IN VITRO DIGESTIBILITY AND NUTRIENT CONTENT OF SELECTED MUNTANT VARIFTIES OF VELVET BEANS (MUCUNA PRURIENS)

BY

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DECLARATION

This dissertation has been composed by myself and has not been accepted in any previous application for a degree qualification. The work is a record of what has been done by myself and all sources of information have been acknowledged by means of references.

Joseph I. Simukoko

May 2009

DEDICATION

This report is dedicated to my Dad, the late Dr Y. T. Simukoko. I wish you were here to see how your son has grown up.

ABSTRACT

The study was conducted to determine the nutrient composition and in vitro digestibility of selected seeds of mutant varieties of *Mucuna pruriens*. The two varieties of Velvet beans used in this study were Sommerset and NIRS. Samples of Velvet Beans were grown at Liempe farm of the University of Zambia located in Choongwe District of Lusaka Province Agro-ecological region II of Zambia. Rumen liquor was obtained from two goats from Chibolya market a local goat slaughter market in Lusaka. Thus the focus of the study was to evaluate the nutritive value of the two varieties and their mutants. These were related to, nutrient content and in vitro digestibility of the beans in goat rumen liquor. Proximate analysis was also carried out on the beans. There were significant differences between velvet bean varieties and treatment means for the following moisture (0.6 - 5.11%), ash (2.9 - 4.75%), phosphorus (1.02 – 2.9%), crude fat (6.3 – 12.25%), and crude protein (11.9 – 27.8%). Calcium only showed significance in the interaction between treatment means, which suggests that all the treated samples contain the similar amounts of Calcium. The grand mean for the Calcium was found to be 1.5%. The *in vitro* Dry Matter Digestibility of velvet beans was not significantly different for all the samples. However, there was a significant difference at $p<0.05$ between the control (soya bean 95.02% digestibility) and the mutant varieties of velvet beans. The velvet beans digestibility results (81 - 93 %) were found to be comparable to those found in sorghum seeds this suggests that velvet beans can be incorporated in goat diets, at some percentage inclusion, with the goats not experiencing any major problems due to their high digestibility. Hence, it can be concluded that the high yielding varieties of velvet beans can be used in place of conventional protein sources such as soya beans in dry season feed supplement for goats.

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ABBREVIATIONS

ANCRASS	Ankara Nuclear Research Center in Agriculture and Animal Sciences
Ca	Calcium
CP	Crude Protein
DB	Determinate bush
ED	Effective Digestibility
HTR	Heat Treated
IVDMD	*in vitro* Dry Matter Digestibility
IVOMD	*in vitro* Organic Matter Digestibility
MB	*Mucuna* beans
ND	Not Determined
NIRS	Near Infra Red Spectrophotometer
NS	Not Significant
NTR	Non- treated
P	Phosphorus
SD	Standard Deviation
SS	Sommerset
TRT	Treatment
VVB	Velvet beans
VVBSM	Velvet Beans Seed Meal

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CHAPTER 1

1.0 INTRODUCTION

There has been an increase in malnutrition in Zambia, mostly in under-five children due to the lack of sufficient protein in their diet. An increase in the production of goats in Zambia can be used to increase food security and protein in the diets of the average Zambian person thus mitigating the problem of malnutrition. However, due to high cost of animal feed most goats are kept at a subsistence level using the extensive system of farming. Furthermore, goats have little and in some cases no dry season feed supplementation when most fodder crops are low in nutrients. In trying to mitigate these problems velvet bean seeds can be used in dry season feed supplements for goats.

Legumes such as Velvet Beans contribute one of the richest and low cost sources of proteins in human and animal diet. However, their utilization is limited by anti- nutritional factors that interfere with the digestibility of nutrients contained in foods. These factors together with protein digestibility are important when food value of different crops is assessed (Janardhanan *et al.* 2003). As a result many small-scale farmers only grow velvet beans for improving soil fertility and the seed goes to waste due to lack of alternative uses.

There have been many efforts to try and address the problem of the anti-nutritional factors present in velvet beans, the most common method used to reduce heat-labile anti-nutritional factors in raw velvet beans and other pulses is heat treatment (Gonzalo *et al.* 2002). Other methods involve soaking the beans and then boiling (Siddhuraju *et al.*, 1996; Del Carmen *et al.*, 1999; Nyirenda *et al.*, 2003). These methods have been found to be successful in reducing the presence of heat and non heat labile anti-nutrients including L-dopa, which is a non-heat labile anti-nutrient.

However, it is difficult to make recommendations as to which method should be used to improve the quality of velvet beans, in particular when economic considerations are taken into account. In no case, can the simple name of a particular process be taken as a guarantee of the quality of the end product. The exact parameters and conditions applied need to be known and the end product involved needs to be assessed chemically. Furthermore, these methods are tedious, time consuming, require a lot of labor and need expensive equipment such as ovens and large industrial stoves. As a result their application is impractical for small-scale farmers and is an unnecessary additional cost for commercial farmers.

It is possible to breed velvet beans seeds with improved digestibility through mutational breeding. Crop improvement using classical induced mutagenesis is now well standardized. A large number of new promising varieties in different crops have successfully been developed world wide using both physical and chemical mutagens. Voluminous literature is now available on basic and applied aspects of mutagenesis. Mutation technique has been refined and holds promise of generating a much wider desirable variability than classical breeding. Recent advances in technology combined with classical mutation breeding offers new and exciting opportunities for development of new varieties of velvet beans that may have a better nutrient profile with low anti-nutrients. Although there is some information available on the nutritional and anti-nutritional properties of velvet beans there has been relatively few systematic collections and evaluation of diverse velvet beans accessions in particular mutant varieties of velvet beans (Janardhanan *et al.* 2000).

By measuring feedstuff digestibility and incorporating this information in an appropriate ration-balancing package, it is possible to determine the digestibility of velvet beans. The determination of feedstuff digestibility is not a simple task. Unlike many routine feedstuff analyses, there is no single, recommended procedure for digestibility analysis. Nutrient digestibility may be determined *in vivo* (in the animal), *in situ* (in place), or *in vitro* (in glass). The Tilley and Terry *in vitro* digestibility test has several advantages. Tests can be conducted with only a very small quantity (0.5 grams) of material. Both rates and extents of digestion can be determined. The most striking advantage for the Tilley and Terry system is its degree of precision. Coefficients of variation in the range of one to two percent are readily attainable. Much of the precision in this system can be attributed to the use of finely ground test samples, which reduces sampling error (Siciliano-Jones, 1997).

It is against this background that this study was done, with the objective of assessing whether the creation of velvet bean mutagens improved the nutritive value of the beans and whether it enhanced the digestibility of the bean. The specific objectives were:

- To determine the nutrient content of the velvet beans.
- To carryout, *in vitro* digestibility trial in goat rumen liquor in order to compare the digestibility of the velvet bean mutagens against the parent bean and against soya beans of known digestibility.

CHAPTER 2

2.0 LITERATURE REVIEW

2.1 Origins and Distribution of Velvet Beans

The culturing of annual velvet beans originates from Southern Africa and distributed throughout the tropics and temperate regions of the world, (Kawonga, 2002; Viera and Carvalho, 1996).

2.2 Botanical Characteristics

Velvet Beans (*Mucuna pruriens*) belongs to the genus *Mucuna* of the family Leguminosaea. This genus encompasses both annuals and short-lived perennials species. In literature, the taxonomy of species in this genus is synonymous. However the main differences among cultivate species are in the character of the pubescence on the pod, seed color and the number of days to harvest the pods. The velvet beans can have non-stingy, oppressed hairs on the pods while other types (commonly known as *co-witch*) have abundant long stingy hairs (Kawonga, 2002; Kumwenda and Gilbert, 1998; Viera and Carvalho, 1996).

2.3 Climatic Requirements and Yield

This crop grows best in warm and wet climatic conditions. The edaphic environment is less restrictive as the crops can grow in a variety of soils and can tolerate poor soils types such as sandy or laterite soils. However, it neither does well in water logged nor frost prone areas. Under favorable conditions, the vines can grow up to 10-14m in length and producing 4-9t/ha dry matter of herbage while depositing about 300kg of nitrogen per hectare (Kawonga, 2002; Kumwenda and Gilbert, 1998).

2.4 Mutation Breeding

In order to come up with varieties of Velvet Beans, which have low anti-nutrient content, yet have high biomass yield, productivity and nutrient content, mutation breeding can be employed. Mutation is the change in the genetic material (i.e. DNA) or the heritable change in the genetic make up of the genetic material (Montelone, 2004). Mutational breeding is therefore a conventional line of genetic science that deals with both heritable and phenotypic changes (traits) intended to bring about new and improved varieties among selected agricultural crops (Lacaundula, 2005).

A variety of procedures may be used. Pollen may be mutagenized and then used in pollination. Dominant mutations will be expressed in the next generation, and further generations of selfing reveal recessives. Alternatively, seeds may be mutagenized. A cell in the enclosed embryo of a seed may become mutant, and then it may become part of germinal tissue or somatic tissue. If the mutation is in somatic tissue, any dominant mutations will show up in the plant derived from that seed, but this generation will be the end of the road for such mutations (Griffiths *et al*, 2000).

Aiming at the aforesaid purposes, a project by the Ankara Nuclear Research Center in Agriculture and Animal Sciences (ANRCAAS) in Turkey was started in 1982 on soybean mutation breeding. This project completed successfully and two mutant soybean varieties were registered in 1994 and named TAEK-A3 and TAEK-C10. The new varieties exhibited higher seed and oil yields per unit area, higher first pod height and protein content than the control (Halitligil, 2008).

Examples of Irradiation Facilities

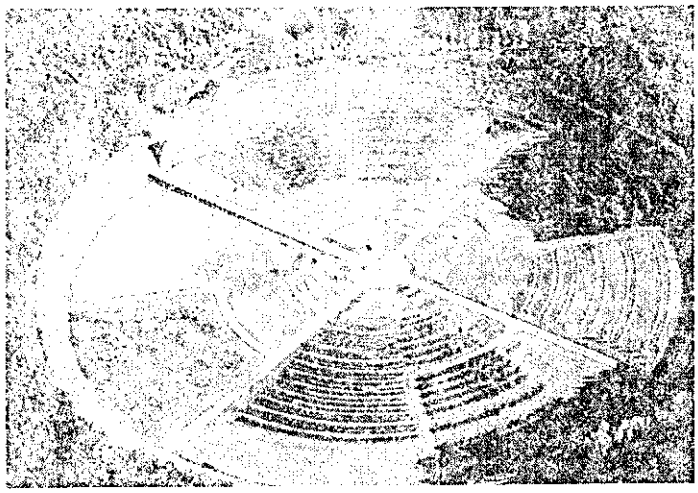


Figure 1: An example of a Gamma irradiation field

Gamma radiation facility

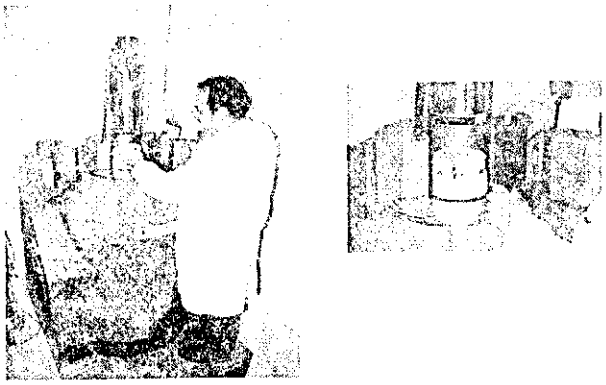


Figure 2: An example of a Gamma radiation facility

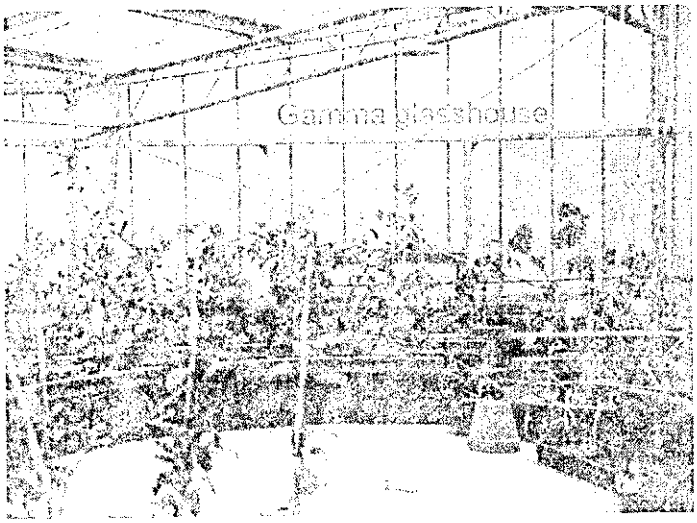


Figure 3: An example of a Gamma glasshouse

FAO/IAEA Mutant Varieties Database

Examples of Irradiation Facilities

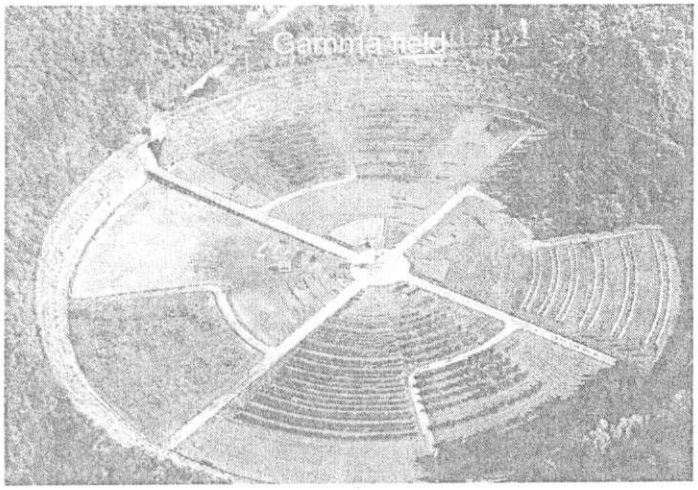


Figure 1: An example of a Gamma irradiation field

Gamma radiation facility

Figure 2: An example of a Gamma radiation facility

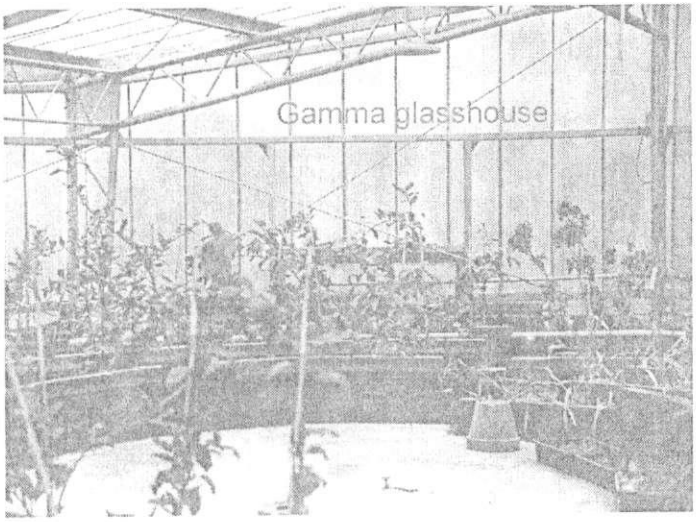


Figure 3: An example of a Gamma glasshouse

FAO/IAEA Mutant Varieties Database

Figure 4: Mutant varieties

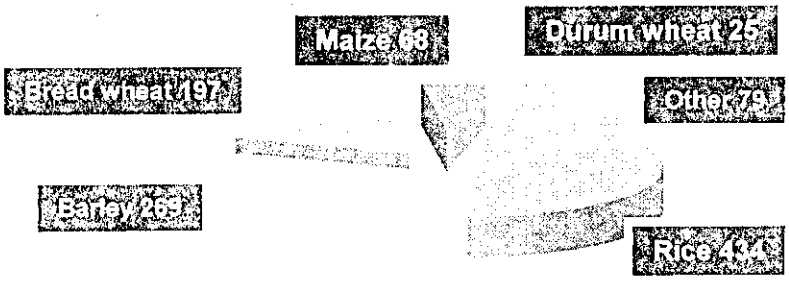


Figure 5: Number of Mutant Varieties Released Per

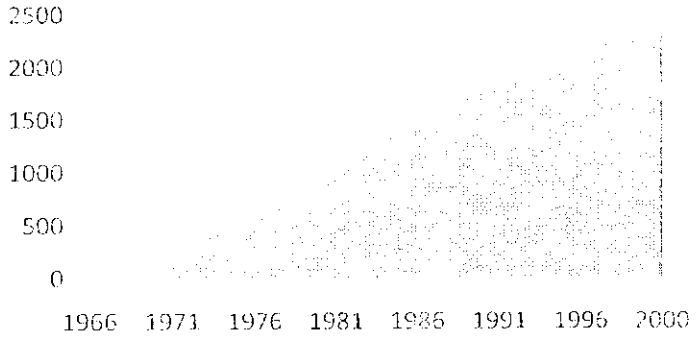


Figure 6: Number of Mutant Varieties of Various Crops

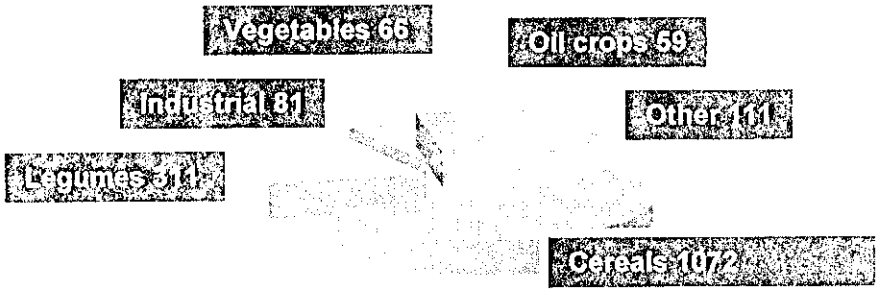
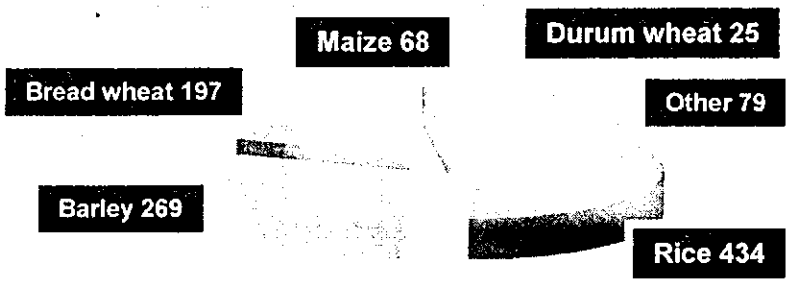


Figure 4: Mutant varieties



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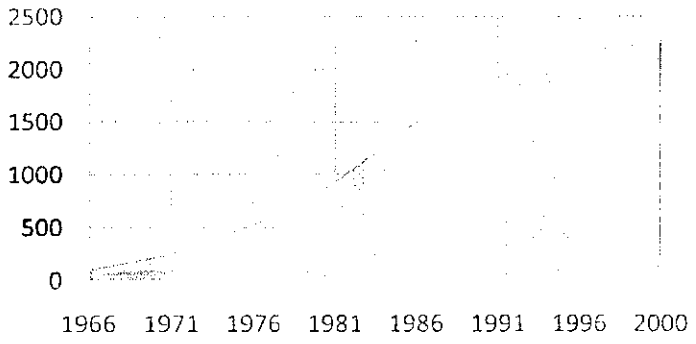
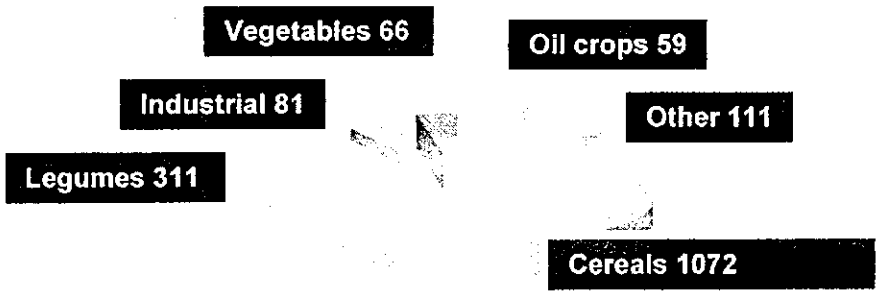


Figure 6: Number of Mutant Varieties of Various Crops



CEREALS - 1072 of 1700 crop accessions (2000)

2.5 Nutrient Composition

The proximate analysis show that Velvet Beans is rich in protein, energy and minerals including Potassium, Calcium and Phosphorus to adequately supplement human and livestock needs (Siddhuraju *et al*, 1996; Laurena *et al*, 1991).

2.5.1 Protein

Velvet beans may contain up to 28%-31% crude protein. While the amino acid profile analysis of the genus *Mucuna* provides most of the essential amino acids that is lysine, cystine, methione, tryptophan and leusine (Laurena *et al*, 1991; Siddhuraju, *et al*, 1996). Even for the deficient amino acids it would still meet between 50% -97% of the FAO/WHO (1990) recommended amino acid requirement. The high levels of significantly complementing cereal crops since cereals are deficient with lysine (Kawonga, 2002).

2.5.2 Energy

The fatty acid (FA) profile show that Velvet Beans are low in fat however, they contain a wide range of FA's in proportion to most common tropical pulses and Soya Bean (*Glycine max*). Distinctively, Velvet Beans is a rich source of oleic acid, the essential fatty acid (EFA). However, it is inferior to Pigeon Pea (*Cajunaus cajan*) and Soya Bean with respect to either the amount or the proportions of Essential Fatty Acids present in the grain legumes (Kawonga, 2002).

2.5.3 Minerals

The composition of minerals present in Velvet Beans make it a rich source of all macro minerals these include Sodium, Potassium, Magnesium, Calcium and Phosphorus. In addition there is adequate amount of Manganese, Iron, Copper and Zinc (Kawonga, 2002).

Table 1. Chemical, Fiber and Mineral Composition of *Mucuna pruriens* Seeds

Component	g/kg DM
Chemical Composition	
Moisture (g/kg FW)	71
Crude Protein (Nx6.25)	314.4
Crude Fiber	51.6
Crude Fat	67.3
Ash	41.1
Carbohydrates (by difference)	525.6
Energy content (kJ/kg DM)	16565.2
Fiber	
ADF	96
NDF	213
Hemicellulose	117
Cellulose	82
Lignin	11.2
Mineral Composition	
Sodium	174.2
Potassium	13304.3
Calcium	2857.1
Magnesium	851.2
Phosphorus	4065.2
Manganese	5.6
Iron	65.4
Copper	23
Zinc	20.4

Siddhuraju, (1996)

Table 2. Chemical composition of *Mucuna pruriens* Grains and Seeds

Chemical fraction	Grain g/kg DM	Husk g/kg DM
Ash	35.3	58.0
Crude protein	278.5	44.0
Gross fat	25.1	11.0
Neutral detergent fiber	259.5	597.7
Acid detergent fiber	88.0	369.5
Lignin	ND	77.5
Acid detergent insoluble N	4.5	0.6
Calcium	4.6	9.5
Phosphorus	2.4	0.3
Nitrogen free extract	394.6	202

ND: Not Determined

Eilittä et al., (2003)

2.6 Anti-Nutritional Factors

All oilseed plants have natural anti-nutritional factors. They are described as non-fibrous naturally occurrence substances exerting negative effects on the performance or health of animals (Willis, 2007).

2.6.1 L -Dopa

L-dopa is a toxic non-protein amino acid that occurs in several higher plants. Consumption of improperly boiled seeds of *Mucuna* led to increase in body temperature and skin eruptions among the traditionally consuming tribes, Kanikkars, in Kerala and this was attributed to the presence of high levels of L-Dopa in the seed (Janardhanan, 2003). Other side effects reported for L –Dopa include state of confusion and vomiting and diarrhea (Afalobi *et al* 1985). Unfortunately L-Dopa is not heat labile. Heat releases the L-Dopa from being held in the seed matrix to a free from that eludes quickly when soaked in water (Nyirenda *et al,* 2003)

2.6.2 Phytic Acid

Most cereal grains, pulses, nuts, and oil seeds contain Phytic acid. It acts as a primary phosphorus reservoir accounting for up to 85% of total phosphorus in grains and legumes. A great deal of research has focused on the unique structure of Phytic acid that bind to minerals, proteins and starch, to lower bioavailability of minerals, to form complexes with proteins and starch, and inhibit enzymatic digestion of both protein and starch (Liener, 1994).

2.6.3 Phenols and Tannins

Broadly defined, Tannins are Polyphenolic substances are chemicals having a molecular weight greater than 500 (Liener, 1994). Phenols are known to decrease the digestibility of proteins, carbohydrates, and minerals in addition they may lower the activity of digestive enzymes and may cause damage to the mucosa of the digestive tract (Liener, 1994). Tannins have negative effects in animal nutrition because they cause the mouth and the digestive tract to reduce the viscosity of the digesta. This property may in turn negatively affect the digestibility of lipids and lipase activity (Liener, 1994).

2.6.4 Hydrogen Cyanide

This acid is liberated through enzymatic action on a cyanogenic glycoside present in the plant tissue. The ingestion of a particular cyanogen can cause acute to chronic cyanide poisoning.

2.6.5 Beherenic Acid

The Beherenic acid content of velvet beans ranges from 0.7-4% of total fatty acid. This means that velvet beans have a potential to cause atherogenicity, which was reported in groundnuts (*Rachis hypogea*) where this acid is between one and three percent only (Kawonga, 2002)

2.6.6 Lectins

These are haemagglutinnins that agglutinate Red Blood Cells. They are also known to reduce protein digestibility and eliminate digestive enzymes from the intestinal membranes hence affect the efficiency of digestion. The absorption of other non –nitrogen containing nutrients may also be impaired by Lectins bound to the intestinal coat surface. Deleterious effects of the Lectins may therefore be a combination of toxicity and malnutrition (Liener, 1994).

2.7 PROCESSING OF VELVET BEAN SEED

Heat treatment is the most common method of processing Velvet Beans. Heat is achieved by various means, e.g., by roasting at 120 °C for 30 min or grilling for an hour after pre-soaking the seed for 24hrs and thereafter dehulling. Another method involves autoclaving for 30mins and then soaking the grits for 24hrs, then boiling for an hour then soaking for 24hrs (Siddhuraju *et al.*, 1996; Del Carmen *et al.*, 1999; Nyirenda *et al.*, 2003).

Siddhuraju *et al.* (1996) found dry heat treatment to be effective in reducing L-Dopa in Velvet Beans. They attributed the reduction in L-Dopa to the racemization under roasting. However, heat treatment through roasting or boiling has been found to be most effective in reducing other anti-nutrients in Velvet Beans (Nyirenda *et al.*, 2003). Heat treatment by thorough roasting and cooking can successfully reduce HCN levels by as much as 68%. Dry heat treatment reduced the content of Phytic acid by 36% and autoclaving reduced phytate content 47% in Velvet Bean (Siddhuraju et al., 1996). However, Beherenic acid is more stable to heat and soaking treatment. Autoclaving at 139°C for one hour and soaking the seed for 20hr only removed 15 and 1.5% respectively, of the acid in winged bean. Trypsin inhibitors (TI) are known to be heat labile as heat disintegrates their structure. Significant reductions of Trypsin Inhibitors, in Velvet Bean seed, of up to 93% by roasting and 96% by autoclaving were reported by Siddhuraju *et al.*, (1996).

A significant reduction of hemagglutinating activity due to Lectins was reported among all blood groups (ABO) when the seed were subjected to both dry heat treatment and autoclaving (Siddhuraju *et al.*, 1996). Roasting can reduce the negative effects of protease and alpha-amylase inhibitors on digestion by 96% and cooking velvet beans removes the negative effects of these anti-nutritional factors completely (Siddhuraju *et al.*, 1996).

2.8 The Feeding Value of Velvet Beans

2.8.1 Poultry

In poultry rations, untreated Velvet Beans was found to replace Soya Beans wheat bran and middlings up to 10% level in the diet without causing significant reduction in the feed intake, loss in market weight of the birds and egg production (Del Carmen *et al*, 1999). On the other hand (Olaboro *et al*, 1999) were able to use double the amount when they used autoclaved beans combined with fishmeal (30%) was used in the diet. The inclusion of fishmeal at that level (30%) could have masked the negative effect of velvet beans beyond the (10%) limit of (Del Carmen *et al.* 1999).

In another study by (Iyayi and Taiwo, 2003) the effect of diets incorporating *Mucuna pruriens* seed meal on the performance of laying hens and broilers was studied. In the first one hundred 18-week old Black Nera birds were randomly allocated on body weight basis to 4 experimental diets with 40% of Soya beans meal was replaced with autoclaved raw velvet bean seed meal (VVBSM). The results of the study suggest that 6% processed VVBSM in diets of layers had no adverse effects on egg qualities. In broilers diets containing 6-12% Roasted Velvet Bean Seed Meal performed as well performed as those fed on sole Soya Bean Meal. Levels higher than 12% caused a reduced performance of the birds because the anti-nutritional factors in *Mucuna* disrupt the digestive tract and organs.

2.8.2 Pigs and Rabbits

In the USA, It was reported that velvet beans were unsatisfactory in swine nutrition as it led to as it led to poor production, even vomiting and diarrhea when large amounts were fed (Viera *et al*, 1996). The leaves of velvet beans, at 50% flowering, fed to rabbits did not cause any observable toxicity symptoms (Viera *et al*, 1996).

2.8.3 Cattle

Velvet Beans inter-cropped with maize is either grazed *in situ* after the harvest of maize or ensiled together with maize to improve quality and yield of the silage (Titteroton and Maasdorp, 1997). Velvet Beans was found more economical when the sorghum was inter-cropped with velvet beans and 100kg N/ha was applied compared with sole sorghum crop. The vines can be cut into hay. However, its product is relatively poor because of its dense and matted growth characteristics that make it difficult not only to cut but also to care the vines resulting in a dark off-colored hay product (Kawonga, 2002).

2.8.4 Goats

A study to assess the impact of *Mucuna* bean (*Mucuna* Spp.) supplementation on milk production of goats was carried out (Mendoza, 2003). The objective of the trial was to determine the impact of supplementation with *Mucuna* bean (*Mucuna* spp.) on milk production of goats during a 28-day period. *Mucuna* bean intake was 872 ± 361 g DM a-1 d-1 and that of Ramon foliage was 1144 ± 38 g DM a-1 d-1. *Mucuna* bean supplementation increased the total dry matter intake in a linear fashion (DMI = 402 + 1.228X, R2 = 0.72).

In a study by Castillo-Caamal *et al*, (2003) the feeding of *Mucuna* beans to small ruminants of Mayan farmers in the Yucatan peninsula, Mexico was assessed. *Mucuna* bean (MB) generally improved animal performance in comparison to the control. Weight changes during the study period for growing lambs, kidding goats, double kids pre-suckling, and single kids pre-suckling were 63 (control) vs. 95 (with MB), -1.40 vs. -0.85, 86 vs. 130, and 110 vs. 214 g a-1 d-1, respectively. For post-suckling kids and non-pregnant goats, no differences in live weight (LW) were observed between control and MB treatments. Farmers generally commented favorably on the MB supplementation, saying that it was useful, helped during dry season, increased animal weight and milk production, and animals consumed MB well. Most farmers found no disadvantages, but two farmers mentioned the same disadvantage: soaked MB tend to become infested with grubs, and animals do not consume them.

2.8.5 Other

The other way of using velvet beans is to grind the pods and the resultant meal was used as protein supplement in sheep, horses, and mule diets replacing cotton seed cake (Kawonga, 2002).

2.9 *In Vitro* Digestibility Studies

The technique first described by Tilley and Terry, (1963) has been the most commonly used in vitro method for predicting digestibility and as a selection tool for improving the nutritional quality of forages. Several modifications of the original procedure have been used to maximize the digestion process because in vitro systems that do not maximize digestion kinetics may not detect differences in substrate digestion (Grant and Mertens, 1992b). Maximizing in vitro digestion depends on several factors, including dilution of the ruminal inoculum, type of buffer used, particle size of the sample, type of mill used for grinding, and type of diet the donor animal is fed.

Ruminal inoculum is typically strained through several layers of cheesecloth and diluted (20:80) in saline solution, artificial saliva, or various buffers. Craig *et al.*, (1984) suggested that strained ruminal fluid alone was not as effective as strained ruminal fluid plus an inoculum of particulate-associated bacteria for simulating ruminal fermentation of fiber from different feedstuffs. Varel and Kreikemeier, (1995) compared the *in situ* and the Tilley and Terry techniques using alfalfa or brome grass as substrates. Differences in lag time, rate of digestion, and extent of digestion were noted between the two techniques. Lag time was shorter, rate was faster, and extent of digestion was greater with the in situ than with the in vitro technique. Differences were attributed to a lower microbial concentration with the in vitro technique compared with microbial concentrations in the rumen of the animal.

Attempts to increase the microbial concentration *in vitro* have not been successful because of a rapid accumulation of end products and a subsequent decrease in pH. The decrease in pH might be of major concern when using the *in vitro* technique to study fiber digestion because cellulolytic bacteria are more sensitive to low pH than are amylolytic species (Therion *et al.*, 1982). However, Terry *et al.*, (1969) demonstrated a minimal decrease in cellulose digestion with an addition of 40% glucose when pH was maintained at 6.8. Grant and Mertens (1992a) developed an in vitro buffering system capable of pH control between 5.8 and 6.8 that has been successfully used to study fiber digestion in vitro (Grant and Mertens, 1992c). Starch digestion also seems to be affected by pH in vitro. Richards *et al.*, (1995) tested different dilutions (1:1, 1:2, 1:3, and 1:4) of strained ruminal fluid and artificial saliva to study starch digestion in vitro. Rate and extent of starch digestion were similar for the 1:2, 1:3, and 1:4 inoculum dilutions but were higher than with the 1:1 dilution. The lower rate and extent of starch digestion with the 1:1 dilution could have resulted from a lower pH noted in vessels when ruminal fluid was less diluted. The authors recommended that dilutions of 1:2 or 1:3 be used when studying starch digestion of grains that are rapidly fermented.

A study was undertaken to evaluate the in vitro gas production and digestibility of Mucuna bean by (Sandoval *et al.*, as cited by Mucuna News, 2002). The results indicated that Mucuna has potential to replace conventional energy sources (e.g. maize and sorghum) in ruminant diets. In addition to the beans, the husks can be without major problems because of their high digestibility (97.94 $\pm$0.35 and 96.02 $\pm$ 1.31% for the beans and 78.96 $\pm$ 1.69 and 78.85$\pm$ 1.75% for the husks).

Table 3. Chemical compositions, *in vitro* dry matter (IVDMD) and organic matter (IVOMD) Digestibility (%) and metabolizable energy content *Mucuna pruriens* beans and husks

Component	Bean	Husk
Dry Matter	98.29	98..85
Crude Protein	27.34	4.84
Ash	3.44	5.78
Ether Extract	2.41	16.82
Neutral Detergent Fibre	40.79	58.87
Acid Detergent Fibre	n.d.	37.49
Lignin	n.d.	11.22
IVDMD	97.94+0.35	78.96+1.69
IVOMD	96.02+1.31	78.96+1.75
ME(MJ.kgDM)	13.9	11.14

Sandoval-Castro *et al.*, (2003)

Table 4. Rumen DM degradability (%) of the velvet bean grain and husk, evaluated in three rumen cannulated cows fed with a basal diet of *Pennisetum purpureum.*

Incubation time (h)	Grain	Husk	Standard Error
3	61.5	ND	---
6	68.0	40.8	2.79
12	78.3	46.7	1.63
24	99.4	61.0	1.03
36	100	73.6	2.65
48	100	81.0	1.53
72	ND	84.8	---

ND: Not Determined
Ayala et *al.*, (2003)

Table 5. Parameters of the rumen DM degradability of the velvet bean grain and husk according to the equation D = a + b (1-exp-ct), and the estimated effective degradability at a rumen outflow rate of 5% (ED5%).

Degradability Parameter (%)	Grain	Husk	Standard Error	Probability
A	45.3	27.7	5.80	NS
B	54.8	66.7	2.79	NS
C	9.7	3.0	1.18	0.04
A+b	100	94.4	2.36	NS
ED5% 81.3	53.2	0.71	0.001	
DM wash loss (SD)	28.1 (7.6)	13.0 (4.5)		
Truly soluble DM (SD)	35.8 (7.6)	26.9 (4.0)		

NS: Not a significant difference (P > 0.05)
SD: Standard deviation

Feeding Meat Goats

In order to raise goats at a low cost, the producer must maximize the use of forage. Feeding of goats cannot be discussed without mentioning the impact of the kidding cycle. Most goats are seasonal breeders, beginning to cycle with the shorter and cooler days of the year. They will continue to cycle (unless they are bred) every 21 days or so, until days lengthen in early summer. The time of kidding determines the period of highest nutritional demand, as late pregnancy and early lactation are critical times for the doe and kid. By manipulating the breeding date, the producer can see to it that peak needs hit when more forages are available, rather than during months when only harvested feed can be used (Pinkerton and Pinkerton, 2000).

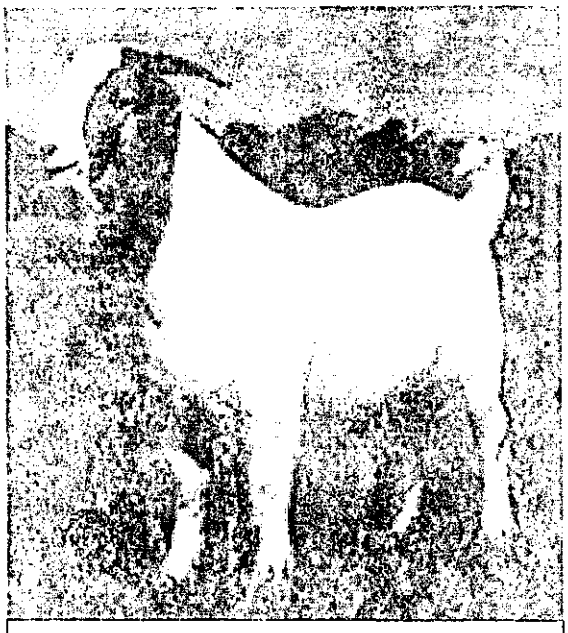


Figure 7: Boer goat

Underfeeding during critical times is not profitable. Neither is feeding large amounts of purchased feed. The manager must plan the production cycle to avoid both these pitfalls. One needs to be aware of the pattern of forage availability in their area, and try to use pasture or browse as much as possible. In addition to pasture or browse, it may be necessary at some times of the year to supplement goats with extra protein and/or energy. To do that efficiently, it is important to understand the requirements of the animal and to meet those needs in the most cost-effective manner (Pinkerton and Pinkerton, 2000).

Table 6: Dietary Protein and Energy Requirements of Goats*

Class of Goat	Ave. feed intake/day, kg[1]	% Crude Protein	% TDN
Growing doeling, 99 kg[a]	5.28	8.8	56
Growing male kid, 145.2 kg[b]	6.38	9	57
Yearling doe, 198 kg[c]	10.12	10	56
3 yr. old doe, 242 kg[d]	11	11.7	69
Mature buck, 484 kg[e]	11.66	9	55
Dairy doe, 330 lb[f]	16.5	11.6	71

*Approximations; based on dry matter in the feeds eaten
[1]Calculated on basis of the dry matter in the feeds eaten
aGrowing at the rate of .55 kg/day
bGrowing at the rate of .726 kg/day
cYearling female, last trimester of pregnancy and growing
dMilking 2 qt/day - enough for twins
eNot gaining weight, moderate activity
fNubian, milking 1 gallon/day of 4.0% butterfat
Pinkerton and Pinkerton, (2000)

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eNot gaining weight, moderate activity
fNubian, milking 1 gallon/day of 4.0% butterfat
Pinkerton and Pinkerton, (2000)

Table 7: Practical Dietary Recommendations for Feeding Goats

Class of Goats	% Protein	% TDN
Growing kids, dry does, and bucks	9 -10	54-58
Growing kids, dry does, and bucks	10 -11	56-60
Lactating goats	12 -13	62-68

Pinkerton and Pinkerton, (2000)

During the warm-season grazing period, goats will very likely meet all their nutritional requirements from whatever combination of forages is available; only a trace-mineralized salt and possibly some phosphorus would be needed in addition (Pinkerton and Pinkerton, 2000)

However, in late fall and winter there is need to supplement. It s important to note that goats are notoriously wasteful hence an addition of about 20 percent more feed is necessary to allow for waste. Goats are choosy, and will refuse feed that is not palatable (Smith and Sherman, 1994)

Finally, when feeding goats it is very important to observe closely and adjust feeding practices based on how the animals are doing. A ration that looks adequate on paper may turn out to be unpalatable, or may need to be increased due to severe weather conditions, or may be overly generous if the goats are finding plenty of browse. A properly nourished animal will be healthier, and more able to handle stress and bad weather. An over-fat animal will have a whole set of problems, and will be a drain on the budget as well (Smith and Sherman, 1994).

CHAPTER 3

3.0 MATERIALS AND METHODS

3.1 Materials

- Velvet Beans Seeds
- Rumen liquor from 2 goats
- Laboratory Chemicals for each procedure as specified in the AOAC procedures AOAC methods, (1998) and for *in vitro* dry matter digestibility (IVDMD) according to Tilley and Terry, (1963).
- Laboratory equipment for each procedure as specified in the AOAC procedures AOAC methods, (1998) and for *in vitro* dry matter digestibility (IVDMD) according to Tilley and Terry, (1963).

3.2 Methods

The processing of the sample was conducted at the field station of the School of Agriculture Sciences, University of Zambia. The two varieties of Velvet beans used in this study were Sommerset and NIRS varieties. Samples of Velvet Beans were grown at Liempe farm of the University of Zambia located in Choongwe District of Lusaka Province Agro-ecological region II of the Zambian Agro-ecological classification system. Rumen liquor was obtained from two goats from Chibolya market a local market in Lusaka.

3.2.1 Processing of the Velvet Beans

3.2.1.1 Dehulling

The samples were soaked in warm water until the seed coat could easily be removed.

3.2.1.2 Drying

The samples were dried on crucibles in a Memmert oven (Model 500, Memmert Co. Schwabach, Germany) at dried at 30°C for 12hrs.

3.2.1.3 Heat Treatment

The samples were roasted 120 °C for 30 minutes in a Memmert oven (Model 500, Memmert Co. Schwabach, Germany)

3.2.1.4 Grit Making

A Wiley Mill with a 5mm sieve was used to produce 4mm grits.

3.2.2 Proximate Chemical Composition Analysis of Velvet Beans

The analysis followed the standard AOAC methods, (1998).

3.2.2.1 Moisture content

This was determined by drying 2g of the sample in a Memmert oven (Model 500, Memmert Co. Schwabach, Germany) at 110°C for 2hours (AOAC methods, 1998).

3.2.2.2 Ash

The determination of ash and mineral extraction was done by combusting 2g of the sample in a Nabertherm Mufile Furnace (Nabertherm Co., West Germany) at 550°C. The ash used to determine Calcium and Phosphorus.

3.2.2.3 Calcium

The minerals in the ash were extracted by boiling as in 10ml of 2N HCl. The solution was then filtered out into a 100ml flask and made up to the 100ml mark by washing the residue with hot distilled water. Calcium was then precipitated with Ammonium Oxalate and titrated to a faint pink with N/10 Potassium Permanganate.

3.2.2.4 Phosphorus

Phosphorus-diluted solutions were read by a colorimeter at 660nm wavelength. A standard curve of the sample solutions was used to determine the concentration of the sample solutions.

3.2.2.5 Crude Protein Analysis

This was carried out by digesting 2g of the sample in a Foss Tecator Digestion System, (Foss Tecator Co., Hoganas, Sweden), at 420 °C for 1hr and distilled in a Markham semi-micro Kjeldahl apparatus.

3.2.2.6 Ether extract

This was determined using 2g of sample placed in extraction thimbles in a Soxhlet flask.

3.3 *In vitro* Digestibility

In vitro dry matter digestibility (IVDMD) was estimated according to Tilley and Terry, (1963). A 0.5g sample of velvet bean seed meal and soya bean meal were incubated with 28ml McDougall's artificial saliva and goat rumen liquor in a thermostatically controlled circulating water bath for 48 hours respectively. Rumen liquor was obtained from two goats from a local market in Lusaka. After incubation the samples were filtered and In vitro dry matter digestibility (IVDMD) was determined.

3.4 Statistical analysis

Analysis of variance (ANOVA) was done using Genstat statistical programme with the completely randomized statistical design with no treament blocking. Means were reported as least square means. Differences between specific mutan. varieties and between mutants and parent plants for chemical composition and IVDMD were tested for significance using Least Significance Difference at 5%.

CHAPTER 4

4.0. RESULTS

4.6 Proximate Analysis

Thirteen (13) velvet bean mutants of Summerset and NIRS together with their parent plant were analyzed for the following moisture, ash, phosphorus, calcium, crude protein and crude fat. There was variation in the treatment means and velvet mutant variety for all parameters with only an exception of calcium.

The most variation was found in crude protein (figure 12) for NIRS profile was with NIRS45-3-7 recording the highest value of crude protein 27.79% accounting for a 14.03% increase from the NIRS Parent which was found to have 23.89%. The lowest crude protein in the NIRS variety was in NIRS 68-6-3 which recorded 18.19% crude protein accounting for 31.33% less crude protein than the NIRS variety. All Sommerset mutant varieties recorded crude protein percentages more than the SS Parent. SS 16-9-9 recorded the highest crude protein of 24.42% accounting for 40.17% increase from the parent that was found to have 14.61% crude protein. The lowest crude protein percentage was in SS40-19-4/9B that recorded 17.84% crude protein accounting for 18.11% increase.

The highest crude fat (figure 13) amongst the Sommerset velvet beans varieties was recorded in SS 40-6-14 that was found to have 10.194% crude fat accounting for a 35.55% increase from the parent that was found to have 6.978% crude fat. SS40-19-4/9B recorded the lowest crude fat % of 6.641%, which was 5.07% lower than the SS parent. Among the NIRS varieties NIRS45-3-7 recorded the highest crude fat % of 12.249% that was 39.46% higher than the NIRS parent, which was found to have 7.416%. NIRS68-6-4 recorded the lowest crude fat % of 7.24%, which was 2.43% lower than the NIRS parent.

NIRS 45-3-7 had the highest moisture (figure 8) content of 4.7% that was 31.5% higher than the parent bean that was found to have 3.2%. Whereas NIRS 52-6-2/11B was found to have the lowest moisture content of 1.851 % that was lower than the parent bean. Among the Sommerset mutant varieties of velvet bean SS 38-25-3 recorded the highest value for moisture of 5.114% that was 27.31% higher than the SS parent which was found to have 3.715%. Whereas SS38-25-3was found to have the lowest moisture content of 2.344% that accounted that was 58.5% lower than the SS parent.

All NIRS mutant varieties had values of ash (figure 9) less than the parent bean, which was found to have 4.7% ash. NIRS68-6-4 had the highest ash percentage of 3.715% accounting for 26% lower ash than the NIRS parent. NIRS 52-6-2/11B had the lowest ash percentage of 3.3% accounting for 42.9% lower ash than the NIRS parent. Sommerset mutant varieties recorded both higher and lower ash percentages from the parent bean, which was found to have 3.95% ash. SS 40-6-14 was found to have the highest ash percentage of 4.75% accounting for a 16.8% increase from the SS parent. SS 38-25-3 had the lowest ash percentage of 3.3% that was 20.2% less than the SS parent.

Among the NIRS varieties all the mutants recorded values higher phosphorus (figure 10) than the parent with the exception of NIRS 52-6-2/3B which had the lowest phosphorus of 1.46% which was 3.8% less than the parent bean which had 1.5%. The highest Phosphorus % for the NIRS varieties was recorded in NIRS 45-3-7 that was 2.748% accounting for a 44.72% higher

highest phosphorus of 1.7% that was 7.7% higher than the parent bean that had 1.56% phosphorus and SS22-4-4/3B recorded the lowest Phosphorus % of 1.091% which was 42.71% less than the SS parent.

Calcium only showed significance in the interaction between treatment means and varieties and the grand mean for the Calcium values was found to be 1.5%.

Figure 20: The relationship between treatment means of velvet beans and Moisture %

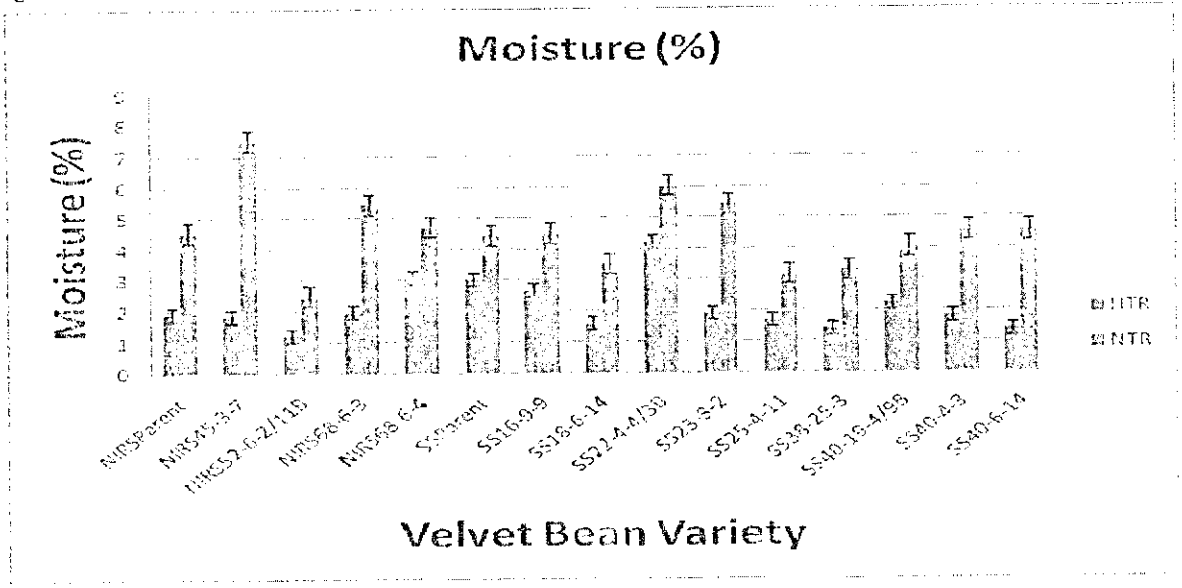
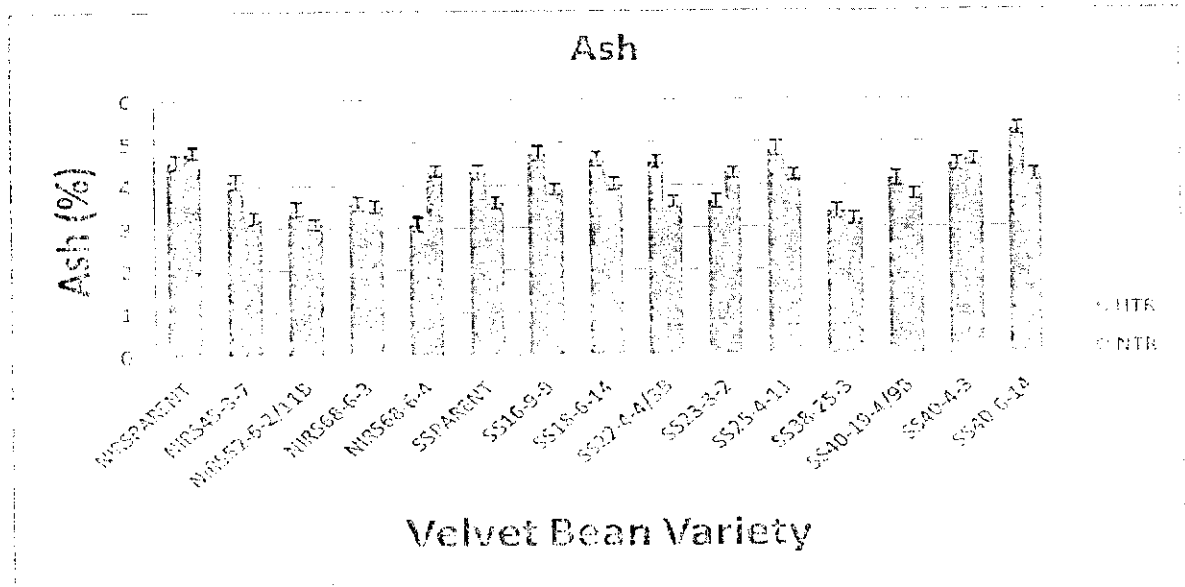


Figure 21: The relationship between treatment means of velvet beans and Ash %



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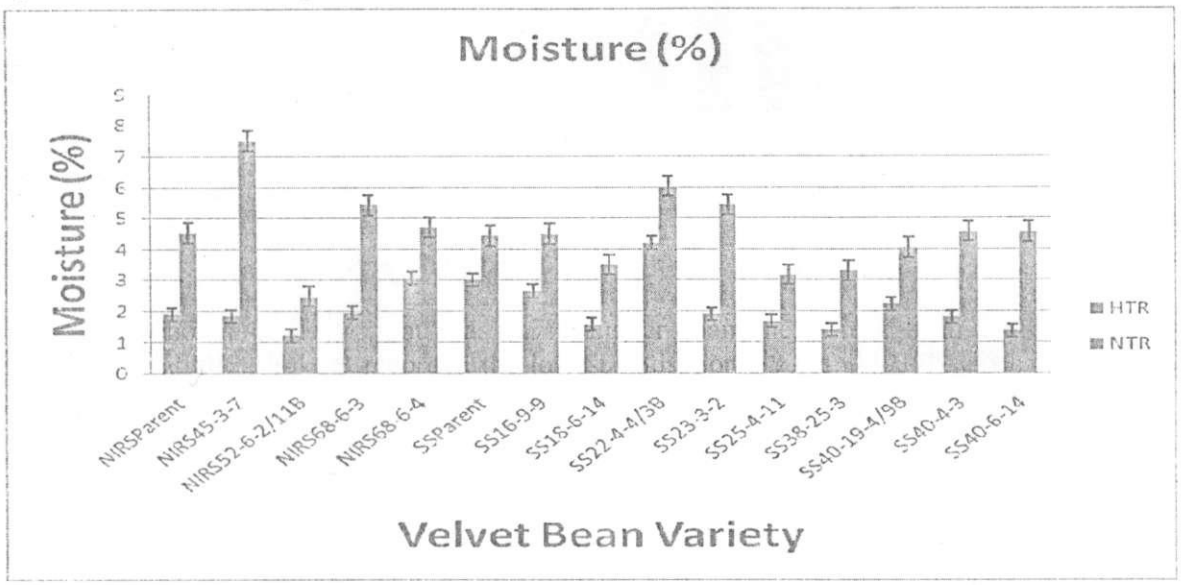


Figure 21: The relationship between treatment means of velvet beans and Ash %

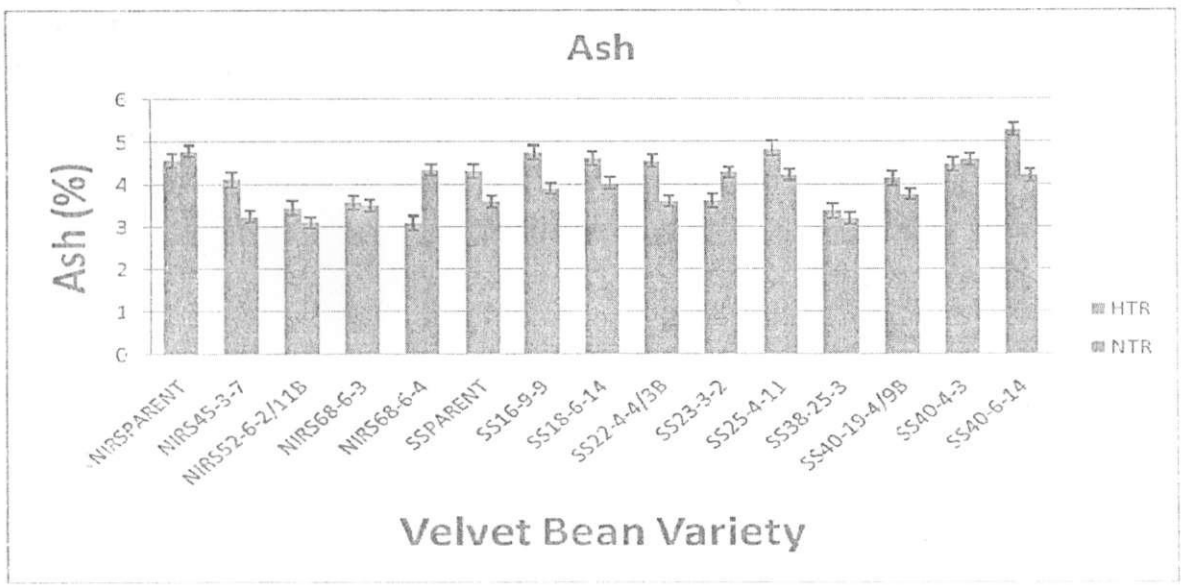


Figure 22: The relationship between treatment means of velvet beans and Phosphorus %

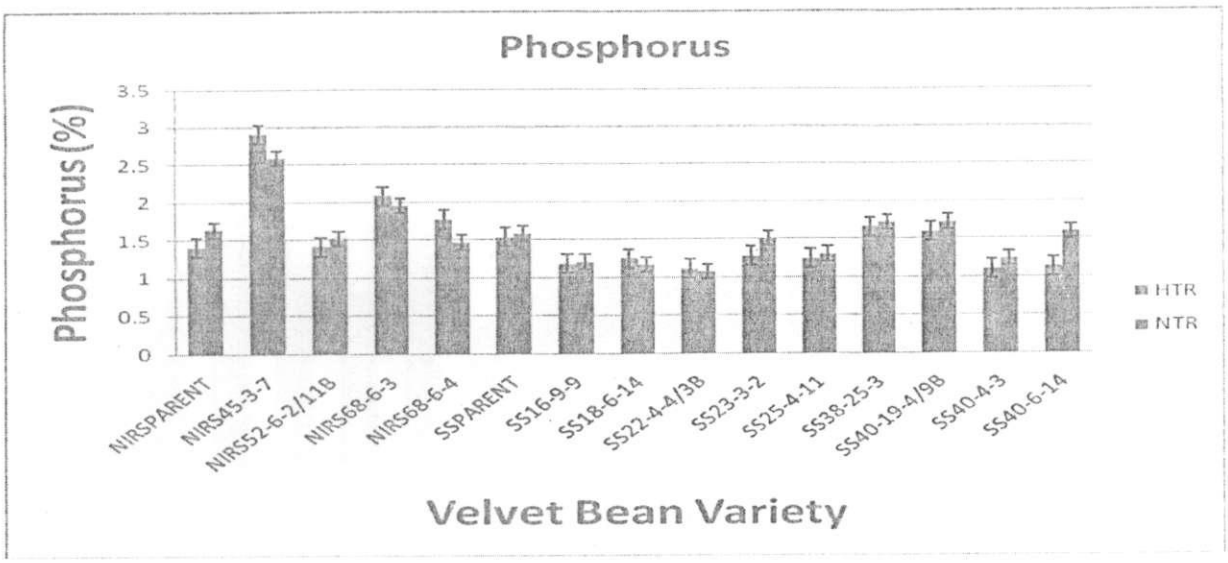


Figure 10: The relationship between treatment means of velvet beans and Calcium%

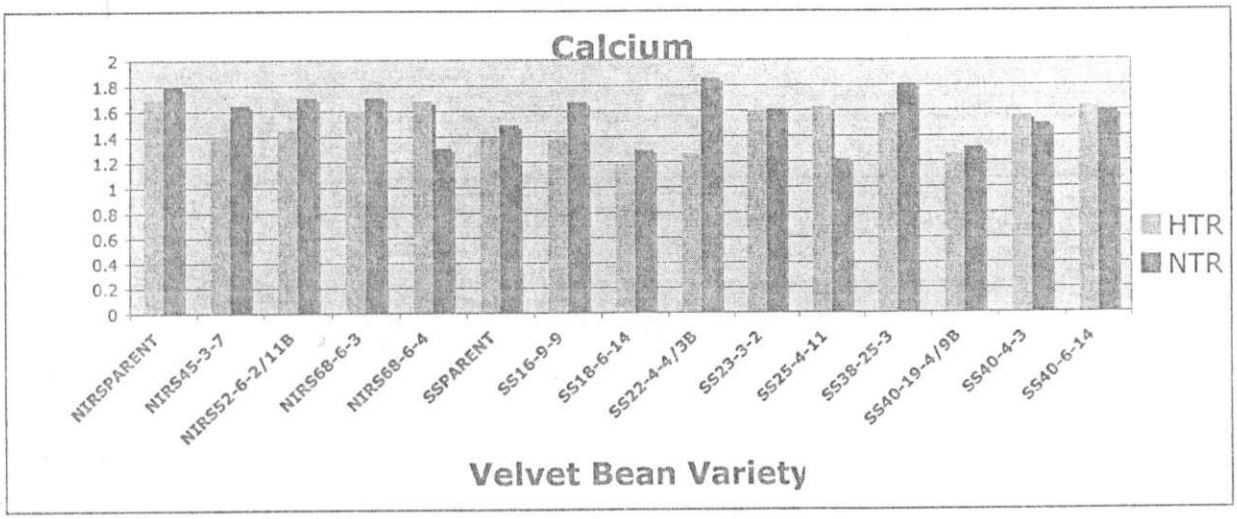


Figure 11: The relationship between treatment means of velvet beans and Crude Protein%

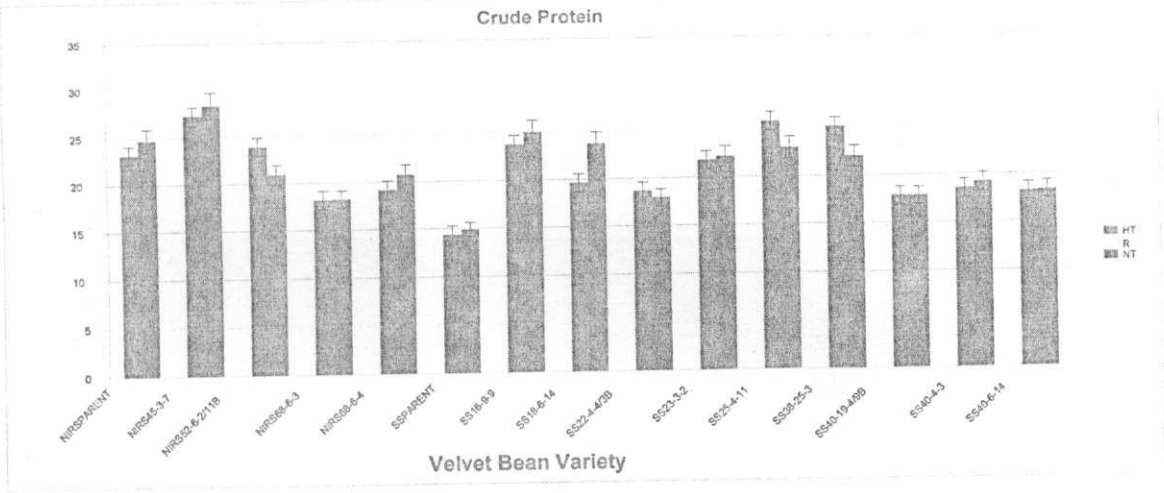
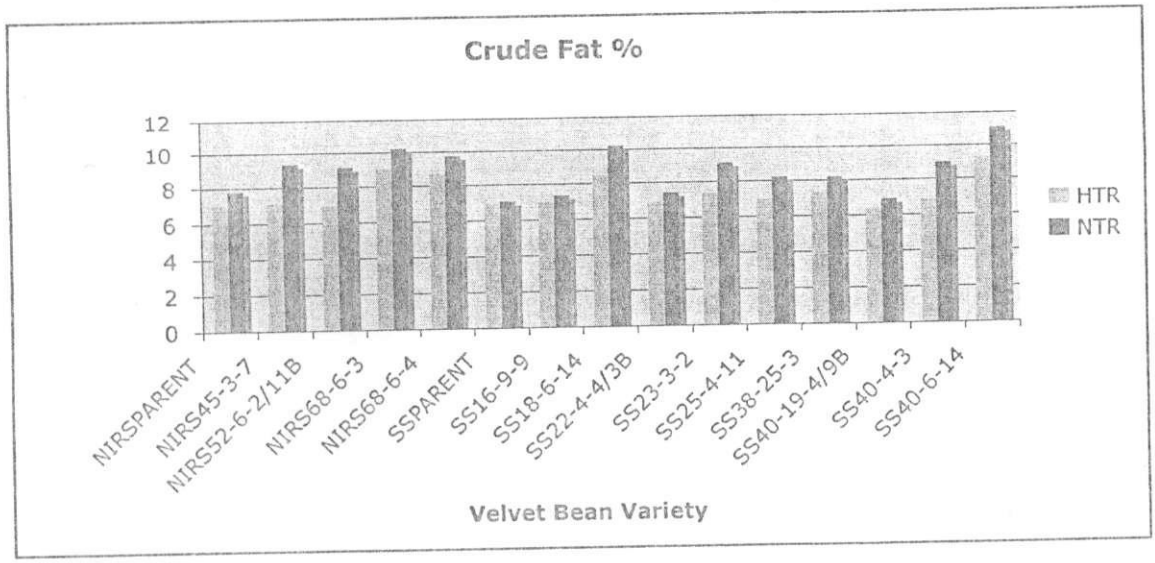


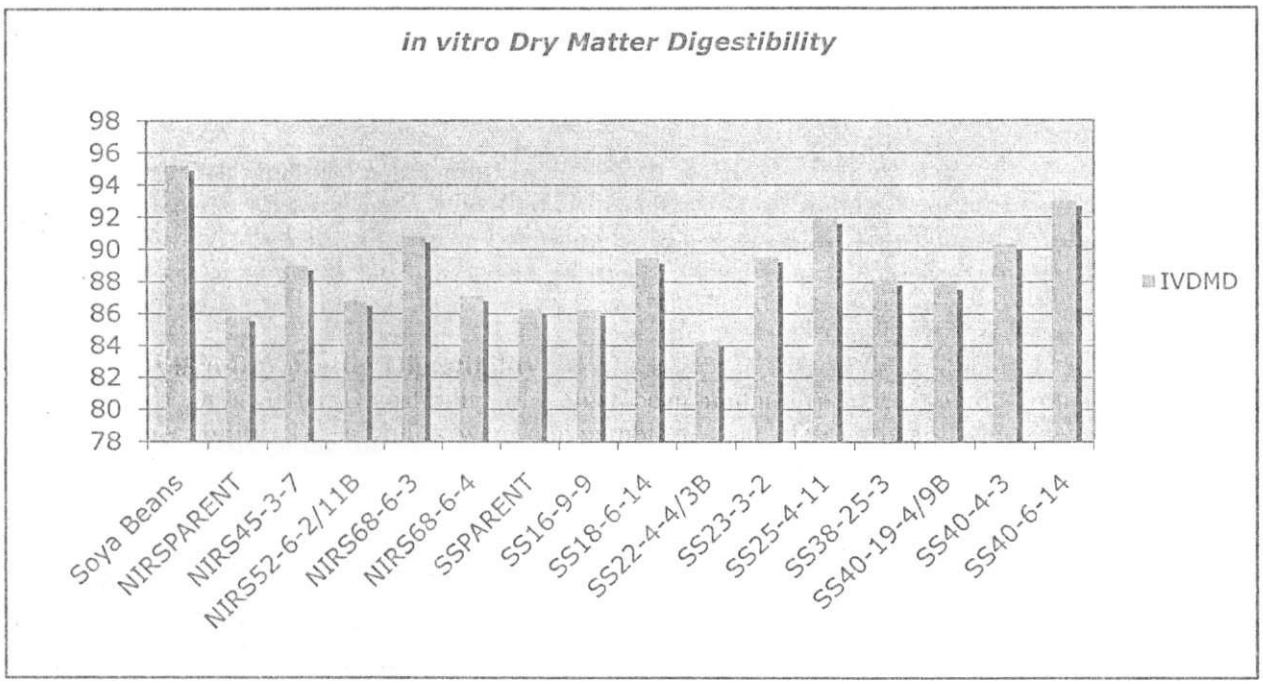
Figure 12: The relationship between treatment means of velvet beans and Crude Fat %



4.7 In Vitro Dry Matter Digestibility

The *in vitro* Dry Matter Digestibility of velvet bean mutant varieties ranged from 84.27% - 93.03% digestibility. These values were comparable to that of soya bean, which was found to have 95.02% digestibility.

Figure 26: in vitro dry matter digestibility of velvet beans %



CHAPTER 5

5.0 DISCUSSION

Induced mutation breeding of Velvet beans resulted in significant changes among the mutant lines in plant type, maturity, plant vigor, color of the flowers, apparent biomass, seed size and seed yield. These traits provided the basis for the screening process. The experiment involved the 13 mutant varieties of velvet beans of which four (4) were NIRS variety and nine (9) were of Sommerset variety. L-Dopa analysis was unfortunately not determined due to lack a standard for L-dopa. Thus the focus of the study shifted to only advance the velvet bean varieties with mutants evaluated only for morphological traits, yield components, seed yield, nutrient content and in vitro digestibility in goat rumen liquor as indicated earlier.

5.1 Observation During Processing

During the processes of dehulling it was found that there is no standard length of time for the soaking of the velvet bean seeds before dehulling can be successful. However, the Sommerset variety proved easier to dehull than the NIRS variety of velvet beans. The seeds of velvet beans are quite hard therefore pounding using a mortar is very difficult.

During the process of heating it was noted that the Memmert oven (Model 500, Memmert Co. Schwabach, Germany) tended to deviate from the set temperature. Temperature was not evenly distributed as the seeds on the top tray tended to roast faster.

5.2 Nutrient Content

The nutrient composition results are presented in Appendix 1. The proximate analysis shows that velvet bean is rich in protein, energy and minerals adequately supplement human and livestock needs (Siddhuraju *et al.* 1996; Laurena *et al.* 1991).

According to the documented velvet beans contain 20%-31% crude protein (Laurena *et al.* 1991; Siddhuraju *et al.* 1996). Despite the significant differences in crude proteins between the mutant varieties and the parents none of the results were significantly higher than the documented values for all the parameters observed.

According to Siddhuraju *et al.* (1996) velvet beans are low in fat. This was found to be true for the mutant varieties of the velvet beans analyzed for crude fat in this study.

Velvet beans is said to be a rich source of all macro minerals including Sodium, Potassium, Magnesium, Calcium and Phosphorus (Kawonga, 2002). However, the analysis only was done for Calcium and Phosphorus. Calcium only showed significance in the interaction between treatment means and varieties, which suggest that all the treated samples contain the similar amount of Calcium. The grand mean for the Calcium was found to be 1.5%.

The differences between heat and non-heat treated samples were due to loss of moisture after heating. This is because of the apparent differences in the dry matter content of the seed. Hence heat-treated samples of the same weight were found to have a significantly higher nutrient content than the non-heat treated samples.

There were significant differences in the nutrient content of all the varieties with an exception of Calcium. They were significant differences between velvet bean variety and treatment means for the following moisture, ash, calcium and phosphorus.

5.6 In vitro Dry Matter Digestibility

The *in vitro* Dry Matter Digestibility of velvet beans was found to be the similar for all the samples. There was a difference between the control (soya bean) and the mutant varieties of velvet beans based on the least significant difference at 5% but this difference was not significant when the treatment means were subjected to analysis of variance ANOVA. There was also no interaction between treatment means and velvet bean varieties. The *in vitro* dry matter digestibility of the mutant varieties of velvet beans was found to be comparable to those achieved by sorghum seeds (85-90%) according to Muleba, (1992). The anti-nutritional factors did not show any detrimental effect on the *in vitro* fermentation. Thus velvet beans can replace conventional energy and protein sources such as maize, and sorghum and soya beans for goats.

CHAPTER 6

6.0 CONCLUSIONS

The chemical profiles of all the mutant varieties were significantly higher than the parent with only the exception of calcium. Thus the rejection of the null hypothesis: that there is no significant difference between the nutrients content of the mutant varieties of velvet beans and the parent bean.

There was no significant difference in the *in vitro* digestibility of mutant varieties of velvet beans and the parent bean. The little difference in the *in vitro* digestibility of soya beans and velvet beans varieties goes to show that velvet beans has the potential to replace soya beans at some percentage inclusion in the diets of goats. Hence, it can be concluded that the high yielding varieties of velvet beans can be used in place of the parent varieties and soya beans in dry season feed supplement for goats.

It is thus hoped that induced mutation breeding in velvet beans could have also resulted in different levels of anti-nutritional factors such as L-Dopa among mutants of the same variety and between the varieties. In addition, it is hoped that the levels of L-Dopa are different in the different plant parts.

6.1 RECOMMENDATIONS

Not analyzing L-Dopa in the earlier generations means that more samples will have to be carried forward and with the inclusion of this year's samples, more samples have been generated without analysis. This has made the analysis of this aspect of the study more expensive as more than twice the original and intended number of samples has now to be analyzed. Despite the high number and consequently high cost of analysis, L-Dopa analysis should still be carried out. Even if promising mutant lines with high plant biomass and seed yield are selected they cannot directly be used for stock feed or human food if they contain high levels of L-Dopa especially in the seed

CHAPTER 7

7.0 REFERENCES

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APPENDIX 1

ANALYSIS OF VARIANCE TABLES

Table 11: Analysis of variance for moisture (H_2O)

Source of variation	d.f.(m.v.)	s.s.	m.s.	v.r.	F pr.
Variety	14	41.7822	2.9844	23.27	< 0.001**
Treatment	1	84.2515	84.2515	656.79	< 0.001**
Replication	1	0.0420	0.0420	0.33	0.572 ns
Variety. Treatment	13(1)	18.1631	1.3972	10.89	< 0.001**
Error	28(1)	3.5918	0.1283		
Total	57(2)	145.0092			

ns	= not significant	P>0.05
*	= Significant	P<0.05
**	= Very significant	P<0.01
Degrees of freedom		28
Standard Error		0.3582
Coefficient of Variation %		10.7
Grand mean		3.360

Table 12: Analysis of variance for Ash

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Variety	14	12.90613	0.92187	52.71	< 0.001**
Treatment	1	1.29685	1.29685	74.15	< 0.001**
Replication	1	0.03407	0.03407	1.95	0.173 ns
Variety. Treatment	14	5.83815	0.41701	23.84	< 0.001**
Error	29	0.50722	0.01749		
Total	59	20.58242			

ns	= not significant	P>0.05
*	= Significant	P<0.05
**	= Very significant	P<0.01
Degrees of freedom		29
Standard Error		0.1323
Coefficient of Variation %		3.3
Grand mean		4.037

Table 13: Analysis of variance for Calcium

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Variety	14	1.08807	0.07772	2.94	0.007 ns
Treatment	1	0.09441	0.09441	3.57	0.069 ns
Replication	1	0.05400	0.05400	2.04	0.164 ns
Variety. Treatment	14	0.86659	0.06190	2.34	0.026 *
Error	29	0.76690	0.02644		
Total	59	2.86997			

ns	= not significant	P>0.05
*	= Significant	P<0.05
**	= Very significant	P<0.01
Degrees of freedom		29
Standard Error		0.1626
Coefficient of Variation %		10.6
Grand mean		1.527

Table 14: Analysis of variance for Phosphorus

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Variety	14	9.73651	0.69546	47.22	< 0.001**
Treatment	1	0.02468	0.02468	1.68	0.206 ns
Replication	1	0.04735	0.04735	3.22	0.083 ns
Variety. Treatment	14	0.56552	0.04039	2.74	0.011*
Error	29	0.42713	0.01473		
Total	59	10.80118			

ns	= not significant	P>0.05
*	= Significant	P<0.05
**	= Very significant	P<0.01
Degrees of freedom		29
Standard Error		0.1214
Coefficient of Variation %		7.9
Grand mean		1.530

Table 15: Analysis of variance for crude protein (CP)

Source of variation	d.f.(m.v.)	s.s.	m.s.	v.r.	F pr.
Variety	14	670.319	47.880	23.50	< 0.001**
Treatment	1	0.213	0.213	0.10	0.749 ns
Replication	1	0.234	0.234	0.11	0.737 ns
Variety. Treatment	13(1)	52.839	4.065	1.99	0.061 ns
Error	28(1)	57.060	2.038		
Total	57(2)	763.271			

ns	= not significant	P>0.05
*	= Significant	P<0.05
**	= Very significant	P<0.01
Degrees of freedom		28
Standard Error		1.428
Coefficient of Variation %		6.8
Grand mean		21.09

Table 16: Analysis of variance for crude fat (CF)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Variety	14	297.0689	21.2192	62.53	< 0.001**
Treatment	1	9.2686	9.2686	27.32	< 0.001**
Replication	1	0.5497	0.5497	1.62	0.213 ns
Variety. Treatment	14	14.2127	1.0152	2.99	0.006 ns
Error	29	9.8403	0.3393		
Total	59	330.9403			

ns	= not significant	P>0.05
*	= Significant	P<0.05
**	= Very significant	P<0.01
Degrees of freedom		29
Standard Error		0.5825
Coefficient of Variation %		6.4
Grand mean		9.08

Table 17: Analysis of variance for *in vitro* Digestibility

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Variety	15	613.75	43.84	2.47	0.019 ns
Treatment	1	5.06	5.06	0.29	0.597 ns
Replication	1	16.97	16.97	0.96	0.336 ns
Variety. Treatment	15	342.12	24.44	1.38	0.225 ns
Error	30	514.17	17.73		
Total	60	1492.07			

ns	= not significant	P>0.05
*	= Significant	P<0.05
**	= Very significant	P<0.01
Degrees of freedom		29
Standard Error		4.211
Coefficient of Variation %		4.5
Grand mean		93.32

APPENDIX 3

TREATMENT MEAN DIFFERENCES BETWEEN PARENT AND MUTANTS

Table 18: Treatment Mean Differences between Parent and Mutants for moisture (H_2O)

Mutant	Mean	Difference	% Difference
NIRSPARENT	3.207	0	0
NIRS45-3-7	4.682	1.475	31.50363093**
NIRS52-6-2/11B	1.851	-1.356	-73.25769854**
NIRS68-6-3	3.695	0.488	13.20703654**
NIRS68-6-4	3.889	0.682	17.53664181**
SSPARENT	3.715	0	0
SS16-9-9	3.568	-0.147	-4.119955157
SS18-6-14	2.538	-1.177	-46.3750985*
SS22-4-4/3B	5.114	1.399	27.35627689**
SS23-3-2	3.669	-0.046	-1.253747615
SS25-4-11	2.424	-1.291	-53.25907591**
SS38-25-3	2.344	-1.371	-58.48976109**
SS40-19-4/9B	3.134	-0.581	-18.53860881**
SS40-4-3	3.192	-0.523	-16.38471178**
SS40-6-14	3.377	-0.338	-10.00888362**

*Least significant differences of means (5% level)
** Least significant differences of means (10% level)

Table 19: Treatment Mean Differences between Parent and Mutants for Ash

Mutant	Mean	Difference	% Difference
NIRSPARENT	4.681	0	0
NIRS45-3-7	3.684	-0.997	-27.06297503**
NIRS52-6-2/11B	3.275	-1.406	-42.93129771**
NIRS68-6-3	3.534	-1.147	-32.45614035**
NIRS68-6-4	3.715	-0.966	-26.00269179**
SSPARENT	3.951	0	0
SS16-9-9	4.333	0.382	8.816062774*
SS18-6-14	4.323	0.372	8.605135323*
SS22-4-4/3B	4.074	0.123	3.019145803
SS23-3-2	3.943	-0.008	-0.2028912
SS25-4-11	4.529	0.578	12.76219916**
SS38-25-3	3.288	-0.663	-20.16423358**
SS40-19-4/9B	3.947	-0.004	-0.101342792
SS40-4-3	4.531	0.58	12.80070625**
SS40-6-14	4.75	0.799	16.82105263**

*Least significant differences of means (5% level)
** Least significant differences of means (10% level)

Table 20: Treatment Mean Differences between Parent and Mutants for Calcium

Mutant	Mean	Difference	% Difference
NIRSPARENT	1.745	0	0
NIRS45-3-7	1.53	-0.215	-14.05228758**
NIRS52-6-2/11B	1.578	-0.167	-10.58301648**
NIRS68-6-3	1.655	-0.09	-5.438066465*
NIRS68-6-4	1.492	-0.253	-16.95710456**
SSPARENT	1.442	0	0
SS16-9-9	1.525	0.083	5.442622951*
SS18-6-14	1.238	-0.204	-16.47819063**
SS22-4-4/3B	1.56	0.118	7.564102564*
SS23-3-2	1.605	0.163	10.15576324**
SS25-4-11	1.423	-0.019	-1.335207309
SS38-25-3	1.695	0.253	14.92625369**
SS40-19-4/9B	1.282	-0.16	-12.48049922**
SS40-4-3	1.528	0.086	5.628272251*
SS40-6-14	1.613	0.171	10.60136392

*Least significant differences of means (5% level)
** Least significant differences of means (10% level)

Table 21: Treatment Mean Differences between Parent and Mutants for Phosphorus

Mutant	Mean	Difference	% Difference
NIRSPARENT	1.519	0	0
NIRS45-3-7	2.748	1.229	44.72343523**
NIRS52-6-2/11B	1.463	-0.056	-3.827751196
NIRS68-6-3	2.016	0.497	24.65277778**
NIRS68-6-4	1.618	0.099	6.118665019
SSPARENT	1.557	0	0
SS16-9-9	1.195	-0.362	-30.29288703**
SS18-6-14	1.207	-0.35	-28.9975145**
SS22-4-4/3B	1.091	-0.466	-42.71310724**
SS23-3-2	1.391	-0.166	-11.93386053**
SS25-4-11	1.269	-0.288	-22.69503546**
SS38-25-3	1.687	0.13	7.705986959*
SS40-19-4/9B	1.659	0.102	6.148282098*
SS40-4-3	1.165	-0.392	-33.64806867**
SS40-6-14	1.358	-0.199	-14.6539028**

*Least significant differences of means (5% level)
** Least significant differences of means (10% level)

Table 22 Treatment Mean Differences between Parent and Mutants for Crude Protein (CP)

Mutant	Mean	Difference	% Difference
NIRSPARENT	23.89	0	0
NIRS45-3-7	27.79	3.9	14.03382512**
NIRS52-6-2/11B	22.41	-1.48	-6.604194556*
NIRS68-6-3	18.19	-5.7	-31.33589885**
NIRS68-6-4	19.95	-3.94	-19.74937343**
SSPARENT	14.61	0	0.00
SS16-9-9	24.42	9.81	40.17**
SS18-6-14	21.71	7.1	32.70**
SS22-4-4/3B	18.31	3.7	20.21**
SS23-3-2	22.01	7.4	33.62**
SS25-4-11	24.45	9.84	40.25**
SS38-25-3	23.64	9.03	38.20**
SS40-19-4/9B	17.84	3.23	18.11**
SS40-4-3	18.88	4.27	22.62**
SS40-6-14	18.25	3.64	19.95**

*Least significant differences of means (5% level)
** Least significant differences of means (10% level)

Table 23: Treatment Mean Differences between Parent and Mutants for Crude Fat (CF)

Mutant	Mean	Difference	% Difference
NIRSPARENT	7.416	0	0
NIRS45-3-7	12.249	4.833	39.45628215**
NIRS52-6-2/11B	8.028	0.612	7.623318386*
NIRS68-6-3	8.298	0.882	10.62906725**
NIRS68-6-4	7.24	-0.176	-2.430939227
SSPARENT	6.978	0	0
SS16-9-9	7.136	0.158	2.214125561
SS18-6-14	8.517	1.539	18.06974287**
SS22-4-4/3B	13.373	6.395	47.8202348**
SS23-3-2	8.908	1.93	21.66591828**
SS25-4-11	10.98	4.002	36.44808743**
SS38-25-3	7.303	0.325	4.450225935
SS40-19-4/9B	6.641	-0.337	-5.074536967*
SS40-4-3	13.008	6.03	46.35608856**
SS40-6-14	10.194	3.216	31.54796939**

*Least significant differences of means (5% level)
** Least significant differences of means (10% level)

Table 24: Treatment Mean Differences between Soya beans and velvet beans for *in vitro* Digestibility

Mutant	Mean	Difference	% Difference
Soya Beans	95.23	0	0
NIRSPARENT	85.84	-9.39	-10.9389562**
NIRS45-3-7	89.03	-6.2	-6.963944738*
NIRS52-6-2/11B	86.81	-8.42	-9.69934339-4*
NIRS68-6-3	90.78	-4.45	-4.90196078-4*
NIRS68-6-4	87.14	-8.09	-9.283910948*
SSPARENT	86.32	-8.91	-10.32205746**
SS16-9-9	86.24	-8.99	-10.42439703**
SS18-6-14	89.45	-5.78	-6.461710453*
SS22-4-4/3B	84.27	-10.96	-13.00581464**
SS23-3-2	89.54	-5.69	-6.354701809*
SS25-4-11	91.95	-3.28	-3.567156063
SS38-25-3	88.13	-7.1	-8.056280495*
SS40-19-4/9B	87.87	-7.36	-8.376010015*
SS40-4-3	90.35	-4.88	-5.401217488*
SS40-6-14	93.03	-2.2	-2.36482855

*Least significant differences of means (5% level)
** Least significant differences of means (10% level)

Table 25: Treatment Mean Differences between Parent and Mutants for *in vitro* Digestibility

Mutant	Mean	Difference	% Difference
NIRSPARENT	85.84	0	0
NIRS45-3-7	89.03	3.19	3.583061889
NIRS52-6-2/11B	86.81	0.97	1.11738279
NIRS68-6-3	90.78	4.94	5.441727253*
NIRS68-6-4	87.14	1.3	1.491852192
SSPARENT	86.32	0	0
SS16-9-9	86.24	-0.08	-0.092764378
SS18-6-14	89.45	3.13	3.499161543
SS22-4-4/3B	84.27	-2.05	-2.432656936
SS23-3-2	89.54	3.22	3.596158142
SS25-4-11	91.95	5.63	6.122892877*
SS38-25-3	88.13	1.81	2.053784182
SS40-19-4/9B	87.87	1.55	1.7639695
SS40-4-3	90.35	4.03	4.460431655
SS40-6-14	93.03	6.71	7.212727077*

*Least significant differences of means (5% level)
** Least significant differences of means (10% level)