error of cross validation (SECV, in parentheses) of 0.98 (0.5) for CP and 0.98 (2.0) for DMD.

Li et al. (2004) developed a calibration equation for predicting the diet quality of sheep using pre-mixed rations from diets collected across the US and fed in Texas. Li et al. (2004) conducted three-week feeding trail using 20 sheep fed with Coastal Bermuda hay during the adaptation period and diets composed of various grasses, forbs and browses during the collection

periods and found R^2 values of 0.95 for CP and 0.80 for DOM, and the corresponding SEC values of the equations were 1.08 and 1.51 for CP and DOM, respectively.

Fecal near infrared spectroscopy technique has also proven to be useful tool outside the US. Stuth (1999) tested the cattle US fecal-NIRS equation on fecal material obtained from cattle grazing in sub-Sahara Africa rangelands. The results indicated that the US equation has a potential to predict the nutritional quality of forages consumed by cattle in this region. Moreover, Ossiya (1999a) assessed the potential of NIRS fecal profiling to predict the crude protein (CP) and digestible organic matter (DOM) in the diet of free-ranging sheep and cattle, and condensed tannins in the diet of sheep in sub-Saharan Africa In this study calibration sets were collected from three SSA countries (Ethiopia, Niger and Nigeria). Ossiya (1999a) developed six sheep CP and DOM equations and two equations for the cattle. The author reported best equations with R^2 and SEC values for cattle CP of 0.95 and 1.04, respectively. The R^2 and SEC values for sheep CP equations were 0.87 and 1.03, and for the sheep DOM equation she found an R^2 of 0.89 and SEC value 3.21 for the combined equation. More recently, Awuma (2003) assessed the feasibility of applying NIRS technique to predict the diet quality of African livestock. Data were collected from trials conducted in four East Africa countries (Tanzania, Kenya, Uganda, Ethiopia) and one West Africa country (Ghana) plus historical data from Ethiopia, Nigeria and Niger. Calibration statistics reported by Awuma (2003) were R^2 of CP for cattle, sheep and goats were 0.92, 0.95 and 0.97 with corresponding SEC values of 0.90, 0.79 and 0.80, respectively. The R^2 and SEC value for DOM were 0.88, 0.94 and 2.82, 1.68 and 2.65 for cattle, sheep and goats, respectively.

In Australia, Coates (2000) examined the application of fecal NIRS profiling for predicting the digestibility and crude protein content of forages in both grazing and stall fed cattle. In their pen fed trials, the author observed high correlation between the NIRS spectrum and the value of

the standard chemistry for nitrogen with R^2 and SEC values of 0.99 and 0.087, respectively, and IVDMD with R^2 and SEC values of 0.97 and 0.022, respectively. Similarly, Gibbs et al. (2000) developed fecal NIRS calibration equation to predict the dietary quality (CP and DOM) of Australian cattle fed forage-based diets with and without supplements. They reported R^2 values of 0.99 and 0.87-0.93 for CP and for DMD, and SEC value of 1.28 and 2.38-2.63, respectively.

In Asia (Japan), Purnomoadi et al. (1996) examined the potential of NIRS to predict the chemical composition of feces and estimated the digestibility and energy values of diets indirectly by using lignin as internal indicator. Purnomoadi et al. (1996) carried out both wet chemistry analysis and NIRS scanning to determine lignin content, and a separate calibration equations were developed for both feed and fecal samples. These equations then were used to predicted lignin concentration in the diets and reported high correlation coefficient between lignin indicator laboratory and lignin indicator NIRS values for crude protein (CP), dry matter (DM), organic matter (OM), acid detergent fiber (ADF), crude fiber (CF), lignin, ether extract (EE) and energy. The authors observed that the in vivo digestibility value for the various chemical constituents and functional properties (DM, OM, CP, ADF, CF and EE) were similar to those obtained through lignin indictor laboratory and lignin indicator NIRS. Purnomoadi et al. (1996) reported R^2 and SEC values of 0.99 and 0.70, respectively for CP and 0.96 and 1.29, respectively for OM content of diets.

In Europe (Portugal), Bruno-Soares et al. (1998) investigated the potential of fecal profiling with NIRS for prediction of crude protein (CP) and other nutritional attributes of various grass species. In feeding trial conducted to develop calibration models for CP, Bruno-Soares and coworkers fed seven temperate grass species to rams. The authors found a high coefficient of determination (R^2=0.98) with low standard error of calibration (SEC=0.63). However; no

validation was performed to evaluate the performance of the equation on other independent samples.

Further, the use of NIRS fecal profile has been assessed in monitoring the nutritional quality of wildlife species. Gallagher (1990) was able to obtain a useful estimate of diet compositions dry matter intake, crude protein, gross energy, digestible dry matter, phosphorus content and phosphorus intake for pen fed white-tailed deer. The author reported R^2 values of 0.84 and 0.75, for CP and DDM, respectively. The corresponding SEC values were 1.42 and 7.1, for CP and DDM respectively. In this particular study, behavioral problems of stall fed deer were cited as one of the reasons for the high SEC for DDM calibration equation. Showers (1997) working with pen fed white-tailed deer also have established NIRS calibration equations for predicting CP and DOM. The author conducted a series of feeding trials using mixed diets blended from 50 different forage species and reported R^2 values of 0.94 for CP and 0.89 for DOM and SEC values of 0.70 and 2.64 for CP and DOM, respectively.

More recently, Keating (2005) developed fecal NIRS profile for monitoring the diet quality of free ranging Rock Mountain Elk in Texas. Keating (2005) used data obtained from in vivo digestion trail covering a wide range of grasses, forbs and browse species obtained from Colorado, Oregon Texas. The author obtained R^2 and SEC values of 0.89 and 1.24, respectively for the CP equation and the R^2 and SEC values of 0.80 and 1.730 respectively for the DOM equation.

Predicting Dry Matter and Organic Matter Digestibility

In addition to its use in predicting the concentration of CP and DOM of forages, near infrared reflectance spectroscopy fecal profiling has also proven to be an accurate method for estimating digestibility. NIRS calibration equation for predicting organic matter in vivo digestibility was

developed by Robert et al. (1986). The authors reported results with high coefficient of determination R^2=0.95 and low standard error of calibration (SEC= 2.05) and. Recently, Lovett et al. (2004) were also able to predict the in vitro digestibility of maize silage with relatively low correlation (R^2=0.60). Another study in Australia by Boval et al. (2004) developed a fecal NIRS equation for predicting OM digestibility using data from in vivo trails of cattle covering only two species of grasses (D*igitaria decumbens* and *Dichanthium spp.)* and obtained a R^2 of 0.72 and SEC of 2.1 (n=87). Boval and coworkers concluded that the relatively high SEC value for OMD equations was partly explained by the high (12%) between animal variations in observed in vivo OMD. Bruno-Soares et al. (1998) also developed calibration equation for predicting digestibility of several green crop cereals fed to rams (sheep). The authors reported R^2 values equal to 0.86 and 0.88 for the DMD and OMD equations respectively. The corresponding SEC values were 2.61 and 2.36, for the DMD and OMD equations, respectively

Predicting Dry Matter and Organic Matter Intake

NIRS has also been used for measuring feed and nutrient intake of ruminants. Agnew et al. (2004) predicted dry matter intake of grazed forages expressed as kg DM/h using NIRS and found R^2=0.76 and standard error of calibration (SEC=0.37). In the same study however, short-term intake parameters expressed as g DM/ per bite, had relatively poor correlation. Similarly, fecal NIRS calibration equation for predicting organic matter intake (OMI) was developed by Boval et al. (2004). Calibration data were generated from Creole steers of Australia (256 ± 35 kg) fed with *Digitaria decumbens* and *Dichanthium* spp. The calibration equation had R^2 and SEC values of 0.61 and 4.62 (g/kgw $^{0.75}$), respectively. Bruno-Soares et al. (1998) working with sheep tried to develop intake calibration equation. Covering a wide range of fresh or green crop cereal species Bruno-Soares et al. (1998) found dry matter intake (IVDMI) calibration equation

with low correlation (R^2=0.41) and high standard error of calibration (SEC= 6.05(g/kgw $^{0.75}$).
The authors concluded that the current equation was less adequate for predicting the intake of green crops by sheep. The study by Gallagher (1990) that carried out feeding trial with White-tailed deer and reported R^2 and SEC values of 0.52 and 12.47 (% BW) for dry mater intake.

Application of NIRS for Predicting the Diet Quality of Non-ruminant Animals

The use of near infrared reflectance spectroscopy to predict the diet quality/ composition was also expanded to non-ruminant herbivores although only in few studies have been reported. Smith et al. (2001) developed a series of calibration equations for poultry. Component measured include nitrogen (crude protein), calcium, phosphorus and gross energy from broiler excreta. Smith et al. (2001) reported high coefficient of determination (R^2=0.88) and low value of standard error of cross validation (SECV= 0.185) for nitrogen, although calibration statistics for the other components were poor. Recently, Xiccato et al. (2003) developed calibration equations for predicting the chemical composition and digestibility of compound feeds for rabbits, a cecal animal like equine. The authors reported coefficient of determination (R^2) and SEC values of 0.84 and 5.7 respectively for CP equation whereas the R^2 and SEC values for the DMD equation were 0.84 and 0.017, respectively.

In the field of human health, several authors (Neumeister et al. 1997; Rivero-Marcotegui et al 1998) have demonstrated the feasibility of NIRS for measuring various chemical constituents from human stool sample. Neumeister et al. (1997) developed near-infrared reflectance spectroscopy calibration equations for predicting the fat and nitrogen concentration. According to the authors, there was a satisfactory correlation between the measurements predicted by NIRS and those produced by standard method with correlation coefficients (R^2) of 0.97 for fat, 0.94 for

nitrogen. Similar values were also found in a study reported by (Benini et al. 1992; Rivero-Marcotegui et al. 1998).

Effect of Species and Physiological State of Animals on NIRS Prediction Capability

A limited study by Lyons and Stuth (1992) examined the effect of physiological status of animals on the calibration. The authors used dry and lactating cows grazed on the same pasture to derive diet-fecal pair data for NIRS calibration. They observed no difference in the calibration statistics with R^2 = 0.70 and SEC=0.1.70 for DOM equations and R^2 = 0.63 and SEC= 0.87 for CP equations. This study demonstrates that physiological stage of animals at least lactation does not affect the predictive ability of fecal NIRS equations.

In another study, Leite and Stuth (1995) applied calibration equation developed for cattle to predict the diet quality of goats and reported that application of the NIRS prediction equations were not successful. The authors concluded that the results indicate that fecal samples from different species of animals vary in their biochemical composition, hence the need for a separate calibration equation for each species

Feeding Behavior of Donkeys

Although available knowledge on the feeding behavior of donkeys is limited, the existing literature has indicated that there is remarkable physiological and behavioral difference between donkeys and other equids (Lamoot et al. 2004) as well as between donkeys and ruminants. Based on the characteristics of the digestive tract, Cork et al. (1999) classified donkeys as hindgut more specifically colon fermenting herbivores and Aganga et al. (2000) characterized donkeys as good grazers and browsers, which eat various species of grasses, forbs and shrubs. In most instances, donkeys graze in communal grazing areas and compete for forages with other livestock both equine (horse) and ruminant (sheep, goats and cattle). Compared with ruminants donkeys have

less selective feeding habit and they eat a variety of plants and plant parts (Jones 1999). During the long dry season when the quantity and quality of the grasses are poor, donkeys browse more and they eat the bark and the succulent layers of trees (Aganga and Tsopito 1998; Canacoo and Avornyo 1998) and maintain a fairly good body condition. Lamoot et al. (2004) studied the grazing behavior of donkeys for two years in the temperate shrub lands. The authors observed that the total diet of grazing donkeys consisted of 19-26 species of graminoid, 38-48 species of forbs and 22-24 woody plant species, which accounts for 80%, 10% and 10% of the diet, respectively.

Despite this flexible feeding habit, seasonal fluctuation in diet quality is still much more pronounced in donkeys than in ruminants. For instance, when sharing the same grazing area, the crude protein content of donkey's diet can be 80% less in dry season conditions than during the wet season, compared only 43% less for goats, 55% for sheep and 68% for cattle (Gebreab et al. 1999). Several authors (Janis 1976; Jones 1999; Mueller et al. 1998) have suggested that donkeys have similar feeding strategy to that of other equids. To compete with ruminants particularly where forage quality is a limiting factor, donkeys use a strategy of high intake, rapid gastro-intestinal transit and low nutrient extraction per unit of feed (Mueller et al. 1998). In addition, the teeth and lips of donkeys permit them to graze close to the ground (Aganga and Tsopito 1998). Another important strategy that donkeys use for compensating their nutritional deficiency is coprophagy. Coprophage in donkeys occurs in young donkeys, which often eat the feces of their dams within a few months after birth, and mature animals when stall-fed on low protein diets (Aganga et al 2000). Coprophagy has been also observed in wild donkeys when forage value, particularly protein is low (Choquenot 1991).

Several authors (Izraely et al. 1989; Cuddeford et al.1995; Pearson et al. 2001) have reported higher digestive efficiency of donkey compared to both ruminant and other equids. Cuddeford et

al. (1995) who compared the digestive efficiency of three breeds of ponies and donkeys reported that feed retention time was relatively longer in donkeys than in ponies and donkeys had higher digestive efficiency than did ponies. These results by Cuddeford et al. (1995) were also confirmed by the work of Pearson et al. (2001). In a study conducted to determine the effect of forage quality on digestibility and gastrointestinal transit time where ponies and donkeys fed oat straw and alfalfa hay, Pearson et al. (2001) found that donkeys retained food residues longer than did ponies (38.8 vs. 29.8 hrs). Similarly Izraely et al. (1989) estimated the mean digesta retention times in donkeys between 36.4 and 37.7 hours. The authors also reported higher digestibility of dry matter, energy, crude protein and fiber fraction in donkeys than in ponies fed the same diet. In another comparative study by Pearson and Merritt (2002), donkeys obtained higher digestibility of dry matter (DM), organic matter (OM) and acid detergent fiber (ADF) than the ponies. Lamoot et al. (2004) concluded that this high digestion efficiency of donkeys compared to other equids enables donkeys to cope more easily in areas where there are adverse nutritive conditions.

In contrast to the above findings, comparative studies between donkeys and ruminant have indicated that donkeys have shorter transit time of digesta and lower digestive efficiency than did ruminants. Izraely et al. (1989) conducted comparative studies between donkeys and Bedouin goats. The authors concluded that the digestive efficiency of donkey was as high as that of the Bedouin goats although the capacity of the donkeys to digest cell wall constituents was lower.

Conclusions

Donkeys play a significant role in providing draught power for the developing world, Despite the obvious socio-economic role that donkeys play in many developing countries, researchers have had neglected these animals for long time and their potential is not fully

exploited, particularly in sub-Saharan Africa. The challenges in using donkeys were found to be shortage of grazing resource and supplements, and inadequate management of these resources. The performance of working donkeys and ultimately their power output is closely linked to the amount of forage consumed (intake) and its quality (crude protein and digestibility). Therefore, an estimation of the quality and quantity of forages consumed by donkeys is extremely useful information for monitoring the nutritional status of the animals and their subsequent management. Most currently used techniques for quantifying the nutritional quality of forages are precise and repeatable for wide variety of forages but they are too laborious, technically complex, time consuming and expensive to be used as management tool under free grazing situation. Therefore, a faster and less laborious and at the same time feasible, repeatable and reliable alternative procedure for nutritive determination is desirable (Andrés et al. 2005)

Prior studies have demonstrated that fecal-NIRS technique could be used as routine tool to generate an objective and reliable indicator for tracking the nutritional status of domestic and wild ruminants. The information obtained via NIRS has been used as the basis for interventions to improve nutritional conditions of free grazing animals both ruminants and non-ruminants. Heretofore, there have been no published reports on NIRS for equines in general and donkeys in particular. Nevertheless today, the world trends in donkey populations and the extensive use of the donkeys for work in the developing countries has led to an awareness of the benefits of studying their nutritional management. It is generally believed that fecal- NIRS as a practical monitoring tool can help to identify trends in the nutritional status of donkeys and improve sustainable management of grazing resources. On the other hand, a study conducted to assess the use of fecal-NIRS calibration equation developed for one species failed to predict the diet quality of forage consumed by another species, indicating the importance of a species-specific calibration equation.

CHAPTER III

DEVELOPING FECAL NIRS EQUATIONS FOR PREDICTING DIET QUALITY OF DONKEYS

Introduction

Donkeys are important sources of draught power for transport and crop production in smallholder agriculture (Pearson et al. 2001), and they play a significant role in the socio-economic life of millions of resource poor people in developing countries (Ghebreab et al. 1999). However, in many countries, the potential of donkeys as draught animals have not been fully utilized (Pearson and Quassat 2000). Nutrition has been a widespread constraint affecting optimum utilization of draught donkeys (Aganga et al. 2001). This problem is mainly due to lack of grazing forage or supplementary feed and inadequate management of those nutrient resources (Muvirimi and Ellis-Jones 1999).

Management of grazing animals generally requires knowledge of the quality and quantity of nutrients that animal can obtain from forage. However, a rapid reliable method of determining the diet quality of grazing equines, particularly donkeys, has been lacking. Prior research has focused on estimating forage quality using various analytical methods, including chemical procedures (Clark et al. 1995), in vitro (Coleman and Moore 2003), in situ (Adesogan et al. 1998) and marker-based in vivo techniques (Van Soest 1982). However, under free grazing situations, analysis of forage samples only provides quality estimates of plant components that the animals could potentially choose from. Human estimation of diet quality via hand plucking plant species and parts has generally been of limited use due to the lack of humans understanding the mix of species that the free ranging animal eats. As an alternative method of visual appraisal of body condition has also been used to monitor the nutritional status of donkeys (Pearson and Quassat 2000) but body condition reflects only past nutrition (Lyons 1990, Stuth et al.1999).

Recent published literatures have indicated that fecal-near infrared reflectance spectroscopy (NIRS) has the potential for predicting the diet quality of free grazing herbivores (Lyons and Stuth 1992; Leite and Stuth 1995). This analysis of feces from grazing animals provides a better idea of what the animals had actually decided to eat and how well they digested the material. Most of prior studies have focused on ruminants and today the technique is used as a routine method for predicting the diet quality of free grazing cattle, sheep, goats, deer and elk (Lyons 1990; Lyons and Stuth 1992; Leite and Stuth 1995; Ossiya 1999; Awuma 2003; Li et al. 2004; Keatin 2005). More recently, several studies indicate a successful prediction of the diet quality via feces of non-ruminant animals, including poultry (Smith et al. 2001), swine (Kemsley et al. 2000), human (Neumeister et al. 1997) and rabbit (Xiccato et al. 1999). However, there are probably few areas of research that have involved free-grazing equines and no attempt has been made to evaluate the potential of the fecal-NIRS technique to predict the diet quality of donkeys. The objective of this study was to develop fecal-NIRS calibration equations for predicting the diet quality of donkeys encountered in free-grazing conditions.

Materials and Methods

Experiment Sites

Data for developing fecal-NIRS calibration equations were generated from two independent studies conducted in United States (Texas), and in Africa (Kenya). Since the same protocol was used during the feeding trials both in the Africa and US studies, the details of the procedures of both studies are presented together. In addition, where there are differences in procedures specific information has been presented for each study.

The Africa Based Experiment

The study was conducted at the Naivasha Research Center, in the facilities of the Kenya Agricultural Research Institute (KARI), in Kenya. The Naivasha Research Center is located at an altitude of 1936-m, lat 0°40'S and long 36°26'E. In Naivasha, the mean annual rainfall is 657 mm (KARI 2004). The experiment was conducted for five weeks, between November and December 2003.

The US Based Experiment

The US based experiment was conducted at the Horse Center, Department of Animal Science at Texas A&M University in College Station, Texas (lat 30°.37' N, long 96°.21' W). College Station has mean annual precipitation of 940mm and varies from 780 to 1100mm, and mean temperature ranges from 10°C in January to 30°C in July (US Department of Commerce 1990 cited in Leite and Stuth 1995).

Experimental Animals

On September 2002, ten mature female donkeys (jennets) ranging from 2 to 6 years of age and mean initial body weight of 196.8±51.9 kg were purchased from private farmers in Texas. Upon purchase, all donkeys were subjected to cogging's test and were shipped to College Station. Donkeys were placed in an approximately 4-hectare native pastureland located in the Rangeland Ecology and Management Field Laboratory, Texas A&M University. Although forage availability was not measured, a visual evaluation of the pasture indicated that there was sufficient forage to keep the donkeys in good condition. On October 2002, all donkeys were subjected to standard quarantine procedures and they were dewormed with Ivermectin and given vaccination against West Nile Virus, Venzuelan Eastern Western Ecephalomyelitis, as well as

Tetanus. In addition, donkeys were subjected to ultra sound for pregnancy test in the Large Animal Clinic, School of Veterinary Medicine. On November 2002, all donkeys were moved to the Horse Center of the Department of Animal Sciences and placed at the Equine Nutrition Laboratory barn. Upon arrival at the Horse Center, the donkeys were identified using a neck bar and confined in a group of 3 to 4 in 6-m x 8-m corrugate sheet-roofed pen. Each group was offered with Coastal Bermuda hay for one week in a common feed trough raised one meter above the ground. Following this period, the donkeys were housed in 3-m x 4-m individual stalls and fed the same hay diet that they had in the previous week and continued for one more week so that they could acclimatize to pen confinement and the new environment. Results from ultra sound diagnosis of the experimental donkeys showed that six of the ten jennies were pregnant. During the first week prior to the initiation of the feeding trial one jenny gave birth to a foal. Since the foal died soon after birth the jenny remained in the experiment and was classified as non-pregnant throughout the trail. As a result five pregnant and five open jennies were used in this study. All experimental procedures and facilities were designed in such way that to fulfill the requirements of the Animal Use Protocol of the Texas A&M University and was approved by the University's Institutional Animal Care and Use Committee.

Diet Preparation

For the US based study, 13 forage and crop residues were collected from different parts of Texas and a total of one hundred composite diets from diverse mixtures of forage and crop residues were hand mixed. For the Africa based study, 40 diets were blended from 12 forage and crop residues collected from Kenya. Each forage source feed was analyzed for its crude protein (CP) level using the standard macro Kjeldahl procedures (AOAC 1995) before blending into forage rations. In these studies the feeds include tropical as well as temperate grasses, legumes

and browse that range in their crude protein (CP) from 3.3% to 21.4 %. Table 3.1 presents the list of different feed types used in the formulation of the experimental diets and their mean crude protein value. The ingredient(s) of each diet then was determined based on the crude protein (CP) level of both the feed (s) and the target diet. In the US study, the most frequently used ingredients were alfalfa hay, coastal bermuda grass hay, bluestem hay and peanut hay. In the Africa study, wheat straw, barley straw, oat hay, maize-stover and lucerne were predominant ingredients (for scientific names see Table 3.1). Additionally, some diets that were previously mixed for sheep and elk experiments were incorporated as ingredients in this study. These diets generally were composed of mainly grasses, forbs and browse widely varying in their crude protein concentration. To minimize difference in physical appearance and thereby prevent sorting by animals each feed type was chopped to 7.5cm length using a grass chopper.

In Vivo Feeding Trial

A series of eleven-week (wk0 to wk10) in vivo digestion trials were conducted between December 2002 and February 2003. The first week was designated as wk_0 or adjustment period. Through out this adjustment period, experimental animals were housed in individual concrete floored stalls and diet, refusal and fecal samples were collected during this week zero. Following the adaptation period, ten, 1-wk periods (wk1 through wk10) were allocated for data collection for in vivo feeding trials. Given the fact that hindgut fermenters such as donkeys and horses need at least four days to balance their intake, clear out previously undigested diets and balance their fecal out put (Gary Potter, personal communication, 2002), each week of in vivo digestion trial consisted of a four days of preliminary period followed by three days of sample collection.

Table 3.1. Common name, scientific name, crude protein concentration (%) and stage of maturity of the major forage and crop residues used in diet formulation in the US and Africa studies

A. Forage used in the US study

No.	Feed type	CP	Scientific name	Stage of maturity of forage
1	Alfalfa concentrate	21.4	*Medicago sativa*	Good quality chopped early mature
2	Alfalfa hay	17.0	*Medicago sativa*	Early mature baled hay
3	Alfalfa mix	15.0	*Medicago sativa*	Late mature sun dried hay
4	Bahai mix	5.4	*Paspalum notatun*	35 days late mature hay
5	Bermuda hay	12.3	*Cynodon dactylon*	30-35 days old sun dried baled hay
6	Corn stalk	4.0	*Zea mays L.*	Late mature crop aftermath
7	Cotton seed hull	4.2	*Gossypium* sp.	Poor quality by product of cotton seed
8	Peanut hay	9.2	*Arachis L. (Fabaceae*)	Good quality early mature hay
9	Ryegrass hay	13.6	*Secale cereale L*	Good quality early to mid mature hay
10	Rice hay	7.8	*Oryza sativa L.*	Late mature sun dried hay
11	Bedding wheat straw	4.6	*Triticum aestivum*	Poor quality crop aftermath
12	Wheat hay	13.5	*Triticum aestivum*	Very good quality early boot stage hay
13	Old world bluestem	3.6	*Schizachyrium scoparium*	Very poor late mature baled for over a year

B. Forage used in the Africa study

No.	Feed type	CP	Scientific name	Stage of maturity of forage
1	Barley straw	7.8	*Hordeum vulgare*	Late mature crop aftermath
2	Blue buffalo hay	3.9	*Cenchrus ciliaris*	Late mature sun dried hay
3	Dried maize stalk	7.1	*Zea mays*	Late mature crop aftermath
4	Lucerne	17.1	*Medicago sativa*	Early to mid mature hay
5	Lucerne mix	13.6	*Medicago sativa*	Late mature sun dried hay
6	Oat straw	6.6	*Avena sativa*	Late mature crop aftermath
7	Oat Hay	7.5	*Avena sativa*	Early mature sun dried hay
8	Poultry waste	10.5	*Na*	Na
9	Red oat	3.3	*Themeda triandra*	Late mature sun dried
10	Rhodes grass	5.5	*Chloris gayana*	Late mature sun dried
11	Rolled Barley	13.6	*Hordeum vulgare*	Processed grain contained late mature
12	Wheat hay	12.9	*Triticum aestivum*	Good quality early mature hay

Feeding Procedures

Animals were fed twice per day at 12-hr intervals (0700 hours-1900 hours) and had free access to feed between successive meal times. The daily feed allowance for each donkey was determined as 2% of the body weight of the animal as recommended by LaCasha et al. (1999) and when necessary adjustment was made based on the previous week intake level. Feed intake of each animal was monitored on a daily basis, calculated from weight of feed offered and feed refused (orts).

During feeding, in an attempt to avoid any discrepancy mainly due to feed intake, a strategy was planned in a manner that feeding experimental donkeys with trial diets started with diets low in crude protein (CP) concentration and gradually increased the concentration across weeks. This procedure was done to avoid aversion selection resulting from a declining diet quality and to promote positive condition through increasing quality of the diet over time. Accordingly during first week (wk1) all donkeys received diets ranging in their crude protein from 5% to 5.9% and then CP level was gradually raised through week nine (wk9). In week nine, donkeys received diets with highest CP level ranging from 18% to 19.4%. However, since the level of protein of diet did not meet the target lowest quality in week one, in week ten all donkeys received diets with lowest (from 4% to 5%) crude protein concentration. No adverse reaction to the low protein diet was noted. Figure 3.1 depicts the mean protein concentration of diets fed to donkeys from wk1 through wk10.

Throughout the experiment period donkeys had free access to trace mineralized lick, and to fresh water. In addition, every day donkeys were turned out from their stall (one at a time) and allowed for 20 minutes exercise under close watch in a large pen with no vegetation or hay present to contaminate the rations. All animals were individually penned during periods of feed access.

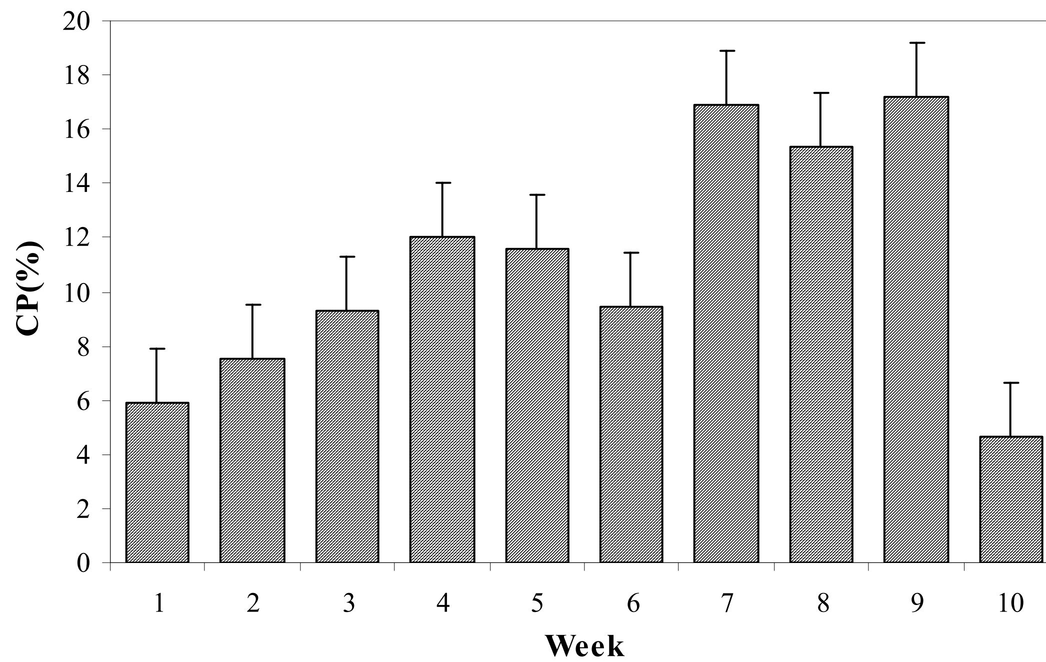

Figure 3.1. Mean crude protein concentration of diets used during the US experiment across weeks. **Error bars are range of means (N=10).

Diet and Ort Samples Collection

In every feeding period, diets were thoroughly mixed and samples were taken before weighing. Feed was measured in a single pan-balance to the nearest gram and feed offered (kg/d) was recorded for each animal. Feed not consumed (kg/d), both from trough and floor were collected twice daily (0700.hours and 1900 hours), and accounted for as orts (refusal). Samples of diets and orts were stored in paper bags at a room temperature for chemical analysis.

Fecal Collection

During the total fecal collection period, sampling was repeated for each diet using the following standard protocol. Throughout the collection period, animals had remained in individual stalls and collection was conducted for three consecutive days (5th, 6th and 7th day). Excreted feces were collected off floor using hand brush and scoop as the event occurred. This 24-hr surveillance procedure was used in order to minimize and/or to prevent any inaccuracy in data collection from coprophage and/or from any other possible contamination by urine, feces (from neighboring animals) and/or feed.

Total feces were collected immediately following each defecation event weighed and sampled at 4 hours interval (a total of six collections per donkey per day). Feces collected during each 4-hour period were stored in plastic tub and weighed using a scientific scale to the nearest gram. After weighing each fecal collection was crumbled, thoroughly mixed, and a 5% (wet weight) representative sample was taken and placed in a sealable plastic zip-lock bag and stored in a refrigerator at -4°C. Where any fecal material was contaminated with either urine, and/or feed, it was saved aside, weighed separately and considered in the total daily output. The total daily fecal output across animal then was computed as sum of the six-four hour collections. For

analysis fecal composites formed by sub sampling from the three-day samples were pooled across animal and week, by diet.

Chemical Analysis

US Based Experiment

For each donkey the three-day composite diet samples and three-day orts were thoroughly mixed and dried in a forced-air oven at $60^{o}C$ for 48 hrs. Samples were ground using a Thomas Mill to pass a 2.00 mm screen, packed in paper bags and stored at a room temperature until used for chemical analysis. Next, each diet and ort sample was analyzed for dry matter (DM), organic matter (OM), and crude protein (CP) contents and corresponding fecal sample was analyzed for DM and OM. Dry matter (DM) and ash were assayed on samples of diet, orts and feces using the standard methods (AOAC, 1995). Dry matter was computed by difference in weight before and after drying in a convection oven at 100-$105^{o}C$ overnight. Organic matter (OM) was determined by ignition each sub sample in a muffle furnace at $500^{o}C$ for 4.5 hours.

The in vivo dry matter digestibility (DMD) across animal by diet was derived using the following model (Osuji et al. 1993).

$$DMD = \frac{(a-b-c)*100}{(a-b)} \qquad [Eq.3.1]$$

Where:

DMD = dry matter digestibility (apparent digestibility)

a = total dry matter offered (kg/d)

b = total dry matter refusal (kg/d)

c = total fecal dry matter (kg/d)

The digestible organic matter (DOM) was computed as grams of organic matter digested for every gram of dry matter ingested and expressed in percentage (Leite and Stuth 1995). Sub-

samples of diet and ort were submitted to the Soil, Water and Forage Test Laboratory at Texas A&M University for nitrogen analysis, which used the standard procedure. Dietary and ort nitrogen was determined on dry matter basis by micro Kjedahl method (AOAC 1995) and then converted to an estimate of crude protein (CP) by multiplying with a coefficient of 6.25. Where ort crude protein level is different from that of diet offered, diet samples values were subjected to correction and the actual crude protein intake by animal was determined. Ort adjusted whole-diet CP values were derived using the following equation by Hack (1987).

$$Y = \frac{(a-(b*d/c)}{(1-d/c)} \qquad \textbf{[Eq.3.2]}$$

Where: Y= ort corrected whole diet CP (%)

a = crude protein of diet (%)

b = crude protein of ort (%)

c = weight of dry matter of diet offered (kg)

d = weight of dry matter of ort (kg)

Africa Based Experiment

In the Africa study, due to loss of the fecal samples before dry matter could be completed on the fecal samples at the Naivasha research center, chemical analysis was carried out only on crude protein and dry matter for the forage samples fed to the animals. Forage samples were also subsequently lost before ashing could be performed due to miscommunication within the Naivasha lab when the fecal samples were accidentally thrown away. Recognizing these potential problems, I chose to attempt to estimate forage ash content based on reported literature of the forage and crop residue species fed in Africa. Nitrogen (crude protein) was analyzed on diet and ort samples using the Kjeldahl procedure while dry matter (DM) was determined on diet and ort samples using standard procedures (AOAC 1995). Both crude protein and forage dry

matter were assayed in the KARI nutritional laboratory in Kenya. Since the fecal samples were lost prior to drying and ashing, fecal dry matter could only be estimating using a value of 27.5% dry matter for all fecal samples. This value represents the average of fecal sample derived in the US study. Organic matter (OM) of diets and ort samples was determined using the standard formula [100-Ash] after dietary ash content was derived from the "book value" ash content of each feed ingredient used in the formulation of that particular diet. Organic matter (OM) of feces was computed by subtracting 2.5 percentage units from the derived fecal dry matter (DM %) value based on the average differences noted in the US experiment.

Digestibility of the dry matter (DDM) and organic matter were computed (OMD) using the standard procedure described by Osuji et al. (1993) (see Equa.1). Digestible organic matter was computed (DOM) using the procedure describe by (Leite and Stuth 1995) as grams of organic matter digested for every gram of dry matter ingested expressed in percentage. Finally the CP, DOM, DDM and OMD values for each diet across animal were used as reference values in the NIRS equation developed.

It should be noted, application of a constant dry matter value to the feces and the derivation of the ash content of the diet from either literature (forage) or lab averages (feces) introduces an error not found in the US digestible study. Therefore, it is expected that there will be considerable detection of significant T outliers in calibration process and subsequent difficulties in conducting validation between the US and African digestibility values.

Fecal Processing and Spectra Collection

Scanning of fecal samples from the US experiment was conducted in the Grazingland Animal Nutrition Laboratory at Texas A&M University and for the Africa samples in KARI in Kenya. Each fecal composite was pooled and dried in a forced-air oven at 60°C for 48 hrs.

Following drying, each sample was ground using a Cyclotec 1093 Sample Mill (FOSS Tecator) to pass a 1-mm screen and saved in paper coin envelope for storage. After grinding, each sample was re-dried in a forced-air oven at 60 ^{o}C overnight (12 hours) to eliminate any recaptured moisture and directly placed in desiccators for one hour to cool to ambient temperature (Lyons et al.1995). Then a sub-sample of approximately 0.75g was packed in a small ring cup (40-mm diameter) and scanned as dry ground powder in reflectance mode (between 400-nm and 2500-nm) using a Pacific Scientific (Neotec) model 6500 monochromator (Perstorp Analytical, Silver Spring, MD, USA). Reflectance data were stored as the logarithm of reciprocal of reflectance (1/R) at every 2-nm interval (Shenk et al. 2001). Inrasoft International software 1.5 version, (Inrasoft International software (ISI) Port Matilda, PA, USA) was used for near infrared spectral data collection, spectral processing and calibration development. At the beginning of each scanning day, a check cell test was performed and if the test did not meet the standard requirements, then instrument diagnostic was carried out. During spectra collection the instrument was operated under conditions of constant temperature (21-24°C) and relative humidity (20-50%). Near infrared spectroscopy analysis of fecal samples from the Africa experiments was carried out in KARI, Kenya using the standard procedures described above on a FOSS 5000 that was calibrated against the NIR spectrophotometer used in the College Station experiment, another source of potential experimental error in the study.

Spectral Pretreatment and Calibration Equation Development

Calibration equations were developed from NIRS spectra derived from fecal samples and equal number of known chemical analytes obtained from conventional chemical analyze of matching diet samples. In developing the most appropriate predictive model, a variety of mathematical transformation functions and smoothing functions were explored. Spectra were

corrected for scattering using Standard Normal Variat (SNV) and detrend. Additionally, mathematical transformation and smoothing functions of the spectra was applied to improve the predictive models. A number of possible combination of derivative (1, 2), gap (4, 8, 12) and smoothing (1) treatments of the spectra were compared. Principal component analysis (PCA) was derived from the spectral data to calculate the distance and samples were viewed graphically (Shenk and Westerhaus 1993). To determine outliers both critical H (10) and critical T (2.5) were explored and number of outlier elimination passes was set 2 times i.e. the program attempted to remove outliers two times before completing the calibration. For calibration the Modified Partial Least-Square regression (MPLS) was used as recommended by several authors (Leite and Stuth 1995; Gordon et al. 1997; Shenk and Westerhaus 1991, 1992; Ruano-Romos et al.1999).

Calibration Equation Selection

In selecting the best calibration equation the following calibration statistics were used: 1) standard error of calibration (SEC), 2) the coefficient of determination in calibration (R^2) and the standard error of cross validation (SECV). The SECV/SD and SECV/Mean ratios were also determined to evaluate the performance of the calibrations (Cazzoloina and Moron 2004). Finally calibration equations were selected based on the lowest SEC and SECV and highest R^2. In addition, the standard error of prediction (SEP), the slope and bias were calculated and considered during equation selection (Shenk and Westerhaus, 1992; Westerhaus et al. 2004). Major wavelengths were selected for each equation and they were examined for their biological significance according to the literature (Awuma 2003). Dominant wavelengths associated with each equation were selected based only on the coefficient of wavelength. Since MPLS model

was used in developing each equation and the model does not compute the F values. Consequently, in the procedure of wavelength selection no F value was t considered. .

Validation of Calibration Equations

Developed equations for all the constituents: CP, DOM, DDM and OMD were validated for their accuracy using an independent dataset that were not part of the population used in calibration development. A subset of 25 percent of the combined US/Africa calibration set was selected using the SELECT program of WINISI software and saved as independent set for validation the performance of the US/Africa equations. Equation validation of the Africa calibration for CP, DOM, DDM and OMD was conducted using the US calibration set while the US calibration equations were validated using the Africa data sets as external sample set. In all these validation exercises, validation was carried out both with and without the samples that were identified as critical T outliers using T value of 2.5 as recommended by Workman (1992).

Results and Discussion

The resultant ranges of chemical composition (CP) and functional properties (DOM, DDM, and OMD) of diets from both the US and Africa studies are presented in Table 3.2.

Nutrient Concentration and Digestibility of the Diets Used in the US Study

In the US study, a total of 100 diets with matching fecal samples were generated. Diets were blended from 13 different forage and crop residues (Table 3.1) and resulted in diverse chemical composition and functional properties. In an attempt to obtain the target diet mixture, a number of feeds ranging from a single feed type per diet to as many as ten feed types were used. The resultant diets for present study ranged in their crude protein content between 4.1% and 19.4%

with a mean of 10.9%±4.3 SD (Table 3.2). The digestible organic matter ranged from 12.1 to 61.9 % (mean value of 44.9%±6.1 SD). The mean apparent dry matter digestibility and organic matter digestibility of diets were 54.2%±8.7 SD and 53.2% ±7.9 SD, respectively.

Nutrient Concentration and Digestibility of the Diets Used in the Africa Study

A total of 40 diet samples with matching fecal data were obtained from the Africa study. Diets were mixed from 11 tropical forage and crop residues. Concentration of dietary crude protein for Africa studies ranged between 3.8% and 14.% with an over all mean value of 8.7% ± 2.9 SD (Table 3.2). The average in vivo digestible organic matter (DOM), dry matter digestibility (DDM) and organic matter digestibility (OMD) was 34.9% ± 12.6, 35.5 ± 12.7 SD and 38.9± 13.4 SD, respectively.

Spectrum Data

Scanning of fecal samples resulted in NIRS spectra over the range from 1100 to 2498nm, yielding a spectrum of 700 data points. Fecal spectral information was first stored as $\log_{10}$ (1/R), where R is the percentage of reflectance and transformed using math treatments (2,4,4,1). The Mahalanobis distance (H statistics) of each spectrum with respect to the average spectrum has shown that of the total sample sets about 97% and 98 % were less than 3 for the US and Africa dataset, respectively, confirming their proximity to the population mean with only 3 and 2 outlier samples for US and Africa, respectively. Even though the outlier numbers was higher for the US fecal spectra than for that of Africa, the US fecal spectra had better distribution of samples than the Africa spectra.

Inclusion of the Africa data set into the US data set further improved the spectra distribution and out of the total 105 samples still only 3 samples were identified as significant H outliers

Table 3.2. Range, mean and standard deviation of the chemical composition and functional properties of mixed diets used in the US and Africa studies expressed as percent

	US				Africa			
Functional property	N	Range	Mean	SD	N	Range	Mean	SD
CP	100	4.1-19.4	11.0	4.3	40	3.8-14.0	8.5	2.73
DOM	100	12.1-61.9	44.8	6.6	40	15.1-74.0	34.9	12.6
DDM	100	14.5-75.8	54.1	8.7	40	16.1-75.7	35.5	12.7
OMD	100	34.1-74.0	53.2	7.9	40	17.5-79.0	38.9	13.4

CP= crude protein
DOM= digestible organic matter
OMD= organic matter digestibility
DDM= dry matter digestibility
N= number of samples
SD= standard deviation

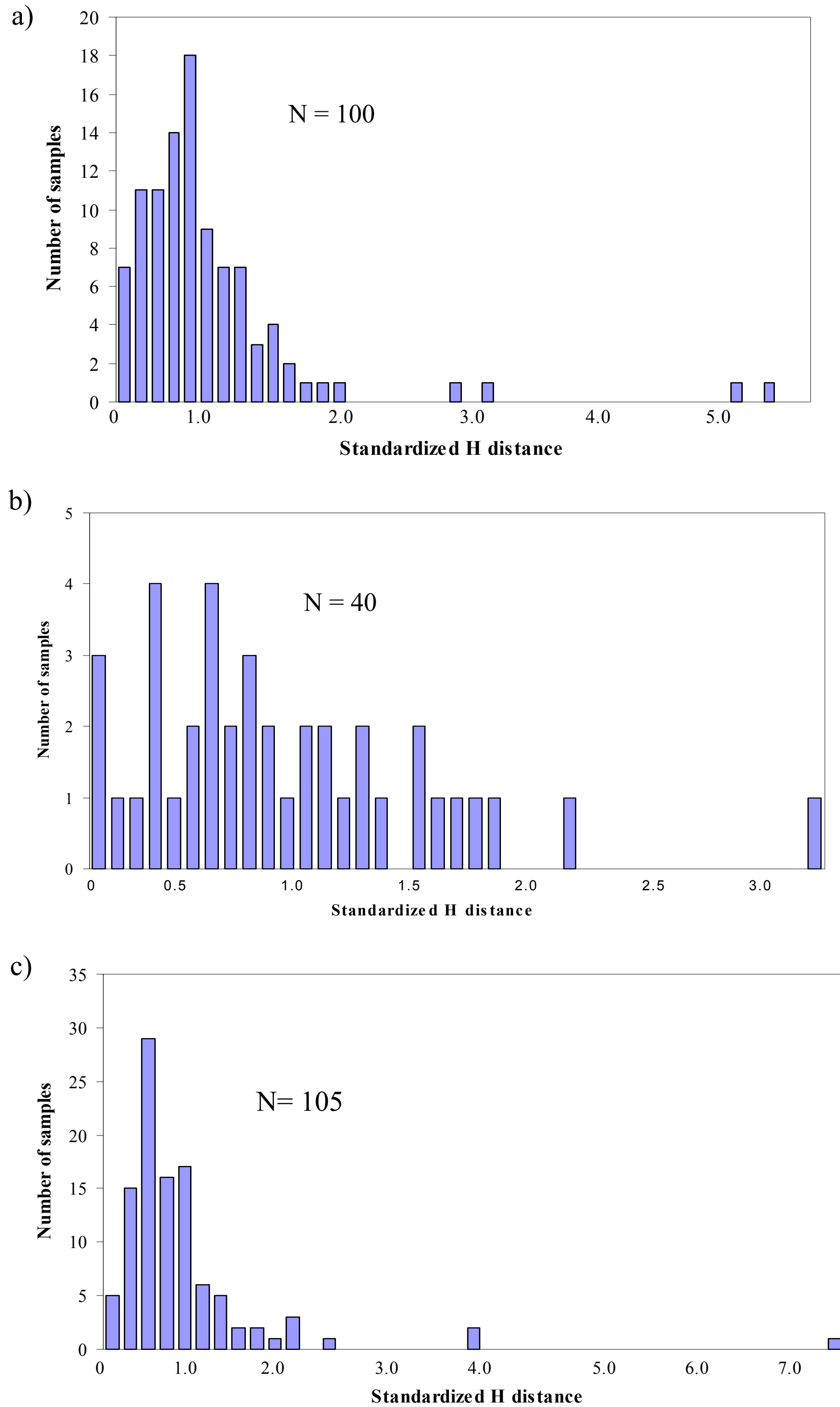

Figure 3.2. The histogram of standardized H values of the a) US, b) Africa and c) US/Africa fecal spectra measured from the mean using second derivative (2,4,4,1), SNV and detrend procedures.

from the US/Africa combined set. These results indicate that majority of the samples were spectrally within the range of the same population of spectra. Figures 3.2a, 3.2b, and 3.2c depict the distribution of the standardized Mahalanobis (H) distance from the sample population mean for the US, Africa and US/Africa combined set, respectively.

Calibration Equations

Crude Protein (CP) Equations

In total, three calibration equations were developed for CP: "US equation" which include data from the US based experiment, an " Africa equation" included data from Africa experiment and "US/Africa equation", that consisted of samples from both the US and Africa experiments. Table 3.3 presents the calibration and cross-validation statistics for US, Africa, and US/Africa CP equations. All CP equations were derived by regressing the crude protein (CP) values obtained by standard chemical analysis of diets samples (reference) against the spectra derived from matching fecal samples using modified partial least square (MPLS) model.

US CP Equation

The US CP equation was developed using 96 diet-fecal pair calibration sets. Diets used in the calibration ranged in CP concentration from 4.1% to 19.4 %. The equation was developed using second derivative (2,8,8,1) data treatment of the calibration spectra $\log_{10}$ (1/R). A critical T-value of 2.5 was set to determine the outlier samples and of the total population only 4 outlier samples were eliminated. The standard error of calibration (SEC) and coefficient of determination (R^2) for the US CP equation were 0.77 and 0.97, respectively (Table 3.3). Also cross validation was used to test the predictive ability of the equation and resulted in a standard error of cross validation (SECV) of 1.194. The three major wavelengths related to the US CP

Table 3.3. Calibration statistics for crude protein (CP%) equations for the US, Africa and US/ Africa calibration sets with SEL value used (0.5)

Equation	N	Mean	SD	SEC	R^2	SECV	SEP (C)	Bias	Math	SECV/SD	SEC/Mean	Dominant Wavelengths (nm)
US-CP	96	11.0	4.3	0.77	0.97	1.19	1.55	-0.72	2	0.28	10.8	1596 1600 1604
AF-CP	39	8.6	2.8	0.88	0.90	1.03	1.35	-0.62	1	0.39	13.0	2204 2200 2332
US/AF-CP	101	10.2	4.2	0.97	0.95	1.25	1.6	-0.75	2	0.40	16.0	1588 1596 1604

US= USA
AF = Africa
CP = crude protein
N= total number of samples included in calibration equation,
SD= standard deviation,
SEC= standard error of calibration,
R^2= coefficient of determination,
SEV= standard error of cross validation,
SEP standard error of prediction,
Bias= mean of differences
Math= 1st or 2nd derivative of log (1/R) spectra
SECV/SD= standard error of cross validation to standard deviation ratio,
SECV/mean = standard error calibration to population mean ratio
SEL = standard error of laboratory reported by Soil, Water and Forage Test Laboratory

equation include 1596, 1600, and 1604-nm. These wavelengths are associated with N-H (1535-1612) and NH- in proteins (1535-1614) and protein fractions i.e. amid I, II, III. Murray and Williams (1987) suggested that these wavelengths are within the range of associates with COO stretch, and combination band of most amino acids.

It was suggested that most of the fecal amino acids and nitrogen compounds were probably originated from microbial protein (cell wall) and from sloughed walls of the large intestine of the donkeys, which is a common event in hindgut fermenters. Several literatures (Van Soest 1994, Cork et al. 1999) indicated that one significant difference of hindgut fermenters such as donkeys from the ruminant strategy is that large quantity of microbial protein generated in the large intestine is excreted as feces because there is no opportunity for absorption of amino acids in the cecum. The present wavelengths are similar to those reported by Awuma (2003). The first wavelength is close to wavelength of 1564-nm and the second and third wavelengths are close to 1620-nm associated with CP equations developed for sheep and cattle, respectively.

Africa CP Equation

The equation was developed using SNV-detrend procedures and treated with second derivative data treatment (2,8,8,1). After eliminating possible outliers via the critical T value (2.5), the equation incorporated 39 of the total calibration set. The standard error of calibration (SEC) for the Africa CP equation was 0.88 with corresponding coefficient of determination (R^2) of 0.90 (Table 3). The Africa CP equation had a standard error of cross validation equal to (SECV) 1.03, which is slightly higher than the standard error of calibration (SEC).

For the Africa CP equation the selected dominate wavelength were 2200, 2204 and 2332-nm. The region 2332-nm is associated with both aliphatic and aromatic amino acids, amide IV, N-H bend and primary amides. The 2332nm-wavelength was slightly higher than those reported

by (Leite and Stuth 1995), who found greater absorbance of high quality samples at 2305-nm for CP equation. The region that appeared more frequently in the Africa CP equation ranges from 2200 to 2204 -nm. These wavelengths fall in the range of wavelengths related with $CH=CH_2$, NH_3+ and NH deformation chemical bonds among others, which are reported in amine and imides plus amino acids II (Murray and Williams 1987). Lyons and Stuth (1992) reported that wavelength 2219 was a major wavelength used in the formation of their CP equation for cattle, which is close to the present wavelength. However, the authors suggested that the wavelength was possibly associated with undigested dietary residues of cell wall carbohydrates, which presents in the feces originated from poor quality forage with high fiber content. Similarly, Coleman and Murray (1993) suggested that the region 2100-2200 associated with dry matter degradability.

As mentioned earlier, the fecal amino acids may be originated from both microbial protein synthesized in the hindgut and sloughed walls of the large intestine of the donkeys. Due to inability to absorb amino acids, microbial proteins manufactured in the large intestine are excreted as feces. This contributes considerably to the fecal protein or amino acid content that partially explain the association of the present wavelengths to CP equation.

US/Africa CP Equation

The US and Africa CP calibration sets were combined to create one US/Africa calibration set (N =140). This total sample set was divided into two sub samples of 105 and 35 diet-fecal pairs, formerly used for calibration and the latter for validation. Inclusion of the Africa sample set into the US sample set expanded the range of the dietary protein. As a result the US/Africa CP equation was developed from diet samples with crude protein concentration ranging from 3.8 % to 19.4%, which exceeded the range for the US and Africa individual calibration sets. In

developing the selected equation second derivative (2,4,4,1) math treatment with SNV-detrend procedures were used. Outliers were identified using the recommended critical T value of 2.5 and in total 5% of the calibration samples were found as outlier samples. The resultant standard error of calibration for the US/Africa CP equation was 0.97 with corresponding coefficient of determination of 0.95. The standard error of cross validation for this equation was 1.25, which is higher than the computed standard error of calibration (SEC) for the equation. The three dominant wavelengths for the combined US/Africa CP equation were 1588, 1596 and 1604-nm. These wavelengths are in accord with those reported for the present US CP equation. As indicated previously the wavelengths are associated with protein and protein fraction materials including amino acids and amines. Fecal amino acids are primarily microbial in origin and some may be from endogenous sloughed intestinal walls (cecum and large intestine walls) excreted from hindgut fermentation. Van Soest (1994) pointed out that in non-ruminant the hindgut fermentation of fiber promotes fecal nitrogen loss in the form of microbial amino acids because fermentation site is past the point of gastric digestion. Therefore it is expected that a considerable amount of microbial amino acid appear in fecal material of donkeys. Further information on the associated chemical and biological values of the wavelengths is presented in Appendix Tables 9- 16.

Discussion of CP Calibration Equations

Present calibration statistics showed that the US CP equation had the lowest SEC value compared to both the Africa and US/Africa CP equations. The highest SEC value was observed for the US/Africa CP equation and an intermediate value was observed for the Africa equation. The relatively high SEC value for the US/Africa equation may be the result of the wide range of calibration sample sets which may explained by a relatively large number of diet mixtures from

different forage species and locations. Highest coefficient of determination (R^2) was obtained for the US CP equation with intermediate for US/Africa and relatively low for the African CP equation. The relatively low R^2 for the Africa CP equation was probably due to the small number of calibration set, i.e. less than fifty (<50) and lower range of CP value within sample sets.

Results of calibration indicated that during the calibration process in all three CP equations less than 5% of the samples were eliminated as T outliers, which is within the acceptable limit. Incorporating the major proportion of the calibration set (>95%) indicates that the calibration equation had covered the full range of attribute values of samples while maintaining acceptable accuracy. On the other hand, the small number of outliers suggests that there was not much discrepancy in the fecal spectrum and matching wet chemistry introduced in sampling of the forage, orts and feces as well as error introduced in lab technique between the two laboratories, consequently the equations covers a wide range of the diets CP values.

In the present work the standard errors of calibration (SEC) for the US, Africa, and US/Africa CP equations were less than twice the laboratory standard error set for crude protein (SEL=0.5, reported by the Soil, Water and Forage Testing Laboratory, at Texas A&M University) indicating acceptable limits for NIRS calibration equations (Hruschka 1987). Moreover, the statistical parameters of calibration for the present US, Africa and US/Africa CP equations appear to be comparable with findings reported in other studies both in domestic and wild ruminants.

In general the US CP equation had the lowest SEC, which was lower than those reported for cattle (Gibbs et al. 2002), for goats (Leite and Stuth 1995) and for elk (Keating 2005). The SEC values for all the three equations were also close to those reported in literature (Lyons et al. 1992; Lyons et al. 1992b; Purnomoadi et al. 1996; Showers 1997; Ossiya 1999a, Awuma 2003) on cattle.

In terms of coefficient of determination the present US, Africa and US/Africa CP equations had R^2 above 0.90 indicating excellent calibration equations. Figures 3.3a, 3.3b and 3.3c depict the relationship between the measured and NIRS predicted crude protein concentration for the US, Africa and US/Africa CP equations, respectively. Particularly, the US and US/Africa CP equations had coefficient of determination higher than those reported by other authors working with ruminant (Lyons et al. 1992b; Leite and Stuth 1995; Purnomoadi et al. 1996; Showers 1997; Ossiya 1999; Gibbs et al. 2002; Awuma 2003; Li et al. 2003; Boval et al. 2004; Keating 2005).

Even though the US CP equation had highest coefficient of determination and lowest standard error of calibration the standard error of cross validation was slightly higher than that of Africa and lower than that of combined US/Africa CP equations. This relatively high standard error of cross validation may be explained partly by high within animal variation. In the present study, a standard error of 0.3 was obtained for protein due to individual animal variation. The present SE is in accord with those reported by Stuth et al. (2003) who obtained (SE) due to animal variation between 0.17 and 0.34 for protein.

The SEC values for the present CP equations were less than twice the standard error of laboratory (2 x 0.5) indicating there was little error introduced due to laboratory procedures. Moreover, the standard error of cross validation (SECV) represents approximately 10.8%, 13% and 16% of the error of the mean crude protein concentration of the reference for the US, Africa, and US/Africa calibration set, respectively.

Recently, Cazzolina and Moron (2004) suggested that in addition to the standard statistical parameters (SEC, SECV and R^2) used in evaluation the performance of calibration equations, the ratio of standard error of cross validation to the standard deviation (SECV/SD) demonstrates how well the calibration models performed for chemical data such as crude protein. They concluded that where the error is close to one-third of the standard deviation (SD) of the

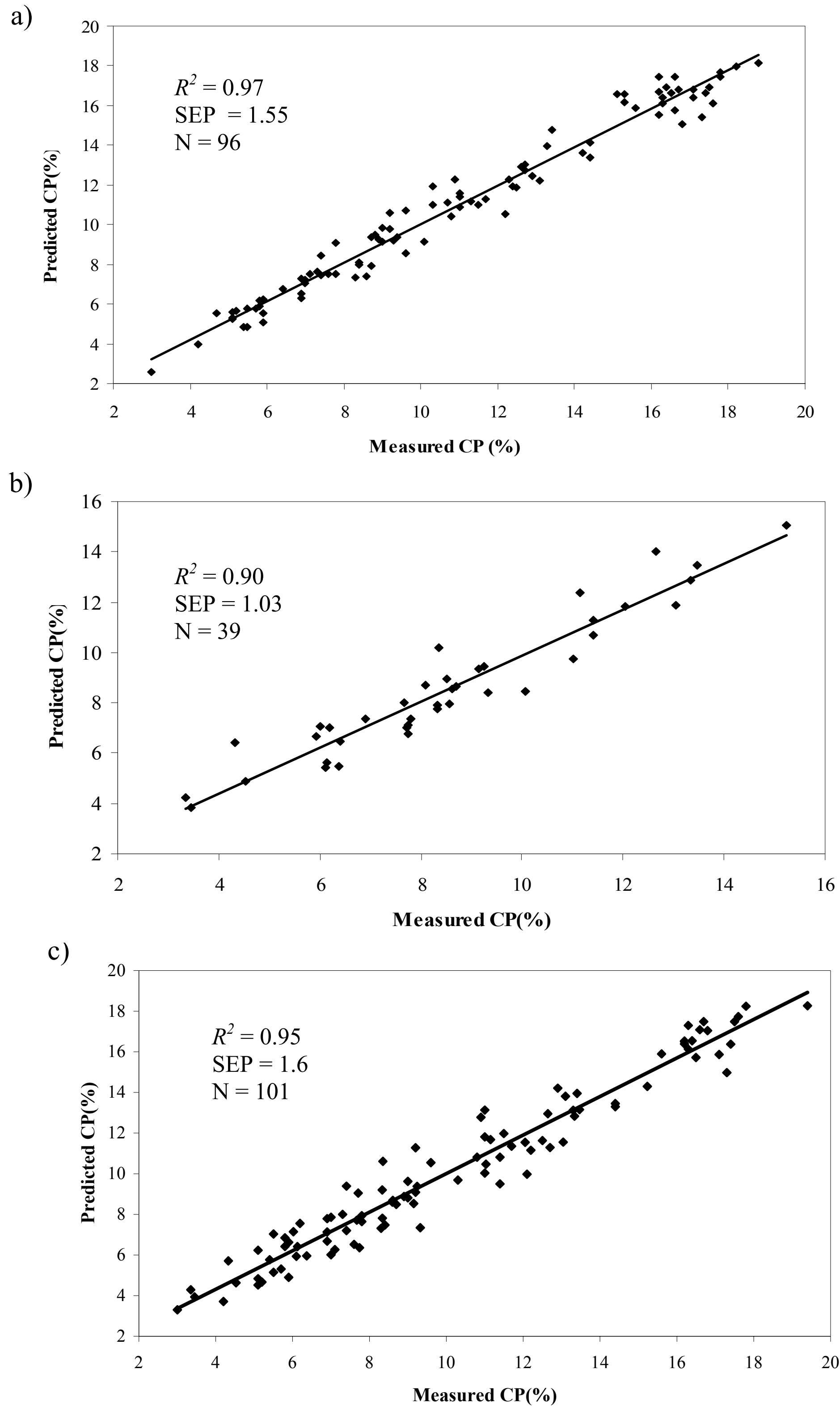

Figure 3.3. Relationship between measured and NIRS predicted crude protein (CP) concentration for a) US, b) Africa and c) US/Africa calibration dataset. The line Y=X represents agreement between predicted and measured CP values.

population in the calibration set, regression indicates good stability of the calibration. Results from the present study showed that SECV/SD ratio were, 0.28, 0.39 and 0.40 for the US, Africa and US/Africa CP equations, respectively, indicating the US CP equations was much more stable than the other two equations with the latter still at intermediate stability. In general, on the basis of the various calibration parameters used to evaluate the performance of the CP equations, present results indicated that the CP equations were successfully developed.

Digestible Organic Matter (DOM) Equations

In total, three calibration equations for digestible organic matter (DOM) were developed for the US, Africa and US/Africa calibration sets. Calibration was carried out by regressing the in vivo derived digestible organic matter (reference) against spectral information derived from NIRS scanning of matching fecal samples. The resultant statistical parameters for the in vivo digestible organic matter (DOM) calibration equations are presented in Table 3.4.

US DOM Equation

Second derivative (2,12,12,1) data treatment along with SNV-D transformation procedure of data points was performed in developing the selected US DOM equation. The US DOM equation was developed with 91 sample sets after nine T outlier samples were removed from the calibration set. The results of the calibration are also presented in Table 4. The standard error of calibration (SEC) for the US DOM equation was 2.58 with corresponding coefficient of determination (R^2) equals to 0.60. This R^2 value indicates that the majority of the variation in the digestible organic matter in the calibration is explained by the equation. Interestingly, in relation to the relatively low SEC value obtained for the DOM equation, the low R^2 was unexpected.

Table 3.4. Calibration statistics for digestible organic matter (DOM %) for the USA, Africa and USA/Africa calibration sets

Equation	N	Mean	SD	SEC	R^2	SECV	SEP (C)	Bias	Math	λ
										1732
US DOM	93	45.4	4.1	2.58	0.60	2.80	3.65	-1.68	2	1796
										1724
AF-DOM	38	33.8	11.2	4.91	0.81	5.60	7.34	-3.39	1	1468
										1148
										1156
US/AF-DOM	98	41.4	8.9	3.51	0.84	4.34	5.64	-2.6	2	2104
										2192
										2272

N=total number of samples used for calibration;
SD= standard deviation
SEC= standard error of calibration
R^2= coefficient of determination
SEV= standard error of cross validation
SEP standard error of prediction
Bias= mean of differences
Math= 1st or 2nd derivative of log(1/R) spectra
λ = wavelength
SEL value of 1.68 reported by Leite and Stuth (995) was used to compare the DOM calibration results

The standard error of cross validation (SECV) for the US DOM equation was 2.8, which is slightly higher than the standard error of calibration (SEC).

The dominant wavelengths selected for the US DOM equation were 1724-nm, 1732-nm, and 1796-nm, which are associated with aromatic and aliphatic compounds. These wavelengths also were associated with CH protein and C-H stretch, NH-bend such as amides, primidines and quinolines among others. Coleman and Murray (1993) suggested that regions at 1724-nm could related to animal lipids (fat and oils) consisting of sloughed intestinal walls excreted from hindgut fermentation. According Wilman et al. (2000) the 1620-1820-nm region associated with low cell wall degradability and the presence of aromatic compound such as lignin that may be characterized by C-H stretch. Awuma (2003) also found 1788-nm associated with DOM equation developed for sheep. The presence of low cell wall degradability and the presence of aromatic of lignin and other fibrous residues in the feces indicates that the release of some of the carbohydrate such as hemicelluloses, lignin and pectin materials that escaped hindgut fermentation. Usually some of the proteins and carbohydrates in the plant or microbial cell are surrounded by more fibrous part of the cell wall.

Africa DOM Equation

The Africa DOM equation was developed using first derivative (1,4,4,1) data treatment and transformed by the standard normal variat and detrend (SNV- D) procedures. A critical T value of 2.5 was used to identify outlier samples and the equation incorporated 38 (90%) of the 40 diet:fecal paired samples. The Africa DOM calibration equation had standard error of calibration SEC = 4.9, and standard error of cross validation value of SECV =5.6 According reports by Lyons and Stuth (1992) and Leite and Stuth (1995) the SEL value for GANLAB were 1.68 and 1.57, respectively. The coefficient of determination (R^2) for the Africa DOM equation

was equal to 0.81, indicating that more than 80% of the variations of the in vivo values were explained by the calibration equation. The major wavelength selected for this particular equation includes 1148-nm, 1156-nm and 1468-nm. These wavelengths are chemically associated with OH- (hydroxayl) phenol, saturated aliphatic carboxyl compound, α-β unsaturated aldehydes, and ketones. According to Leite and Stuth (1995) these chemical structures associated with the presented selected wavelengths are reported to be in all starch and cellulose containing substances. In addition, NH_2 amine and primary amine, CH3 and =CH2 groups also associated in this regions. The present wavelengths 1148-nm and 1156-nm fall in the same region to those reported by Awuma (2003) who found 1154-nm was associated with DOM equation developed for sheep using fecal samples. Deaville and Givens (1998) suggested that the region 1430-1630-nm was associated with low cell wall degradability of forage whereas Coleman and Murray (1993) associated the region with degradability of dry matter of forage in cattle.

Absorption of aliphatic bonds in the present DOM equation could be associated with aliphatic amino acids such as glycine, and essential amino acids such valine, alanine and luecine and isoleucine as suggested by Showers (1997) as little of these amino acids is taken up by the large intestine of hindgut fermenters like donkeys.

US/Africa DOM Equation

The DOM equation for the US/Africa combined calibration set was developed from samples sets with values ranging from 12.1% to 74.0 % and a mean of 41.4%± 8.9 SD. Data points were transformed using the standard normal variat and detrend procedures. Second derivative (2,4,4,1,) data treatment was also used. After eliminating 5 possible T outlier samples the selected DOM equation was based on 100 (97%) calibration sets. Calibration and cross validation statistics for the US/Africa equation is also present in Table 3.4.

The US/Africa DOM equation had a standard error of calibration (SEC) and coefficient of determination (R^2) of 3.5 and 0.84, respectively. Cross validation was also performed to determine the predictability of the equation and the resultant standard error of cross validation (SECV) for the calibration set was 4.3.

For the combined US/Africa DOM equation dominant wavelengths were 2104-nm, 2192-nm and 2272-nm. The wavelength 2104-nm is associated with all amino acids, secondary amides, amines, and carbonyls, the 2192-nm is associated with urea and amide I, whereas the 2272-nm is associated mostly with pyrimidines and quinolines. As mentioned earlier, most of the fecal amino acids may probably be originated from the microbial proteins in the cecum and colon of the animals that escaped absorption. Literature has indicated that loss of microbial amino acid nutrients in the feces is greater in the hindgut ferementers than ruminants. The presence of microbial amino acids in the associated chemical bond for DOM could be expained by the fact that total organic matter fraction of feces is a composite of microbial cell wall (Awuma 2003) from the hindgut that escaped absorption. The wavelength 2192-nm was close to 2184-nm, which are reported to associate with crude fiber (Purnomoadi et al. 1996). The same wavelength was similar to 2188-nm reported by Ruano-Ramos et al (1999) and considered to relate to minerals.

Discussion of DOM Calibration Equations

The US/Africa DOM equation had the highest coefficient of determination (R^2=0.84) followed by the Africa DOM equation (0.81). Inclusion of the Africa samples into the US calibration set resulted in increased standard error of calibration and coefficient of determination by 1.5 and 0.24 units, respectively (Table 3.4). In addition, the BIAS value was inflated by almost 1% in the US/Africa DOM calibration equation as compared to the US DOM calibration.

Even though there was fairly high SEC value, the relatively high coefficient of determination for the Africa and US/Africa calibration indicated that there was good correlation between the measured and NIRS predicted values of the DOM for the calibration set. The high coefficient of determination for the US/Africa data was probably due to wide range of samples in the calibration set. On the other hand, despite the low SEC value in the present study, lowest coefficient of determination was obtained for the US DOM equation. Coates (1998) and Workman (2001) noted that R^2 could be influenced by the range of values within the calibration set. The US DOM equation range (12.1-61.9%) had only 3% below 30 and 13 above 50%. This wide range of values with less number of samples at extreme could contribute to the low R^2 for the US DOM equation.

Although there has been no NIRS equation established for prediction the in vivo or in vitro digestible organic matter in equines, in comparison with previously published data set for ruminant, the present DOM equations showed comparable precision with relatively intermediate standard error of calibration (SEC). The US DOM equation when compared to the literature the SEC value was considerably lower than those reported for cattle DOM equations by Ossiya (1999a) (SEC= 3.39), and Awuma (2003) (SEC =3.02), and also lower than those reported for sheep by Ossiya, (1999a) (SEC= 3.21) and Awuma (2003) SEC =2.86). In other studies conducted in Australia, Coates (1998) and Gibbs et al. (2002) developed a calibration equation for predicting the in vitro digestible organic matter for cattle and reported SEC values ranging from 2.2 to 3.3 and 2.38 to 2.63, respectively. Awuma (2003) also developed calibration equation for predicting the in vitro DOM for sheep in Africa and found SEC value 2.4. In contrast to present results, other authors working with ruminant had shown lower SEC values. These include Lyons and Stuth (1992) (SEC=1.75) who reported on cattle, and Li et al. 2004 (SEC=1.51) who reported on sheep.

Even though the present results seem somewhat inferior to those reported by Lyons and Stuth (1992), and Li et al. (2004), compared with other earlier works, the precision of the estimate achieved in the present US DOM equation appeared to be comparable particularly with most reports on cattle (Coates 1998; Gibbs et al. 2002; Awuma 2003). Lyons and Stuth (1992) working with cattle and Leite and Stuth (1995) working with goats reported that the standard error of laboratory (SEL) for in vitro DOM in the GANLAB was 1.68 and 1.57, respectively. In the present study, the SEC (2.58) for the US DOM equation was slightly lower than twice the reported SEL in GANLAB (SEL= 1.68), indicating insignificant error was introduced due to laboratory/experimental procedures.

In terms of coefficient of determination, both the US/Africa (R^2 =0.84) and Africa (R^2 = 0.81) DOM equations were quite satisfactory although relatively low and unexplained coefficient of determination was obtained for the US DOM. Figures 3.4a, 3.4b, and 3.4c depict the mathematical relationship between the measured and NIRS predicted digestible organic matter for the US, Africa and US/Africa calibration sets, respectively. The resultant coefficient of determination for the Africa and US/Africa DOM equations deemed to be comparable with those findings by Lyons and Stuth. (1992), Ossiya (1999a), and Keating (2005) who reported lower coefficient of determination for *in vitro* DOM in cattle and elk, respectively.

From the knowledge of the author, yet there is no data available on equines to compare the present results with therefore, most comparisons would be with reports on ruminants. Comparison of the present result with some of the prior works in ruminants has shown that the present calibrations seem less accurate. However, it is a noteworthy fact that earlier published prediction equations for DOM were derived from in vitro or in situ digestion trials and have

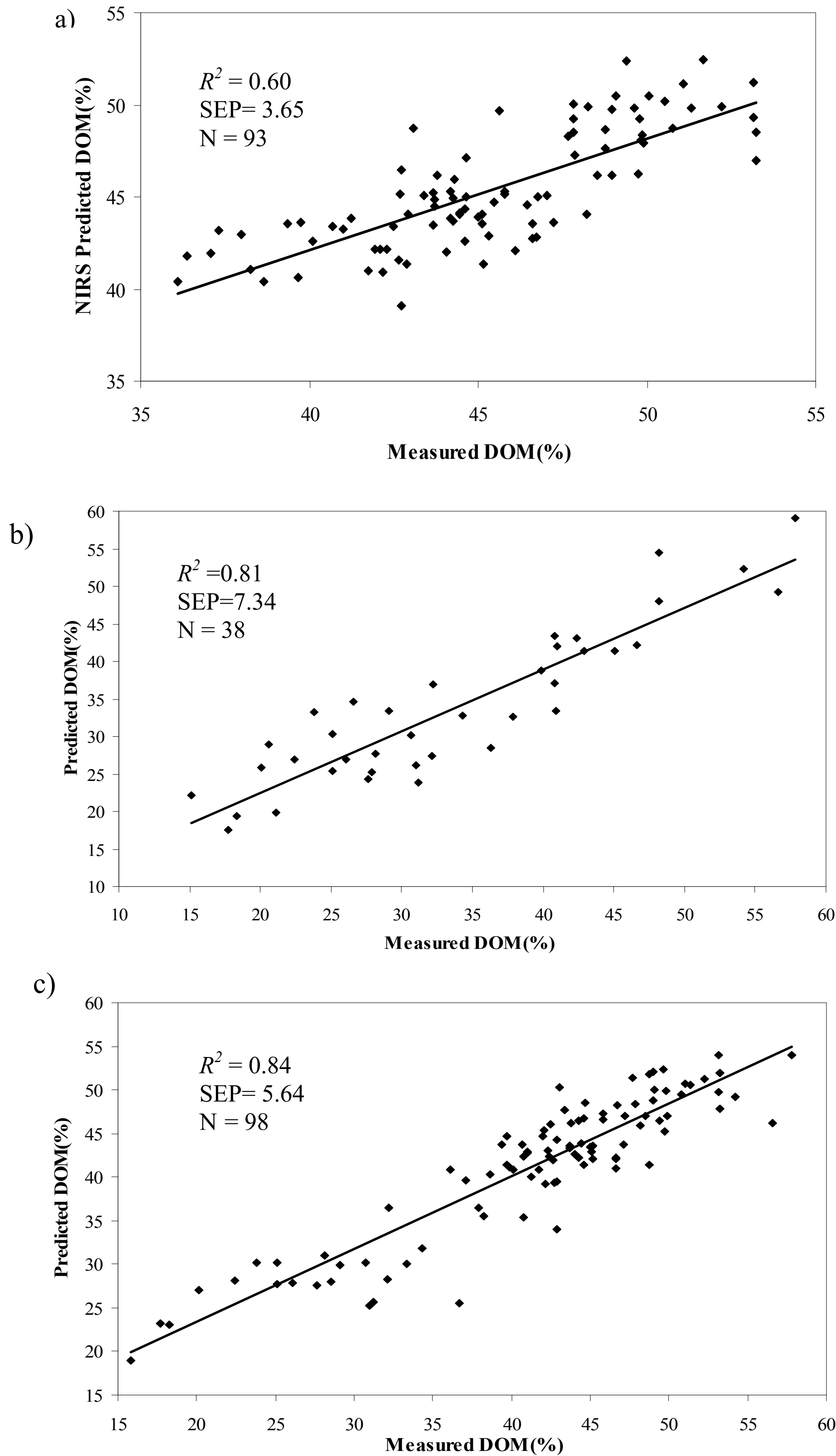

Figure 3.4. Relationship between measured and NIRS predicted digestible organic matter concentration for a) US, b) Africa and c) US/Africa calibration datasets after outlier samples were removed. The line Y=X represents agreement between predicted and measured DOM values.

relatively less source of error than those data derived from in vivo trials (Coates 1998, 2000b). On the other hand, in vivo estimates of digestibility derived from total fecal collection are subjected to a wide range of variations due to both animal and diet factors (Van Soest 1994), which had considerable influence in the prediction accuracy of the present calibration equations. Agnew et al. (2004) concluded that the first obstacle to the development of useful predictive equations was the effect of within and among animal variations. This conclusion was supported by data reported from feeding trial carried out with ruminants. Recently, Boval et al. (2004) observed that between animal variations as high as 12% for DOM. Similarly Li et al. (2004) who developed DOM equation for sheep reported that 25-30% error in prediction was attributed to within animal variation. Stuth et al. (2003) working with steers reported standard error (SE) values associated with individual animal variation was varied from 0.22-0,35 units of DOM. In the present study (unpublished data) donkeys were fed with week zero diet (Coastal Bermuda hay, for scientific name see Table 1,with 9% CP, for one week) and DOM was predicted using the selected equation and an SE value of 0.30 units of DOM, agreeing with the Stuth et al. (2003). Boval et al. (2004) noted that these errors due to animal variation could contribute to the increase in SEC and decreased R^2 values.

Another possible factor for the higher SEC values for present in vivo DOM equations could be the heterogeneous nature of the diets used in the experiment. Recently, Boval et al. (2004) indicated that NIRS measures the associate effects of diets and diet type can have great impact on the accuracy of the calibration equations. The present equations were developed from diet-fecal-pair calibration sets that derived from diverse forage and crop residue feeds. This diversity therefore can influence the accuracy of the DOM equations through its effect on spectral characteristics.

Despite the relatively high SEC value in the present study, the standard error of cross validation (SECV) to population mean ratio indicated that SECV account approximately an error of 6.3%, 10.3 % and 16% of the mean value of the in vivo DOM data set, respectively. The results suggested that the US DOM equation has acceptable error compared to the other two equations. In all the DOM analyzed the SEC values were considerably lower than the standard deviation (SD) for their respective population, indicating the present DOM calibration equations (US, and US/Africa) could be used to determine the concentration of DOM. When standard errors of cross validation to standard deviation ratio (SECV/SD) was determined as measure of model stability, the US, Africa and US/Africa DOM equation had a value of <0.42, indicating an intermediate stability of the equations.

An interesting observation in the present study was that while a remarkable improvement in terms of R^2 was obtained by combining the US and Africa calibration sets, there still was a reduction in the SEC value. The main contributor factor for the cause of deficiency in calibration of the US/Africa equation is the type of reference DOM from Africa which is in turn attributed to the poor technique used in deriving dietary and fecal attributes (DM, OM). The improvement in R^2 may be partially explained by an increase in sample number (Coates 2000b), expanding data range and improving the distribution over data range (Lyons and Stuth 1992). In general, results have indicated that both US and US/Africa models were in acceptable level of accuracy although the Africa equation appeared to be poor in terms of SEC. From a practical standpoint, the calibration sets used in developing the present equations have advantages over the earlier works. The present US and US/Africa DOM equations have incorporated a large number of the reference data obtained from wide range of forage and crop residue, which can partly explain the reason for the relatively moderate calibration statistics, reflect the actual situation of grazing lands that the animals face in reality. Therefore the equation can be used to predict the DOM

concentration within a wide varied population botanically and ecologically both in the US and Africa.

Dry Matter Digestibility (DDM) and Organic Matter Digestibility (OMD) Calibration Equations

The calibration equations for the DDM and OMD were developed for the US, Africa, and US/Africa combined, calibration set. However, the best calibration statistics were obtained for those equations derived from the US and US/Africa calibration sets. Whereas calibration equations derived from the Africa sample showed poor calibration statistics. As clearly mentioned in previous section this poor performance of the Africa DOM equation is mainly due to the problem of deriving accurate laboratory reference data particularly dietary OM, fecal DM, and OM. Table 3.5 presents the calibration and cross validation statistics for the dry matter (DDM) and organic matter (OMD) digestibility equations from the three calibration sets.

US DDM Equation

The US dry matter digestibility calibration equation was developed with a total of 95 in vivo dry matter digestibility reference values regressed against the matching fecal NIRS spectra. First derivative (1,12,12,1), and SNV_D data treatment on fecal spectra were performed. The standard error of calibration (SEC) for the in vivo dry matter digestibility (DDM) was 3.28 with a corresponding coefficient of determination (R^2) of 0.79. The standard error of cross validation for the DDM equation was 3.60, which is 3% higher than the standard error of calibration (SEC=3.28). Nevertheless, the SECV accounts only about 6.8% of the error of the mean value of DDM, which is similar to those reported by Brno-Soares et al. (1998). Brno-Soares et al. (1998) found 5.7% DDM in sheep fed green crop cereals. The standard errors of cross validation to

Table 3.5. Calibration statistics for organic matter digestibility (OMD %) and dry matter digestibility (DDM%) for the US, Africa and US/Africa calibration sets

Equation	N	Mean	SD	SEC	R^2	SECV	SEP (C)	Bias	Math	λ
US										
OMD	94	54.7	6.2	3.25	0.72	3.61	4.71	-2.17	1	1364 1372 2108
DDM	95	53.2	7.2	3.28	0.79	3.60	4.72	-2.18	1	1572 2108 2276
Africa										
OMD	38	37.8	12.0	5.53	0.79	6.36	8.3	-3.82	1	1460 1468 1476
DDM	39	34.5	11.0	6.31	0.67	7.19	9.4	-4.3	2	1940 1980 1992
US/Africa										
OMD	100	49.4	11.0	4.2	0.85	5.2	6.8	-3.1	2	1152 2192 2272
DDM	100	47.0	11.8	4.3	0.87	5.1	6.6	-3.1	2	1148 1152 2272

N= number samples included in calibration equation
SD= standard deviation
SEC= standard error of calibration
R^2= coefficient of determination
SEV= standard error of cross validation
SEP standard error of prediction
Bias= mean of differences
Math= 1st or 2nd derivative of log(1/R) spectra
λ = wavelengths (nm)

standard deviation (SECV/SD) ratios were 0.46 DDM indicating intermediate stability of the equation. Figure 3.5a and 3.5b depict the relationship between measured and NIRS predicted US OMD and DDM values, respectively.

The standard error of calibration for the present US DDM equation was slightly higher than those reported by Purnomoadi et al. (1996) and Coates (1998). In developing NIRS predicting equation for in vivo dry matter digestibility, Purnomoadi et al. (1996) found a SEC of 2.93 and Coates (1998) reported SEC=2.5, which are equivalent to the present standard error of calibration.

The wavelengths 1572 -nm, 2108 -nm, 2276-nm were identified as dominant wavelengths for the US DDM equation. The wavelengths are associated with molecular NH group NH protein, CH-aldehydes phenols. In addition, they are associated with NH amines and imides amino acids, urea, and I. Deaville and Givens (1998) demonstrated that the region 1500-1640-nm associated with high degradability of grass. Park et al. (1997) also suggested the 2280-nm region was associated with digestibility of silage.

US OMD Equation

The in vivo organic matter digestibility (OMD) equation was based on 94 sample sets ranging in their OMD values from 39.45%-66.85%. From a total of 100 samples varied in OMD value 14.47%-75.77%, six samples with value less than 33.84 and greater than 66.4 were identified as T outliers using a value of 2.5. First derivative (1,4,4,1) math treatment and SNV-D transformation procedures were performed on spectra data before calibration is carried out. The standard error of calibration (SEC) for the US OMD equation was 3.25 with corresponding coefficient of determination (R^2) of 0.72 (Fig. 3.5b).Cross validation results have also shown that the OMD equation had SECV value of 3.60 which is higher than the SEC value.

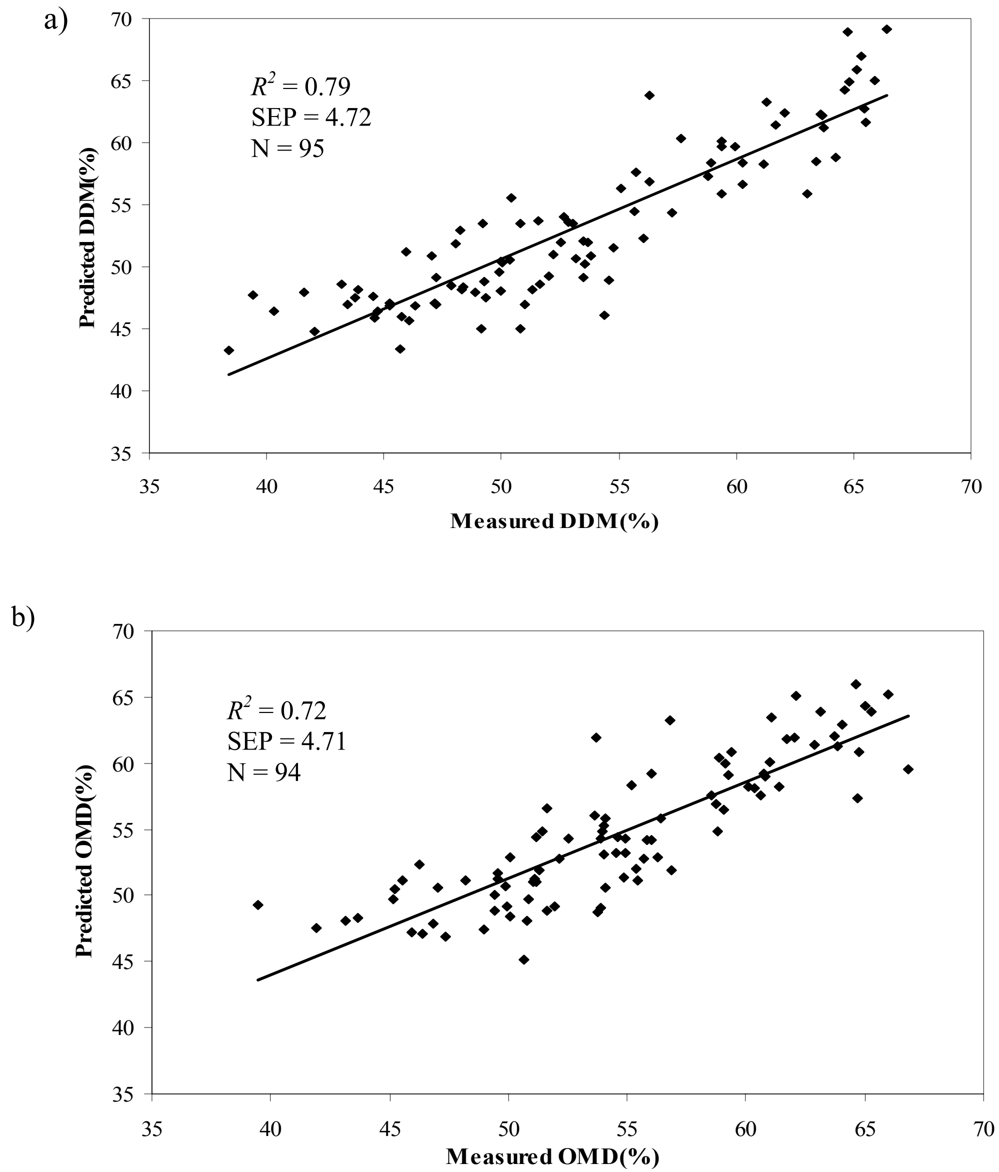

Figure 3.5. Relationship between measured and NIRS predicted a) dry matter digestibility and b) organic matter digestibility for the US calibration dataset. The line Y=X represents agreement between predicted and measured variables.

The present US OMD equation had intermediate SEC, which is within the range reported by Baker et al. (1994). The authors developed 20 calibration equations for predicting the in vivo organic matter digestibility and found SEC values ranging from 1.99 to 4.23. The coefficient of determination (R^2) for the US OMD equation was good in which more than two-third of the variations in measured OMD were explained by the calibration equation. See Figure 3.5b for the relation between measured and NIRS predicted US OMD. The R^2 is comparable with values reported for cattle OMD equations by Boval et al. (2004). Boval et al. (2004) developed a fecal NIRS equation for predicting OMD using data from 10 in vivo trials covering only two forage species (*Dichanthium spp and Digitaria decumbens*) and obtained R^2 of 0.72 with corresponding SEC=2.1.

Although the standard error of cross validation was considerably higher than the corresponding standard error of calibration the standard errors of cross validation to standard deviation (SECV/SD) ratios were 0.41, indicating the OMD equation has good stability. The present data also have shown that the SEC accounts 6.7% of the error of the mean value of OMD, which is similar to those reported by Brno-Soares et al. (1998) who obtained 5.2% error of the mean for the OMD, in sheep. For selected IVOMD equations, Boval et al. (2004) reported standard error of calibration value (SEC =2.1) lower than the present result (SEC=3.36). But it must be noted that as mentioned in earlier section, the in vivo estimates of digestibility derived from total collection are themselves subjected to appreciable error due to high individual animal variations (citation) and the imperfect collection during the in vivo digestibility trials, which is another contributory factor to the low performance of digestibility equations.

The three dominant wavelengths for the US OMD equation were 1364-nm, 1372-nm and 2108-nm. These wavelengths chemically associated with CH- aldehyde, OH- alcohol, phenols, NH amine and imides indicating the presence of both carbohydrate and protein molecules in the

diets. The wavelength 2108-nm falls with the region 2100-2200-nm where Coleman and Murray (1993) suggested that the region associated with dry matter degradability.

US/Africa OMD Equation

The calibration set used in developing the US/Africa OMD equation was a subset of the US and Africa combined calibration sets. The equation was based 100 samples ranging from 21.2% to 66.85 % derived from a population value ranging 14.5% to 80%. The equation was derived using a second derivative (2,4,4,1), SNV and detrend procedures. The calibration and cross validation statistics for the OMD equation are also presented in Table 3.5.

The standard error of calibration (SEC) and standard error of cross validation (SECV) were 4.2 and 5.2, respectively. Similar to other US/Africa digestible equation (DDM) relatively high coefficient of determination R^2 =0.85 was observed for the OMD equation (Fig. 3.6a).

The three dominant wavelengths identified for the US/Africa OMD equation include 1152 -nm, 2192-nm and 2272-nm. The wavelength 1152-nm associates with CH_2 group, CH_3 group, CH- stretch carboxyl compound and was in accord with the wavelength reported by Awuma (2003). The wavelength 2192-nm was also related to crude fiber (Purnomoadi et al. 1996) and minerals (Ruano-Ramos et al. 1999).

The wavelength 1152-nm was similar to 1154-nm, which Awuma (2003) identified as dominant wavelength for one of the fecal DOM equations developed for sheep. Both 2192-nm and 2272-nm are associated with NH amine plus amides, esters, aliphatic and aromatic compound. According to Lister et al. (1995) the region 2120-2240-nm, was associated with high degradability of dry matter of forage equations. One of the present dominant wavelengths i.e. 2192-nm falls within this region indicating its association with digestibility.

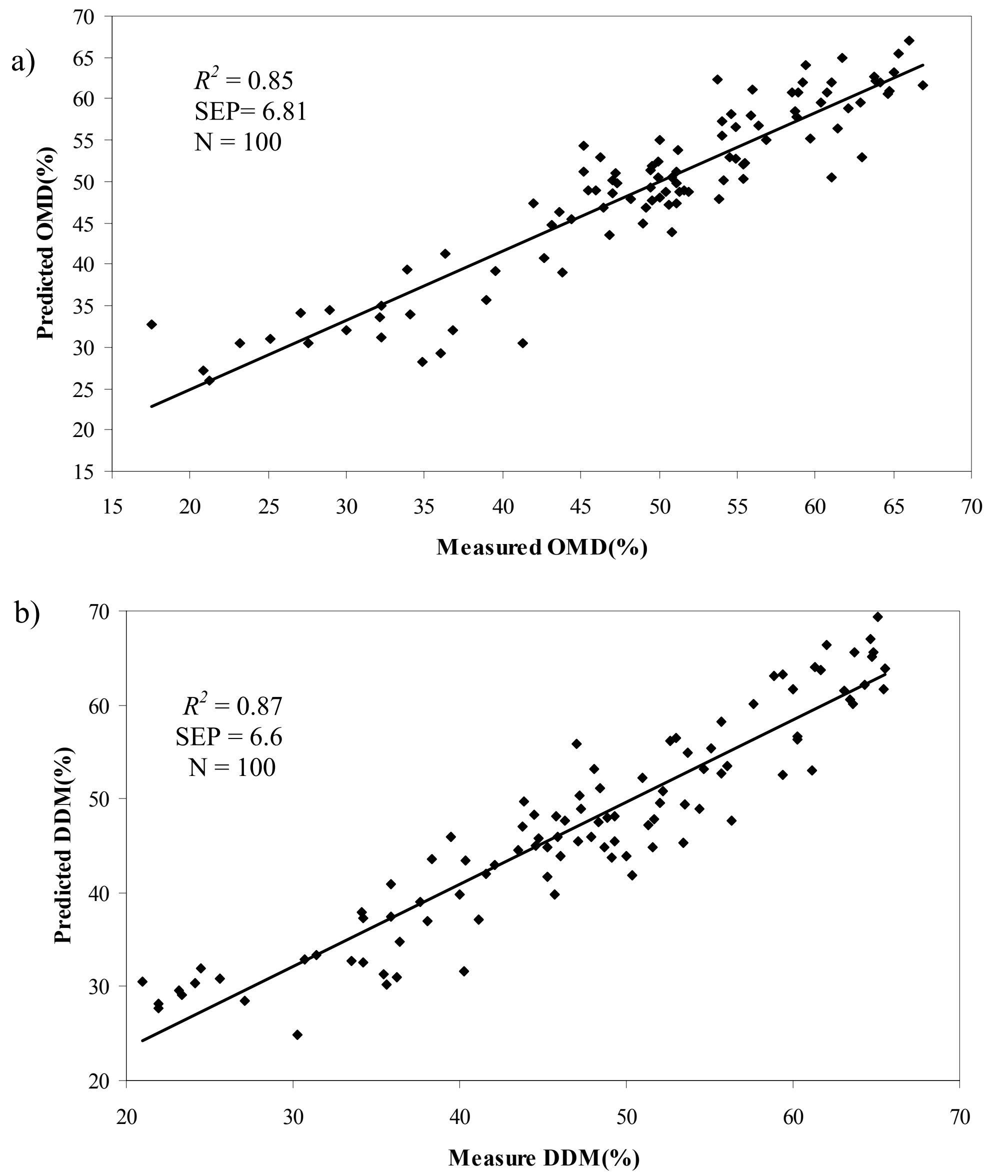

Figure 3.6. Relationship between measured and NIRS predicted a) organic matter digestibility and b) dry matter digestibility for the US/Africa calibration datasets. The line Y=X represents agreement between predicted and measured variables.

US/Africa DDM Equation

The US/Africa DDM equation was derived from 100 calibration set with value ranging between 21.0% and 65.55 % DDM. Five samples were removed due to significant T values greater than 2.5. The equation had standard error of calibration (SEC) of 4.3 with a corresponding coefficient of determination (R^2) equals to 0.87. The standard error of cross validation obtained from the equation was 5.1, which is also higher than the observed corresponding SEC value.

Compared to the US DDM equation, the present equation had higher R^2 although the SEC value slightly deteriorated in the present equation (Fig. 3.6b). The relatively higher R^2 value may be due to the increased in number of samples as well as increased in the range of DDM values. The US/Africa DDM had dominant wavelength included 1148-nm, 1152-nm and 2272-nm. The last two wavelenths1152-nm and 2272-nm were also dominant in the OMD equation at and as indicated previously these wavelengths are associated with both protein and carbohydrates molecules.

Present calibration results indicated that inclusion of the Africa sample set into the US data set resulted in considerable increase in coefficient of determination values both for the organic matter digestibility and dry matter digestibility equations. This improvement in calibration statistics indicates the introduction of variation that enhances the amount of the variance in the calibration set that could be explained by the equation.

Validation of Prediction Equations

Data Acquisition

The present preliminary CP, DOM, DDM and OMD calibration equations for the US/Africa calibration set were validated using a randomly selected subset of samples derived from the combined US /Africa sample sets. The validation set consisted of a total of 35 diet-fecal pairs including 78% and 22% from the US and Africa data sets, respectively exhibiting a wide variation in constituents. Since no independent population was available for validating the US and Africa equations from the respective sites, the US data set was used as independent data to validate the Africa calibration equations and the Africa data set was used to validate the US equations.

Validation Results for the US/Africa Equations

US/Africa CP Equation

In the validation set, crude protein (CP) averaged 11.2 % with a standard deviation of 3.7, a minimum of 5.9% and a maximum of 18.8%. The crude protein concentration of diets in the validation set was within the range of the crude protein concentration of samples in the calibration set. Table 3.6 presents the mean, range and standard deviation of the constituents.

The standard error of prediction (SEP) for crude protein was 1.79 with coefficient of simple correlation (r^2) equals to 0.82. The relatively high correlation coefficient indicated that the association between measured and near infrared spectroscopy predicted crude protein was quite strong. In addition, when the CP equation was applied to the same validation set after eliminating one critical T outlier samples (n=34) considerable improvements were observed in terms of both SEP (1.60) and r^2 (0.87) indicating the effect of error with wet chemistry or sample

Table 3.6.The mean, range, and standard deviation of crude protein, digestible organic matter, dry matter digestibility and organic matter digestibility of diets in the validation dataset and corresponding NIRS predicted values

	Measured (validation)					Predicted				
Constituent	N	Mean	Minimum	Maximum	SD	N	Mean	Minimum	Maximum	SD
CP	35	11.24	5.9	18.8	3.75	35	11.65	4.06	19.98	4.05
DOM	35	43.51	20.60	61.88	8.49	35	43.62	21.87	54.76	8.38
DDM	35	50.53	16.10	73.99	12.11	35	50.13	24.86	71.56	11.89
OMD	35	52.35	22.40	75.77	11.25	35	52.16	26.42	67.74	11.06

CP = crude protein
DOM = digestible organic matter
DDM = dry matter digestibility
OMD = organic matter digestibility
SD = standard deviation
N = number of samples

preparation on the predictive ability of the equations. In both cases, however, the SEP between reference and NIRS predicted for the validation set was relatively high compared with the SEC value obtained for calibration. A summary of the validation statistics is presented in Table 3.7.

Discussion of the US/Africa CP Equation Validation

High coefficient of correlation between the measured (reference) and NIRS predicted values were found for the protein equations. The coefficient of simple correlation (r^2 =0.82) was relatively high, indicating that the NIRS calibration has been successfully developed for the prediction of the crude protein concentration of unknown or independent diets both from US and Africa although the SEP between measured and the NIRS predicted crude protein concentration was relatively high compared with the required accuracy for protein. Many factors can cause the NIRS analysis error to exceed the level computed when calibration was developed (Shenk and Westerhaus 1998). One possible reason for the large SEP in the present CP equation may be the low variation in the chemical composition of the diets indicating there has been small range of value among the validation set. Nevertheless, in comparison with previously published data for ruminants, the present standard error of prediction (SEP) was considerably low. In validating four CP equations developed for sheep, Ossiya (1999a) reported SEP values of 3.42, 2.17, 4.09 and 3.445 with corresponding r^2 of 0.48, 0.11, 0.52 and 0.30, respectively.

The CP validation had lowest BIAS, under-predicted the validation set by a mean of only 0.41, indicating by far largest part of the error was due to random variation (Table 3.7). Comparison of predicted and measured crude protein demonstrated that there was an accurate fit for the average CP values (11.65 vs. 11.24), although the predicted CP range was slightly larger than the range in validation set (for comparison see Table 3.6).

Table 3.7. Validation statistics for the US/Africa CP, DOM, DDM and OMD calibration equations using 25% of the combined US/Africa calibration sets before and after removing the critical T outlier samples

		Validation statistics					
Equation		N	SEP	r^2	SEP (C)	Bias	Slope
CP							
	a	35	1.79	0.82	1.74	0.41	0.84
	b	34	1.56	0.87	1.52	-0.56	0.81
DOM							
	a	35	5.19	0.65	5.27	-0.11	0.82
	b	34	4.66	0.70	4.70	-0.54	0.79
DDM							
	a	35	7.34	0.65	7.44	0.40	0.82
	b	34	6.21	0.74	6.30	-0.30	0.82
OMD							
	a	35	6.70	0.66	6.80	0.20	0.83
	b	34	5.89	0.73	5.96	-0.39	0.81

CP = crude protein
DOM = digestible organic matter
DDM = digestibility of dry matter
OMD= organic matter digestibility
N= number of samples used in validation
SEP= standard error of prediction
SEP(C)= bias corrected standard error of prediction
r^2 = coefficient of simple correlation
a = validation before removal of outlier samples
b = validation after removal of outlier samples

The coefficient of simple correlation (r^2) obtained for the present US/Africa CP validation for donkeys (r^2 = 0.82, see Fig. 3.7a) was also higher than those reported by Ossiya (1999a), comparable with values reported by Awuma (2003) and Keating (2005) but lower than those values reported by Lyons and Stuth (1992) who studied ruminants. Even though the coefficient of correlation was lower than the coefficient of determination for calibration, the former still indicated that a high percent of the variability in the predicted values was due to variation with the selected CP equation. As a result, the selected CP equation can be an important tool in predicting the crude protein concentration of forage consumed by donkeys both in Africa and US with acceptable degree of accuracy.

US/Africa DOM Equation

The range and standard deviation of digestible organic matter (DOM) of the validation set before and after removal of T outlier samples were 20.6% to 61.9% and 8.5 (SD), respectively, which is within the range of the values in the calibration set (see Table 3.6 above). The standard error of prediction (SEP) for the DOM validation was 5.19, which is much higher than the standard error of calibration. The coefficient of simple correlation (r^2) for prediction was 0.65.indicating relatively intermediate association between measured and NIRS predicted DOM (see Fig. 3.7b). During validation, effect of T outlier samples was also investigated by applying the DOM equation to validation set after outlier samples were removed. The result indicate that with SEP value of 4.66 and r^2 value of 0.70, the equation had better performance in predicting the DOM concentration of the independent diets with outliers removed than the validation with outliers.

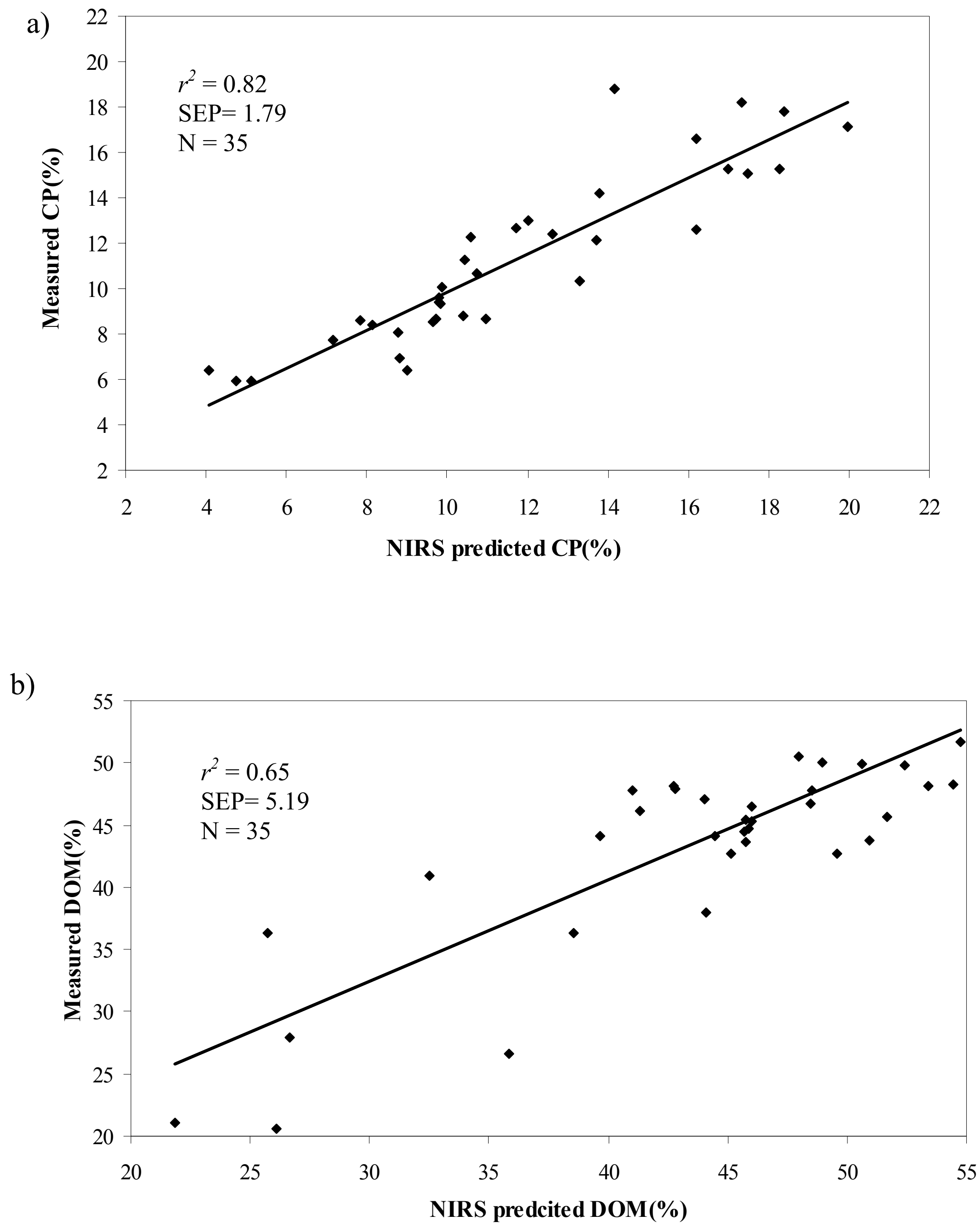

Figure 3.7.Relationship between NIRS predicted and measured a) crude protein and b) digestible organic matter for the US/Africa combined validation set before removal of T outlier samples. The line Y=X represents agreement between predicted and measured variables

US/Africa DDM Equation

There was a large variation in dry matter digestibility (DDM) among sample sets for validation. The mean DDM was 50.5 with a standard deviation (SD) of 12.1 units, a minimum of 16.1 and a maximum of 74.0%. In this case the range was larger in the validation set than that was in the calibration set. Consequently, estimation of the dry matter digestibility in the validation set resulted in a standard error of prediction (SEP) of 7.34 and coefficient of simple correlation (r^2) of 0.65. Despite the relatively high correlation coefficient for the DDM equation, the value of SEP was much higher than the calibration error indicating that the equation performance was generally poor to predict the values in the validation set. Application of the DDM equation to validation set after removal of one sample identified as outlier resulted in better accuracy of prediction, albeit much too high for practical use. The SEP was reduced to 6.21 while the r^2 was increased to 0.74, indicating the effect of outlier samples on the performance of calibration equations was remarkable. The removal of one outlier had shown considerable improvement in the predictive performance of the DDM equation indicating the presence of variation between the NIRS value and the reference DDM value that was derived with different method and used in the US study.

US/Africa OMD Equation

The mean organic matter digestibility (OMD) of diet sample in the present validation set before removal of T outlier samples was 52.4 with a minimum and maximum values of 22.4% and 75.5%, respectively. The distribution of OMD in the validation set was within the range of the values in the calibration set (see Table 3.6). Prediction results indicated that the standard error of prediction (SEP) was 6.7 with corresponding coefficient of simple correlation (r^2) equals to 0.66. The values of SEP and r^2 obtained for the prediction sets were inferior to those results

for calibration sets, indicating the calibration equation was insufficient for prediction of OMD from the validation set. However, improvement in validation statistics was observed when validation set without outlier samples was used. The SEP and r^2 values were 5.89 and 0.73 when one sample identified as critical T outlier was removed from the validation set.

Discussion of the US/Africa DOM, DDM and OMD Equations Validation

Independent validation of the DOM equation resulted in a lowest standard error of prediction (SEP) compared to results obtained for OMD and DDM equations. The standard error of prediction (SEP) achieved in the present DOM equation was also lower than those reported by Ossiya (1999a). Ossiya (1999a) working with sheep reported SEP value of 6.7 and 9.8 for in vivo and in vitro DOM validations, with corresponding coefficient of simple correlation (r^2) values of 0.11 and 0.20 respectively. The author noted that the poor performance of the calibration equations in predicting external validation sets the main source of error for validation was difference in lab value due to problems with laboratory procedures. We feel that this is a major contributor to the differences noted in this study.

The present DOM equation had the smallest BIAS values, over-predicted validation set by a mean of only 0.11, while Ossiya (1999a) reported BIAS values greater than 3.0. The results indicated that the relationship between measured and predicted DOM concentration was much stronger in the present work than that of the previous reports at least those by Ossiya (1999a).

Among the digestibility equations, the DDM had the highest SEP value compared to those values for DOM and OMD. On the other hand, the DDM equation had correlation coefficient (r^2=0.65), which is equal to the value for DOM. This relative high magnitude of coefficient of simple correlation with relatively high SEP could be caused due to the wide variation in digestibility values among validation diets. The predicted DDM ranged from 24.9 to 71.6 %,

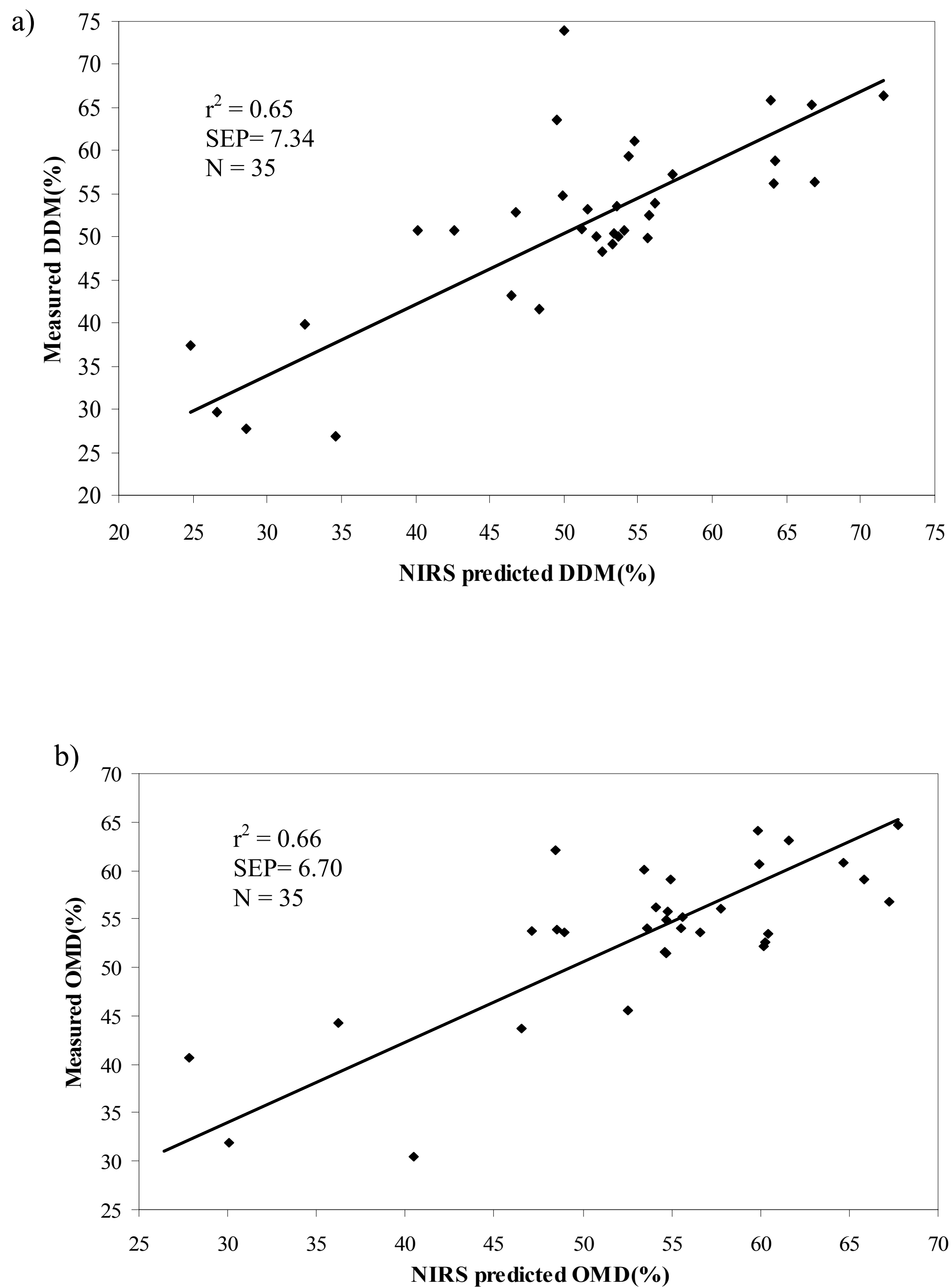

Figure 3.8. Relationship between NIRS predicted and measured a) dry matter digestibility and b) organic matter digestibility for the US/Africa combined validation set before removal of T outlier samples. The line Y=X represents agreement between predicted and measured variables

which was narrower than the range in the validation set. The prediction equation for OMD had superior performance to that of DDM in terms of both standard error of prediction (SEP) and coefficient of simple correlation *(r^2)* (Figs. 3.8a and 3.8b*)*. This disparity in prediction performance between DDM and OMD was expected particularly when the nature of chemical constituent of the attributes is considered. The dry matter (DM) of forage consists of both organic compounds (cell content and cell wall) and inorganic elements (minerals) whereas OM consists only organic components. Since minerals do not absorb in the near infrared region (Stuth 2003) normally NIRS works best for determining organic compounds and less effectively for the analysis of minerals (Coates 2000a), which could partly explain the better performance of both DOM and OMD than DDM equations. In general, the independent validation of the calibration equations indicated that NIR spectroscopy predicted feed digestibility with lower precision than for crude protein although more than two-third of the total variation in digestibility data being explained by the NIRS equations. The superior performance of protein equations over digestibility equations could be due to the noticeable difference between determination of the digestibility and crude protein reference values. Shenk and Westerhaus (1991) grouped crude protein and digestibility with highest and lower accuracy categories, respectively. Analyzing of the reference value for crude protein concentration of diets by standard Kjeldahl procedures is direct and it simply measures single chemical entity i.e. the N content of a sample with high precision (Coleman 1989). Further crude protein (CP) is easily repeated across labs and in this case two separate labs conducted the CP as well as the in vivo digestibility trials and contribute too much of the anomalies observed in digestibility. In addition, Roberts et al. (2004) suggested that the accuracy and precision for CP equations could occur because of the strong –NH absorption in the NIR region. Unlike crude protein, the digestibility analysis procedures do not measure a single entity (Shenk et al.1992) and can be influenced by

both feed factors (NDF, ADF, pattern of chemical boding between hemicelluloses and lignin, associated effects (Orskov 2000) and non-feed factors (level of feed intake, appetite drive, and rate of passage). Some of these factors may not be predicted well by NIRS technique and partially explain the lower precision of the NIRS prediction for digestibility. More importantly is that the validation set for the US/Africa equations was derived from both Africa and US population and quality of the technique between the two locations could be the biggest source of error and subsequent poor validation performance. The difficulties associated with deriving accurate reference value particularly for the Africa digestibility was that the laboratory references value were not derived directly from the forage samples subjected to NIRS. Instead all digestibility attributes were determined using average values derived from literature. As already mentioned this method could result in poor experimental matching between diet samples and feces.

Validation Results for the US Calibration Equations

In this validation a total of 40 samples were used from the Africa study to test the performance of the US CP, DOM, DDM and OMD equations. Equation validation was carried out both before and after eliminating samples with critical T value above 2.5 as recommended by Workman (1992). Table 3.8 presents the result of the validation statistics in terms of SEP, r^2, bias and slope values.

US CP Equation

The standard error of prediction (SEP) and coefficient of simple correlation (r^2) for the CP equation were, 2.73 (2.05) and 0.69 (0.79) respectively (values in parenthesis are validation statistics with out critical outlier samples.). In both cases, the SEP and r^2 values were much

Table 3.8.Validation statistics for the US CP, DOM, DDM and OMD calibration equations using the Africa diet-fecal data as independent validation sets both with and without removing critical T (2.5) outlier samples

Equation	N	SEP	r^2	SEP(C)	Bias	Slope
CP						
a	40	2.73	0.69	1.64	2.2	0.89
b	32	2.05	0.79	1.27	1.62	0.98
DOM						
a	40	15.56	0.20	11.63	-10.5	2.00
b	15	4.79	0.73	4.81	-1.14	2.10
DDM						
a	40	18.60	0.095	13.59	-12.87	1.64
b	11	6.70	0.178	6.33	-2.90	2.17
OMD						
a	40	18.68	0.22	12.40	-14.11	2.36
b	15	6.68	0.63	5.40	-4.17	1.90

CP = crude protein
DOM = digestible organic matter
DDM = digestibility of dry matter
OMD= organic matter digestibility N= number of samples used in validation
SEP= standard error of prediction
r^2 = coefficient of simple correlation
a = validation before removal of outlier samples
b = validation after removal of outlier samples

inferior to the SEC and R^2 values obtained for calibration. The bias value greater than 1% was relatively high indicating that the US calibration equation under predicted the CP concentration of diets from Africa.

Discussion of the US CP Equation Validation

Predicting the Africa CP concentration using the US CP equation has shown moderate success particularly when the equation was applied to validation set before eliminating T outlier samples. The SEP value however, considerably improved when T outlier sample were removed. The presence of about 20% outlier samples in the Africa data set indicates the incompatibility between validation and calibration sets. As can be seen from Table 3.8, there was considerable large BIAS value (1.62-2.2) indicating the difference in lab technique between the Africa and US studies albeit the predictive ability of the US CP equation is still reasonable.

US DOM, DDM and OMD Equations

Predicting digestibility attribute of Africa diets using US equations was also unsatisfactory. The SEP and r^2 vales before eliminating outlier samples were respectively 15.56 and 0.20 for the DOM, 18.60 and 0.095 for the DDM and 18.68 and 0.22 for the OMD equations. Bias with values greater than 10% were considerably high for all equations indicating the equations over predicted the digestibility parameters of the Africa diets. Nevertheless, when the DOM, DDM and OMD equations were applied for validation sets after eliminating the outlier samples, which accounts for more than 62% of the population, considerable improvement in validation statistics (SEP, r^2 and bias) was observed (Table 3.8 above). This presence of large number of T outlier samples indicates that a major challenge to the use of the chemical analysis was the variability across the two laboratories (Africa vs. US). Particularly, the reference value derived for the

Africa data set was not acceptable for the prediction of the US equations mainly due to the methodology used to derive the Africa reference value. This can be clearly seen when over 50% of the value reported for the Africa are less than the lowest value for the US dataset, which was extremely poor quality wheat bedding straw.

Discussion of the US DOM, DDM and OMD Equations Validation

This less sufficient prediction performance of US DOM, DDM and OMD equations using the Africa data set was largely due to the incompatibility between calibration and validation samples which attribute to differences in lab technique used to derive the African reference values. Particularly, the examination of the BIAS and SEP values clearly indicates there was considerable experimental error introduced due to difference in methods used to derive the DOM, DDM and OMD attributes in the Africa dataset. In addition, as mentioned earlier, various animal, diet, and environmental variations could be contributing factors for the poor predictive performance of the equations. Achievement of high predicting equations by removal of the major portion of the population from the validation set, however, does not imply that the equations are not useful, particularly when the application of the equations for grazing animal under the complex grazing land is considered**.** We feel that elimination of outliers in this case was a reflection of elimination of poor laboratory technique at the Naivasha lab.

Validation Results for the Africa Equations

The validation results for the Africa CP, DOM, DDM and OMD calibration equations using US data both before and after T outlier samples were removed are presented in Table 3.9.

Table 3.9. Validation statistics for the Africa CP, DOM, DDM and OMD calibration equations using the US diet-fecal datasets as independent validation sets both with and without eliminating critical T (2.5) outlier samples

	Validation statistics					
Equation	N	SEP	r^2	SEP(C)	Bias	Slope
CP						
a	100	2.91	0.66	2.78	0.92	0.76
b	79	1.88	0.85	1.77	0.64	0.86
DOM						
a	100	19.92	0.03	8.2	18.16	0.183
b	47	12.3	0.55	4.92	11.24	1.024
DDM						
a	99	18.55	0.04	8.64	16.43	0.29
b	69	13.77	0.16	6.45	12.19	0.42
OMD						
a	100	28.87	0.03	13.08	25.77	0.19
b	31	12.52	0.63	5.85	11.12	1.20

CP = crude protein
DOM = digestible organic matter
DDM = digestibility of dry matter
OMD= organic matter digestibility
N= number of samples used in validation
SEP= standard error of prediction
r^2 = coefficient of simple correlation
a = validation before removal of outlier samples
b = validation after removal of outlier samples

Africa CP Equation

When the Africa CP equation was applied to the US data set the resultant SEP was 2.91 with a corresponding coefficient of simple correlation (r^2) value of 0.66. The SEP value was higher than the SEC obtained in calibration, indicating the present Africa CP equation was less successful to predict the CP concentration of US diets. The coefficient of simple correlation (r^2) value of 0.66 indicates that two-third of the variation in the US CP value were expressed by the Africa CP model. Improvement both in terms of SEP (1.88) and r^2 (0.85) was observed when the Africa CP equation was applied to the US data set after the removal of samples with higher critical T value. This result indicates the presence of error in reference values inflate the error associated with NIRS prediction equations and better performance of the equation could be obtained if the equation is applied to validation set without outlier samples. Application of the CP equation both before and after removal of T (2.5) outlier samples results in a bias values of less than 1%. These values indicate that the Africa CP equation systematically under-predicted the CP content of diets obtained from the US study, probably a result in differences in lab technique between the two labs.

Discussion of the Africa CP Equation Validation

Validation of Africa CP equation with the US independent data set resulted in moderately successful results. This relatively good performance of the CP equation was expected, particularly when the precise chemical analysis of crude protein was considered between the two laboratories (US and Africa). However, the relatively high SEP value may be attributed to the slight difference in the range of CP between validation and calibration sets. As mentioned earlier the Africa equation was developed based on calibration set with relatively narrow range whereas the US validation set had greater range of CP value (for comparison see Table 3.2).

Africa DOM, DDM and OMD Equations

The SEP and r^2 for the DOM equation were 19.92 and 0.03, respectively before removal of T outlier samples were 12.3 and 0.55, respectively after removal of T outlier samples. In both cases the SEP values were much higher than SEC values obtained for the calibration while the R^2 were much lower for validation set than for the calibration set. As expected better r^2 was observed after eliminating more than 50% of the population as outlier samples. The BIAS with values greater than 10% was an indicative of poor performance of the equation in prediction the DOM concentration of the US diets.

Validation of the Africa DDM equation resulted in SEP and r^2 values of 13.77 (18.55) and 0.16 (0.042), respectively (value in parenthesis are with outlier samples). OMD equation validation resulted in SEP of 12.52 (28.87) and r^2 of 0.63 (0.033). Both in vivo digestibility equations tested in this study performed less accurate. BIAS values ranging from 12.19 to 16.43 for the DDM and from 11.1 to 28.87 for the OMD indicate that Africa digestibility equations under predicted the US data sets. When DDM and OMD equations were also applied to the separate US non-pregnant and pregnant sample sets, improvements both in SEP and r^2 values were only marginal. In general validation statistics with high SEP and low r^2-values were mostly unacceptable.

Discussion of the Africa DOM, DDM and OMD Equations Validation

The validation statistics for the in vivo digestible parameters (DDM, DOM and OMD) suggested that techniques used to derived DM and OM values for the fecal samples used in the Africa equations were relatively poor for predicting the independent US parameters. One reason may be that, digestibility unlike crude protein, is a property of forage or feed rather than a chemical parameter (Stuth et al. 2003) and can be influenced by various factors including animal

and dietary factors as well as conduct of the experiment. In addition, the range of all the digestibility attributes were different for the calibration set (Africa) and validation sets (US), with difference that accounts for more than 10%, indicating the samples sets were incomparable. Further more, spectra diversity associated with different plant species and/or mixture together with environmental influences such as soil and climate factors (Boval et al. 2004) may be another cause for the poor performance of the Africa equations. In the present study, out of the total 25 feed or forage types used to generate diet samples (reference) only four forage types were commonly found in both studies. These results indicate that validation of calibration equation developed from a different population than the one being predicted (validation set) could result in considerably reduced precision and accuracy of prediction, in this case due to large differences in methods to derived digestibility and quality control by the research personnel. Validation of most equations by excluding T outlier samples resulted in considerable improvement in accuracy suggesting that the reference values for the validation samples were erroneous. Since near infrared reflectance technique is strictly correlative, the accuracy of its prediction can never exceed the accuracy with which the measurement is determined (Norris and Barnes 1976). Therefore, from the present Africa study’s method of data collection and diverse samples used in creating the reference value in both locations, it is fair to expect some deficiencies in validation performance of the digestibility equations.

Conclusions

The present studies have generated first order useable calibration equations to predict the diet quality of free grazing donkeys with various successes. The primary results and conclusions of this study are as follows:

1. The study demonstrated that NIRS has good reliability in the prediction of crude protein (CP) concentration of mixed diets consisting grasses, forbs and crop residue for donkeys. The calibration and validation analysis for CP equations have indicated that CP equations have precision equivalent to that of conventional wet chemistry methods. Moreover, comparison in performance made between present CP equations and published ruminant equations have shown that the performance of the present CP equations for donkeys (equids) were even better than some of those reported for ruminant. Thus, despite the variation in prediction accuracy among the present equations, the US, Africa and US/Africa CP equations have shown excellent performance, and they can be used for routine analysis for predicting the crude protein concentration of unknown diets, both in US and Africa with great degree of accuracy.

2. Data from the study also suggested that fecal NIRS is a viable technique for predicting the functional properties (DOM, DDM and OMD) of diets consumed by donkeys. Good prediction performance with an intermediate accuracy was observed for the US and US/Africa DOM equations although the Africa DOM equation lacked relative precision due to the DM and OM approximation techniques introduced to overcome the lack of this information. One of the major problems with developing robust calibration equation for predicting the digestibility of diets particularly with the Africa dataset was the inability to determine accurate reference values from in vivo trials. Nevertheless, the DOM equations still have shown comparable accuracy with those reported in literature for ruminant, particularly for the US dataset. Hence, application of the present DOM equation (US only) for predicting the DOM concentration of unknown diets

from fecal sample can give acceptable degree of accuracy although both OMD and DDM equations did not meet the acceptable level of accuracy for prediction.

3. The present study confirms the potential of NIRS for predicting the diet quality of donkey and possibly other equines that should capture the quality of diets in free-grazing conditions based on the wide variety of plant species fed in the pen trials. It also confirmed that performance of the fecal-NIRS calibration for equines (hindgut fermenters) was as robust as those for ruminant animals (foregut fermenters).

4. It is clear that calibration equations are not static and will always be subjected to continued expansion and refinement. Therefore, research with more diet and fecal samples particularly from Africa, is needed to be done to further improve the prediction accuracy of the present digestibility equations and/or expand them by incorporating a broad base data that enables to create more robust equations.

CHAPTER IV

EFFECT OF PREGNANCY STATUS OF DONKEYS ON THE PERFORMANCE OF FECAL NIRS CALIBRATION EQUATIONS

Introduction

The advent of fecal-NIR spectroscopy technique has enabled extraction of useful information from fecal samples for predicting the diet quality of free grazing animals. Today, the technology is routinely used as a nutritional status-monitoring tool in grazing livestock management. Recent studies (Tolleson et al. 2001) have demonstrated that NIRS analysis of fecal samples can provide information not only about the animal's diet quality but also about the animal itself.

Prior works have provided evidence that fecal chemistry of animals differs with the physiological state, sex and feed type of animals. Several authors (Tolleson et al. 2000; Godfrye et al. 2001) have explored the potential for fecal NIRS to determine animal physiological status. Recently, Tolleson et al. (2001) working with cattle were able to diagnose the pregnancy status of cows. The study by Godfrye et al. (2001) also demonstrated a successfully discrimination between pregnant and lactating sheep. In addition, fecal- NIRS was used to determining the gender of various animals, including white-tailed deer (Osborn et al. 2002), red deer (Tolleson et al. 2004), cattle (Tolleson et al. 2004) and sheep (Godfrey et al. 2001). On the other hand, the same fecal NIRS calibration equations have been used to predict the nutritional quality of animals under different physiological status. Information about whether physiological state of animal affects the performance of fecal NIRS calibration equation for predicting the nutritional quality of animals is scarce. A limited study by Lyons and Stuth (1992), working with dry and lactating cows, indicated that calibration statistics of NIRS equations were not influenced by the physiological difference of the animal. Since this last work of Lyons and Stuth (1992) no attempt

has been made to examine the issue. The objective of the study was to investigate the effect of pregnancy state on the performance of fecal-NIRS for predicting the diet quality of donkeys.

Materials and Methods

Feeding Trial

Data were obtained from a previous 10-week in vivo feeding trial conducted in College Station, Texas (See Chapter III). Ten female donkeys, five pregnant and five open, were fed on mixed diets of forage and crop residue to generate dietary reference data and corresponding fecal spectra. Pregnancy status of donkeys was determined using ultra sound scanning technique.

Each feeding trial lasted seven days with four adaptation and three collection days. Forage based rations were offered twice a day (0700 hours and 1900 hours) and feed refusal was collected before each feeding time. Representative samples were collected for each diet and ort, and saved for chemical analysis. During the three days of total fecal collection, sub samples of feces were collected ever four hours and was weighed and mixed thoroughly. A representative sample (5% of wet weight) was pooled for the three days and saved for analysis. Each donkey received feed equivalent to 2% of its body weight with some adjustments made following the preceding intake rate.

Chemical Analysis

Each diet, ort (refusal) and fecal sample was pooled for the three days, dried on forced air oven at 60°C for 48 hours, and ground using a Wiley Mill to pass through a 2-mm sieve. Ground samples were submitted to the Forage, Soil and Water Testing Laboratory, at Texas A&M University, which follows the standard procedures (AOAC 1990) for analysis of crude protein (CP). Fecal samples were also dried in a forced- air oven as described in the above and ground to

pass a 1-mm sieve and stored in a paper coin envelope for chemical and spectral analysis. Organic matter (OM%) of diet, ort and fecal samples were analyzed according the AOAC (2000). Dry matter digestibility (DDM) and organic matter digestibility (OMD) were determined according (Merchen 1988). Digestible organic matter as gram of organic matter digested per gram of dry matter ingested was determined according to Lyons and Stuth (1992).

Fecal Spectra Collection

NIRS analysis on fecal samples was done using NIR Systems reflectance monochromator, Model 6500, equipped with Win ISI Intrasoft International version 1.50. Spectra were collected from 1100 to 2500 nm in steps of 2-nm. Scanning was conducted in the Grazingland Animal Nutrition Laboratory (GANLAB) at Texas A&M University. The details of the methodology used in deriving the spectra data was described in depth in the materials and method section of Chapter III.

Calibration Development

One hundred samples were divided into two equal subsets each of 50 samples, and categorized as pregnant and non-pregnant calibration sets. The data sets obtained for each category were treated separately using different combinations of math treatments and scatter correction (SNV-D) as described by Shenk and Westerhaus (1991) and (Duckworth 2004). Calibration equations for each category were derived using the dietary reference value as dependent variable and regressed it against the fecal spectra (independent variable). Modified partial least square (MPLS) regression analysis was used to develop the calibration equation (Shenk and Westerhaus 1991) for crude protein (CP), digestible organic matter (DOM), digestibility of dry matter (DDM), and digestibility of organic matter (OMD). Cross validation

was also carried out by taking a series of randomly selected subsets of seven samples of the calibration set and examine the distribution of difference between the predicted and reference values for each remain set (Shenk and Westerhaus 1991; Shenk et al. 2004).

Equation Selection

Calibration equations for each constituent were selected based on the magnitude of the standard error of calibration (SEC), coefficient of determination (R^2), and standard error of cross validation (SECV) and wavelength characteristics were used (William 1987; Shenk and Westerhaus 1991; Lyons and Stuth 1992; Shenk et al. 2004). In addition validation statistics, including standard of error of prediction (SEP), bias, and slope were considered in describing the predictive ability (performance) of each calibration equation.

Predicting the Pregnant and Non-pregnant Data Sets Using the Combined US Equations

As reported earlier in Chapter III, calibration equations for CP, DOM, OMD and DDM were developed using the combined pregnant and non-pregnant calibration sets. To evaluate the performance of the combined US equations prediction of the pregnant and non-pregnant datasets obtained from the US study was carried out. Prediction was conducted both before and after elimination of potential outlier from both data sets. In addition, all math treatments, SNV and detrend procedures were used using value similar to those used during calibration of the combined equation. Since both data sets were part of the calibration set that created the combined equation we expect that the combined equation will predict the two datasets at the same level of accuracy.

Results and Discussion

Chemical composition and functional properties of the 100 diets both for the pregnant and non-pregnant calibration sets are presented in table 1. Splitting the calibration set into pregnant and non-pregnant sets resulted in a different range in calibration sets for the pregnant and non-pregnant groups. The range of CP concentrations for the pregnant and non-pregnant calibration sets were from 4.1% to 18.2 % and from 4.7 % to 19.44 %, respectively. DOM values ranged from 33.7 % to 53.3 % for pregnant and from 12.1 % to 62.1% for non-pregnant. Similarly the OMD and DDM values ranged from 39.5% to 66.0%, and from 37.7% to 66.4% for the pregnant calibration set, respectively and from 14.5% to 75.8 % and from 34.1% to 74.0% for the non-pregnant calibration set, respectively. In all the constituents wider range was observed in non-pregnant calibration sets than pregnant calibration sets.

Performance of the Combined US Equations

Prediction of the CP concentration of diet consumed by the pregnant and non-pregnant group using the combined US calibration equation before removal of outlier samples have resulted in standard error of prediction (SEP) values of 1.1 1 and 1.19, and coefficient of simple correlation (r^2) values of o.937 and 0.935, respectively The DOM was predicted with an SEP value of 3.01 and r^2 of 0.573 for pregnant and 7.28 and 0.183 for the non-pregnant data sets, respectively. Similarly, the combined DDM equation predicted the DDM with an SEP and r^2 value of 3.47 and 0.797 for the pregnant and 5.47 and 0.533 for the non-pregnant data sets. Application of the combined OMD equation also resulted in an SEP and r^2 values of 3.38 and 0.732 respectively for the pregnant, and 8.38 and 0.361 for the non-pregnant data sets.

After the removal of potential outliers, one from the non-pregnant and two from the pregnant data sets, a considerable improvement in prediction accuracy of the combined CP equation was

observed. The CP concentration of diets from the non-pregnant data set was predicted with SEP and r^2 values of 0.769 and 0.967, respectively and for the pregnant data set the SEP and r^2 were 0.826 and 0.961, respectively. Prediction of the DOM equation after removal of five potential outliers resulted in SEP and r^2 values of 2.71 and 0.485 for the pregnant and with one outlier removed the DOM of the non-pregnant had SEP and r^2 values of 2.58 and 0.0.64. Improvement in prediction accuracy was also observed when the combined equation was applied to predict both DDM and OMD of diets. The SEP and r^2 value were 3.428, 0.756 and 3.31 and 0.67 were for the pregnant DDM and OMD respectively. The DDM of the non-pregnant data sets were predicted with SEP and r^2 values of 3.03 and 0.833. However, since the pregnant OMD had no potential outliers, no difference was observed in prediction accuracy.

Since all combined equations were created after the removal of potential outlier samples from the calibration set, therefore, it is believed that the actual performance of the equations was reflected in the latter results. Accordingly, the prediction performance statistics indicated that the CP equation predicted both data sets with almost the same level of accuracy before and after elimination of outlier samples. However, prediction of DOM, DDM and OMD resulted in slight different level of accuracy. In all the predictions, the pregnant data were better predicted than the non-pregnant ones. Therefore, the combined US equations have shown difference in its predictive capability for the two data sets and calls for further investigation if pregnant and non-pregnant separate equations provide better results.

Calibration Results

The calibration analysis for CP, DOM, OMD and DDM resulted in different calibrations statistic for the pregnant and non-pregnant data sets. Table 4.1 presents the calibration statistics CP, DOM, OMD and DDM equations.

Table 4.1. Calibration statistics for digestible organic matter (DOM), crude protein (CP), organic matter digestibility (OMD) and dry Matter digestibility (DDM) equations of pregnant and non-pregnant data sets. SEL of 0.5 and 1.68 were used for CP and DOM

Equation	N	Range	Mean	SEC	R^2	SECV	SEP (C)	Bias	Math	λ
CP	50	4.1-18.2	10.7±4.4	0.91	0.95	1.12	1.46	-0.67	2	1428
a) PRG	(49)									1716
										2348
	50	4.7-19.4	11.2±4.3	0.78	0.97	1.36	1.76	-0.81	2	1412
b) NPG	(49)									1892
										1908
DOM	50	33.7-53.3	45.5±4.6	2.34	0.71	3.02	3.92	-1.81	2	2076
a) PRG	(49)									2276
										2340
b) NPG	50	12.1-62.1	44.0±8.1	1.59	0.82	2.55	3.31	-1.53	2	2324
	(45)									2364
										2484
OMD	50	39.5-66.0	54.9±6.5	2.63	0.84	4.27	5.56	-2.56	2	1876
a) PRG	(50)									2076
										2364
	50	14.5-75.8	53.3±10.5	1.98	0.88	3.39	4.40	-2.03	2	1932
b) NPG	(49)									2324
										2364
DDM	50	37.7-66.4	53.4±7.8	2.81	0.86	3.44	4.47	-2.06	2	2076
a) PRG	(45)									2276
										2340
b) NPG	50	34.1-74.0	53.0±8.1	3.55	0.75	3.90	5.08	-2.34	1	1740
	(45)									1772
										2316

NPG=non-pregnant, PRG = pregnant, N= total number of samples in calibration equation, number in bracket indicate number of samples incorporated in the calibration equation after outlier elimination, SD= Standard deviation, SEC= Standard error of calibration, R^2= coefficient of determination, SECV= standard error of cross validation, SEP = standard error of prediction, Bias= Mean of differences Math= 1st or 2nd derivative of log(1/R) spectra. SEL= standard error of laboratory.

CP Equations

The SEC value for the crude protein (CP) calibration equation for non-pregnant group was 0.78 and R^2 was 0.97, whereas for the pregnant group the SEC and R^2 were 0.91 and 0.95, respectively. The standard error of cross validation for pregnant CP equation was 1.12 and for the non-pregnant equation it was 1.36. Higher bias was observed for non-pregnant than pregnant data set (-.81 vs. -.61).

DOM equations

The standard error of calibration (SEC) and coefficient of determination (R^2) values for non-pregnant DOM equation were 1.59 and 0.82, respectively, whereas for the pregnant group the SEC was 2.38 with a corresponding R^2 value equals to 0.71. The standard errors of cross validation for pregnant and non-pregnant DOM equations were 3.02 and 2.55, respectively.

DDM Equations

The standard error of calibration (SEC) and coefficient of determination (R^2) values for pregnant DDM equation were 2.81 and 0.86, respectively and for the non-pregnant group SEC=3.55 and R^2 =0.75, respectively. Higher standard error of cross validation (SECV=3.90) was obtained for the non-pregnant DDM equation than the pregnant equations (SECV=3.44) although both equations had a bias values equal to -2.1.

OMD Equations

The OMD equation for the pregnant group had standard error of calibration, coefficient of determination and standard error of cross validation of 2.63, 0.84 and 4.27, respectively.

The standard error of calibration and coefficient of determination for the non-pregnant OMD equation were 1.98 and 0.88, respectively and the standard error of cross validation was 3.39.

Discussion

In both the pregnant (PRG) and non-pregnant (NPG) equations, there was a good correlation (R^2 >0.95) between CP concentration measurements produced by NIRS and those measured using standard laboratory methods. Calibration statistics analysis has shown that there was some slight difference in precision and accuracy between the two CP equations. Relatively higher coefficient of determination was obtained for the NPG CP equation than those for PGN equations. Similarly, the standard error of calibration (SEC) was lower for non-pregnant CP equation than those for pregnant equations. Figures 4.1a and 4.1b depict the relationship between measured and NIRS predicted crude protein for the PRG and NPG calibration sets. The SEC value for non-pregnant group was lower by 0.13 % and accounts only 7% of the error of the mean while the SEC for the pregnant account for 8.5% of the error of the mean. In terms of standard error of cross validation, the non-pregnant CP equation had relatively higher SECV value compared to those for non-pregnant group.

In comparison with previous results found for the combined US CP equation reported in Chapter III, little difference in calibration statistics was observed. In addition, the present calibration results for NPG and PGN CP equations were also comparable with those previously reported by other authors (Lyons 1990; Leite and Stuth 1995; Ossiya 1999a; Awuma 2003; Li et al. 2004; Keating 2005).

Difference in calibration statistics was observed among the digestibility equations, including DOM, DDM and OMD. In general lower standard error of calibration (SEC) was obtained for the NPG DOM and OMD equations than those for PRG Equations. The standard error of

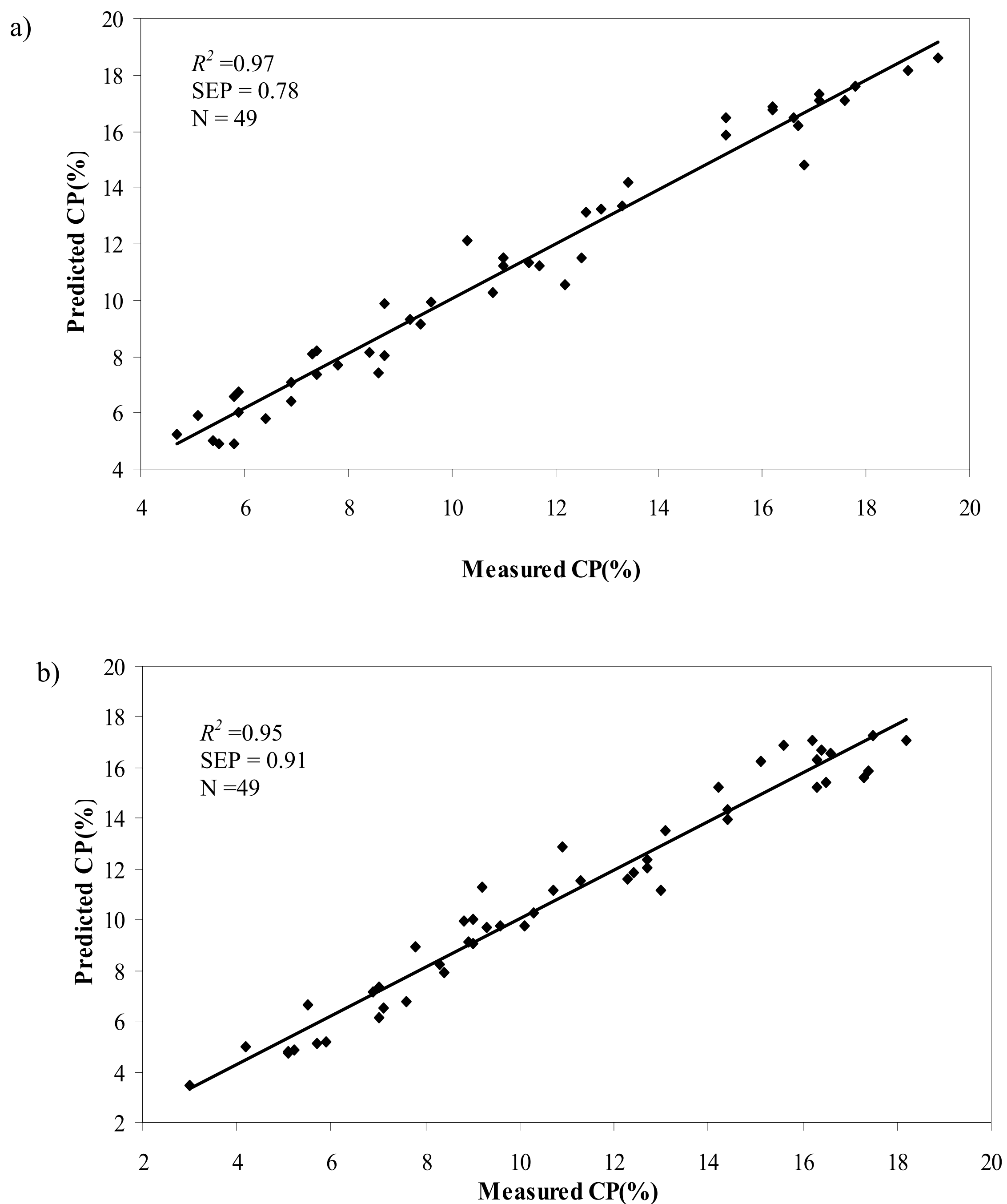

Figure 4.1. Relationship between measured and NIRS predicted crude protein concentration of diets for a) non-pregnant and b) pregnant calibration set. The line Y=X represents agreement between predicted and measured CP values

calibration for DDM was lower for PRG than NPG equations. Higher coefficient of determination (R^2) was obtained for NPG DOM and OMD equations than the PRG DOM and OMD equations. Figures 4.2a and 4.2b depict the relationship between measured and NIRS predicted digestible organic matter (DOM) of diets for the non-pregnant and pregnant calibration set, respectively. These high coefficient of determination results indicated that NIRS calibration explained the variation better for the non-pregnant data set than that of pregnant. One interesting result of this study was that despite the decrease in size of calibration set (number of samples) and variation, both NPN and PRG DOM equations were superior to the combined calibration equation reported in Chapter III.

In other words, splitting up the total calibration set into pregnant and non-pregnant subsets had shown some improvement in R^2 values. In addition, SEC value was decreased by almost 1.0% for non-pregnant DOM calibration although only marginal improvement was observed for the pregnant calibration sets (0.03%) and account for only 3.6 and 5.1% % of the error of the mean DOM for the non-pregnant and pregnant DOM, respectively. These values are much lower than was reported for the combined DOM equation.

Validation of the Pregnant and Non-pregnant Donkeys Equations

To test the accuracy of the equations developed for pregnant and non-pregnant donkeys, a validation was performed on calibration equation for all the constituents. Equation validation was performed using the pregnant calibration set as independent data to test the performance of non-pregnant equations and vice versa. Validation statistics for the CP, DOM, DDM and OMD are presented in Table 4.2.

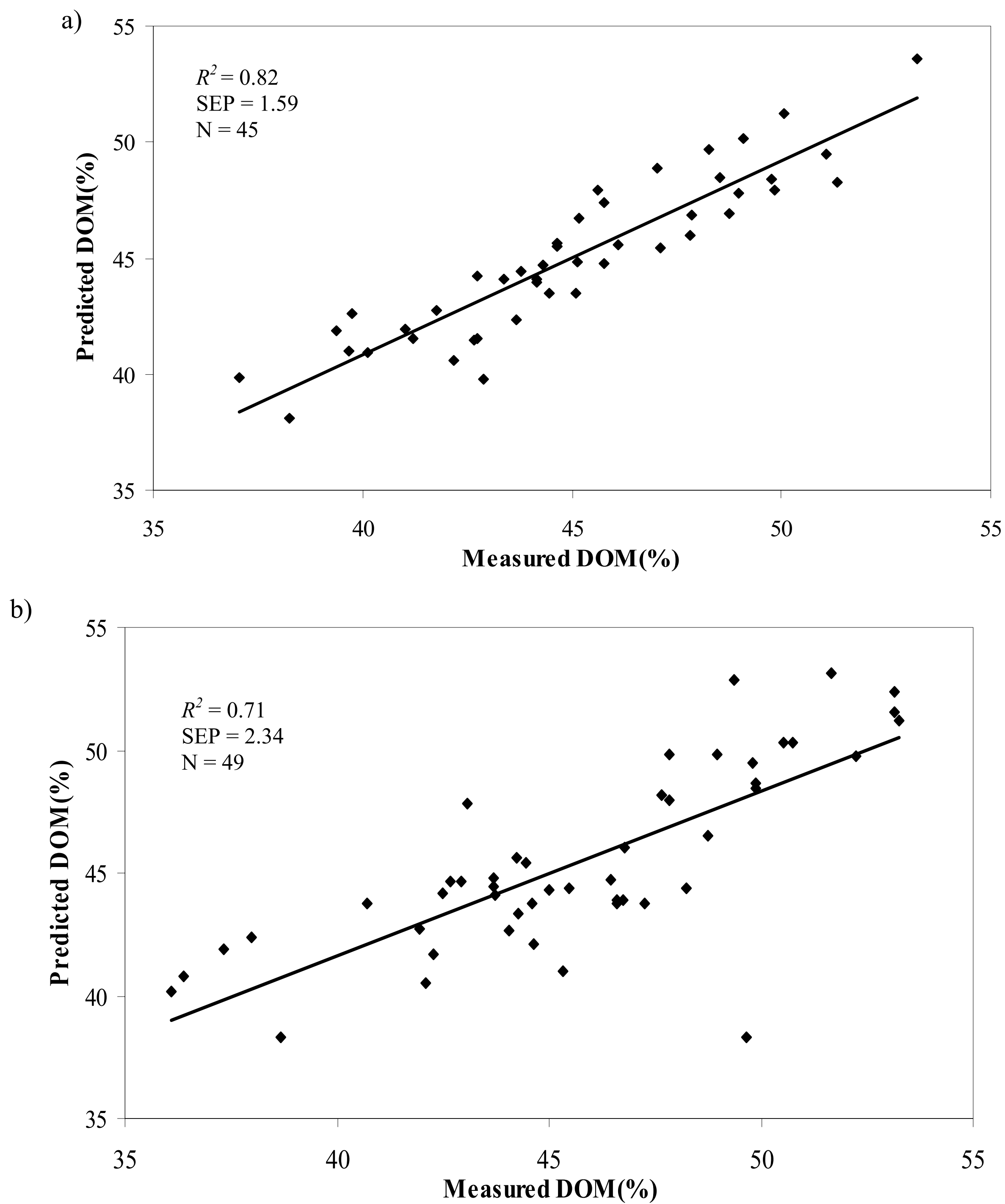

Figure 4.2. Relationship between measured and NIRS predicted digestible organic matter of diets for a) non-pregnant and b) pregnant calibration set. The line Y=X represents agreement between predicted and measured DOM values.

Table 4.2 Validation statistics after the non-pregnant and pregnant CP, DOM, DDM and OMD calibration equations were applied to pregnant and non-pregnant datasets, after the removal of critical T outlier samples, respectively

Equation	N	Mean	SEP	r^2	SEP (C)	Bias	Slope
PRG CP	48	11.5	1.27	0.91	1.27	-0.20	0.97
NPG CP	48	10.8	1.38	0.90	1.35	0.33	0.91
PRG DOM	44	44.9	3.15	0.41	3.19	0.03	0.64
NPG DOM	45	45.9	2.94	0.53	2.96	-0.3	0.90
PRG DDM	45	52.7	3.89	0.70	3.93	-0.17	0.90
NPG DDM	49	53.2	3.91	0.74	3.95	-0.59	1.03
PRG OMD	46	54.1	4.19	0.58	4.23	0.14	0.87
NPG OMD	47	55.1	4.0	0.63	4.34	0.17	0.89

PRG= pregnant
NPG= non-pregnant
N= number of samples in validation set
SEP= standard error of prediction
r^2= coefficient of simple correlation
SEP (C)= standard error of prediction corrected for bias
Bias= mean of differences
Slope= rate of change

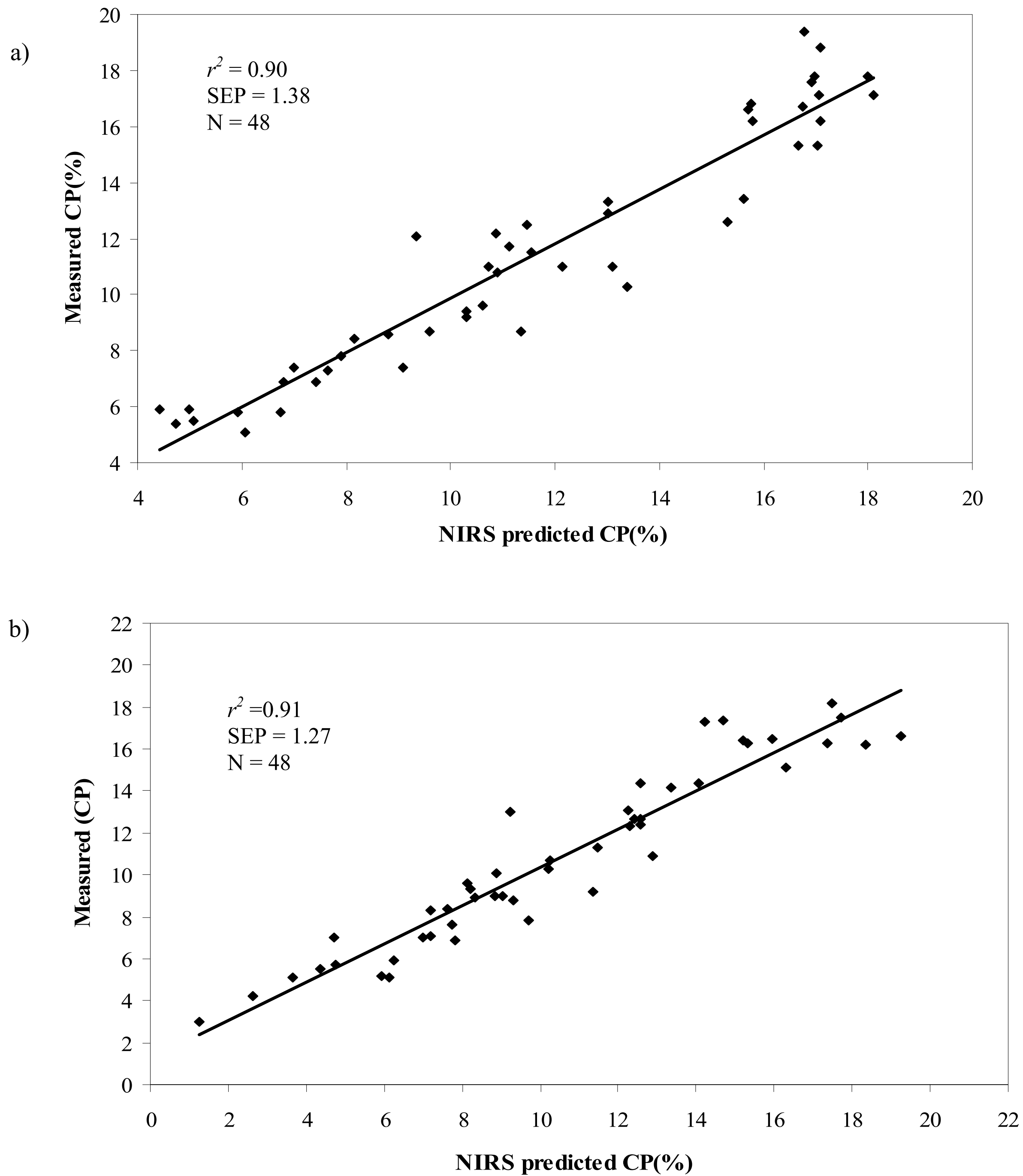

Figure 4.3. Relationship between NIRS predicted and measured crude protein concentration when a) non-pregnant equation applied to pregnant dataset and b) pregnant equation applied to the non-pregnant dataset. The line Y=X represents agreement between predicted and measured CP values.

CP Equations

The standard errors of prediction (SEP) for PRG and NPG CP equations were 1.27 and 1.38 with the corresponding coefficient of simple correlation (r^2) of 0.91 and 0.90, respectively. Very strong correlation between the measured (reference) and NIRS predicted values were observed in both the pregnant and non-pregnant prediction. Figures 4.3a and 4.3b depict the relationship between measured and NIRS predicted CP for non-pregnant and pregnant sample sets, respectively. Compared to the performance of the combined equation in predicting the pregnant and non-pregnant data set, the performance of both NPG and PRG were also comparable both in terms of SEP and r^2. The result indicates that the three CP equations (PRG, NPG and combined) did not differ much in their predictive capability.

DOM, DDM and OMD Equations

The coefficients of simple correlation (r^2) for the pregnant (PRG) and non- pregnant (NPG) DOM were 0.41 and 0.53, respectively with corresponding standard error of prediction values of 3.15 and 2.94. For both equations the values of SEP and r^2 obtained for prediction were inferior to those values of their respective calibration. In both PRG and NPG equations high SEP and low r^2 were observed for the prediction of DDM and organic OMD. However, relatively more accurate result of prediction for DOM was observed for NPG equations than those for the PRG. Figures 4.4a and 4.4b depict the relationship between NIRS predicted and measured DOM concentration of diets using pregnant and non-pregnant DOM equations. Compared to the combined DOM equation, the combined DOM equation had better prediction performance than both NPG and PRG equations. For instance, using the pregnant data set as independent validation set for both the combined and NPG equation, the SEP value was much lower for combined equation than the NPG. Similarly the PRG equation had higher SEP than the

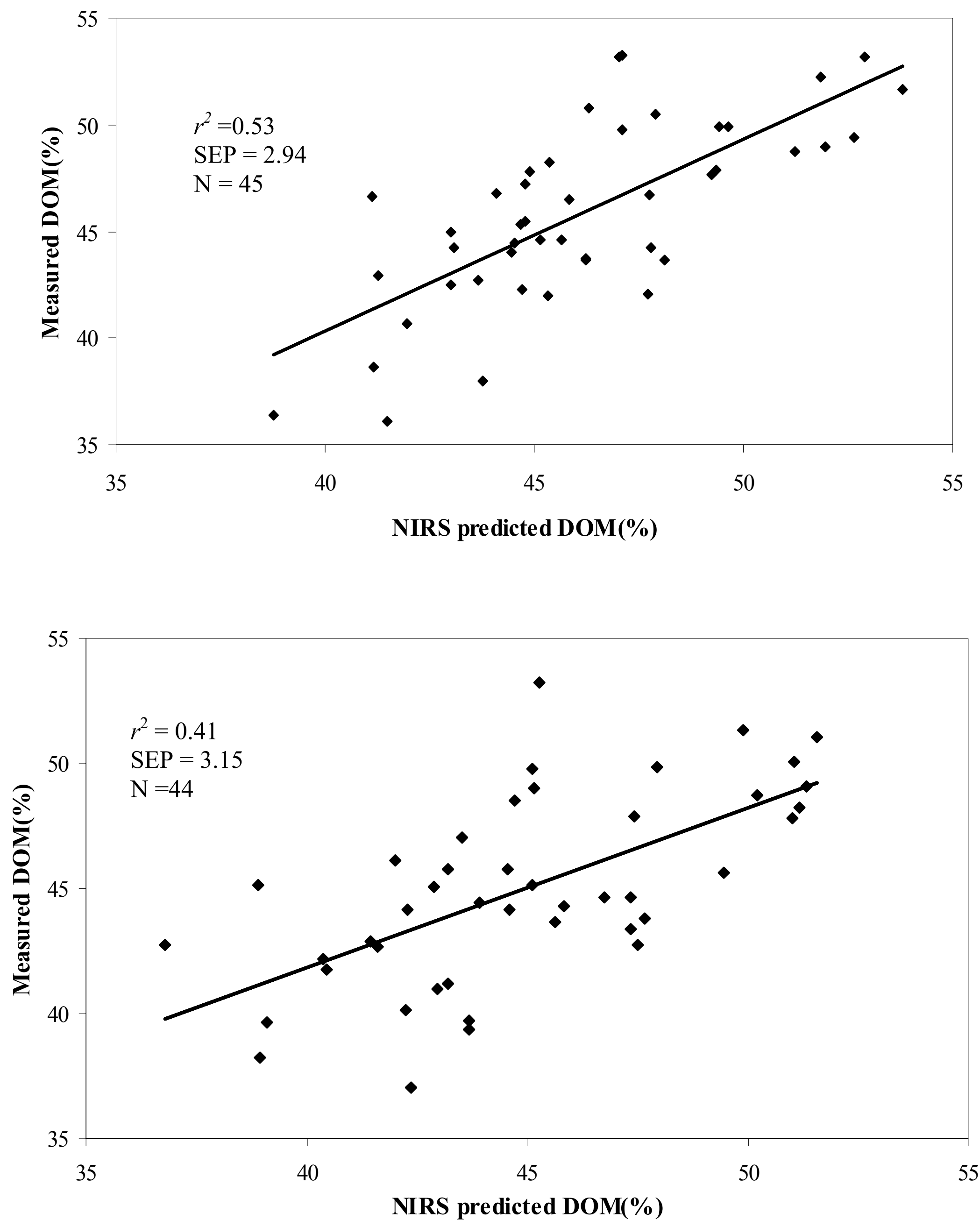

Figure 4.4. Relationship between NIRS predicted and measured DOM when a) non-pregnant equation applied to pregnant dataset and b) pregnant equation applied to the non-pregnant datasets. The line Y=X represents agreement between predicted and measured DOM values.

combined equation when both equations were applied to predict the non-pregnant data set. These results indicate that the separate calibration equations perform less than the combined equation developed from combined pregnant and non-pregnant calibration datasets and they can predict to a higher level of accuracy for the other physiological state than when separate them into a two different equations. However, it should be noted, the superior performance of the combined equations stems from the fact that both pregnant and non-pregnant data sets were part of the calibration set while the two separate equations were validated with independent data set that were not part of the calibration set.

Conclusions

In this study it was possible to developed separate calibration equation for pregnant (PRG) and non-pregnant (NPG) donkeys with excellent calibration and validation performances. Calibration results have indicated that both PRG and NPG CP equations with coefficients of determination (R^2)-values greater than 0.95 and SEC values less than 1.0 (2x SEL) had acceptable performance and were comparable with those found for the combined CP equation. However, differences both in calibration and validation statistics were observed among the three DOM equations (NPG, PRG and combined). Despite the superior calibration result observed in both the pregnant and non-pregnant equations the better prediction capacity was observed for the combined equation. Nevertheless, because of the problem of the method used (lack of independent data set) to evaluate the performance of these three equations, it was not possible to identify the significance of this difference and nor was possible to conclude that the cause of this disparity was physiological difference of the animals. The differences could simple be the choice of the original calibration equation and will require further investigation to determine how predictive capacity of NIRS is affected by animal physiological state.

CHAPTER V

RELATIONSHIP BETWEEN DIET QUALITY AND DRY MATTER INTAKE OF DONKEYS FED MIXED DIETS

Introduction

Free grazing animals exist in a highly dynamic situation in which their performance (growth, milk production or draught power) is determined not only by the quality of the diet but also by the quantity of forage available and its subsequent intake (Reid and Jung 1981; Minson 1982). In the past few decades, remarkable progresses have been made on developing fecal-NIRS calibration equations for predicting the diet quality of various free grazing herbivores. Consequently, monitoring of the nutritional status of cattle, goats, sheep, deer, elk, and currently donkeys coupled with improved nutrition models is providing a viable mechanism to monitor the nutritional well being of free-ranging livestock. The NIRS technique provides information mainly about the nutritional quality; chemical composition (crude protein and mineral elements) and functional properties (digestibility) of forages consumed by the animal. Such rapid reliable prediction of diet quality in the grazing situation is necessary for proper animal as well as forage resources management.

However, estimates of the nutritional qualities are often of little value or incomplete, with out information regarding the amounts of forage that is consumed. As a result depending on the system of animal production various direct and indirect approaches have been used to predict the quantity of forage (feed) eaten by animals. In the case of stall feeding intake is simply determined as the daily feed offered less the feed refusal (ort). Direct measures of intake in free-ranging animals has proved difficult (Ferri et al. 2003), posing one of the most difficult challenges in addressing nutritional management of animals in grazing environments.

There have been attempts by several authors to identify dietary factors related to intake so that an indirect prediction method could be applied. Studies with sheep, cattle and horses (Hodgson 1982; Minson, 1982; NRC 1987, 1989, 1996; Van Soest 1994; LaCasha et al. 1999) have confirmed the existence of a correlation between intake and digestibility but not are adequate for prediction of intake. Most of the findings demonstrated that intake of forages has been positively related to dry matter digestibility. Additionally, reports by NRC (1987) Van Soest (1994) and LaCasha (et al. 1999) have indicated that protein content of diets has a major effect on dry matter intake and it is generally believed that deficiencies or excesses of crude protein depresses feed intake. In addition, DOM/CP ratio, metabolic modifiers, associate effects and physiological state of the animals affects the intake of animals (Hogan 1979, cited in Stuth et al. 1999).

Based on the relationships exist between diet quality and dry matter intake a number of regression equations have been published relating intake of pasture by sheep (Minson 1982), cattle (Van Soest 1994) and horses (NRC 1989). Additionally, prior literature review works by Mayes and Dove (2000) have indicated that fecal output has been a relevant variable to predict feed intake of free grazing ruminant if you know the indigestible fraction of the diet.

Compared with ruminants, few attempts have been made to measure intake in free-ranging non-ruminant herbivores (Mayes and Dove 2000), especially donkeys. Consequently the question whether diet factors affect the dry matter intake of donkeys has not been clearly established. The objective of this study was to determine the relationship between various diet qualities and dry matter intake of donkeys.

Materials and Methods

Data Acquisition

Dietary crude protein (CP), in vivo dry matter digestibility (DDM), in vivo digestible organic matter (DOM), organic matter digestibility (OMD), total digestible nutrient (TDN) and fecal dry matter output (FDMP) were determined from a previous in vivo feeding trail through total fecal collection procedure (see Chapter III). Fecal and dietary samples were obtained from 10 consecutive install feeding trials conducted in College Station, Texas, USA. The feeding trial was conducted between December 2002 and February 2003. Before the initiation of the experiment donkey were subjected to pregnancy test using ultrasound-scanning technique. Ten female donkeys (five pregnant and five open) with mean initial body weight of 196.8 ± 51.9kg were used in these experiments. To represent the variation in grazing situations, diets used in this experiment were specially mixed from several feed types consisting legumes, grasses and crop residues. Diets range in their ingredient number from a single feed per diet to as many as ten feed types per ration. A total of 100 diets mixed from 13 forage species and crop residues were used during the eleven weeks feeding trial.

Sample Collection

Donkeys were fed twice daily at 12-hrs interval (07.00hour and 19.00hour) and feed refusal (ort) from each donkey was collected twice daily before each meal. Feed was offered as 2% of the body weight of the animal and adjustment was made based on the rate of intake during the first four adaptation periods. During the digestion trial, total excreted feces were collected off the ground by hand immediately after each defecation event via observers watching the animals 24 hours a day for the entire collection period and weighed at 4-hours interval for 3-consecutive measurement days. Feces collected during each 4-hours period were stored in plastic tub,

weighed, and mixed thoroughly. A 5% sub-sample was obtained by weight (wet weight) after weighing. Total fecal output was measured daily for three consecutive days and mean daily output was determined by dividing the total output by number of days (3 days). During each three days of intake and fecal output measurement, samples of the diet offered and diet refusal (ort from trough and floor) were collected for laboratory analysis.

Chemical Analysis

At the end of the three days collection period, each diet, refusal and fecal sample was sub sampled, dried at 60°C over night, and ground to pass though 2-mm sieve. Ground samples were subjected to dry matter analysis and percentage of dry matter was computed as [weight of sample after drying/weight of sample before drying] x 100. For crude protein analysis, diet and ort samples were submitted to the Soil, Water and Forage Testing Laboratory at Texas A&M University. Nitrogen was determined by the standard Kjeldahl method (AOAC 1995) and was multiplied by a coefficient 6.25 to derive crude protein concentration. In vivo dry matter digestibility was determined by the conventional equation described by (Merchen 1988) DMD = 1-FDM/DMI] x100. In vivo organic matter digestibility (OMD) was estimated as described by Ferri et al. (2003) OMD= [1-FOM/OMI] x 100. In vivo digestible organic matter (DOM) was determined as gram of organic matter digested per gram of dry matter ingested as described by Lyons and Stuth (1992). Dry matter intake (DMI) was calculated by the conventional method [DM offered- DM refused] (Merchen 1988). Total digestible nutrient (TDN) was determined multiplying DOM times 1.05 (NRC 1996) and metabolizable energy (ME) was calculated by DOM times 0.15MJ/kgDM (NRC 1987, 1996). Details of the digestibility experiment, chemical analysis and calculations were reported in Chapter III.

Animal Body Measurements

Body weight of donkeys was taken at the beginning (November 15, 2002); middle (January 10, 2003) and end (February 12, 2003) of the experiment and an average of these three body weight measurements were considered as mean body weight for each donkey used in the study. Mean body weight of each animal was standardized to metabolic body weight (kg^{075}) (NRC 1989).

Statistical Analysis

Regression equations were determined using the linear regression model (SAS 2000) to evaluate predictive relationship between the dependent variables intake and the independent variables crude protein (CP), dry matter digestibility (DDM), digestible organic matter (DOM) and DOM/CP ratio. In addition, the relationship between fecal dry matter output and dry matter intake, and fecal output and body weigh was determined using the above statistical model. A linear regression model:

$$y = \beta_0 + \beta_1 x + \varepsilon, \quad \text{[Eq.5.1]}$$

where : ***x*** is the explanatory variable, diet quality attributes

y is the dependent variable dry matter intake.

β_0 is the intercept (the value of ***y*** when ***x*** = 0).

β_1 is the slope of the line

ε is a random error, was used for data analysis

Results

Table 5.1 presents the chemical composition, functional properties of diets used in the experiment and dry matter intake averaged across week. Minimum and maximum dietary crude protein concentrations were 5.2% and 16.5, respectively. Weekly average dry matter digestibility ranged from 47.0% to 64.3%. Daily dry matter intake per donkeys was averaged as 3.1 kg or 1.6 % body weight and the average daily fecal dry matter output per donkey was 1.5 kg/d or 0.8% body weight. The minimum and maximum fecal outputs were 0.5kg/d and 2.6kg/d, respectively. The mean body weight of donkeys computed from 3 consecutive measurements was 196.3±51.8kg.

Dry Matter Intake (DMI) and Crude Protein (CP)

Simple linear regressions between DM intake and crude protein concentration have shown mixed results. The relationship between dry matter intake and crude protein concentration of diets for non-pregnant (NPGR), pregnant (PRG) and combined pregnant and non-pregnant (COMB) data set was fitted using a graphics and curve- expert fitting program (Curveexpert 1.3). Scattered plots of the data suggested that the relation between the parameters requires non-linear regression. In order to derive the best-fit model, the original CP data was fitted to linear model. Simple linear regression results indicate that there was a positive relationship ($p< 0.0001$) between DMI and CP for the NPG, PRG, and COMB datasets, with the r^2 values being 0.37-0.40. The independent CP data were then were fitted using a quadratic model. All relationships were significant ($p< 0.0001$) and each data group (NPG, PRG and COMB) had higher coefficient of determination with r^2 values being between 0.60 and 0.65.

Table 5.1. The mean crude protein, dry matter digestibility, digestible organic matter, dry matter intake, total digestible nutrient, metabolizable energy and fecal dry matter output across weeks

Week No.	CP (% DM)	DDM (%)	DOM (%)	TDN (%)	ME (MJ/kg DM)	DMI (kg/d)	FDMP (kg/d)
1	5.5	47.4	44.2	46.4	6.6	2.9	1.5
2	7.0	44.4	41.9	44.0	6.3	3.1	1.7
3	8.6	54.9	47.1	49.5	7.1	3.0	1.4
4	11.1	49.7	43.2	45.5	6.5	3.3	1.6
5	11.1	52.7	45.2	47.5	6.8	3.3	1.6
6	8.9	49.0	42.9	45.0	6.4	3.4	1.7
7	16.0	64.3	49.6	52.1	7.4	3.4	1.3
8	14.0	57.3	45.8	48.1	6.9	3.6	1.5
9	16.5	61.3	49.3	51.8	7.4	3.5	1.4
10	5.2	47.0	38.3	40.2	5.7	1.6	0.9
Mean	10.4	52.8	44.8	47.0	6.7	3.1	1.5
SD	4.1	6.6	3.5	3.7	0.53	0.57	0.24

CP= crude protein,
DDM= dry matter digestibility,
DOM= digestible organic matter,
TDN= total digestible nutrient,
ME= metabolizable energy,
DMI= dry matter intake
FDMP= fecal dry matter output

The simple coefficients of determination (r^2) derived from the linear and quadratic relationships between DMI and CP data are presented in Table 5.2. The results indicate that the variation in DMI intake due to difference in CP concentration was better explained when data were fitted with non-linear models. However, in both cases it was shown that large portion of the variation in intake was explained by the difference in crude protein concentration of diets.

Dry Matter Intake (DMI) and Dry Matter Digestibility (DDM)

Dry matter intake (DMI) was regressed against dry matter digestibility (DDM) for the NPG, PRG and COMB data sets. There was no significant relationship ($p > 0.05$) between DMI and DDM. The simple coefficients of determination (r^2) values in the relationship for the three categories (NPG, PRG and COMB) were low, ranging between 0.036-0.044 (see Table 5.2). The results indicate that the level of dry matter digestibility of diets poorly explained the variation in dry matter intake.

Dry Matter Intake (DMI) and Digestible Organic Matter (DOM)

There was no positive linear relationship ($p>0.05$) between DMI and DOM for NPG, PRG and COMB data sets ($P< 0.0005$). The r^2-values were 0.058 for the NPGR, 0.052 for the PRG and 0.09 for COMB datasets (see Table 5.2).

Dry Matter Intake (DMI) and DOM/CP Ratio

A negative linear relationship between DMI and DOM/CP ratio was observed for the combined data set. The simple coefficient of determination (r^2) was 0.39, indicating DMI relates relatively better with DOM/CP ratio and intake decreases at rate of 3.59 g/kgw$0^{.75}$ for every unit increase in the DOM/CP ratio (see Table 5.2).

Table 5.2. Regression results for the relationship between crude protein, dry matter digestibility, fecal dry matter output and dry matter intake of non-pregnant, pregnant and combined datasets

Relationships	Statistical Parameters						
	N	a	b	SE_b	r^2	p	F
DMI vs. CP							
NPG	50	38.58	1.99	0.36	0.38	0.0001	29.9
PRG	50	45.70	1.45	0.26	0.40	0.0001	31.8
COMB	100	41.99	1.73	0.22	0.37	0.0001	59.7
DMI vs. DDM							
NPG	50	37.41	0.43	0.3	0.048	0.119	2.5
PRG	50	49.26	0.23	0.17	0.036	0.189	1.8
COMB	100	43.54	0.32	0.16	0.044	0.0384	4.4
DMI vs. DOM							
NPG	50	53.29	0.16	0.30	0.058	0.60	0.3
PRG	50	17.82	0.97	0.26	0.052	0.50	4.6
COMB	100	36.07	0.56	0.18	0.09	0.200	9.7
DOM/CP vs. DMI							
COMB	100	78.01	-3.585	0.457	0.38	0.0001	61.45
DMI vs. FDMP*							
NPG	50	19.7	1.42	0.19	0.53	0.0001	54.5
PRG	50	30.7	1.10	0.24	0.30	0.0001	21.0
COMB	100	23.8	1.31	0.15	0.45	0.0001	79.0
DMI vs. FDMP**							
NPG	50	0.43	1.56	0.18	0.59	0.0001	72.7
PRG	50	0.70	1.37	0.23	0.38	0.0001	29.2
COMB	100	0.53	1.46	0.14	0.52	0.0001	108.7
FDMP vs. BWT							
COMB	10	0.61	0.005	0.0003	0.96	0.0001	198.6

DMI = dry matter intake (g/kg $^{0.75}$)
CP = crude protein (%DM)
DDM = dry matter digestibility ((%)
DOM = digestible organic matter (%)
FDMP = fecal dry matter output
DOM/CP= digestible organic matter to crude protein ratio
NPG = non-pregnant group
PRG = pregnant group
COMB = combined data set
a= intercept
b = regression coefficient or slope
r^2 = simple coefficient of determination
SE_b = standard error of slope
p = probability
F = fisher's test
N = number of observations
* = (metabolic body weight (g/kg $^{0.75}$)
**= (% body weight)

Dry Matter Intake (DMI) and Fecal Dry Matter Output (FDMP)

Dry matter intake and fecal dry matter output relationship was examined into two ways (1) DMI and FDMP expressed as gram per kilogram metabolic body weight (g/kgw$0^{.75}$) and (2) FDMP as percentage of body weight (%BW). In both cases there was significant positive relationship (p<0.0001) between DMI and FDMP with relatively high simple coefficient of correlation. For all three data sets NPG, PRG and COMB) the r^2 values (0.30-0.59) were higher when FDMP was expressed as % BW than as (g/kgw$0^{.75}$) (0.38-0.52). However, in both cases the result indicated that relatively large percent of the variation in dry matter intake was accounted for by the fecal dry matter output.

Body Weight and Fecal Dry Matter Output

Regression of fecal dry matter output and live body weigh of donkeys have also shown significant positive relationship between the parameters (P<0.0001). The simple coefficient determination (r^2) was 0.96 indicating strong correlation between body weight and FDMP with 95%of the variation in fecal dry matter output well expressed by the model.

Discussion

Dry Matter Intake

Figures 5.1a, 5.1b, 5.1c and 5.1d depict the distribution of the various dietary and animal attributes obtained from the study. In general the results indicate that there was a wide variation in chemical composition and functional properties of diets fed to donkeys. The distribution of the nutrient concentration and digestibility of diets used in the present study reflects the situation which donkeys encountered in grazing areas. During the time period measurement carried out, dry matter intake by donkeys was on average 59.2g/kgw $^{0.75}$, which is equivalent to 15.8g of DM/kg LW, or 3.1kg/d. The present intake rate for donkeys were comparable with those values

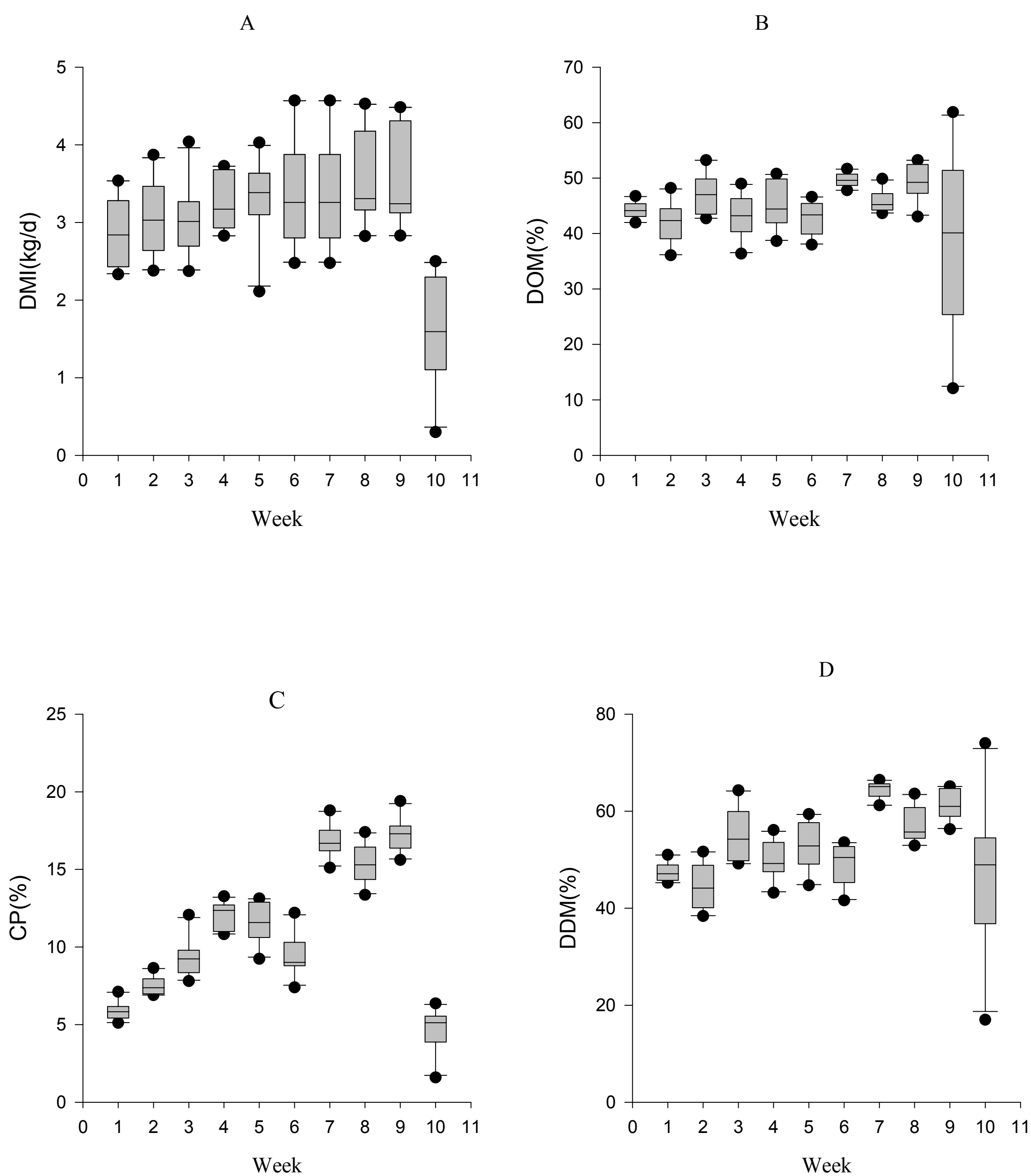

Figure 5.1.Distribution of (a) dry matter intake (DMI), (b) digestible organic matter (DOM), (c) crude protein concentration (CP %) and (d) dry matter digestibility (DDM) of diets across weeks.

reported in the literature. Pearson et al. (2001) reported that daily dry matter intake of donkeys fed alfalfa and oat mixed diets amounts to 18.1g of DM/kg LW or 3.3kg/d. In another study Cuddeford et al. (1995) working with donkeys reported a lower dry matter intake of donkeys. When donkeys fed on alfalfa and oat diets mixed at different proportions, intake was 13.3g of DM/kg LW, or 2.3 kg of DM/d, which is relatively lower than values obtained in the present study. However, another study in Africa by Aganga et al. (2000) has reported higher daily dry matter intake (4kg /d).

Dry Matter Intake and Crude Protein

In the present study, the most important variable for estimating dry matter intake was dietary crude protein. Dry matter intake and crude protein curves of donkeys were readily fit to both linear model and quadratic models.

However, more significant relationship with relatively better accuracy was found between CP and DMI when the data were fitted to quadratic model. In both models similar accuracy of prediction was observed among the relationships developed from the NPG, PRG and COMB. Despite the increase in number of samples, inclusion of the NPG data into the PRG data to develop the combined equation did not enhance the relationship between intake and CP. Figures 5.2a, 5.2b and 5.2c depict the relationship between DMI and CP for NPG, PRG and combined respectively.

Looking at the scattered plot of the original data (Figs.5.2a, 5.2b, and 5.2c) indeed indicated that dry matter intake of donkeys was low on diets with low concentration of dietary crude protein and rapidly increased up to a level of 6-8% CP in the diet. There was little or no positive increase in intake when CP values were greater than 10%.

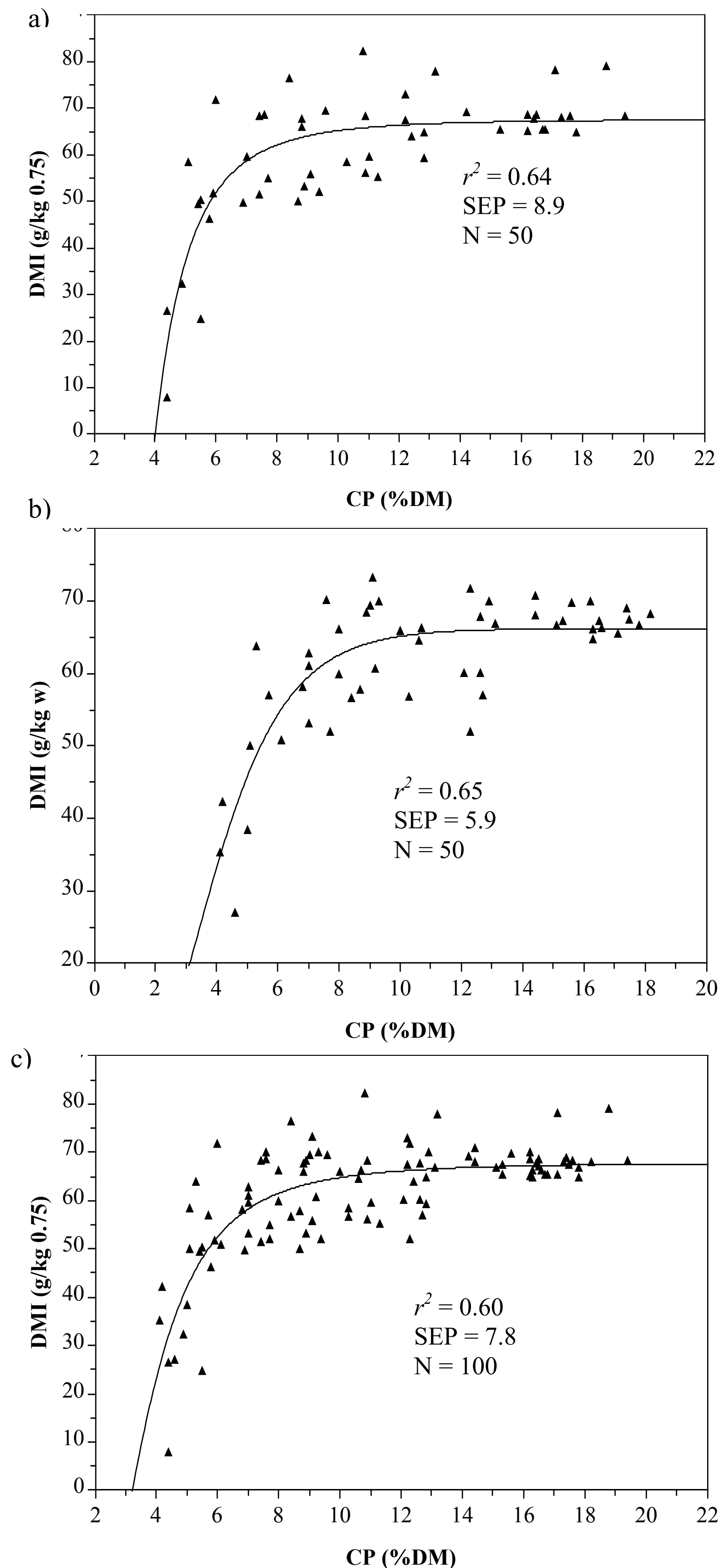

Figure 5.2.Relationship between dry matter intake (g/kg w.^{075}) and crude protein concentration of diets observed on a) non-pregnant, b) pregnant and c) combined datasets.

The low CP content of the diets may be a contributing factor to the low intake by the donkeys through inhibition of microbial digestion in the hindgut (Pearson et al. 2001) but whether the downward trend in intake with increasing CP level was associated with excessive amount of nitrogen in the digestive tract or other factor is not clearly established in donkeys and needs further consideration.

Reports in the literature are inconsistent regarding the relationship between crude protein concentration and feed intake in equines. For instance, Boulot (1987) cited in Dulphy et al (1997a) reported a poor linear relationship (r^2= 0.18) between CP concentration and DMI by donkeys. In review of forage intake by horse, Dulphy et al. (1997b) also noted that dry matter intake was not derived by crude protein content of diets and they concluded that the variation in CP do not seem to be very important in equids. However, results from the present study were in agreement with the work of Dulphy et al. (1997a). Dulphy and coworkers established relationship between crude protein (CP) and dry matter intake in horse and found a curvilinear relationship with moderate correlation coefficient. Another study by Lachica et al. (2001) working with ponies also reported curvilinear relationship between intake and crude protein concentration of mixed diets.

Dry Matter Intake and Dry Matter Digestibility

In the present study no significant linear relationship between dry matter intake and digestibility parameters was observed. All prediction equations develop for DMI using digestibility variables had low correlation coefficient values and slopes not significantly different from zero. For instance the regression equation for the COMB data set, DMI= 43.54 + 0.32DDM was insufficient predictor of dry matter intake (p=0.20). Most of the data points are clustered towards the x=y line of the plot (with y value near constant) and there are a few points, which lie

well below the main cluster of the data indicating the digestibility parameters were not useful variables in prediction dry matter intake. Figures 5.3a, 5.3b, and 5.3c depict the relationship between dry matter intake and digestibility for the NPN, PRG and combined (COMB) respectively. In the combined data set, five of the six outliers (4 for non-pregnant and 1 for pregnant group) with dry matter intake well below 40g/kgw $^{0.75}$ were observed on week -10 of the experiment where different donkeys received low quality diets with CP level ranged from 4.1% to 5.5%. A poor relationship between the dry matter digestibility and intake was reported in equines. A limited study by McMeniman (2003) working with young horses showed there was poor correlation (r^2 =0.232) between dry matter digestibility of pasture and dry matter intake of horses. Like the DDM the relationship between DMI and DOM was also poor indicating dry mater intake was not explained by variation in DOM.

The failure of digestibility parameters to accurately predict dry matter intake of donkeys may be partly explained by experimental error due to occasional behavioral problems with some of the donkeys. The fast passage rate (shorter retention) of digesta through the digestive tract of donkeys coupled with appetite drive differences could help contribute to this problem. Even though no measurement was taken on digesta passage rate in the present study, published literature have indicated that the transit of digesta is faster in donkeys than in ruminant (Izraely et al. 1989) but slower than in other equids (Pearson et al. 2001). For instance, Izraely et al. (1989) found mean retention time of digesta in the gastro-intestinal tract of donkey 38.8 hours. Pearson et al. (2001) reported a mean retention time of 36.4 hours opposing to reported values in sheep 44.8 hours (Melaku et al. 2004) and 72.2-91.2 hours in cattle (Aharoni et al. 1997). Results from comparative study between donkeys and goats also has shown that the mean retention time in the donkey was shorter than the goats and was consistent with its capacity to compensate for a low quality diet by increasing its intake rate (Izraely et al. 1989).

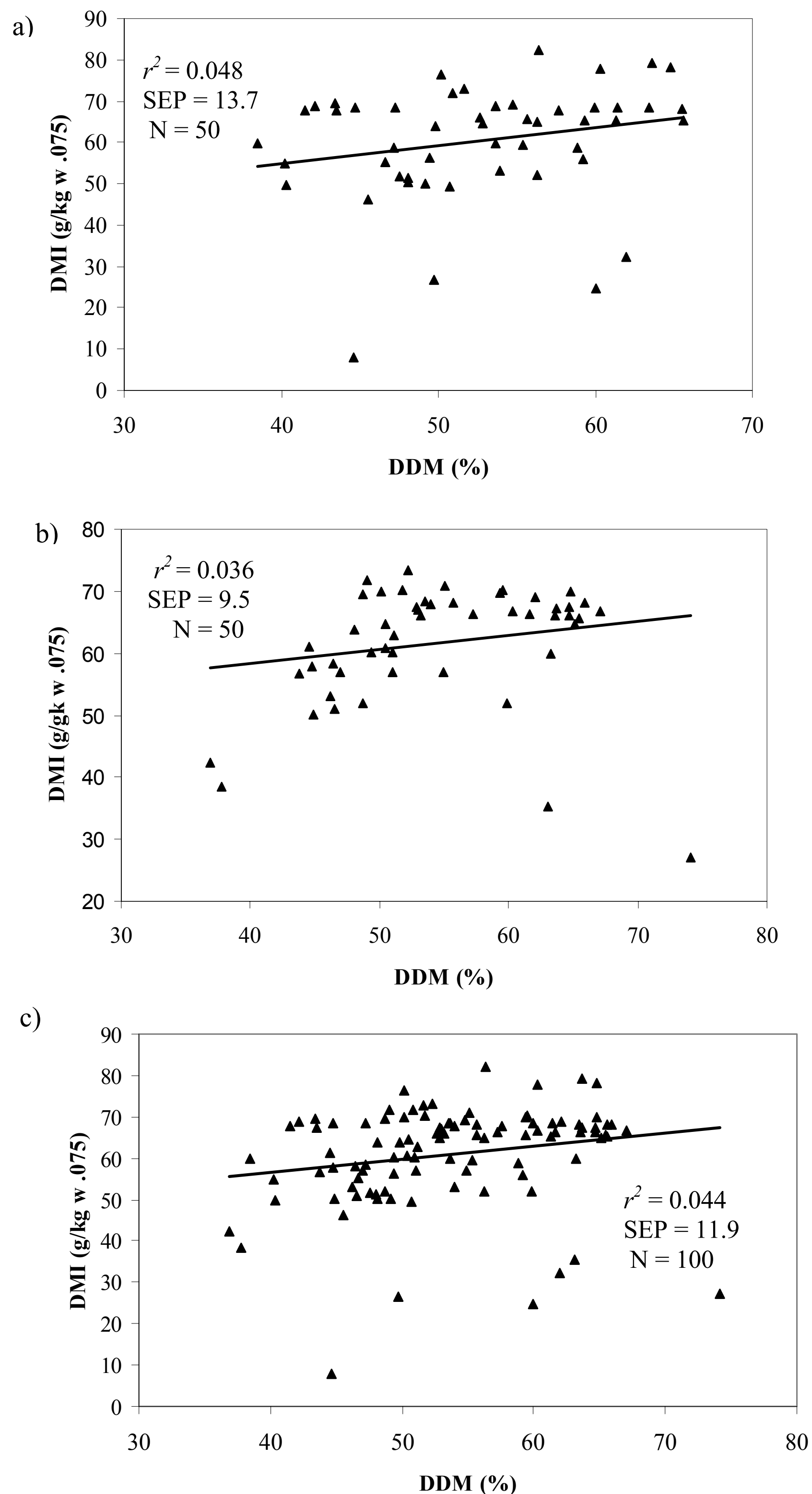

Figure 5.3. Relationship between dry matter intake (DMI g/kg w.^{075}) and digestibility of dry matter (DDM%) for a) non-pregnant, b) pregnant and c) combined datasets. Y=X perfect relationship between variables.

Another possible reason for the poor relationship between intake and digestibility parameters in donkeys may be the architecture of the gastrointestinal tract of donkeys. Unlike ruminant, donkeys' stomach is a small organ (Janis 1976; Ensminger and Olentine 1978) and possesses no reticulo-omasal orfice that selectively retain large forage particles. This may enable the donkeys to discharge indigested residue faster and allow the animals ingest larger amount of forage even low quality diets. However, the effect of extent of filling of the digestive tract (stomach and large intestine) on the control of appetite in equids, especially donkeys are not yet well known (Pearson et al. 2001) although in ruminant it has been believed that the amount of forage eaten at a meal is limited by the capacity of the rumen (NRC 1987; Van Soest 1994) particularly when the meal consists of low quality diets with high roughage contents.

Dry Matter Intake and DOM/CP ratio

Linear regression of the DOM/CP ratio and DMI resulted in a good correlation ($r^2 = 0.39$) with negative slope. This result indicates that dry matter intake is negatively related to the DOM/CP ratio meaning DMI decrease with increasing the DOM/CP ratio. The present findings were comparable to those reported by Moore and Kunkle (1995) in ruminant. The authors reported negative relationship between intake and DOM /CP ratio with relatively higher R^2(0.69). Moore and Kunkle (1995) and Moore et al. (1999) suggested that the optimum DOM/CP ratio for ruminant is 4. In the present study high DM intake was observed when diet DOM/CP falls between 2.5 and 4.0 (65g/kgw $^{0.75}$) and depression in intake started as diet DOM/CP ratio becomes greater than 4. More rapid decline in intake was observed when diet DM/CP ratio approaches 8. The two outliers well below 30g/kgw$^{0.75}$ were observed when donkeys received poor bedding wheat straw diets with CP level ranged from 4.4% to 4.6%. Fitting the data to linear regression model after the removal of these outliers resulted in

improved simple coefficient of determination (r^2 = 0.49). Figure 5.4 depicts the relationship between digestible organic matter to crude protein ratio (DOM/CP) and dry matter intake DMI (g/kg w $^{.075}$). The results indicate that intake in donkeys can be derived more by the energy and protein balance than by DOM or other digestibility attributes. The effect of DOM/CP ratio in intake in ruminant is through its associated effects on microbial growth, digestibility and pH level of the rumen (Stuth et al 1999). However, whether such effect is apparent in hindgut fermenters equine such as donkey has not been established yet.

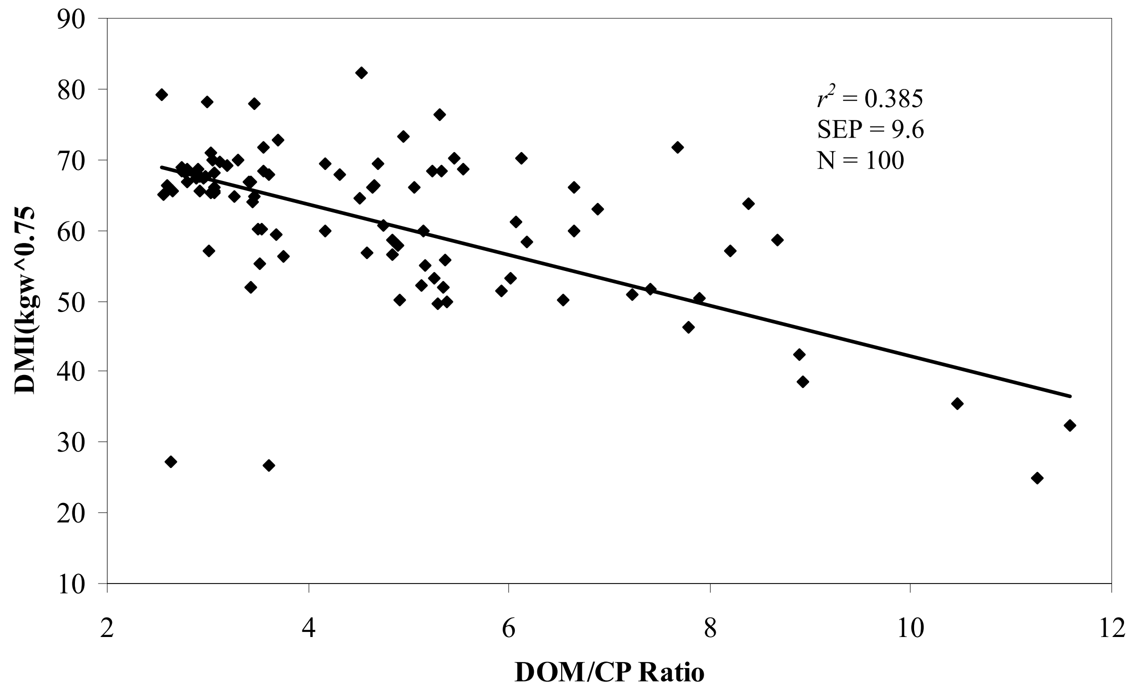

Figure 5.4. Relationship between digestible organic matter to crude protein ratio (DOM/CP) and dry matter intake (DMI g/kg w $^{.075}$). The line Y=X prefect agreement between the variables.

Dry Matter Intake and Fecal Dry Matter Output

The present study also provided data concerning the relationship between dry matter intake and fecal dry matter output. Intake of dry matter was positively correlated with fecal output. The regression equation DMI (g/kg$^{0.75}$) = 23.8 ± 1.31FDMP (g/kg$^{0.75}$) and DMI (kg/d) = 0.53 ± 1.46 FDMP (kg/d) seem sufficient predictors of dry matter intake with relatively high coefficient of correlation. The regression equation suggested that dry matter intake of donkeys increased by 1.31 ± 0.15 and 1.46 ± 0.14 with every unit rise in fecal dry matter output ($p < 0.0001$). Figures 5.5a, 5.5b and 5.5c depict the relationship between dry matter intake and fecal dry matter output both expressed as g/kg w .^{075} for non-pregnant, pregnant and combined pregnant and non-pregnant, respectively.

In the present study the computed mean daily fecal output expressed as percentage of live body weight and metabolic body weight for donkeys were lower than values reported for horse and ruminants. Mayes and Dove (2000) reported mean daily fecal out for horse 1.5% of the body weigh and recent reports in cattle had shown that daily fecal output was 1.67% of the body weight of the cows (Aharoni et al. 1999). Thus the present data for donkey was about one half of the value reported for horse and cows. This result may be partly explained by the fact that donkeys have more efficient digestion than both horse and ruminant as reported by various authors (Izraely et al. 1989; Pearson et al. 2001).

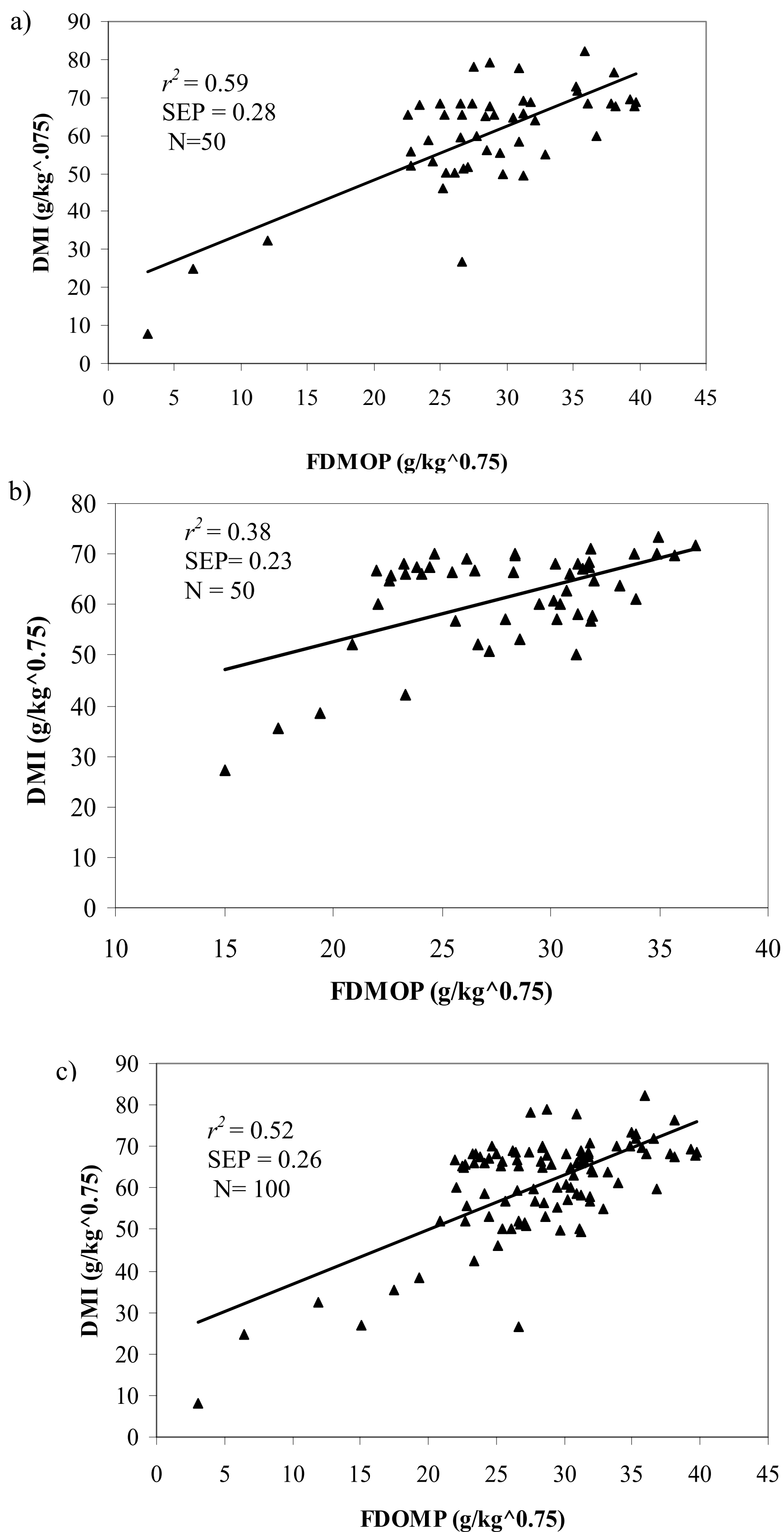

Figure 5.5.Relationship between dry matter intake (g/kg w.^{075}) and fecal dry matter output (g/kg w.^{075}) for (a) non-pregnant, (b) pregnant and (c) combined datasets. The line Y=X perfect agreement between the variables.

Fecal Dry Matter Output and DOM/CP Ratio

A curvilinear quadratic relationship was found between fecal dry matter output (g/kgw $^{0.75}$) and DOM/CP ratio. The coefficient of simple correlation r^2=0.448 was quite high indicating a strong relationship between fecal dry matter output and DOM/CP ratio. Fecal dry matter output increase as the DOM/CP ratio is between 3 and 4, and reaches maximum at about 5, which is slightly greater than the point where ruminants reach maximum fecal output. Figure 5.6 depicts the relationship between digestible organic matter to crude protein ration/ (DOM/CP) and fecal dry matter output (FDMOP).

The present DOM/CP ratio and fecal dry matter output relationship supports our findings that dry matter intake decreases when donkeys fed on high crude protein. Stuth et al. (1999) based on the model Intake = FDMP/Indigestible (Ellis et al 1988, cited in Stuth et al. 1999), indicated that dry matter intake is directly proportional to fecal dry matter output. The higher the fecal dry matter output the higher rate of intake. However, fecal output is affected by several factors, including crude protein due to its effect on appetite. At high CP level fecal output decreases and then intake depressed. The curvilinear relationship between fecal dry matter output and DOM/CP ratio, and the significant negative linear relationship between intake and DOM/CP ratio, suggest decreased intake with increasing crude protein and lowered DOM values or increasing CP relative to moderate levels of DOM beyond the upper limit of fecal output. .

Fecal Dry Matter Output and Body Weight

The study also demonstrated a positive relationship between fecal outputs (kg/d) and body weight (kg) with excellent correlation and low standard error of prediction. Figure 5.7 depicts the relationship between body weigh and fecal dry matter out put. The positive slope indicates that fecal output increases at a rate of 0.005 ± 0.00031kg for every unit increase in body weight of

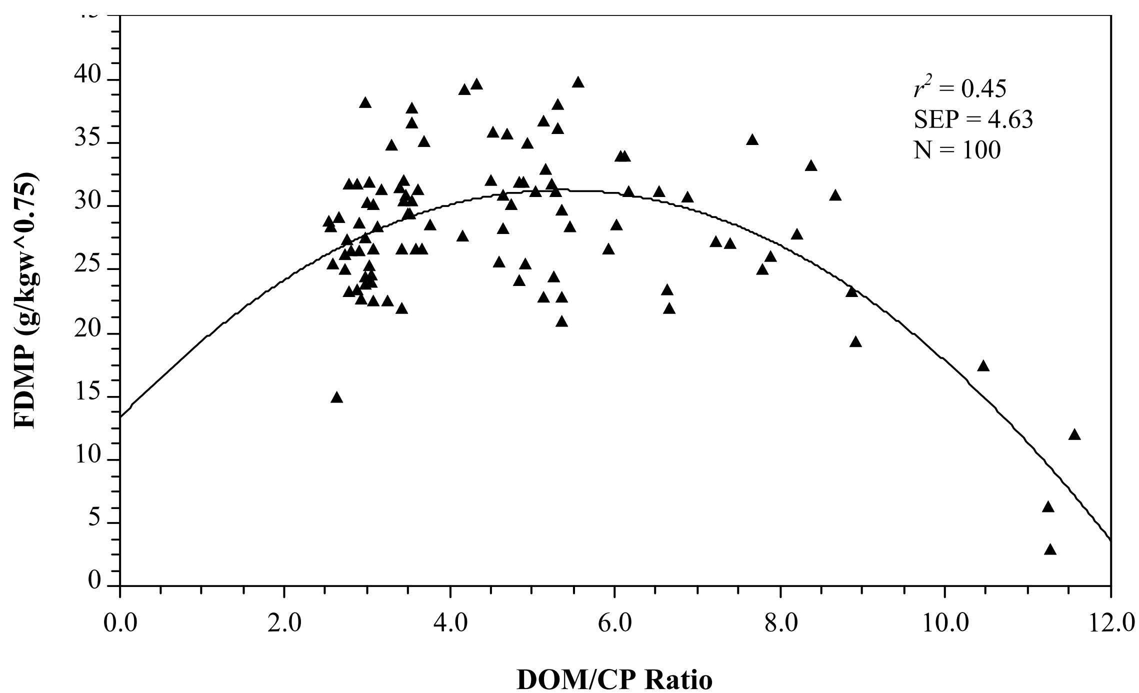

Figure 5.6. Relationship between digestible organic matter to crude protein ratio and fecal dry matter output (g/kg w $^{0.75}$).

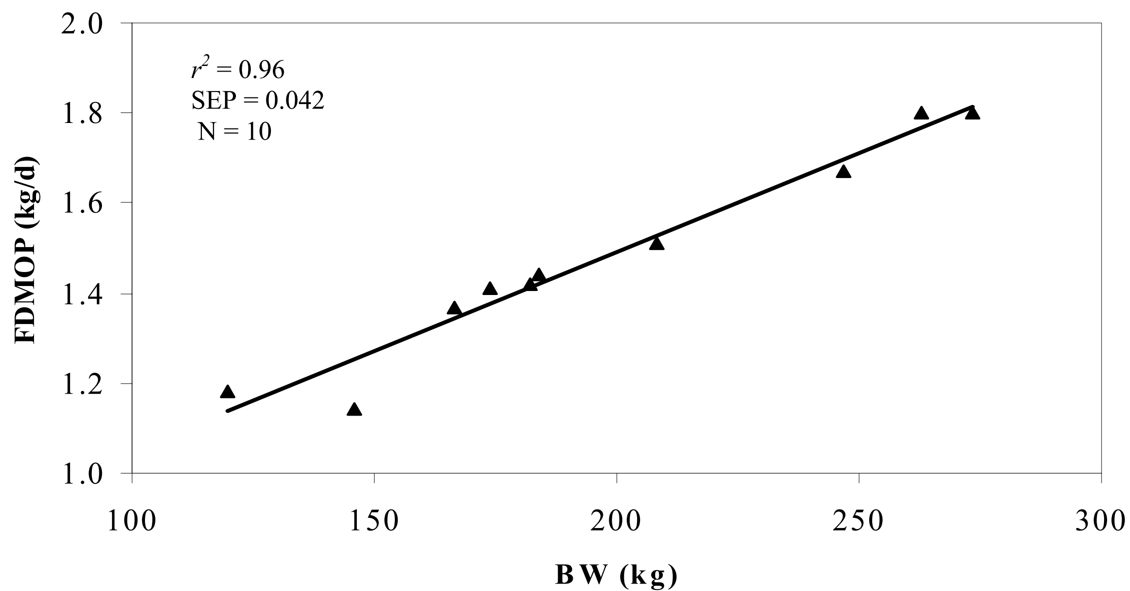

Figure 5.7. Relationship between fecal dry matter output and body weight of donkeys fed on mixed diets. The line Y = X perfect agreement between the variables.

the animal. This supports the reports that fecal output is higher in large animal than smaller and was consistent with dry matter intake.

Conclusions

The present study has established relationship between various diet qualities and dry matter intake of donkeys with mixed successes. In general correlation between crude protein concentration of diets and dry matter intake was good. Such regression equations with high correlation and low standard error of prediction can be useful for predicting the dry matter intake of donkeys. However, because feed intake can vary greatly with environmental conditions, and management factors, any equation should be viewed as providing a rough guideline for nutritional management rather than an absolute prediction of intake (NRC 1996).

Correlation between dry matter intake (DMI) and digestibility parameters (DDM and DOM) was poor and neither of the digestibility regression equations could adequately relate with dry matter intake. Hence prediction of intake in donkeys using simple digestibility criteria is unreliable, as already reported by Dulphy et al. (1997a).

However, a better correlation was observed between DMI and DOM/CP ratio than DMI and DOM indicating the intake of donkeys is derived more by the energy-protein balance than by digestibility attributes. A good correlation was also obtained between dry matter intake and fecal dry matter output, and between fecal dry matter out put and DOM/CP ratio, particularly the combined equation with relatively high simple coefficient of determination and low SEP. Correlation between fecal dry matter output and body weight was also excellent. Based on the present findings, further research to establish more reliable relationship between intake and diet characteristics is recommended.

CHAPTER VI

PREDICTING DRY MATTER INTAKE AND FECAL OUTPUT OF DONKEYS USING FECAL NEAR-INFRARED REFLECTANCE SPECTROSCOPY (NIRS)

Introduction

Knowledge of the nutrient intake by animals is one of the requirements for successful animal and rangeland resources management. In free grazing animals measuring intake has been a challenge. Fecal-near infrared reflectance has been widely used as an indirect method for rapid and accurate prediction of the chemical composition and functional properties of forages consumed by various grazing animals. Furthermore, several studies have demonstrated that using near-infrared reflectance spectroscopy (NIRS) could potentially determine the dry matter or organic matter intake of ruminants, including cattle (Ward et al. 1982;Parker et al. 1997; Gordon et al. 1998) and sheep (Abreu et al. 1992; Bruno-Soare et al. 1998) from forages (hay, silage). To our knowledge no attempt has been made to measure intake via NIRS in equines in general and donkeys in particular. The over all objective of this study was determine the potential for fecal NIRS profiling for predicting the intake of donkeys fed on mixed diets. The specific objective was to develop NIRS calibration equation for predicting dry matter intake, organic matter intake and fecal dry matter output.

Materials and Methods

Data Acquisition

Data for this study were obtained from a previous 10-week in vivo feeding trial conducted by our research program. The details of the experimental protocol can be found in Chapter III.

Intake data were collected from ten donkeys (five pregnant and five open jennies) with mean initial body weight of 196.3 ± 50.1kg. Test of pregnancy status of donkeys was determined by subjecting donkeys to ultrasound scanning technique.

During the study donkeys were fed one hundred mixed diets blended from 13 forages and crop residues over seven days, four adaptations and three collection days per period. Feed, ort and fecal samples were collected for each diet across week and donkey. The forage based rations were offered twice a day (7.00hours and 19.00 hours) while refusals (orts) were collected twice a day before each meal. For donkey feed was offered as 2% of the body weight and adjustment was made based on the intake rate of the animal during the first four days adaptation period. Total feces excreted were collected for three consecutive days where a 5% (wet weight) sample was collected in 4 hours interval. Diet, ort and fecal samples were chemically analyzed following the standard protocol by (AOAC 1995) and described elsewhere herein Chapter III.

Body weight of donkeys was taken three times during the study, i.e. at the beginning, mid and end of the experiment and an average of the three measures over the 90 days was considered as mean body weight of each donkey used in the experiment. Intake both dry matter and organic matter of diets were determined using the following formula. DMI = DM offered- DM refused (Mechngen 1988; Van Soest 1994). To minimize variation in intake and fecal output due to animal size, both intake (dry matter and organic matter intake) and fecal dry matter output were standardized to metabolic body weight (g/DM kg $w^{0.75}$) (Parker et al. 1997; Gordon et al. 1998).

Spectral Data and Treatments

Scanning of dried ground fecal samples was carried out using FOSS NIRSystem reflectance monochromator, Model 6500. Spectral data were collected in the range from 1100-nm to 2498-nm at 2 nm intervals and recoded as log 1/R (where R is the reflectance). Two derivative

treatments namely, first and second derivatives of the spectra were carried out using gap (4,8,12), smooth I (4,8,12) and smooth II (1) on log 1/R data as described by (Agnew et al. 2004) to get as many treatment combinations as possible. In addition, spectral data were corrected for scatter and particle size using SNV and detrend procedures (Williams 1987). As recommended by (Workman 1992) a critical T value of 2.5 and H value of 10, were used to eliminate samples with spectral distance too far from the population mean. The number of outlier eliminating passes was set 2, to allow the software to remove outliers twice before completing the final calibration (this means that the program made two attempts to remove outliers before completing the calibration). NIRS calibrations were developed for dry mater intake (DMI), organic matter intake (OMI) and fecal dry matter output (FDMP) for pregnant (PRG), non-pregnant (NPG) and combined (COMB) data sets. All calibration equations were developed on the treated spectral data using modified partial least square model (MPLS) of FOSS-Tecator Intrasoft International LLC (Win ISI II, version. 1.5) (Gordon et al. 1998). Selection of calibration equations was carried out on the basis of calibration statistics, including low standard error of calibration (SEC) and standard error of cross validation (SECV), and high coefficient of determination (R^2) (Lyons and Stuth 1992; Ruano-Ramos et al. 1999; Awuma 2003).

Validation

As measure of performance (predictive ability) of the selected equations, cross validation of equations was conducted. The cross validation groups was set N times (where N = number of samples in calibration set) so that each sample in the calibration to be sequentially excluded from calibration set and would be predicted by an equation developed with the remaining N-1 samples. In addition, independent validations of the calibration equations were also undertaken using the pregnant data set to validate the non-pregnant equation and *vice versa*. Whereas the

calibration equations developed from the combined data sets (COMB) were validated using the pregnant and non-pregnant data sets separately although both data sets were used in building up the equation. Furthermore, in the exercise of validation to determine the effect of T outlier samples on the performance of the equations, validation was carried out both before and after critical T outlier samples were removed from the validation set. As recommended by Workman (1992) a critical T value of 2.5 was used in this study.

Results

Intake Measurements

Based on the three consecutive measures of live body weight the mean and standard deviation (SD) metabolic body weight of donkeys (kg $w^{0.75}$) were 52.1 and 10.2 (SD) respectively indicating donkeys were representative of a wide range in body size. The mean dry matter and organic matter intake of donkeys were 61.7g /kg $w^{0.75}$ ± 10.25 (SD) and 50. 9 (g/kg $w^{0.75}$) ± 8.1 (SD). The mean and standard deviation for the FDMP were 28.5 (g/kg $w^{0.75}$) and 5.7 (SD), respectively. The mean and standard deviation values for dry matter intake (DMI), organic matter intake (OMI) and fecal dry matter output (FDMP) used in the calibration set are presented in Table 6.1.

Calibration Results

Dry Matter Intake (DMI) Equations

Calibration equations were developed for the dry matter intake (DMI), organic matter intake (OMI) and fecal dry matter output (FDMP) using the whole US diet-fecal pairs. In addition, to see if better predictive equations can be created from the combined dataset calibration was carried out by splitting the US dataset into pregnant and non-pregnant calibration sets.

Table 6.1. Calibration statistics for the dry matter intake (DMI), organic matter intake (OMI) and fecal dry matter output (FDMP) of pregnant (PRG), non-pregnant (NPG) and combined (COMB) datasets expressed as (g DM/kg $w^{0.75}$)

Equation		N	Mean	SD	SEC	R^2	SECV	SEP	BAIS	Math
DMI										
	NPG	45	61.58	10.84	3.70	0.88	6.50	3.77	0.246	1,12,4,1
	PRG	46	62.60	8.42	2.24	0.93	2.65	4.02	-0.242	2,4,4,1
	COMB	94	61.70	10.25	3.45	0.89	5.36	3.30	0.000	2,4,4,1
OMI										
	NPG	45	50.76	8.39	3.11	0.86	5.4	3.6	0.408	2,8,8,1
	PRG	45	51.47	6.60	1.88	0.92	3.38	1.75	0.00	2,4,41
	COMB	95	50.94	8.10	3.21	0.84	5.02	3.1	0.00	2,4,4,1
FDMP										
	PRG	47	28.70	4.10	3.33	0.36	3.70	3.25	-0.211	1,4,4,1
	NPG	45	28.98	6.44	2.17	0.88	4.31	1.97	0.090	2,8,8,1
	COMB	99	28.54	5.70	2.16	0.86	3.78	2.07	0.00	2,4,4,1

DMI = dry matter intake
OMI = organic matter intake
FDMP = fecal dry matter output
NPG = non-pregnant
PRG = pregnant
COMB = combined
N= number of samples
SD = standard deviation
SEC = standard error of calibration
R^2 = coefficient of determination
SECV = standard error of cross validation
SEP = standard error of prediction
Bails = mean of difference
Math = math treatment (1st and 2nd)

Calibration statistic for the dry matter intake (DMI), organic matter intake (OMI) and fecal dry matter output FDMP equations for the PRG, NPG and combined data sets are presented in Table 6.1.

The standard error of calibration (SEC) for the PRG, NPG and combined DMI equations were 2.24, 3.45 and 3.70 g/kgw $^{0.75}$, respectively with corresponding coefficient of determination (R^2) values equal to 0.93, 0.89 and 0.88, respectively. The resultant crosses validation for the DMI has shown that lowest SECV was obtained for the PRG DMI equation (SECV = 2.65) followed by the combined equation (SECV = 5.36) whereas the SECV values for the NPG DMI equation was relatively high (6.5). The standard error of calibration (SEC) accounts for an error of the mean dry matter intake of 3.6%, 5.6% and 6.0%, respectively for the PRG, combined and NPG equations. Figure 6.1a depicts the relationship between measured and NIRS predicted DMI for the combined calibration set.

Organic Matter Intake (OMI) Equations

The OMI calibration equations had SEC values of 1.88 for the PRG, 3.11 for NPG and 3.21 for the COMB with corresponding R^2 values of 0.92, 0.86 and 0.84, respectively. The SEC values represent an error of the mean of % 3.7, 6.1 % and 6.3 %, for the PRG, NPG and combined, respectively. Cross validation results have indicated that SECV values were 3.38 for PRG, 5.02 5.40 for NPG and for combined equations. Figure 6.1b depicts the relationship between the measured and NIRS predicted organic matter intake for the combined calibration set.

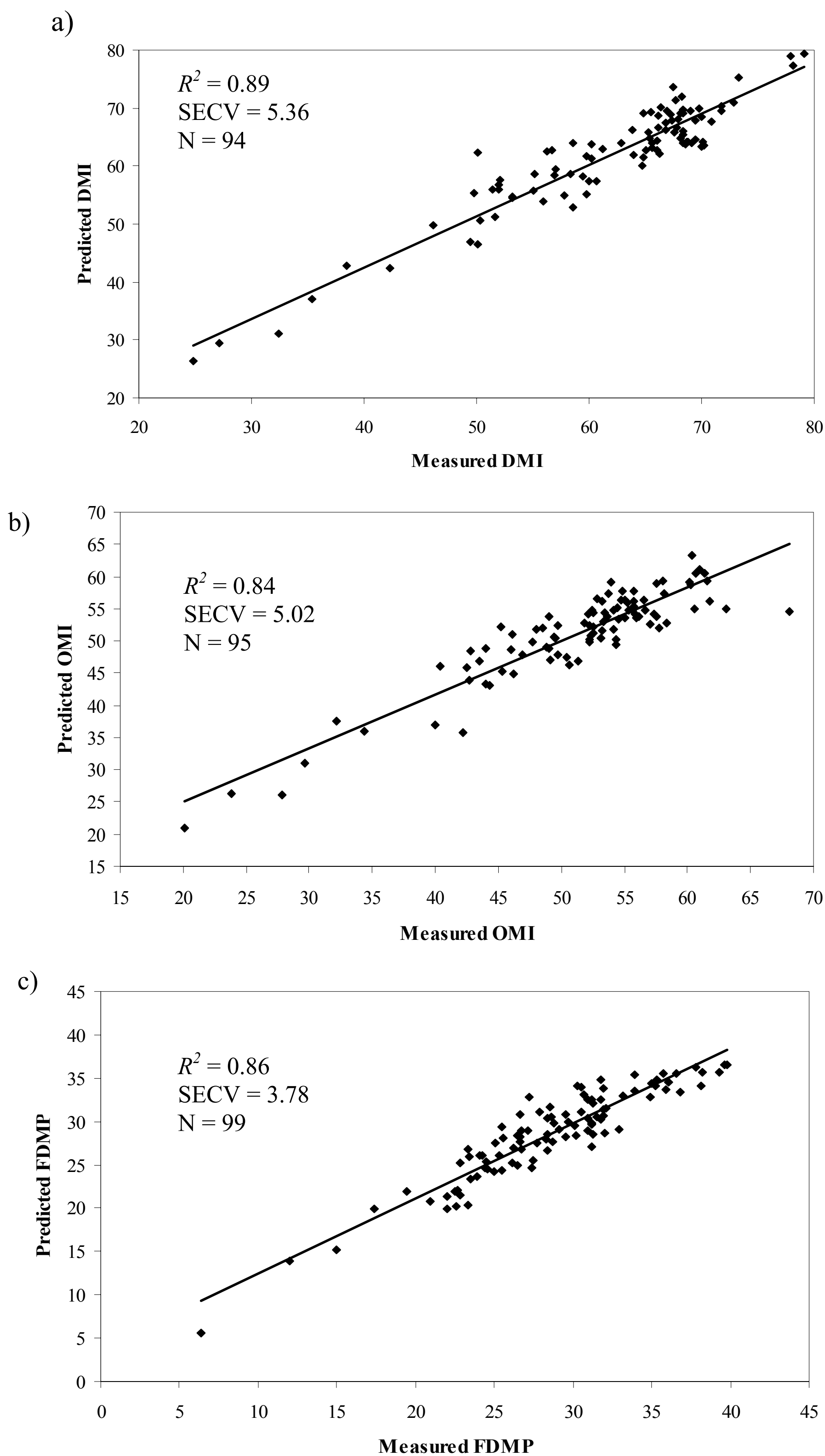

Figure 6.1. Relationship between measured and NIRS predicted a) dry matter intake (DMI) b) organic matter intake (OMI) and c) fecal dry matter output (FDMP) expressed as g /kg w $^{0.75}$ for the combined calibration dataset. The line Y = X perfect agreement between variables.

Fecal Dry Matter Output (FDMP) Equations

The fecal dry matter output (FDMP) calibration equation for the PRG, NPG and combined data sets had SEC values of 3.33, 2.17, and 2.16 (g DM/ kg $w^{0.75}$), respectively with corresponding R^2 values of 0.33,0.88, and 0.86, respectively. The SEC values have represented a standard error of the mean of fecal output 7.6% for PRG, 7.5% for NPG, and 11.6% for combined data sets. Results from cross validation have also shown that the PRG, NPG, and combined had SECV values of, 3.7, 4.31and 3.8, respectively. Figure 6.1c depicts the relationship between measured and NIRS predicted value of fecal dry matter out put for the combined calibration set.

Validation of Prediction Equations

Table 6.2 presents the validation statistics for the dry matter intake (DMI), organic matter intake (OMI) and fecal dry matter output (FDMP) for the pregnant, non pregnant and combined datasets both with and without the removal of the critical T outlier samples.

Dry Matter Intake (DMI) Equations Validation

The independent validation of DMI equations resulted in standard errors of prediction (SEP) (figures in parenthesis are values for validation after T outlier samples removed) of 12.17 (6.1) for PRG, 8.1 (7.4) for the NPG, DMI data sets. For the combined equation SEP values were 7.24 (4.36) for the non-pregnant data and 5.66 (4.06) for the pregnant data sets. The coefficients of simple correlations (r^2) for validation have varied by pregnancy status with values of 0.61 (0.62) for NPG, and 0.33 (0.39) for PGN. The combined equation had r^2 value of 0.75 (0.84) and 0.76 (0.77) when the equation was applied to predict the non-pregnant and pregnant data sets, respectively. The result have indicated that the prediction performance of the combined equation

Table 6.2. Validation statistics in terms of SEP, r^2 , bias and slope for the dry matter intake (DMI), organic matter intake (OMI) and fecal dry matter output (FDMP) of donkeys expressed as (g/ $kgw^{0.75}$).

Equation		N	SEP	Bias	r^2	SEP(C)	Slope
DMI							
NPG							
	a)	50	8.08	2.13	0.61	7.88	0.61
	b)	49	7.40	1.68	0.62	7.29	0.65
PRG							
	a)	50	12.17	-3.25	0.33	11.85	1.28
	b)	39	6.10	-0.89	0.39	6.10	0.74
COMB[1]							
	a)	50	7.24	-0.04	0.75	7.32	1.16
	b)	46	4.36	0.22	0.84	4.42	1.00
COMB[2]							
	a)	50	5.66	-0.26	0.76	5.71	0.74
	b)	48	4.06	-0.42	0.77	4.08	0.91
OMI							
NPG							
	a)	50	7.08	2.14	0.50	6.82	0.69
	b)	49	6.64	2.57	0.57	6.19	0.70
PGR							
	a)	50	9.91	-2.72	0.30	9.63	1.25
	b)	40	5.16	-0.51	0.33	5.20	0.72
COMB[1]							
	a)	50	5.68	0.12	0.77	5.73	1.2
	b)	49	4.09	0.69	0.82	4.07	1.07
COMB[2]							
	a)	50	5.17	-1.00	0.70	5.13	0.81
	b)	48	3.28	-0.22	0.87	3.31	0.87
FDMP							
NPG							
	a)	50	4.84	-0.08	0.44	4.89	1.25
	b)	48	3.76	-0.02	0.48	3.80	0.72
PGR							
	a)	50	7.14	-0.97	0.063	7.15	0.70
	b)	47	4.37	0.45	0.22	4.40	0.86
COMB[1]							
	a)	50	4.19	0.47	0.67	4.20	1.03
	b)	48	2.80	0.35	0.80	2.81	1.01
COMB[2]							
	a)	50	4.52	-0.27	0.57	4.56	0.53
	b)	40	2.42	-0.41	0.74	2.40	0.84

DMI = dry matter intake, OMI = organic matter intake, FDMP = fecal dry matter output, NPG = non-pregnant, PRG = pregnant, COMB = combined, N= number of samples, r^2 = coefficient of simple correlation, SEP = standard error of prediction, Bias = mean of difference, a = validation before removal of outlier samples, b= validation after removal of T outlier samples, [1] validation with non-pregnant data set, [2] validation with pregnant data set.

was similar for predicting both the pregnant and non-pregnant data sets before T outliers were removed. However, improvement in linearity *(r^2*) occurred for the non-pregnant data set when more outlier samples were removed from the validation sets suggesting a problem with sample preparation or chemistry of the sample (Workman 1992).

Organic Matter Intake (OMI) Equations Validation

The SEP values were 7.08 (6.64) for the NPG and 9.91 (5.16) for the PGR data sets for the validation of the OMI. When the combined calibration equation was applied to non-pregnant and pregnant data sets SEP values of 5.68 (4.9) and 5.17 (3.28) respectively, were observed. The coefficient of simple correlation (r^2) values were 0.50 (0.57), and 0.3 (0.33) for the NPG and PRG equations, whereas the combined equation had r^2 values 0.77 (0.83) and 0.70 (0.87) when the equation was applied to non-pregnant and pregnant data sets, respectively. The relatively superior performance of the combined equation with pregnant dataset can be partly explained by the removal of two significant T outliers samples compared to others.

Fecal Dry Matter Output (FDMP) Equations Validation

The standard errors of prediction (SEP) were generally lowest for the COMB equations. The combined equations had SEP values varied from 2.4 - 4.5 and the coefficients of simple correlation (r^2) varied from 0.57-0.80, followed by the NPG with SEP 4.83 (3.76) and r^2 0.44 (0.48). Validation performance for the PRG equations was relatively inferior both in terms of SEP and r^2 with values 7.14 (4.37) and 0.063(0.22), respectively.

Discussion

Calibration statistics for the DMI and OMI equation have suggested that there were acceptable accuracy of prediction of intake. In all the DMI equations the R^2 was close to 0.90, indicting that the greatest portion of the variations in dry matter intake were explained by the models, especially the PRG DMI which had the strongest correlation between measured and NIRS predicted values. In terms of SEC values, relatively high value was obtained for the NPG data set and lowest for the PRG. This disparity in accuracy indicates the presence of variation between lab reference and the NIRS predicted values more in the non-pregnant dataset. When the PGN and NPG data sets were merged and a new calibration, i.e. combined was generated, the SEC value was reduced by 0.25 units while the coefficient of determination (R^2) value increased by 1%. This decrease in SEC in one hand and the increase in R^2 value on the other for the combined DMI equation could be explained by the increase in number of samples for calibration and introduction of variation that enhanced the predictive ability of the combined equations. For all the DMI equations, the SEC values represent less than 5% of the error of the mean of dry matter intake indicating high accuracy of prediction of dry matter intake within the population.

The present results were comparable with or superior to some prior findings. For example Bruno-Soare et al. (1998) developed calibration equations for predicting the voluntary dry matter intake (IVDMI) by sheep and reported SEC and R^2 value of 6.05g/kg $w^{0.75}$ and 0.41, respectively. Another study by Gordon et al. (1998) who developed calibration equation for predicting dry matter in intake of silage by cattle on metabolic live weight basis and reported SEC ranging 4.2-8.0 g/kg $w^{0.75}$ and R^2 ranging 0.55- 0.88. Abreu et al. (1992) using intake data from rams developed calibration equation and obtained an SEC value of 6.62 (g/kg.$^{0.73}$) with a corresponding $R^2$of 0.86. Parker et al. (1997) working with cattle and sheep investigated the potential of NIRS for predicting the voluntary dry matter intake of silage. These authors reported

an SEC of 3.43 (g/kg$0.^{75}$) and R^2 value equal to 0.90, which is close to those results obtained for the present combined DMI calibration.

In the present study validation statistics for intake (DMI and OMI) have indicated that, both the standard error of prediction (SEP) and coefficient of simple correlation (r^2) for the PRG equations were inferior to the SEC and R^2 obtained for calibration. However, the validation statistics for the combined (COMB) equation with SEP varied from 4.36 to7.24 (g/kg$0.^{75}$) and r^2 varied from 0.76 to 0.84 indicates better performance of the equations particularly when T outlier samples were excluded from the validation set. Figures 6.2a 6.2b and 6.2c, respectively, depict the relationship between the NIRS predicted and measured DMI, OMI and FDMP, for the COMB equations. Improvement in performance of equations due to removal of T outlier samples indicates that there was incompatibility between spectral data and the reference due to difference in determination technique. The present validation results for DMI were superior to prior reports by Parker et al. (1997) who found an SEP of 5.42 g/kg $^{0.75}$ with corresponding coefficient of simple correlation (r^2) value of 0.72. In general the bias values were relatively low for the combined equations, which slightly over predicted, but high for the other two equations. The low bias values for the combined may be due to the use of pregnant and non-pregnant sample sets (which were part of the calibration set that used for developing the combined calibration equations), as validation set.

Examination of the relationship between OMI equations showed that among the three equations better calibration statistics were obtained for the PRG data. The PRG equation had lowest SEC, and SECV values with highest R^2value and would predict the OMI of donkey with accuracy of ± 3.38g/kg $w^{0.75}$, which is better than those of Boval et al (200) who reported higher SECV (5.29). This translates to a practical value of < 0.1% of body weight that is not fat

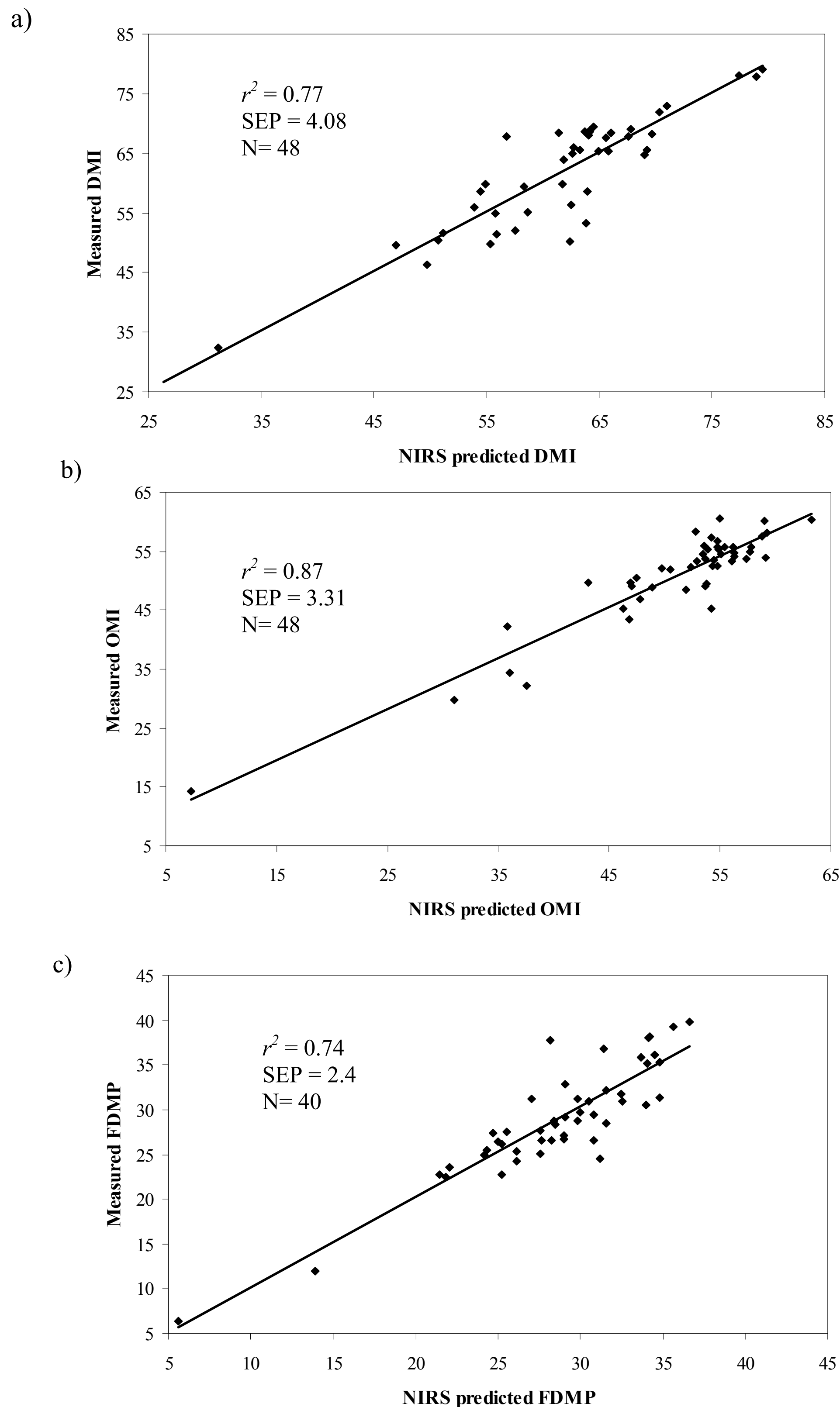

Figure 6.2. Relationship between NIRS predicted and measured a) dry matter intake (DMI) b) organic matter intake (OMI) and c) fecal dry matter output (FDMP) expressed as g/kg w $^{0.75}$ when the COMB equations were applied to a) and c) non-pregnant datasets and b) pregnant dataset. The line Y=X prefect agreement between the variables.

adjusted. Compared with prior works, the present equations had lower SEC values, especially than those reported by Ward et al. (1982). Ward et al. (1982) developed NIRS calibration equations for predicting OMI of cattle fed on fresh forages and reported an SEC of 9.6g/kgw $^{0.75}$ and R^2 equals to 0.72. More recently, Boval et al. (2004) developed fecal NIRS calibration equation for predicting OMI of cattle grazing tropical grasses and obtained SEC and R^2 values of 4.62g/kgw $^{0.75}$ and 0.61, respectively. In the present study, the calibration statistics both for the OMI and DMI were similar, especially when the percentage of error to the mean (SEC/mean) term is considered.

The validation statistics for the DMI had indicated that there were a wide range of SEP value (3.3-9.9) and r^2 value (0.30-0.87) for the equations. The coefficient of simple correlation t (r^2) for the PRG equation was inferior to those of NPG, as well as combined.

In all equations better performances were observed when the equations were applied to validation sets after eliminating significant T outlier samples. For instance, removal of 20% of the pregnant validation samples resulted in considerable improvement in the performance of the DMI and OMI equations. The presence of T outlier samples indicate that there was variation between the NIRS values and its primary chemical values (Workman 1992) which have considerable effect on the predictive capacity of equations.

In general the present data have demonstrated that with SEC value of less 4g/kg w $^{0.75}$ and R^2 above 0.85, fecal NIRS calibration equation could be developed for intake both dry matter and organic matter donkeys at accuracy levels comparable to those reported for ruminants.
The present study also explored the potential for fecal NIRS for predicting the fecal dry matter output. From the authors' knowledge, this is the first study that used NIRS to calibrate FDMP. With SEC value less than 2.2 and R^2 above 0.85 both the NPG and combined calibration results have demonstrated that the FDMP could successfully be calibrated. Although no prior data are

available to compare the present result with, calibration statistics have suggested that best model demonstrated the FDMP in donkeys can be predicted with an accuracy of ± 3.7 g/kg $w^{0.75}$, which accounts only for 0.098% of the live body weight of an average donkey.

Conclusions

The present study demonstrated that the fecal NIRS could be used to predict the intake by donkeys with acceptable level of accuracy once calibrations are developed. The preliminary conclusions include:

1. The intake equations were successfully calibrated and showed a good performance in predicting dry matter (DMI) and organic matter intake (OMI) particularly when the calibration statistics (low SEC and high R^2) values are considered.

2. The performance statistics of equation developed for predicting the fecal dry matter output (FDMP) suggested that fecal dry matter output can be predicted with acceptable level of accuracy.

3. In general, a considerable improvement in validation statistics could be obtained when equations were applied to validation sets after eliminating significant T outliers, indicating the influence of the variation between an NIRS value and its reference on the performance of calibration equations. This suggests the need to objective eliminate experimental error when driving NIRS calibration equations for real world application.

4. The present study has shown that NIRS can potentially be used to predict intake with a precision that is better than obtained with a regression technique based on any chemical or functional parameters of diets.

5. Based on the present findings, additional study to further investigate the potential of NIRS for predicting intake in equines with a broader range of feed types needs consideration.

CHAPTER VII

SUMMARY AND CONCLUSIONS

The present studies have explored the potential for fecal-near infrared reflectance spectroscopy (NIRS) profiling for predicting the diets qualities: crude protein (CP), digestible organic matter (DOM), dry matter digestibility (DDM), organic matter digestibility (OMD), and intake: dry matter (DM) and organic matter (OMI) of pen fed donkeys. The data from these studies indicate that calibration equations were developed with mixed successes.

Both calibration and validation results have demonstrated that the present CP equations were successfully developed. The US, Africa and US/Africa CP calibration equations have excellent prediction capacity, which is equivalent to the conventional wet chemistry methods. On the basis of the various calibration and validation parameters used to evaluate the performance of calibration equations, the present CP equations were also comparable with and in some cases better than those reported in literature on ruminant.

A good calibration equation was also derived from the US DOM calibration set and relatively acceptable accuracy was obtained for US/Africa DOM data set. The precision of the estimate achieved in the present DOM equations appeared to be comparable with those reported in literature on ruminant and had error values less than twice the SEL. However, performance of Africa DOM, DDM and OMD equations own to the poor technique used to derive dietary and fecal attributes such as dry matter (DM) and organic matter (OM) were not successful. Because derivation of digestibility attributes from a constant dry matter value to the feces and the derivation of the ash content of the diet and feces from literature or lab averages introduces an unnecessary additional error of calibration.

Data from the study also suggested that fecal NIRS is a viable technique for predicting the dry matter intake (DMI) and organic matter intake (OMI) of donkeys. Both DMI and OMI

equations showed good prediction performance with high accuracy and were comparable with previous reports on ruminant.

The presented study also investigated the relationship between dry matter intake and various diet qualities, including DDM, CP, DOM, and DOM/CP. In addition, relationship between DMI and fecal dry matter output was also examined. Results have indicated that the CP and DOM/CP ratio have higher correlation than other digestibility attributes. Investigation on the effect of pregnancy of donkeys on predictive capacity of NIRS has shown that although differences in calibration statistics were observed with better result for non-pregnant than both combined and pregnant equations, no difference could be observed in validation statistics especially for CP equations.

In general, the present studies have generated first order useable calibration equations to predict the nutritional status of free grazing donkeys and in view of the importance of the present calibration equations practical applicability it was concluded that:

1. All US, Africa and US/Africa CP calibration equations could be used as a useful tool for routine analysis for predicting the crude protein concentration of diets both in the US, Africa and probably in other part of the world.

2. Application of the US and US/Africa DOM calibration equations for predicting the digestible organic matter concentration (energy) of forages would give good accuracy with marginal errors and well recommended.

3. Dry matter (DMI) and organic matter (OM) intake equations had also shown good prediction accuracy and could be used as a tool for predicting the intake of free grazing donkey with great accuracy.

4. Prediction of intake using single digestibility attributes such as DDM and DOM could not give reliable estimate at least for donkeys.

5. In the present study, the major problems with developing robust calibration equation for predicting the digestibility of diets with the Africa dataset was the inability to determine accurate reference values from the in vivo trial in Naivasha.

6. The nature of calibration equations is dynamic and always is subjected to continue expansion and refinement. Therefore, research with more diet and fecal samples, particularly from Africa to further enhance the predictive capacity of the present digestibility equations needs consideration.

7. Since fecal near-infrared reflectance technique is strictly correlative, the accuracy of its prediction can never exceed the accuracy with which the measurement is calibrated. Therefore, dietary and fecal attributes derived from multiple literatures or average lab values can introduce unexpected error and are unreliable to use them in developing calibration equations.

LITERATURE CITED

Abrue, J.M.E., A.B. Soares, I. Murray, T. Acamovic, and R.M. Paterson. 1992. The value of Portuguese pulse straws. *In:* I. Murray and I. A. Cowe [eds.]. Making light work: advances in near infrared spectroscopy. Weinheim, Federal Republic of Germany: VCH. p 319-322.

Adesogan, A.T., E. Owen, and D.I. Givens. 1998. Prediction of the in vivo digestibility of whole crop wheat from in vitro digestibility, chemical composition, in situ rumen degradability, in vitro gas production and near infrared reflectance spectroscopy. *Animal Feed Science and Technology* 74: 259-272.

Adesogan, A.T., D.I. Givens, and E. Owen. 2000. *In*: L.'t Mannetje and R.M. Jones [eds.]. Measuring chemical composition and nutritive value in forages. Field and laboratory methods for grassland and animal production research. Wallingford, Oxon, UK: CABI Publishing. p12-25.

Aganga, A.A., and C.M. Tsopito. 1998. A note on the feeding behaviour of domestic donkeys: a Botswana case study. *Applied Animal Behaviour Science* 60: 235–239.

Aganga, A.A., M. Letso, and A.O. Aganga. 2000. Feeding donkeys. *Livestock Research for Rural Development* 12:1-5.

Agnew, R.E., R.S. Park, C.S. Mayne, and A.S. Laidlaw. 2004. Potential of near infrared spectroscopy to predict the voluntary intake of grazed grass. *Animal Feed Science and Technology* 115:169-178.

Aharoni, Y., A. Brosh, and Z. Holzer. 1999. Comparison of models estimating digesta kinetics and fecal output in cattle from fecal concentrations of single-dosed markers of particles and solutes. *Journal of Animal Science* 77:2291–2304.

Andrés, S., A. Calleja, S. López, À. R. Mantecón and F. J. Giráldez. 2005a. Nutritive evaluation of herbage from permanent meadows by near-infrared reflectance spectroscopy: 2. Prediction crude protein and dry matter degradability. *Journal of Science of Food and Agriculture* (in press).

Andrés, S., F. J. Giráldez, S. Lopez, À.R. Mantecón, and A. Calleja. 2005b. Nutritive evaluation of herbage from permanent meadows by near-infrared reflectance spectroscopy: 1. Prediction of chemical composition and in vitro digestibility. *Journal of Science of Food and Agriculture* (in press).

Antoniewicz, A.M., J. Kowalczk, J. Kanski, Z. Gorska Matusiak, and M. Nalepka. 1995. Rumen degradability of crude protein of dried grass and Lucerne forage measured by in sacco incubation and predicted by near infrared spectroscopy. *Animal Feed Sciences and Technology* 54:203-216.

AOAC. 1995. Official methods of analysis. 16th edition. Washington, DC: Association of Official Analytical Chemists. p 30.

Awuma, K.S. 2003. Application of NIRS fecal profiling and geostatistics to predict diet quality of African livestock . Ph.D. Dissertation. College Station, TX: Texas A&M University.161p.

Baker, C.W., D.I. Givens, and E.R. Deaville. 1994. Prediction of organic matter digestibility in vivo of grass silage by near infrared reflectance spectroscopy; effect of calibration method, residual moisture and particle size. *Animal Feed Science and Technology* 50:17-26.

Barton, F.E. 2002. Theory and principles of near infrared spectroscopy. *Spectroscopy Europe*. Available at. http://www.spectroscopyeurope.com/NIR_14_01 , Accessed 10 November 2004.

Bekers, O., C. Postma, J.C. Fischer, P.F. Franck, and A.J. Lombarts. 1996. Fecal nitrogen determination by near infrared spectroscopy. *European Journal of Clinical Biochemistry* 34: 561-3.

Benini, L., S. Caliari, F. Bonfante, C. G. Guidi, T. M. Brentegani, G. Castellani, C. Sembenini, E. Bardelli, and I. Vantini. 1992. Near infrared reflectance measurement of nitrogen faecal losses. *Gut* 3:749-52

Birth, S.G., and G.H. Hecht. 1987. The physics of near-infrared reflectance. *In:* P.Williams, and K. Norris [eds.]. Near-infrared technology in the auricular and food industries. St. Paul, MN: American Association of Cereal Chemists. p 1-16.

Blench, R. 1999. Animal traction in Africa: analyzing its environment impact. *In:* P. Starkey, and P. Kaumbutho [eds.]. Meeting the challenges of animal traction. A resource book of the Animal Traction Network for Eastern and Southern Africa (ATNESA). London, UK: Intermediate Technology Publications. p 52-59.

Blench, R. 2001. The history and spread of donkeys in Africa. *In*: P. Starkey, and D. Filding [eds.]. Donkeys, people and development. A resource book of the Animal Traction Network for Eastern and Southern Africa (ATNESA). Wageningen, The Netherlands: CTA. p 22-30.

Boever, J.L., B.G. Cottyn, J.M. Vanacker, and Ch. V. Boucque'. 1995. The use of NIRS to predict the chemical composition and energy value of compound feeds for cattle. *Animal Feed Science and Technology* 51:243-253.

Boval, M., D.B. Coates, P. Lecomte, V. Decruyenaere, and H. Archimede. 2004. Faecal near infrared reflectance spectroscopy (NIRS) to assess chemical composition, in vivo digestibility and intake of tropical grass by Creole cattle. *Animal Feed Science and Technology* 114:19-29.

Brooks J. III, M. Anderson, and P.J. Urness. 1984. Infrared reflectance analysis of forage quality for elk. *Journal of Wildlife Management* 48:254-258.

Bruno-Soares, A.M., I. Murray, R.M. Paterson, and J.M.F. Abreu. 1998. Use of near infrared reflectance spectroscopy (NIRS) for the prediction of the chemical composition and nutritional attributes of green crop cereals. *Animal Feed Science and Technology* 75:15-25.

Buvanendran, V. 1989. Constraints to improving reproductive efficiency of ruminant livestock in developing countries. *In*: Feeding strategies for improving productivity of ruminant livestock in developing countries. *In*: Proceedings of Combined Advisory Group Meeting and a Research Co-ordination Meeting; 13-17 March 1989; Vienna, Austria: IAEA (International Atomic Energy Agency). p 51-61.

Canacoo, E.A., and F.K. Avornyo.1998. Daytime activities of donkeys at range in the coastal savanna of Ghana. *Applied Animal Behaviour Science* 60: 229–234.

CapMunday, W.L., C. Zaapf, and J. Reeves III. 2004. Testing and validating instrument performance. *In:* C.A. Roberts, J. Workman Jr., and J.B. Reeves III [eds.]. Near infrared spectroscopy in agriculture. Agronomy Monograph No. 44. Madison, WI: ASA, CSSA, SSSA, Inc. Publishers. p 33-48.

Choquenot, D. 1991. Density depend growth, body condition and demography in feral donkeys. Testing the food hypothesis. *Ecology* 72: 805:813.

Clark, D.H., and R.E. Short. 1993. Comparison of AOAC and light spectroscopy analysis of uncooked, ground beef. *Journal of Animal Science* 72: 925-931.

Clark, D.H., D.A. Johnson, K.D. Kephart, and N.A. Jackson. 1995. Near infrared reflectance spectroscopy estimation of ^{13}C discrimination in forage. *Journal of Range Management* 48:132-136.

Coates, D.B. 1998. Predicting feed digestibility and crude protein content from the feces of grazing cattle. CSIRO Tropical Agricultural, Davies Laboratory Report CS.253.Townsville, Australia: CSIRO Tropical Agriculture. 27p.

Coates, D.B. 1999. Fecal spectroscopy (NIRS) for nutritional profiling of grazing cattle. *In*: D. Eldridge, and D. Freudenberger [eds.]. People and rangelands: building the future. Proceedings of the VIth International Rangeland Congress: Townsville Queensland, Australia: p. 466-467.

Coates, B.D. 2000a. Fecal NIRS-what does it offer today's grazier? *Tropical Grasslands* 34:230-239.

Coates, D.B. 2000b. Predicting the digestibility and crude protein content from the faeces of grazing cattle. Executive summary part two. A research project conducted by CSIRO Tropical Agriculture. CS.253.Townsville, Australia: CSIRO. 30p.

Coleman, S.W. 1989. In vivo and in vitro measurements of forage quality. In G.C. Marten, J.S. Shenk, and F.E. Barton II (eds). Near infrared reflectance spectroscopy (NIRS): Analysis of forage quality. Agric. Handbook No. 643. Washington, DC. ARS. USDA. p 83-95.

Coleman, S.W., J.W. Stuth, and J.W. Holloway. 1989. Monitoring the nutritional status of grazing cattle with near-infrared analysis of feces. Proceedings of the XVI International Grasslands Congress, Nice, France. p 881-882.

Coleman, S. W., and I. Murray. 1993. The use of near-infrared reflectance spectroscopy to define nutrient digestion of hay by cattle. *Animal Feed Science Technology* 44:237-249.

Coleman, S.W., H. Lippke, and M. Gill. 1999. Estimating the nutritive potential of forages. *In*: H.G. Jung and C.G. Fahey Jr. [eds.]. Nutritional ecology of herbivores. Proceedings of the Vth International Symposium on the Nutrition of Herbivores. Savoy, IL: American Society of Animal Sciences. p 647-751.

Coleman, S.W., and J.E. Moore. 2003. Feed quality and animal performance. *Field Crop Research* 84:17-29.

Combs, D.K., E.P. Beyer-Neumann, M.T. Rodriques, D.J.Undersander, and P.C. Hoffman.1998. Current research in forage utilization at the University of Wisconsin-Dairy Sciences Department. Proceedings of the Forage Utilization. Available at: http.//www.edu/dysci/dairydays/98Proceedings/ForageUtilization.htm. Accessed 20 November 2004.

Cork, S.J., I.D. Hume, and G.J. Faichney. 1999. Digestive strategies of nonruminant herbivores: the role of the hindgut. *In:* G. J. Hans-Joachim and G. C. Fahey, Jr. [eds.]. Nutritional ecology of herbivores. Proceedings of the Vth International Symposium on the Nutrition of Herbivores. Savoy, IL: American Society of Animal Science. p 210-260.

Cowe, I. A., J.W. McNicol, and D. C. Cuthbertson. 1992. A comparison of the shapes of principal component and partial least square factors obtained from near infrared spectra..*In:* I. Marray, and I. A. Cowe. [eds.]. Making light work: advances in near infrared spectroscopy. Weinheim, Federal Republic of Germany: VCH. p 191-199.

Cozzolino, D., and M. Labandera. 2002. Determination of dry matter and crude protein contents of undried forages by near infrared reflectance spectroscopy. *Journal of Science of Food and Agriculture* 82: 380-384.

Cozzolino, D. A., and A. Moron. 2004. Exploring the use of near infrared reflectance spectroscopy (NIRS) to predict trace minerals in legumes. *Animal Feed Science and Technology* 111: 161–173.

Crooks, J.E. 1978. The spectrum in chemistry. London, UK: Academic Press. 313p.

Cuddeford, D., R.A. Pearson, R.F. Archibald, and R.H. Muirhead. 1995. Digestibility and gastro-intestinal transit time of diets containing different proportions of alfalfa and oat straw given to Thoroughbreds, Shetland ponies, Highland ponies and donkeys. *Animal Science* 61:407-417.

Deaville, E.R., and I.D. Givens. 1998. Regions of normalized near infrared reflectance difference spectra related to the rumen degradation of fresh grass, grass silage and maize silage. *Animal Feed Science Technology* 72:41-51.

Deaville, E.R., and P.C. Flinn. 2000. Near infrared (NIR) spectroscopy : an alternative approach for the estimation of forage quality and voluntary intake. *In:* D.I. Givens, E. Owen, R.F.E Axford, and H.M. Omed [eds.]. Forage evaluation in ruminant nutrition. Wallingford, Oxon, UK: CAB International. p 301-320.

Devendra, C. 1987. Herbivores in the arid and wet tropics. *In*: J.B. Hacker, and D. Ternouth [eds.]. Nutrition of herbivores. New York: Academic Press. p 23-46.

Devendra, C., and C.C. Sevilla. 2002. Availability and use of feed resources in crop-animal systems in Asia. *Agricultural Systems* 71: 59-73.

Dryden, G. M. 2003. Near infrared reflectance spectroscopy: applications in deer nutrition. A report for the Rural Industries Research and Development Corporation. Rural Industries Research and Development Corporation Publication No W03/007. RIDRC Project No UQ-109A. Australia: RIRDC. 46p.

Duckworth, J. 2004. Mathematical data preprocessing. *In:* C.A. Roberts, J. Workman Jr., and J.B. Reeves III [eds.]. Near infrared spectroscopy in agriculture. Near infrared spectroscopy. Agronomy Monograph No. 44. Madison, WI: ASA, CSSA, SSSA, Inc. p 115-132.

Dulphy, J.P., W. Martin-Rosset, H. Dubroeucq, and M. Jailler. 1997a. Evaluation of voluntary intake of forages trough-fed to light horse. Comparison with sheep. Factors of variation and prediction, *Livestock Production Science* 52: 97-104.

Dulphy, J.P., W. Martin-Rosset, H. Dubroeucq, J.M. Ballet, A. Detour, and M. Jailler. 1997b. Compared feeding pattern in ad libitum intake of dry forage by horses and sheep. *Livestock Production Science* 52:49-56

Ensminger, M.E. and C.G. Olentine, Jr. 1978. Feeds and nutrition-abridged. 1st edition. Clovis, NM: The Ensminger Publishing Company. 824p.

Faber, K., and B. R. Kowalski. 1997. Propagation of measurement errors for the validation of predictions obtained by principal component regression and partial least squares. *Journal of Chemometrics* 11: 181-238.

FAO. 2001. Pastoralism in the new millennium. FAO Animal Production and Health Paper No. 150. Rome, Italy: Food and Agriculture Organization. 104p.

FAO. 2003. Food and Agriculture Organization Stat Database. Available at http://faostat.fao.org/faostat/form. Accessed January 2004.

Fearn, T.1992. Shrinking calibrations. *In:* I. Marray, and I. A. Cowe [eds.]. Making light work: advances in near infrared spectroscopy. Weinheim, Federal Republic of Germany: VCH. p 147-152.

Fernando, P., and P. Strakey. 1998. Donkeys and development: socio-economic aspects of donkey use in Africa. *In:* P. Starkey, and D. Filding [eds). Donkeys, people and development. A resource book of the Animal Traction Network for Eastern and Southern Africa (ATNESA).Wageningen, The Netherlands: CTA. p 31-44.

Ferri, M.C., N. P. Stritzier, M.A., Brizuela, and H.J. Petruzzi. 2003. In vivo digestibility of kleingrass from fecal nitrogen excretion. *Journal of Range Management* 56:52-55.

Foley, W.J., A. McIlwee, I.van Lawler, L Aragones, A. P. Woolnough, and N. Berding. 1998. Ecological application of near infrared reflectance spectroscopy a tool for rapid, cost-effective prediction of the composition of plant and animal tissues and aspects of animal performance. *Oecologia* 116:293-305.

France, J. M., K. Theodrou, R.S. Lowmand, and D.E Beever. 2000. Feed evaluation for animal production *In*: K.M. Theodorou, and J. France [eds.]. Feeding systems and feed evaluation models. Wallingford, Oxon, UK: CABI Publishing. p 25-35.

Gallagher, J.F. 1990. Fecal indices of nutritional status of white tailed deer based on near infrared reflectance. Ph.D. Dissertation. College Station, TX: Texas A&M University. 62p.

Gebreab, F. A. Gebre Wold, F. Kelemu, A. Ibro, and K. Yilm.1999. Donkey utilisation and management in Ethiopia. *In:* P. Starkey and D. Fielding [eds]. Animal Traction Network for Eastern and Southern Africa. Improving Donkey Utilisation and Management; 5-9 May 1997; Debre Zeit, Ethiopia: ATENSA. p 46-52.

Gebre Wold, A., A. Tegegne, and A. Yami. 1999. Research needs of donkey utilization in Ethiopia. *In*: P. Starkey and D. Fielding [eds.]. Donkeys, people and development. A resource book of the Animal Traction Network for Eastern and Southern Africa (ATENSA). Wageningen, The Netherlands: CTA. p 77-81.

Gibbs, S.J., D.A. Coates, D.P. Poppi, S.R. McLennan, and R.M. Dixon. 2002. The use of fecal near infrared spectroscopy to predict dietary digestibility and crude protein content for cattle fed supplements. *Animal Production Australia* 24:299-305.

Givens, D.I., W.C. Baker, and B. Zamime. 1992. Regions of normalized near infrared reflectance difference spectra related to rumen digestion of straw. *Animal Feed Science and Technology* 36:1-12.

Godfrey, R.W., E., R Dodson, K J. Bultman, D.R. Tolleson, J.W. Stuth, and J. A. Norman. 2001. Use of near infrared reflectance spectroscopy to differentiate pregnancy status and gender of hair sheep in the tropics. *Journal of Animal Science* 79 (Suppl. I): 26.

Gordon, F.J., K.M. Cooper, R.S. Park, and R.W.J. Steen. 1998. The prediction of intake potential and organic matter digestibility of grass silage by near infrared spectroscopy analysis of un-dried samples. *Animal Feed Science and Technology* 70: 339-351.

Hach, Co.1987. Feed analysis manual. Ames, IA: Hach Company. 256p.

Hodgson, J. 1982. Influence of sward characteristics on diet selection and herbage intake by grazing animal. *In*. J.B. Hacker [ed.] Nutritional limitation to animal production from pastures. Proceedings of the International Symposium; 24-28 August 1981; St. Lucia, Queensland, Australia. p 12-30.

Holechek, J.L., M. Vavra, and R.D. Pieper.1982. Method of determining the nutritive quality of range ruminant diets: a review. *Journal of Animal Science* 54: 364-376.

Holechek, J.L., R.D. Pieper, and C.H. Herbel. 1998. Rangeland management and principles. Englewood Cliffs, NJ: Prentice-Hall. 542p.

Hruschka, W.R. 1987. Wavelength analysis methods. *In.* P. Williams, and K. Norris [eds.]. Near-infrared technology in the agricultural and food industries. St. Paul, MN: American Association of Cereal Chemists, Inc. p 35-55.

Hruschka, W.R., and K.H. Rasmussen. 1982. Least squares curve fitting of near infrared spectra predicted protein and moisture content of ground wheat. *Applied Spectroscopy* 36:261-265.

Izraely, H., I. Chosniak, C.E. Stevens, W.M. Demment, and A. Shkolnik .1989. Factors determining the digestive efficiency of the domesticated donkey (*Equus asinus*). *Quarterly Journal of Experimental Physiology* 74:1-6.

Janis, C. 1976. The evolutionary strategy of the equidae and the origins of rumen and cecal digestion. *Evolution* 30:757-774.

Jones, D.I.H., and M.K. Theodorou. 2000. enzyme techniques for estimating digestibility. *In*: D.I. Givens, E. Owen, R.F.E Axford and H.M. Omed [eds.]. Forage evaluation in ruminant nutrition. Wallingford, Oxon, UK: CABI Publishing. p 155-173.

Jones, P. 1999. Calculating the true cost of donkey ownership. *In*: Proceedings of the Animal Traction Network for Eastern and Southern Africa (ATENSA) Workshop; 20-24 September 1999; Moumalanga, South Africa: ATENSA. p 235-239.

Journet, M. E., H. M. Grenet, Farce, M. Theriez., and C. Demarquilly [eds.]. 1995. Recent developments in the nutrition of herbivores. *In*: Proceedings of the IVth International Symposium on the nutrition of herbivores; 11-15 September, 1995; Clermont-Ferrand, France. p 200-207.

KARI. 2004. Kenya Agricultural Research Institute Centers. The Naivasha Research Center. Available at http://www.kari.org/Naivasha/NaivashaMore.htm. Accessed on September 2004.

Keating, M.S. 2005. Prediction of the diet quality parameters of Rocky Mountain elk via near infrared reflectance spectroscopy (NIRS) fecal profiling. Ph.D. Dissertation. College Station, TX: Texas A&M University.118p.

Kemsley, E.K., H.S. Tapp, A.J. Scarlett, R. Hammond, and R.H. Wilson. 2001. Comparison of spectroscopy techniques for the determination of Kjeldahl and ammoniacal nitrogen content of farmyard manure. *Journal of Agricultural and Food Chemistry* 49: 603-609.

LaCasha, P.A., H.A. Brady, V.G. Allen, C.R. Richardson, and K.R. Pond. 1999. Voluntary intake, digestibility, and subsequent selection of Mutua Bromegrass, Coastal Bermudagrass, and Alfalfa Hays by yearling horses. *Journal of Animal Science* 77:2766-2773.

Lachica, M., and J.F. Aguilera. 2003. Estimation of energy needs in the free-ranging goats with particular reference to the assessment of its energy expenditure by the ^{13}C-bicarbonate method. *Small Ruminant Research* 49: 303-318.

Lamoot, I., J. Callebaut, E. Demeulenaere, C. Vandenberghe, and M. Hoffmann. 2005. Foraging behaviour of donkeys grazing in a coastal dune area in temperate climate conditions. *Applied Behaviour Science* 92:93-112

Landau, S., T. Glasser, L. Dvash, and A. Perevolotsky. 2004. Faecal NIRS to monitor the diet of Mediterranean goats. *South African Journal of Animal Science* 34 (Suppl.I):76-80

Landau, S., T. Glasser, and L. Dvash. 2005. Monitoring nutrition in small ruminants with the aid of near infrared reflectance spectroscopy (NIRS) technology: a review. *Small Ruminant Research* (in press).

Leite, E.R., and J.W. Stuth. 1995. Fecal NIRS equations to assess diet quality of free-ranging goats. *Small Ruminant Research* 15: 223-230.

Li, H., J.W. Stuth., D.R. Tolleson., and S.L. Kronberg. 2004. Near infrared reflectance spectroscopy (NIRS) of feces to determine dietary crude protein and digestible organic matter in sheep. Abstract. Annual Meeting of the Society for Range Management; 25-30 January 2004; Salt Lake City, UT. p 120.

Lister, S.J., R. Sanderson, S.M. Dhanoa, and M.E. Gill.1995.Use of near infrared reflectance spectroscopy to investigate the effect of polyethylene glycol on changes in *Leucaena leucocephala and Caliandra sp*. incubated in vitro. *Animal Science* 60:543-553.

Lopez, S., J. Dijkstra, and J. France. 2000. Prediction of energy supply in ruminants, with emphasis on forages. *In:* D.I Givens, E. Owen, R.F.E Axford and H.M. Omed [eds.]. Forage evaluation in ruminant nutrition. Wallingford, Oxon, UK: CABI Publishing. p 63-111.

Lovett, D.K., E.R. Deavill, F. Mould, D.I, Givens, and E. Owen. 2004. Using near infrared reflectance spectroscopy (NIRS) to predict the biological parameters of maize silage. *Animal Feed Science and Technology* 115:179-187.

Lyons, R.K. 1990. Fecal indices of nutritional status of free-ranging cattle using near infrared reflectance spectroscopy. Ph.D. Dissertation. College Station, TX: Texas A&M University.140p.

Lyons, R.K., and J.W. Stuth. 1992. Fecal NIRS equations for predicting diet quality of free ranging cattle. *Journal of Range Management* 45:238243.

Lyons, R.K., J.W. Stuth, J.E. Huston, and J.P. Angere.1993. Prediction of the nutrient composition of the diets of supplemented versus unsupplemented grazing beef cows based on near infrared reflectance spectroscopy of feces. *Journal of Animal Science*. 71:530-538.

Lyons, R. K., J.W. Stuth, and J.P. Angerer.1995. Technical note: fecal NIRS question field validation. *Journal of Range Management* 48:380-382.

Marais, J.P. 2000. Use of markers. *In:* D'Mello J.P.F [ed.]. Farm animal metabolism and nutrition. Wallingford, Oxon, UK: CABI Publishing. p 255-277.

Martens, H., and T. Naes.1987. Multivariate calibration by data compression. *In:* P. Williams, and Norris, K. [eds.]. Near-infrared technology in the agricultural and food industries. St. Paul, MN: American Association of Cereal Chemists, Inc. p 59-88.

Maxwell, T.J., and J.A. Milne.1995.Role of herbivores in sustainable land production systems. Principles and practice-some of the issues. *In*: M. Journet, E. Grenet, M-H. Farce. M. The'riez, and C. Demarquilly [eds.]. Recent developments in the nutrition of herbivores. Proceedings of the IVth International Symposium on the Nutrition of Herbivores. Paris, France: Paris INRA. p 17-31.

Mayes, R.W., and H. Dove. 2000. Measurement of dietary nutrient intake in free-ranging mammalian herbivores. *Nutrition Research Review* 13:107-138

McMeniman, N.P. 2003. Pasture intake by young horses. RIRDC Publication No W03/005. RIRDC Project No UQ-72A. Australia: RIRDC. 20p.

Melaku, S., K.J. Peters, and A. Tegegne. 2005. Intake, digestibility and passage rate in Menz sheep fed tef (*Eragrostis tef*) straw supplemented with dried leaves of selected multipurpose trees, their mixtures or wheat bran. *Small Ruminant Research* 56: 139-149.

Merchen, N. R. 1988. Digestion, absorption and excretion in ruminants *In:* D.C. Church [ed.]. The ruminant animal: Digestive physiology and nutrition. Englewood Cliffs, NJ: Prentice Hall. p 172-201.

Minson, D.J. 1982. Effect of chemical and physical composition of herbage eaten upon intake. *In*: J.B. Hacker [ed.]. Nutritional limitation to animal production from pastures. Proceedings of the International Symposium; 24-28 August 1981; St Lucia, Queensland, Australia. Farnham Royal, UK: CAB. p 167-182.

Mofareh, M. M., R. F. Beck, and A. G. Schenerberger. 1997. Comparing techniques for determining steer diets in northern Chihuahuan Desert. *Journal of Range Management* 50:27-32.

Montalvo, Jr. J.G., and T.Von Hoven. 2004. Analysis of cotton. *In:* C.A. Roberts, J. Workman Jr., and J.B. Reeves III [eds.]. Near infrared spectroscopy in agriculture. Near infrared spectroscopy. Agronomy Monograph, No. 44. Madison. WI: ASA, CSSA, SSSA, Inc. p 231-267.

Moore, J.E., and W.E. Kunkle. 1995. Improving forage supplementation programs for beef cattle. *In*. G.C Fahey, Jr. [ed.]. Forage quality evaluation and utilization. Madison, WI: ASA, CSSA, SSCA, Inc. p 967-998.

Moore, J. E., M. H. Brant, W. E. Kunkle, and D. I. Hopkins. 1999. Effects of supplementation of voluntary forage intake, diet digestibility, and animal performance. *Journal of Animal Science* 77 *(*Suppl. II):122-135.

Mueller, P. J., P. Protos, K. A. Houpt, and P. J. Van Soest. 1998. Chewing behaviour in the domestic donkey (*Equus asinus*) fed fibrous forage. *Applied Animal Behavour Science* 60: 241-251.

Murray, I., and P.C. Williams. 1987. Chemical principles of near infrared technology. *In*: P. Williams, and K. Norris [eds.]. Near infrared technology in the agricultural and food industries. St. Paul, MN: American Association of Cereal Chemists, Inc. p 17-34.

Muvirimi, F., and J. Ellis-Jones. 1999. A farming system approach to improving draft animal power in sub-Saharan Africa. *In.* P. Starkey, and P. Kaumbutho [eds.]. Meeting the challenges of animal traction. A resource book of Animal Traction Network for Eastern and Southern Africa. London, UK: Intermediate Technology Publication. p 10-19.

Naes, T., T. Isaksson, T. Fearn, and T. Davies. 2002. A userfriendly guide to multivariate calibration and classification. Chichester, UK: NIR Publication. 350p.

Nahm, K.H. 1992. Practical guide to feed, forage and water analysis. Accurate analysis with minimal equipment. Seoul, Korea: Yoo Han Publishing Inc. 269p

Ndikumana, J., J. Stuth, R. Kamidi, S. Ossiya, R. Marambii, and P. Hamlett. 2000. Coping mechanisms and their efficacy in disaster prone pastoral systems of the Greater Horn of Africa. Nairobi, Kenya: International Livestock Research Institute. 112p.

Neumeister, V., J. Henker, G. Kaltenborn, C. Sprossig, and W. Jaross. 1997.Simultaneous determination of fecal fat, nitrogen, and water by near-infrared reflectance spectroscopy. *Journal of Pediatric Gastroenterology Nutrition* 25:388-93.

Niamir-Fuller, F. 1998. The resilience of pastoral herding in Sahelian Africa. *In:* F. Berkes , C. Folke, and J. Colding [eds.]. Linking social and ecological systems: Management practices and social mechanisms for building resilience. Cambridge, UK: Cambridge University Press. p 250–284.

Nikolich, K., C. Sergides, and A. Pittas. 2001. The application of near infrared reflectance spectroscopy (NIRS) for the quantitative analysis of hydrocortisone in primary materials. *Journal of Serb Chemical Society* 66:189-198.

Nolan, T., and A. Nastis. 2000. Some aspects of the use of vegetation by grazing sheep and goats. Available at; http://ressources.ciheam.org/om/. Accessed 15 December 2004.

Norris, H.K., R. F. Barnes, J.E. Moore, and J.S. Shenk. 1976. Predicting forage quality by near infrared reflectance spectroscopy. *Journal of Animal Science* 43: 889-897.

NRC (National Research Council). 1981. Nutrient requirements of goats. Angora, Dairy, and Meat Goats in Temperate and Tropical Countries. Washington, DC: National Academy Press.

NRC (National Research Council). 1982. Nutrient requirements of goats. Washington, DC: National Academy Press.

NRC (National Research Council). 1987. Predicting feed intake of food-producing animals. Washington, DC: National Academy Press.

NRC (National Research Council). 1989. Nutrient requirements of horse. Washington, DC: National Academy Press.

NRC (National Research Council). 1996. Nutrient requirements of beef cattle. 7th edition. Washington, DC: National Academy Press.

Nsahlai, I.V., P.O. Osuji, and N.N. Umunna. 2000. Effect of form and quality of feed on the concentration of purine derivatives in urinary spot samples, daily microbial N supply and predictability of intake. *Animal Feed Sciences and Technology*. 85:223-238.

Olsson, N.O. 1969. The nutrition of the horse. *In*: D. Cuthbertson [ed.]. Nutrition of animal of agricultural importance. Part II. International Encyclopedia of Food and Nutrition. Oxford, UK: Pergamon Press. p 830-840.

Ørskov, E.R. 2000. The in situ technique for the estimation of forage degradability in ruminants. *In:* Givens, D.I., E. Owen, R.F.E. Axford, and H.M. Omed [eds.]. Forage evaluation in ruminant nutrition. Wallingtonford, UK: CAB International. p 175-188.

Ørskov, E.R., and M. Ryle. 1990. Energy nutrition in ruminants. London, UK: Elsevier Applied Science Publishers. 149p.

Osborn, R., D. Tolleson, T. Ginnett, S. Cooper, and J. Stuth. 2002. Determination of gender and age in white-tailed deer by near infrared reflectance spectroscopy of feces. Proceedings of the 82nd annual conference of the American Society of Mammalogists; 15–19 June 2002; Lake Charles, LA: American Society of Mammalogists. p 44-45.

Ossiya, S. 1999a. Development of a nutritional profiling system for free grazing livestock in major agro-ecological zones of Sub-Saharan Africa. Ph.D. Dissertation. College Station, TX: Texas A&M University.150p.

Ossiya, S. 1999b. Viewpoint: a strategy to adapt the use of near infrared reflectance spectroscopy fecal profiling into pastoral communities of Sub-Saharan Africa. Linking Policy Makers with Livestock Early Warning Systems in East Africa. Proceedings of Workshop; 2-4 March 1999; Entebbe, Uganda: Global Livestock Cooperative Research Program (GL-CRSP). p 47-59.

Osuji, P.O., Nsahlai I.V., and H. Khalili. 1993. Feed evaluation. ILCA Manual No.5. Addis Ababa, Ethiopia: International Livestock Center for Africa. 40p.

Panin, A., and J, Elis-Jones.1994. Increasing the profitability of draught animal power. *In:* P. Starkey, E. Mwenya, and J. Stares [eds.]. Improving animal traction technology. Wageningen, The Netherlands: Technical Centre for Agricultural and Rural Cooperation (CTA). p 94-103.

Park, S.R., F.J. Gordon, R.E. Agnew, R.J. Barnes, and R.W.J Steen. 1997. The use of near-infrared reflectance spectroscopy on dried samples to predict biological parameters of grass silage. *Animal Feed Science Technology* 68:235-246.

Pasikatan, M.C., J.L. Steele, C. K. Spillman, and E. Haque. 2001 Review. Near infrared reflectance spectroscopy for online particle size analysis of powders and ground materials. *Journal of Near Infrared Spectroscopy* 9: 153-164.

Pearson, R.A., and J.Merritt. 1991. Intake, digestion and gastrointestinal transit time in resting donkeys and ponies and exercised donkeys given *ad libitum* hay and straw diets. *Equine Veterinary Journal* 23:339-343.

Pearson, R.A., and A. J. Smith. 1992. Improving draft animal management. *In*. P. Starkey, E. Mwenya and J. Stares [eds.]. Improving animal traction technology. Animal Traction Network for Eastern and Southern Africa Workshop; 18- 23 January 1992; Lusaka, Zambia: ATNESA. p 122-129

Pearson, R.A., and E. Vall. 1998. Performance and management of draught animals in agriculture in sub-Saharan Africa.: a review. *Tropical Animal Health Production*. 30:309-24.

Pearson, R.A., and E. Nengomasha, and R.C. Krecek. 1999. The challenges in using donkeys for work in Africa. *In:* P. Starkey, and P. Kaumbutho [eds.]. A resource book of Animal Traction Network for Eastern and Southern Africa. London, UK: Intermediate Technology Publication. p 190-198.

Pearson, R.A., and M. Quassat. 2000. A guide to live weight estimation and body condition scoring of donkeys. Midlothia, UK: Center for Tropical Veterinary Medicine, University of Edinburgh. 25p.

Pearson, R. A., R.F. Archibald, and R.H. Muirhead. 2001.The effect of forage quality and level of feeding on digestibility and gastrointestinal transit time of oat straw and alfalfa given to ponies and donkeys. *British Journal of Nutrition* 85: 599-606.

Pritchard, J.C., A.C. Lindberg, D.C.J. Main, and H.R. Whay. 2005. Assessment of the welfare of working horse, mules and donkeys, using health and behaviour parameters. *Preventive Veterinary Medicine* (in press).

Purnomoadi, A., M. Kurihara, T. Nishida, M. Shibata A. Abe, and K. Kameoka.1996. Application of near infrared reflectance spectroscopy to predicted fecal composition and its use for digestibility estimation. *Animal Sciences Technology* (Japan) 67: 851-861.

Ramaswamy, N. S. 1998. Draft animal welfare. *Applied Animal Behaviour Science* 59: 73–84.

Reeves, J.B. 2000. Use of near infrared reflectance spectroscopy. *In:* J.P.F. D'Mell [ed.]. Farm animal metabolism and nutrition. Wallingford, Oxon, UK: CABI Publishing. p 185-207.

Reeves, J.B., III, and J.S. Van Kessel. 2000. Near infrared spectroscopic determination of carbon, total nitrogen, and ammonium-N in dairy manures. *Journal of Diary Science* 83:1829-1836.

Reid, R.L., and G.A. Jung. 1981. Problems of animal production from temperate pastures. *In*: J.B. Hacker [ed.]. Nutritional limitation to animal production from pastures. Proceedings of an International Symposium; 24-28 August1981.St Lucia, Queensland, Australia: Commonwealth Agricultural Bureaux. p 25-35.

Rivero-Marcotegui, A., J. E. Olivera-Olmedo, F. S. Valverde-Visus, M. Palacios-Sarrasqueta, A. Grijalba-Uche, and S García-Merlo. 1998. Water, fat, nitrogen, and sugar content in feces: reference intervals in children. *Clinical Chemistry* 44:1540-1544.

Robert, P., D. Bertrand, and C. Demarquilly. 1986. Prediction of forage digestibility by principal component analysis of near infrared reflectance spectra*. Animal Feed Science and Technology* 16:215-224.

Roberts, C.A., J. Stuth, and P. Flinn. 2004. Analysis of forage and feedstuffs. *In*: C.A. Robetrts, J. Workman Jr., and J.B. Reeves III [eds.]. Near infrared spectroscopy in agriculture. Near Infrared Spectroscopy. Agronomy Monograph No. 44. Madison, WI: ASA, CSSA, SSSA, Inc. p 231-267.

Ruano-Romos, A., A. Gracia-Ciudad, and B. Gracia-Criado. 1999. Near infrared spectroscopy prediction of mineral content in botanical fraction from semi-arid grasslands. *Animal Feed Science and Technology* 77: 331-343.

SAS. 2000. Statistical Analysis System SAS software package. Version 6. Cary, NC: SAS Institute Inc.

Saul, C., L. Siefert, and J. Opuda-Asibo. 1997.Diease and health problems of donkeys: a case study from eastern Uganda. Improving Donkey Utilization and Management. ATNESA workshop; 5-9 May 1997; Debre Ziet, Ethiopia: ATNESA. p 58-63.

Schulz H. 2004. Analysis of coffee, tea, coca, tobacco, spices, medicinal and aromatic plants and related products. *In*: C.A. Roberts, J. Workman Jr., and J.B. Reeves III [eds.]. Near infrared spectroscopy in agriculture. Near infrared spectroscopy. Agronomy Monograph No. 44. Madison, WI: ASA, CSSA, SSSA. Inc. p 345-376.

Shenk, J.S., and M.O. Westerhaus. 1991. Population definition, sample selection and calibration procedures for near infrared reflectance spectroscopy. *Crop Science* 31:469-474.

Shenk, J.S., J.J. Workman Jr., and M.O. Westerhaus. 1992. Application of NIR spectroscopy to agricultural product. *In*: A.D. Burns and E.W. Ciurczak [eds.]. Handbook of near-infrared analysis. New York: Marcel Dekker, Inc. p 383-431.

Shenk, J.S., J.J. Workman, Jr., and M.O. Westerhaus. 2001. Application of NIR spectroscopy to agricultural products. *In:* D.A. Burns and E.M Ciurczak [eds.]. Handbook of near-infrared analysis. Second Edition. New York: Marcel Dekker, Inc. p 385-435.

Shenk, J.S. 2004. Standardization and calibration transfer. *In*. C.A. Roberts,., J. Workman Jr., and J.B. Reeves III [eds.]. Near infrared spectroscopy in agriculture. Near infrared spectroscopy. Agronomy Monograph No. 44. Madison, WI: ASA, CSSA, SSSA Inc. p 175-206.

Showers, S.E. 1997. Prediction of Diet parameters of White-tailed deer via near infrared reflectance spectroscopy (NIRS) fecal profiling. M.S. Thesis. College Station. TX: Texas A&M University. 102p.

Skoog, D.A., E.J.Holler, and T.A. Nieman. 1998. Principles of instrumental analysis. 5th Edition. Philadelphia: Saunders College Publishing. 700p.

Smith, T.N., G.M. Pesti, R.I. Bakalli, J.Kilburn, and H.M. Edward, Jr. 2001. The use of near-infrared reflectance spectroscopy to predict the moisture, nitrogen, calcium, total phosphorus, gross energy, and phytate phosphorus contents of broiler excreta. *Poultry Science* 80:314-319.

Starkey, P., and M. Starkey 2001. Regional and world trends in donkey populations. Animal Traction Network for Eastern and Southern Africa Improving Donkey Utilisation and Management; 5-9 May 1997; Debre Zeit, Ethiopia: ATENSA. p 230-237.

Starkey, P.H. 1994. A world-wide view of animal traction highlighting some of the key issues in eastern and southern Africa. *In*: P. Starkey, E. Mwenya and J. Stares [eds.]. Improving animal traction technology. Proceedings of the First Workshop of the Animal Traction Network for Eastern and Southern Africa; 18-23 January; Lusaka, Zambia. Wageningen, The Netherlands: Technical Centre for Agricultural Co-operation (CTA). p 6-81.

Stern, M.D., A. Bach, and S. Calsamiglia. 1997. Alternative techniques for measuring nutrient digestion in ruminants. *Journal of Animal Science* 75:2256-2276.

Stuth, J. W. 1998. Nutrition of brood cows in the South Texas rush country: using the NIRS/NUTBAL nutritional management system. *In*: A. Gonzales, J.C.M Gonzales, E.G. Valenzuela, and D.A Tewolde [eds.]. Tqller de Granaderia de bovinosde carne del norte de Mexico y sure de Texas. Tamps, Mexico: C.D. Victoria. p 161-169.

Stuth, J.W., R.K. Lyons, J.P. Angerer, and C.D. McKown. 1991. Role of NIRS based nutritional monitoring systems for grazing and nutritional management of range livestock. *In*: Proceedings of the Grazing Livestock Nutrition Conference, Steamboat Springs, CO: Oklahoma Agricultural Experimental Station. p 83-89.

Stuth, J. W., P. Dyke, A. A. Jama, and J. Corbett. 1998. The use of NIRS/NUTBAL, PHYGROW, and APEX in a meta-modeling environment for early warning system to monitor livestock nutrition and health. National Workshop on Early Warning Systems for Monitoring Livestock Nutrition and Health; 4 February 1998; Addis Ababa, Ethiopia: LEWS. p 41-56.

Stuth W. J. M. Freer, H. Dove and R.K. Lyons. 1999. Nutritional management of free-ranging livestock. *In*: Hans-Joachim G. J and George C. Fahey, jr. [eds.]. Nutritional ecology of herbivores. Proceedings of the Vth International Symposium of the Nutrition of Herbivores. 11-17 April 1999, San Antonio. Savoy, IL. American Society of Animal Science. p 696-751.

Stuth, J. W., A. Jama, and D. Tolleson. 2003. Direct and indirect means of predicting forage quality through near infrared reflectance spectroscopy. *Field Crops Research* 84:45-56.

Sullivan, G.M., K.W. Stokes, D.E. Farris, T.C. Nelsen, and T.C. Cartwright. 1980. Transforming a traditional forage/livestock system to improve human nutrition in Tropical Africa. *Journal of Range Management* 33:174-178.

Tamminga, S., and X.B. Chen. 2000. Animal based techniques for the estimation of protein value of forages. *In*. D.I Givens, E. Owen, R.F.E Axford and H.M. Omed [eds.]. Forage evaluation in ruminant nutrition. Wallingford, Oxon, UK: CABI Publishing. p 215-231.

Tilley, J.M.A and R.A. Terry. 1963. A two stage technique for in vitro digestion of forage crop. *Journal of British Grassland Society* 18:104-111.

Tolleson, D.R., T.W. Willson, R.D. Randel, D.A. Neuendorff, A.W. Lewis, and J.W. Stuth. 2000. Discrimination between physiologically different groups of cattle via near infrared reflectance of feces. *Journal of Animal Science* 78 (Suppl. I): 14.

Tolleson, D.R., R.D. Randel, J.W. Stuth, T.S. Willard, and S.B. Gandy. 2001. Determination of pregnancy in cattle using near infrared reflectance spectroscopy of feces. *Journal of Animal Science* 79: 21-25.

Tolleson, D.R., R.D. Randel, J.W. Stuth, and D.A. Neuendorff. 2004. Determination of sex and species in red and fallow deer by near infrared reflectance spectroscopy of the faeces. *Small Ruminant Research* 57:141-150.

Uden, P., and P.J. Van Soest.1982. The determination of digesta particle size in some herbivores. *Animal Feed Science and Technology* 7:35-44.

Van Kempen, T., R. Poulenc and A. France (1996). NIRS technology: can we measure amino acids digestibility and energy values? 12th Annual Carolina Swine Nutrition Conference; 13 November, 1996. Available at htt:/mark.asci.ncsu.edu/nutrit~1/Miscellaneous/theo96csnc.htm. Accessed 23 February 2005.

Van Soest, P. J. 1982. Nutritional ecology of the ruminant. Corvallis, OR: O&B Books. 373p.

Van Soest, P.J. 1994. Nutritional ecology of the ruminant. Second edition. Ithaca, NY: Comstock Publishing Associates. 476p.

Volesky, D. J., and S.W. Coleman. 1996. Estimation of botanical composition of esophageal extrusa samples using near infrared reflectance spectroscopy. *Journal of Range Management* 49: 163-166.

Walker, J.W., S.D. McCoy, K.L. Launchbaugh, and M.J. Fraker. 2000. Near infrared spectroscopy of sheep feces for predicting botanical composition of diets. Sheep and Goat, Wool and Mohair. Research Reports, CPR 4923-4949. College station, TX. The Texas Agricultural Experiment Station. p 81-94.

Ward, R.G., G. S. Smith, J.D. Wallace, N.S. Urquhart, and J. S. Shenk. 1982. Estimates of intake and quality of grazed range forage by near infrared reflectance spectroscopy. *Journal of Animal Science* 54:399-402.

Westerhaus, M., J. Workman, Jr., J.B. Reeves III, and H. Mark. 2004. Quantitative analysis. *In.* C.A. Roberts, J. Workman Jr., and J.B. Reeves III [eds.]. Near infrared spectroscopy in agriculture. Near infrared spectroscopy. Agronomy Monograph No. 44. Madison,WI: ASA, CSSA, SSSA Inc. p 133-174.

Westerhaus, O.M. 1992. Computer simulation of partial least squares. *In:* I. Murray, and I. A. Cowe [eds.]. Making light work: advances in near infrared spectroscopy. Weinheim, Federal Republic of Germany: VCH. p 134-139.

Weston, R.H. 1982. Animal factors affecting intake. *In*: J.B. Hacker [ed.]. Nutritional limitation to animal production from pastures. Proceedings of the International Symposium; 24-28 August 1981; St. Lucia, Queensland, Australia: Commonwealth Agricultural Bureaux. p183-198.

Williams, P.C. 1987. Variables affecting near-infrared reflectance spectroscopic analysis. *In:* P. Williams, and K. Norris [eds.]. Near-infrared technology in the agriculture and food industries. St. Paul, MN: American Association of Cereal Chemists, Inc. p 143-167.

Wilman, D., M. Field, S.J. Lister, and D.I. Givens. 2000. The use of near infrared spectroscopy to investigate the composition of silages and the rate and extent of cell-wall degradation. *Animal Feed Science and Technology* 88: 139-151.

Wilson, R. T. 1981. Distribution and importance of the domestic donkey in circumsaharan Africa. *Singapore Journal of Tropical Geography* 2: 136-143.

Workman, J., Jr. 1992. NIR spectroscopy calibration basics. *In:* A. Burns and E.M Ciurczak [eds.]. Handbook of near-infrared analysis. Second Edition. New York: Marcel Dekker Inc. p 247-280.

Workman, J., Jr, and J. Shenk. 2004. Understanding and using the near-infrared spectrum as an analytical method. *In*: C.A. Roberts, J.,Workman Jr., and J.B. Reeves, III [eds.]. Near infrared spectroscopy in agriculture. Near infrared spectroscopy. Agronomy Monograph No. 44. Madison, WI: ASA, CSSA, SSSA, Inc. p 3-48.

Xiccato, G., A. Trocino, A. Carazzolo, M. Meurens, L. Maertens, and R. Carabano. 1999. Nutritive evaluation and ingredient prediction of compound feeds for rabbit by near infrared reflectance spectroscopy. *Animal Feed Science and Technology* 77:201-212.

APPENDIX

Appendix Table 1. Dry matter intake (DMI kg/d) of donkey during the US experiment period

	Week No.									
Animal ID	1	2	3	4	5	6	7	8	9	10
1	2.4	2.4	2.4	2.8	2.8	2.9	2.9	2.9	2.8	1.6
2	2.9	3.5	3.3	3.5	3.5	3.7	3.7	3.3	3.2	2.5
3	3.5	3.5	3.3	2.8	3.6	3.6	3.6	3.7	3.6	2.3
4	2.3	2.8	2.8	3.0	3.2	3.2	3.2	3.3	3.2	1.6
5	2.8	2.9	2.5	3.2	3.3	3.3	3.3	3.3	3.2	0.3
6	2.6	2.5	2.8	3.0	2.1	2.5	2.5	2.8	2.8	1.0
7	2.4	2.7	2.7	3.1	3.2	3.3	3.3	3.3	3.2	2.3
8	3.0	3.3	3.3	3.7	3.6	4.5	4.5	4.5	4.5	2.1
9	3.5	3.9	4.0	3.7	4.0	2.6	2.6	4.5	4.5	1.2
10	3.2	3.2	3.2	3.7	3.7	4.6	4.6	4.1	4.3	1.5

Appendix Table 2. Dry matter digestibility (DMD %)of diets used in the US experiment

	Week									
Animal ID	1	2	3	4	5	6	7	8	9	10
1	50.8	42.1	50.0	56.1	54.4	47.2	63.6	60.3	64.8	17.0
2	51.0	43.7	54.6	53.5	53.0	53.6	65.9	62.0	64.6	47.9
3	45.8	51.6	64.3	48.3	59.4	52.2	66.4	63.6	65.1	37.7
4	48.3	51.3	63.0	48.4	57.3	53.1	63.7	55.7	61.7	43.9
5	47.0	39.4	58.9	49.1	52.6	52.6	65.5	55.7	56.3	34.1
6	46.1	44.5	49.9	50.5	50.0	48.9	64.8	55.1	59.4	50.4
7	45.2	40.3	49.2	49.3	46.3	43.5	61.2	53.5	60.0	63.4
8	47.1	38.4	53.8	43.2	44.7	41.6	65.5	54.7	57.6	51.6
9	45.7	44.6	49.3	45.3	50.8	45.9	65.3	52.9	60.3	50.0
10	47.3	48.0	56.3	53.7	58.8	52.0	61.3	59.4	63.4	74.0

Appendix Table 3. Organic matter digestibility (OMD%) of diets used in the US experiment

	Week									
Animal ID	1	2	3	4	5	6	7	8	9	10
1	46.1	42.2	44.7	49.0	47.10	39.4	47.8	45.8	51.1	15.9
2	46.7	40.7	47.2	45.5	44.59	46.6	50.5	47.7	52.2	44.6
3	42.0	46.6	53.2	43.7	50.75	45.0	51.7	49.9	53.2	33.3
4	44.5	48.2	53.2	42.1	49.89	46.5	49.0	44.3	43.1	37.3
5	43.4	39.7	48.8	42.7	44.29	44.4	51.3	44.6	45.6	28.5
6	44.0	42.5	43.7	47.8	42.68	42.3	49.4	43.7	48.8	42.9
7	45.1	37.1	42.7	41.0	39.68	40.1	47.9	45.2	48.5	56.7
8	44.2	36.1	46.8	36.4	38.66	38.0	49.8	45.3	47.8	49.6
9	42.2	42.7	42.9	38.3	44.16	41.2	50.1	44.2	49.8	12.1
10	43.7	43.8	48.3	45.8	49.84	45.1	49.1	47.0	53.2	61.9

Appendix Table 4. Ort corrected crude protein concentration (CP%) of diets used in the US experiment

	Week									
Animal ID	1	2	3	4	5	6	7	8	9	10
1	5.9	7.3	8.4	10.8	11.7	7.4	18.8	13.4	17.1	5.4
2	5.7	8.4	10.3	12.7	13.1	8.9	18.2	17.4	17.5	5.5
3	7.1	7.6	7.8	12.4	9.2	9.0	15.1	16.3	16.3	5.1
4	5.2	6.9	8.3	12.7	10.7	10.1	16.5	14.4	16.6	4.2
5	5.5	7.8	9.2	12.5	12.9	8.7	16.7	16.8	17.8	5.8
6	5.9	7.0	9.3	11.3	13.0	9.0	16.2	14.4	15.6	3.0
7	5.8	6.9	8.7	11.0	11.5	9.6	16.6	16.2	17.6	5.1
8	5.1	7.0	9.6	12.3	10.9	8.8	17.3	14.2	16.4	1.6
9	6.9	8.6	12.1	13.3	12.6	11.0	17.1	15.3	17.8	4.7
10	5.9	7.4	9.4	11.0	10.3	12.2	16.2	15.3	19.4	6.4

Appendix Table 5. Fecal dry matter output (FDOP kg/d) of donkeys fed on mixed in the US experiment

	Week									
Animal ID	1	2	3	4	5	6	7	8	9	10
1	1.3	1.4	1.4	1.3	1.0	1.3	1.0	1.1	1.0	1.0
2	1.2	1.3	1.1	1.3	1.3	1.3	1.0	1.1	1.0	0.8
3	1.6	1.7	1.2	1.8	1.4	1.7	1.1	1.2	1.1	1.6
4	1.8	1.7	1.2	1.5	1.6	1.7	1.3	1.7	1.4	1.3
5	1.3	1.6	1.1	1.6	1.5	1.5	1.1	1.4	1.4	1.5
6	1.3	1.6	1.4	1.5	1.6	1.7	1.1	1.5	1.3	0.8
7	1.6	1.9	1.7	1.9	1.9	2.6	1.7	2.1	1.8	0.8
8	1.5	1.8	1.2	1.8	1.8	1.9	1.1	1.5	1.4	0.1
9	1.9	2.1	2.0	2.0	2.0	1.4	1.5	2.1	1.8	1.0
10	1.7	1.7	1.4	1.7	1.5	2.2	1.6	1.7	1.6	0.4

Appendix Table 6. Dry matter intake (DMI) and fecal dry matter output (FDMO) of donkeys fed on mixed diet during the Africa study

	DMI (kg/d)				FDMO (kg/d)			
	Week				Week			
Animal ID	1	2	3	4	1	2	3	4
1	3.62	3.90	3.50	4.06	1.76	2.52	1.52	3.38
2	3.65	4.41	2.46	3.83	1.40	3.21	0.81	3.01
3	3.72	4.47	3.30	4.44	1.87	3.15	1.34	3.41
4	4.32	4.58	3.98	4.86	2.59	3.18	2.54	3.10
5	4.55	4.51	4.71	4.32	2.65	3.29	2.91	3.21
6	4.39	4.62	3.87	4.99	2.82	3.51	2.54	3.92
7	4.10	4.67	4.44	5.13	2.10	3.01	2.82	3.52
8	4.11	4.73	4.65	4.68	3.21	3.68	2.91	3.79
9	3.93	4.94	4.65	4.93	2.31	2.95	2.80	3.28
10	4.43	4.57	4.66	5.16	2.91	3.30	3.25	3.73

Appendix Table 7. Calibration statistics for CP, DOM, DDM OMD for the US calibration set (n=100) using T=1.5

						Calibration statistics					
Constituent	N	OL	Mean	SEC	R^2	SECV	1-VR	T	H	Math treatment	Regression type
CP	77	23	10.886	0.308	0.995	0.64	0.977	1.5	10	2,4,4,1	MPLS
DOM	80	20	45.276	1.67	0.857	2.08	0.79	1.5	10	1,12,12,1	MPLS
OMD	80	20	55.08	2.21	0.859	2.586	0.807	1.5	10	2,12,4,1	MPLS
DDM	77	22	54.242	2.13	0.901	2.47	0.866	1.5	10	2,4,4,1	MPLS

N = number of samples
OL = number of outliers
SD = standard deviation
R^2 = coefficient of determination
SEC = standard error of calibration
SECV = standard error of cross validation
1-VR= coefficient of determination for cross validation
SEP (C) = Standard error of prediction
T= critical t value
H = critical H value
MPLS = modified partial least square

Appendix Table 8. Calibration statistics for CP, DOM, DDM, and OMD for the US/Africa combined calibration set (N=140) using T= 1.65 and Modified Partial Least Square.

Constituent	N	OL	Mean	SD	SEC	R^2	SECV	1-VR	SEP	Bias	T	H	Math
CP	113	27	10.17	3.95	0.67	0.97	0.93	0.95	1.21	-0.56	1.65	10	2,4,4,1
DOM	115	25	43.24	6.93	2.84	0.83	3.25	0.78	4.22	-1.95	1.65	10	2,4,4,1
OMD	115	25	51.29	9.38	3.36	0.87	3.77	0.88	4.90	-2.26	1.65	10	2,4,4,1
DDM	115	25	48.77	10.67	3.42	0.897	3.68	0.88	4.74	-2.19	1.65	10	1,12,12,1

N number of samples
OL = number of outliers
SD = standard deviation
SEC = standard error of calibration
R^2 = coefficient of determination
SECV = standard error of cross validation
1-VR= coefficient of determination for cross validation
SEP (C) = Standard error of prediction
T= critical t value
H = critical H value

Appendix Table 9. Dominant wavelengths and associated chemical bonds for the CP equations

Equation	Major dominant Wavelength (nm)	Associated chemical bonds
US	1596	NH group, N-H deformation, primary and secondary amines C= O stretch, Urea, amid I, C-O bending, COŌ zwitterions, C-O stretch, amino acids, Amide II, N-H deformation, NH_4+
	1600	N-H bend, *trans*-secondary amides, NH_4+, N-H deformation, primary and secondary amines, Amide II, N-H deformation, ring deformation, pyrimidines, quinolines,
	1604	Amide II, N-H deformation, ring deformation, pyrimidines, quinolines, N-H bend, *trans*-secondary amides, N-H deformation, primary and secondary amines, NH_4+
Africa	2200	Benzene ring deformation, C-H stretching, *cis* unsaturation, combination C-H stretching
	2204	Combination C-H stretching, Benzene ring deformation, C-H stretching, *cis* unsaturation
	2332	COŌ- stretch, or combination band, most amino acids, combination band ionized amino acids, C-H stretch aliphatic compound, C-O stretch, primary alcohol, Coupled C-O stretch, carboxylic acid
US/Africa	1588	Urea, amide I, amino acids, Hydrogen bonding, peptide links, protein helices, NH- stretch, secondary amides, *cis* –bonded NH, C-O stretching, COŌ zwitterions, C-O
	1596	NH group, N-H deformation, primary and secondary amines C=O stretch, Urea, amid I, C-O bending, COŌ- zwitterions, C-O stretch, amino acids, Amide II, N-H deformation, NH_4+
	1604	Amide II, N-H deformation, benzene ring deformation, pyrimidines, quinolines, N-H bend, *trans*-secondary amides, N-H deformation, primary and secondary amines, NH_4+

Appendix Table 10. Dominant wavelengths and associated chemical bonds for the CP equations

Equation	Major dominant Wavelength (nm)	Associated chemical bonds
Pregnant	1428	C=O vibrations, open-chain acid anhydrides O-H stretch, internal OH bonds, single bridge, O-H stretch, COOH group P-H stretching,
	1716	N-H stretch, symmetrical, all amino acids and hydrochlorides, N-H bend, *cis* secondary amides, C-O stretch, amino acid ionized, carbonyls, C-H stretch, carbonyl compounds, CH stretch, CH_3 group (A_1), C-H stretch CH2 group (A_2), C-H stretch, -CH=(A_3)
	2348	Amide IV, N-H bend, primary amides, C-N stretch, -N=C=N-, coupled C-O and O-H stretch, carboxylic acids, C-O stretch, amino acid ionized, carbonyls, COŌ stretch, or combination band most amino acids, C-H stretch, aliphatic compounds
Non- Pregnant	2348	Amide IV, N-H bend, primary amides, C-N stretch, -N=C=N-, coupled C-O and O-H stretch, carboxylic acids, C-O stretch, amino acid ionized, carbonyls, COŌ stretch, or combination band most amino acids, C-H stretch, aliphatic compounds
	1412	O-H stretch, internal OH bonds, single bridge, COOH group, intramolecular OH bonds, single bridge, carbonates, P-H stretching
	1892	Unknown absorbers in most amino acids, C-N stretch, acrylamines, alkyl amines, primary-tertiary, *cis*-secondary amides, coupled C-O and O-H stretch, carboxylic acids, phosphates, PO_4
	1908	Unknown absorbers in most amino acids, N-H stretch, symmetrical, all amino acids and hydrochloride, C-H bend, CH in long chain fatty acids –CH=CH-: $CH=CH_2$ in phase deformation, P-OH stretching, phosphate, PO_4

Appendix Table 11. Dominant wavelength and associated chemical bonds for the DOM equations

DOM	Major dominant Wavelength (nm)	Associated chemical bonds
US	1732	C-H stretch, carbonyl compounds, CH stretch, CH_3 group (A_1), C-H stretch CH2 group (A_2), C-H stretch, -CH=(A_3), C-H in-phase deformation, CHO groups, C-CH3; CH asymmetrical deformation, -CH2 -
	1724	C-CH3; CH asymmetrical deformation, CH stretch, CH_3 group (A_1), C-H stretch CH_2 group (A_2), C-H stretch, -CH=(A_3), C-H stretch, carbonyl compounds
	1796	Amide IV: N-H bend, primary amides, C-N stretch, amides with no N substitution, coupled C-O and O-H stretch, carboxylic acids, C-O symmetrical vibrations, zwitterions, C-H in-phase deformation C-H stretch aliphatic compounds,
Africa	1468	P-H stretching, C=O stretch, α-β unsaturated aldehydes ketones
	1148	N-H stretch, symmetrical, all amino acids and hydrochlorides, O-H stretch, carboxylic acid dimmers, O-H stretch, intramolecular OH bonds, polymers
	1156	N-H stretch, symmetrical, all amino acids and hydrochlorides, C-N stretch, N=C=N-,
US/Africa	2104	N-H stretch, symmetrical, all amino acids, hydrochlorides, N=N stretching, unsaturated nitrogen compounds, NH3 deformation; amino acid I, N-H deformation, primary and secondary amines, C=O stretch, amid III combination, secondary amides, C-O stretch, long chain fatty acids, primary alcohol
	2192	C-H stretching *cis* instauration, combination, skeletal in-plane deformation, C-O stretch, amino acids ionized carbonyls, Urea, amide I,
	2272	N-H bend, cis-secondary amides, C-O stretch, amino acid ionized carbonyls, C-O stretch, primary alcohol, C-CH_3; CH asymmetrical deformation, C-CH_2; CH asymmetrical deformation, pyrimidines and quinolines, ring deformation

Appendix Table 12. Dominant wavelength and associated chemical bonds for the DOM equations

Equations	Major dominant Wavelength (nm)	Associated chemical bonds
Pregnant	2076	Ring deformation, pyrimidines, quinolines, N=N stretching, , unsaturated nitrogen compounds, NH_3 deformation, amino acid I, N-H deformation, primary and secondary amines, C-O bending, COŌ zwitterions, amino acids, C=O stretch, solid primary amines, amides I, C-O, O-H stretching combination, primary alcohols
	2276	N-H bend, cis-secondary amides, C-O stretch, primary alcohols, C-CH3; CH asymmetrical deformation, C=N, SCN=
	2340	Amides IV: N-H bend, primary amides, C=N stretch, -N=C=N- C-N stretch, primary amines, primary alpha-carbon atoms. Coupled C-O and O-H stretch, carboxylic acids, C-O stretch, primary alcohol, C-H stretch aliphatic compounds
Non-Pregnant	2324	Amides IV: N-H bend, primary amides, C=N stretch, -N=C=N-, C-N stretch, primary amines, primary alpha-carbon atoms. C-O stretch, primary alcohol, C-H stretch aliphatic compounds
	2364	Amides IV: N-H bend, primary amides, C-O stretch, primary alcohol, C-O/O-H stretch coupled, carboxylic acids, C-H stretch aliphatic compounds
	2484	C-N stretch, acryalmines, alkyl amines, primary-tertiary, cis-secondary amides, COŌ stretch, or combination band, most amino acids, O-H deformation, secondary alcohols C-H bend CH in long-chain fatty acids, NO3-, P=O free. NH_4+

Appendix Table 13. Dominant wavelength and associated chemical bonds for the DDM equations

Equation	Major dominant Wavelength (nm)	Associated chemical bonds
US	1572	N-H stretch, secondary amides, *cis*-bonded NH, hydrogen bonding, peptide links, proteins helices, ring deformation, pyrimidines, quinolines, amide II, N-H deformation coupled with C-H stretching, secondary amides, especially peptides, N=N stretching, unsaturated nitrogen compounds-H deformation, primary and secondary amines, C=O stretch, urea, amide I, C-O bending, COŌ zwitterions, C-O stretch, COOH, amino acids
	2108	N-H stretch, symmetrical, all amino acids, hydrochlorides, N=N stretching, unsaturated nitrogen compounds, NH3 deformation; amino acid I, N-H deformation, primary and secondary amines, C=O stretch, amid III combination, secondary amides, C-O stretch, long chain fatty acids
	2276	N-H bend, *cis*-secondary amides, C-O stretch, amino acid ionized carbonyls, C=N SCN=, C-O stretch, primary alcohol, C-CH_3; CH asymmetrical deformation, C-CH_2; CH asymmetrical deformation, pyrimidines and quinolines, ring deformation,
Africa	1940	Unknown absorber in most amino acids, C=O stretch, ketones
	1980	Amide III, combination N-H stretch with C-O stretch, secondary amides, *trans*-secondary amides, C-N stretch, unsaturated nitrogen compounds, C=O stretch, α-β unsaturated aldehydes , ketones, O-H deformation, secondary alcohols, C-O/O-H stretch coupled, carboxylic acids
	1992	Amide III, combination N-H stretch with C-O stretch, secondary amides, C-N stretch, unsaturated nitrogen compounds, C=O stretch, internally bonded, saturated aliphatic carboxylic acids, C=O stretch, α-β unsaturated ketones, O-H deformation, secondary alcohols

Appendix Table 13. Continued

Equation	Major dominant Wavelength (nm)	Associated chemicals
US/Africa	1148	-H stretch, symmetrical, all amino acids and hydrochlorides, O-H stretch, carboxylic acid dimmers, O-H stretch, intramolecular OH bonds, polymers, N-H stretch, symmetrical, all amino acids and hydrochlorides, C-N stretch
	1152	N=C=N-, C-H stretch, carbonyl compounds, CH_3 group (A_1), C-H stretch CH2 group (A_2), C-H stretch, -CH=(A_3)
	2272	N-H bend, cis-secondary amides, C-O stretch, amino acid ionized carbonyls, C-O stretch, primary alcohol, C-CH_3; CH asymmetrical deformation, C-CH_2; CH asymmetrical deformation, pyrimidines and quinolines, ring deformation

Appendix Table 14. Dominant wavelength and associated chemical bonds for the DDM equations

Equation	Major dominant Wavelength (nm)	Associated chemical bonds
Pregnant	2076	Ring deformation, pyrimidines, quinolines, N=N stretching, , unsaturated nitrogen compounds, NH_3 deformation, amino acid I, N-H deformation, primary and secondary amines, C-O bending, $CO\bar{O}$ zwitterions, amino acids, C=O stretch, solid primary amines, amides I, C-O, O-H stretching combination, primary alcohols
	2276	N-H bend, *cis*-secondary amides, C-O stretch, amino acid ionized carbonyls, C=N SCN=, C-O stretch, primary alcohol, $C\text{-}CH_3$; CH asymmetrical deformation, $C\text{-}CH_2$; CH asymmetrical deformation, pyrimidines and quinolines, ring deformation,
	2340	Amides IV: N-H bend, primary amides, C=N stretch, -N=C=N- C-N stretch, primary amines, primary alpha-carbon atoms. Coupled C-O and O-H stretch, carboxylic acids, C-O stretch, primary alcohol, C-H stretch aliphatic compounds
Non-Pregnant	1740	N-H bend, *cis*-secondary amides, C-H stretch, carbonyl compound, C_H stretch, CH3 group (A_1), C-H stretch CH2 group (A_2), C-H stretch, -CH=(A_3), C-H stretching, aliphatic compounds, $C\text{-}CH_3$; CH asymmetrical deformation, pyrimidines and quinolines, ring deformation, P=O hydrogen bonded, Phosphates, PO_2-, P=O hydrogen bonded
	1772	C-N stretch, amides with no N substitution-H in-phase deformation, CHO groups, -CH=CH2 in phase CH2 deformation, C-H stretch aliphatic compounds-SH stretch, P=O hydrogen bonded, , Phosphates, $PO_{2,}$ Phosphates, PO_3=
	2316	Amide IV; N-H bend, primary amides, N-H bend, Cis-secondary amides, C-N stretch, -N=C=N-, C-N stretch, primary amines, primary alpha-carbon atoms, C-H stretching, methylene groups, combination primidines and quinolines, ring formation, C=N, SCN=, P-(phenyl ring)

Appendix Table 15. Dominant wavelength and associated chemical bonds for the OMD equations

Equation	Major dominant Wavelength (nm)	Associated chemical bonds
US	1364	O-H stretch, tertiary alcohol and secondary alcohol, P-H stretching
	1372	O-H stretch, primary alcohol and secondary alcohol, P-H stretching,
	2108	N-H stretch, symmetrical, all amino acids, hydrochlorides, N=N stretching, unsaturated nitrogen compounds, NH3 deformation; amino acid I, N-H deformation, primary and secondary amines, C=O stretch, amid III combination, secondary amides, C-O stretch, long chain fatty acids
Africa	1460	P-H stretching, C=O stretch, α-β unsaturated aldehydes, C=O stretch, α-β unsaturated ketones, ketones, saturated aliphatic carboxylic acids
	1468	P-H stretching, C=O stretch, α-β unsaturated aldehydes, ketones, saturated aliphatic carboxylic acids, C=O stretch,, ketones
	1476	C=O stretch, α-β unsaturated aldehydes, ketones
US/Africa	1152	N-H stretch, symmetrical, all amino acids and hydrochlorides, C-N stretch, N=C=N-, C-H stretch, carbonyl compounds, CH_3 group (A_1), C-H stretch CH2 group (A_2), C-H stretch, -CH=(A_3)
	2192	C-N stretch, secondary amines, secondary carbon atoms, C-O stretch, amino acid ionized carbonyls, long chain fatty acids, C=O stretch, urea, amide I, ,
	2272	N-H bend, cis-secondary amides, C-O stretch, amino acid ionized carbonyls, C-O stretch, primary alcohol, C-CH_3; CH asymmetrical deformation, C-CH_2; CH asymmetrical deformation, pyrimidines and quinolines, ring deformation,

Appendix Table 16. Dominant wavelength and associated chemical bonds for the OMD equations

Equation	Major dominant Wavelength (nm)	Associated chemical bonds
Pregnant	1876	Unknown absorbers in most amino acids, C-N stretch, acrylamines, alkyl amines, primary-tertiary, C-N stretch, amides with no N substitution C=O vibration, open-chain acid anhydrides, C-O/O-H stretch coupled, primary alcohols, O-H deformation, primary alcohols. , C_H bend, CH in long chain fatty acids, -CH-; CH deformation, C-H stretch aliphatic compounds, P-OH stretching, silicates, phosphates, $PO_{4,}$ phosphates, $PO_{3.}$
	2076	Ring deformation, pyrimidines, quinolines, N=N stretching, unsaturated nitrogen compounds, NH_3 deformation, amino acid I, N-H deformation, primary and secondary amines, C-O bending, COŌ zwitterions, amino acids, C=O stretch, solid primary amines, amides I, C-O, O-H stretching combination, primary alcohols
	2364	Amides IV: N-H bend, primary amides, C-O stretch, primary alcohol, C-O/O-H stretch coupled, carboxylic acids, C-H stretch aliphatic compounds
Non-Pregnant	1932	Unknown absorbers in most amino acids, amide III: combination N-H stretch with C-O stretch, secondary amides, amides III combination, C-N stretch, acrylamine, alkyl amines, primary-tertiary, C=O stretch, ketones, saturated aliphatic carboxylic acids, , carboxyl acids, C-H bend, CH –in long chain fatty acids, P-OH stretching, silicates, phosphates, $PO_{4,}$
	2324	Amides IV: N-H bend, primary amides, C=N stretch, -N=C=N-, C-N stretch, primary amines, primary alpha-carbon atoms. C-O stretch, primary alcohol, C-H stretch aliphatic compounds
	2364	Amides IV: N-H bend, primary amides, C-O stretch, primary alcohol, C-O/O-H stretch coupled, carboxylic acids, C-H stretch aliphatic compounds

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