DEVELOPMENT OF A NUTRITIONAL PROFILING SYSTEM FOR FREE-RANGING LIVESTOCK IN MAJOR AGRO-ECOLOGICAL ZONES OF SUB-SAHARAN AFRICA

A Dissertation

by

SARAH OSSIYA

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

August 1999

Major Subject: Rangeland Ecology and Management

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ABSTRACT

Development of a Nutritional Profiling System for Free-Ranging Livestock in Major Agro-Ecological Zones of Sub-Saharan Africa. (August 1999)

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The potential of NIRS fecal profiling to predict 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 (SSA), was examined. The calibration sets comprised of samples from the semi-arid, humid, and highland zones of SSA. USA samples were incorporated into the cattle calibration. Validation samples were independently derived. Cluster analysis was conducted to aid interpretation of results.

Six sheep CP and DOM equations were created according to the combination of samples ("All locations", "Ethiopia", "Ethiopia and Nigeria", "Niger", "Extrusa" (CP) or "*In vitro*" (DOM), and "Stall" (CP) or "*In vivo*" (DOM). Calibration statistics were satisfactory, (SEC within twice the SEL, and R^2>0.80), except for the "Niger" CP equation (R^2 0.70). Selected wavelengths were biologically significant. Only the "All locations" CP validation had a R^2 >0.80, (R^2 0.88), indicating the potential for development of a robust CP sheep equation. DOM validation results were poor; the "Ethiopia and Nigeria"

equation performed best (R^2 0.40). Spectral diversity indicated between the Niger and validation samples was confirmed by cluster analysis.

The sheep tannin calibration statistics were satisfactory (SEC 9.02, R^2 0.91). Selected wavelengths were highly indicative of tannin-protein complexes. Validation statistics were less satisfactory: a high SEP 57.79, indicated laboratory technique differences, a R^2 of 0.62 was below the required R^2 0.80, but was indicative of the potential of the methodology to predict tannin in the diet of sheep. Spectral diversity was confirmed by a cluster analysis.

Two equations were formulated for the cattle CP and DOM calibration: "Africa", and "USA and Africa". Both CP and DOM calibration statistics were deemed satisfactory. The CP validation statistics were similar to each other though not of predictive capacity. The DOM validation statistics were poor; SEP and R^2 ("Africa" 12.27 and 0.09) and ("USA and Africa" 11.49 and 0.06), respectively. T outliers indicated differences in determination techniques.

The findings substantiate the potential of NIRS fecal profiling as a methodology to predict the diet of free-ranging livestock across SSA. More diverse calibration sets, and standardization of laboratory techniques is required.

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Above all, I stand in awe of God Almighty, whose will is good, pleasing and perfect, whose ways unfathomable, and whose grace is immeasurable.

For all that has been — thanks

To all that shall be – yes

(Dag Hammarskjold)

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CHAPTER I

INTRODUCTION

Livestock contribute significantly to the agricultural Gross Domestic Product (GDP) as well as national GDP of most sub-Saharan Africa (SSA) countries, across agro-ecological zones. In 1988, for the region as a whole, livestock constituted 8% of the total GDP and 25% of the agricultural GDP (Jabbar 1993). For some individual countries, these figures are as high as over 50% for the agricultural GDP and more than 20 % of the total GDP (Sansoucy et al. 1995). The contribution of livestock in SSA, however, goes far beyond direct food supply to ensuring varying degrees of sustainable farming and economic stability, but this is not reflected in the GDP statistics (Sansoucy et al. 1995). The significance of the contribution of livestock increases with a decline in rainfall and soil fertility; indeed pastoralism is the dominant mode of agricultural production in the arid and drier areas of the semi-arid zone (Coppock 1993, Niamir-Fuller 1998).

The vast majority of the livestock depend upon rangelands consisting of native vegetation (Coppock 1993). These rangelands are diverse, extending across the five major agro-ecological zones: highland (montane),

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This dissertation conforms to the style set forth in the Journal of Range Management.

sub-humid, humid, semi-arid and arid (Attah-Krah 1989). The demarcations of these agro-ecological zones are related to the climate (especially rainfall amount and distribution), altitude as it affects temperature, and the length of the annual growing period (Adegbola 1985, Ehui et al. 1994). Across zones, rangelands are relegated to marginal land and remote land (access constrained), and are extensive in nature.

One of the major challenges to livestock production on these rangelands is the fluctuating forage production in terms of both quality and quantity due to seasonal effects and recurrent drought (Coppock et al. 1986). These fluctuations are reflected in the livestock production losses (Hashim and Fadlalla 1989, Coppock 1993). In semi-arid regions, drought is a regular phenomenon and is central to the economic planning of subsistence livestock owners (Swinton, 1988).

While mitigation in developed countries commonly takes the strategy of supplementation to maintain and or increase productivity during the seasonal periods of low range forage quality and quantity, this is an uneconomical option for most of SSA. Mitigation in SSA rangelands is opportunistic taking the form of mobility, utilization of crop residues, and diversification among other strategies (Sanford 1982, Coppock et al. 1986, Behnke and Kerven 1995). The later is a risk management strategy that is built into the herds (Sanford 1982).

Where pastoralism is practiced, during and immediately after the rainy season, the most productive strategy is to move animals into the drier areas to

take advantage of the flush of high quality forage produced by the annual grasses (Sanford 1982, Hashim and Fadlalla 1989), In the dry season, the most productive strategy is to access enough water and low quality forage in the wetter areas, to maintain the productive capacity of the herd (Sanford 1982, Hashim and Fadlalla 1989). Mobility has decreased due to economic development and land tenure schemes in pastoral areas (Coppock et al. 1986), and with increase in land pressure as cropland encroaches on rangeland.

Opportunistic shifts in herd structure, as is practiced by the semi-transhumant Borana people of Ethiopia, may entail division of livestock into core and satellite herds on a physiological basis to conserve local resources for the breeding and lactating cows and calves (Coppock 1993). The satellite herds consist of hardy immatures, dry cows and bulls (Coppock 1993). Opportunism is also exemplified in spatial segregation of livestock species according to divergent patterns of habitat utilization to exploit the landscape heterogeneity (Coppock et al. 1986).

Crop residue utilization increases in importance as livestock feed as annual rainfall increases (Coppock 1993). However, crop residues are also a key feed resources in some semi-arid regions. In Niger, for example, livestock are moved to cropping areas in the dry season where they are fed, first on the standing crop residues by arrangement with crop farmers, then later moved back onto rangeland in a effort to maximize utilization of all available resources (Fernandez-Rivera et al. 1994, Sere et al. 1995).

While the modes of mitigation differ between SSA countries and developed countries, the key critical decisions are common, i.e., when to initiate and terminate the mitigation strategy in a cost-effective manner. In the precarious SSA rangelands, and especially where there is an almost total dependence on livestock, as in the case of the Borana people of southern Ethiopia where 55% of the human diet is milk (Coppock 1993), the gravity of these decisions is greatly amplified. Survival and not production may be the key issue (Swinton 1988, Connor 1991, Behnke and Kerven 1995). Importance of objective, timely, and cost-effective interventions is further emphasized given the community, national and even regional ramifications that may result, such as the social, ecological, and epidemiological impacts of movement of livestock across internal and international boundaries.

Lyons (1990) noted that mitigation is often based on current perceptions of nutritional status of the grazing animal, which are based on factors (body condition) that are actually indications of past nutrition. Body condition of range animals in SSA may also reflect factors that are non-related to the nutritive value of the range forage such as long distance movements and parasites (Hashim and Fadlalla 1989).

Visual appraisal of forage, as is a common practice by pastoralists in SSA (Niamir-Fuller 1998), to provide qualitative information is subjective and can not be reliably used to mimic the selectivity of free-ranging animals (Theurer 1970). Galt et al. (1969) observed that dietary botanical composition as determined by

visual observation resulted in considerable differences in quantities of species when compared with corresponding rumen samples. Dyson-Hudson (1991) noted that pastoralists are equally inept at tracking the environment (and thus the nutritional status of forage) in a predictive manner as ecologists, even though they can describe the environment (appearance of the forage).

The most pertinent variables in assessment of the nutritional status of free-ranging animals are the quality of the diet selected by the animals as defined by the level of crude protein (CP) (Hobbs et al.1983), digestible organic matter (DOM) (Holechek et al. 1982b), and the quantity consumed (intake). Intake is closely related to animal performance (Van Soest 1994), but is also dependent on a number of factors including animal physiology and ambient temperature, which increases specificity of intake measures per individual animal, thus reducing predictability of intake across animals, and reliability of intake as a practical indicator of nutritional status.

The contribution of one other factor, tannins, can not be underestimated nor overlooked in SSA. Leguminous trees and shrubs contribute heavily to the diets of range animal in all agro-ecological zones of SSA. Their importance increases as one approaches the more arid areas. In arid regions, leguminous trees and shrubs may contribute well over 50% of the biomass production of rangeland, and up to 40% of the feed available to animals (Kaitho 1997). The use of these leguminous trees and shrubs is limited by the presence of anti-nutritional factors of which tannins form the major group. Tannins, especially

condensed tannins, affect intake and digestibility negatively and affect protein utilization.

Current methodologies for profiling the nutritional status of free-ranging livestock in reference to these indicators are hampered by limitations that deem them incompatible with the nature of SSA rangelands and the livestock production systems utilized there. Accessibility, low cost, reliability, rapid turnaround, and compatibility with extension capabilities are all desirable qualities of a suitable method to profile the nutritional status of livestock in SSA. Since rangelands and their manner of use in SSA cut across national boundaries (Hodgson 1975), cooperative effort is required for successful efforts at rangeland production, development and mitigation. A suitable method should be amendable to networking facilities, and have early warning capabilities

Recent studies have indicated the usefulness of near infrared reflectance spectroscopy (NIRS) as a tool for nutritional profiling of free-roaming ruminants (Lyons and Stuth 1992, Leite and Stuth 1995, Showers et al.1999, Coates 1998). This method has potential for utilization in research and management, and for use in remote regions characteristic of SSA rangelands. Interfacing of this monitoring technology with decision support systems such as NUTBAL (Nutritional Balance Analyzer) allows for creation of a viable management and mitigation tool. NIRS can also be interfaced with geographical information systems (GIS) to create databases and facilitate networking.

The studies of Lyons and Stuth (1992), and Leite and Stuth (1995) produced NIRS calibration equations for cattle and goats, respectively. These studies were conducted at locations in sub-tropical regions of Texas. Sub-Saharan Africa has five major agro-ecological zones with different vegetation diversity.

Sheep are an important livestock in SSA contributing about a third to the livestock numbers, and playing an important role in balancing and maximizing feed utilization. Walker et al. (1998) developed NIRS fecal profiling equations to quantify leafy spurge (*Euphorbia esula* L) in the diets of sheep. However no NIRS fecal profiling equations for predicting the diet consumed by sheep exist in the scientific literature.

Windham et al. (1988), Roberts et al. (1993), and Goodchild et al. (1998) found that NIRS could analyze the tannin concentration of forage varieties with an accuracy similar to reference methods. Therefore it may be possible to develop calibration equations to predict tannins in diets of free-ranging livestock by fecal NIRS profiling, thereby improving predictive capacity of nutritional decision support systems.

CHAPTER II

LITERATURE REVIEW

Forage Quality Indicators of Importance in Sub-Saharan Africa

SSA rangelands are diverse, displaying heterogeneity in vegetation and landscape across the agro-ecological zones (Coppock et al. 1986). Generally, SSA rangelands are characterized by high temperatures, and long nights in the growing season, and dominated by C4 grasses and C3 shrubs (Van Soest 1994). These factors lead to a generally lower nutritive value as a result of greater lignification, low soluble carbohydrates, and high cell wall levels, including higher neutral detergent fibre (NDF) concentrations, than in temperate regions (Van Soest 1994). As a result of the lower forage quality, forage intake is generally less than considered optimal, with fibrous bulk being the limiting factor (Van Soest 1994).

The most pertinent indicators of the quality of the diet selected by the animal in SSA are the level of CP (Hobbs et al. 1983), the digestible organic matter (Holechek et al. 1982b, Van Soest 1994), and intake (Van Soest 1994). Digestibility, which indicates the portion of the grazing animal's diet that can actually be utilized, provides the best practical indicator of the quality of the diet (Holechek et al. 1982b), and is more often measured than intake, even though intake is more responsible for total animal responses (Van Soest 1994). Intake demonstrates more inter-animal variation being complicated by animal physiology, forage palatability, and environment among other factors (Van Soest

1994). Digestibility reported on an organic matter basis has an advantage over dry matter basis, as it is not confounded by the effects of consumption of inorganic matter such as soil.

CP is also a crucial practical indicator of forage quality in SSA. Milford and Minson (1964) found that intake of tropical forages by ruminants decreased dramatically when CP levels fell below 7%. While grasses contribute the largest portion of the diet of range animals in SSA rangelands, they rapidly loose quality with the onset of the dry season. Le Houerou (1978) reported that dry mature grass may have very low to zero digestible protein.

Compared to tropical grasses, green leaves of leguminous browse are generally richer in protein and minerals, especially in the dry season (Adegbola 1985) and contain double the amount of energy as dry grass (Le Houerou 1980). Kaitho (1997) noted that in the drier agro-ecological zones, leguminous browse contributed as much as and even more than 40% to the rangeland biomass production. Leguminous browse therefore plays a significant carry-over role in ensuring survival and sustainability of livestock in SSA (Attah-Krah 1989). The use of leguminous browse is however limited by the presence of anti-nutritional factors of which tannins form the major group. Mueller-Harvey et al. (1987) reported that numerous browse species that are important forage sources contain appreciable quantities of condensed tannins. Palo and Robbins (1991) suggest that selective browsing to avoid anti-nutritional factors is a more important cause of selective browsing than selection for nutrients or energy.

Tannins are a chemically diverse group of water soluble polyphenolic compounds that form complexes with proteins, carbohydrates, alkaloids, vitamins, and minerals (Hagerman et al. 1992, Makkar et al. 1996). Tannins are broadly classified into two groups (Haslam 1989); hydrolyzable tannins which are gallic or hexahydroxydiphenic acid-derived (gallo or ellagitannins), and condensed tannins which are flavan-3,4 diol-derived (Lewis and Yamamoto 1989). The latter are more commonly referred to as proanthocyanidins or polyflavanoids and are more widely encountered in nature (Haslam 1989, Lewis and Yamamoto 1989). Hydrolyzable tannins are more susceptible to enzymatic and non-enzymatic hydrolysis than proanthocyanidins and are usually more soluble in water (Reed 1995). Proanthocyanidins are polymers of flavan-3-ols linked through an interflavan carbon bond that is not susceptible to hydrolysis (Markham 1982, Reed 1995).

Dietary tannins have been reported to be responsible for a variety of nutritional problems including mortality (Butler 1989). However, the ability to form strong complexes with proteins is the most important aspect of their nutritional and toxicological effects (Hagerman and Butler 1981). While CP of the different dietary components is used as a basis for assessing the nutritional value of a given diet (Hobbs et al. 1983), the methodology assumes that protein availability is unaffected by tannins (Robbins et al. 1991, Kaitho 1997).

Tannins may affect intake and digestibility negatively, but may also act to protect protein from degradation in the rumen (Van Soest 1994). Tannins may

reduce intake of forage legumes by decreasing palatability or negatively affecting digestion (Reed 1995). Rate of growth for growing animals may also be affected as it reflects total intake and availability of nutrients in the diet (Reed 1995). Tannins may increase the efficiency of protein utilization by ruminants by increasing rumen escape and hydrolysis in the abomasum; increasing the efficiency of urea recycling to the rumen by lowering the rate of protein degradation and deamination in the rumen therefore lowering ruminal NH3, and increasing the glycoprotein content and excretion of saliva leading to more N recycled to the rumen (Robbins et al.1987, Reed 1995). The positive effects of tannins on protein utilization are usually attributed to hydrolysable tannins, and low levels of condensed tannins (1-3% kg^{-1} DM) (Barry 1989).

High levels of condensed tannins, (5-10% kg^{-1} DM) (Barry 1989), may be responsible for negative effects. Digestible protein in plants containing significant tannins was lower than predicted from regressions for low-tannin plants, the reduction being proportional to the protein-precipitating capacity of the plant tannins (Robbins et al. 1987, Robbins et al. 1991). Increases in fecal nitrogen output have been noted in animals consuming tanniferous forages (Van Soest 1994). In a study, Hagerman et al. (1992) found that none of the ingested tannic acid (a hydrolysable tannin) was excreted in the feces of deer or sheep because the tannin was highly hydrolyzed soon after ingestion and gallic acid that was produced was absorbed and excreted in urine. The metabolic fate of the condensed tannins is more complex. Robbins et al. (1991), found that deer

excreted 100% of the ingested quebracho tannin (a condensed tannin) in feces consistent with formation of indigestible complexes with protein. Sheep, in the same study, excreted only about 60% of ingested quebracho tannin (Robbins et al. 1991). Given the effects of tannins, and the high contribution of leguminous browse to the diets of range animals in SSA, tannins are therefore an important factor to consider in the assessment of nutritional status of livestock in SSA.

Current Methodology to Assess the Nutritional Status of Free-ranging Livestock in Sub-Saharan Africa

Many methods have been developed to try and assess the nutritional status of free-ranging livestock. These include bulk sampling by clipping or mowing, collection of new growth from selected plant species, plucking samples believed to represent the grazing animal's diet, and use of cages or micro-plots which exclude grazing animals and can be clipped to represent the diet (Holechek et al. 1982a). Body condition scoring and the use of fistulated animals are also common practices. All these methods have limitations.

Body condition score is based on an assessment of the animal's body fatness on a scale subject to user judgement. Body condition is the end result of past nutrition, precluding timely intervention (Lyons 1990). However, body condition may also reflect other factors that are non-related to forage quality, such as parasite load and the effects of long distance movements (Hashim and Fadlalla 1989). There is a lack of standardization of body condition scoring in SSA, especially for indigenous small ruminants. Hand plucking of forage to

simulate that harvested by the animal is also subject to experience and complicated by the selectivity of free-ranging livestock.

The use of fistulated animals gives accurate results for the area sampled, especially where esophageally fistulated or rumen evacuated animals are used (Holechek et al. 1982b). However, use of fistulated animals requires skill and special care, and is limited to research applications which preclude the use of this method as a management tool for the remote and extensive areas characteristic of SSA rangelands.

Wet chemistry can provide accurate analyses of the nutritive value of samples derived from fistulated animals; however the extended turn around time reduces the reliability of this method (Norris et al. 1976), and expense reduces scope for utilization of this method across SSA. Wet chemistry and other methods also do not reflect the associative effects of dietary component synergistic and antagonistic relationships in the animals' gut.

Tannin content of range animal's diets is usually assessed by chemical analysis. There are numerous quantitative assays for tannins, but Reed (1995) observed that no single method will give satisfactory results in relationship to nutritional effects because the chemical properties that are involved in the reactivity of tannins in assays may differ from the properties that underlie their nutritional or toxic effects. Difficulties arise from sample preparation as temperature influences determination of tannins, and there are also difficulties in extraction of tannins (Reed 1995). There is generally a lack of standardization of

procedures across laboratories. Assays are generally expensive and costly in terms of labor and time to carry out.

Fecal Indices of Forage Quality

Because of the shortcomings of the other methodologies commonly used, considerable effort has been directed towards the development of indices between known diets and fecal samples (Holechek et al. 1982a, Nunez-Hernandez et al. 1992). Langlands (1967) and Holloway et al. (1981) suggested that the use of a fecal index may be the only technique for evaluating the nutritional status of grazing animals that has potential for application under extensive pasture conditions, allowing quick and easy sampling across extensive areas and into remote regions. However, interpretation of fecal indices remains uncertain (Wehausen 1995). Reliability of fecal indicators for monitoring range animal nutritional status has also been a highly criticized and controversial subject with numerous advocates on either side. Workers who noted the potential of fecal indices include Lancaster (1949), Langlands (1967), Allden and Jennings (1969), Hinnant (1979), Holloway et al. (1981), Van Soest (1982), Squires and Siebert (1983), Wofford et al.(1985), Leslie and Starkey (1987), Nunez-Hernandez et al. (1992), Van Soest (1994), Wehausen (1995), and Hodgman et al. (1996). Workers who noted the limited reliability of fecal indices include Hobbs (1987), Hashim and Fadlalla (1989), and Leite and Stuth (1990). Lancaster (1949) developed the first fecal indices recorded in literature.

Feces are composed of undigested forage, (composed almost entirely of dietary cell wall constituents), plus metabolic excretions (Van Soest 1994). Metabolic excretions are mostly microbial matter, derived from the fermentation of feed ingredients by microbes, plus a small amount of endogenous secretions that consist of digestive tract cells and digestive secretions (Van Soest 1994). Fecal nitrogen (FN) content therefore represents a combination of unabsorbed dietary nitrogen (DN), undigested microbial N and endogenous N diluted by undigested dietary dry matter and endogenous material (Leslie and Starkey 1987). Virtanen (1966), Mason (1969), Wofford et al. (1985), and Van Soest (1994) assert that nearly all nitrogen in ruminant feces is of microbial origin and very little is from the feed. Mason (1969) noted that the concentration of microbial matter in the ruminant feces increases as the quality of the diet improves. This would make the concentration of microbial matter (N or other) an indicator from which diet quality can be predicted. This is the basis of fecal indices.

The value of fecal indices lies in their predictive capacity. Basic criteria for whether or not an equation should be used for predictive and therefore management purposes, is that the independent variable(s) (fecal variables) should account for at least 80% of variation in the dependent variable (dietary variable) (Nunez-Hernandez et al. 1992, Hodgman et al. 1996). Furthermore, the equation should not be used to predict samples outside the range used to build the equation (Hodgman et al. 1996).

Fecal indices have been used to try and predict forage intake. However, regardless of foraging strategy, Hodgman et al. (1996) noted that forage intake may be difficult to model using only fecal chemistry because it is a behavioral, environmental, and biological phenomenon. Most workers found that intake and in vivo digestibility are not accurately predicted with any single variable or combination of independent variables.

Fecal N has repeatedly been shown as the principal variable used for predicting diet quality of ruminants. For example, Wofford et al. (1985) found that FN was highly correlated to DN (R^2 0.81), and showed the potential of detecting N deficiencies in steer diets from fecal N% on an organic matter (OM) basis, when steer diets contained low levels of soluble phenolics. Wofford et al. (1985) found that grasses showed a higher correlation between FN and DN (R^2 0.91), compared to non-grasses (R^2 0.81). Hodgman et al. (1996) found similar results. Leslie and Starkey (1987) concluded that FN as a single variable, represents a viable index of dietary N changes of a single population, comparison of disjunct populations with similar habitats, and a comparison between years, with the condition that no secondary metabolites are present in the forage being consumed.

FN has also been positively correlated with weight changes (Allden and Jennings 1969, Gates and Hudson 1981, Squires and Siebert 1983) which is a bio-energetic expression of overall animal condition, though Hashim and Fadlalla (1989) found a negative correlation.

However, the reliability of fecal indices has been questioned. Leite and Stuth (1990) found that no fecal parameter by itself had a high correlation with dietary variables when expressed on a proportion or concentration basis. While a combination of fecal indexes accounted for more variation in dietary parameters than fecal nitrogen, still multiple fecal indices used in this study were of limited value in predicting diet quality and intake. Fecal tannins were not related to dietary CP (R^2 -0.38). Total N (N conc. % in fecal OM) and N proportion (g N/g fecal OM) were both poorly related to CP (for both R^2 0.59). Fecal tannins were also poorly related to DOM (R^2 –0.42), and total N accounted for only 0.48% variability in DOM. Leite and Stuth (1990) concluded that there are difficulties in seeking wet chemistry techniques for predicting dietary quality and nutrient intake from fecal parameters. Hobbs (1987) reviewing the work of Leslie and Starkey (1987) and Sinclair et al. (1982) found no significant relationships between DN and FN even though both original studies claimed significant relationships. Hobbs (1987) noted that large variation in fecal N was associated with relatively small variation in diet quality. Leite and Stuth (1990) suggested that repeatability and accuracy of fecal indices is hampered due to the wide array of plant species consumed by ruminants across landscapes and between seasons.

One of the central issues in the debate on the validity and usefulness of fecal indices of dietary quality has been the potential confounding influence of tannins on fecal nitrogen through reduced protein digestibility (Mould and

Robbins 1981, Robbins et al. 1987, Hobbs 1987, Hodgman et al. 1996). Protein-binding phenolics can cause excess forage protein to reach the feces thereby decreasing the ability of FN to index microbial fecal protein (MFP) by increasing the proportion of N in undigested feed (UF) (Wehausen 1995). The important consideration here is that high fecal N may result from high levels of tannins rather than high levels of DN or digestibility; the investigator will not know which influence predominates (Hobbs 1987). Because 80% of all shrubs and 15% of forbs contain tannins (Rhoades 1979) all diets containing dicotyledonous plants must be assumed to contain tannins until proven otherwise (Hobbs 1987). The confounding influence explains why N in feces of herbivores consuming shrubs and forbs frequently is elevated and variable (Arman et al. 1975, Reassumes et al. 1978 and Holechek et al. 1982a).

The study by Wofford et al. (1995) demonstrates the influence of tannins on fecal indices. Wofford et al. (1985) carefully reviewed a number of studies relating dietary nitrogen to fecal nitrogen and concluded that the best associations occurred when diet nitrogen concentrations were not over 2.4% (equivalent to 15% CP). They then developed a regression equation combining data from their study with those from Hinnant (1979) and Arthun et al. (1981), using only samples with dietary nitrogen concentrations of 2.4% and below. Allden and Jennings (1969) suggested that when fecal nitrogen concentrations fell below 1.65%, then CP deficiencies in the diet could be suspected. Wofford et al. (1985), using the equation they developed, predicted that a fecal nitrogen

concentration of 1.65% would give a diet nitrogen concentration of 1.06% equivalent to 6.64% CP, a level close to the limiting dietary CP value (7% CP). Wofford et al. (1985) also found that when fecal NDF-N concentrations exceeded 1.00%, their equation appeared unreliable and suggested that the presence of soluble phenolics in the diet should be suspected.

Determination of Forage Quality and Anti-nutritive Factors in Forage Material by NIRS

The basis for using NIRS to determine diet quality is that materials absorb near-infrared light at wavelengths commensurate with the absorption properties of bonds of functional groups in their constitution. NIRS has successfully been used to predict the nutritive value of forages and hays through direct scanning of the forage samples or the extrusa obtained from esophageally fistulated animals (Holechek et al. 1982a, Volesky and Coleman 1996).

NIRS has also been used in the analysis of anti-nutritive factors in forage (Windham et al. 1988, Roberts et al. 1993, Goodchild et al. 1998). The ability to analyze tannin concentration by NIRS is based on the fact that the major chemical components of tannin or in the protein-tannin complex have NIR absorption properties at given wavelengths (Windham et al. 1988). Goodchild et al. (1998) formulated NIRS calibrations for various measures of tannins on a set of 40 hays and straws of *Vicia* and *Lathyrus spp.* They successfully predicted, in the drymatter, 4.6–34.1 g /kg total phenolics with a cross-validation R^2 of 0.95 and a SECV (standard error of cross-validation) of 1.68 g /kg; 1.3–23.1 g/ kg

total tannins (R^2 = 0.89, SECV = 1.84 g/ kg) and 0.5–30.3 g/ kg condensed tannins (R^2 = 0.93, SECV = 2.34 g/ kg). Windham et al. (1988) had comparable results, and concluded that NIRS can analyze the tannin concentration of forage material with an accuracy similar to the reference method.

Both Windham et al. (1988) and Goodchild et al. (1998) found that the significant wavelengths had meaningful relationships to condensed tannins. The first wavelength found by Windham et al. (1988) was 1664nm, the first overtone of a C-H stretching fundamental from the aromatic polymers flavan-3-ols and flavan-3,4-diols found in condensed tannins. The second wavelength was 2024nm. This region contains –OH phenols (1955-2035), and a protein combination region from 2000. The occurrence of both phenols and protein are in agreement with the structure expected for tannins that form complexes with proteins (Hagerman and Butler 1981, Haslam 1989). Goodchild et al. (1998) found that a wavelength close to 2144 nm was common to all measures of tannins, and was attributed to condensed tannins and its flavanoid precursors. Windham et al. (1988) concluded that although the primary wavelengths may have been obtained empirically, the establishment of a relationship between the wavelengths and functional groups implies that the NIRS determination may be partly analytical.

NIRS and Fecal Profiling

The validity and usefulness of fecal NIRS profiling to determine the nutritional status of animals has been demonstrated for different purposes and

different livestock and wildlife species. Czarnik–Matusewicz and Korniewick (1996) successfully used NIRS fecal profiling to determine the crude fat levels in fecal samples of sows. The NIRS equations generated gave accurate predictions in comparison with the reference method (Soxhlet's method, a six-hour extraction by petroleum ether). Czarnik–Matusewicz and Korniewick (1996) concluded that this method had validity for use in the measurement of crude fat content in zootechnical experiments.

Purnomoadi et al. (1996), working with dairy cattle, used NIRS to predict the chemical composition of feces and to estimate digestibility based on a lignin indicator method. They compared three methods to determine feed digestibility: *in vivo* (digestibility trials), chemical analysis of the lignin indicator (LIGLAB), and NIRS prediction of the lignin indicator (LIGNIR). Separate calibrations equations to predict the concentration of lignin in the feed and in the fecal samples were developed.

The digestibility estimates with LIGNIR were similar to those obtained *in vivo*, however, the *in vivo* values were closer to the LIGLAB figures. Purnomoadi et al. (1996) suggested that the differences between the lignin indicator methods and the *in vivo* method was related to the fact that lignin was partially digestible. Purnomoadi et al. (1996) concluded that digestion estimation by LIGNIR shows the potential for individual and routine measurement on a practical farm basis, provided some correction factors or equations are devised to minimize the difference between *in vivo* and that estimated by LIGNIR.

The method employed by Purnomoadi et al. (1996) requires double NIRS calibration for both the feeds and the fecal samples, and a reliable indicator to estimate digestibility. NIRS has been used to predict the digestibility of diets by use of equations derived by regressing spectra derived from NIRS scanning of fecal samples against wet chemistry values of the diets consumed , i.e., prediction of primary products from secondary sources.

Lyons and Stuth (1992) successfully examined the feasibility of predicting diets of grazing cattle with NIRS fecal profiling. Data from two distinctively different sites was combined to formulate a robust equation, since the data range for the first site was limited. Fecal samples were derived from intact grazing animals and diet samples from esophageally fistulated animals. Digestibility was determined *in vitro* and corrected to *in vivo* values. Cows, at different physiological stages, and steers were used in the study.

Lyons and Stuth (1992) were able to successfully obtain predictive equations for DOM and CP, (R^2 = 0.80 and 0.92), respectively. A SEC (standard error of calibration) and SEV (standard error of validation) of 1.66 and 1.65 for DOM which were comparable to an SEL (standard laboratory error) of 1.68. A SEC and SEV of 0.89 and 0.92 were acceptable for an SEL of 0.44 for CP. No effects of physiological stage of animals on calibration were noted (Lyons and Stuth 1992). Lyons et al. (1993) found that supplementation did not affect prediction of the diet quality of the base range forage by fecal NIRS profiling if fecal sampling occurred 36 and 56 h after supplemental feeding had

ceased for CP and DOM, respectively. Lyons (1990) was unable to predict intake by NIRS fecal profiling using a marker (ytterbium acetate).

Application of fecal profiling equations developed by Lyons and Stuth (1992) for cattle were unsuccessful for goats, indicating that goat feces are biochemically different from cattle feces (Leite and Stuth 1995). Leite and Stuth (1995) suggested that the differences in digestive physiology and diet selection may result in spectral diversity precluding prediction across animal species. Leite and Stuth (1995) successfully formulated equations to predict CP and DOM in goat diets. Successful validation with data from two sites: the original site used to build the calibration equation and another distinctively different site, show the possibility of developing robust equations that can be used across agro-ecological zones. Showers et al. (1999) working with white tail deer, show the feasibility of using fecal NIRS profiling for predicting the nutritional status of wildlife, a break through that could have implications for wildlife management.

All the above studies were conducted in Texas locations with sub-tropical climates. Coates (1998) examined the application of fecal NIRS profiling for the tropical region of North Australia. Data from both grazing and stall experiments were used to formulate equations. Diversity in the grazed samples was achieved by sampling from different sites with different soil type, over different seasons through three years, and at different stages of plant growth (Coates 1998). Stall samples were derived from animals fed a range of diets. The study was also used to examine fundamental facets of the estimation of *in vivo* digestibility from

in vitro analysis of dietary forages and extrusa. Prediction of intake by NIRS scans of feces was also examined.

Coates (1998) found that stall experiments gave better calibration statistics than grazing experiments. He suggested that the difference may reflect the greater accuracy in derivation of reference values for stall experiments. However, a combination of stall and grazing experiments still gave satisfactory predictive ability. As with other authors (Lyons and Stuth 1992, Leite and Stuth 1995, and Showers et al. 1999), Coates (1998) found better predictive statistics for CP than DOM. Coates (1998) was better able to predict *in vitro* digestibility than *in vivo* in the stall experiments (R^2 = 0.97 compared to R^2= 0.89). He suggested that this is due to the fact that *in vivo* values incorporated animal variables and therefore made *in vivo* an inferior predictor of forage quality compared to *in vitro* digestibility which is a function of the feed only. While Coates (1998) had better statistics for prediction of intake by NIRS fecal profiling (R^2 = 0.79), compared to Lyons (1990) (R^2= 0.67), the most significant results were the successful prediction of digestible dry matter intake (DDMI), (R^2 = 0.89). DDMI (g/kg LW) integrates digestibility and intake to give a measure of digestible energy, which is closely related with animal performance (Coates 1998).

Coates (1998) in examining the relevance of using *in vitro* digestibility for estimating *in vivo* digestibility, found that neither *in vitro* values of feed nor extrusa were closely related to *in vivo* values (R^2 = 0.73 (extrusa)) compared to

(R^2 = 0.64 (feed)). These results indicate that estimates of *in vivo* digestibility derived from *in vitro* analysis of tropical forages may not be accurate (Coates 1998). While there were prospects for using NIRS to predict *in vivo* values (R^2 = 0.89), *in vivo* digestibility was also found not to be well correlated to forage intake and it was concluded that *in vivo* digestibility is not a good for predicting intake of tropical forages (Coates 1998). These findings may have implications both for the choice of which digestibility method to use in defining the nutritional status of tropical forages and for use in the development of NIRS calibration equations.

Preliminary tests on fecal material derived from cattle grazing in *Acacia senegal* and *Commiphera sp.* savanna rangelands in southern Kenya indicate the potential of the current cattle equation utilized in USA sub-tropical to temperate regions for use in SSA (Stuth, personal communication). The equations were, however, unable to predict some samples. This could indicate a probable spectral difference due to differences in species diversity and nutrient range (Westerhaus 1985a). The equations may need to be further assessed and a more robust set of equations for cattle developed to encompass the complexity of vegetation found across the different agro-ecological zones of SSA

Mixed species herds, comprising most frequently of cattle, sheep and goats, are a common risk management strategy in SSA. Coppock et al. (1986) noted that mixed species herds in aggregate provide a very broad opportunistic, and temporally stable trophic niche that results from equitable use of all forage

classes. Sheep comprise, by number 35% of the livestock in SSA, compared to 32% and 30% for cattle and goats, respectively (FAO 1989) and are therefore an important livestock species across all the agro-ecological of sub-Saharan Africa. No NIRS fecal profiling equation for sheep exists in literature. NIRS calibration equations to address nutritional profiling of this species are needed.

Windham et al. (1988), Roberts et al. (1993), and Goodchild et al. (1998) demonstrated that condensed tannins can be predicted in forages by NIRS. NIRS calibration is inherently dependent upon the accuracy and precision (standard laboratory error (SEL)) of, and validity of the reference method, but NIRS has been shown to give lower analytical errors within and among laboratories than is obtained with the reference method (Windham and Baron 1988). Therefore, while NIRS may inherently be limited by dependence on the reference method, it may solve the issue of standardization within and across labs, reduce cost of analysis, and provide a means for quick and simple analysis for management purposes. It may be possible to profile the tannin status of the diet of grazing animals by fecal NIRS profiling.

Hypotheses

1. NIRS fecal profiling can be used to predict the CP and DOM of forages consumed by free-ranging cattle and sheep in SSA.
2. NIRS fecal profiling can be used to predict the level of condensed tannins in the diet of sheep.

3. Robust NIRS fecal profiling equations can predict dietary CP and DOM of sheep and cattle across the agro-ecological zones of SSA.
4. Enhanced USA equations can predict dietary CP and DOM of sheep and cattle grazing rangeland in SSA.

CHAPTER III

METHODOLOGY: DEVELOPMENT OF ROBUST NIRS FECAL PROFILING EQUATIONS FOR MANAGEMENT PURPOSES IN SUB-SAHARAN AFRICA

Introduction

The method of near infrared reflectance spectroscopy (NIRS) fecal profiling to predict the diet of free-ranging livestock involves the utilization of a regression between diet reference data (wet chemistry values for the analyte) and the NIRS spectra data derived from scans of matching fecal samples. Viable predictive equations are developed through the processes of calibration and validation. This chapter briefly discusses the physics and chemistry of NIRS as a background to the method of NIRS fecal profiling, followed by the general methodology used in the calibration and validation processes. Descriptions of the agro-ecological zones (AEZ's), and the locations therein from which calibration and validation data were derived, are given as a background to the samples and data used in the development of NIRS fecal profiling equations for Sub-Saharan Africa (SSA).

The Physics and Chemistry of NIRS

NIRS is used both in a qualitative and quantitative manner to elucidate and quantify the chemical constituents of an organic substance. The basis of NIRS analysis is that constituents of the substance being scanned contain chemical bonds that absorb radiant energy at specific wavelengths commensurate with their properties (Windham and Baron 1988). Atoms or

molecules absorb radiation of frequency *v* if they can thereby gain a quantum of energy E, where E= h*v*, h being Planck's constant (Crooks 1978). The molecule may store energy by virtue of vibration of its bonds, the quantum size found to correspond to absorption around 10^{13} Hz, the infrared region (Crooks 1978, Osbourne and Fearn 1986). Absorption of energy from one vibrational level to an adjacent one results in a fundamental transition, which principally occurs in the mid infrared region (Crooks 1978, Ciurczak 1992). Weaker absorption's are observed when transitions occur from the ground state to a none-adjacent vibrational level resulting in overtones (Crooks 1978, Ciurczak 1992). The overtone frequency is slightly less than twice the fundamental, falling in the near infrared region. The absorption bands in the near infrared region are therefore the result of overtones or combinations of overtones originating in the fundamental mid-infrared region (Ciurczak 1992). The absorption spectrum of a chemical substance is thus a display of the fractional amount of radiation absorbed at each wavelength (Crooks 1978).

Qualitative Analysis

Qualitative analysis by NIRS is based on the fact that each of the chemical functional groups have bonds with near infrared absorption properties which can be used to differentiate one functional group from another (Norris 1985). In NIRS, Crooks (1978) noted that it is possible to consider a particular bond as vibrating independently of the rest of the molecule. Crooks (1978) explains this phenomenon: for example, the mass of the H atom is so much less

than the mass of the adjacent C atom in an organic molecule. Consequently, the adjacent C atom behaves as if it were the whole remainder of the molecule whose skeleton is formed of C atoms. The C-H vibration can thus be considered as separate from the other vibrations of the molecule. In other words, the C-H vibrations are not coupled to the others. Crooks (1978) noted that the C-C bonds, which form the skeleton of organic molecules, are closely coupled. The C=O bond, however, is more rigid than adjacent C-C bonds, so that C=O vibrations are separate from the rest of the molecule (Crooks 1978). An unknown substance is therefore identified by looking for group frequencies (signature wavelengths) and comparing with reference spectra. A group frequency is associated with a particular functional group and is signature to all molecules containing that group; wavelengths therefore have biological significance.

Quantitative Analysis: The Beer-Lambert Law

NIRS harnesses measurement of the intensity of absorption, as I/R (the inverse of reflectance) to quantify a particular chemical constituent of a substance (Crooks 1978). Quantitative analysis by NIRS is based on the Beer-Lambert Law, which assumes that all the molecules of the same type absorb radiation of a given frequency to the same extent (Crooks 1978). Doubling the concentration of the molecules will therefore double the amount of light absorbed (Crooks 1978). Likewise, each succeeding layer of the molecules will

absorb a proportional, though not equal amount of light, due to the amount of light absorbed by the preceding layer(s) (Crooks 1978).

If each layer is of thickness l_0, and there are n layers, the total path, l, through the sample is nl_0 (Crooks 1978). The intensity of light emerging from the ith layer is I_i, and is a fraction f of the intensity of radiation incident on that layer, equivalent to the radiation emerging from the (i-1)th (previous) layer, thus $I_i = fI_{(i-1)}$ (Crooks 1978). Therefore, the intensity of radiation transmitted by the sample as a whole is given by:

$$I_n = fI_{(n-1)} = f^2 I_{(n-2)} = \ldots = f^n I_0 \qquad \text{(Equation I) (Crooks 1978)}$$

I_0 being the intensity of the radiation incident on the sample. Hence:

$$\log_{10} (I_0/I_n) = n \log_{10} f \qquad \text{(Equation II) (Crooks 1978)}$$

When the concentration, c, is doubled, and the path length is halved, there is no change in the number of molecules in the path of radiation (Crooks 1978). Therefore, for a fixed path length, the variation of n with concentration is $n = c/c_0$ where c_0 is a standard concentration (Crooks 1978). In combination with the thickness of the layer l,

$$n = cl/c_0 l_0, \qquad \text{(Equation III) (Crooks 1978)}$$

therefore, substituting into equation II:

$$\log_{10} (I_0/I_n) = (cl/c_0 l_0) \log 10 f \qquad \text{(Equation IV) (Crooks 1978)}$$

Which may be rewritten as:

$$A = \epsilon cl \qquad \text{(Equation V) (Crooks 1978)}$$

the Beer-Lambert law, which states the variation of absorption with concentration at constant path length. Where:

A = absorbance which is equal to $\log_{10}(I_o/I_n)$

ϵ = absorbtivity (the extinction coefficient) = $(\log_{10} f)/c_o l_o$, ϵ is usually referred to have a standard concentration of c_o of one mol l^{-1}, and standard length l_o of 1 cm (Crooks 1978, Osbourne and Fearn 1986).

Because transmittance, T is I_n/I_o, the ratio of energy transmitted to the incident radiation, then A can be written as: A = log (1/T). In NIRS transmittance is negligible and thus reflectance R, is measured, therefore A = log (1/R), where R is the amount of reflectance emerging from a sample.

Calibration

NIRS fecal profiling is a methodology that utilizes the qualitative and quantitative properties of spectra generated from scans of fecal samples to predict the diet consumed. The practical application of this relationship requires the formulation of predictive equations, from a set of diet-fecal pairs that are representative of the population, through calibration and validation.

Calibration is conducted to select the best fitting equation with wavelength coefficients that best explain the variation in the constituent of interest in the sample set (Westerhaus 1985b). Calibration is achieved by regressing the spectrally derived values against the reference data. Modified stepwise multiple regression is used to estimate the equation coefficients and measure the goodness of fit (Westerhaus 1985b). At each step, the best fitting wavelength is

selected and rejected until no other improvements are made in explaining variation. Different math treatments (data transformations), such as running average smoothing, are used to reduce noise. First and second derivatives of the spectra are used to reduce the noise due to combination overtones that result in overlapping bands. Derivatives, especially second derivatives, present a clear separation between peaks that overlap substantially in the original spectra (Osbourne and Fearn 1986). Because the derivatives are linearly derived, they can be used quantitatively in the same way as the original spectra (Osbourne and Fearn 1986).

A number of criteria are used to select the best equations. These include an examination of the SEC (the Standard Error of Calibration) against the SEL (the Standard Laboratory Error; which is the error of repeatability of the reference method): the SEC describes how well the calibration samples fit the reference data (Westerhaus 1985b). The lower the SEC the better, however, a suitable equation with a SEC between one and two times twice the SEL is selected. R^2, the coefficient of determination, is another factor examined, equations that are selected have to meet the minimum requirement of an R^2 of 0.80 (Westerhaus 1985b). The F value of the coefficients are examined. The rule is to select F values over 10, indicative of a significant contribution of the wavelength to explanation of variation in the constituent (Westerhaus 1985b). Finally, the biological significance of the wavelengths of the selected equations is examined. In general first and second terms are of most importance, and

should reflect the constituent of interest. However, the wavelengths should be assessed to eliminate selection of equations with wavelengths that signify the presence of water (Westerhaus 1985b).

Validation

Validation of the calibration equations is conducted to assess the predictive ability and stability of the selected calibration equations. Validation entails prediction of an independently derived set of samples, with known reference values, using the selected calibration equations. A number of validation statistics are used to examine the predictive ability of the calibration equations. The t and H values of the residuals are examined: many validation samples with large t values would indicate that overfitting occurred, making the equation specific to those samples in the calibration set (Westerhaus 1985c, Mark 1992). The BIAS, which is the mean of the differences between the NIR predictions and the chemical analysis and estimates the systematic errors, is also examined. The standard deviation (S_D), known as the standard error of prediction (SEP), of the differences (D) between the NIR-measured and chemical analysis, which estimates the random errors is also examined: the rule is to select the equation with the smallest SEP (Westerhaus 1985c, Hruschka 1987). The final criterion is the slope of the regression relating the NIRS determinations to the reference values. Equations are selected for slopes tending towards 1.0 (Westerhaus 1985c).

Acquisition of the Fecal Samples and Dietary Data

Development of a robust NIRS fecal profiling calibration equation for management purposes is contingent upon using well matched diet-fecal samples that are representative of the heterogeneity (chemical, physical and botanical) in the population of interest (Abrams 1985). SSA has five major agro-ecological zones that are demarcated based on climatic factors and altitude (as it affects temperature) that have bearing on the type and class of, and thus the diversity of vegetation in each zone. Establishment of a data set that is representative of the heterogeneity across SSA, for the explicit purpose of formulating viable and robust calibration equations, would be costly in terms of the resources of time and money. However, the International Livestock Research Institute (ILRI), which has the mandate to improve livestock production across the agro-ecological zones of SSA, has such a sample set and database. ILRI has research centers located strategically to cater for the needs of the different major agro-ecological zones: semi-arid and arid - Sadore, Niger; highland - Debre Zeit, Ethiopia; and tropical and sub-tropical, Ibadan, Nigeria. Extensive livestock research by scientists at these locations has produced numerous well-matched diet-fecal samples that are carefully preserved for a number of years after the actual experiments. One of the strengths of NIRS is that once fecal samples are stabilized, (through timely collection to avoid contamination by exposure to elements of nature, proper moisture stabilization by drying at 60^0C, grinding and moisture controlled storage), the samples can be

used in future analyses (Lyons 1990). Data for the calibrations were taken from ILRI sample and data sets of the period from 1992 through 1997. Data from Nigeria and Ethiopia were all from stall trials therefore, no vegetation information is included in the site descriptions.

Description of the Sample Collection Locations

Calibration and validation samples and data were collected across three agro-ecological zones of SSA: semi-arid, humid, and highland. Agro-ecological classification is based on rainfall (quantity), and the length of the growing period (LGP), and attitude as it affects temperature. The LGP is defined as the period in days during the year when rainfed available soil moisture supply is greater than half the potential evapo-transpiration (PET) (Seré et al. 1995). The agro-ecological zones are therefore demarcated as: arid: LGP less than 75 days; semi-arid, LGP in the range 75-180 days; sub-humid: LGP in the range 180-270; and humid: LGP greater than 270 days (Seré et al. 1995). Highland areas, where altitude modifies climate, are defined by mean monthly temperatures of less than 20^0C during the growing season (Ehui et al. 1994, Seré et al. 1995).

The Semi-arid Zone

The semi-arid zone is characterized by rangelands of low and highly variable production, a reflection of climate; rainfall is between 500 and 1000mm (Ehui et al. 1994, Seré et al. 1995). Livestock keeping is the dominant form of land use, and 10% or less of the dry matter eaten by animals is provided by crop production (stubble, crop by-products or annual forage crops) (Seré et al. 1995).

Managing the production risk caused by the variability of feed availability is the central economic issue (Swinton 1988, Seré et al. 1995) and is factored into planning. Data for the semi-arid agro-ecological zone was collected in Niger ($11^0$33'N and $23^0$33'N), a typical Sahelian country dominated by the arid and semi arid zones. Twenty percent of export earnings are due to livestock products, second only to uranium. 29% of the Niger population is directly dependent on livestock for their livelihood (Seré et al. 1995).

The basic feature of Niger is massiveness and ruggedness (Sivakumar et al. 1993). Most soils are poor, sandy or clayey-sands (Sivakumar et al. 1993). In the semi-arid zone, between 14^0 and 18^0 N, the rainfall is concentrated in a short period of a maximum of 3 months and the long dry season is exaggerated by the drying continental winds (Sivakumar et al. 1993). The semi-arid zone gives way to the arid zone where there is little vegetation due to extreme aridity. Data collected was from ILRI experiments conducted at three locations: Sadore, Bundu and Toukounous

a. Sadore

The ILRI semi-arid and arid research center is located at Sadore, ($13^0$14'N, $2^0$16'E), 216 m above sea level, 20 km northeast of Niamey. Average rainfall is 575mm yr^{-1}(SD= 138), with a LGP of 109 days (variance 21.7 days) beginning in June and ending in September (Sivakumar et al. 1993). During the dry season between December and May, the mean temperature varies between 11 and 41^0C (Sivakumar et al. 1993). During the rainy season (June-

September), the temperature ranges between 21 and 39^0 C (Sivakumar et al. 1993). Potential evapo-transpiration ranges from 163.5 mm in December to 238.4mm in May. The main grasses are *Ctenium elegans, Pennisetum pedicellatum, Diheteropogon hagerupii* The main forbs are *Borreria stachydea* and *Hibiscus sabdariffa*, and the main legumes are *Guiera senegalensis* and *Cassia mimosoide* (Ayantunde 1998, Hiernaux, unpubl).

b. Toukounous

Some ILRI-Niger experiments were carried out at the government owned Toukounous Sahelian Experimental Station located 200 km north-east of Niamey and 20 km North of Filingué between 14^0 30'N 3^0 18' E. The station is situated in the Dallol Bosse valley at an altitude of about 200 m above sea level (Achard 1991). Average rainfall is 330.2 mm (SD=105mm), with a growing season of 76 days (variance of 24.9 days) starting in May and ending in the middle of September. The dominant vegetation species are annuals. In this area, there are differences between the vegetation of the dunary and interdunary zones: in the dunes, the herbaceous layer is dominated by the grasses *Schoenefeldia gracilis, Aristida mutabilis, Cenchrus biflorus* and *Dactyloctenium aegyptium.* Dominant leguminous shrub species include *Indigofera aspera, Indigofera secundiflora* and *Zornia glochidiala*. The dominant trees are *Balanites aegyptiaca* and *Macrua crassifolia*. In the interdunary areas, the dominant grass species is *Panicum lactum*; the dominant shrubs are *Sesbania leptocarpo*, and

Aeschynomene indica, and *Acacia radiana* is the most common tree (Achard 1991).

c. Bundu

Bundu is 60 km north-east of Niamey, close to Hamdalaye. It is located at approximately 14^0N, with an average rainfall of 400mm/year (SD 120mm). Major range grass species include *Aristida spp, Cenchrus biflorus,* and *Adnropogan gayanus*. Common leguminous species are *Cassia mimosoides, Jacaranda spp.* and *Zornia glochidiala* (Hiernaux unpubl.).

The Humid Zone

The humid zone is characterized by high temperatures and humidity, these conditions, and the prevalence of trypanosomiasis in some areas, make this zone a challenge for livestock (Ehui et al. 1994, Seré et al. 1995). Mixed farming systems dominate, in which livestock have multiple roles (Seré et al. 1995). However, the system is strongly based on rangeland as the main feed resource. Forage quality and quantity are more dependent on soils than on rainfall (Seré et al. 1995). Data for the humid zone was collected from Ibadan (4^0E, and 7^0N), Nigeria, average temperature 26^0C, with a range of 11 - 38^0C (IWS 1998). Average rainfall 1500 mm y^{-1}, LGP greater than 270 days (Atta-Krah 1989 and Seré et al. 1995).

The Highland Zone

The major common feature of the highland zone is the fact that cold temperatures during all or part of the year determine the predominant

vegetation, which is quite distinct from that of other tropical environments i.e. more C_3 plant species are prevalent in this zone than in other tropical zones (Seré et al. 1995). Livestock, which provide a range of services, are important but tend to be of secondary importance to cropping (Seré et al. 1995). Rangeland is the primary feed resource, but quality varies widely, crop residues may provide 10% or more of the livestock feed (Seré et al. 1995). There is a definite dry season, during which crop residues are the main feed resource. Data and samples for the highland zone were collected in Ethiopia at the ILRI research station in Debre-Zeit, found at 1850 m above sea level, temperature ranging from 5^0C to 34^0C, with an average rainfall of 713mm yr^{-1}. In Ethiopia, which owns about 16.9% of the livestock of SSA, livestock contribute 33% of the agricultural GDP and 15% of the total GDP.

CHAPTER IV

DEVELOPMENT OF A NUTRITIONAL PROFILING SYSTEM FOR FREE-RANGING SHEEP IN MAJOR AGRO-ECOLOGICAL ZONES OF SUB-SAHARAN AFRICA

Introduction

Sheep are an important livestock species in Sub-Saharan Africa (SSA) where they comprise 35% of the livestock population (FAO 1989). Diversification in terms of species type is a common strategy used by livestock owners to indirectly achieve environmental tracking and manipulation in a precarious environment (Coppock et al. 1986, Dyson-Hudson 1991, Niamir-Fuller 1998). While cattle, which are valued for their superior products such as milk, traction power, and a higher monetary value, would be the livestock of choice in more favorable conditions, episodic climatic variations demand that risk be mitigated by incorporation of smaller ruminants in livestock herds.

Mixed species herds are a common risk management strategy to ensure complementary forage utilization so as to optimize feed resources and minimize animal production losses (Coppock 1993). Sheep, like goats, are classified as intermediate feeders, demonstrating feeding habit flexibility although they are preferential grazers (Van Soest 1994). A grazing preference means that sheep overlap with cattle in their feeding habits, however, the flexibility of sheep allows them to utilize habitats that cattle are less adapted to (Migongo-Bake and Hansen 1987, Coppock 1993).

Survival of a reproductive stock is a very important consideration in livestock production in sub-Saharan Africa where livestock numbers are often depleted by episodic catastrophic events (Swinton 1988, Dyson-Hudson 1991). Sheep, which are small ruminants with a high reproductive rate, therefore play an essential role in building up livestock numbers after such devastating occurrences. This role is greatly emphasized in the arid and semi-arid regions where livelihood is dependent on livestock.

Most sheep in Sub-Saharan Africa are dependent on the rangelands, where the major challenge is to track the nutritional status of livestock so as to achieve timely mitigation of the environmental fluctuations. A suitable method to track the nutritional status of range based livestock needs to be client-orientated, reliable, easy to use, and accessible for use in remote regions. The methodology should also have early warning capabilities, and facilitate networking given that the use of rangelands in Sub-Saharan Africa cuts across internal and international borders. NIRS fecal profiling is a methodology that has demonstrated amenability to these requirements. Lyons and Stuth (1992), Leite and Stuth (1995), Coates (1998), and Showers et al. (1999) demonstrated the potential of NIRS fecal profiling for use in assessing the nutritional status of free-ranging ruminants. Equations to quantify leafy spurge (*Euphorbia esula* L) in the diet of sheep exist (Walker et al. 1998), but no NIRS fecal profiling equations to predict crude protein (CP) and digestible organic matter (DOM) in sheep diets

exist in the scientific literature. This chapter discusses the development of NIRS fecal profiling equations for sheep.

Currently, two separate NIRS fecal profiling equations for cattle are being used in the USA. The equations are based on plant diversity in the two major agro-ecological zones of the USA: the sub-tropical zone, where there is a greater proportion of C4 plants to C3 plants, and the temperate zone where the greater proportion of forage vegetation is C3 plants. SSA has five major agro-ecological zones (arid, semi-arid, sub-humid, humid and highland) with a differing vegetation diversity across zones. This chapter examines the possibility of developing robust CP and DOM equations that can be utilized across the agro-ecological zones of SSA.

Methodology

Development of a robust predictive NIRS fecal profiling equation entails calibration and validation before the equation can be used for management purposes. A fundamental statistical requirement of a robust equation is that the calibration set must be representative of the diversity in the population to be predicted. In order to adhere to this requirement a legacy set of fecal samples and matching dietary data (reference data) were obtained from the International Livestock Research Institute (ILRI) which has the mandate to conduct livestock research across the agro-ecological zones of Sub-Saharan Africa. Validation entails examining the ability of the calibration equation to predict a sample set

that was independently derived from the same population. The validation sample set was derived from an independently conducted digestibility trial.

The Calibration Process

Sample and Data Collection

Sheep fecal samples and matching dietary data were obtained from three stations of ILRI in different agro-ecological zones of SSA; semi-Arid: Niger; humid: Nigeria, and highlands: Ethiopia. Samples and data from Niger were obtained from experiments conducted at two locations:

(a) Sadore (216 m above sea level), length of growing period (LGP) 109 days, temperature range 11-41^{0}C, average rainfall 575 mm yr^{-1}.

(b) Bundu LGP 109 days, average rainfall 400 mm yr^{-1}.

All the samples from the highland zone of Ethiopia were obtained from experiments conducted at Debre-Zeit (1850 m above sea level), temperature range 5-34^{0}C, average rainfall 713 mm yr^{-1}. The fecal samples and matching dietary data for the humid zone of Nigeria were obtained from Ibadan: LGP more than 270 days, temp range 11-38^{0}C, mean temperature 26^{0}C, average rainfall 1500 mm yr^{-1}.

A total of 439 fecal samples with matching dietary data were obtained; 246 from Niger, 9 from Nigeria, and 184 from Ethiopia. Table 1 summaries the details of the samples obtained for CP and DOM. Appendix Table 1 summaries the publications, (where available), or the experimental

Table 1. Summary of the sheep CP and DOM calibration data: site of collection, year and month of experiment, experiment type, CP and DOM range and mean, and feed type.

Site	Experiment	Month[1]/ year of collection	N	Type of expt.	Variable	Range	Mean	Feed type
DZ	Umunna et. al. (1995)	1994	26	Stall/ *in vivo*	CP DOM	4.93-10.09 47.28-61.10	7.76 54.28	Basal: Oat hay and Oat straw. Supplements: *Lablab sp.*, Wheat middlings, *Sesbania sp., Chamaecytisus palmensis*
DZ	Nsahlai and Umunna (1996)	1995	18	Stall/ *in vivo*	CP DOM	7.93-13.95 51.06-55.61	10.42 53.27	Oat hay *ad libitum* with 250 g of lablab or *Sesbania sp.* with or without 91 g crushed maize grain.
DZ	Umunna et al. (Unpubl)	1995	24	Stall/ *in vivo*	CP DOM	10.81-18.22 48.33-58.15	15.36 54.36	Basal diets of Bird Resistant (BR) sorghum or Non-Bird Resistant (NBR) sorghum with or without supplements of various *Sesbania sp.* of varying tannin content
DZ	Boitumelo et al. (Unpubl)	1997	48	Stall/ *in vivo*	DOM	37.88-58.88	47.51	Basal diets of Bird Resistant (BR) or Non-Bird Resistant Sorghum with or without a supplement of various *Sesbania spp*
DZ	Kaitho et al. 1997	1996	68	Stall/ *in vivo*	CP DOM	4.65-10.89 46.96-79.12	8.75 58.66	Teff straw (*Eragrotis tef*), and teff straw with one of the supplements: *Leucaena leucocephala, L. pallida, Chamaecytisus palmensis*, and *Sesbania sesban*
IB	Larbi (unpubl)	1996	9	Stall/ *In vitro*	CP DOM	15.63-16.96 64.55-68.12	16.37 66.97	Sorghum supplemented with either shade or sun dried *Senna siamea*

Locations: DZ Debre Zeit, Ethiopia; IB Ibadan, Nigeria; SA Sadore, Niger; BU, Bundu, Niger. CP Crude Protein, DOM Digestible organic matter. [1] Month of collection is noted only for the grazing experiments that are directly affected by climate and vegetation characteristics at the time of the experiment. [a, b,] etc., experiments with the same letter are experiments that have the same protocol with trials conducted in consecutive years

Table 1. Continued.

Site	Experiment	Month[1]/ year of collection	N	Type of expt.	Variable	Range	Mean	Feed type
SA	Fernandez-Rivera et al. (unpubl)[a]	Nov. 1992	36	Grazing/ *in sacco*	CP DOM	8.25-16.22 23.63-39.33	14.43 30.81	Natural pasture, Sadore
SA	Fernandez-Rivera et al. (unpubl.) [a]	Oct 1993	48	Grazing/ *in sacco*	CP DOM	7.24-11.45 20.43-43.25	9.39 36.12	Native pasture, Sadore
BU	Sangaré & Fernandez-Rivera (unpubl.) [b]	Jul, Sept, Nov & Dec 1994	32	Grazing/ *in sacco*	CP DOM	15.20-22.11 43.86-48.45	17.83 45.23	Native pasture, Bundu
BU	Sangaré & Fernandez-Rivera (unpubl.) [b]	Jan, Apr, Jun, Jul, Sep, Oct, Dec 1995	56	Grazing/ *in sacco*	CP DOM	9.6-23.55 32.48-60.84	15.85 44.72	Native pasture, Bundu
BU	Sangaré & Fernandez-Rivera (unpubl.) [b]	Jan, & Apr 1996	24	Grazing/ *in sacco*	CP DOM	10.55-18 40.14-47.03	14.28 43.59	Native pasture, Bundu
SA	Fernandez-Rivera et al.	1994	12	Stall/ *in vivo*	CP DOM			Bush hay (*Eragrostis tremula*)
SA	Zouladeini et al. (unpubl.)	1993	9	Stall / *in vivo*	CP DOM	9.47-9.61 28.79-44.84	9.54 35.82	Various browse leaves from the tree species *Guiera senegalensis, Mitragyna inermis, Ficus gnaphalocarpa* and *Anogeissus leiocarpus.*
SA	Schlect (Unpubl.)	1995	24	Stall/ *in vivo*	CP DOM	11.10–18.74 53.00 – 2.63	14.92 57.82	Basal feeds: groundnut hay and cowpea hay with or with out supplements *Combretum aculeatum* and *Pterocarpus lucens*
SA	Fernandez-Rivera et al. 1994.	1994	5	Stall/ *in vivo*	CP DOM	4.65 42.95	4.65 42.95	Pearl millet stover leaves (*Pennisetum glaucum*)

protocol of the experiments from which the samples were obtained. Menz sheep were used in all the experiments conducted in Ethiopia. In Nigeria, West African Dwarf sheep were used, while in Niger, Oudah Pedlah sheep were used in all trials.

Sample Processing

Fecal samples were catalogued to ensure proper matching to dietary data (reference or wet chemistry data). Five grams of each sample was packed into a sealable plastic bag and shipped from the ILRI location in SSA via an irradiation facility in New Jersey (where the samples were exposed to two, 3-MRAD doses), to the Grazingland Animal Nutrition Lab (GANLab) at Texas A&M University. The fecal samples were dried overnight at 60^0C in a forced-air oven to remove moisture. The fecal samples were then re-ground, through a Udy cyclone mill to pass a 1-mm sieve, to ensure uniform particle size. NIRS is sensitive to moisture content (Lyons 1990), therefore to stabilize moisture content before scanning, the samples were re-dried in a forced air oven at 60^0C for 48 hours. The fecal samples were allowed to cool for one hour, in a dessicator, packed into NIRS sample cups with quartz windows, and scanned on a NIRSystems Inc. Model 6500 scanning reflectance monochromator, equipped with three tilting filters and a spinning sample cup, that reads reflectance in the range of 1100-2500 nm at 2 nm intervals. NIR spectra were stored in a microcomputer interfaced with the spectrophotometer, as log (1/R) values for the different wavelengths.

Reflectance data was regressed to the dietary (reference) data by multiple stepwise regression in which individual wavelengths were added to maximize R^2 (Walker et al. 1998). A maximum of 12 wavelength terms were selected to explain the variability in the reference data. Eleven math treatments were applied to the calibration process. The treatments used are explained by the expression (a, b, c, d) (Windham et al. 1988, Shenk et al 1992), where:

a = the derivative function i.e., a value of 0 being log 1/R values, values of 1 and 2 etc., first and second derivatives, respectively, and so on.

b = the gap between points used to calculate the derivative

c = the segment length over which the above function was smoothed

d = the segment length over which the smooth function was subject to a second smooth.

The treatments used are listed in Appendix Table 2. One or two iterations were conducted through the data set to eliminate outliers.

A number of criteria were used in selection of the equations. The SEC (standard error of calibration) was assessed in comparison to the SEL (the standard laboratory error). A SEC within twice the SEL is desirable. The SEL's were calculated according to the formula of Workman (1992). There were a number of experiments combined from three different laboratories, requiring a unique protocol to calculate the SEL for each group of equations. Repeated data are needed to calculate the SEL; this was not available for all the samples

Table 2. Rations used in the sheep validation trial: basal and supplementary feeds, and average CP and DOM values for the rations.

Treatments	Number of animals	Basal Feed	Supplement(s) Drymatter basis (grms)	CP	DOM (*in vivo*)
1	4	Oat hay	Maize grain 54.18 Lablab *hay (Lablab purpureus)* 126.12	10.27	58.65
2	4	Oat hay	Maize grain 54.18 *Sesbania sesban* 10865 118.30	10.46	59.10
3	4	Oat hay	Tagasaste (*Chamaecytisus palmensis*) 189.80	9.52	59.69
4	4	Oat hay	Wheat middlings 188.00	4.86	59.17
5	4	Maize (stover)-lablab stover	Lablab hay 150.99	9.28	50.90
6	4	Teff straw (*Eragrostis teff*)	*Sesbania sesban* 2024 183.94	8.15	59.19

In vivo digestibility was derived as the differential between the organic matter (OM), and dry matter (DM) in the feed and feces.

Five grams of each fecal sample was placed in a sealable plastic bag and shipped via an irradiation facility in New Jersey, USA, to the GANLab at Texas A&M University. The samples were prepared and scanned using the same procedure as for the calibration samples, and NIRS spectra for the validation samples stored in NIRS files.

The validation samples were predicted with the previously selected calibration equations. The performance of the calibration equations was assessed based on a number of criteria; in the order of: the BIAS, the SEP, the coefficient of correlation R^2, and the slope (close to 1).

Calibration and Validation Results

CP Calibration Results and Discussion

Table 3 presents the sheep CP calibration results. All the SEC's were within the limit stipulated in equation selection, i.e., within twice the SEL. The "Ethiopia and Nigeria CP" equation has the lowest SEL, and the "Ethiopia CP" the lowest SEC. The SEC, which is the standard deviation for the residuals due to differences between reference values and the NIR predicted values for samples within the calibration set, is an estimate of the theoretical best accuracy obtainable for a specified set of wavelength terms (Workman 1992). Inclusion of the Nigeria samples into the Ethiopia set, increased the SEC. The "Stall CP" SEC was lower than the "Extrusa CP" SEC value. The "Niger CP" equation, that

Table 3. The sheep CP calibration results: SEL, SEC, R^2, the three major wavelengths, and their associated chemical bonds

Equation	N	Treatment	SEL	SEC	R^2	# Terms	F	Wave Lengths	Associated Chemical Bonds
All Locations	317	1, 10, 5, 1	1.03	1.46	0.87	12	216.36	1916	Nitrites, H-O-H water, carbonyl bond
							193.82	2356	-CH protein bonds, -CH aromatic, -CH3 groups, -CH vibrations, =CH2 groups, -CH
							172.48	1972	nylon Nitrites, -OH phenol, -NH2 group, H-O-H water, carbonyl group, =CO terminal
Extrusa CP	136	1,5,5,1	1.38	1.54	0.88	8	387.53	1328	-CH aromatic, =CH2 terminal
							226.69	1760	-CH proteins, -CH aromatic, -CH vibration, =CH2 groups, -CH3 groups, =CH3 aliphatic
							189.31	1140	=CH2 groups, -CH vibrations, -CH-O-CH2 terminal, =CH2 terminal groups, -CH3 groups
Stall CP	134	2, 10, 10, 1	1.38	1.45	0.85	5	444.92	1388	-CH3=CH2
							230.15	1668	-CH vibration, =CH unsaturated, =CH2 terminal group, -CH aromatic, =CH2 groups, =CH2 terminal
							150.80	2420	-CH aromatic, -CH protein bonds, =CH2 groups, -CH nylon,

Table 3. Continued.

Equation	N	Treatment	SEL	SEC	R^2	# Terms	F	Wave Lengths	Associated Chemical Bonds
Ethiopia	103	2,20,10,1	0.60	0.81	0.93	8	334.41	1764	-CH proteins, -CH vibration, =CH2 terminal, -CH3 groups, =CH groups, =CH3 aliphatic
							101.25	1472	-OH phenols, -COOH carboxylic, -NH2 amines, primary amines
							71.32	2040	Nitrites, -NH2 groups, =NH amines+imides, -SH groups, -OH hydro peroxides
Ethiopia and Nigeria	98	1,15,5,1	0.58	1.01	0.88	4	312	1476	-OH phenols, -COOH carboxylic, -NH2 amines, primary amines
							263	1644	-CH vibrations, =CH2 terminal group, =CH2 terminal, -CH unsaturated,
							55	2332	-CH protein bonds, -CH3 aliphatic, -CH aromatic, -CH3 groups, -CH vibrations, =CH2 groups, -CH nylon
Niger	223	1,15,5,1	1.38	2.10	0.76	5	140.11	1312	-CH aromatics, =CH2 terminals
							108.92	2100	-OH alcohol+phenol, =NH amines+imides, -CH aldehydes, =CH2 terminal groups
							77.46	2348	-CH protein bonds, -CH3 aliphatic, -CH aromatic, -CH3 groups, -CH vibrations, =CH2 groups, -CH nylon

had more than half the samples determined from extrusa, had the highest SEC. Lyons and Stuth (1992) reported a SEC of 0.89 for an equation combining two locations (La Copita, and College-Station, Texas) and 0.88 for the College Station location alone. Showers et al. (1999) reported a SEC of 0.70 for a deer CP equation.

R^2, which determines the amount of variation in that data that is adequately modeled by the calibration equation, demonstrated a trend similar to that of SEC. The exception was a higher R^2 for the "Extrusa CP" equation in relation to that of the "Stall CP" equation. The "Ethiopia CP" equation was the only equation with a R^2 above 0.90, comparable to the results obtained by Lyons and Stuth (1992) for cattle equation combining two locations (0.92) and Showers et al. (1999) for deer (0.94). The "Niger CP" equation had a R^2 below 0.80 (0.76).

Wavelength assessment was conducted on the basis of two guidelines: firstly, biological significance, that is the selected wavelengths should indicate absorption by functional groups in the feces that are signatures of CP in the diet. Secondly, the wavelengths were examined in light of the derivative function, which guide's visual interpretation of spectra in relation to log (1/R) spectra, and thus interpretation of the coefficient sign. This latter guideline is important when comparing absorbance by fecal samples derived from diets with high CP against those of low CP content.

Feces are composed of undigested diet material (10-40%), made up almost entirely of cell wall constituents, metabolic matter (50-80%), and a small amount of endogenous secretions (10-15%) (Van Soest 1994). The metabolic component is largely microbial at the time of excretion (Van Soest 1994). Microbial matter consists of undigested cell walls of rumen microbes, and microbial cells from the cecum and large intestine (Merchen 1998). Mason (1969) noted that concentration of microbial matter, including nitrogen, in the ruminant feces is proportional to the quality of the diet. This would make the concentration of microbial matter in the feces an indicator from which diet quality can be predicted.

Bonds of functional groups that are indicative of microbial activity include among others amino groups and nitrites (Merchen 1988), and carbonyls, phenols and methyl groups found in the indigestible cell wall of microbes (Van Soest 1994). Mason (1969) reported that the amino acid profile of feces as a whole was very similar to that of isolated gastro-intestinal microbes. Nitrites are the reduction product of nitrates, which sometimes occur at high concentrations in forage (Emerick 1988). Nitrates are known to accumulate in plants under the influence of various factors, especially those that limit growth of plants, the most important being drought (Emerick 1988). However, the conversion of nitrates to nitrites may also occur in the rumen through microbial reduction of nitrate, which may be used as a source of nitrogen for protein synthesis (Emerick 1988).

Virtanen (1966), Mason (1969), Wofford et al. (1985) and Van Soest (1994) assert that nearly all nitrogen in ruminant feces is of microbial origin and very little is from feed. However, this assertion may not hold true when livestock consume diets containing high amounts of tannins. Tannins, which bind plant protein, have a confounding influence on fecal nitrogen through reduced digestibility. Protein-binding phenolics can cause excess forage protein to be defecated. Many of the diets from which the fecal samples used in the calibration were derived, contained a browse component, fed either as a supplement, or accessed by the sheep grazing natural pasture. Aromatic, phenolic and alcoholic bonds indicate anti-nutritive factors including tannins and lignin (Fahey and Berger 1988). Carbon-hydrogen bonds and nitrogen-hydrogen bonds protein bonds may indicate the presence of undigested protein, characteristic of lignin-protein and or tannin-protein complexes. These protein bonds may also indicate undigested or whole microbial cells. Wavelength significance is discussed in light of these observations.

Visual analysis of spectra provides clues of absorption peak interpretation. However it is difficult to visually interpret first derivative spectra because band peaks and valleys do not follow the log (1/R) spectral pattern (Shenk et al. 1992). Second order spectra are more visually interpretable. Although peaks are inverted in relation to those of log (1/R) spectra, the pattern is maintained relative to the log (1/R) spectra (Shenk 1992). Band resolution in the second derivative is also enhanced so that overlap is reduced, and negative

coefficients signify greater absorption in the second derivative (Shenk 1992). Visual interpretation, to compare the absorbencies of spectra of samples from high and low CP diets will be conducted only for the "Stall CP", and the "Ethiopia CP" equations that are second derivative equations. A general overview of the significance of the selected wavelengths in all the equations is given, followed by an assessment of the two major wavelengths of each equation.

There are three major absorption bands for proteins, 1536 –1614 nm for –NH proteins, 1716-1764, and 2276-2492 indicative of –CH proteins; the latter band being the widest of the three. All the CP equations have at least one wavelength within these bands; the "Extrusa CP", and "Ethiopia CP" in the 1716-1764 band, all the others in the 2276-2492 nm band. All the equations have wavelengths indicating –CH aromatic, and –OH phenol bonds which are indicators of anti-nutritive quality factors including tannins. Some of the equations indicate nitrites and amino groups, indicative of microbial activity.

"All locations CP" Equation

The best wavelength of 1916nm is indicative of nitrites, H-O-H (water), and carbonyl bonds. Showers et al. (1999) reported a wavelength of 1972 nm that is in the same band as 1916 nm, for their deer CP equation. Carbonyl bonds are the characteristic functional group of aldehydes , carboxylic acids, derivatives of carboxylic acids including esters and amides, and ketones (Gessiman 1977). Rumen microbes metabolize dietary protein through hydrolysis into amino acids, which they use for protein synthesis (Van Soest

1994). Amino acids in excess of microbial needs are oxidatively deaminated to ammonia and carboxylic acids (Van Soest 1994).

The second major wavelength, 2356 nm, is indicative of CH proteins, aromatic bonds and various carbon-hydrogen bonds. These bonds are indicative of the presence of anti-nutritive factors, undigested forage protein, and or microbial activity (whole microbial cells). The combination of protein and aromatic bonds may indicate a tannin-protein complex. Williams and Norris (1987) report 2355 to be highly significant for cellulose, and 2345 nm for protein. Purnomoadi et al.(1995) reported a wavelength of 2396 nm in their protein equation, which falls in the same band as 2356 nm.

The indication of H-O-H water bonds by the first wavelength in this equation indicates susceptibility to moisture control. However, water is not indicated in any of the other equations, which were all derived using the same spectral data. The third major wavelength, 1972 nm, also indicates water, however, Williams and Norris (1987) noted that 1972 nm is one of the most significant wavelengths for protein.

"Extrusa CP" Equation

1328 nm is indicative of carbon-hydrogen aromatic bonds and carbon-hydrogen double terminal groups. The presence of aromatics could indicate anti-nutritive factors such as lignins and tannins. All the extrusa samples were derived from grazing experiments in which sheep were exposed to rangeland with a browse component. The second major wavelength, 1760 nm, is indicative

of carbon-hydrogen proteins, and carbon-hydrogen aromatic bonds, among other carbon-hydrogen functional groups. The former group is indicative of undigested protein and the latter of anti-nutritive factors, such as tannins and lignins. A combination of these groups could indicate tannin-protein or lignin-protein complexes, thus supporting the interpretation of the major wavelength.

"Stall CP" Equation

The major wavelength of 1388 nm is indicative of –CH3=CH2. Showers et al. (1999) reported absorption by the deer CP equation at 1344 nm, which is banded together with 1388 nm. Carbon-carbon double bonds are associated with protein, starch and cellulose. The second wavelength of 1668 nm is indicative of CH aromatic bonds indicative of anti-nutritive factors, and various carbon-hydrogen groups that may signify proteins and carbohydrates. The aromatic bonds are indicative of anti-nutritive factors.

"Ethiopia CP" Equation

The major wavelength of 1764 nm is indicative of carbon-hydrogen proteins, carbon-hydrogen groups and CH3 aliphatics. The second wavelength of 1472 nm is indicative of OH phenols, -NH amines, primary amines and carboxylic groups. OH phenols are indicative of the hydroxyl function (Hill and Rendell 1975) in the structure of anti-nutritive factors such as tannins and lignin. Carboxylic groups, NH2 amines, and primary amines indicate microbial activity, and or a forage source high in amines .

"Ethiopia and Nigeria CP" Equation

The major wavelength of 1476 nm is indicative of OH phenols, -COOH carboxylic groups and amine groups. The OH groups are indicative of the hydroxyl function, and suggest presence of anti-nutritive factors. The carboxylic groups are indicative of amino groups, which together with the amine groups indicate microbial activity.

The second major wavelength 1644 nm, is indicative of various carbon-hydrogen bonds which are generally related to protein, starch and cellulose, whose presence indicates undigested material in the feces, probably due to binding by anti-nutritive factors.

"Niger CP" Equation

The major wavelength of 1312 nm is indicative of carbon-hydrogen aromatics, and CH2 terminal groups. The aromatic bonds are associated with anti-nutritive quality factors such as the polyphenolic lignins and tannins (Hill and Rendell 1975). The CH2 bonds are associated with starch, protein and cellulose which may be bound by anti-nutritive factors. The second major wavelength of 2100 nm indicates – OH alcohol+phenol bonds associated with anti-nutritive factors. Goodchild et al. (1998) identified 2150 nm, which is banded together with 2100 nm, as a wavelength that was consistently related to condensed tannins. Other functional groups indicated by 2100 nm include = NH amines+imides, -CH aldehydes, and = CH2 terminal groups. Amines and aldehydes are products of oxidative deamination of amino acids in excess of the

needs for protein synthesis by microbes (Van Soest 1994, Gessiman 1977). The indication of both protein and anti-nutritive factors may denote that this equation infers the presence of tannin-protein complexes.

CP Validation Results and Discussion

CP validation results are presented in Table 4. The "All locations CP" equation had the lowest BIAS and the "Niger CP" equation the highest BIAS, over estimating the validation samples by a mean of 2.869.

The SEP of the "All locations CP" equation was the lowest, followed by that of the "Ethiopia and Nigeria CP" equation, the "Stall CP", the "Extrusa CP", and the "Niger CP" equation. The "Ethiopia CP" equation had the highest SEP.

The "All locations CP" equation had the best R^2, 0.88, which was the only R^2 above 0.80 required for predictive purposes; this equation also has the best slope (0.915). The second best R^2 was that of the "Ethiopia CP" equation (R^2 0.52), which had the only negative slope (-0.577). The "Ethiopia and Nigeria CP" equation and the "Extrusa CP" equation had similar R^2's (0.46 and 0.48) respectively, but very different slopes (0.299 and 0.666) respectively. The "Stall CP" equation had the poorest R^2 performance.

In general, the "All locations CP" equation had the best over all performance. The performance of the "Ethiopia CP" equation is weaken by the high SEP; which is mitigated in the "Ethiopia and Nigeria CP" equation by the inclusion of the Nigeria samples. The "Extrusa CP", "Stall CP" and "Niger CP" equations generally performed poorly. The fact that addition of the Niger

Table 4. The sheep CP validation results: the BIAS, SEP, R^2, and slope of the different CP equations

Equation	[1]n	Math	BIAS	SEP	R^2	Slope
All locations	24	1,10,5,1	0.497	0.853	0.88	0.915
Extrusa CP	24	1,5,5,1	0.543	3.424	0.48	0.299
Stall CP	24	2,10,10,1	-0.872	2.165	0.11	0.433
Ethiopia only	24	2,20,10,1	-0.910	4.090	0.52	-0.577
Ethiopia and Nigeria	24	1,15,5,1	-1.501	2.155	0.46	0.666
Niger	24	1,15,5,1	-2.869	3.445	0.30	0.501

[1]n is the number of samples in the validation set

equations to the "Ethiopia and Nigeria CP" equation greatly improved performance, suggests that the Niger samples added variability that strengthened the "Ethiopia and Nigeria CP" calibration set. The performance of the "All Locations CP" equation in predicting the validation set is illustrated in Figure 1.

DOM Calibration Results and Discussion

DOM calibration results are presented in Table 5. The "Ethiopia DOM" and the "Ethiopia and Nigeria DOM" SEC's were within twice their respective SEL's. The "Ethiopia DOM" equation had the lowest SEC (1.55), which was lower than those reported for cattle DOM equations by Lyons and Stuth (1992) 1.75 and 1.66 for a single location (La Copita, Texas) and two locations (La Copita and College-Station, Texas), respectively, and lower than 2.64 reported by Showers et al. (1999) for a deer DOM equation. The "*In vitro* DOM" equation had the highest SEC (3.39). All the equations had R^2 above 0.80, with the "Ethiopia DOM" equation performing the best, R^2 0.92.

Wavelength assessment was conducted using the same guidelines applied to the CP calibrations i.e., on the basis of biological significance, visual interpretation of spectra, and interpretation of the coefficient sign. All the equations except the "*In vitro* DOM" are second derivative equations. Inference of wavelengths that are of biological significance in indicating DOM in the diet is not as straightforward as for CP. DOM is a term that encompasses all the feed components that are digested, the feces are technically the balance. Feces are

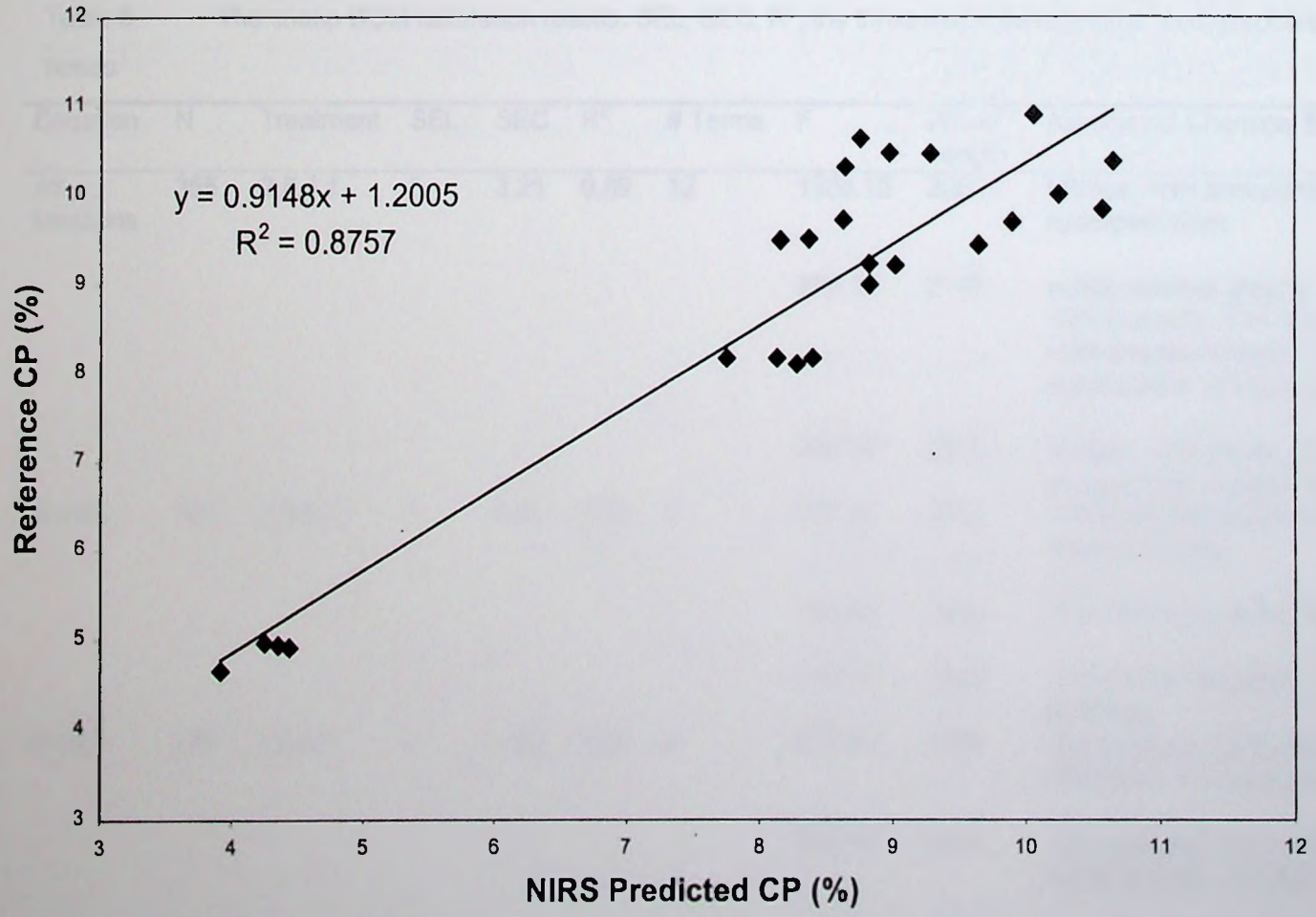


Figure 1. Reference CP (%) of the validation set against that predicted by the "All Locations CP" NIRS equation

Table 5. The sheep DOM calibration results: SEL, SEC, R^2, the three major wavelengths, and associated chemical bonds

Equation	N	Treatment	SEL	SEC	R^2	# Terms	F	Wave Length	Associated Chemical Bonds
All locations	355	2,8,4,1	*	3.21	0.89	12	1338.15	2060	Nitrites, =NH amines+imides, -OH hydroperoxides
							372.19	2140	=CH2 terminal groups, -CH-CH groups, -CH aromatic, -OH alcohol+phenol, =NH amines+imides, =CH,=CH2 unsaturated, =CH2 groups
							235.74	2016	Nitrites, -OH phenol, -NH2 groups, -SH groups, =NH amines+imides
In vitro	155	2,8,6,1	*	3.39	0.83	9	157.47	2052	-OH hydroperoxides, Nitrites, =NH amines+imides
							150.84	1844	HCL hydrogen acids, Nitrites
							150.71	1364	-CH-O-CH2 terminals, -CH-CH2-CH2 terminals
In vivo	179	1,4,4,1	*	1.82	0.89	9	277.87	1700	-CH aromatic, CH2 groups, -CH vibrations, =CH groups, CH2 terminal
							229.10	2416	-CH aromatic, -CH protein bonds, =CH2 groups, -CH nylon, -CH aromatic
							112.06	2388	-CH vibration, CH2 groups, -CH aromatic, -CH protein bonds, =CH2 groups, -CH nylon

* insufficient data to calculate this variable

Table 5. Continued.

Equation	N	Treatment	SEL	SEC	R^2	# Terms	F	Wave Length	Associated Chemical Bonds
Ethiopia	104	2,10,10,1	0.91	1.55	0.92	3	810.55	1536	-NH2 amines, primary amines, secondary amines, =CH terminal, =NH groups, =NH proteins
							233.73	2044	-OH hydroperoxides, nitrites, -NH2 groups, =NH amines+imides
							35.94	2232	=CH,=CH2, =CH groups, =C=O esters, -CH vibrations
Ethiopia and Nigeria	113	2,8,6,1	0.89	1.89	0.87	4	635.41	1536	-NH2 amines, primary amines, secondary amines, =CH terminal, =NH groups, =NH proteins
							127.66	2028	Nitrites, -OH phenol, -NH2 groups, -SH groups, =NH amines+imides
							20.51	2396	-CH vibrations, =CH2 groups, -CH aromatics, -CH proteins, -CH nylon
Niger	189	2,10,10,1	*	3.16	0.89	11	271.61	2056	-OH hydroperoxides, nitrites, =NH amines+imides
							140.12	1708	-CH vibration, =CH unsaturated, -CH aromatic, =CH2 groups, =CH2 aliphatic
							106.13	1540	Primary amines, secondary amines, =CH terminal, =NH groups, =NH proteins

composed of, between 10 - 40 %, of undigested feed material, 50 - 85 % metabolic material (of which 85 - 90% is of microbial origin), and 10 - 15% material of endogenous origin (Van Soest 1994). Inferences of the level of DOM in the diet can thus be drawn from the signatures of undigested forage and from microbial material that dominate feces. The indigestible and poorly or incompletely digested fed components includes lignin, cellulose, hemi-cellulose and tannin-protein complexes. Lignin, a polyphenolic compound, is indicated by phenolic, alcoholic, and aromatic functional groups among others. Tannin complexes are indicated by phenolic, aromatic, and protein bonds.

Microbial activity is also indicative of DOM. Mason (1969) noted that the concentration of microbial matter in ruminant feces increased as the quality of the diet improves. Lyons and Stuth (1992) suggested that greater absorbance by fecal samples derived from ruminants feed high quality diets, is a reflection of bonds in undigested microbial cells due to the proliferation of microbial populations. Some bonds associated with microbial activity are amino acid groups and nitrites, and in the indigestible cell wall, carbonyls, phenols, and methyl groups (Merchen 1988, Van Soest 1994)

In all the equations with second derivative math, all the two major wavelengths had negative coefficients, indicating greater absorption at those wavelengths.

"All locations DOM" Equation

The major wavelength, 2060 nm, is indicative of amino groups and nitrites, indicating microbial activity. The second major wavelength of 2140 nm indicating carbon-hydrogen aromatic bonds and OH alcohol+phenol bonds, which denote the presence of anti-nutritive factors like lignin and tannins. However, this second wavelength is of far less significance as compared to the major wavelength: F statistic 372.19 against an F statistic of 1338 for the major wavelength. Williams and Norris (1987) indicate 2055 nm as a very significant wavelength for protein, and 2140 nm as strongly significant for protein. Goodchild et al. (1998) report 2150 nm as highly significant for condensed tannins.

"In vitro DOM" Equation

Nitrites are indicated by both the major wavelengths of the "In vitro DOM" equation i.e., 2052 nm and 1844 nm. Nitrites are reduction products of nitrates which sometimes occur at high concentrations in forage (Emerick 1988). The conversion of nitrates to nitrites may also occur in the rumen from microbial reduction of nitrate, which may be used as a source of nitrogen from which protein is synthesized (Emerick 1988). Williams and Norris (1987) indicate 2055 nm, which is close to 2052nm, as a very significant wavelength for proteins.

"In vivo DOM " Equation

The "*in vivo DOM*" equation is the only first derivative equation. Absorbance at 1700 nm, the major wavelength, indicates carbon-hydrogen

aromatic bonds, denoting anti-nutritive factors, and various carbon-hydrogen groups, which may indicate undigested carbohydrates and protein. Showers et al. (1999) reported a second derivative DOM equation with greater absorption for the lower quality samples at 1656 nm. A cursory appraisal of the spectra of high quality and low quality samples showed that the lower quality sample absorbed more than the high quality sample (i.e., had greater valleys), an observation that collaborates the findings of Showers et al. (1999) working with a second derivative equation. The second major wavelength, 2416 nm, indicates a combination of carbon-hydrogen aromatic bonds and carbon-hydrogen protein bonds, possibly denoting the presence anti-nutritive factors and undigested protein, which could indicate tannin-protein complexes.

"Ethiopia DOM" and "Ethiopia and Nigeria DOM" Equations

The "Ethiopia DOM" and the "Ethiopia and Nigeria DOM" equations have similar major wavelengths, 1536 nm and 2044 nm for Ethiopia, and 1536 nm and 2028 nm for Ethiopia and Nigeria. The major and second wavelengths of both equations indicate similar functional groups i.e., nitrites, various amine groups, nitrogen-hydrogen groups and protein. Williams and Norris (1987) report that 2055 nm, which is banded with 2028nm and 2044 nm, as very highly significant for protein.

"Niger DOM" Equation

The major wavelength, 2056 nm is indicative of nitrites, NH amines+imides, which denote microbial activity. Williams and Norris (1987)

indicate that 2055 nm is highly significant for proteins. The second wavelength, 1708 nm indicates CH aromatics, and CH2 aliphatic groups denoting the presence of anti-nutritive factors. The second wavelength could indicate the presence of a browse component in the diet of Niger sheep.

Williams and Norris (1987) indicate 2055 nm as highly indicative of proteins, wavelengths close to 2055 nm, are present in all the equations except the "*In vivo DOM*" equation. The "*In vivo* DOM" equation was the only first derivative equation. In general, the equations except the "All locations", Niger, and "*In vivo* DOM", did not indicate absorption by anti-nutritive factors associated with undigested material. In the "All locations" equation, the significance of the wavelength indicating anti-nutritive factors was greatly over-shadowed by the major wavelength indicating microbial activity. This indicates that the selected wavelengths are representative of DOM. The greater absorption by the higher quality samples at the major wavelengths, substantiates that the wavelengths may be diagnostic of DOM.

DOM Validation Results and Discussion

The DOM validation results are presented in Table 6. The DOM equations performed poorly compared to the CP equations. The "Ethiopia and Nigeria DOM" equation had the lowest BIAS, followed by the "All locations DOM" equation. The "Ethiopia DOM", "*In vitro* DOM," and "*In vivo* DOM" equations have a BIAS of greater than 3.00. All equations had high SEP's, between 4.774 for "Ethiopia and Nigeria DOM", and 9.826 for "*In vitro* DOM". The best R^2 and

Table 6. The sheep DOM validation results: the BIAS, SEP, R^2, and slope of the different DOM equations

Equation	N[1]	Math	BIAS	SEP	R^2	Slope
All locations	24	2,8,4,1	0.422	6.250	0.21	0.489
In vitro	24	2,8,6,1	-3.608	9.826	0.11	0.226
In vivo	24	1,5,5,1	3.814	6.724	0.20	0.846
Ethiopia	24	2,10,10,1	3.538	6.232	0.31	1.025
Ethiopia and Nigeria	24	2,8,6,1	-0.281	4.774	0.41	0.950
Niger	24	2,10,10,1	0.702	9.110	0.07	0.193

[1] N is the number of samples that were in the validation set

slope were those for the "Ethiopia and Nigeria DOM" equation, which out performed the "Ethiopia DOM" equation R^2 (0.41 and 0.31) and slope (0.950 and 1.025), respectively. The predictive performance of the "Ethiopia and Nigeria DOM" in predicting the validation samples is illustrated in Figure 2. The inclusion of 9 Nigeria samples increased the predictive capacity of the Ethiopia calibration set. The Niger only equation had the poorest overall performance in terms of SEP, R^2, and slope.

As in the CP equations, the addition of Nigeria samples added variability to the "Ethiopia DOM" equation, which enhanced its predictive capacity. However, unlike CP, addition of Niger samples did not enhance the "Ethiopia and Nigeria DOM" equation further. In predicting the validation set with the "All Locations DOM" equation, all the validation samples were indicated as global H outliers, signifying that the validation samples are spectrally different from the calibration set. Inclusion of the validation set into the "All locations" calibration equation, and subsequent prediction of the validation set, a standard exploratory procedure, did not improve the predictive capacity of the "All locations DOM" equation. Inclusion of samples from an experiment that was originally included in the calibration, and had a wide DOM diversity (low to high DOM values), improved the validation statistics of the "All locations" DOM equation: (before: 6.250, 0.21 and 0.49) and (after: 5.167, 0.52 and 0.90) for SEP, R^2 and slope respectively.

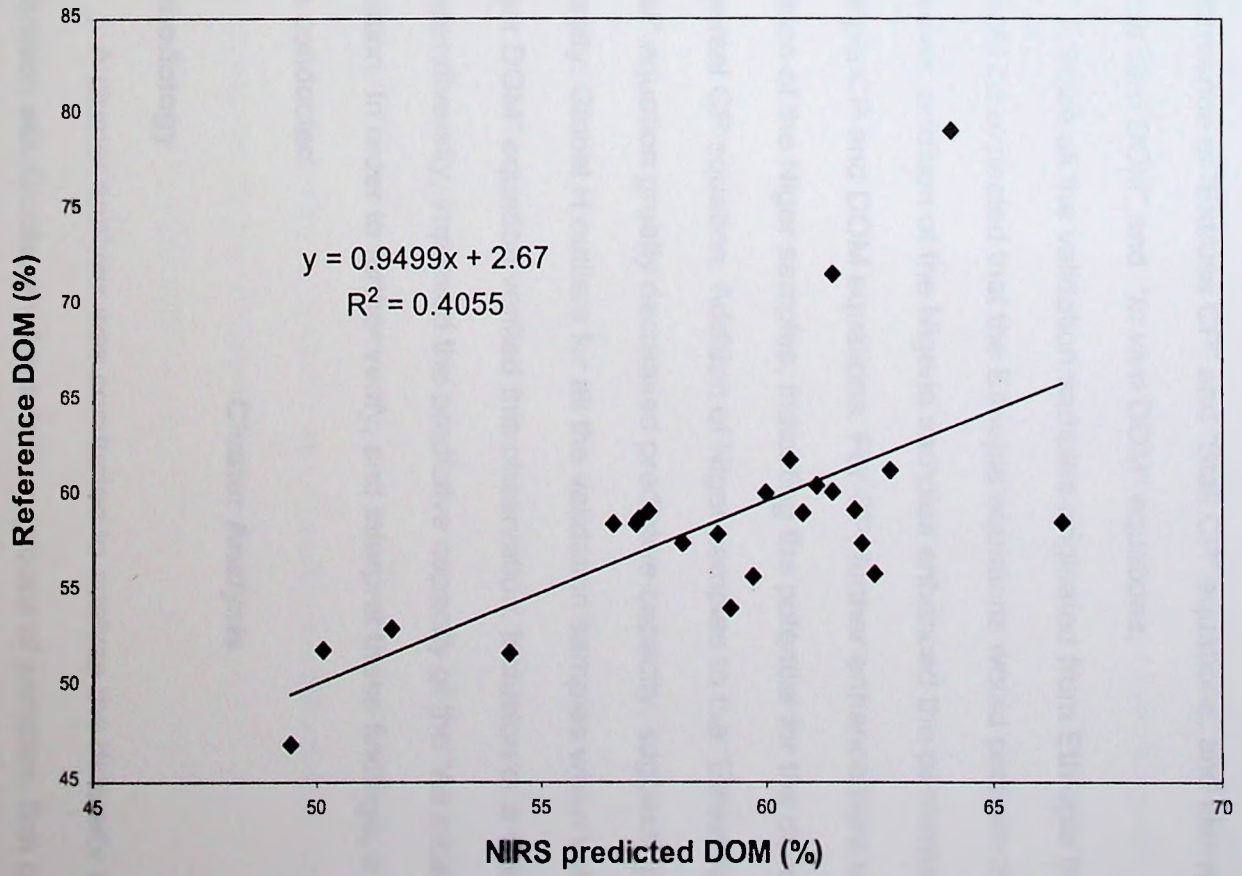


Figure 2. Reference DOM (%) of the validation set against that predicted by the "Ethiopia and Nigeria DOM" NIRS equation

Conclusion

In concurrence with the findings of Lyons and Stuth (1992), Showers et al. (1999) and Coates (1998), CP equations in general exhibited better calibration and validation statistics. There was apparently no difference in the performance of "Extrusa CP" and "Stall CP" equations, and like wise between the "*In vitro* DOM" and "*In vivo* DOM" equations.

Since all the validation samples originated from Ethiopia (highland zone), it would be expected that the Ethiopia equations would perform the best. However, addition of the Nigeria samples enhanced the performance of both the Ethiopia CP and DOM equations. For CP, further enhancement was achieved by addition of the Niger samples, indicating the potential for the development of a universal CP equation. Addition of Niger samples to the "Ethiopia and Nigeria DOM" equation greatly decreased predictive capacity, suggesting spectral diversity. Global H outliers for all the validation samples when validated by the "Niger DOM" equation verified this observation. Inclusion of a validation set with a wider diversity, improved the predictive capacity of the "All locations DOM" equation. In order to further verify, and interpret these findings, a cluster analysis was conducted.

Cluster Analysis

Methodology

A cluster analysis was conducted to analyze the diversity in the calibration set. Cluster analysis defines groups of samples, first on the basis of

similarities or homogeneity in respect to specified variables, and secondly, by difference from other groups in respect to those same variables (McCune and Mefford 1995, Sharma 1995). Of the 436 samples used in the sheep CP and DOM calibrations, 117 had only one constituent, i.e., either only CP or DOM and so were not included in the cluster analysis as both CP and DOM were designated as clustering variables. Of the 346 samples included in the cluster analysis, 204 were from Niger, 113 from Ethiopia, 5 from Nigeria. The 24 validation samples were also included in the cluster analysis.

The software PC-Ord (version 2) by MjM Software Design, software for multivariate analysis of ecological data (McCune and Mefford 1995), was used to cluster the sample set. Data was formatted in a Lotus-1-2-3 spreadsheet, and imported into PC-Ord. Three variables were used: CP and DOM, which were entered as quantitative variables, and location as a categorical variable. Each sample was also given a row name; the calibration samples were identified by the location followed by the number i.e., all Niger samples had NIG and a number, all Nigeria had Nii and a number, and all Ethiopia had ETH and a number, the validation samples were identified by VAL and a number .

The measure of similarity used was squared Euclidean distance between samples given by the formula (Sharma 1995):

$$D^2_{ij} = \Sigma^{p}_{k=1} (x_{ik} - x_{jk})^2$$

Where D^2_{ij} is the squared distance between samples *i* and *j*,

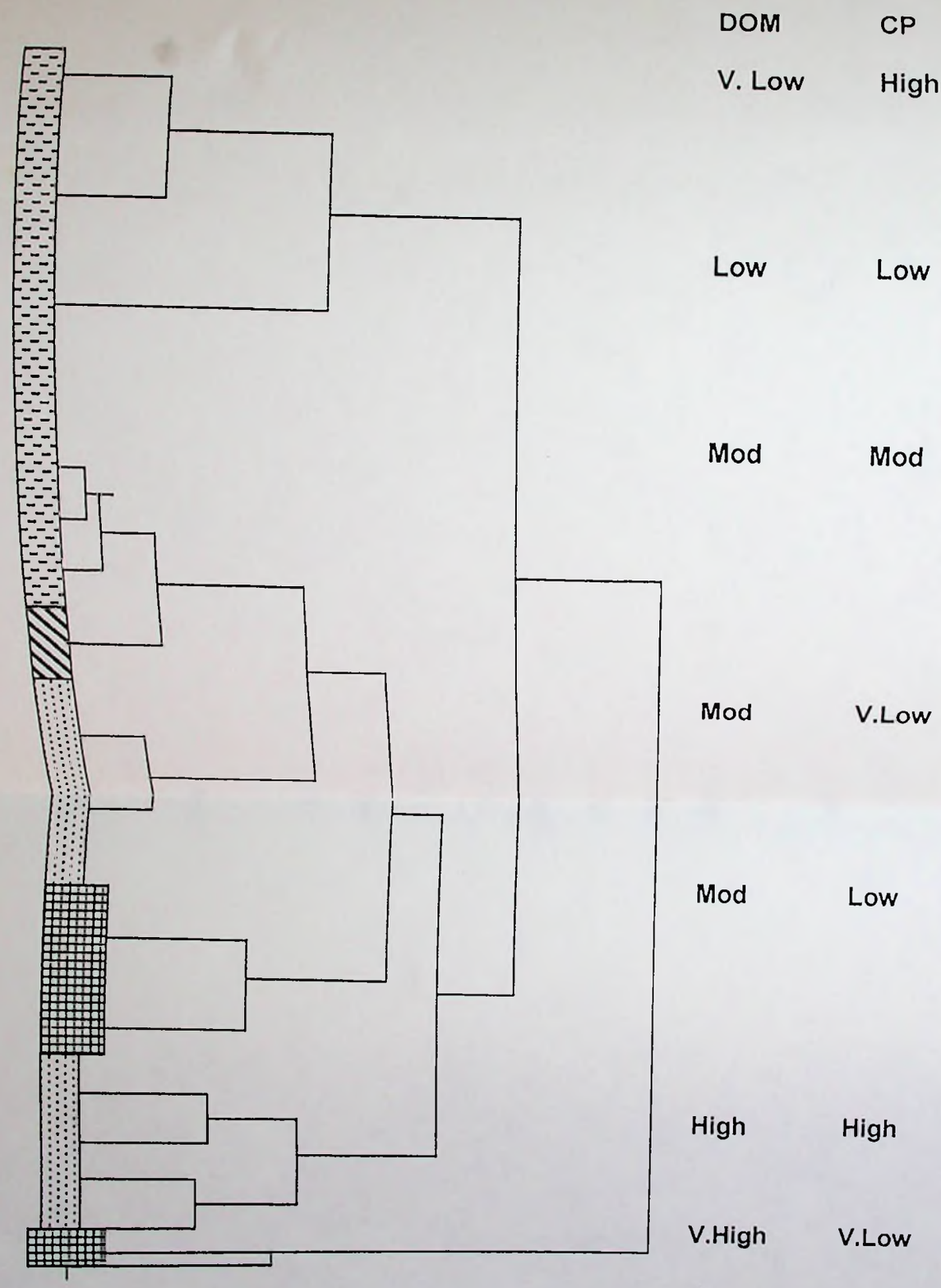
X_{ij} is the value of the *k*th variable for the *i*th subject

X_{jk} is the value of the *k*th variable for the *j*th sample,

p is the number of variables

With the squared Euclidean algorithm, the more similar the samples, the smaller the distance between then and vice versa (Sharma 1995). Hierarchical cluster analysis was used, in which larger clusters were composed of smaller clusters (McCune and Mefford 1995). To link the groups, the unweighted Centroid method was employed, in which each group was replaced by an average sample, the centroid of that group (Sharma 1995). The groups, that had the smallest distance between them, were then clustered together in the next hierarchy, and so on till the whole sample set formed one group. The result of the cluster analysis was displayed as a dendogram, in which the single-width spacing was used to produce a compact dendogram given the large number of samples, nevertheless the full dendogram is too long to present here. Figure 3 presents an outline illustrating the salient features of the dendogram.

In order to facilitate interpretation of the dendogram, the quantitative variables were categorized. A dietary CP value of 7% is known to be limiting (Mason 1969). However, categorization of diets with higher CP values is not well defined in the scientific literature. Neither is there a clear definition of the DOM categories in the scientific literature. The method of using quartiles was employed to ensure objective categorization. In this methodology, the sample set is treated as a "population" and divided into quartiles on the basis of the sample deviation around the mean of the sample set.



Validation samples

Niger samples

Ethiopia samples

Nigeria samples

Figure 3. The sheep CP and DOM dendogram indicating the CP and DOM trends, the location of the validation samples, and the areas of concentration of the Niger, Nigeria and Ethiopia samples.

Table 7 presents the results of the categorization. The samples with values that fell within the first quartile were designated low for that variable, those that fell in the second and third quartiles were designated moderate, and those in the fourth quartile were designated high for that constituent. Every third sample in the dendogram was then assigned a category for both CP and DOM on the basis of these categories to aid elucidation of the CP and DOM patterns.

Cluster Analysis Results and Discussion

The categories derived through the use of quartiles may be deemed biologically acceptable, especially in reference to CP in which the limiting CP level of 7% is included in the low CP category. A pattern emerged for both variables as the samples in the dendogram where assigned levels according to the categories (see Figure 3). The DOM pattern was more prominent than the CP pattern, forming the backbone of the dendogram. DOM varied from low to very high, running from the top to the bottom of the dendogram. CP exhibited a wave-like pattern (going from one category to another) along the dendogram. Since DOM appeared to be the major determinant in placement of samples along the dendogram, a further analysis to elucidate the proportion of samples from each location within each DOM category was conducted, the results are presented in Table 7.

A second pattern, on the basis of agro-ecological zones emerged. Most of the Niger samples (65.69%) were in the low DOM category, 21.57% in the moderate DOM category, for a total of 87.26% of the Niger samples in the low

Table 7. Categorization of CP and DOM to aid analysis of the sheep CP and DOM dendogram

Category	Variable		Number and percentage of samples in each category by location (in terms of DOM categories)				Category Total
	CP	DOM	Niger	Ethiopia	Nigeria	Validation	
Low	3.80-9.20	24.68-44.16	(134/204)[1]	(19/113)	(0/5)	(0/24)	153
			65.69%	**16.81%**	**0%**	**0%**	
Moderate	9.21-15.74	44.17-55.48	(44/204)	(70/113)	(5/5)	(7/24)	126
			21.57%	**61.95%**	**100%**	**29.17%**	
High	15.75-23.55	55.49-79.12	(26/204)	(24/113)	(0/5)	(17/24)	67
			12.74%	**21.24%**	**0%**	**70.83%**	
Location/Group Total			204	113	5	24	346

[1] the figures in brackets are the proportions of samples from each location that fall with in a given category

and moderate categories. All the Nigeria samples were in the moderate DOM category. 83.09% of the Ethiopia samples were in the high or the moderate DOM category (61.95% in the moderate, and 21.24% in the high). 70.83% of the validation samples were in the high DOM category, and the remainder in the moderate category; no validation samples were in the low DOM category. This meant that two-thirds of the Niger samples were skewed towards the top end of the dendogram, while two thirds of the Ethiopia samples were skewed toward the center and bottom end of the dendogram.

A third observation is that of a spectral diversity illustrated by the combinations of CP and DOM levels. Because the DOM trend is well defined (a strong one-directional trend from low to high along the dendogram), it eliminates repetition of a particular CP-DOM combination along the dendogram. Since the agro-ecological trend has already been demonstrated, this may substantiate that there is vegetation diversity among the agro-ecological zones, influencing the DOM equations.

Conclusion

The findings of the cluster analysis help to explain the generally poor performance of the DOM equations in comparison to the CP equations. The validation samples were concentrated in the bottom of the dendogram in the only area in which high DOM samples were represented. The Niger calibration set is highly skewed toward the low DOM end of the dendogram, greatly reducing its ability to predict the DOM of the validation samples. Trends in DOM

calibration were opposite to those of the CP equation. The "All locations" CP equation had the best performance as it added variation since all the CP categories are represented throughout the dendogram.

The fact that the Niger and Ethiopia samples are skewed in different directions supports the inference of spectral diversity demonstrated by global H outliers for all the validation samples when they were predicted with the Niger DOM equation.

These findings suggest that for sheep there is greater potential of developing a robust CP equation, that can predict across the agro-ecological zones of SSA, than of developing a robust DOM equation. However, verification of vegetation diversity based on agro-ecological zones needs to be conducted using a larger calibration set with incorporation of more samples from other zones.

CHAPTER V

UTILIZATION OF NIRS FECAL PROFILING TO DETERMINE THE CONDENSED TANNIN CONCENTRATION OF DIETS CONSUMED BY SHEEP

Introduction

Leguminous browse species (designated browse in this chapter) contribute significantly to the diet of livestock across the agro-ecological zones of SSA. Livestock access browse either directly on rangeland or as supplemental feed offered in cut-and-carry systems or forage banks. The importance of browse in the sustenance of livestock in SSA is underscored by the more stable nutritive value of the green leaves of browse across the seasons and through climatic fluctuations as compared to grasses (Saleem et al. 1978, Adegbola 1985, Kaitho 1997).

Sheep, which are classified as grass-preferring intermediate feeders, are known to adapt readily to the higher intake of browse plants when grass availability, and or, nutritive value is restricting (Van Soest 1994). A major limitation to the utilization of browse is the presence of high levels of anti-nutritive factors in the plant material, primarily tannins. Numerous important browse species contain tannins (Mueller-Harvey et al. 1987, Kaitho 1997). Tannins have beneficial properties such as the capacity to reduce bloat and protect protein from degradation in the rumen. Goodchild et al. (1998) noted that up to 30 or 40 g kg^{-1} DM (Dry Matter) of reactive condensed tannins is beneficial

for livestock, reducing protein degradation and thus losses in the rumen. Higher levels are prohibitive to fibre digestion. Livestock are known to practice selective browsing to avoid toxic levels of anti-nutritive factors (Palo and Robbins 1991). It is postulated that rangeland livestock, free to select their own diet, will combine forage sources i.e., avoid exclusive consumption of tanniferous browse material, so as to mitigate the detrimental effects of tannins (Palo and Robbins 1991, Provenza 1995). Maceration and drying of plant material may promote the binding of tannins and plant protein exacerbating the effects of tannins (Terrill et al. 1992, Van Soest 1994). Anti-nutritive factors, of which tannins are a part, are known to increase in plant material due to environmental stress such as disease, fungal and insect attack, defoliation and drought (Bryant et al. 1992, Van Soest 1994). A methodology to track and predict the fluctuations of tannins in the diet of rangeland livestock is needed.

Windham et al. (1988), Roberts et al. (1993), and Goodchild et al. (1998) have successfully demonstrated the ability of NIRS to determine the tannin content of forages. These findings indicate that there may be the potential for successful tracking and prediction of dietary tannins through the methodology of NIRS fecal profiling.

There are several methods for the analysis of tannins dependent of the type of tannin and the purpose of the analysis; no single method is satisfactory for all needs (Reed 1995). Variability in interpretation and application of the methodologies, such as the use of varying standards, has made repeatability of

the methods across laboratories low. Windham and Baron (1988) noted that while calibration for tannin analysis using NIRS is dependent on the accuracy of the reference method, the NIRS methodology has been shown to give lower analytical errors within and among laboratories than is obtained with the reference method.

Tannins are broadly classified into two categories: hydrolyzable (ellagitannins) and condensed tannins (proanthocyanidins). Hydrolyzable tannins are potentially less problematic for livestock, being readily susceptible to hydrolysis in the abomasum, making them more readily absorbed (Reed 1995). This paper examines the use of NIRS fecal profiling to predict proanthocyanidins, (interchangeably referred to as condensed tannins) in dietary material as quantified by the method of Reed et al. (1982), a modification of the method of Bate-Smith (1975) i.e., the n-butanol methodology, which is specific to the proanthocyanidins, and the rarely occurring flavan-3, 4-diols. This method quantifies extractable condensed tannins (proanthocyanidins) in the neutral detergent fibre residue. Proanthocyanidins have been noted to increase fecal nitrogen output (Van Soest 1994). Robbins et al. (1991) reported excretion in the feces of the condensed tannin (quebracho tannin) to the magnitude of 100% and 60% of what was consumed by deer and sheep, respectively.

Calibration

Calibration Methodology

Calibration samples for the development of NIRS fecal profiling equations to predict condensed tannin content in diets of sheep were acquired from an experiment conducted by Kaitho et al. (1997) at the ILRI station at Debre-Zeit in Ethiopia (Highland zone, 1850 mm above sea-level, mean rainfall 713 mm yr^{-1}, average daily temperature range 5^0C to 34^0C). Forty-seven fecal samples were collected and catalogued in order to match them with dietary reference values including neutral detergent insoluble proanthocyanidins (designated NBUT in this chapter), CP, and DOM values. Table 8 summaries the calibration fecal samples collected and the matching dietary data. A list of the samples used in the calibration, and their matching data is presented in Appendix Table 4.

Dry matter, ash and kjeldahl nitrogen were determined according to AOAC (1990) standard procedures. Neutral detergent fibre (NDF) was analyzed by the method of Van Soest and Robertson (1985). Kaitho et al. (1997) determined the NDF insoluble proanthocyanidins according to the method of Reed et al. (1982). Two grams NDF samples were heated at 95^0C for one hour in n-butanol containing 5% concentrated HCL and then absorbance was read at 550nm.

Five grams of fecal samples were placed in sealable plastic bags and shipped from ILRI-Ethiopia, via an irradiation facility in New Jersey where the samples were exposed to two 3-MRAD of irradiation, before being forwarded to

Table 8. The samples used in the tannin calibration: site of collection, month and year, n-butanol measured proanthocyanidins, DOM and CP ranges and means, and feed type

Site	Experiment	Month/ year Of collection	N	Type of expt.	Variable	Range	Mean	Feed type
DZ	Kaitho et al. 1997	1996	47	Stall/ in vivo	NBUT CP DOM	0-103.40 4.65-10.89 46.96-79.12	29.30 8.75 58.66	Teff straw only *ad libitum*, and teff straw *ad libitum* with 190 grams of one of the following the supplements: *Leucaena leucocephala*, *Dolichus lablab*, *Chamaecytisus palmensis*, and *Sesbania goetzei*, or one of the accessions of *Sesbania sesban* i.e., 1190, 1198, 10865, 15019, 15036 and 2024

the GANLab at Texas A&M University. The samples were dried overnight in a forced-air oven and ground with a Udy mill to pass a 1-mm screen. The samples were then left to dry in the forced air oven for 48 hrs to stabilize the moisture content. NIRS predictions are sensitive to the moisture content of the sample (Lyons 1990). The samples were taken out of the oven and left in a dessicator to cool before they were put into NIRS sample cups with quartz windows. The samples were read using a NIRSystems Inc. Model 6500 scanning reflectance monochromator, equipped with a spinning sample cup, that reads wavelengths between 1100 and 2500 nm at 2-nm intervals. NIR spectra were stored in a microcomputer, interfaced with the spectrophotometer, as log 1/R values for the different wavelengths.

The spectra data were related to the reference data by multiple regression in which individual wavelengths were added to maximize R^2 (Walker et al. 1998). Eleven math treatments denoted by the expression (a,b,c,d) were used, where (Windham et al. 1988, Shenk et al. 1992):

a = the math derivative 1^{st} to 4^{th}

b = the gap between points used to calculate the derivative

c = the segment length over which the above function was smoothed

d = the segment length over which the smooth function was subject to a second smooth;

The treatments used are listed in Appendix Table 2.

Outlier passes were conducted once or twice and a scatter correction, standard normal variate and detrend, was used. Each combination of treatments produced a different equation. The best equation was selected, using a number of criteria, in the order of: a SEC between one and two times the SEL, the highest R^2, wavelength coefficients that were not above 10 000, F values for the wavelengths that were all above 10, and finally, the biological significance of the predictive wavelengths.

Calibration Results and Discussion

Table 9 presents the calibration results. The best equation had a first derivative math. Thirty-nine samples were incorporated into the equation, eight samples were eliminated as outliers.

The SEC, of 9.02, was lower than that reported by Windham et al. (1988) who had a SEC of 14.0 g kg DM for a calibration conducted for the Vanillin-HCL procedure. An R^2 of 0.91 was the same as that reported by Windham et al. (1988).

The 1st order derivative of log (1/R), which has a spectra curve that is visually difficult to interpret because the band peaks and curves do not follow the log (1/R) spectra pattern (Shenk et al. 1992). This is unlike the 2nd derivative spectra in which absorption curves are inverted from log (1/R) and a spectral pattern is maintained (Shenk et al. 1992). Visual interpretation will thus be limited to an appreciation of the differences in the curves for the low and high tannin samples. In the 2nd derivative, a negative coefficient indicates a positive

Table 9. Tannin calibration statistics: SEL, SEC, R^2, selected wavelengths, and associated chemical bonds

Equation	N	Treatment	SEL	SEC	R^2	# Terms	F	Wave Lengths	Associated Chemical Bonds
N-butanol	39	1, 15, 5, 1	*	9.02	0.91	4	158.56	2092	=NH amines+imides, -CH aldehydes, =CH2 terminal groups
							81.32	2172	-OH alcohol+phenol, +NH amines+imides, =CH,=CH2 unsaturated, =CH2 groups
							45.69	2028	nitrites, -OH phenol, -NH2 groups, -SH groups, =NH amines+imides
							19.42	2388	-CH aromatic, -CH protein bonds, -CH vibrations, =CH2 groups, -CH aromatic, -CH nylon

* insufficient data to calculate SEL

absorbance peak, this interpretation can not be applied to the 1st derivative. Further interpretation of the wavelengths will focus on examining the biological relevance of the selected wavelengths. The magnitude of the F-statistic is used as a guideline to the significance of the wavelength in contributing to explaining the variability in the equation. All four wavelengths are discussed.

All the wavelengths are in congruence with the known structure of tannin-protein complexes in forage material. Tannins are flavonoid derived (Markham 1982). Flavonoids constitute one of the largest groups of naturally occurring phenols (Markham 1982). The basic unit of proanthocyanidins is the flavonoid nucleus (15 carbon atoms) consisting of two aromatic rings: a phenyl-propanoid unit and another attached phenyl ring linked by a three-carbon unit (Markham 1982, Hemmingway 1989, Lewis and Yamamoto 1989, Van Soest 1994). The biphenyl linkage is resistant to cleavage by hydrolysis (Van Soest 1994). According to Hill and Rendell (1975), phenols have 3 vibrations in the mid-infrared region pertaining to the hydroxyl function:

(i) the C-O-H bend which is closely related with skeletal vibrations and thus does not produce a band diagnostic of hydroxyl,

(ii) the C-O stretch is also associated with the skeletal vibrations and is consequently not diagnostic of hydroxyl, but bands involving changes in this functional group are identifiable

(iii) the free O-H stretching vibration, which is diagnostic of hydroxyl

Aromaticity is diagnosed by the separate C-H or C=C vibrations (Socrates 1980). Overtones of the –C=O stretch, the O-H stretch characteristic of phenols, and the C-H stretch originating in the mid-infrared zone are evident in the near infrared zone. Proanthocyanidins are known to form large complexes with protein molecules, which are excreted and detectable in the feces (Wehausen 1995).

The major wavelength of 2092 nm is characteristic of absorption by NH amides+imides, -CH aldehdyes, and CH2 terminal groups. Amides and aldehydes are evidence of microbial activity, being reduction products from the process of protein synthesis in the rumen. This wavelength is also attributed to absorption by stretching bending of the O-H bond, characteristic of phenols. Evidence of bonds pertaining to both the hydroxyl function and to protein is in congruence with the structure of tannin-protein complexes.

The second wavelength is 2172 nm, characteristic of phenols, amides and imides, and various CH groups. Phenols are characteristic of anti-nutritive factors such as lignin and tannins. Amides are reduction products of microbial synthesis of protein. Goodchild et al. (1998) identified wavelengths close to 2150 nm as consistently the major wavelength present in all of the measures of tannins they conducted, including condensed tannins quantified by the n-butanol method. 2172 nm, is banded together with 2150 nm i.e., a broad absorption band encompasses the two wavelengths. Goodchild et al. (1998) suggest that this band is characteristic of condensed tannins.

The final wavelength of 2388 nm is indicative of CH aromatics and CH proteins, among other bonds, indicating the presence of anti-nutritive factors and undigested proteins. The combination which is in conformity with the known structure of tannin-protein complexes. Figures 4 illustrates absorption spectra of samples with different CP and DOM levels and similar tannin levels, Figure 5 illustrates the differences in absorption at the major wavelengths by samples with different tannin levels. Windham et al. (1988) suggested that although wavelengths were obtained by an empirical approach, establishment of a relationship between the wavelengths and the functional groups implies that NIRS determination may be partly analytical. The findings here seem to support this suggestion.

Validation

Validation Methodology

Validation samples were acquired from three experiments conducted at the ILRI station for research in the semi-arid zone located at Sadore, Niger (216 m above sea level, mean rainfall 575 mm, average daily temperature range 11-41^{0}C). Thirty-eight fecal samples with matching dietary data: NBUT, CP and DOM (obtained by similar methodology as the calibration samples), were collected, catalogued and shipped to the GANLab at Texas A&M University via an irradiation facility in New Jersey where the samples were exposed to two 3-MRAD doses.

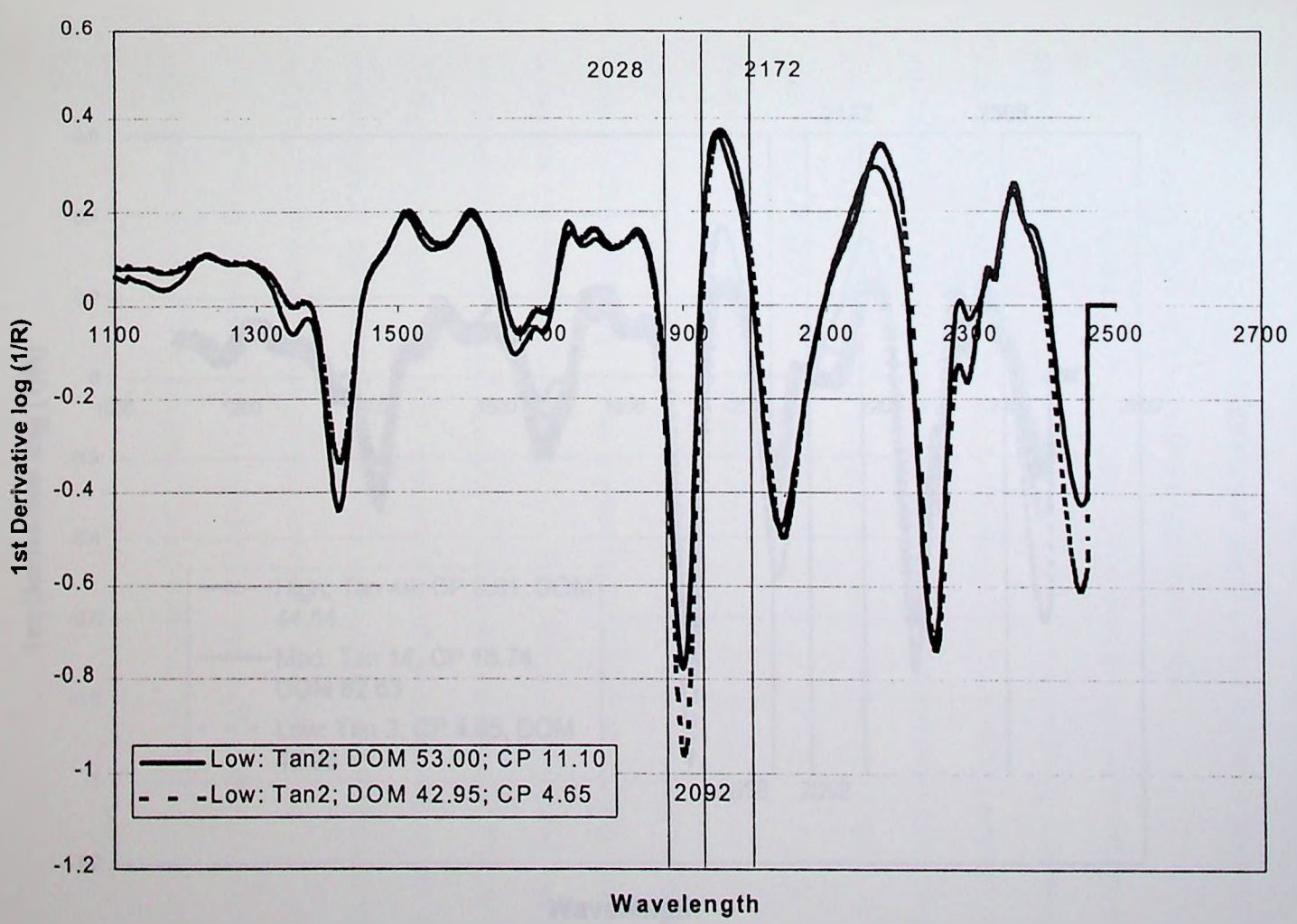


Figure 4 . Spectra of two samples with different CP and DOM levels and similar tannin levels illustrating their similar absorption at the major wavelengths

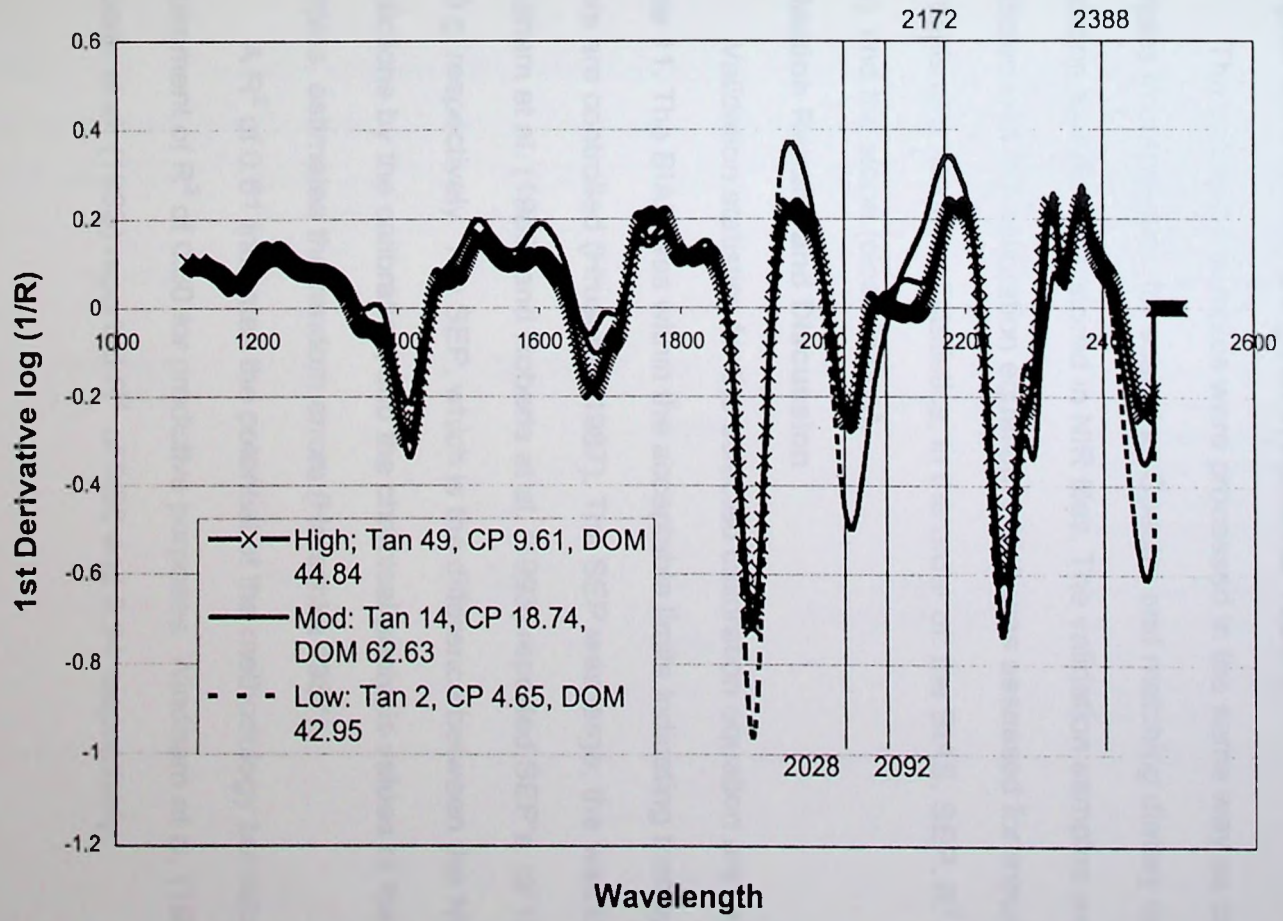


Figure 5. Spectra of three samples with different tannin levels depicting the differences in their absorption at the major wavelengths in the selected 1st derivative equation.

Table 10 presents a summary of the validation data, and Appendix IV the individual sample data. Experimental protocols for the trials from which the samples were derived are given in Appendix Table I.

The validation samples were processed in the same way as calibration samples in preparation for scanning. Spectra and matching dietary data for the validation sample were stored in NIR files. The validation samples were predicted with the calibration equation, which was assessed for robustness on the basis of a number of statistics, in the order of: the BIAS, SEP, R^2 (close to one), and the slope (close to one).

Validation Results and Discussion

Validation statistics for the selected calibration equation are presented in Table 11. The BIAS was within the acceptable limits indicating that systematic errors are controlled (Hruschka 1987). The SEP was large, the was large, 57.79. Windham et al. (1988) and Roberts et al. (1993) reported SEP's of 15.0 g and 11.0 g respectively. The SEP, which is the difference between the NIR predictions by the calibration, and the chemical analysis values of the validation samples, estimates the random errors (Hruschka 1987).

A R^2 of 0.61 indicates the potential of the methodology but falls below the requirement of R^2 of 0.80 for predictive purposes. Windham et al. (1988) and Roberts et al. (1993) reported R^2 of 0.96 and 0.94 respectively.

Table 10. The tannin validation data: site of collection, month and year, n-butanol measured proanthocyanidins, DOM and CP ranges and means, and feed type

Site	Experiment	Month/ year of collection	N	Type of expt.	Variable	Range	Mean	Feed type
SA	Fernandez-Rivera et al. 1994	1992	5	Stall/in vivo	NBUT CP DOM	57.00 4.65 42.95	57.00 4.65 42.95	Pearl millet stover leaves (*Pennisetum glaucum*)
SA	Zouladeini et al. (unpubl.)	1993	9	Stall./in vivo	NBUT CP DOM	9.47-9.61 28.79-44.84	9.54 35.82	Various browse leaves from the tree species *Guiera senegalensis, Mitragyna inermis, Ficus gnaphalocarpa* and *Anogeissus leiocarpus*
SA	Schelt (Unpubl.)	1995	24	Stall	N-BUT CP DOM	11.10-18.74 53.00-62.63	14.92 57.82	Basal feeds: groundnut hay and cowpea hay with or without the supplements *Combretum aculeatum* and *Pterocarpus lucens*

Table 11. The tannin validation statistics; the BIAS, SEP, R^2, and slope

Equation	# validation samples	SEP	R^2	BIAS	Slope	Comments
N-butanol	36	57.79	0.61	1.860	0.219	Global H outliers indicating spectral diversity

The slope of 0.219 was low, indicating that the calibration equation would consistently under or over estimate the low and high values (Westerhaus 1985b). Roberts et al. (1993) reported a slope of 1.02. Figure 6 presents tannin values predicted by the selected equation against the reference tannin values

The validation statistics are generally not as good as those obtained by other workers i.e., Windham et al. (1988) and Roberts et al. (1993). This may be a reflection of the vegetation diversity in the calibration and validation sample sets, a fact sustained by the many large global H values for the validation. Large global H values indicate spectral diversity between the calibration and validation sample sets (Westerhaus 1985b). Windham et al. (1988) and Roberts et al. (1993) both worked with different accessions of a single species thus limiting spectral diversity in their calibration and validation sets. Roberts et al. (1993) who worked with a set of diverse accessions of a single species birdsfoot trefoil (*Lotus corniculatus*), reported that there were no H outliers in their validation process.

Cluster Analysis

Methodology

A cluster analysis was done to examine the diversity of the samples used in both the calibration and validation, and to aid understanding of the calibration and validation results. The software PC-ORD (Version 2) for multivariate analysis of ecological data (McCune and Mefford 1995) was used to cluster

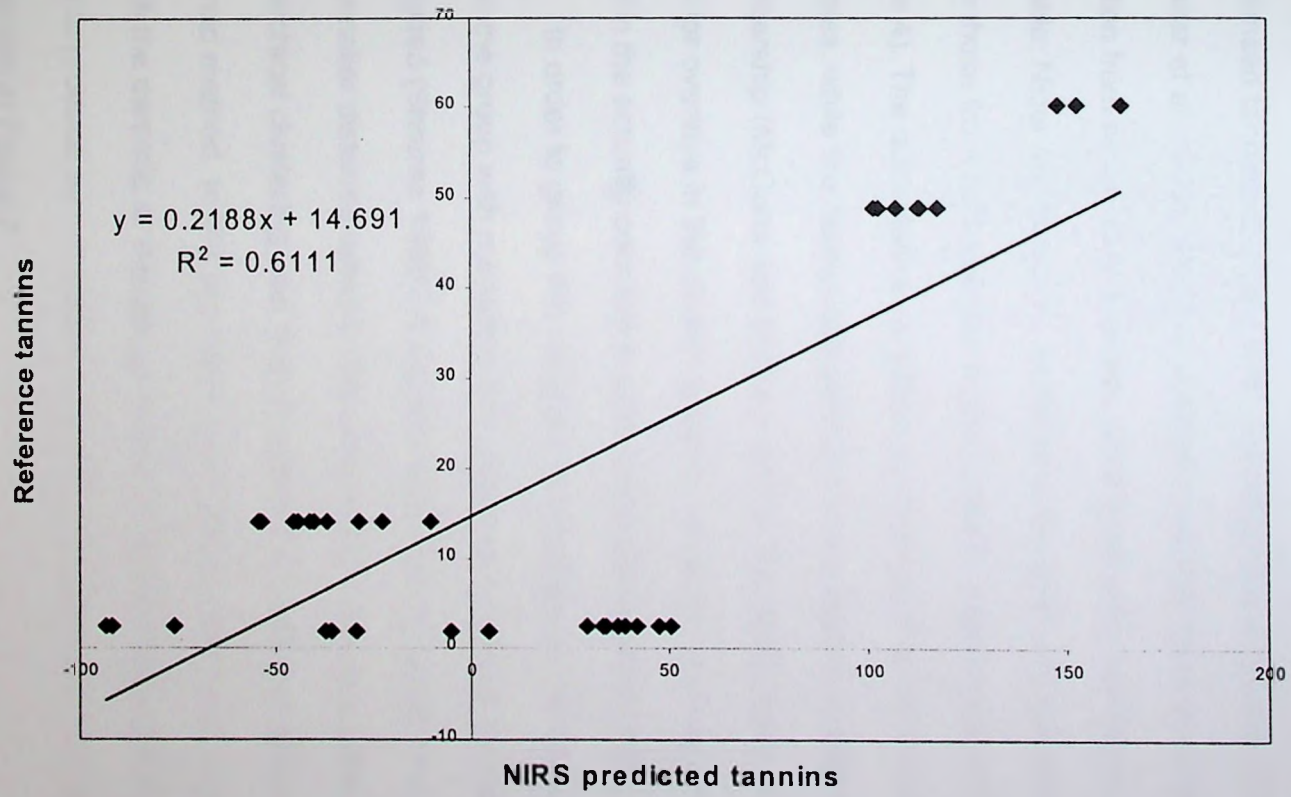


Figure 6. Reference tannin values of the validation set against those predicted by the selected equation

the samples. Sample dietary data NBUT, CP and DOM were imported from Lotus 1-2-3 into PC-ORD. CP and DOM were included in the cluster analysis as condensed tannins complex with, and affect the utilization of both CP and DOM (Makkar et al. 1996). All three variables were denoted quantitative variables. The location from which data samples were taken was denoted as a categorical variable; Niger samples (i.e., those from the semi-arid zone) are denoted NIG, while those from Ethiopia (the highland zone) were denoted ETH (see Appendix Table 4). The quantitative variables are used by PC-ORD to rank or arrange the samples, while the categorical variables are qualitative statements of group membership (McCune and Mefford 1995). The categorical variables are thus used for overlays in the cluster analysis, while the quantitative variables are used in the actually correlation's (McCune and Mefford 1995).

In order to group the samples so that they are homogenous as possible within the group with respect to the clustering variables, a measure of similarity is needed (Sharma 1995). A squared euclidean distance was used which results in a smaller distance between the more similar samples (Sharma 1995). Hierarchical clustering was done in which the clustering algorithm was the centroid method. In this algorithm each group is replaced by an average subject that is the centroid of that group (Sharma 1995). Single dendogram width was used to produce as compact as possible a dendogram. The full dendogram is presented in Figure 7.

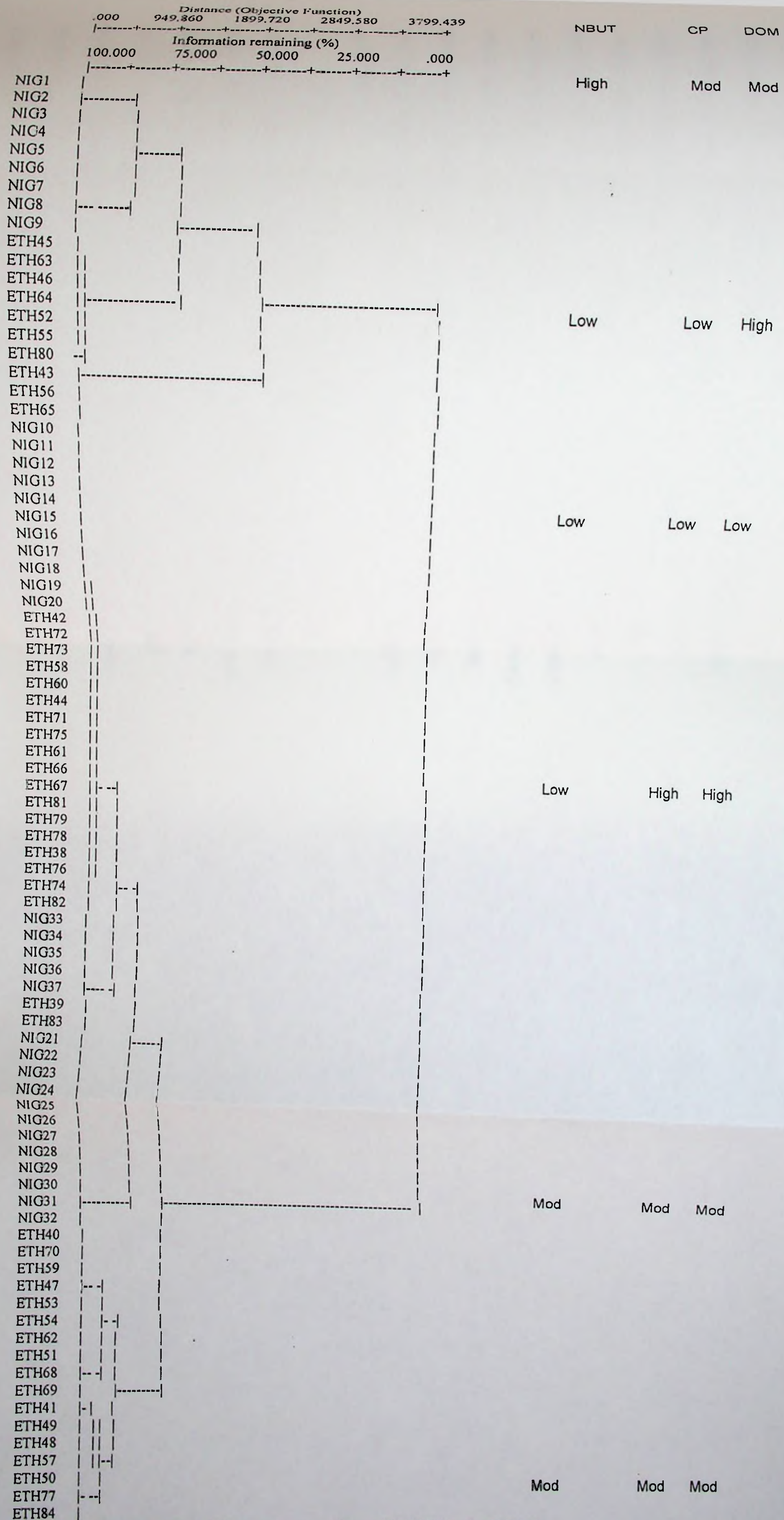


Figure 7. The tannin (N-butanol measured proanthocyanidins) dendogram, illustrating: the position of the calibration samples (ETH and number; derived from Ethiopia), the validation samples (NIG and number; derived from Niger), and the trend in tannin (NBUT), CP, and DOM levels along the dendogram

Cluster Analysis Results and Discussion

The dendogram was analyzed to aid interpretation. The range of each variable was taken and the inter-quartile ranges calculated to delineate categories to enable detailed assessment of the cluster results. The use of inter-quartile ranges was preferred over delineation on the basis of low and high values of each variable as the data set was small and potentially not a true representation of the population especially given the fact group and not individual animal dietary values were used. The inter-quartile method treats the sample set as normally distributed. The first inter-quartile range was assigned as the low category, the second and third as the moderate category, and the fourth as the high category (see Table 12).

It appears that the categories derived objectively through the use of quartiles are biologically meaningful. Goodchild et al. (1998) suggested that up to 30 or 40 g kg^{-1} reactive condensed tannins in the diet might be beneficial, and higher levels, anti-nutritional. Barry (1989) indicated 1-3% condensed tannins kg^{-1} DM, (equivalent to 10-30g kg^{-1} DM) as beneficial, but 5-10% condensed tannins kg^{-1} DM (equivalent to 50-100g kg^{-1}DM) as a high and detrimental level of condensed tannins in *Lotus sp*. The categorization using inter-quartiles indicates 0-2.5 g kg^{-1} DM as low, and between 2.51 and 35.10 as moderate levels, and more that 35.11 as high. 7% CP is known to be limiting to ruminant livestock (Milford and Minson 1964). The inter-quartile method designates diets with CP values up to 7.97% as low CP diets. Table 12 presents the categories

Table 12. The categories used in the analysis of the tannin dendogram

Category	Variable		
	N-butanol	CP	DOM
Low	0 – 2.5	3.8 - 7.97	28.79 - 45.14
Moderate	2.51 – 35.10	8.0 - 11.10	45.14 – 53.17
High	35.11 – 103.40	11.11 – 18.74	53.18 – 62.63
Range	0 – 103.40	3.80 – 18.74	28.79 – 62.63

used in elucidating the trends of the three variables in the cluster analysis. The categorization results are presented in the outline of the dendogram (see Figure 7). Further analysis of the classification using single variables to cluster i.e., NBUT alone, CP or DOM alone, and using two variables at a time i.e., NBUT and CP, and NBUT and DOM indicated that the most influential variables in specifying the groups in the cluster analysis were N-BUT and DOM. The most defined and significant trend is that of tannin. There was a very strong trend from high through low to moderate tannin levels along the dendogram. The trends for CP and DOM where not as pronounced or well defined.

There is no clearly defined agro-ecological zone partitioning; i.e., the Niger (semi-arid) and the Ethiopia (highland) samples are not prominent in any section of the dendogram. However, the greater number of Ethiopia samples are located in the lower two-thirds of the dendogram, while the Niger samples are in the upper two-thirds of the dendogram. The data is clumped for zones as sheep were assigned a group value for a diet in the Niger data i.e., a group dietary value. The intermixing of the Niger and Ethiopia data indicate that there is a potential for prediction across agro-ecological zones using these data sets. The lack of diet diversity reduces the robustness of the calibration equation, and thus it's capability to predict the validation equations. The fact that there is only a partial overlap between the calibration and validation data may partly account for the low slope and the high SEP in the validation.

Conclusion

The reasonable calibration statistics, and the valid biological significance of the calibration wavelengths, in combination with the current validation statistics, even in light of the limited calibration sample set and the spectral diversity between the calibration and validation sets, suggest the potential of NIRS fecal profiling to predict the condensed tannin content of diets consumed by sheep. The cluster analysis aided explanation of the limited capacity of the calibration equation to predict the validation samples. A more diverse calibration set needs to be used to examine the potential of NIRS fecal profiling to predict dietary tannins.

CHAPTER VI

DEVELOPMENT OF ROBUST NIRS FECAL PROFILING EQUATIONS TO PREDICT THE DIET OF FREE-RANGING CATTLE IN SUB-SAHARAN AFRICA

Introduction

Lyons and Stuth (1992) and Lyons et al. (1993) have successfully demonstrated the use of fecal profiling to predict the diet of free-ranging cattle in the USA. Coates (1998) demonstrated the potential for use of fecal NIRS profiling to predict the diets of range cattle in tropical Australia. Currently, a tandem of two NIRS fecal profiling equations is in use in USA. The first equation was formulated to predict fecal samples derived from cattle fed diets with a greater C3 vegetation component in comparison to C4 i.e., samples characteristic of temperate regions. The second equation was formulated to predict the diets from fecal samples that were derived from cattle that were fed diets with a greater C4 vegetation component, characteristic of the sub-tropical region. Preliminary tests, using these equations, on fecal material derived from cattle grazing *Acacia senegal* and *Commiphera sp.* savanna rangelands in southern Kenya, indicate the potential of the USA equations for use in sub-Saharan Africa (SSA) (Stuth, personal communication). However, the equations were unable to predict some of the Kenyan samples. This may reflect a spectral difference due to species diversity, and or, a limitation in the predictive capacity of the equations due to a difference in nutrient range (Westerhaus 1985c).

This chapter examines the possibility of developing robust cattle crude protein (CP) and digestible organic matter (DOM) NIRS fecal profiling equations that can be used across Africa and USA. Successful development of such equations could facilitate transfer of the NIRS technology, which is calibration dependent, by allowing pre-calibration of NIRS equipment. This would ensure standardization across laboratories and thus facilitate collaborative efforts in use of the NIRS fecal profiling technique for management, or research.

Methodology

Development of robust equations is contingent upon incorporation of a large set of samples reflective of the diversity (chemical, physical, botanical etc.,) in the target population. Legacy fecal samples and data were used to formulate the calibration set. The Africa fecal samples and matching dietary data were derived from the International Livestock Research Institute (ILRI) which has the mandate to conduct livestock research across the agro-ecological zones of SSA. Cattle fecal samples and matching dietary data was obtained from three agro-ecological zones: highland represented by Debre Zeit Ethiopia (1850 m above sea level, temp range 5 – 34^{0}C, average rainfall 713 mm yr^{-1}). Samples representative of the semi-arid zone were obtained from three locations in Niger: Sadore (average rainfall 574 mm yr^{-1}), Bundu (average rainfall 400 mm yr^{-1}), and Toukounous (average rainfall 330 mm yr^{-1}). Fecal samples and matching dietary data for the humid zone were obtained from Ibadan, Nigeria (average rainfall

1500 mm yr^{-1}). Fecal samples and matching dietary data were collected from different experiments conducted over a period of years.

Fecal samples and matching dietary data from the USA were obtained from various grazing trials conducted over a number of years, in various locations of the nation. Table 13 presents the summary of the samples used in the calibration set.

The Calibration Process

Five grams of each fecal sample from Africa was sealed in a plastic bag, and the samples were shipped from the ILRI locations via an irradiation facility in New Jersey, USA, where they received two 3-MRAD doses, to the Grazingland Animal Nutrition Lab (GANLab) at Texas A & M University. The Africa fecal samples were dried overnight at 60^0C in a forced-air oven to remove moisture, and ground through a Udy cyclone mill to pass a 1-mm sieve to ensure uniform particle size.

All the fecal samples (USA and Africa) were dried over 48 hours in a forced-air oven at 60^0C, to stabilize moisture content. The samples were let to cool for an hour in a dessicator, packed into NIRS sample caps with quartz windows, and scanned on a NIRS Systems Inc. Model 6500 scanning reflectance monochromator equipped with three tilting filters, and a spinning sample cup, that reads reflectance in the range of 1100-2500 nm at 2 nm intervals. NIR spectra were stored in a microcomputer interfaced with the spectrophotometer, as log (1/R) values for the different wavelengths.

Table 13. The calibration set used in the development of the Africa and USA and Africa cattle equations: the number of samples from each location, CP and DOM ranges and means

Location		N	Crude Protein		Digestible Organic matter	
			Range	Mean	Range	Mean
Africa	Niger (Sadore, Bundu and Toukounous)	226	6.08 – 29.26	12.47	33.73 – 66.40	46.72
	Nigeria (Ibadan)	18	14.52 – 23.03	18.51	62.87 – 69.26	67.04
	Ethiopia (Debre Zeit)	200	2.88 – 12.92	8.51	35.06 – 71.91	60.34
	All Africa data	444	2.88 – 29.26	11.02	33.73 – 71.91	53.35
USA	Various locations	316	3.55 – 20.18	8.65	50.50 – 70.03	61.08
All Samples		760	2.88 – 29.26	10.02	33.73 – 71.91	56.59

Reflectance data were regressed against the dietary (reference) data by multiple stepwise regression in which individual wavelengths were added to maximize R^2 (Walker et al. 1998). A maximum of 12 wavelength terms were selected to explain the variability in the reference data. Eleven math treatments were applied to the calibration process. The treatments used are explained by the expression (a, b, c, d) (Windham et al. 1988, Shenk et al 1992), where:

a = the derivative function i.e., a value of 0 being log 1/R values, values of 1 and 2 etc., first and second derivatives, respectively, and so on.

b = the gap between points used to calculate the derivative

c = the segment length over which the above function was smoothed

d = the segment length over which the smooth function was subject to a second smooth.

The treatments used are listed in Appendix Table 2. One or two iterations were conducted through the data set to eliminate outliers.

A number of selection criteria were used in selection of the equations. The SEC (standard error of calibration) was assessed in comparison to the SEL (the standard laboratory error). A SEC within twice the SEL is desirable. The SEL's were calculated according to the formula of Workman (1992). There were a number of experiments combined from three different laboratories, therefore, there was a need to formulate a protocol to calculate the SEL for each equation. Repeated data are needed to calculate the SEL; this was not available for all the

samples collected. However, the SEL is the repeatability constant for the wet chemistry analysis for a given constituent, in a particular lab (Workman 1992) therefore, SEL's calculated from available data were deemed representative of the lab. A weighted average, based on the proportional number of samples contributed per lab to an equation, was used to calculate the SEL for equations with samples from two or more locations.

Equation selection was also conducted on the basis of the R^2, the coefficient of determination; an R^2 of 0.80 is required for predictive purposes. Equations were also selected for F values >10, indicating a significant contribution of the wavelength term to the explanation of the variability in the constituent (Westerhaus 1985a). The coefficients of the wavelength terms were also examined, and coefficients greater than 10,000 were rejected. The final selection criterion was the biological significance of the wavelengths in the equations in relation to the constituent being predicted.

Two equations were created for each variable (CP and DOM); an equation incorporating only samples from Africa, and a second one incorporating samples from USA and Africa.

The Validation Process

Fecal samples and matching dietary data for the validation were collected through a metabolism trial conducted at the ILRI station located in Debre-Zeit

Ethiopia. Eight steers, four *Bos indicus* (Borana breed) and four crossbreeds (Borana X Friesian) were weighed, and their body condition score taken. The steers were blocked by breed, and assigned a group within block by weight. A randomized complete block (RCB) design was used to assign treatments within group. Table 14 presents the rations fed in the validation trial .

The steers were allowed 12 days to adjust to the rations, the basal diet being offered *ad libitum*. Water also provided *ad libitum*. Thereafter, total fecal collection was conducted for a 7-day period, during which the amount of feed offered and refused were also quantified. Feed and refusal was sub-sampled for later analysis. Aliquot fecal samples (10%) were collected and frozen on a daily basis. At the end of the collection period, the fecal samples of individual animals was thawed overnight, pooled and sub-sampled. The samples were oven dried (60^0C to constant weight) and ground through a Udy cyclone mill to pass a 1-mm sieve.

Digestibility was derived as the differential between the organic matter (OM), and dry matter (DM) in the diet and the feces. Dry matter assays were conducted on the feed offered and refused, and the feces using the method described by the AOAC (1990). Analyses for ash, and Kjeldahl nitrogen (N) in the rations, refusals, and feces were conducted according to AOAC (1990) standard procedures.

Table 14. The rations used in the cattle validation trial: basal feeds and supplements, and CP and DOM means per ration

Number of animals	Basal Feed	Supplement(s)	CP	DOM
2	Teff straw (*Chamaecytisus palmensis*)	None	4.60	53.94
2	Teff straw	Wheat middlings	7.79	61.69
2	Teff straw	*Sesbania sesban* 10865	12.86	67.62
2	Native grass hay (Debre-Zeit[1])	None	4.58	73.21
2	Native grass hay (Debre-Zeit)	Lablab hay	10.10	54.61
2	Native grass hay (Debre-Zeit)	*Acacia siberiana* pods	14.68	65.04
2	Wheat trifolium	None	4.45	51.54
2	Native grass hay (Suluta[1])	None	5.67	41.92
2	Native grass hay (Suluta)	Cottonseed cake	12.85	57.80
2	Native grass hay (Suluta)	*Desmodium sp.*	7.64	52.20
2	Maize Stover	None	3.64	60.56
Mean			8.08	58.19

[1] The place from which the hay was obtained

Calibration Results and Discussion

CP calibration results are presented in Table 15. The SEC for the "Africa CP" equation was close to the SEL. The SEC of the "USA and Africa CP" equation was slightly higher than twice the SEL. The "Africa CP" equation had a lower SEC than the "USA and Africa CP" equation. The SEC's for both equations are higher that those derived by Lyons and Stuth (1992) for cattle at a single location (College Station, Texas) 0.88, and two locations (La Copita, and College Station) 0.89. Both equations had high R^2 values, 0.95 and 0.90 for the "Africa CP" and "Africa and USA CP" equations, respectively. The R^2 were comparable to those derived by Lyons and Stuth (1992) 0.92 for two locations, and Showers et al. (1999) for deer (0.94). Both CP equations have the same second derivative maths, 2 ,8, 4, 1. Visual interpretation was conducted to compare absorbance spectra of high and low quality samples.

The "Africa CP" Equation

The major wavelength for the "Africa CP" equation, 2168 nm, is indicates bonds of OH alcohol+phenol, NH amines+imides and various carbon-hydrogen groups. Amines are reduction products of microbial synthesis of protein in the rumen. Alcohols and phenols are bonds associated with anti-nutritive factors like lignin and tannins, and the carbon-hydrogen groups signify the presence of undigested proteins, starch and cellulose. The presence of anti-nutritive factors and undigested protein is in congruence with tannin-protein or lignin-protein complexes. Goodchild et al. (1998) identified wavelengths close to 2150 nm as

Table 15. The cattle CP calibration results: SEC, R^2, the three major wavelengths, and the associated chemical bonds

Equation	N	Treatment	SEL	SEC	R^2	# Terms	F	Wave Lengths	Associated Chemical Bonds
Africa CP	294	2, 8, 4, 1	*	1.04	0.95	12	56.83	2168	-OH alcohol+phenol, =NH amines+imides, =CH,+CH2 unsaturated, =CH2 groups
							405.66	1452	-OH alcohols, -OH phenols, =NOH oxine, -COOH carboxylic, -NH2 amines
							356.71	2004	Nitrites, carbonyl bond, -OH phenol, =CO terminal, -NH2 groups, -SH groups, -OH alc0hols, =NH amines+imides
USA and Africa CP	630	2, 8, 4, 1		1.26	0.90	12	1010	1656	-CH vibration, =CH unsaturated, =CH2 terminal group, -CH aromatic, =CH2 groups, =CH2 terminal
							836.07	1456	-OH alcohols, -OH phenols, -COOH carboxylic, -NH2 amines, primary amines
							417	1632	-CH vibrations, olefins, =CH2 terminal groups, =CH2 terminal, -CH unsaturated

* insufficient data to calculate this variable

significant for condensed tannins. Williams and Norris (1987) indicate 2140 nm as significant for protein. 2140 nm and 2150 nm are banded together with 2168 nm.

The "USA and Africa CP" Equation

The major wavelength, 1656 nm is indicative of various CH2 groups and CH aromatic bonds. The CH bonds could indicate carbohydrates, and the aromatic bonds, the presence of anti-nutritive factors. The CP equations have similar second wavelengths: 1452 nm for the Africa CP equation, and 1456 nm for the combined "USA and Africa CP" equation. These equations indicate anti-nutritive factors signified by –OH alcoholic bonds and –OH phenolic bonds.

Table 16 presents the DOM calibration results. The "USA and Africa DOM" equation had a lower SEC, and higher R^2, compared to that of the "Africa DOM" equation, (SEC 2.39 and 3.4), and (R2 0.91 and 0.85) for "USA and Africa DOM", and "Africa DOM" equations, respectively.

The "Africa DOM" Equation

The major wavelength 2028 indicates nitrites, and amines, which are indicative of microbial activity (i.e., by products of protein synthesis). Phenolic bonds are indicative of anti-nutritive factors. SH groups, which contain sulfur, indicate protein. Williams and Norris (1987) report 2055 nm, which is banded with 2088 nm, as a highly significant wavelength for proteins. Greater absorbance at this wavelength by the high quality samples, may indicate enhanced microbial activity.

Table 16. The cattle DOM calibration results: SEC, SEL, R^2, the three major wavelengths, and the associated chemical bonds

Equation	N	Treatment	SEL	SEC	R^2	# Terms	F	Wave Lengths	Associated Chemical Bonds
Africa DOM	339	2, 20, 10, 1	*	3.39	0.91	12	480.08	2028	Nitrites, -OH phenol, -NH2 groups, -SH groups, =NH amines+imides
							463.83	2236	=CH,=CH2 unsaturated, =CH2 groups, =C=O esters, -CH vibrations, -CH aromatic
							392.14	1604	=NH groups, =NH proteins
USA and Africa DOM	595	2, 8, 6, 1	*	3.4	0.85	12	276.84	1124	=CH2 groups, -CH vibrations, -CH-O-CH2 terminal, =CH2 terminal groups
							219	1524	-NH2 amines, primary amines, secondary amines, =CH terminal
							201	1740	-CH vibration, -CH aromatic, =CH2 groups, =CH2 terminal, -CH3 groups, =CH groups, =CH aliphatic, -CH proteins

* insufficient data to calculate the SEL

There was less absorbance by the high quality sample at 2236 nm, the second major wavelength. This wavelength indicates various carbon-hydrogen groups associated with undigested starch, protein and cellulose, and carbon-hydrogen aromatic bonds indicative of anti-nutritive quality factors.

The "USA and Africa DOM" Equation

The major wavelength, 1124 nm indicates carbon-hydrogen groups associated with undigested starch, protein and cellulose in the feces. The second wavelength, 1524 nm, indicates NH2 amines, primary amines, and secondary amines, all of which are reduction products of the microbial synthesis of protein from ammonia (Geissman 1977, Van Soest 1994).

Validation Results and Discussion

Validation statistics for both CP and DOM are presented in Table 17. The "Africa CP" equation had a lower BIAS (0.217) compared to the "USA and Africa CP" equation. The SEP's are similar, as are both the R^2 (0.60) and (0.59) for the "Africa CP" equation and the "USA and Africa CP" equations, respectively. The R^2 are below the required level (0.80) for predictive purposes. The two equations differ in slope, 0.820 and 1.554 for the "Africa CP" and the "USA and Africa CP" equations, respectively. A high slope indicates that the equation will over-predict or underestimate the low and high samples. The performance of the "Africa CP" NIRS equation in predicting the validation samples is illustrated in Figure 8.

The performance of the DOM equations was not comparable to that of the CP equations. The "USA and Africa DOM" equation had a lower BIAS than the

Table 17. The cattle CP and DOM validation results: the BIAS, SEP, R^2, and slope of the "Africa" and "Africa and USA" equations

Equation	N	Math	BIAS	SEP	R^2	Slope
Africa CP	22	2, 8, 4, 1	0.217	2.489	0.60	0.820
USA and Africa CP	22	2, 8, 4, 1	0.570	2.695	0.59	1.554
Africa DOM	22	2, 8, 6, 1	-2.01	12.27	0.09	0.440
USA and Africa DOM	22	2, 20, 10, 1	0.593	11.497	0.06	0.884

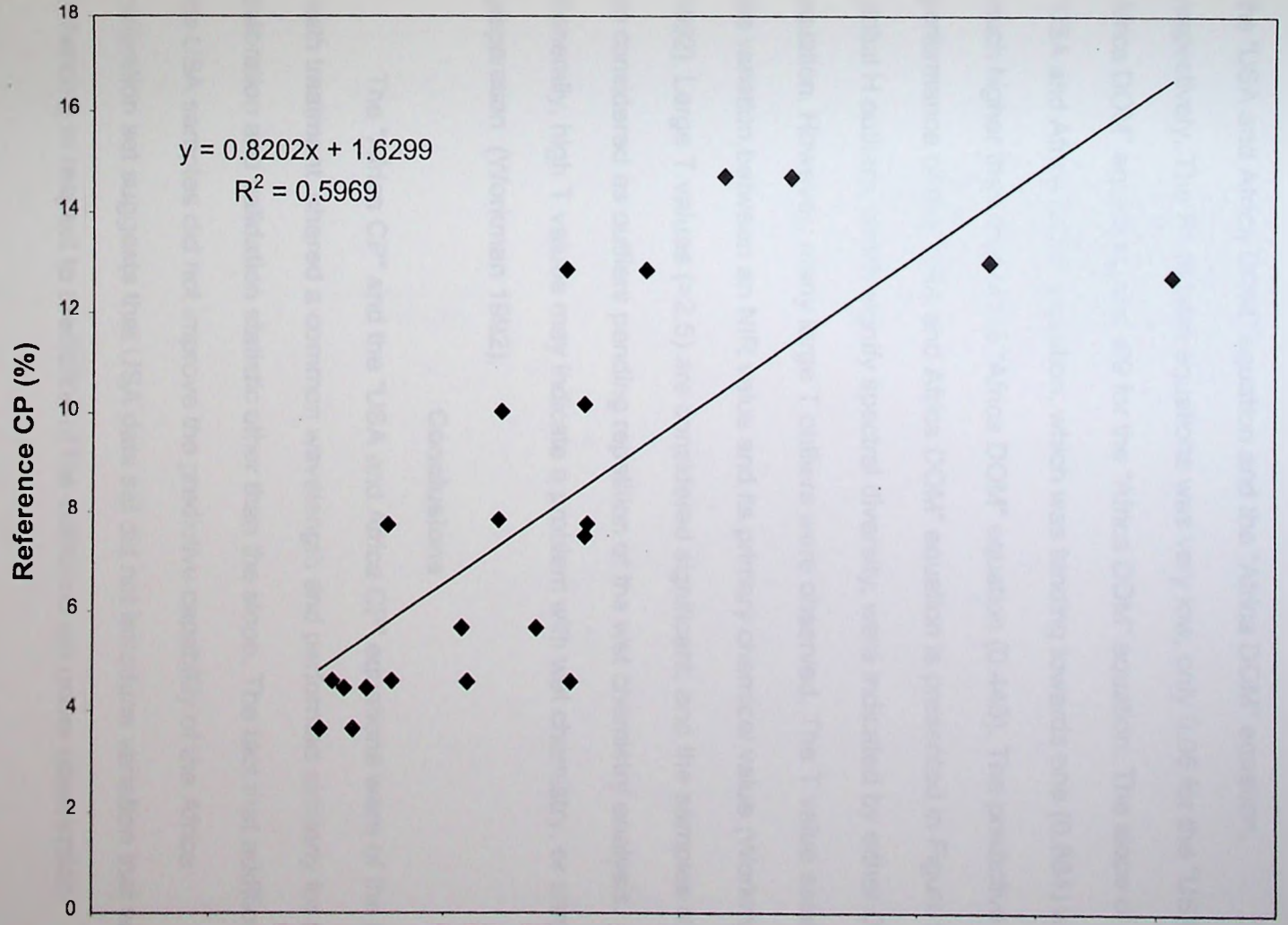


Figure 8. Reference CP (%) of the validation set against those predicted by the "Africa CP" equation

"Africa DOM" equation. Both equations had high SEP's (11.497 and 12.27) for the "USA and Africa DOM" equation and the "Africa DOM" equation, respectively. The R^2 of both equations was very low, only 0.06 for the "USA and Africa DOM" equation, and .09 for the "Africa DOM" equation. The slope of the "USA and Africa DOM" equation, which was tending towards one (0.884) was much higher than that of the "Africa DOM" equation (0.440). The predictive performance of the "USA and Africa DOM" equation is presented in Figure 9. No global H outliers, which signify spectral diversity, were indicated by either DOM equation. However, many large T outliers were observed. The T value assesses the variation between an NIR value and its primary chemical value (Workman 1992). Large T values (>2.5) are considered significant, and the samples should be considered as outliers pending repetition of the wet chemistry analysis. Generally, high T values may indicate a problem with wet chemistry, or sample preparation (Workman 1992).

Conclusions

The "Africa CP" and the "USA and Africa CP" equations were of the same math treatment, shared a common wavelength and performed similarly for all calibration and validation statistic other than the slope. The fact that addition of the USA samples did not improve the predictive capability of the Africa calibration set suggests that USA data set did not introduce variation that was enhancing in respect to prediction of the validation set under observation.

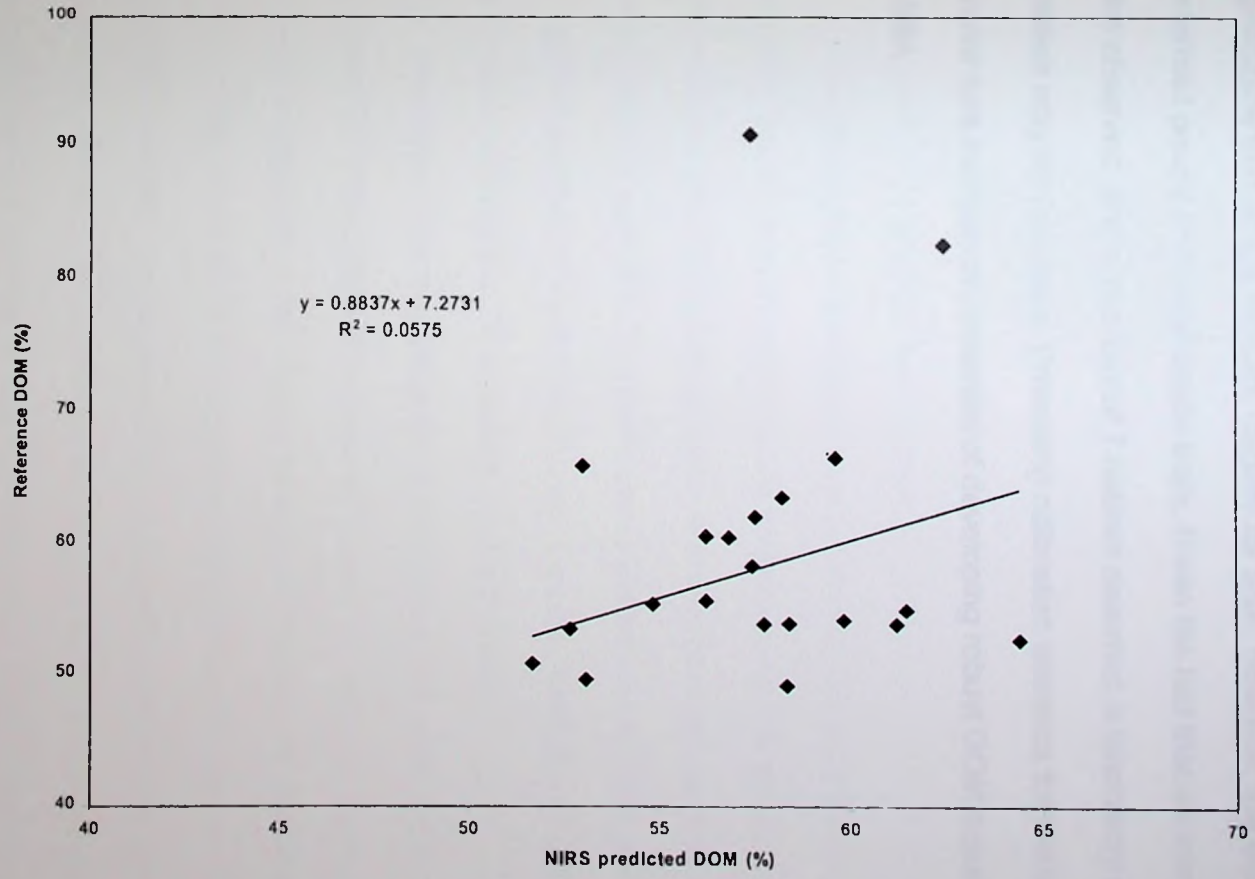


Figure 9. Reference DOM (%) of the validation set against those predicted by the "USA and Africa DOM" equation

Both the DOM equations, the "Africa DOM" and the "USA and Africa DOM" equations produce calibration statistics and wavelengths of biological significance that would indicate viability for SSA. However, the equations performed poorly in the validation trials. Given the fact that no spectral outliers were observed, and a number of T outliers occurred, a laboratory determination problem may be indicated. Promising calibration statistics for the large and diverse sets indicate the potential of developing robust DOM equations for cattle in SSA.

CHAPTER VII

CONCLUSIONS

The findings of this research indicate the potential for development of a robust NIRS fecal profiling equation for predicting CP in the diets of free-ranging sheep. The superior performance of the CP equation that combined data from across the agro-ecological zones, over that of Ethiopia, in predicting the validation samples from Ethiopia, indicates that it may be advantageous to create a universal CP equation for SSA.

The possibility of creating robust (universal) CP equations for sheep was mirrored in the findings for cattle. Good calibration results were derived for the combined USA and Africa CP equation, which encompassed a wide, and diverse range of samples. The comparable performance of the USA and Africa CP equation to that of the Africa equation for most validation statistics, suggests that a robust USA-Africa CP equation can be created.

There was poor performance by the sheep and cattle DOM equations. The sheep DOM equations demonstrated poor validation statistics, however, the fact that the inclusion of the Nigeria samples enhanced the performance of the Ethiopia DOM sheep equation, indicates that the Nigeria samples added predictive variability. Reduction of the predictive capacity of the Ethiopia-Nigeria sheep DOM equation on addition of the Niger sheep samples suggested spectral diversity in relation to DOM between the Ethiopia and Niger sheep samples. This observation was substantiated by a cluster analysis, which

demonstrated an agro-ecological trend in the sheep sample set. It is necessary to verify these findings with a larger and more diverse calibration set. However, cluster analysis aided interpretation of the NIRS validation results, and may have potential for use in selecting samples for inclusion into equations for specific purposes.

The Cattle DOM equations also performed poorly. However, indication of T outliers which signify lab determination problems, and no global H outliers which signify spectral diversity, suggests that the cattle DOM equations may perform better when predicting other validation sets.

There is indication that NIRS fecal profiling may be applicable for predicting the condensed tannin content of the diet of free-ranging sheep. Wavelengths that were incorporated into the selected equation are highly biologically significant in relation to the known structure of condensed tannins that bind protein, and have been reported in the scientific literature by other workers using NIRS to quantify tannins in forage material.

A major challenge to the use of chemical analysis is the variability across laboratories. The results of the analyses conducted may not be acceptable for predictive purposes, but they demonstrate the possibility of using NIRS across agro-ecological zones. In general the findings substantiate the potential of NIRS fecal profiling for predicting constituents in the diets of free-ranging livestock.

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APPENDIX

Appendix Table 1. Sources of calibration samples and data for sheep: publications and protocols

Location	Experiment	Publication or Protocol Title	Experimental Design
DZ	Umunna et al. 1995[1]	Umunna, N.N., P.O. Osuji, I.V. Nsahlai, H. Khalili and M. A. Mohamed-Saleem. 1995. Effect of supplementing oat hay with lablab, sesbania, tagasaste or wheat middlings on voluntary intake, N utilization and weight gain of Ethiopian Menz sheep. Small Ruminant Research.18:113-120	Metabolism trial with 30 sheep given one of the following diets: Oat hay *ad libitum*, Oat hay + 250 g of one of the following supplements: *Lablab purpureus* hay, Wheat middlings, *Sesbania sesban*, or *Chamaecytisus palmensis*, and Oat straw ad libitum with *Lablab purpureus* hay. Prior 80 day growth trial, 6 days fecal collection.
DZ	Nsahlai and Umunna 1996[1]	Nsahlai, I. V., and N.N. Umunna. 1996. Sesbania and lablab supplementation of oat hay basal diet fed to sheep with or without maize grain. Ani. Feed Sci. Tech. 941:	Metabolism trial with 20 sheep assigned to four diets: Oat hay *ad libitum* with 250 g of lablab or sesbania with or without 91 g crushed maize grain. 6 day collection period; Prior 70 day growth trial.
DZ	Umunna et al. Unpubl.	(Umunna, N.N, P.O. Osuji, I. Hasque and I. Nsahlai.) Responses of sheep and cattle to Bird Resistant (BR) and Non-Bird Resistant (NBR) sorghum stover supplemented *with S. sesban* of varying tannin content and the influence of diet on the partition of excreta nutrients for plant growth.	Metabolism trial with cattle and sheep fed basal diets of either Bird Resistant or Non-Bird Resistant sorghum stover , with or without a *Sesbania sesban* supplement
DZ	Boitumelo et al. (unpubl)		Metabolism trial with cattle and sheep fed basal diets of either Bird Resistant or Non-Bird Resistant sorghum stover , with or without a *Sesbania sesban* supplement
DZ	Kaitho et al. 1997[1]	R. J. Kaitho, N. N. Umunna, I. V. Nsahlai, S. Tamminga and J. Van Bruchem. 1997. Utilization by sheep of browse supplements with varying tannin levels: 1. Intake, digestibility and live weight changes	Sixty-six sheep fed basal diets of teff straw alone, or with a 190 grams of a supplement: lablab, tagasaste, leucaena, *S. goetzei*, or one of six accessions of *Sesbania sesban* (S 1190, S 1198, S2024, S10865, S15019 and S15019)

[1] Published articles, the rest are experimental protocols

Appendix Table 1. Continued

Location	Experiment	Publication or Protocol Title	Experimental Design
IB	Larbi (Unpubl.) 1996	Predicting intake and apparent digestibility by west African dwarf sheep and goats fed browse: effect of drying method on intake, digestibility and N utilization	Metabolism trial with 20 sheep fed four diets: a basal diet of Sorghum with shade or sun dried *Sienna siamea*; 17 day trial with 5 day collection period
SA	Fernandez-Rivera et al. (unpubl.)[a] 1993	Stocking rate and goat:sheep ration effects on livestock output and vegetation	Three year experiment, (1992-1994). Two blocks , 18 pastures, 2 of which are controls. Two stocking rates (0.25 and 0.5 TLU/ha) by four goat: sheep ratios (0:6, 2:4, 4:2 and 6:0) in two seasons (dry and wet), intact and esophageally fistulated animals used
BU	Sangaré & Fernandez-Rivera(unpubl.)[b] 1994 - 1996	Seasonal variation in rumen function, forage intake, fecal output, and quality of the diet selected by grazing cattle, goats and sheep	Three year experiment, (1994 - 1996). Eight intact, six rumen-fistulated, and six esophageally fistulated sheep grazed in Bundu with farmers livestock
SA	Fernandez-Rivera et al. (unpubl.)[a] 1994	Fernandez-Rivera, S., F. Mahler, P. Hiernaux and M. Turner. Evaluation of polyamid grains as markers to estimates the fecal excretion by cattle, sheep and goats.	Metabolism trial with fourteen rams fed *Eragrostis tremula ad libitum*, with or without a supplement consisting of wheat bran and salt. 15-day trial followed by 7-day collection period.
SA	Zouladeini et al. (unpubl.) 1993	Zouladeini, H., S. Fernandez-Rivera and J. M. Watier. The chemical composition, voluntary intake and digestibility of tree species by sheep in Niger.	Tree species are *Guiera senegalensis*, *Mitragyna inermis*, *Ficus gnaphalocarpa* and *Anogeissus leiocarpus*.
SA	Schlect (Unpubl.) 1995		Metabolism trial with thirty sheep fed five diets: basal feeds were groundnut hay and cowpea hay with or with out supplements *Combretum aculeatum* and *Pterocarpus lucens*.
SA	Fernandez-Rivera et al. 1994	Fernandez-Rivera, S., A. Midou and H. Marichatou. 1994. Effect of food allowance on diet selectivity and intake of pearl millet (*Pennisetum glaucum*) stover leaves on sheep. Anim. Prod. 58:249-256.	Metabolism trial with thirty Peulh Oudah rams fed millet stover *ad libitum*.

Appendix Table 2. The maths treatments used in the calibration process

Treatment	a = the derivative function	b = the gap	c = the first smooth segment	d = the second smooth segment
1	1	5	5	1
2	1	10	5	1
3	1	15	5	1
4	2	10	10	1
5	2	20	10	1
6	3	10	10	1
7	4	10	10	1
8	1	4	4	1
9	2	8	6	1
10	2	8	4	1
11	3	5	5	1

Appendix Table 3. Samples incorporated into the tannin calibration and validation

ID	SITE	NBUT	DOM	CP
NIG1	1	59.9	28.79	9.47
NIG2	1	59.9	28.79	9.47
NIG3	1	59.9	28.79	9.47
NIG4	1	48.7	44.84	9.61
NIG5	1	48.7	44.84	9.61
NIG6	1	48.7	44.84	9.61
NIG7	1	48.7	44.84	9.61
NIG8	1	48.7	44.84	9.61
NIG9	1	48.7	44.84	9.61
NIG10	1	2.4	53	11.1
NIG11	1	2.4	53	11.1
NIG12	1	2.4	53	11.1
NIG13	1	2.4	53	11.1
NIG14	1	2.4	53	11.1
NIG15	1	2.4	53	11.1
NIG16	1	2.4	53	11.1
NIG17	1	2.4	53	11.1
NIG18	1	2.4	53	11.1
NIG19	1	2.4	53	11.1
NIG20	1	2.4	53	11.1
NIG21	1	14.1	62.63	18.74
NIG22	1	14.1	62.63	18.74
NIG23	1	14.1	62.63	18.74
NIG24	1	14.1	62.63	18.74
NIG25	1	14.1	62.63	18.74
NIG26	1	14.1	62.63	18.74
NIG27	1	14.1	62.63	18.74
NIG28	1	14.1	62.63	18.74
NIG29	1	14.1	62.63	18.74
NIG30	1	14.1	62.63	18.74
NIG31	1	14.1	62.63	18.74
NIG32	1	14.1	62.63	18.74
NIG33	1	1.8	42.95	4.65
NIG34	1	1.8	42.95	4.65
NIG35	1	1.8	42.95	4.65
NIG36	1	1.8	42.95	4.65
NIG37	1	1.8	42.95	4.65
ETH38	2	6.6	46.16	9.43
ETH39	2	0	44.85	3.8
ETH40	2	35.1	45.09	10.04
ETH41	2	16.4	47.43	8.86

Appendix Table 3. Continued

ID	SITE	NBUT	DOM	CP
ETH42	2	7.4	52.39	7.75
ETH43	2	103.4	50.83	7.89
ETH44	2	7.4	54.71	8.79
ETH45	2	71.9	44.5	7.98
ETH46	2	71.9	49.22	7.9
ETH47	2	30.5	47.05	8.26
ETH48	2	23.1	45.16	9.88
ETH49	2	16.4	48.45	9.19
ETH50	2	23.1	52.26	9.45
ETH51	2	35.1	54.21	9.59
ETH52	2	71.8	50.97	9.76
ETH53	2	30.5	46.62	7.83
ETH54	2	30.5	48.13	7.92
ETH55	2	71.8	52.09	9.53
ETH56	2	103.4	49.26	7.67
ETH57	2	23.1	48.61	9.67
ETH58	2	6.6	52.31	8.87
ETH59	2	35.1	43.66	9.46
ETH60	2	6.6	50.69	9.68
ETH61	2	2.5	50.63	7.02
ETH62	2	30.5	44.04	8.55
ETH63	2	71.8	46.62	9.69
ETH64	2	71.9	50.29	8.36
ETH65	2	103.4	48.12	8.08
ETH66	2	2.5	50.39	6.72
ETH67	2	2.5	49.54	6.86
ETH68	2	35.1	55.43	9.49
ETH69	2	35.1	51.46	9.7
ETH70	2	35.1	44.61	9.28
ETH71	2	7.4	54.72	8.29
ETH72	2	7.4	51.14	7.52
ETH73	2	7.4	51.64	7.66
ETH74	2	6.6	48.93	8.38
ETH75	2	6.6	55.8	8.98
ETH76	2	6.6	45.07	8.88
ETH77	2	16.4	56.6	8.99
ETH78	2	0	50.72	3.8
ETH79	2	2.5	53.69	6.88
ETH80	2	71.9	56.58	8.51
ETH81	2	2.5	47.93	6.67
ETH82	2	7.4	47.56	8.17
ETH83	2	0	45.16	3.8
ETH84	2	16.4	55.18	10.52

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