DETOXIFICATION, NUTRITIVE VALUE, AND ANTHELMINTIC PROPERTIES OF *Mucuna pruriens*

By

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To my family:
The love of my life, my soul mate, and wife, Andrea Feaster Huisden, and our four wonderful sons, Raoul Max Franklin Huisden (12), Christiaan Henry Huisden (5), John Franklin “Sjaak” Huisden (2), and Carlo Dennis Huisden (1). A special word of thanks to my precious wife, Andrea; without her unfailing love, patience, wisdom, support, and encouragement this accomplishment would not exist.

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The *Mucuna pruriens* bean has high protein and starch contents, but also contains 3, 4 - dihydroxy-L-phenylalanine (L-Dopa), which has pharmaceutical properties, but is toxic when ingested by monogastrics. Experiments were conducted to detoxify *Mucuna* for monogastrics, and to evaluate its anthelmintic effect in ruminants. Experiment 1a) examined how long it takes to decrease the pH of ensiled *Mucuna* to $\leq$ 4.6, the typical minimum pH for ensiled legumes. Crushed beans (6 mm) were ensiled for 0, 3, 7, 21, and 28 days. A pH of 4.5 and an L-Dopa concentration of 1.3% (54% reduction) were recorded after 28 days of ensiling. Experiment 1b) determined the effect of particle size (2, 4, or 6 mm) of ensiled *Mucuna* on L-Dopa concentration and on fermentation and nutritional characteristics. Ensiling 1- or 6-mm particles reduced the L-Dopa concentration by about 60% while preserving most nutrients. Experiment 2 studied the effects of extraction in solutions of acetic acid (ACD, pH 3) or sodium hydroxide (ALK, pH 11) for 8 hours or sonication (SON) for 5 minutes on the L-Dopa concentration and nutritional composition of finely (1 mm) or coarsely (6 mm) ground *Mucuna* beans. All extraction methods reduced the L-Dopa concentration of fine particles to safe levels ($\leq$ 0.4%) but increased their neutral detergent fiber (NDF) and starch concentrations and decreased their water-soluble carbohydrate (WSC) and crude protein (CP) concentrations. Extraction methods were less

effective at reducing the L-Dopa in coarse particles and had inconsisent effects on their nutritional composition. Experiment 3 evaluated the effect of feeding detoxified *Mucuna* beans on the performance, behavior, and health of 60 Sprague-Dawley rats randomly assigned to five treatments. Dietary treatments consisted of a commercial rat chow (CON) or diets in which 10% of the rat chow was replaced with either undetoxified *Mucuna* (MUC), or *Mucuna* detoxified by ACD or ALK extraction, or ensiling for 28 days (SIL). Compared to CON, *Mucuna*-based diets gave similar feed intake and weight gain. No behavioral abnormalities were caused by any of the diets in open field analyses on days 3 and 10 but when data for both days was collectively analyzed, all *Mucuna*-based diets exhibited less locomotion than control rats. The decrease in activity was numerically less in rats fed ACD and SIL diets than in those fed MUC and ALK. Feeding MUC caused splenomegaly and monocytosis, and reduced blood phosphorus concentrations relative to CON, but detoxification of *Mucuna* prevented these effects. Experiment 4 determined if ingestion of *Mucuna* beans reduces helminth parasite infestation in lambs. Thirty-six Dorper x Katahdin ram lambs (28.8 $\pm$ 5 kg body weight) were randomly allocated to three treatments: a cottonseed meal control diet, a diet in which *Mucuna* replaced cottonseed meal, and a treatment that involved administering levamisole (2 ml/45.4 kg) to lambs fed the control diet. Diets were formulated to be isonitrogenous (14% CP) and isocaloric (64% total digestible nutrients). Lambs were challenged 3 times per week for 2 weeks by gavage with infectious *H. contortus* larvae. Unlike levamisole treatment, *Mucuna* intake did not affect (P<0.05) fecal egg counts (412 vs. 445 eggs/g) or abomasal worm counts (958 vs. 1170 total worms), though a numerical (P > 0.10) reduction was evident. Neither levamisole nor *Mucuna* treatment affected anemia indicators, daily feed intake, weight gain or dressing. In conclusion, *Mucuna* intake did not reduce helminth parasite infection in lambs.

CHAPTER 1
INTRODUCTION

Mucuna pruriens is an annual climbing legume indigenous to tropical regions with numerous uses as a food, feed, and nutraceutical. Like many other legumes, however, it has molecular components that can adversely affect its nutritional value, but the ability of these molecules to inhibit enzymes, to selectively bind and enter the circulatory system may be useful in pharmacology. Therefore, nutritionists and medical researchers have contrasting views about the toxicity of the genus.

According to Szabo and Tebbett (2002) the major drawback of *Mucuna*, which has compromised its usefulness as a food source for either humans or livestock, is associated with its chemical concentration. *Mucuna* contains novel alkaloids, saponins, and sterols (Manyam et al., 2004) and a high concentration of L-Dopa. In addition, serotonin and a number of indolic alkaloids structurally related to serotonin have been reported in various parts of the *Mucuna* plant, several of which have hallucinogenic properties of considerable strength (Szabo and Tebbett, 2002). It would, however, be unlikely for these low-level alkaloids to have any effect on human and animal consumers because their absorption across the gastrointestinal tract is negligible (Szabo, 2003).

According to Taylor (2004) beans of *Mucuna* are not only high in protein, but also in non-structural carbohydrates, lipids, and minerals. In South and Mid America, *Mucuna* beans have been roasted and ground to make a coffee substitute and the bean is also cooked and eaten as a vegetable. *Mucuna* is also one of the most remarkable green manures as it can add up to 30 ton/ha of organic matter to soils (Pretty et al., 1998).

The crop has a long history of use in Indian Ayurvedic medicine and traditional medical practice in several countries where it is used to treat a wide variety of ailments including

Parkinson's disease (Manyam and Sanchez-Ramos, 1999; Nagashayana et al., 2001). However, many of such uses are based on anecdotal healing properties that require verification and scientific validation.

The literature review (Chapter 2) provides an introduction to *Mucuna pruriens*, its taxonomy and characteristics, its use as a food and feed source, its toxic and antinutrient properties, various methods used to detoxify the bean, and *Mucuna's* nutraceutical potential. The research described in subsequent chapters is aimed at enhancing the use of *Mucuna* as a food and feed source for monogastrics and to exploit its nutraceutical use as an anthelmintic. Four experiments were conducted. These included detoxification experiments during which ensiling, acid and alkali solvent extractions, and sonication were used to reduce the L-Dopa concentration. The primary objectives were to detoxify the bean and to evaluate the nutritional value of the detoxified product (Chapters 3 and 4). In the next experiment, beans produced from three detoxification methods were fed to rats and their behavior, performance, and physiological responses were monitored (Chapter 5). The aim of the final experiment was to determine if incorporation of *Mucuna* beans in the diet reduces helminth parasite infection in lambs (Chapter 6).

CHAPTER 2
LITERATURE REVIEW

Introduction

Mucuna pruriens is an annual climbing legume that has been used for centuries for a wide array of nutraceutical, nutritional, and other purposes. It is indigenous to Asia and now grows in many tropical regions, including Africa, South America, and the West Indies. It was grown throughout the southeastern United States as a livestock feed and green manure until the 1950s when the advent of cheaper inorganic fertilizers and soybean meal sources led to its demise (Eilitta and Carsky, 2003). In many tropical countries, *Mucuna* beans are processed into flour or a coffee substitute, or eaten as a vegetable. *Mucuna* plants are also used as a highly effective green manure, adding up to 30 ton/ha of organic matter to soils (Pretty et al., 1998). According to Taylor (2004) *Mucuna* beans are high in starch (39-41%; Ezeagu et al., 2003) and protein (25-38%; Ezeagu et al., 2003; Adebowale et al., 2003b). *Mucuna*, like other legumes, contains both beneficial and problematic molecular components. The challenge in promoting *Mucuna* for human or livestock consumption is identifying cost-effective techniques to reduce its antinutritional properties while preserving its nutrients. The most important antinutrient in *Mucuna* is 3, 4 dihydroxy-L-phenylalanine (L-Dopa; Szabo, 2003). The beans of most *Mucuna* species contain a high concentration (2-7%) of L-Dopa. Ingestion of *Mucuna* has been associated with reduced performance and health in livestock and humans, and most of such problems are thought to result from L-Dopa ingestion. Several additional naturally occurring compounds that share tryptamine as a base structure and have hallucinogenic properties of considerable strength (Szabo, 2003) like serotonin and indolic alkaloids structurally related to serotonin have been reported in various parts of the plant However, it is unlikely for these alkaloids to adversely

affect human or animal consumers because their concentrations is low and their absorption across the gastrointestinal tract is negligible (Flores et al., 2002; Szabo, 2003).

Mucuna is used as a treatment for various health problems. There are reports of its use in Indian Ayurvedic medicine as a treatment for worms, dysentery, diarrhea, snakebite, sexual debility, tuberculosis, impotence, rheumatic disorders, muscular pain, gonorrhea, sterility, gout, cancer, delirium, dysmenorrhea, diabetes etc. However, many of these claims derive from common use and have not been verified scientifically.

This review focuses on the following aspects of *Mucuna pruriens*: taxonomy and physical characteristics, nutritional value, antinutritional and toxic properties, methods for detoxifying the bean and their impact on its nutritional value.

Taxonomy and Characteristics of *Mucuna pruriens*

Classification

Mucuna pruriens is part of the tribe Phaseoleae and family Fabaceae (Taylor, 2004). Currently the genus accounts for 137 species, subspecies or varieties (St-Laurent et al., 2002). The most commonly cited species include *M. deeringiana* Merrill, *M. utilis* Wallich (Bengal velvetbean), *M. pruriens*, *M. nivea*, *M.* Hassjoo (Yokohama velvetbean), *M. aterrima* Holland (Mauritius and Bourbon velvetbean), *M. capitata*, and *M. diabolica* (Pretty et al., 1998). Table 2-1 shows additional species and their common names. However, the taxonomy of these species has been confused, and some designations may be synonymous (Capo-chichi et al., 2003). More recent taxonomists have considered all cultivars of the velvetbean as *Mucuna pruriens* variety *utilis* (St-Laurent et al., 2002).

Table 2-1. Scientific and common names of *Mucuna pruriens*

Scientific Names	Common Name
Carpopogon pruriens	Nescafé
Dolichos pruriens	*Mucuna*
M. aterrima	Pó de mico
M. atropurpurea	Fava-coceira
M. cochinchinensis	Cabeca-de-frade
M. cyanosperma	Cowage
M. deeringiana	Cowhage
M. esquirolii	Cow-itch
M. prurita	Velvetbean
M. utilis	Bengal bean
Stizolobium aterrimum	Mauritius bean
S. deeringianum	Itchy bean
S. pruriens	Krame
S. pruritum	Picapica
S. niveum	Chiporro
Negretia pruriens	Buffalo bean

Taylor (2004)

Species Description

The genus *Mucuna* comprises a species of annual and perennial legumes with vigorously climbing habits that originated in southern China and eastern India, where the plants were widely cultivated as a green vegetable crop (Pretty et al., 1998). *Mucuna* is self-pollinating hence natural out-crossing is rare; the life cycles range from 100 to 300 days to harvest of the pod and the genus thrives best under warm, moist conditions, below 1500 m above sea level, and in areas with plentiful rainfall (Pretty et al., 1998). The Food and Agriculture Organization (FAO) established climatological growing conditions to be temperatures of 20-30°C throughout the growing period with 1200-1500 mm/year or more of rainfall (Bachmann, 2008).

According to Taylor (2004), the plant grows 3 to 18 meters in length and its flowers are white to dark purple and hang in long clusters or racemes. There are differences among cultivated species in the character of the pubescence on the pod (stinging versus non-stinging), bean color (white, beige, mottled, grey, and black), and number of days (110-123 days after

planting) until pod maturity (Pretty et al., 1998; Bachmann, 2008; Chikagwa-Malunga et al., 2008a). "Cowitch" and "cowhage" are common English names of *Mucuna* cultivars whose pods are covered with long reddish-orange hairs that cause intense irritation to the skin on contact. The itch is possibly caused by mucunain, which is a proteolytic enzyme and serotonin, which is a neurotransmitter (Pretty et al., 1998; Szabo and Tebbett, 2002). According to Taylor (2004), the species name derives from the Latin for "itching sensation" and refers to the results of contact with the hairs on the pod. The hair of non-stinging cultivars, known by the common English name velvetbean are silky and lay flat against the pod's surface.

Current Uses of *Mucuna pruriens*

Mucuna is used throughout the world for a variety of purposes. For example, it is used as an ornamental crop that forms a dense canopy with visually appealing purple blossoms. *Mucuna pruriens* is also one of the most popular multi-purpose legumes among small farmers in the tropics because it is an excellent source of green manure, in part because of its ability to fix atmospheric nitrogen (N) thereby restoring soil fertility. It also adds significant quantities of organic matter ($\leq$ 30 tons) to the soil (Gilbert, 2002). *Mucuna's* abundant shallow roots and dense leaves and vines reduce soil erosion, suppress weeds and conserve soil moisture.

Nutraceutical Versatility

Mucuna pruriens has been used for wide-ranging nutraceutical applications for centuries. Its beneficial effects are due to its content of pharmacologically active compounds, such as L-Dopa which is used extensively in the treatment of Parkinson's disease. According to Taylor (2004), numerous countries and various cultures claim that *Mucuna* has various healing properties but most of these ethno-pharmacological claims require scientific validation as they are often linked to specific cultural traditions and beliefs. Taylor (2004) provided a useful overview of uses of *Mucuna* in different countries (Table 2-2).

Table 2-2. Ethnobotany: worldwide uses or functions of *Mucuna pruriens*

Country	Uses and ailments or conditions for which *Mucuna* is used
Brazil	Anthelmintic, aphrodisiac, diuretic, food, hydropsy, nerve tonic, Parkinson's disease, poison
Germany	Carminative, cholesterol, hypotensive, hypoglycemic, muscle pain, rheumatism, rubefacient, anthelmintic
India	Abortion, alterative, anthelmintic, antivenin, aphrodisiac, cancer, catarrh, cholera, cough, debility, delirium, diabetes, diarrhea, diuretic, dropsy, dysentery, dysmenorrhea, emmenogogue, fertility, gout, impotency, irritant, lithiasis, nerve tonic, nervine, night dreams, scorpion sting, spermatorrhea, sterility, tuberculosis, uterine stimulant, worms
Nigeria	Antivenin
Pakistan	Aphrodisiac, diabetes
Elsewhere	Anasarca, anodyne, anthelmintic, antidotal, aphrodisiac, asthma, burns, cancer, cholera, cough, cuts, diarrhea, diuretic, dog bite, dropsy, emmenagogue, insanity, intestinal parasites, mumps, nervine, paralysis, pleuritis, resolvent, ringworm, rubefacient, snakebite, sores, syphilis, tumors, vermifuge, wind-burns, worms

(Houghton and Skari, 1994; Taylor, 2004)

Mucuna sap has reportedly been used against pests, as an insect repellent, and its L-Dopa concentration may make it an effective anthelmintic (Faridah Hanum and van der Maesen, 1996).

Antioxidant property

Some of the espoused healing properties are yet to be verified scientifically but research has validated some historical claims. Tripathi and Upadhyay (2002) conducted *in vitro* and *in vivo* studies with an alcohol extract of the beans of *Mucuna pruriens* to investigate its antioxidant properties. The effect was also studied on iron-induced lipid peroxidation, oxidation of glutathione, and its interaction with hydroxyl and superoxide radicals. There was no change in the rate of aerial oxidation of glutathione but the extract inhibited iron sulfate-induced lipid peroxidation. It also inhibited the specific chemical reactions induced by superoxides and hydroxyl radicals. Tripathi and Upadhyay (2002) concluded that the alcohol extract of the beans

of *Mucuna pruriens* has an antilipid peroxidation property, which is mediated through the removal of superoxides and hydroxyl radicals.

Antivenin property

The *Mucuna*-based coffee substitute Nescafé has been used as an antivenin and a tonic for nervous system disorders (Pretty et al., 1998; Taylor, 2004). *Mucuna* leaf extracts act on thrombin and fibrinogen to enhance blood clotting, which makes such extracts a useful treatment against snake venom-induced hemorrhage (Houghton and Skari, 1994). Several *in vivo* studies validate this traditional use. Guerranti et al. (2002) demonstrated that the observed antivenin activity has an immune mechanism. Antibodies of mice treated with non-lethal doses of venom reacted against some proteins of *Mucuna pruriens* extract. Proteins of *Echis carinatus* venom and *Mucuna pruriens* extract have at least one epitope in common. The antivenin properties of an extract of *Mucuna* beans were also demonstrated *in vivo* by Guerranti et al. (2001). *Echis carinatus* venom (EV) contains a mixture of proteins that inhibit the coagulative cascade, causing severe bleeding and hemorrhage. The effect of this *Mucuna* extract on prothrombin activation after EV administration *in vitro* was studied and an increase in procoagulant activity was found, potentially explaining the protective effect *in vivo*.

Fertility-enhancing property

Misra and Wagner (2007), studied how to best extract L-Dopa from *Mucuna* for its use in reducing male impotency, as an aphrodisiac, and a nerve tonic. They found that L-Dopa can best be extracted with a 1:1 EtOH-H_2O mixture using ascorbic acid as protector, while thin layer chromatography (TLC) fingerprinting may be used to authenticate the plant material in the herbal industry. The use of *Mucuna* as an aphrodisiac was validated through clinical studies in India. *Mucuna* increases sexual potency, partly because it increases sperm count and testosterone levels (Siddhuraju et al., 1996). Due to the presence of L-Dopa, *Mucuna pruriens* can reportedly

be used as an aphrodisiac and prophylactic agent in patients suffering from oligospermia to elevate sperm count in men and improve ovulation in women (Lorenzetti et al., 1998; Sridhar and Bhat, 2007). The *Mucuna* bean also improves sperm motility (Sridhar and Bhat, 2007). *Mucuna*-derived L-Dopa and dopamine are also effective inhibitors of prolactin, a hormone released by the pituitary gland that is considered responsible for 70–80% of erection failures in males (Vaidya et al., 1978a,b). In one study, oral intake of *Mucuna* beans in 56 human males improved erection, duration of coitus, and post-coital satisfaction after 4 weeks of treatment.

Growth-promoting property

Mucuna also has anabolic and growth-hormone stimulating properties. The presence of L-Dopa and thus dopamine in the human system stimulates the release of growth hormone by the pituitary gland (Mesko, 1999). The anabolic effect of the bean is due to its ability to increase testosterone production. In 2002, a U.S. patent was filed (Patent No. 6340474; Anon, 2002) on the use of *Mucuna pruriens* to stimulate the release of growth hormone in humans.

Hypoglycemic property

Several *in vivo* studies of Nescafé's hypoglycemia-inducing effect validate the traditional use of the *Mucuna* plant for diabetes treatment (Grover et al., 2001, 2002). Feeding of a *Mucuna pruriens* seed diet for 1 week to rats reduced fasting blood glucose levels by 39% (Grover et al., 2002). Plasma glucose concentrations in mice were reduced by 9% when *Mucuna* was administered (Grover et al., 2001). Furthermore, decoction of the leaf (5 g/kg) or bean reduced total cholesterol concentration in rats (Taylor, 2004).

Anthelmintic property

Sources from various countries claim that *Mucuna* has anthelmintic properties (Taylor, 2004) but there is inadequate evidence to support this claim. Jalalpure et al. (2007) reported a significant increase in paralysis of worms due to application of a *Mucuna pruriens* oil extract .

Research in which *Mucuna* was substituted for soybean meal in the ration of sheep (Chikagwa-Malunga et al., 2008d) indicated lower coccidian oocyst scores ($P < 0.05$) and a 52% numerical reduction in fecal egg counts (FEC) in lambs fed a high *Mucuna* diet relative to a soybean diet. This emphasizes the need for further scientific investigation of the anthelmintic properties of *Mucuna*. Such studies are particularly important given the increasing problem of parasite resistance to antiparasitic drugs and the increased concern about drug residues in animal products and the environment. These problems make the search for biological anthelmintics a priority.

The most notorious helminth in tropical and sub-tropical small ruminant production is *Haemonchus contortus*. *Haemonchus contortus* infects sheep, goats, deer and other ruminants and has been a significant cause of economic loss to small ruminant-producers worldwide (Lange et al., 2006). It is therefore imperative to examine the anthelmintic effect of *Mucuna* on this problematic nematode. The considerable egg-laying capacity of *H. contortus* is maintained by adults feeding on blood. The late stage immature larvae also feed on blood. Blood loss can result in anemia, anorexia, depression, loss of condition, and eventually death of the host animal (Miller and Horohov, 2006).

The purported anthelmintic properties of *Mucuna* require scientific validation. If such claims are proven valid, research would also be needed to identify the active components to increase the therapeutic use of *Mucuna* components. Impacts of chronic ingestion of high levels of *Mucuna* L-Dopa also warrant further research (Pretty et al., 1998).

Mucuna pruriens as a Food and Feed Source

One of the most important uses of *Mucuna pruriens* is as a source of dietary protein. Dietary *Mucuna* has played an important role in preventing malnutrition in Central American countries such as Honduras (Eilitta et al., 2002) and African countries such as Benin, Nigeria (Versteeg et al., 1998), Malawi (Gilbert, 2002) and Guinea (Diallo et al., 2002). The use of

Mucuna as a food crop has also been reported in Ghana and Mozambique; during the 18th and 19th centuries, *Mucuna* was grown widely as a green vegetable in the foothills of the eastern Himalayas and in Mauritius, and both green pods and mature beans were boiled and eaten (Sridhar and Bhat, 2007). *Mucuna* was eventually replaced as a vegetable in Asia by more palatable legumes, but it is generally still eaten during famines. In northeastern India it is used as a specialty food.

In the mid 1980's, the women of the World Neighbors' development program in El Rosario, Honduras used *Mucuna* to make substitutes for wheat flour, coffee, and cocoa (Bunch, 2002) and developed 22 recipes that were inexpensive, easy to prepare, highly nutritional, and based on locally available ingredients. The program documented, among other benefits, the positive impact of *Mucuna*-based nutrichocolate on the milk production of nursing mothers whose breastfed infants progressed in two months from having second-degree malnutrition to no malnutrition at all (Bunch, 2002). In Guatemala and Mexico, *Mucuna pruriens* has traditionally been roasted and ground to make Nescafé, the main coffee in much of Central America (Pretty et al., 1998; Taylor, 2004). Nevertheless, concerns about the pharmacological properties of compounds in *Mucuna* have mitigated against wider use of *Mucuna* as a food source.

It is not clear if ingested L-Dopa accumulates in the tissues of monogastric livestock consumed by humans, but they do not seem to accumulate in ruminant tissues. Chikagwa-Malunga et al. (2008b) fed forty Rambouillet wether lambs on *Mucuna* or soybean meal diets and showed that muscle L-Dopa concentrations of all lambs were low and within the normal range (< 5 ng L-Dopa/g), indicating that ingested *Mucuna* L-Dopa did not accumulate in the animals' muscle tissue. The authors concluded that meat products from sheep fed *Mucuna* beans

containing about 2% L-Dopa 15 h prior to slaughter was safe for human consumption. Similar studies are required on monogastric livestock.

Nutritional Value

Various species of *Mucuna* are grown as a food crop in many parts of the world because of the nutrient density of the beans (Pretty et al., 1998). Table 2-3 shows the nutrient composition of *Mucuna* bean meal from Nigeria.

Table 2-3. Chemical composition of *Mucuna* bean meal

Parameter	Concentration
Proximate composition (g/kg DM)	
Crude protein	354
Crude fiber	77
Ether extract	32
Ash	36
Nitrogen-free extract	479
Major minerals (g/kg DM)	
Potassium	14
Calcium	10
Magnesium	19
Phosphorus	8
Trace minerals (mg/kg DM)	
Zinc	13
Manganese	27
Iron	129
Copper	25
Antinutritional *factors (mg/kg DM)*	
Hydrocyanic acid	82
Tannins	21
Phytic acid	21

DM=dry matter. Iyayi and Egharevba (1998), Iyayi and Taiwo (2003).

Protein Concentration

The crude protein (CP) concentration of raw *Mucuna* bean has been reported to be as low as 21% (Flores et al., 2002) and as high as 38% (Adebowale et al., 2005b). These differences are due to factors like variety, growth environment, and maturity. Protein concentration may decline as the bean matures, due to nitrogen availability during bean filling and the final bean size.

Immature beans have been reported to have a concentration of 37% CP while mature beans had 24% (Wanjekeche et al., 2003). Table 2-4 shows the difference in CP concentrations of different cultivars of *Mucuna*.

Table 2-4. Proximate crude protein concentration (g/100 g DM) of beans of 12 *Mucuna* accessions from Nigeria

	Crude protein
M. utilis	29.6
M. cochinchinensis	29.8
M. veracruz (white)	29.4
M. veracruz (mottled)	26.8
M. veracruz (black)	24.5
M. georgia	29.3
M. rajada	29.3
M. ghana	29.2
M. preta	28.0
M. jaspeada	27.6
M. pruriens	27.2
M. deeringiana	27.7
Mean ± SD	28.2 ± 1.6

SD=standard deviation. Adapted from Ezeagu et al. (2003).

The protein concentration of *Mucuna* beans is similar to that of other food grain legume beans, which can vary from 18 to 44% on a dry weight basis (Kay, 1979). Some studies show that *Mucuna* protein is made up mainly of albumin and globulins, which typically have a favorable pattern of essential amino acids (Bressani, 2002). Because of their high lysine concentration, *Mucuna* beans are good sources of supplementary protein for monogastric diets based on cereal grains and root crops, which are low in protein and lysine (Bressani, 2002; Adebowale et al., 2007).

Mucuna contains appreciable amounts of most amino acids (Ukachukwu et al., 2002), with the exception of sulfur amino acids (SAA). The methionine levels in *Mucuna* are 1.3 g/16 g N on average, according to Bressani (2002); this value is similar to other beans such as Jack bean (1.2), lima bean (1.5), and 1.2 in pigeon pea (Ukachukwu et al., 2002). The relatively low

methionine concentration of *Mucuna* is also evident from Table 2-5, which compares the amino acid concentration of *Mucuna* to the nutritional standards defined for human consumption by the Food and Agriculture Organization of the United Nations (FAO) and the World Health Organization (WHO).

Supplementation is an effective method for improving *Mucuna's* amino acid profile. The addition of 0.2-0.3% of the limiting SAA typically increases the protein quality of grain legumes, however digestibility was not improved (Bressani, 2002). The usual level of methionine supplementation is 0.3%, if a diet of 10% protein is derived exclusively from cooked beans. This added methionine should raise the total SAA concentration of the food to the recommended FAO/WHO level.

Mucuna protein digestibility is similar to that of other grain legumes (Bressani, 2002). *Mucuna* beans have an apparent protein digestibility ranging from 69-82% in rats (Siddhuraju et al., 1995). Bressani (2002) summarized protein digestibility studies conducted in human subjects and showed that digestibility is a problem in grain legumes due to enzyme-inhibiting polyphenols and the increases in dietary fiber concentration during the cooking of beans. Such polyphenols probably also explain the variability in the color of *Mucuna* beans. Colors ranging from white to black reflect differences in protein digestibility. In general, white beans have the highest digestibility (62.1%) followed by black and red *Mucuna* beans (49.6 and 55.7%), respectively (Bressani, 2002). This is because greater concentrations of the digestibility-inhibiting polyphenolic compounds in the seed coat are present in black or red than in white beans (Sridhar and Bhat, 2007).

Although *Mucuna* is high in protein as well as starch content, it is most valuable as a food or feed when used as a protein source to complement cereal-based diets of monogastrics that are

deficient in key amino acids (Adebowale et al., 2007). Diets used to alleviate hunger in developing countries are often characterized by bulky cereal-based porridge which lacks key amino acids (Adebowale et al., 2005a). *Mucuna* is a particularly useful protein supplement to such diets because its high lysine content complements the lysine deficiency in such cereal-based diets (Bressani, 2002; Adebowale et al., 2007).

Table 2-5. Amino acid concentration of *Mucuna* bean (g/16 g N) and Food and Agriculture Organization/World Health Organization (FAO/WHO) standard values for human diets.

Amino acid	Average *Mucuna*[a]	*Mucuna utilis*[b]	FAO/WHO standard[c]
Lysine	6.6	6.4	5.5
Histidine	3.1	2.2	-
Arginine	7.2	5.9	-
Tryptophan	1.4	ND	1.0
Aspartic acid	8.2	8.9	-
Threonine	3.6	4.2	4.0
Serine	4.1	4.1	-
Glutamic acid	17.2	14.4	-
Proline	ND	5.3	-
Glycine	5.1	3.9	-
Alanine	2.8	3.3	-
Cystine	0.8	ND	-
Methionine	1.3	1.9	3.53
Valine	5.6	5.3	5.0
Isoleucine	4.1	4.7	4.0
Leucine	7.9	7.2	7.0
Tyrosine	4.7	4.8	-
Phenylalanine	3.9	5.2	6.0

Adapted from Bressani (2002). ND = not determined; [a] Kay (1979); [b] Ravindran and Ravindran (1988); [c] FAO/WHO reference standard for human amino acid requirements

Other Nutrients

Mucuna beans are rich in minerals, especially potassium, magnesium, calcium, and iron (Table 2-6; Duke, 1981). Kay (1979) reported that the bean contains thiamine and riboflavin at low levels of 13.9 and 1.8 ppm. It contains low amounts of calcium, phosphorus, magnesium and sodium. The relatively high potassium (K) concentration in *Mucuna* is especially noteworthy.

Iron concentration is of special interest because the diets of many in developing countries where *Mucuna* is grown are deficient in this mineral (Bressani, 2002).

The lipid concentration of *Mucuna* beans varies widely. Some researchers reported low ranges of 2.8-4.9% (Siddhuraju et al., 2000) but others reported higher ranges of 8.5-14.0% (Vijayakumari, et al., 2002). Adebowale et al (2005b) showed that the ether extract of whole seed, cotyledon and seed coat consists of 9.6, 9.8 and 3.0% lipid, respectively.

Comparison to Other Legumes

According to Bressani (2002), *Mucuna* beans are similar to common beans and other edible grain legumes in proximate composition, amino acid concentration, micronutrients, content of antinutrients (e.g., enzyme inhibitors, lectins, phenolic acid, tannin compounds, phytic acid, and sugars). However, only *Mucuna*, and to a lesser extent *V. faba* (Faba bean), contain L-Dopa.

Table 2-6. Mineral concentration of different cultivars of *Mucuna* (mg/100g DM)

Mineral	*Mucuna*			
	pruriens[a]	*utilis*[b]	*gigantea*[c]	*pruriens*[d]
Sodium	17.4	70.0	35.3	4.1
Potassium	1330.4	1110.0	2295.6	2537.0
Calcium	285.7	250.0	518.3	247.0
Magnesium	85.1	110.0	506.5	72.4
Phosphorus	406.5	220.0	194.3	459.0
Manganese	0.56	1.00	2.36	0.31
Iron	6.54	1.30	9.42	5.19
Copper	2.30	0.60	1.18	0.47
Zinc	2.04	1.00	8.24	1.71

[a] Siddhuraju et al. (1996); [b] Ravindran and Ravindran (1988); [c] Rajaram and Janardhanan (1991); [d] Mary Josephine and Janardhanan (1992).

The lysine concentration of grain legumes ranges from 223 mg/g N for the peanut (*A. hypogaea*) to 492 mg/g N in Dolichos (*D. lablab*). The concentration of lysine in the *Mucuna* bean ranges from 327 to 412 mg/g N (Bressani, 2002). Gross energy (GE) ranges from 16.6-17.2

KJ/g (Ezeagu et al., 2003). *Mucuna* is nutritionally similar to other known legume crops, such as Jack bean (*Canavalia ensiformis*) and Yam bean (*Sphenostylis stenocarpa*). It also compares closely with other legumes of West Africa, such as Kidney bean (*Phaseolus vulgaris*), Lima bean (*Phaseolus lunatus*), Pigeonpea (*Cajanus cajan*), and Bambara nut (*Voandzeia subterranea*) (Bressani, 2002).

The nutritive value of *Mucuna* bean is similar to that of soybean in many respects (Ukachukwu et al., 2002; Kay, 1979). However, important differences also exist; *Mucuna* has much higher starch content, lower fat and protein concentrations, and lower SAA concentrations than soybean (Adebowale et al., 2007). The SAA concentration of grain legumes ranges from 96 to 224 mg/g N, and the FAO/WHO reference value of SAA in humans for grain legumes is 220 mg/g N. Values of SAA in *Mucuna* in the literature range from 116 to 132 mg/g N (Bressani, 2002), which accounts for only 53-60% of the FAO/WHO reference value for amino acids in human diets. Therefore, methionine supplementation is advisable when *Mucuna* is the main dietary protein source.

Various studies have shown that consumption of other grain legume beans reduced plasma cholesterol levels and slowly increased blood glucose levels due to fiber intake from the beans. In general, the acid detergent fiber (ADF) and neutral detergent fiber (NDF) concentrations of *Mucuna* are at least as great as those of other grain legumes (Bressani, 2002), as shown in Table 2-7. Therefore, *Mucuna* bean intake will likely decrease plasma cholesterol and slowly increase blood glucose levels.

Table 2-7. Fiber fractions in various grain legumes (g/100g DM)

Component	*Canavalia ensiformis*[a]	*Cajanus cajan*[b]	*Vigna sp.1*	*Mucuna*	
				guatemala	*utilis*
Acid detergent fiber	11.0	9.1	7.6-7.9	8.9	10.4±0.6
Neutral detergent fiber	13.6	15.1	9.5-10.7	14.7	20.4±0.7
Hemicellulose	2.6	6.0	1.6-3.1	5.8	10.0±0.9
Cellulose	8.1	6.9	5.0-5.7	7.1	9.3±0.9
Lignin	2.9	2.2	2.2-2.6	1.8	0.8±0.06

[a] Bressani and Chon (unpublished data); [b] Ravindran and Ravindran (1988). Bressani (2002).

Antinutritional and Toxic Properties

Antinutritional compounds in *Mucuna* beans include L-Dopa, tannins, lectins, phytic acid, and trypsin and amylase inhibitors (Sridhar and Bhat, 2007). *Mucuna* is also rich in indolic alkaloids, saponins, and sterols (Manyam et al., 2004). Concentrations of these antinutrients in *Mucuna* are shown in Table 2-8. The stinging hairs of the seed pods contain the phytochemical mucunain, which causes skin irritation and itching (Pretty, 1998). This severe itching could also be due to the serotonin found in *Mucuna* pods (Szabo, 2003).

Because of their common tryptamine base structure and hallucinogenic properties, the indolic alkaloids in *Mucuna* have also been of concern (Szabo, 2003). Hallucinogenic indoles such as N,N-dimethyltryptamine, bufotenine and other tryptamines, including serotonin have been detected in various parts of the *Mucuna* plant (Daxenbichler et al., 1972; Lorenzetti et al., 1998). Szabo and Tebbett (2002) measured alkaloids in *Mucuna* roots, stems, leaves, pods, and bean and reported low concentrations of approximately 0.001%. Serotonin was found only in fresh leaves and stems (~0.001%) and not in the *Mucuna* bean. However, more recently Szabo (2003), analyzed roots, pods, stems, leaves, and beans for various indolealkylamines and reported indoles to be present at roughly 0.0001% by weight; these levels are lower than previously measured. Many plants contain serotonin including commonly eaten fruits such as apples (17 mg/g) and bananas (15 mg/g). By comparison these levels are much higher than those

Table 2-8. Antinutritional components of beans of 12 *Mucuna* accessions from Nigeria

Component	*M. utilis*	*M. cochin-chinensis*	*M. veracruz* (white)	*M. veracruz* (mottled)	*M. veracruz* (black)	*M. georgia*	*M. rajada*	*M. ghana*	*M. preta*	*M. jaspeada*	*M. pruriens*	*M. deerin-giana*	Mean ± SD
L-Dopa (g 100 g-1)	6.82	6.35	5.75	6.43	8.34	7.24	4.00	5.35	7.50	6.57	6.30	8.18	6.57 ± 1.21
Trypsin Inhibitors (TUI/mg)	30.81	42.12	36.97	51.55	36.64	45.97	43.02	38.32	43.27	46.55	45.04	47.63	42.32 ± 5.74
Phytate (g 100 g-1)	0.85	0.79	0.85	0.90	0.26	0.32	0.29	0.53	0.26	0.45	0.37	0.66	0.54 ± 0.25
Phytate-P (g 100 g-1)	0.24	0.10	0.24	0.25	0.07	0.09	0.08	0.15	0.07	0.13	0.11	0.19	0.14 ± 0.07
Phytate-P (as % Total P)	47.89	22.41	49.38	45.26	14.18	16.57	18.43	33.79	14.57	27.10	24.44	34.38	29.03 ± 12.97
Total Oxalate (mg 100 g-1)	1.35	2.48	2.08	1.98	1.13	1.53	1.98	2.95	2.31	1.81	1.26	2.85	1.98 ± 0.60
Soluble Oxalate (mg 100 g-1)	1.12	1.19	1.71	1.08	1.08	1.08	1.76	1.89	1.85	1.08	1.17	2.01	1.42 ± 0.38
Soluble oxalate (% total)	82.96	78.63	82.21	54.55	95.58	70.59	88.89	64.07	80.09	91.53	92.86	70.53	79.37 ± 12.47
Cyanide (mg 100 g-1)	0.12	0.12	0.12	0.10	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12 ± 0.01
Nitrate-N (mg 100 g-1)	1.80	ND	ND	1.60	ND	ND	ND	ND	ND	ND	5.20	2.40	2.75 ± 1.67
Tannin (g 100 g-1)	1.62	1.63	1.62	1.60	1.59	1.63	1.66	1.70	1.65	1.57	1.62	1.56	1.62 ± 0.04

The values are means of two independent determinations. ND=Not Detected. Ezeagu et al., 2003.

reported in *Mucuna* bean (Szabo and Tebbett, 2002). Most tryptamine derivatives are also characterized by poor absorption and rapid peripheral metabolism; thus the presence of low level indolealkylamines is unlikely to affect the use of *Mucuna* as a food and feed source (Szabo, 2003).

Tannins

It has been reported that tannic acid consumption negatively impacts the protein efficiency ratio and apparent protein digestibility of red *P. vulgaris* (Bressani, 2002); as intake of tannins increases, protein quality and protein digestibility decreases, therefore the tannins in *Mucuna* may affect its viability as a protein source.

Tannins are natural defense compounds found in plants that make the plant unpalatable due to their astringency. Tannins, like other phenolic compounds, vary widely in type, concentration and astringency. In addition to these factors, tannin-binding effects depend on animal physiological status, sex, exposure to pathogens, anticipated productive performance and environmental conditions (Waghorn et al., 2003). The structural diversity of tannins within and between plant species causes variation in their biological activity.

According to Reed (1995), tannins are water-soluble polymeric phenolics that form strong complexes with proteins. The strength of these complexes depends on characteristics of both tannin and protein (molecular weight, tertiary structure, iso-electric point, and compatibility of binding sites; Waghorn et al., 2003). Horvath (1981) gave a more comprehensive definition that reflects the fact that tannins form complexes with starch, cellulose, minerals as well as protein.

The antinutrient activity of tannins starts early in the digestive tract where they form complexes with salivary glycoproteins resulting in reduced feed intake. This tannin–mucoprotein complex diminishes the lubricant property of saliva, leading to dryness of the oral cavity. Tannins reduce feed digestibility by binding bacterial enzymes and forming complexes with cell

wall carbohydrates; they also exert inhibitory effects on the growth and activity of rumen microbes and can be carcinogenic (Wang et al., 1976). The two major classes of tannins are condensed tannins or proanthocyanidins (PA) and hydrolyzable tannins (HT; Reed, 1994).

Proanthocyanidins

Proanthocyanidins, the most common type of tannin found in forage legumes, have been considered non-toxic; however, they are associated with lesions of the gut mucosa and can result in extensive binding of proteins, including microbes, salivary, and enzyme sources (Waghorn et al., 2003). Proanthocyanidins are flavonoid polymers confined to intracellular vacuoles and are essentially un-reactive until released by cell rupture (Waghorn et al., 2003). Tannins reduce cell wall digestibility by binding bacterial enzymes and forming indigestible complexes with cell wall carbohydrates. Digestibility of organic matter and fiber fractions is low for high PA diets (Reed, 1994).

Chewing initiates binding between PA and plant and salivary proteins causing protein aggregation instead of the normal solubilization. Proanthocyanidins are indiscriminate in the protein to which they bind and easily create a protein (N) deficiency, especially when poor-quality grasses make up a substantial portion of the diet (Waghorn et al., 2003).

Hydrolyzable tannins

Hydrolyzable tannins are especially toxic to ruminants. Pyrogallol, a hepatotoxin and nephrotoxin, is a product of HT degradation by ruminal microbes. Hervas et al. (2002) reported striking lesions in the digestive tract, distension of abomasum and small intestine, and dense mucous material in the caecum, and changes in plasma biochemistry in sheep fed high (3 g quebracho tannin extract/kg live-weight) tannin levels. The major lesions associated with HT poisoning are hemorrhagic gastroenteritis, necrosis of the liver, and kidney damage with proximal tubular necrosis.

Tannins can be used to increase post-ruminal protein availability in ruminants. Preston and Leng (1990) demonstrated that inclusion of tannins in low amounts in ruminant diets reduced ruminal fermentation and allowed protein to bypass the rumen to the lower intestine where it was more readily available. However, the release of the protein post-ruminally depends on the nature of the tannin-protein complex and the prevailing pH (Salawu et al.,1999).

The *Mucuna* plant contains up to 8% tannins, mostly concentrated in the leaves (4.4-7.4%; Chikagwa-Malunga et al., 2008a); such levels can reduce DM digestibility and protein utilization (Makkar et al., 1988; Mangan, 1988; Orskov and Miller, 1988; Dalzell et al., 1998). As shown in Table 2-8, the bean regularly contains lower concentrations of tannins (1.6-1.7%; Ezeagu et al., 2003). These levels may be beneficial in ruminant diets since they can promote the bypassing of the rumen. Levels considered beneficial for this purpose range from 2-4% of DM while higher levels have been associated with reduced digestibility in livestock (Mueller-Harvey and McAllan, 1992). Although the concentrations in *Mucuna* beans and pods are reportedly low and the majority of tannins in legumes are non-toxic proanthocyanidins, more research is needed to ascertain the hydrolysable tannin content of *Mucuna* beans.

L-Dopa

The beans of most *Mucuna* species contain relatively high concentrations of 3, 4 dihydroxy-L-phenylalanine (L-Dopa), the aromatic amino acid precursor of the neurotransmitter dopamine. *Mucuna* beans from various sources and cultivars contain 2.3-7.6% L-Dopa (St-Laurent et al., 2002); this is in agreement with the reported range of 3.1-6.7% by Daxenbichler et al. (1972). However, one study reported as little as 1.5% L-Dopa in the bean of *M. gigantea* in southern India (Rajaram and Janardhanan, 1991). The wide range and discrepancies may reflect the improper taxonomy of *Mucuna pruriens* cultivars, though variations among cultivars may

also be due to genotypic variation, maturity, latitude, and environmental factors (St-Laurent et al., 2002).

Due to the abundance and toxic nature of L-Dopa, it is of greater concern than other antinutrient components of *Mucuna* (Szabo, 2003). The consequences of consuming toxic levels of these compounds can be severe. For instance, an outbreak of acute psychosis in Mozambique was attributed to excessive consumption of velvetbean L-Dopa (Infante et al., 1990). This resulted because, during a famine and drought, the water used to boil and detoxify the bean was consumed rather than discarded as it normally is, and thus a higher than normal volume of the toxins was ingested (Pretty et al., 1998).

In one study, L-Dopa was assayed in the roots, stems, leaves, and pods of dried *Mucuna pruriens,* variety *utilis* plants, the stems and leaves of fresh plant material, in raw bean samples, and in bean prepared according to four different recipes (Szabo and Tebbett, 2002). Concentrations were 0.15% in dried leaves and pods, 0.49% in dried stems 4.47 to 5.39% in the raw bean, 0.10% in beans boiled repetitively, and 2.38% in roasted beans. Chikagwa-Malunga (2008a), reported that L-Dopa concentrations in the stems (0.1-0.2%) and whole plant (0.1-1.8%) peaked at 110 days after planting while the concentration in the leaves remained constant (0.1-0.3%) during plant maturation of *Mucuna pruriens,* variety *utilis*. Whole pods contained up to 4% L-Dopa and this concentration decreased with maturity. It is evident from the literature that the highest concentration of L-Dopa is present in the seed, which is the main part of the plant used in monogastric diets.

Pharmacodynamics of L-Dopa in humans

Most of the adverse effects of *Mucuna* consumption by humans are associated with L-Dopa, which is an intermediary product in the enzymatic synthesis of dopamine from L-tyrosine (Szabo and Tebbett, 2002). Dopamine regulates functions in the brain (neurotransmitter), heart

(inotropic increase of cardiac output), vascular system (vessel dilator), and kidney (diuretic) (Grossman et al., 1999; Grover et al., 2001). L-Dopa is widely distributed in muscle, kidney and liver, and present across the blood-brain barrier in the central nervous system due to de novo synthesis (Szabo and Tebbett, 2002). Many of these physiological effects are correlated with the de novo synthesis and level of intake of L-Dopa.

Szabo and Tebbett (2002) provided the following description of the pharmacokinetics in humans: Approximately 33% of an orally administered dose of L-Dopa is absorbed from the human gastrointestinal tract, primarily from the jejunum. Peak plasma concentrations occur within 1 to 3 hours of ingestion with levels that may vary as much as tenfold among individuals. L-Dopa undergoes decarboxylation into dopamine extra- and intracerebrally. A majority of the successfully absorbed L-Dopa is converted to dopamine in the periphery, mainly in the intestinal mucosa via decarboxylation by the enzyme L-aromatic amino acid decarboxylase (LAAD). In addition to dopamine, peripheral L-Dopa is metabolized to melanin, norepinephrine, 3-methoxytyramine, methyldopa, 3,4-dihydroxyphenylacetic acid, and 3-methoxy-4-hydroxyphenylacetic acid (homovanillic acid or HVA). These metabolites are rapidly excreted in the urine such that about 80% of an administered dose of L-Dopa is excreted within 24 hours; less than 1% of which consists of the original compound (Dollery, 1999; Szabo and Tebbett, 2002). Of the L-Dopa metabolites in the urine, roughly 50% consist of dihydroxyphenylacetic acid (DOPAC) and HVA, 10% of dopamine, while less than 30% occurs as 3-O-methyldopa (Dollery, 1999).

Dopamine produced by decarboxylation of L-Dopa by LAAD in the periphery does not cross the blood-brain barrier, but is further metabolized by mono amine oxidase (MAO) in endothelial cells into DOPAC (Szabo and Tebbett, 2002). Less than 1% of the administered dose

actually crosses the blood-brain barrier into the central nervous system and the basal ganglia. In the brain's basal ganglia it is converted to dopamine by LAAD or dopa decarboxylase. Dopamine subsequently undergoes enzymatic inactivation catalyzed by MAO and catechol-O methyltransferase (COMT). The COMT in the glial cells, methylate dopamine to 3-methoxytyramine, while MAO oxidizes dopamine to DOPAC. The COMT also methylates about 10% of oral L-Dopa to 3-O-methyldopa in the red blood cells and in the liver (Dollery, 1999). The COMT and MAO together convert dopamine to 3-methoxy-4-hydroxyphenyl acetic acid and HVA.

Adverse effects of L-Dopa on humans

Most side effects of L-Dopa ingestion in humans arise directly from dopamine's activity as a neurotransmitter involved in the regulation of the heart, vascular system, digestive tract, and excretory system, rather than from its well-known effect on receptors in the brain. Some consequences of increased peripheral dopamine include orthostatic hypotension resulting in dizziness and in some cases staggering and increased heart rate (Szabo and Tebbett, 2002). Although tolerance to the effects develops in individuals ingesting L-Dopa over a few months, the heart-related effects could pose a serious problem for those who have a predisposing cardiac condition (Szabo and Tebbett, 2002). In contrast, some studies report improved cardiac function after L-Dopa administration to patients with refractory or congestive heart failure (Grossman et al., 1999). This was because oral L-Dopa treatment produced beneficial natriuretic and diuretic effects because the resulting dopamine increases renal blood flow and increases the force of cardiac contraction. These beneficial effects temporarily relieve the symptoms and signs of heart failure.

Psychiatric disturbances have also been reported in patients receiving high doses of L-Dopa in a dose-dependent manner (Szabo and Tebbett, 2002). These include mild nervousness,

anxiety and agitation, insomnia, and vivid dreams. About 15% of Parkinson's patients have experienced serious psychiatric manifestations as a result of L-Dopa therapy. Confusion, delirium, depression and, in extreme cases, psychotic reactions with hallucinations have been reported (Szabo and Tebbett, 2002), suggesting that L-Dopa should be avoided by patients with a history of psychosis or epilepsy. Also, due to its inotropic and vasodilating activity, L-Dopa should be avoided by patients with glaucoma, asthma, renal, hepatic, cardiovascular or pulmonary disease (Dollery, 1999). Since it is a precursor of melanin, which is associated with melanoma formation, L-Dopa could promote the formation of skin cancers (Dollery, 1999).

Based on anecdotal evidence, the *Mucuna* bean could potentially cause contraction of the womb (Taylor, 2004); likely due to its inotropic property and this could be useful in inducing or assisting during labor. However, *Mucuna's* usefulness as a uterine stimulant is jeopardized by its potentially harmful impact on the unborn baby. Szabo and Tebbett (2002) report that L-Dopa consumed by nursing mothers can also appear in breast milk, causing harmful side effects in infants.

Nausea, vomiting, and anorexia have been reported as side effects of *Mucuna* ingestion, and are associated with the effects of peripherally produced dopamine on the dopamine receptors in the area postrene of the brain stem. Nausea and vomiting may occur on consumption of as little as 250 mg of L-Dopa if the patient is unaccustomed to such an exposure (Szabo and Tebbett, 2002). These adverse effects emphasize the need for detoxification of *Mucuna* before it is fed to humans.

Adverse effects of L-Dopa on monogastric livestock

As in humans, most adverse effects of *Mucuna* intake in monogastric livestock are attributable to its L-Dopa concentration. Symptoms in broilers and pigs include reduced feed intake, weight loss, and low feed conversion (Del Carmen et al., 2002; Flores et al., 2002). In one

study, Flores et al. (2002) reported reduced palatability, daily weight gains, feed intake, and feed conversion with substitution of *Mucuna* beans for soybean meal in pig diets. Necropsy revealed acute toxic hepatitis and advanced necrosis in the pigs fed the *Mucuna* bean. Liver failure was also evident as blood analysis showed abnormal concentrations of glutamic pyruvic transferase, aspartate amino transferase, and bilirubin.

Relative to control animals, abnormalities have been observed in animal growth, carcass and individual organ weight, and blood plasma levels of triiodothyronine, cholesterol and creatine in chickens fed raw *Mucuna* beans (Carew et al., 2002; Del Carmen et al., 1999). According to Del Carmen et al. (1999), reduction in growth and carcass weight of broilers resulted from inclusion of 10 – 30% of raw *Mucuna* in the diet. Feed intake decreased at the 30% inclusion rate while feed efficiency fell at both 20 and 30% rates. Feed intake was less severely affected than growth, indicating that reduced feed intake was not the major cause of growth decrease. Rather, the authors suggested that *Mucuna* bean inhibited metabolic processes, leading to growth reduction. This was in agreement with Carew et al. (2002), who observed similar reductions in growth and feed intake when *Mucuna* levels were increased in chick diets. Additionally, Carew et al. (2002) reported reduced growth, increased weight of the gizzard and pancreas, and lengthening of the small intestine and ceca in chicks fed raw *Mucuna* beans. The increased gizzard and pancreatic weight were attributed to *Mucuna's* poor digestibility and trypsin-inhibitor concentration, which increased the demand on these organs to secrete more exocrine digestive enzymes. Low concentrations of plasma triiodothyronine (T3), blood cholesterol and plasma creatine were also associated with *Mucuna* bean intake, and these reflected increased glomerular filtration rate in the kidney, as well as low muscle mass. The concentration of the cytoplasmic enzyme, alanine aminotransferase increased with *Mucuna* bean

intake, indicating either liver or muscle damage. Decreased plasma concentrations of creatine provided strong evidence of muscle damage but alkaline phosphatase, another indicator of liver damage, did not confirm the damage in chickens. The foregoing emphasizes the need to investigate methods of reducing the L-Dopa concentration of *Mucuna* to safe levels.

Safe levels of dietary *Mucuna* L-Dopa

The dose at which a chemical becomes toxic is highly dependent on the prevailing conditions and the applications; compounds that are considered toxic at certain levels of exposure may help sustain, improve, or restore health at other dosages. All chemicals can be considered toxic if the dose is high enough and even highly toxic chemicals may be used safely if exposure is kept low enough (Williams et al., 2000). *Mucuna* L-Dopa has long been used in the treatment of Parkinson's disease (Manyam and Sanchez-Ramos, 1999; Nagashayana et al., 2001) and other ailments (Taylor, 2004), yet the toxicity of *Mucuna* L-Dopa has limited its use for dietary purposes (Szabo and Tebbett, 2002).

Szabo and Tebbett (2002) suggested that *Mucuna* L-Dopa can be transferred to milk after consumption of *Mucuna*-based products. However, recent research indicates that ingested *Mucuna* L-Dopa does not accumulate in ruminant tissues (Chikagwa-Malunga et al., 2008b). Published studies on accumulation of *Mucuna* L-Dopa in monogastric tissues were not found. Therefore, although guidelines on safe *Mucuna* L-Dopa concentrations for monogastric diets exist, it is unclear if ingestion of L-Dopa at such levels results in L-Dopa accumulation in the tissues of monogastric livestock consumed by humans.

Guidelines on safe *Mucuna* L-Dopa concentrations for monogastric diets are based on *in vivo* experiments (Carew et al., 2003; Ferriera et al., 2003; Iyayi and Taiwo, 2003; Ukachukwu and Szabo, 2003) as well as data gathered from use of L-Dopa in the treatment of Parkinson's Disease (Szabo and Tebbett, 2002). Several researchers have agreed that L-Dopa concentrations

of 0.3 – 0.4% of *Mucuna* (DM basis) are safe for monogastrics based on multiple studies with poultry (Eilitta et al., 2003). However, this safe limit for poultry should be considered a guideline for other monogastric species because the level of L-Dopa toxicity probably varies with animal species, metabolic rate, performance level, body and health condition, and the level of intake relative to body weight.

According to Lorenzetti et al. (1998), the maximum daily dose of *Mucuna* L-Dopa that can be tolerated by an adult person without causing side effects is approximately 1500 mg per day. Therefore, a healthy adult should be able to safely consume 500 g of *Mucuna*-based food containing 0.1% L-Dopa, and dietary *Mucuna* or *Mucuna* products should contain no more than 0.1% L-Dopa. However, this guideline may not apply to long-term L-Dopa ingestion or to *Mucuna* consumption by children, pregnant women, and people with a medical condition (Szabo and Tebbett, 2002). This safety level is in agreement with Teixeira et al. (2003) who concluded that 0.1% L-Dopa is an acceptable target level, based on the fact that Faba bean and Broad bean *(Vicia faba)* contain 0.2 – 0.5% L-Dopa, yet they have been safely consumed for generations by people around the world.

Although these safety targets are invaluable, future research should focus on obtaining more specific guidelines that account for species and physiological stage. In order to determine the dose-response relationship of *Mucuna* L-Dopa on monogastrics, detailed toxicity testing is required for a significant duration of time. The response to these dosages should range from a lethal dose (e.g. LD_{50}) to a dose that shows no observable effect levels (NOEL) or side effects under the established time frame. Using established rules of risk assessment, these findings can then be extrapolated to other species, including the human (Williams et al., 2002).

Detoxification of *Mucuna pruriens*

Processing methods have been developed to facilitate nutrient utilization of many food grain legumes for both human and animal consumption. Table 2-9 shows the nutritional concentration of soybean and *Mucuna* processed by some of such procedures. In the case of *Mucuna*, processing methods can serve the additional purpose of detoxifying the bean by decreasing the L-Dopa concentration to a safe level while maintaining its nutritional benefits (Bressani, 2002). Some of the processing methods used with *Mucuna* include soaking in water, alkalis or acids, and various cooking methods including dry heating (roasting), boiling and frying, as well as germinating or fermenting. These processing methods are discussed below.

Table 2-9. Composition of soybean meal and *Mucuna* beans processed by different methods (DM basis).

Meal	DM %	CP %	CF %	Ash %	Ca %	P %	L-Dopa %
Soybean meal	90.15	48.00	6.28	5.73	0.25	0.60	NA
Toasted *Mucuna*	92.65	22.31	6.16	3.48	0.14	0.35	2.76
Cooked *Mucuna*	88.45	22.10	5.63	3.16	0.14	0.36	2.58
Soaked *Mucuna*	89.16	21.9	6.16	3.27	0.16	0.36	2.55
Raw *Mucuna*	88.98	21.77	4.69	3.17	0.17	0.42	3.99

DM: Dry Matter; CP: Crude Protein; CF: Crude Fiber; Ca: Calcium; P: Phosphorus; NA: not applicable; Toasted = dry heated for 30 min. at 130^{o}C; Cooked = boiled for 30 min.; Soaked = immersed in water at room temperature for 48 hours; Raw = untreated. Flores et al. (2002)

Thermal processing methods include cooking beans at atmospheric pressure or pressure-cooking, without or with previous soaking in water, which typically reduces cooking time. Although such methods can reduce L-Dopa concentrations, cooking increases dietary fiber concentration from approximately 19 to 26% and the fiber traps protein and probably makes it unavailable (Bressani, 2002). Cooking also induces losses of vitamins (25-30%) and minerals (10-15%). Specific thermal processing methods include the following:

Boiling

According to Teixeira et al. (2003), L-Dopa extraction from ground *Mucuna* beans (1 mm particle size) can be achieved by increasing the water temperature. The extraction time required for reducing the L-Dopa concentration to 0.1% can be reduced from 55 hours at 20^{o}C to < 1 hour at 100^{o}C. Attempts to replace boiling in water by extraction with acidic or alkaline solvents or to reduce boiling time by presoaking before boiling have yielded different results. Nyirenda et al. (2003) soaked *Mucuna* grits (4 mm particle size) in water with and without sodium bicarbonate for 24 hours, and then they boiled it for 1 hour and soaked it again for 24 hours in order to achieve an 88% L-Dopa reduction (0.4% L-Dopa). They concluded that boiling alone was the principal method for eliminating L-Dopa and that the soaking steps may not be worthwhile. Ukachukwu and Szabo (2003) used wet heating (boiling for 30-45 min) of beans, with or without additives (4% wood ash, sodium carbonate, or calcium hydroxide) to detoxify *Mucuna* and tested the product in broiler diets. Broilers fed on beans previously treated with wood ash and boiled for 45 minutes performed best and such treated beans could be included at levels of up to 30% of the ration of broilers without causing adverse effects on weight gain, feed conversion ratio, and protein efficiency ratio relative to a maize-soybean dietary control group. Nyirenda et al. (2003) fed broilers on diets in which 50% of the dietary CP was from *Mucuna* beans detoxified through boiling for 60 minutes and subsequently drying at 50^{o}C for 18 hours or soaking in 0.25% and 0.50% baking soda for 24 hours. They found that the rations produced similar feed intake, weight gains, and feed conversion ratios as control diets consisting of maize and soybean.

When Ukachukwu and Obioha (1997) compared the efficacy of dry and wet thermal processing methods, such as toasting (dry heating) or boiling in water at a temperature of 100^{o}C, they found that boiling was more effective as it reduced hydrocyanic acid concentration by 25%,

hemagglutinin activity by 50%, trypsin inhibitor activity by 43%, tannin concentration by 10%, and L-Dopa concentration by 31-41% (Ukachukwu and Obioha, 2000).

Roasting

Roasting beans enhances the flavor and reduces the L-Dopa concentration up to 45% but reduces their nutritive value by lowering the levels of available lysine and other amino acids (Bressani, 2002). Heating at 130^{o}C for 30 minutes reversed many adverse effects of L-Dopa on blood chemistry and anatomy in poultry. Inclusion of 10% roasted *Mucuna* beans in the diet of chickens resulted in better growth and carcass yield relative to a raw *Mucuna* treatment since trypsin inhibitor activity was eliminated by heating (Del Carmen et al., 1999). Heating also eliminated amylase inhibitor activity.

Iyayi and Taiwo (2003) reported that layers fed 6% of the diet as roasted *Mucuna* beans maintained good egg quality relative to a soybean-based control diet. The beans were roasted with sand over an open fire for about 40 minutes till their shiny seed coats became dull. They also reported that replacing 33% of dietary soybean meal with *Mucuna* beans did not reduce feed intake or weight gain but did cause kidney damage. From an animal production standpoint, this 33% replacement may seem acceptable since it may not negatively affect the production and economic gain during the animals' productive life. From an animal health standpoint, however, the adverse changes in physiology are important since they can cause unnecessary discomfort to the animal. Further research is needed to determine the appropriate inclusion rate of roasted *Mucuna* beans for maintaining animal health.

Diallo et al. (2002), report that the most effective detoxification method was to roast the beans for 20-30 minutes, crack them, leave them in water for 48 hours while changing fresh water every 8-12 hours, and then cook them for about 2 hours. This technique removed L-Dopa to a level below 0.1% but it is more labor intensive than many others.

Of the processing methods mentioned thus far, only those methods that utilized boiling decreased the L-Dopa concentration to or near the target level. While roasting removed about half the L-Dopa, permeation of the seed body with hot water was necessary to extract >75% of this water-soluble compound. Therefore, boiling is preferable to roasting for L-Dopa removal.

Although many thermal processing methods are effective in reducing L-Dopa levels and rendering the bean safe for monogastric consumption, applying sufficient heat requires time, energy and expense. Many energy sources for rapidly heating *Mucuna* would be costly or unavailable in developing countries where *Mucuna* consumption is likely to be most beneficial. Therefore it is important to investigate alternative methods.

Ensiling

Mucuna has been fermented with *Rhizopus oligosporus* to make food products such as tempe (Egounlety, 2003). The fermentation did not reduce CP concentration after 48 hours but increased the concentration of water-soluble proteins from 1.22% to 19.42%, indicating increased proteolysis. Matenga et al. (2003) studied effects of ensiling different mixtures of maize and *Mucuna* and reported a reduction in L-Dopa content of 10% for a 100% *Mucuna* with 0% maize mixture, and a reduction of 48% for a 30% *Mucuna* with 70% maize mixture. Therefore, fermentation might be a useful *Mucuna* detoxification strategy and the efficacy seemed to increase as more fermentable carbohydrates were supplied in the mixture. However, no other studies examining effects of fermenting *Mucuna* by itself or without supplemental micro organisms were found in the literature. Therefore this method of detoxifying *Mucuna* requires further study.

Solvent Extraction

Solvent extraction is widely used in the food, pharmaceutical and chemical industries to extract a soluble constituent from a solid. Solvent extraction involves three steps: (1) submerging

the solute in the solvent; (2) separating the solution formed from the spent residue; and (3) washing the spent residue (Balaban and Teixeira, 2002). High temperatures can be used to facilitate this process. However, for the extraction to be successful, the beans must be broken into fragments and must be in contact with the water for a sufficient amount of time.

Solubility in water

Water is the most readily available solvent for smallholders, but according to the Merck Index (1983), L-Dopa has only limited solubility in water (66 mg in 40 mL). Assuming an initial concentration of L-Dopa in *Mucuna* bean of 6-7% dry weight, this translates into the need for 40 parts of water to one part bean by weight (40 liters of water per kg of beans; Teixeira et al., 2003). This may be unrealistic for smallholders in places where copious amounts of water are not readily available or where clean water is expensive.

Nyirenda et al. (2003) soaked *Mucuna* grits (4 mm particle size) in water for 24 hours (1:1 ratio by weight of water to bean), they then boiled it for 1 hour and soaked it again. They came to the conclusion that boiling was effective at reducing L-Dopa but the soaking steps were not. Their observation was likely based on the fact that not enough water was used to satisfy the solubility requirements and that the particle size might have been insufficiently small. Teixeira et al. (2003), observed a reduction in L-Dopa concentration to below 1% within 24 hours of extraction in water alone for 1-mm but not in 2-, 4-, or 8-mm *Mucuna* bean particles. They used a 28 L water bath to submerge 45 five gram samples under continuous water circulation of 15 L per minute. Therefore, extraction rates can be quite high provided 1) sufficient water is used, 2) the *Mucuna* bean is ground into a sufficiently small particle size, and 3) agitation is performed.

Two solvent extraction processing methods using water and technology more readily available to smallholders in developing countries with access to rudimentary food preparation tools are the Sack in Stream and Overflowing Trough and Rake methods (Diallo et al., 2002).

Sack in Stream

In the Sack and Stream method, a strong, porous sack filled with cracked and de-hulled *Mucuna* beans is submerged in a flowing stream or river (Diallo et al., 2002). This method brings a constant supply of continuous-flowing pure solvent in contact with the bean particles with little energy, labor, or equipment required. The force of the water flowing through the sack enhances L-Dopa extraction at the particle/solvent interface, and the constant supply of fresh water maintains a maximum concentration gradient for effective extraction. Immersing *Mucuna* beans within a porous bag in a flowing river for 3 days reduced L-Dopa concentration of cracked and whole beans to 0.2 and 0.72%, respectively (Diallo and Berhe, 2003). Depending on the size and flow rate of the river and the amount of L-Dopa released from the beans, adverse impacts on marine life could occur. Long-term damage would be unlikely however, given the short half life (1 hour) of L-Dopa (Murata, 2006), modest *Mucuna* volumes processed by smallholders, and the low concentrations of L-Dopa due to constant dilution.

Overflowing Trough and Rake

This method involves submerging cracked, de-hulled *Mucuna* beans in a watering trough or other such vessel, and filling it to overflowing while stirring the beans with a rake (Diallo et al., 2002). When extraction is completed, the trough is drained, and the beans recovered for use. This method allows for relative velocity at the particle/solvent interface as well as for agitation, both of which are required for proper extraction (Teixeira et al., 2003). However, the constant need for fresh water and the labor intensive stirring would limit its adoption. The efficacy of the method at reducing L-Dopa has not been studied. The procedure needs to be standardized with respect to particle size, water temperature, intensity and duration of agitation, and amount and velocity of the water supply.

Solubility in alkaline and acid solvents

Alkaline conditions may facilitate the inactivation of L-Dopa in *Mucuna* beans (Wanjekeche et al., 2003). Experiments have demonstrated that soaking cracked *Mucuna* beans in a solution of lye (calcium hydroxide, $Ca(OH)_2$) reduced the concentration of L-Dopa to less than 0.01% (Diallo et al., 2002). However, the treated material was remarkably dark in color; therefore further research was advocated to determine its acceptability to humans. Diallo et al. (2002) indicated that soaking *Mucuna* beans in 4% Ca $(OH)_2$ solution for 48 hours effectively detoxified the bean to a level of 0.001% L-Dopa. This is in agreement with Teixeira et al. (2003), who reported that a NaOH solution extraction of *Mucuna* beans (1-mm particle size) at pH 11 reduced the L-Dopa concentration to < 0.1% in less than 8 hours. However, these authors also noted that the consistently dark color of the alkali-extracted bean may reduce its acceptability to consumers. This dark coloration is due to production of melanin from the L-Dopa (Latellier et al., 1999; Teixeira et al., 2003; Wanjekeche et al., 2003).

L-Dopa is also readily soluble in dilute solutions of acetic acid (Teixeira et al., 2003). In fact, most traditional laboratory analytical methods for quantifying L-Dopa begin with extraction in hydrochloric acid (Daxenbichler et al., 1972). Soaking *Mucuna* beans in acidified water (pH 3) reduced the L-Dopa in *Mucuna* beans (1-mm particle size) to safe levels (<0.4 mm) in less than 8 hours (Teixeira et al., 2003). According to Wanjekeche (2003), beans cooked in acid solutions are darker than raw beans but lighter than beans cooked in alkaline solution. This is presumably due to less melanin formation.

Siddhuraju and Becker (2005), soaked cracked *Mucuna* beans for 24 hours at room temperature in either 0.07% sodium bicarbonate or 0.1% ascorbic acid before autoclaving the beans for 20 minutes at 121^{o}C. The L-Dopa content was reduced to 1.2 and 1.5% in the alkaline and acidic solutions respectively. It is, however, not certain how much of this effect was due to

the solvent extraction since the extracted beans were autoclaved before analysis, and pressure-cooking is one of the most effective methods of L-Dopa removal (Bressani, 2002; Teixeira et al., 2003; Nyirenda et al., 2003). Therefore, research is still needed to investigate if acid extraction is more effective than alkaline extraction, and to determine the nutritional value of the extracted beans.

Sonication

Agitation by stirring, raking, or shaking is effective at partially reducing L-Dopa levels (Diallo et al., 2002; Teixeira et al., 2003) but St-Laurent et al. (2002) reported complete extraction of L-Dopa from *Mucuna* in less than 5 minutes by placing 0.1 g of the powdered bean in 15 g of distilled water (150 parts water to 1 part bean) and placing it in an ultrasonication bath for 5 minutes. Compared to more traditional agitation methods, sonication apparently makes L-Dopa more available to the solvent by rupturing the cellular structures in the bean. This method of L-Dopa extraction has not been researched for practical use. If this method were to be adapted for medium- to large-scale production of *Mucuna*, it would require investment in sonication water baths and safety equipment for the protection of the operators (e.g. sound barriers and silencers).

Statement of Objectives

Mucuna has great potential as a high-protein, high-starch food and feed for humans and monogastric livestock. However, the high levels of L-Dopa in this bean have limited its adoption for these purposes. Various methods of detoxifying *Mucuna* have been examined, but relatively little attention has been paid to the effects of methods on the nutritional value. Some important experiments have validated certain aspects of *Mucuna's* nutraceutical promise but additional research is required to validate many other ethno-pharmacological claims of its effectiveness.

Therefore the aims of this dissertation are to compare different methods of detoxifying the *Mucuna* bean for monogastric consumption, to evaluate the nutritional value of the detoxified bean, and to examine effects of feeding the detoxified bean on the performance and health of monogastrics. An additional aim was to examine the anthelmintic potential of *Mucuna.*

Specific objectives of the dissertation, examined in respective experiments, were as follows:

Objective 1a: To determine the effect of ensiling duration on the fermentation of *Mucuna*;

Objective 1b: To study the effect of particle size of ensiled *Mucuna* on L-Dopa concentration, nutritive value, and fermentation characteristics;

Objective 2: To determine the effect of sonication, or acid or alkaline solvent extraction on the L-Dopa concentration and nutritive value of *Mucuna* beans;

Objective 3: To determine the effect of feeding ensiled, acid extracted or alkali-extracted *Mucuna* bean on the performance, physiology and behavior of Sprague-Dawley rats;

Objective 4: To determine if ingestion of *Mucuna* beans reduces helminth parasite infection in lambs.

CHAPTER 3
EFFECT OF ENSILING ON L-DOPA AND NUTRITIONAL VALUE OF *Mucuna pruriens*

Introduction

Mucuna pruriens is a legume indigenous to Asia that grows in many tropical regions including Africa and the West Indies. According to Ezeagu et al. (2003), *Mucuna* beans are high in protein (24-29%) and starch (39-41%). Adebowale et al. (2005b) reported a higher protein range for *Mucuna* (33 to 38%), reflecting variation in growth environment. These nutritional attributes explain why various species of *Mucuna* are grown as a minor food crop in tropical countries despite their toxic properties. The major toxic component of *Mucuna* compromising its usefulness as a food source for humans or livestock is its content of antinutritional compounds, particularly 3,4-dihydroxy-L-phenylalanine (L-Dopa), the chemical precursor to the neurotransmitter dopamine. Szabo and Tebbett (2002) reported L-Dopa concentrations ranging from 4.47 to 5.39% L-Dopa in the bean, but wider ranges have been reported (3.1 to 6.7%; Daxenbichler et al., 1972).

Although ruminants are not adversely affected by ingestion of *Mucuna* (Burgos et al., 2002; Perez-Hernandez, 2003; Nyambati and Sollenberger, 2003; Castillo-Caamal, 2003ab; Eilitta et al., 2003; Matenga et al., 2003; Mendoza-Castillo et al., 2003; Muinga et al., 2003; Chikagwa-Malunga et al., 2008b), numerous publications report its toxic effects on monogastrics (Carew et al., 2002; Del Carmen et al., 2002; Flores et al., 2002). Most toxic effects in monogastrics arise directly from dopamine's activity as a neurotransmitter involved in the regulation of the heart, vascular system, digestive tract, and excretory system, rather than from its well-known effect as a neurotransmitter in the brain (Szabo and Tebbett, 2002). Some consequences of increased peripheral dopamine in humans include orthostatic hypotension resulting in dizziness and in some cases staggering and increased heart rate, nausea, vomiting,

and anorexia are also common side effects of excess L-Dopa ingestion, and they are associated with the effects of peripherally produced dopamine on the dopamine receptors (Szabo and Tebbett, 2002). Safe levels of L-Dopa in monogastric livestock diets are considered to be 0.4% or less (Eilitta et al., 2003; Carew et al., 2003; Ferriera et al., 2003; Iyayi and Taiwo, 2003; Ukachukwu and Szabo, 2003).

Studies indicate that processing techniques can reduce the L-Dopa concentration of *Mucuna* beans to a safe level, enabling it to be used as a food source for monogastrics (Bressani, 2002). Processing methods that utilize heat decrease the L-Dopa concentration to or near a 1% level; permeation of the bean with hot water removes over 75% of the water-soluble L-Dopa, and boiling eliminates almost all (>99%) of the L-Dopa (Szabo and Tebbett, 2002). Extraction rates increase with increasing water temperature, allowing safe levels to be reached within 13 h at 40°C, 3 h at 66°C, and 40 min in boiling water (Teixeira and Rich, 2003). However, this method is not economically feasible for widespread use in developing countries (Gilbert, 2002) because heating fuel is expensive and copious amounts of water are required. Other treatment methods such as solvent extraction and ensiling are more practicable for developing countries. Since it is readily soluble in dilute acid solutions, assays for determining the concentration of L-Dopa in plants begin with hydrochloric acid extraction. Acid-solvent extraction can be just as effective as boiling water extraction (Myhrman, 2002), but this method depends on the availability of acids.

Mucuna has been boiled and fermented to produce food products such as tempe (Egounlety, 2003), and mixtures of *Mucuna* and corn have also been ensiled (Matenga et al., 2003). In both instances the digestibility of the bean increased after fermentation. According to Egounlety (2003), the crude protein (CP) concentration was unaffected when tempe was made from *Mucuna,* but fermentation increased the protein digestibility. Matenga et al. (2003) ensiled

various mixtures of *Mucuna* and maize grain for 21 days and reported that the L-Dopa concentration was reduced by 10% for a 100% *Mucuna* sample and by 47% for a 30% *Mucuna* 70% maize mixture. The decrease in L-Dopa concentration due to *Mucuna* fermentation might be a useful strategy for making *Mucuna* safe for feeding to non-ruminants. However, the ensiling duration that is required for fermentation of *Mucuna* alone has not been determined and little is known about the nutritive value of fermented *Mucuna.*

During ensiling, anaerobic microorganisms convert plant sugars into acids, thereby decreasing the pH. Quality silage is achieved when lactic acid is the predominant acid produced, as it is the strongest fermentation acid and it rapidly reduces the pH, ensuring efficient nutrient preservation. When the initial concentration of water-soluble carbohydrates (WSC) exceeds 7%, a favorable homolactic fermentation usually occurs (Hong Yan Yang et al., 2006). Since *Mucuna* bean contains over 18% WSC, it is likely to be a good substrate for anaerobic microbial growth.

Removal of L-Dopa from *Mucuna* beans depends on the particle size; smaller particles generally increase the surface area and promote the rate of interaction with extraction solvents (Teixeira et al., 2003). Larger particles, however, can be obtained by cracking the bean by blunt force; this requires less preparation and less use of expensive equipment such as grinders. This study had two objectives. The first one was to determine the effect of ensiling duration on the fermentation characteristics of *Mucuna* and the second to study the effect of particle size of ensiled *Mucuna* on L-Dopa concentration, nutritive value and fermentation characteristics.

Materials and Methods

Mucuna pruriens cv. Georgia bush, containing 25% CP, 4.6% ether extract (EE), 17.3% neutral detergent fiber (NDF), 18.1% WSC, 38.2% starch, and 2.8% L-Dopa was obtained from the University of Georgia, Tifton, GA, USA.

Effects of Ensiling Duration

In the first of two experiments, beans were crushed in a roller mill (model 10004; Peerless International, Missouri, USA), collected in dark plastic bags, mixed thoroughly and 1500 g sub-samples were weighed into individual vacuum mini silo bags (26.5cm x 38.5cm; VacLoc Vacuum Packaging Rolls, FoodSaver, Neosho, MO, USA; Figure 3-1) in quadruplicate. To provide sufficient moisture for the fermentation, 900 ml of double-distilled water were added to the beans in each bag. A vacuum sealer (V2220, FoodSaver, Neosho, MO, USA) was used to remove residual air from the bags. Individual mini-silos were wrapped in brown paper bags and kept in a dark room at room temperature (18 to 25^{o}C) for up to 28 days. The dark ensiling conditions were used to prevent the degradation of light-sensitive L-Dopa.

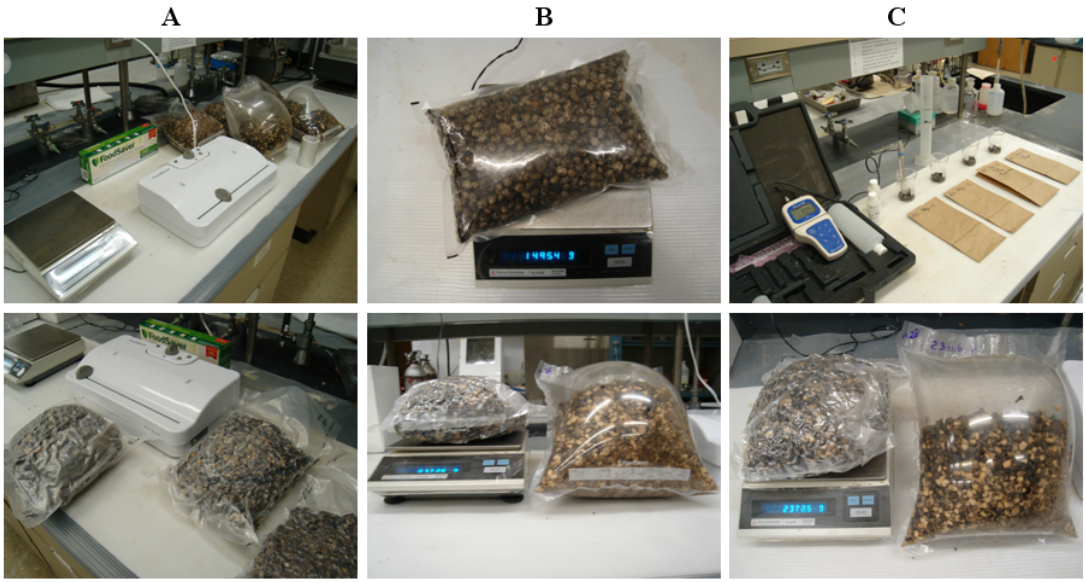


Figure 3-1. Method of ensiling *Mucuna* beans: A) vacuum sealing mini silos, B) weighing before and after ensiling, C) measuring the pH; note excessive gas produced.

The mini-silos were inspected daily and manually vented by pricking with a pin to remove excessive gas accumulation when necessary (Figure 3-3). Pin holes were immediately sealed with silo-tape after venting. Four bags containing *Mucuna* were opened after 0, 3, 7, 21, and 28

days of ensiling. After ensiling, the contents of each bag were analyzed for dry matter (DM), pH, and concentrations of volatile fatty acids (VFA), lactic acid, CP, and ammonia nitrogen (NH_3-N). A pH of 4.6 or lower was taken to indicate adequate fermentation because this pH represents the typical minimum value for ensiled legumes (Heinrichs and Ishler, 2000). Samples with a pH of 4.6 or lower were also tested for L-Dopa.

Effects of Particle Size of Ensiled *Mucuna*

Crushed *Mucuna* beans were sieved through a 6-mm (coarse) screen (USA Standard Testing Sieve, Fisher Scientific) or ground in a Wiley mill to pass through a 4-mm (medium) or a 2-mm (fine) screen (Arthur H. Thomas Company, Philadelphia, PA, USA). Samples (1500 g) of each particle size were weighed into vacuum plastic bags in quadruplicate. Double-distilled water (900 ml) was added to each bag and the bags were sealed and ensiled for 28 days based on the results of Experiment 1.

Upon opening, mini-silos were subject to pH measurement, then subsampled for analyses of DM, NDF, EE, gross energy (GE), L-Dopa, CP, and NH_3-N, WSC, starch, mold and yeast counts, VFA, and aerobic stability (AS).

Chemical Analysis

Mucuna silage extract was obtained by blending 20 g of the ensiled bean with 200 ml of distilled water for 30 s at high speed in a blender (31BL91 Waring Commercial Blender, Dynamics Corporation of America, New Hartford, Connecticut, USA). The mixture was filtered through two layers of cheesecloth and the pH measured (Accumet pH meter, model HP-71, Fischer Scientific, Pittsburg, PA, USA). The filtrate was centrifuged (1369 × g for 20 min at 4^oC) and the supernatant stored at -20^oC for subsequent determination of VFA and NH_3-N concentration.

Mucuna silage samples were dispatched in a cooler (4°C) to the American Bacteriological & Chemical Research Corporation (Gainesville, Florida) for yeast and mold counts. Yeasts and molds were enumerated by pour plating with standard methods agar (SMA) to which 4% of chloramphenicol and chlortetracycline were added (Tournas et al., 1999). Dry matter concentration was determined after drying at 60°C for 72 hours and ash was measured by combustion in a muffle furnace at 550°C overnight.

Dried samples were ground to pass through a 1-mm screen in a Wiley mill (Arthur H. Thomas Company, Philadelphia, PA, USA). Total N was determined by rapid combustion using a macro elemental N analyzer (Elementar, vario MAX CN, Elementar Americas, Mount Laurel, NJ, USA) and used to compute CP (CP = N × 6.25). The neutral detergent fiber (NDF) concentration was measured using the method of Van Soest et al. (1991) in an ANKOM 200 fiber analyzer (ANKOM Technologies, Macedon, NY, USA). Amylase was used in the analysis and the results were expressed on a DM basis. An adaptation of the Noel and Hambleton (1976) procedure that involved colorimetric quantification of N was used to determine NH_3-N concentration with an ALPKEM auto analyzer (ALPKEM Corporation, Clackamas, OR, USA). Volatile fatty acids were measured using the method of Muck and Dickerson (1988) and a high performance liquid chromatograph (Hitachi, FL 7485, Tokyo, Japan) coupled to a UV Detector (Spectroflow 757, ABI Analytical Kratos Division, Ramsey, NJ, USA) set at 210 nm. The column was a Bio-Rad Aminex HPX-87H (Bio-Rad laboratories, Hercules, CA, USA) with 0.015M H_2SO_4 mobile phase and a flow rate of 0.7 ml/min at 45ºC.

To measure AS, thermocouple wires were placed at the center of a mini-silo bag containing 1000 g of silage and each bag was placed in an open-top polystyrene container covered with a brown paper bag to maintain dark conditions while allowing adequate aeration.

The thermocouple wires were connected to data loggers (Campbell Scientific Inc. North Logan, UT, USA) that recorded the temperature every 30 minutes for 657 hours. The time that elapsed prior to a 2ºC rise in silage temperature above ambient temperature was denoted AS. Ambient temperature was monitored every 30 minutes with three thermocouple wires. Concentration of L-Dopa was measured using the method of Siddhuraju and Becker (2001b) and a high performance liquid chromatography system (Hewlett Packard HP1100) and variable wavelength UV detector set at 280 nm. The column used was an Apollo C18 (4.6 x 250 mm) column with a 19.5 ml methanol: 1 ml phosphoric acid: 975.5 ml water (pH 2; v/v/v) mobile phase flowing at 1 ml/min at 25ºC. Water-soluble carbohydrates were quantified by the anthrone method (Ministry of Agriculture, Fisheries and Food, 1986). Starch was measured by a modification (Hall, 2001) of the glucose-oxidase-peroxidase (GOP) method of Holm et al. (1986). Ether extract was determined using the soxhlet procedure (Association of Official Analytical Chemists, 1984). Gross energy (GE) was determined by an adiabatic bomb calorimeter (1261 isoperibol bomb calorimeter, Parr Instrument Company, Moline, Illinois, USA), using benzoic acid as a standard.

Statistical Analysis

Each experiment had a completely randomized design with 4 replicates. Data were analyzed with the MIXED procedure (SAS 9.1, SAS Inst. Inc., Cary, NC, USA). The following model was used to analyze the results:

$Y_{ij} = \mu + T_i + E_{ij}$
Where:
Y_{ij} = dependent variable
μ = general mean
T_i = effect of the i^{th} treatment
E_{ij} = experimental error

When the treatment effect was significant ($P < 0.05$), means were separated with a PDIFF statement. Tendencies were declared at $P > 0.05$ and ≤ 0.10. Orthogonal polynomial contrasts

(linear, quadratic and cubic) were used to evaluate the effects of ensiling duration in Experiment 1 and increasing the particle size (2, 4, and 6 mm) in Experiment 2 on silage quality measures.

Results

Effects of Ensiling Duration

A pH of 4.5 (Table 3-1) and an L-Dopa concentration of 1.3% (Figure 3-2) were obtained after 28 days of ensiling. During the fermentation, the pH decreased cubically, whereas concentrations of NH_3-N, lactate, isobutyrate and isovalerate increased non-linearly. Butyrate was not detected in the silages. The NH_3-N concentration remained below the threshold of 10% of total N throughout the ensiling period, lactate concentration had increased by 74% by day 28 and therefore lactate was the predominant fermentation acid. The lactate:acetate ratio increased cubically from 1.12 at day 0 to 3.60 at day 28. Dry matter losses were not detected in any treatment.

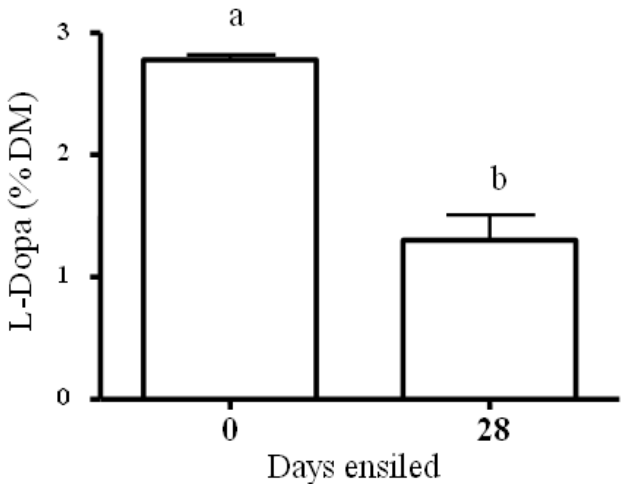


Figure 3-2. The L-Dopa concentration of unensiled and ensiled *Mucuna* bean. Means without a common superscript letter differ ($P < 0.05$); error bars denote standard error.

Table 3-1. Fermentation characteristics and L-Dopa concentration of *Mucuna* silage after various ensiling durations

	Days						
Item	0	3	7	21	28	SEM[A]	Polynomial contrasts
pH	6.2[a]	6.1[a]	5.4[b]	4.7[c]	4.5[c]	0.1	C
NH_3-N, % DM	0.19[a]	0.26[a]	0.29[a]	0.31[a]	0.39[b]	0.02	C
NH_3-N,% total N	4.9[d]	6.8[c]	8.1[b]	7.8[bc]	9.4[a]	0.4	C
Lactate, % DM	0.66[cd]	0.29[c]	1.97[bcd]	2.06[bc]	2.57[ab]	0.57	C
Acetate, % DM	0.73	0.43	0.61	0.53	0.85	0.17	NS
Propionate, % DM	0.15	0.93	0.64	0.09	0.21	0.33	NS
Iso-butyrate, % DM	0.47[c]	0.26[c]	0.32[c]	0.84[b]	1.35[a]	0.10	C
Iso-valerate, % DM	0.23[d]	0.61[c]	0.95[a]	0.59[c]	0.64[bc]	0.08	C
Total VFA, % DM	1.00	1.62	2.30	1.49	2.62	0.62	NS
Lactate:acetate	1.12[bc]	0.65[c]	3.09[abc]	3.80[ab]	3.60[abc]	1.00	C

[A] SEM = standard error of mean; C = cubic; NS = not significant; within a row, means without a common superscript letter differ ($P < 0.05$).

Effects of Particle Size of Ensiled *Mucuna*

The L-Dopa concentration was reduced by 64, 42 and 57% in coarse, medium and fine particles, respectively (Figure 3-3). Particle size did not affect CP, starch, fat, or NDF concentrations or DM-losses (Table 3-2). The WSC concentration of ensiled coarse/medium and fine particles was reduced by 45 and 73%, respectively. Gross energy values were unaffected by ensiling, but ensiled medium and fine particles had 6-7% less GE than ensiled coarse particles. The ash concentration of coarse and control particles were similar but ash concentration increased by more than 44% by ensiling medium and fine particles. Ensiling decreased the pH by 22-26%, but particle size did not affect this reduction (Table 3-3). Ensiling increased lactate, isobutyrate, total VFA, valerate, and NH_3-N concentrations. Ensiling also increased acetate, propionate and isovalerate concentrations of fine and coarse but not medium particles. Lactate was the main fermentation acid that was produced and the lactate:acetate ratio exceeded 3.0 in all ensiled treatments. Among ensiled samples, coarse particles had less lactate and more NH_3-N

than fine or medium particles, and a lower lactate to acetate ratio. Mold and yeast counts (Table 3-4) were unaffected by particle size, and due to low numbers of these microbes, AS was maintained beyond 657 hours in all treatments.

Table 3-2. Chemical composition of unensiled *Mucuna* (CON) and *Mucuna* ensiled at various particle sizes for 28 days

		Ensiled				Polynomial contrasts
Item	CON	2 mm	4 mm	6 mm	SEM	
DM, %	91.2[a]	37.6[b]	39.8[b]	38.9[b]	1.0	NS
DM-loss, %	NA	1.1	0.9	0.8	0.4	NS
CP, % DM	25.0	23.2	23.7	24.4	0.4	NS
Ash, % DM	6.0[c]	13.4[a]	10.7[ab]	8.8[bc]	1.0	L
GE, cal/g	4055[ab]	3859[b]	3903[b]	4135[a]	71	NS
Starch, % DM	38.2	38.0	39.6	38.4	1.0	NS
WSC, % DM	18.1[a]	4.8[c]	10.1[b]	10.0[b]	1.2	L
Fat, % DM	4.6	4.8	4.7	4.9	0.2	NS
NDF, % DM	17.3	19.9	18.7	18.1	1.2	NS

SEM = standard error of mean; L = linear effect; NS = not significant; within a row, means without a common superscript letter differ ($P < 0.05$).

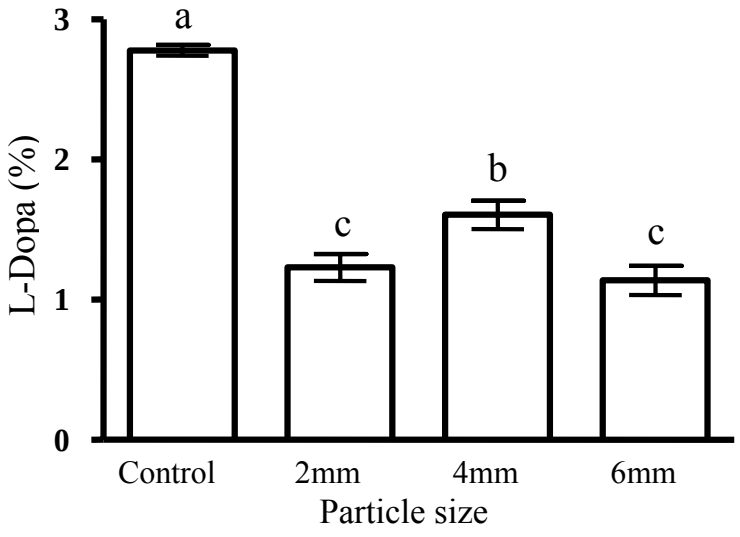


Figure 3-3. Effect of particle size on L-Dopa concentration of ensiled *Mucuna*. Means without a common superscript letter differ ($P < 0.05$); error bars denote the standard error.

Table 3-3. Fermentation characteristics of unensiled *Mucuna* (CON) and *Mucuna* ensiled at various particle sizes for 28 days

		Ensiled				Polynomial
Item	CON	2 mm	4 mm	6 mm	SEM[A]	contrasts
pH	6.18[a]	4.58[b]	4.80[b]	4.73[b]	0.11	NS
Lactate, % DM	0.66[c]	6.40[a]	6.44[a]	4.42[b]	0.56	NS
Lactate, % total acids	42.33	53.66	57.57	46.23	13.62	NS
Acetate, % DM	0.73[b]	1.28[a]	1.05[ab]	1.30[a]	0.15	Q
Propionate, % DM	0.15[b]	0.46[a]	0.31[ab]	0.45[a]	0.09	NS
Iso-butyrate, % DM	0.47[b]	3.01[a]	2.76[a]	2.61[a]	0.21	NS
Butyrate, % DM	0.00[b]	0.47[a]	0.37[ab]	0.19[ab]	0.12	NS
Iso-valerate, % DM	0.23[b]	0.88[a]	0.73[ab]	1.07[a]	0.17	NS
Valerate, % DM	0.00[b]	0.01[a]	0.01[a]	0.01[a]	0.00	NS
Total VFA, % DM	1.00[b]	5.50[a]	4.70[a]	5.07[a]	0.36	NS
Lactate:acetate	1.12[c]	5.03[ab]	6.16[a]	3.41[b]	0.64	Q
NH_3-N,% DM	0.18[c]	0.51[b]	0.51[b]	0.56[a]	0.01	NS
NH_3-N,% total N	4.41[c]	11.56[b]	11.88[b]	13.03[a]	0.28	L

[A] SEM = standard error of mean; L = linear effect; Q = quadratic effect; NS = not significant; within a row, means without a common superscript letter differ ($P < 0.05$).

Table 3-4. Microbial counts and aerobic stability (AS) of unensiled *Mucuna* (CON) and *Mucuna* ensiled at various particle sizes for 28 days

		Ensiled			
Item	CON	2 mm	4 mm	6 mm	SEM
Yeasts, log cfu/g[A]	<1.0	3.0	<1.0	<1.0	N/A
Molds, log cfu/g[A]	<1.0	2.8	2.5	3.0	0.3
Aerobic stability, hours	N/A	>657	>657	>657	N/A

[A] cfu/g = Colony-forming units per g of *Mucuna* silage; within a row, means without a common superscript letter differ ($P < 0.05$).

Discussion

Characteristics of well preserved silages include a pH below 4.0, an NH_3-N concentration below 10% of total N, and a lactate:acetate ratio above 2.0 (Owens et al., 1999). Legume silages tend to have a pH range of 4.6 to 5.2 (Heinrichs and Ishler, 2000). Therefore, it took 28 days to achieve the minimum typical pH of legume silages. The NH_3-N concentration in Experiment 1 increased to 9.4% of total N after 28 days of ensiling, indicating that proteolysis was not

excessive. The predominance of lactate in the total acid concentration indicates that a homolactic fermentation occurred and reflects the utilization of WSC by lactic acid bacteria. This lactate accumulation led to the 27% drop in pH to a value of 4.5 after 28 days of fermentation. Since legume silages typically do not achieve a lower pH, this ensiling duration was used in Experiment 2. Ensiling for 28 days resulted in a 54% decline in L-Dopa concentration to 1.2% of DM. This was higher than the generally accepted safety threshold ($\leq$ 0.4%) for consumption of *Mucuna* by monogastric livestock (Eilitta et al., 2003; Carew et al., 2003; Ferriera et al., 2003; Iyayi and Taiwo, 2003; Ukachukwu and Szabo, 2003).

In Experiment 2, concentrations of NH_3-N were slightly above the threshold of 10% indicating that minimal proteolysis occurred (Seglar, 2003). The relatively high lactate concentrations and lactate:acetate ratios beyond the critical value of 2.0 in all ensiled treatments indicate the predominance of efficient homo-fermentative pathways during ensiling of *Mucuna*.

Ensiling decreased the L-Dopa concentration by 61% relative to the control, exceeding the 10 to 47% decrease reported by Matenga et al. (2003) after ensiling different mixtures of *Mucuna* with maize grain for 21 days. The 10% reduction in L-Dopa content occurred in a 100% *Mucuna* with 0% maize mixture, and the 47% reduction occurred for a 30% *Mucuna* with 70% maize mixture. More L-Dopa was lost as more maize was included in the mixture probably because more fermentable sugars that enhanced the fermentation were supplied as the proportion of maize increased. The reason why fermentation of *Mucuna* was more efficient at L-Dopa extraction in this study than in the previous one is probably attributable to differences in soluble carbohydrate concentrations of *Mucuna,* as well as the longer ensiling duration in this study.

Ensiling for 28 days reduced L-Dopa concentrations to about 1%, which was higher than the threshold of 0.4% considered safe for consumption by monogastric livestock. This ensiled

Mucuna should be fed with other ingredients in diets of monogastrics to dilute the L-Dopa ingested and avoid excessive L-Dopa intake.

Particle size affected L-Dopa concentration of ensiled beans in a quadratic manner. Inexplicably, the medium sized particles had higher residual levels of L-Dopa relative to the fine and coarse particles. Nevertheless, L-Dopa concentration was markedly reduced by ensiling without affecting CP concentration at each particle size. Apart from that of Matenga et al. (2003), no published studies were found on the effects of ensiling *Mucuna* alone on its L-Dopa concentration and nutritional value. However, Egounlety (2003) also reported that the CP content of *Mucuna* tempe did not change after several boiling steps and a fermentation process that collectively reduced the L-Dopa content and increased proteolysis. Greater ammonia-N concentrations in ensiled versus unensiled *Mucuna* in thus study, also indicate that ensiling increased proteolysis.

The fact that ensiling produced normal fermentation characteristics and decreased the L-Dopa concentration without adversely affecting concentrations of most nutrients indicates that *Mucuna* silage can be of value as a food and feed component in the diet of monogastrics. However, care should be taken to ensure that total L-Dopa intake does not exceed levels associated with side effects. In humans, a daily L-Dopa intake of 0.25 g/day is used as a starting dose for Parkinson's patients in order to minimize side effects (Szabo and Tebbett, 2002). The body progressively adjusts to ingested L-Dopa, therefore this starting dose could be increased by 0.5 g every 7-10 days up to a maximum therapeutic dose of 8 g L-Dopa/day.

Relative to other detoxification methods, such as solvent extraction at variable pH (Diallo et al., 2002 and Teixeira et al., 2003) and thermal processing (Wanjekeche et al., 2003), ensiling is a simpler, relatively inexpensive procedure that does not require purchasing heating fuel, acid

or alkaline solvents, or using copious amounts of water. Furthermore, unlike solvent extraction, ensiling does not lower the CP content or make the bean much darker due to formation of melanin (Teixeira et al., 2003; Wanjekeche et al., 2003). However, ensiling for 28 days did not reduce the L-Dopa concentration as much as these more conventional detoxification methods. Future research should investigate if longer ensiling durations produce greater reductions in the L-Dopa concentration.

Coarse particles of *Mucuna* ensiled well and resulted in as much L-Dopa removal as fine particles. This implies that for ensiling, beans can be crushed to an approximate particle size of 6 mm and this can easily be achieved by applying a blunt force to crack the beans into a few pieces. Therefore, no mechanical grinders are required and this represents an additional advantage over the solvent extraction method where a fine particle size must be achieved for successful detoxification.

Conclusion

In the current study, the pH of ensiled *Mucuna* was reduced to 4.5 within 28 days, which is the typical minimum pH value for legume silages. This ensiling duration decreased the L-Dopa concentration from 2.8 to 1.3% (Experiment 1) or 2.8 to 1.2% (Experiment 2). The lactate:acetate ratio of the ensiled bean was high because lactate dominated the fermentation. Except for decreasing the WSC concentration, the chemical concentration of the bean was preserved during ensiling. Mold and yeast counts were low and AS was maintained for over 657 hours. Based on the fermentation characteristics, good nutritional composition, extensive AS, and reduction of L-Dopa concentration, ensiling is a promising method of processing *Mucuna* beans for monogastric consumption. The aerobic stability of beyond 657 hours indicates that the ensiled bean can be stored for long periods at room temperature. Coarse particles of *Mucuna* ensiled well and resulted in as much L-Dopa removal as fine particles, and had higher energy

and WSC concentrations. Therefore, mechanical or electrical grinders are not required for processing beans to be ensiled; application of a blunt force that will split the beans into a few pieces is sufficient for ensiling. Ensiled *Mucuna* should be fed with other dietary ingredients to moderate L-Dopa ingestion by monogastrics because ensiling does not completely reduce the L-Dopa concentration to safe levels. Future research should investigate if longer ensiling durations (>28 days) produce greater reductions in the L-Dopa concentration.

CHAPTER 4
EFFECT OF SONICATION AND SOLVENT EXTRACTION ON L-DOPA AND NUTRITIONAL VALUE OF *Mucuna pruriens*

Introduction

Malnutrition in developing countries is due in part to insufficient access to affordable protein sources. Diets of many children in such countries lack protein and instead consist mainly of cereal-based porridge that is bulky, low in energy and nutrients, and high in antinutrient concentration (Adebowale et al., 2005a). *Mucuna pruriens,* a legume indigenous to tropical regions, can be used to increase the dietary protein for such children. The beans of *Mucuna pruriens* are high in nutrients including protein (25-38%), starch (39-41%), and fiber (4%; Ezeagu et al., 2003; Adebowale et al., 2005b). Adebowale et al. (2007) compared the amino acid profile of *Mucuna* with human protein requirements suggested by the Food and Agriculture Organization (FAO), World Health Organization (WHO), and the United Nations (UN/ONU) and reported that the bioavailability and amino acid concentrations of *Mucuna* protein isolates exceeded recommended levels for all but methionine and cysteine. The lysine concentration of *Mucuna* is particularly high (Bressani, 2002), therefore *Mucuna* is a valuable supplementary protein source to cereal-based diets which are known to be lysine deficient. The chemical composition of the beans varies with cultivar, geographical location, maturity at harvest, and bean color (St-Laurent et al., 2002; Ezeagu et al., 2003).

Mucuna contains anti-nutritive factors (ANF) and the most potent and problematic ANF in the *Mucuna* bean is L-Dopa (Ukachukwu et al., 2002), the concentration of which ranges from 3 to 7% on a dry basis (Daxenbichler et al., 1972). Symptoms of *Mucuna* intake in humans and monogastric livestock include reduced feed intake, weight loss, diarrhea, vomiting, and skin lesions (Del Carmen et al., 2002; Flores et al., 2002; Szabo and Tebbett, 2002).

Studies indicate that some processing techniques can reduce *Mucuna's* L-Dopa concentration to the safe threshold of $\leq$ 0.4% L-Dopa (Eilitta et al., 2003). L-Dopa is readily soluble in dilute solutions of hydrochloric acid and traditional assay techniques for determining the level of L-Dopa in a sample begin by performing a total extraction in hydrochloric acid (Daxenbichler et al., 1972). Acidification of water to pH 3 allows extraction of the L-Dopa in *Mucuna* beans at 1-mm particle size to safe levels in less than 8 hours (Teixeira et al., 2003). However, this treatment could result in protein loss because of the increased protein solubility at pH less than the isoelectric point (pH 4.0-5.0) of *Mucuna* protein (Adebowale et al., 2007).

Alkaline conditions may also facilitate the inactivation of L-Dopa in *Mucuna* beans. Although limited information has been published on solubility of L-Dopa in alkaline solutions, Diallo et al. (2002) reported that a calcium hydroxide solution was more effective than water for removing L-Dopa from *Mucuna* bean. Soaking the beans in 4% calcium hydroxide solution for 48 hours reduced the L-Dopa concentration to 0.001%. Teixeira et al. (2003) also reported that extraction of *Mucuna* beans (1 mm particle size) in NaOH solution at pH 11 reduced L-Dopa to safe levels (<0.4%) in less than 8 hours. However, Teixeira et al. (2003) and Wanjekeche et al. (2003) reported that melanin is produced when *Mucuna* L-Dopa is extracted at alkaline pH and this makes the beans black. Melanin has been anecdotally associated with the formation of melanoma in some studies (Dollery, 1999; Letellier et al., 1999; Siple et al., 2000), but no evidence for this association was found in other studies (Weiner et al., 1993; Pfutzner and Przybilla, 1997; Fiala et al., 2002). Nevertheless, the black color of the acid or alkali-extracted bean may reduce its acceptability. According to Wanjekeche, beans cooked in acid solutions are a lighter shade of black than beans cooked in alkaline solution, therefore they may be viewed as

more acceptable. Effective L-Dopa detoxification methods that don't adversely affect the color or nutritional value of the bean are needed.

Sonication is a method used in more recent laboratory L-Dopa extraction procedures (St. Laurent et al., 2000) and it is not associated with discoloration of the beans. St-Laurent et al. (2002) reported 5 minutes to be the most effective duration for sonication. However, effects of sonication on the nutritive value of *Mucuna* are unknown.

Successful removal of L-Dopa from *Mucuna* beans with solvents depends on the particle size; smaller particles generally increase the surface area and the solid-liquid interaction, promoting the rate of L-Dopa transfer (Teixeira et al., 2003). In contrast, larger particles require less preparation and less expensive equipment such as grinders. The objective of this study was to examine the effects of method of extraction of finely (1 mm) or coarsely (6 mm) ground *Mucuna* beans on the L-Dopa content and nutritional composition. Methods examined included extraction in either acetic acid (pH 3) or sodium hydroxide (pH 11) for 8 hours or extraction by sonication (SON) in water (pH 7) for 5 minutes.

Materials and Methods

Extraction Methods

Mucuna pruriens cv. Georgia bush was obtained from the University of Georgia, Tifton, GA, USA. *Mucuna* beans were crushed (Roller Mill model 10004, Peerless International, Missouri, USA) and either sieved to pass through a 6-mm screen (USA Standard Testing Sieve, Fisher Scientific, Pittsburgh, PA, USA) or ground in a Wiley mill to pass through a 1-mm screen (Arthur H. Thomas Company, Philadelphia, PA, USA). Twenty-four representative 50-g samples (8 per treatment) of fine (1 mm) or coarse (6 mm) particles were subjected to sonication (SON) in water (neutral pH) or soaked in acidic (ACD) or alkaline (BAS) solutions. The ACD solution was brought to pH 3 by diluting 0.8 ml of a 25% (v/v) acetic acid solution in 2 L of distilled

water. The alkaline solution was brought to pH 11 by dissolving 0.1 g of sodium hydroxide in 2 L of distilled water. Each solution was shaken (Eberbach shaker, Michigan, USA) at room temperature for 8 hours (Figure 4-2), then sieved through four layers of cheesecloth and a Whatman #1 filter paper (1001-240, Fisher Scientific, Pittsburgh, PA, USA). The residue was subsequently rinsed with 1 liter of distilled-deionized water. Samples were also submerged in 2 L of water (pH 7.3) within a sonication bath (Branson Ultrasonics, Connecticut) and sonicated for 5 minutes at room temperature. For each treatment, pairs of replicate residues were composited to provide sufficient sample for chemical analysis (n=4).

Chemical Analysis

Sonicated and solvent-extracted residues were dried at 55 ^{o}C to 97% DM prior to further analysis. Dried samples were ground to pass through a 1-mm screen in a Wiley mill (Arthur H. Thomas Company, Philadelphia, PA). Dry matter concentration was determined after drying at 60^{o}C for 72 hours and ash was measured by combustion in a muffle furnace at 550^{o}C overnight. Gross energy levels were determined by an adiabatic bomb calorimeter (1261 isoperibol bomb calorimeter, Parr Instrument Company, Moline, Illinois, USA), using benzoic acid as a standard.

Total N was determined by rapid combustion using a macro elemental N analyzer (Elementar, vario MAX CN, Elementar Americas, Mount Laurel, NJ) and used to compute CP (CP = N × 6.25). The neutral detergent fiber (NDF) concentration was measured using the method of Van Soest et al. (1991) in an ANKOM 200 Fiber Analyzer (ANKOM Technologies, Macedon, NY). Amylase was used in the analysis and the results were expressed on a DM basis. The anthrone method (Ministry of Agriculture, Fisheries and Food, 1986) was used to quantify water-soluble carbohydrate (WSC). Starch was measured by a modification (Hall, 2001) of the

glucose-oxidase-peroxidase method of Holm et al. (1986). Ether extract (EE) was determined using the soxhlet procedure (Association of Official Analytical Chemists, 1984).

The L-Dopa concentration of the *Mucuna* beans was measured using the method of Siddhuraju and Becker (2001b) and a high-performance liquid chromatography system (Hewlett Packard HP1100) with an autosampler, degasser, binary pump modules, and variable wavelength UV detector set at 280 nm. The column used was an Apollo C18 (4.6 x 250 mm) column with a 19.5 ml methanol: 1 ml phosphoric acid: 975.5 ml water (pH 2; v/v/v) mobile phase flowing at 1 ml/min at 25ºC.

Statistical Analysis

The experiment had a completely randomized design involving 7 treatments: untreated control, and acid, alkali, or sonication treatments of 1- and 6-mm beans. Each treatment had 4 replicates (n=4) and all values reported are least squares means. The following model was used to analyze the results:

$Y_{ij} = \mu + T_i + E_{ij}$
Where:
Y_{ij} = dependent variable
μ = general mean
T_i = treatment effect (fixed effect representing *Mucuna* processing method and particle size)
E_{ij} = experimental error

Data were analyzed with the MIXED procedure (SAS 9.1, SAS Inst. Inc., Cary, NC, USA). Significance was declared at $P < 0.05$ and means were separated with a PDIFF statement. Tendencies were declared at $P > 0.05$ and ≤ 0.10.

Results

All processing methods reduced L-Dopa concentrations of fine *Mucuna* particles from 2.8% to less than 0.2% (Figure 4-1). Acid and alkali treatments made the solvents and extracted bean residues darker (Figure 4-2).

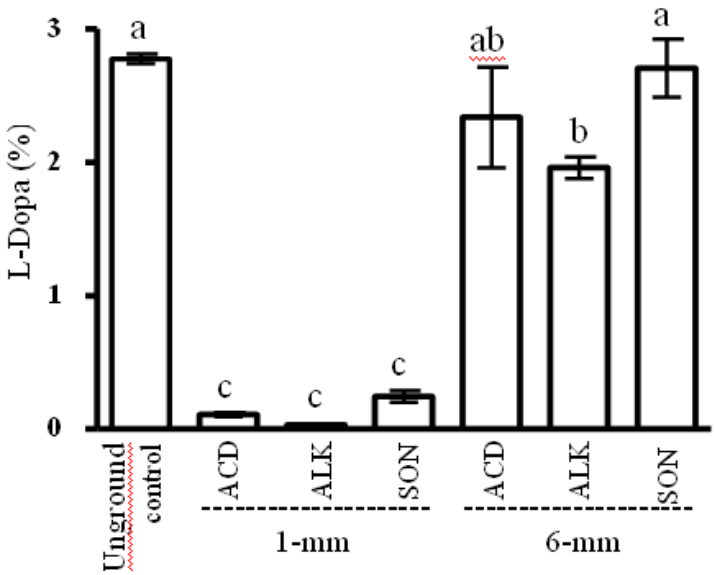


Figure 4-1. The L-Dopa concentration of fine (1 mm) or coarse (6 mm) *Mucuna* particles subjected to acid extraction (ACD), alkali extraction (ALK), or sonication (SON). Means without common letters differ ($P < 0.05$); error bars denote standard error.

All methods also reduced CP and WSC of fine particles by 24-31% and 78-81%, respectively (Table 4-1) and increased their NDF and starch concentrations by at least 62 and 14%, respectively. Fat concentration of fine particles was reduced from 5.5% to 4.2% by SON, whereas ACD and ALK reduced their GE values by approximately 10%. The ash concentration of fine particles was increased by 88% and 35% by ALK and SON, respectively. Sonication and ACD did not reduce L-Dopa concentration of coarsely ground beans but ALK reduced it from 2.8% to 2%. Sonication reduced CP, WSC, and fat concentration of coarse particles by 6, 17, and 27%, respectively. The ALK treatment increased their starch concentration by 17% but decreased their WSC concentration by 78%. The ACD treatment increased the NDF concentration of coarse particles by 35% but decreased their WSC and fat concentrations by 51% and 31%. Ash concentration and GE of coarse particles were not affected by any of the treatments.

Table 4-1. Effect of processing method on the chemical composition of fine (1 mm) and coarse (6 mm) *Mucuna* beans

Item	Unground Control[A]	ACD[B] 1 mm	ALK[C] 1 mm	SON[D] 1 mm	ACD[B] 6 mm	ALK[C] 6 mm	SON[D] 6 mm	SEM
Dry matter, %	95.4	94.9	95.3	95.6	95.1	95.5	95.1	0.2
Crude protein, % DM	25.4^{a}	19.3^{c}	17.9^{cd}	17.4^{d}	25.3^{ab}	24.6^{ab}	23.9^{b}	0.5
Ash, % DM	6.0^{c}	7.9^{bc}	11.3^{a}	8.1^{b}	6.6^{bc}	6.9^{bc}	7.3^{bc}	0.7
Gross energy, Kcal/g	4.1^{a}	3.6^{b}	3.6^{b}	3.8^{ab}	4.0^{ab}	3.9^{ab}	3.9^{ab}	0.13
Starch, % DM	38.2^{b}	45.9^{a}	46.2^{a}	43.7^{a}	36.8^{b}	44.8^{a}	34.6^{b}	1.5
WSC, % DM	18.1^{a}	3.8^{d}	3.9^{d}	3.5^{d}	8.8^{c}	13.3^{b}	15.0^{b}	0.8
Fat, % DM	5.5^{a}	5.9^{a}	5.6^{a}	4.2^{b}	3.8^{c}	3.6^{c}	4.0^{b}	0.4
NDF, % DM	17.3^{e}	32.0^{b}	38.0^{a}	28.1^{bc}	23.4^{c}	21.0^{de}	20.3^{de}	1.9

Within a row, means without a common superscript letter differ ($P < 0.05$); [A] untreated beans; [B] acid-treated beans; [C] alkali-treated beans; [D] sonicated beans; WSC = water-soluble carbohydrate; NDF = neutral detergent fiber.

Discussion

Successful removal of L-Dopa from *Mucuna* beans with solvents depends on the particle size because smaller particles increase the surface area and the solid-liquid interaction, which promotes the rate of transfer of solute at the particle surface (Teixeira et al., 2003). Safe L-Dopa levels in *Mucuna* beans destined for monogastric livestock consumption are considered to be 0.4% or less (Eilitta et al., 2003). All extraction methods were equally effective in reducing the L-Dopa content of fine *Mucuna* particles to ≤ 0.2% and thus making them safe for consumption by monogastrics. However, the L-Dopa content of coarse *Mucuna* particles was not decreased by ACD and SON and the 29% reduction by ALK treatment was far less than that of the ALK treatment of fine particles (100%). These particle-size dependent responses are in agreement with Teixeira et al. (2003), who showed that L-Dopa removal at pH 3 or 11 depended on particle size was more effective in beans ground to a 1-mm particle size versus that ground to 2-, 4-, and 8-mm sizes. This is because larger particles have less surface area, and therefore result in less efficient extraction of L-Dopa. The efficacy of the acid and alkali-treatment of fine particles also agrees with the observations of Teixeira et al. (2003).

The CP concentration of fine particles was reduced by 24-31% in all treatments but that of coarse particles was only reduced by SON. The latter was likely because of the cell rupturing effect of sonication which would have exposed more of the protein to the solvent and thus increased solvent penetration. Most (67.5%) of the protein in *Mucuna* is water-soluble (Adebowale et al., 2007). Greater CP and WSC losses in finer particles were because of the greater surface area exposed to the solvents. This is in agreement with Myhrman (2002) and Teixeira et al. (2003) who reported CP losses of 11% and up to 50%, respectively due to leaching after soaking of finely ground bean samples in acid or alkaline solutions. Losses of CP from ACD and ALK-treated fine particles were also facilitated by the solubility of *Mucuna* protein. High protein solubility in water and alkaline pH conditions can increase protein losses in solutions, disrupt protein structure and lead to degradation of certain amino acids (Adebowale et al., 2007). Water-soluble albumin is the dominant protein in *Mucuna* bean and *Mucuna* protein has its highest solubility at alkaline pH (8-12) with a maximum value of 96% at pH 12, whereas solubility at acidic pH (2-6) is less and minimum protein solubility in most solutions is at pH 4.0-5.0, which corresponds to the isoelectric pH of *Mucuna* protein (Adebowale et al., 2003; 2005a). For unknown reasons, the alkaline extraction of *Mucuna* did not lead to greater CP losses than the acidic extraction. More work should be done on effects of these extraction methods on the true protein concentration of *Mucuna.*

The increased starch content of fine particles due to ACD, BAS or SON treatments of fine particles agrees with responses to acid or alkali extraction of *Mucuna* reported by Siddhuraju and Becker (2005). The increased starch content was due to partial loss of soluble components including WSC, protein, and L-Dopa, all of which decreased with solvent extraction relative to CON. The reduction in concentration of these components and the energy value of the bean, and

the concomitant increases in starch and fiber concentration imply that solvent extraction and sonication modified the nutritive value of the bean and resulted in losses of key components.

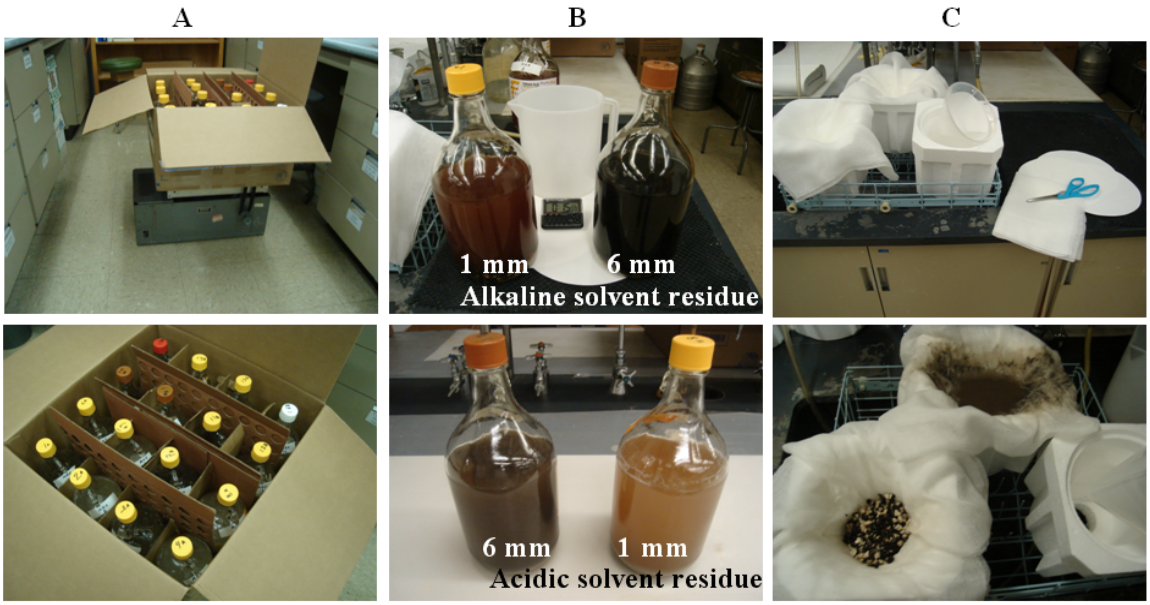


Figure 4-2. Detoxification of *Mucuna* bean through acid or alkali solvent extraction: A) shaking for 8 hours, B) color changes resulting from solvent pH, and C) washing and filtering the detoxified beans.

Although methionine and cysteine are limiting amino acids in the *Mucuna* bean, a key nutritional attribute is its high lysine concentration and bioavailability (Adebowale et al., 2007). Cereal grains are typically used to alleviate hunger in developing countries but they are known to be deficient in lysine and thus not very effective in reducing malnutrition (Adebowale et al., 2005b). The lysine and amino acid composition of *Mucuna* reportedly exceeds the recommended FAO/WHO/UN reference values for human diets, indicating that it is an excellent source of essential amino acids, and can be used to fortify cereal-based foods deficient in lysine (FAO/WHO/ONU, 1985). Further research should determine effects of the processing methods employed in this experiment on concentrations of lysine and other amino acids in *Mucuna.*

The pH of the solvent is an important factor that can affect the success of L-Dopa removal from *Mucuna* and the residual nutritional quality. Several authors mention that due to formation of melanin, *Mucuna* beans are darker after acid or alkali extraction (Teixeira et al., 2003; Wanjekeche et al., 2003). The darker color in the acid and alkali extracts was in agreement with such observations (Figure 4-3). Melanin is a metabolite of L-Dopa characterized by its dark color. The conversion of L-Dopa into melanin requires specific conditions and is most evident at alkaline pH (Teixeira et al., 2003; Wanjekeche et al., 2003); for example if bicarbonate is added to the cooking or soaking water of the *Mucuna* bean (Eilitta et al., 2003).

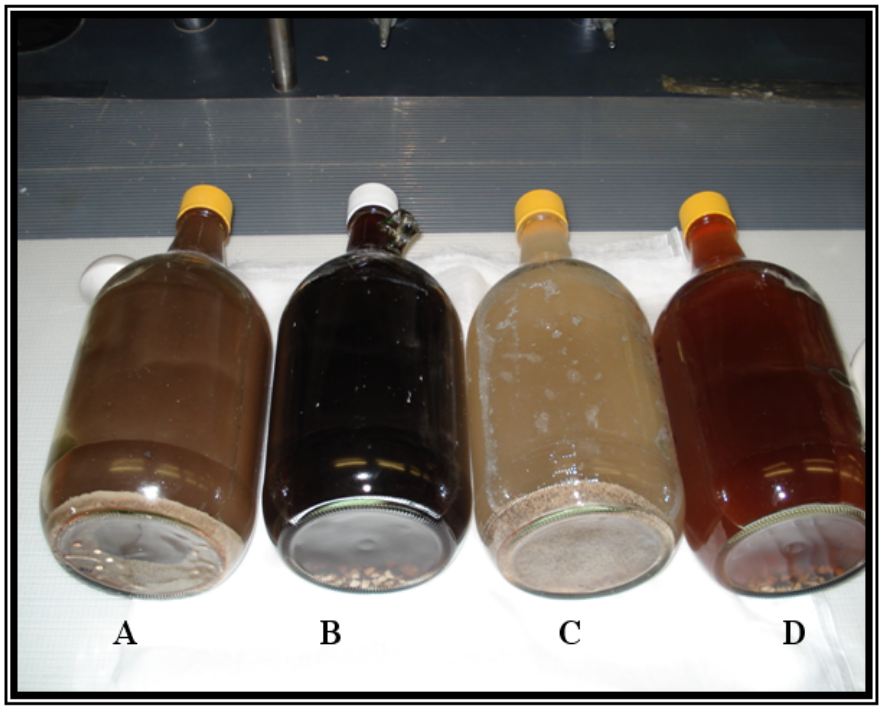


Figure 4-3. Color changes after detoxification of *Mucuna* bean through A) Alkaline extraction at 1 mm particle size, B) Alkaline extraction at 6 mm, C) Acid extraction at 1 mm, D) Acid extraction at 6 mm particle size.

Diallo et al. (2002) successfully reduced the L-Dopa concentration to 0.001% after 48 hours of soaking cracked *Mucuna* beans in calcium hydroxide solution, but noted the remarkably dark coloration upon treatment. Beans cooked in acid solutions are lighter in color than beans

cooked in alkaline solutions (Wanjekeche et al., 2003). Adebowale et al. (2007) noted that darker colors occurred in sodium hydroxide solutions of pH 11 relative to less alkaline solutions. This causes concern because the effects of melanin on health are controversial. Melanin has been anecdotally associated with the formation of melanoma in some studies (Dollery, 1999; Letellier et al., 1999; Siple et al., 2000), but no evidence for this association was found in other studies (Weiner et al., 1993; Pfutzner and Przybilla, 1997; Fiala et al., 2002). Discarding the solvent residue as in the current study, may reduce this concern. Nevertheless, the darker color of the extracted bean indicates the need for further investigation of concentrations of melanin residues in the detoxified bean.

Conclusion

The efficiency of L-Dopa removal in the solvent extracts was mainly affected by particle size. Both acidic and alkaline solvents performed equally well at detoxifying fine particles of *Mucuna* bean to safe levels (<0.4% L-Dopa) but also reduced their WSC and CP concentrations and increased their starch and NDF concentrations. However, these methods were not effective at detoxifying coarse *Mucuna* particles and they had less consistent effects on their nutritive value. Effective detoxification of fine particles occurred at the expense of CP and WSC concentration. Acidic and alkaline solvent extraction darkened the bean, suggesting that they increased the formation of melanin, a metabolite of L-Dopa characterized by its dark color. Future research should determine melanin concentrations in acid- or alkali-extracted beans as well as their concentrations of true protein and amino acids.

Sonication prevented discoloration of *Mucuna* due to acid or alkaline extraction and it was also an effective method of detoxifying fine but not coarse particles of *Mucuna* to safe levels (<0.4% L-Dopa). Sonic waves rupture cells, thereby increasing solvent penetration and particle dispersal while promoting the accessibility of the solvent to L-Dopa. The limited surface area of

the coarse (6 mm) particles combined with the lack of penetration of the sonic waves through the larger particles were likely responsible for the limited effectiveness of sonication on coarse particles. Sonication generally resulted in similar modifications to the nutritive value of the bean as acid or alkali solvent extraction but caused greater losses of fat from fine particles.

CHAPTER 5
BEHAVIORAL, PERFORMANCE, AND PHYSIOLOGICAL RESPONSES OF RATS FED DETOXIFIED *Mucuna pruriens*

Introduction

The major problem that has compromised the usefulness of *Mucuna pruriens* as a food source is its concentration of antinutrients. According to Szabo and Tebbett (2002) and Ukachukwu et al. (2002), 3,4-dihydroxy-L-phenylalanine (L-Dopa) is the most potent toxic compound in the *Mucuna* bean, which contains between 3.1 and 6.7% L-Dopa (Daxenbichler et al., 1972).

Szabo and Tebbett (2002) described the following pharmacokinetic profile of L-Dopa, which illustrates the danger of introducing undetoxified *Mucuna* into the human diet. Approximately 33% of an orally administered dose of L-Dopa is absorbed from the gastrointestinal tract, primarily from the jejunum in the small intestine. Peak plasma concentrations occur within 1 to 3 hours. Most of the successfully absorbed L-Dopa is converted to dopamine in the periphery via action of the enzyme L-aromatic amino acid decarboxylase (LAAD). Less than 1% of the administered dose enters the brain where it is converted to dopamine in the basal ganglia. In addition to dopamine, peripheral L-Dopa is also metabolized to melanin, norepinephrine, 3-methoxytyramine, methyldopa, 3,4-dihydroxyphenylacetic acid, and 3-methoxy-4-hydroxyphenylacetic acid (homovanillic acid). These metabolites are rapidly excreted in the urine such that approximately 80% of an administered dose is excreted within 24 hours, less than 1% of which remains unchanged. Consequences of increased peripheral dopamine in humans can include nausea, vomiting, and anorexia, orthostatic hypotension resulting in dizziness, staggering, and increased heart rate. Psychiatric disturbances have also been reported in patients receiving high doses of L-Dopa in a dose-dependent manner (Szabo and

Tebbett, 2002). These can include nervousness, anxiety and agitation, insomnia, vivid dreams, confusion, delirium, depression and psychotic reactions with hallucinations.

Cotzias et al. (1974) reported that feeding rodents high levels of L-Dopa affected behavior; they expressed motor hyperactivity, muscle jerks, stereotyped movements, jumping, gnawing, corkscrew tails, ataxia, salivation, piloerection, red muzzle, and convulsions. Most side effects arise directly from dopamine's activity as a neurotransmitter involved in the regulation of the heart, vascular system, digestive tract, and excretory system, rather than from its well-known effect on receptors in the brain (Szabo and Tebbett, 2002). Symptoms of *Mucuna* intake in broilers and pigs include weight loss, reduced feed intake, and feed conversion efficiency (Flores et al., 2002; Del Carmen et al., 2002).

Despite the health hazards caused by L-Dopa, the *Mucuna* bean's high protein concentration makes it an important part of the diet in Asia, Africa, and South/mid-America. According to Ezeagu et al. (2003), *Mucuna* beans are not only high in protein (25-30%), but also in starch (39-41%). Adebowale et al. (2007) showed that except for methionine and cysteine, concentrations of bioavailable amino acids in *Mucuna* protein isolates exceeded the values for human diets recommended by the Food and Agricultural Organization (FAO), World Health Organization (WHO), United Nations (UN/ONU). *Mucuna's* high lysine concentration makes it a suitable supplementary protein to cereal-based diets which are known to be lysine deficient (Adebowale, 2007). *Mucuna* could thus be used to alleviate malnutrition in developing countries, provided its L-Dopa concentration is effectively reduced (Bressani, 2002; Teixeira et al., 2003).

The safety threshold is a bean L-Dopa concentration of less than 0.4% (Eilitta et al., 2003; Carew et al., 2003; Ferriera et al., 2003; Iyayi and Taiwo, 2003; Ukachukwu and Szabo, 2003). Processing techniques have been evaluated that reduce the *Mucuna* L-Dopa concentration to safe

levels (Bressani, 2002), but these techniques are often costly as they require expensive fuel to generate heat, copious amounts of water that is not always readily available, and they are labor intensive and time consuming. Few studies have examined the residual nutritional value of detoxified *Mucuna* bean and the effects of feeding it to monogastrics.

A few promising detoxification methods were identified in preliminary studies. Ensiling the *Mucuna* bean for 28 days reduced the L-Dopa concentration by 54%, preserved the starch and protein concentrations, and resulted in a product that had not deteriorated after 657 h of storage (Chapter 3). Solvent extractions for 8 hours at pH 3 or 11 almost eliminated (> 90% removal) *Mucuna* L-Dopa but also reduced the protein concentration by 24-31% (Chapter 4). The purpose of this study was to evaluate the effect of feeding detoxified *Mucuna* beans on performance, physiology and behavior of Sprague-Dawley rats.

Materials and Methods

Mucuna Detoxification

Mucuna pruriens cv. Georgia bush, were obtained from Dr. Sharad Phatak at the University of Georgia, Tifton, GA, USA. The three detoxification methods evaluated consisted of acid extraction, alkaline extraction and ensiling.

Detoxification through acid or alkali solvent extraction

Mucuna beans were ground in a Wiley mill to pass through a 1-mm screen (Arthur H. Thomas Company, Philadelphia, PA, USA). An acidic solution was brought to pH 3 by diluting 0.8 ml of a 25% (v/v) acetic acid solution in 2 L of distilled water. The alkali solution was brought to pH 11 by dissolving 0.1 g of sodium hydroxide in 2 L of distilled water. Suspensions (25 g/l) of *Mucuna* in the acid and alkaline solutions were shaken (Eberbach shaker, Michigan, USA) at room temperature for 8 hours, then filtered through four layers of cheesecloth and a

Whatman #1 filter paper (1001-240, Fisher Scientific, Pittsburgh, PA, USA). The residue was subsequently rinsed with one liter of distilled-deionized water and dried at 55°C to 97% DM.

Detoxification through ensiling

Mucuna beans were ground in a Wiley mill to pass through a 6-mm screen (Arthur H. Thomas Company, Philadelphia, PA, USA). Ground beans were weighed (1500 g) into individual vacuum bags (26.5 x 38.5 cm, VacLoc Vacuum Packaging Rolls, FoodSaver, Neosho, MO, USA) and 900 ml of double distilled water were added to provide sufficient moisture for fermentation. A vacuum sealer (V2220, FoodSaver, Neosho, MO, USA) was used to remove residual air from the bags and to heat seal them. Bags were placed in brown paper bags and kept in a dark room at room temperature (18 to 25°C) for 28 days. The bags were inspected daily and manually vented by pricking with a pin to remove excessive gas accumulation when necessary. Pin holes were immediately sealed with silo-tape after venting. After ensiling, the concentrations of each bag were dried at 55°C to 97% DM. All procedures were performed under conditions of limited lighting since L-Dopa is light sensitive. Upon detoxification, representative samples were analyzed for L-Dopa and nutritional value (Table 5-1).

Table 5-1. Chemical composition of undetoxified (control) and detoxified *Mucuna* beans

Item	Control	Detoxification method[a]		
		Ensiling	Acid extraction	Alkaline extraction
Crude protein, % DM	25.0	23.2	19.3	18.1
Ash, % DM	6.0	13.4	7.9	11.3
Gross energy, Kcal/g	4.1	3.9	3.6	3.6
Starch, % DM	38.2	38.0	45.9	46.2
WSC, % DM	18.1	4.8	3.8	3.9
Fat, % DM	4.6	4.8	5.9	5.6
NDF, % DM	17.3	19.9	32.0	38.0
pH	6.2	4.5	3.0	11.0
L-Dopa, % DM	2.8	1.2	0.1	0.0

WSC = water-soluble carbohydrate; NDF = neutral detergent fiber; [a] Chapters 3 and 4.

Analysis of L-Dopa

The L-Dopa concentration of detoxified *Mucuna* beans was measured using the method of Siddhuraju and Becker (2001b) and a high performance liquid chromatography system (Hewlett Packard HP1100) with a variable wavelength UV detector set at 280 nm. The column used was an Apollo C18 (4.6 x 250 mm) column with a 19.5 ml methanol: 1 ml phosphoric acid: 975.5 ml water (pH 2; v/v/v) mobile phase flowing at one ml/min at 25ºC.

Nutritional Value Analysis

Dried samples were ground to pass through a 1-mm screen in a Wiley mill (Arthur H. Thomas Company, Philadelphia, PA), and ash was measured by combustion in a muffle furnace at 550^{o}C overnight. Total N was determined by rapid combustion using a macro elemental N analyzer (Elementar, vario MAX CN, Elementar Americas, Mount Laurel, NJ) and used to compute CP (CP = N × 6.25). NDF concentration was measured using the method of Van Soest et al. (1991) in an ANKOM 200 Fiber Analyzer (ANKOM Technologies, Macedon, NY). Amylase was used in the analysis and the results were expressed on a DM basis. The anthrone method as described by the Ministry of Agriculture, Fisheries and Food (1986) was used to quantify water-soluble carbohydrate (WSC). Starch was measured by a modification (Hall, 2001) of the glucose-oxidase-peroxidase (GOP) method of Holm et al. (1986). Ether extract (EE) was determined using the soxhlet procedure (Association of Official Analytical Chemists, 1984). Gross energy levels were determined by an adiabatic bomb calorimeter (1261 isoperibol bomb calorimeter, Parr Instrument Company, Moline, Illinois, USA), using benzoic acid as a standard. *Mucuna* silage extract was obtained by blending 20 g of the ensiled bean with 200 ml of distilled water for 30 s at high speed in a blender (31BL91 Waring Commercial Blender, Dynamics Corporation of America, New Hartford, Connecticut, USA). The mixture was filtered through

two layers of cheesecloth and the pH measured (Accumet pH meter, model HP-71, Fischer Scientific, Pittsburg, PA, USA).

Dietary Treatments

The diets for each of the five treatments were prepared by Harlan Teklad (Madison, WI, USA) and each consisted of 1.2 cm pellets and contained 25-26% CP, 9% ash, 37% carbohydrates, 4% fat and 2.7 Kcal/g GE. The treatments consisted of one control diet (CON) consisting of a commercial rat chow (8604 rodent diet, Harlan Teklad, Madison, WI, USA) and four *Mucuna*-based diets in which 10% of the commercial rat chow was replaced with either untreated *Mucuna* (MUC), or *Mucuna* beans detoxified by acetic acid extraction (ACD), sodium hydroxide extraction (BAS), or ensiling (SIL).

Animals and Measurements

Sixty 6- to 8-week-old male Sprague-Dawley rats with an initial body weight of 200 grams were purchased from Harlan (Indianapolis, IN, USA). Rodents are commonly used as a suitable animal model for monogastrics in food safety and toxicology studies. Rats were individually-housed (Figure 5-1) in 40 x 30 x 20 cm cages and randomly assigned to the five treatments (n=12). All animals were housed at 20 $\pm$ 1^{o}C in a 12-h light/dark cycle with an *ad libitum* tap water and food supply. Experiments were performed according to the policies and guidelines of the Institutional Animal Care and Use Committee of the University of Florida, Gainesville, USA.

Open field behavior analysis

Animal behavior was evaluated on day 3 (Week 1) and day 10 (Week 2) using the open field test. The open field consisted of a round grey plastic arena measuring 70 cm in diameter surrounded by a grey plastic wall 34 cm high, lit with three 40W bulbs (Figure 5-1). The floor of the arena was divided into several concentric units by black painted lines, dividing the arena into 19 fields. This method is used to evaluate rodent behavior (Carlini et al., 1986). Open field

activity helps determine locomotion and motor activity as well as speed (Vogel, 2002). Each week, the 60 rats were placed one at a time in the center of the arena for 5 minutes. The open field test was videotaped using a high-resolution video camera WV-CP244 (Panasonic, Secaucus, NJ, USA). Video analysis was performed using TopScan, Top View Animal Behavior Analyzing System (version 1.00, Clever Sys Inc., Preston, VA, USA) by an unbiased and treatment-blinded technician.

Performance and physiological analysis

Rats were handled in a laminar flow hood (Figure 5-1). Feed intake during the first 10 days of the trial was calculated on a daily basis by weighing the remaining feed (orts) left over from what was offered on the previous day. Animals were also weighed daily during the first 12 days of the trial and growth records used to determine average daily gain and total weight gain. After 14 days the rats were necropsied and the heart, liver, kidneys, spleen, and gonads were weighed. Organ weights were normalized to reflect percent of body weight.

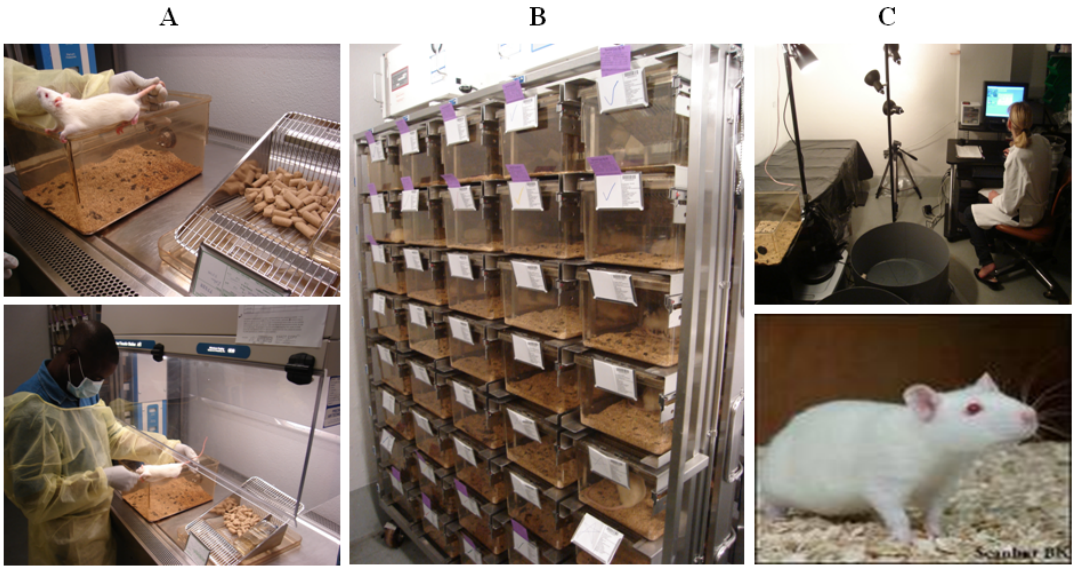


Figure 5-1. Experimental conditions for rat feeding trial: A) Rats were handled under the laminar flow hood and B) housed in individual cages; C) A Sprague-Dawley rat and the open field test set-up.

Clinical pathology analysis

At the end of the trial (day 14), blood was collected post-anesthesia through cardiac puncture and stored in serum and EDTA vacutainer tubes (Vacuette, Greiner Bio-One NA, Inc, Monroe, NC, USA) for testing in a clinical pathology laboratory that performed a chemistry panel (vet-20 chemistry profile for rodents) and complete blood counts (CBC).

Statistical analysis

Statistical analysis was performed with GraphPad Prism (version 4.00, GraphPad Software Inc., San Diego, CA, USA) and one-way analysis of variance (ANOVA) followed by Student–Newman–Keul's multiple comparison test. In all cases differences were considered significant if $P < 0.05$.

Results

Performance

Table 5-3 shows that dietary treatments did not affect DM intake or weight gain. Total DM intake, average daily DM intake, and total DM intake as a percent of body weight, average daily DM intake as a percent of body weight, total weight gain, and average daily weight gain were not different among treatments ($P > 0.05$). However, rats fed MUC had numerically ($P > 0.1$) lower values than those fed other diets.

Behavior

Dietary treatments did not affect distance traveled or number of line crossings in the open field test during day 3 (Week 1) or day 10 (Week 2; Figure 5-2). Nevertheless, rats fed *Mucuna* consistently had numerically ($P>0.05$) lower line crossings and distance traveled compared to CON and this trend was most evident in rats fed MUC and BAS on day 10. A malfunction of the Top View analyzing system allowed analysis of only six animals per treatment during day 3 (n=6) and three animals per treatment on day 10 (n=3).

Table 5-2. Effects of feeding unprocessed or detoxified *Mucuna pruriens* on DM intake and growth of rats

	CON[a]	MUC[b]	ACD[c]	BAS[d]	SIL[e]
Feed intake, g/11d	228.1 ± 6.3	212.4 ± 5.8	224.6 ± 3.4	230.8 ± 7.2	223.4 ± 6.2
Daily DM intake, g	20.7 ± 0.6	19.3 ± 0.5	20.4 ± 0.3	21.0 ± 0.7	20.3 ± 0.6
Feed intake, % BW	86.1 ± 2.1	81.9 ± 1.4	84.6 ± 1.1	85.6 ± 2.2	82.1 ± 2.0
Daily DM intake, % BW	8.6 ± 0.2	8.2 ± 0.1	8.5 ± 0.1	8.6 ± 0.2	8.2 ± 0.2
Weight gain, g/10d	59.5 ± 3.3	58.2 ± 5.3	61.8 ± 2.6	65.7 ± 2.2	66.9 ± 2.5
Daily weight gain, g	5.9 ± 0.3	5.8 ± 0.5	6.2 ± 0.3	6.6 ± 0.2	6.7 ± 0.2

Mean ± standard error; within a row, means without a common superscript letter differ ($P < 0.05$); [a] control diet, standard rat chow without *Mucuna*; [b] untreated *Mucuna* diet; [c] *Mucuna* beans detoxified by acetic acid extraction; [d] *Mucuna* beans detoxified by sodium hydroxide extraction; [e] *Mucuna* beans detoxified by ensiling; BW = body weight; DM = dry matter.

Compared to CON, feeding *Mucuna* diets significantly ($P < 0.01$ and $P < 0.05$) reduced total (day 3 and day 10) distance traveled and total number of line crossings in the open field test. The reduction was more statistically significant ($P < 0.01$) in rats fed MUC versus those fed detoxified *Mucuna* diets ($P < 0.05$). There was a numerical indication ($P > 0.05$) that the reduction was less severe in rats fed ACD and SIL.

Physiology

Necropsy revealed that in all treatments the heart, liver, kidney and testicular weights remained unchanged relative to CON (Table 5-3). Levels of red blood cells were not different among treatments either. Feeding MUC increased spleen weight (splenomegaly) and monocyte occurrence (monocytosis) relative to CON, but feeding the detoxified beans did not (Figure 5-3). Concentrations of alkaline phosphatase were increased by 11-17% due to feeding detoxified beans instead of MUC, but all *Mucuna* treatments resulted in similar alkaline phosphatase concentrations as CON. Blood phosphorus concentration was decreased by feeding MUC relative to CON (9.78 vs 10.74 mg/dl) but it was similar in rats fed CON and detoxified diets.

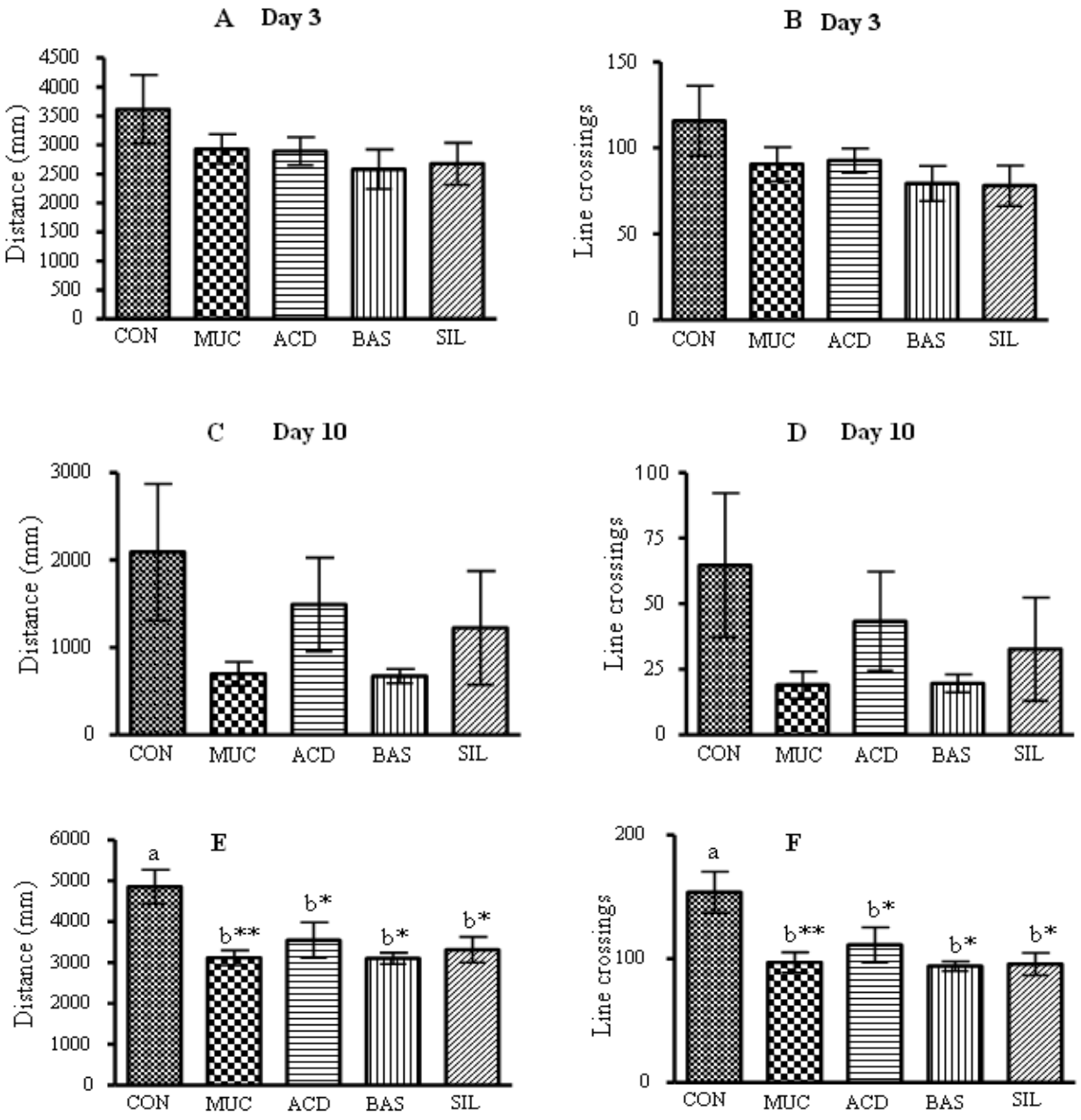


Figure 5-2. Effect of feeding detoxified *Mucuna pruriens* on A) distance traveled on day 3; B) open field line crossings on day 3; C) distance traveled on day 10; D) open field line crossings on day 10; E) total distance traveled on both days; and F) total line crossings on both days. Means without a common superscript letter differ ($P < 0.05$ *, $P < 0.01$ **). CON = control diet; MUC = untreated *Mucuna* diet; ACD = *Mucuna* beans detoxified by acetic acid extraction; BAS = *Mucuna* beans detoxified by sodium hydroxide extraction; SIL = *Mucuna* beans detoxified by ensiling. Error bars denote standard error.

Table 5-3. Effects of feeding unprocessed or detoxified *Mucuna pruriens* on organ weights and concentrations of monocytes, alkaline phosphatase, and phosphorus in the blood

	CON[A]	MUC[B]	ACD[C]	BAS[D]	SIL[E]
Heart, % BW	3.84 ± 0.04	3.76 ± 0.05	3.88 ± 0.06	3.68 ± 0.05	3.71 ± 0.07
Liver, % BW	45.2 ± 0.6	44.8 ± 1.6	45.1 ± 1.1	44.2 ± 1.0	44.6 ± 0.9
Kidney, % BW	7.7 ± 0.4	7.6 ± 0.3	7.6 ± 0.2	7.2 ± 0.1	7.6 ± 0.2
Testicles, % BW	12.4 ± 0.7	13.6 ± 0.3	13.2 ± 0.2	12.9 ± 0.3	12.8 ± 0.3
Spleen, % BW	2.48^{b} ± 0.05	2.79^{a} ± 0.08	2.67^{ab} ± 0.05	2.54^{b} ± 0.06	2.64^{ab} ± 0.06
Red blood cells, $x10^6$/ul	7.4 ± 0.1	7.3 ± 0.1	7.4 ± 0.1	7.3 ± 0.1	7.1 ± 0.1

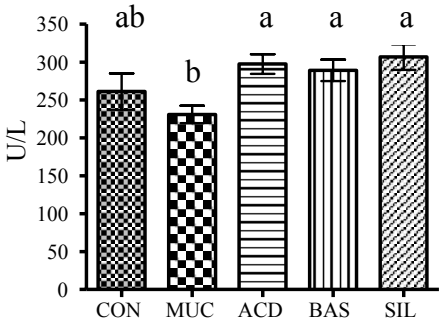
Mean ± standard error; within a row, means without a common superscript letter differ ($P < 0.05$); [A] control diet, standard rat chow; [B] untreated *Mucuna* diet; [C] *Mucuna* beans detoxified by acetic acid extraction; [D] *Mucuna* beans detoxified by sodium hydroxide extraction; [E] *Mucuna* beans detoxified by ensiling.

Discussion

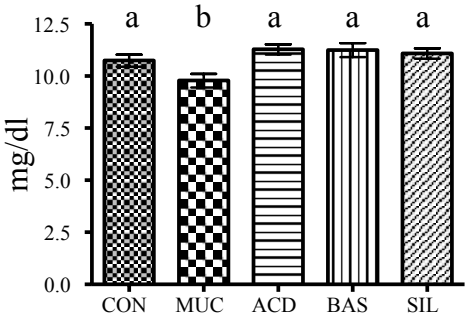
In the current study, the feed intake, growth, behavioral and physiological effects on organ weight, complete blood counts, and blood chemistry profile were determined to better understand food safety aspects of detoxified *Mucuna* bean. At the 10% of DM inclusion rate, feeding *Mucuna* did not significantly affect rat behavior in the open field on days 3 and 10. The open field is an unfamiliar environment to the rat that naturally tends to explore novel situations. A rat that is sedated or ill will travel less when exposed to the new environment such as the open field, which allows for rat locomotion and motor activity (Vogel, 2002). In contrast, an animal that is stimulated and healthy will spend more time exploring the center of the open field and will travel further. During the latter part of the open field test, technical difficulties prevented computer analysis of data from nine animals per treatment. Greater treatment replication may have amplified the numerical differences reported.

The fact that none of the *Mucuna* treatments affected behavior in the open field on day 3 or 10 may have been because of the relatively low *Mucuna* inclusion rate and also because oral *ad libitum* L-Dopa administration produces a more gradual and lower maximum concentration

A: Alkaline phosphatase



B: Phosphorus



C: Monocytes

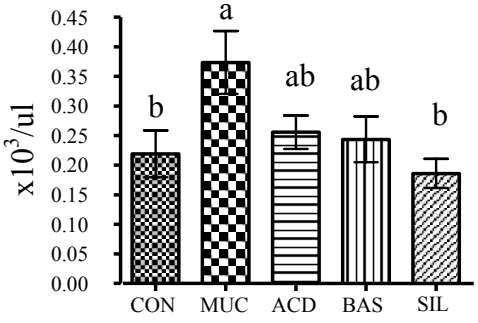


Figure 5-3. Effects of feeding detoxified *Mucuna pruriens* on blood levels of A) alkaline phosphatase; B) phosphorus; and C) monocytes. CON = control diet; MUC = untreated *Mucuna* diet; ACD = *Mucuna* beans detoxified by acetic acid extraction; BAS = *Mucuna* beans detoxified by sodium hydroxide extraction; SIL = *Mucuna* beans detoxified by ensiling. Means without a common superscript letter differ ($P < 0.05$); error bars denote standard errors.

(C_{max}) in the plasma (Bartus et al., 2004). Rats were fed *ad libitum* and thus continuously exposed to L-Dopa. The numerically greater distance travelled and line crossings of rats fed ACD and SIL versus MUC on day 10 suggest that detoxification procedures that acidify the beans had less adverse effects on behavior. Both ACD and SIL had lower pH (pH 3.0 and pH 4.5 respectively) than BAS (11.0) and MUC (6.2). The lower pH may have played a role in reducing adverse effects of the MUC diet on behavior. The trend toward less abnormal behavior in rats fed ACD and SIL could also be partially due to their lower L-Dopa concentrations relative to MUC.

Despite the relatively low dietary *Mucuna* inclusion rate of 10% DM and the *ad libitum* feeding, the total distance traveled and total line crossings for days 3 and 10 were reduced by feeding *Mucuna* diets, indicating increased abnormal behavior relative to CON. Abnormal behavior in rats fed MUC may be related to side effects caused by *Mucuna*-derived hallucinogens (Szabo and Tebbett, 2002). Cotzias et al. (1974) reported that feeding rodents high levels of L-Dopa adversely affected behavior and caused motor hyperactivity, muscle jerks, and stereotyped movements. Intake of L-Dopa in humans is also associated with psychiatric disturbances, which can lead to nervousness, anxiety and agitation, insomnia, confusion, delirium, depression as well as psychotic reactions with hallucinations and anorexia (Szabo and Tebbett, 2002). However, because the reductions in total line crossing and distance travelled also occurred in detoxified *Mucuna* diets, they may have been due to components other than L-Dopa.

According to Sato et al. (1994), chronic L-Dopa exposure is often associated with a gradual decline in efficacy. Murata (2006) reported that Parkinson's disease patients who showed wearing-off phenomenon had higher C_{max} and shorter L-Dopa half life ($T_{1/2}$) than patients not displaying wearing-off. This suggests that over time neurotoxic signs such as confusion and agitation would give way to peripheral toxicity such as gastrointestinal upset

(reduced intake or anorexia). Although feed intake was not reduced during this study, numerically lower travel and line crossings in rats fed ACD and SIL versus MUC on day 10 may have been signs of the initial stage of neurotoxicity (confusion, nervousness, depression).

The fact feed intake and weight gain did not differ among treatments, suggests that acceptability and nutrient bioavailability of control and *Mucuna*-based diets were similar. *Mucuna*-based diets have reportedly been associated with a decrease in acceptability and intake compared to soybean based diets (Del Carmen et al., 1999; Flores et al., 2002). The relatively low *Mucuna* inclusion rate (10% of diet DM) in this study may explain why intake was not affected by feeding MUC in this study.

Although the detoxification methods resulted in different L-Dopa and CP concentrations, similar performance and clinical data suggest the CP bioavailability and food safety were comparable among detoxified treatment diets. Solvent extraction typically disrupts the protein structure and degrades AA in *Mucuna* (Adebowale et al., 2007), nevertheless, feeding BAS and ACD did not adversely affect growth and performance, likely due to the relatively low dietary *Mucuna* inclusion rate.

Adverse effects due to MUC consumption evidenced by splenomegaly and monocytosis were not evident when detoxified diets were fed. The splenomegaly caused by MUC agrees with studies where spleen enlargement occurred when poultry was fed *Mucuna* beans (Iyayi and Taiwo, 2003; Iyayi et al., 2005; Pugalenthi et al., 2005; Carew and Gernat, 2006). The spleen is the largest collection of lymphoid tissue in the body and splenomegaly resulting from feeding MUC probably reflects increased workload or hyper-function of the organ. Splenomegaly is associated with red blood cell destruction in the spleen, congestion due to portal hypertension and infiltration by leukemias and lymphomas, obstruction of blood flow or antigenic stimulation,

and infection (Grover et al., 1993). Carew et al. (2003) observed lymphoid necrosis, macrophage proliferation and lympho-phagocytosis of the spleen at a 12% *Mucuna* inclusion in the diet of broilers. Iyayi et al. (2005) reported that lymphoid depopulation in the spleen is indicative of the degenerative effects associated with feeding raw *Mucuna* beans.

Relative to CON, the dietary inclusion of undetoxified *Mucuna* bean (MUC) also caused monocytosis, a state of excess monocytes in the peripheral blood indicative of various disease states. Monocytes are leukocytes that replenish macrophages and dendritic cells and elicit an immune response at infection sites. In the tissues, monocytes mature into different types of macrophages that are responsible for phagocytosis of foreign substances in the body. Monocytosis can indicate inflammation, stress due to disease, hyperadrenocorticism, immune-mediated disease, and malignant tumors (Meuten, 2008).

The immediate causes of splenomegaly and monocytosis in the current study are not clearly evident though both conditions reflect a response to a clinical condition. Interestingly, differences with respect to spleen weight and concentrations of phosphorus and monocytes counts between rats fed MUC versus CON were not evident when those fed CON versus detoxified diets were compared. Since the detoxified diets contained reduced levels of L-Dopa, the main toxic compound of concern in *Mucuna*, it is likely that L-Dopa toxicity was at least partially responsible for these clinical conditions in rats fed MUC.

Alkaline phosphatases remove phosphate groups by dephosphorylation, and they are most effective in an alkaline environment (Coleman, 1992). Feeding undetoxified *Mucuna* resulted in lower plasma alkaline phosphatase (hypophosphatasemia) and phosphorus concentrations relative to detoxified *Mucuna* treatments. Phosphatases are involved in signal transduction because they regulate the action of proteins to which they are attached (Steelman et al., 2008; Yi

and Lindner, 2008). To reverse the regulatory effect, phosphate is removed on its own by hydrolysis or through mediation of protein phosphatases. Hypophosphatasemia can occur in cases of malnutrition, hypothyroidism, anemia, and chronic myelogenous leukemia. Given the fact that diets were manufactured by a renowned feed company specialized in rodent diets, Harlan Teklad, and formulated to supply similar quantities of nutrients it is not likely that malnutrition caused the hypophospatasemia. In support of this statement, rats were fed *ad libitum* and no differences in feed intake among treatments were observed. The rats were not anemic at the time of necropsy as shown by their red blood cell counts (7 x 10^6/ul) that were within the normal range (3-8 x 10^3/ul) as indicated by the clinical pathology laboratory.

Conclusion

It can be concluded that dietary inclusion of detoxified or undetoxified *Mucuna* at 10% of diet DM did not affect any performance measure. Compared to feeding CON, feeding MUC decreased blood phosphorus concentration and caused splenomegaly and monocytosis but feeding detoxified *Mucuna*-based diets did not have these effects. Feeding MUC also decreased alkaline phosphatase levels relative to feeding detoxified *Mucuna* diets. Therefore, the detoxification processes improved the safety of *Mucuna*.

The behavior of rats fed all *Mucuna* diets instead of CON was characterized by decreased total travel distance and decreased total line crossings. These reductions were numerically less pronounced in rats fed ACD and SIL versus MUC or BAS in an open field test, suggesting that these detoxification methods are more promising than the BAS method. This aspect of the study was partly compromised by an equipment problem. Therefore, future research should repeat the open field test to provide more conclusive results on effects of detoxifying *Mucuna* on the behavior of rats.

Because feeding detoxified *Mucuna* increased the concentration of alkaline phosphatase, which is a key enzyme regulating gene expression, future research should examine effects of feeding *Mucuna* on gene expression. Follow up research should also focus on long term effects of feeding the detoxified diets to multiple monogastric species while taking into account that a larger number of animals (n>12) may allow better detection of treatment effects.

CHAPTER 6
EFFECT OF FEEDING *Mucuna pruriens* OR LEVAMISOLE INJECTION ON *Haemonchus contortus* INFECTION IN LAMBS

Introduction

Infections caused by gastrointestinal nematodes are the major constraint in small ruminant production. A helminth of particular concern, especially in tropical and sub-tropical regions worldwide, is *Haemonchus contortus*. The considerable egg-laying capacity of this nematode is maintained by adults feeding on blood. The late stage immature larvae also feed on blood. Blood loss can result in anemia, anorexia, loss of condition, and eventual death in the host animal (Miller and Horohov, 2006). *Haemonchus contortus* infects sheep, goats, deer, and other small ruminants and has been a significant cause of economic loss to small ruminant producers worldwide (Lange et al., 2006).

The prophylaxis of *haemonchosis* has been jeopardized by selection for nematodes with resistance to anthelmintics (Bricarello et al., 2005). Extensive use of anthelmintics for the control of helminth infections has resulted in drug resistance, which is usually manifested by poor clinical response to anthelmintic treatments, though the latter may also be due to other factors. Taylor et al. (2002) described several other conditions that may cause clinical signs similar to those normally associated with parasitism as well as a variety of reasons why anthelmintics fail to control nematodes. Due to the increasing problem of parasite resistance to antiparasitic drugs and the increased concern about drug residues in animal products and the environment, the application of nutraceutical and other biological approaches to parasite control has become more urgent. Ethnoveterinary sources have advocated exploiting antiparasitic properties of plant secondary metabolites with prophylactic or therapeutic properties (Athanasiadou and Kyriazakis, 2004). Further incentives to investigate alternative solutions are the economic loss that occurs due to decreased production, the costs of prophylaxis and

treatment, and the deaths of valuable livestock. Recently several studies have been conducted to study alternative methods of internal parasite control (Albonico, 2003; Kerboeuf et al., 2003; Bricarello et al., 2005; Louvandini et al., 2006).

Mucuna pruriens is a legume indigenous to tropical regions, especially Africa, India, and the West Indies. Szabo and Tebbett (2002) stated that the major drawback of *Mucuna*, which has compromised its usefulness as a food/feed source for humans and other monogastrics is associated with its L-Dopa concentration. *Mucuna* however, has been successfully used in the diet of ruminants that can digest the L-Dopa it contains (Muinga et al., 2003, Mendoza-Castillo et al., 2003, Matenga et al., 2003; Chikagwa-Malunga et al., 2008b). *Mucuna* beans contain varying levels of L-Dopa (St-Laurent et al., 2002; Daxenbichler et al., 1972; Tebbett, 2002; Szabo and Tebbett, 2002). One study reported as little as 1.5% to as much as 9% L-Dopa in the bean of *M. gigantea* in southern India (Rajaram and Janardhanan, 1991).

Sources from various countries claim that *Mucuna* has anthelmintic properties (Taylor, 2004) but there is only anecdotal evidence to support this claim. Research in which *Mucuna* was substituted for soybean meal in the ration of sheep (Chikagwa-Malunga et al. 2008d) indicated lower coccidial oocyst scores ($P < 0.05$) and a 52% numerical reduction in fecal egg counts (FEC) in lambs fed a high *Mucuna* diet. When Jalalpure et al. (2007) applied ether-extracted oil from *Mucuna pruriens* beans to intestinal worms, they reported a significant increase in the paralysis of worms. Conroy and Thakur (2005) reported that *Mucuna pruriens* trichomes treatment was as effective against gastro-intestinal parasites in pregnant does as the commercial anthelmintic medicine fenbendazole. These results emphasize the need for further scientific investigation of the anthelmintic properties of *Mucuna*. The aim of this study was to determine if incorporation of *Mucuna* beans in the diet reduces helminth parasite infection in lambs.

Materials and Methods

Animals

The study was conducted during the summer of 2007 at the University of Florida's sheep research unit, Gainesville, FL, USA (30^{o} N lat.). The investigation involved a 7-week trial. Thirty-six, 6-month-old, Dorper x Katahdin ram lambs weighing 28.8 $\pm$ 5 kg were de-wormed subcutaneously with levamisole (0.4 mg/kg), balanced for body weight and FEC, and randomly allocated to three treatment groups. The 12 lambs in each treatment group were randomly assigned to four pens, each within one of four blocks representing different areas of the barn. Each pen contained three randomly chosen lambs from a specific treatment. Pen was the experimental unit for feed intake, but sheep was the experimental unit for other measurements. The randomized complete block design consisted of 12 lambs in each treatment group, randomly assigned to four blocks, with three lambs in each pen.

Treatments

Mucuna pruriens cv. Aterrima beans were imported from Brazil (Wolf & Wolf Seeds Inc. FL, USA). The beans contained 31% CP and an L-Dopa concentration of 5.3%. All lambs were fed *ad libitum* amounts of total mixed rations formulated to be isonitrogenous (14% CP) and isocaloric (64% total digestible nutrients). The control diet contained 44% corn grain, 17% cottonseed meal, 0% *Mucuna* meal, 34% cottonseed hulls, and 3% molasses (dry matter (DM) basis). The *Mucuna* diet contained 53% corn grain, 0% cottonseed meal, 36% *Mucuna* meal, 6.5% cottonseed hulls, and 3% molasses (DM basis). Treatments consisted of lambs fed 1.) the control diet (CON) and no anthelmintic, 2.) the diet in which *Mucuna* replaced cottonseed meal as the main protein source with no anthelmintic (MUC), and 3.) the control diet with weekly subcutaneous injections of levamisole (0.4 mg/kg; ANT). Lambs were allowed a 2-week

adaptation period to adjust to their new diets before experimental challenge with *H. contortus* larvae for 3 weeks.

Animals were kept on the assigned regimen for a total of 5 weeks. Subsequently, two lambs per pen were harvested for dressing estimation and the third lamb was grazed on bahiagrass pasture for 19 days and then harvested for abomasal worm counts and dressing estimation.

Figure 6-1. Experimental conditions for anthelmintic trial: A) Customized diaper designed to collect *H. contortus* eggs from a donor goat; B) the Baermann technique was used to harvest larvae before microscopically identifying and quantifying them.

Haemonchus contortus **Challenge**

Daily fecal outputs were collected from a naturally infected donor goat with fecal egg counts of up to 1900 eggs/g using a customized collection diaper (Figure 6-1). Fecal matter was incubated at 37^{o}C in pans covered with cheesecloth for a 2-week period. During incubation, regular moistening with double distilled water prevented drying, and kneading provided

additional aeration. Microscopic identification and quantification confirmed an abundance (>200/ml) of infectious L3 larvae. Larvae were harvested from the fecal matter (Figure 6-1) via the Baermann technique (Baermann, 1917). Larvae were stored under a thin layer of water in culture flasks at room temperature. During the third through the fifth week of the trial, animals were challenged 3 times per week by gavage (Figure 6-2) with infectious *H. contortus* larvae (2000 larvae/lamb in 10 ml of distilled water).

Figure 6-2. Introduction of infectious larvae from donor goat through gavage: A) *Haemonchus contortus* eggs were collected from a donor goat, "Jack," then incubated and larvae cultured; B) Lambs were dosed by gavage with resulting infective-stage larvae.

Measurements

Animal performance measures included feed intake, body weight gain, and dressing. Measurements were taken to monitor both animal production and clinical status. Clinical evaluation involved weekly FEC and anemia scoring with abomasal worm counts (AWC) upon necropsy. For FECs, feces were collected directly from the rectum of each lamb and analyzed using the McMaster technique (Lange et al., 2006). To test for anemia (Figure 6-3), the color of the ocular conjunctivae was scored using the FAMACHA Anemia Guide (Kaplan et al., 2004). In addition, 7 ml of blood was collected by jugular venipuncture into vaccutainer tubes

containing EDTA for refractometric analysis of blood protein levels. Capillary tube specimens of blood were spun in a microhematocrit centrifuge to determine packed cell volume (PCV).

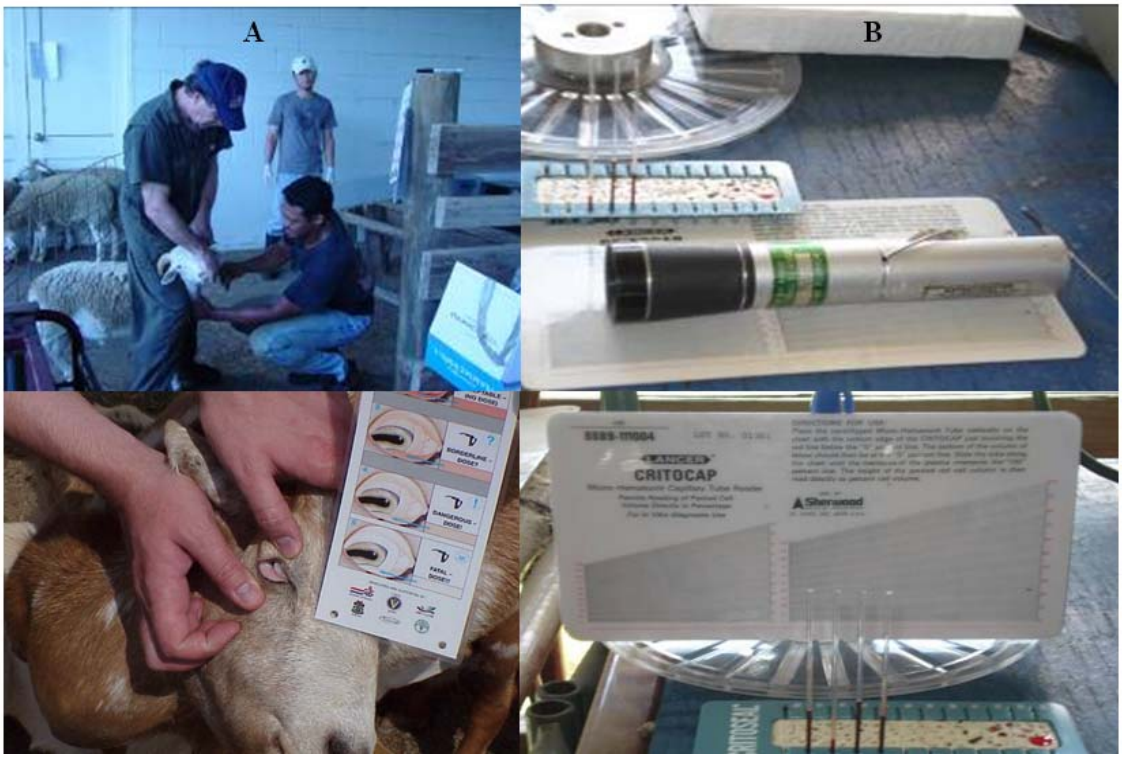


Figure 6-3. Anemia indicators were used to determine clinical signs of haemonchosis: A) Blood collection by jugular venipuncture and examination of the ocular membrane for FAMACHA scoring, B) Blood protein was measured by refractometer and packed cell volume determined after spinning capillary tubes in a microhematocrit centrifuge.

Statistical Analysis

The experimental design was a randomized complete block with 36 lambs, four blocks, three treatments, 12 pens, and three lambs per pen. The models used to analyze individual treatment effects were:

For one time measurements: $Y_{ij} = \mu + T_i + B_j + E_{ijk}$
Where:
Y_{ij} = dependent variable
μ = general mean
T_i = treatment effect (nutritional measurements, abomasal worm counts, dressing)
B_j = block effect (random effect)
E_{ijk} = experimental error

For weekly measurements: $Y_{ijk} = \mu + T_i + B_j + TB_{ij} + W_k + TW_{ik} + BW_{jk} + TBW_{ijk} + E_{ijkl}$

Where:

Y_{ij} = dependent variable

μ = general mean

T_i = treatment effect (FEC, PCV, blood protein, FAMACHA score, weight gain, fixed effects)

B_j = block effect (random effect)

TB_{ij} = treatment x block interaction

W_k = week (repeated measurement)

TW_{ik} = treatment x week interaction

BW_{jk} = block x week interaction

TBW_{ijk} = treatment x block x week interaction

E_{ijkl} = experimental error

Significance was declared at $P < 0.05$ and tendencies at $P < 0.10$.

Statistical analysis was performed with the MIXED model procedure of SAS (SAS 9.1, SAS Inst. Inc., Cary, NC, USA). The GLIMMIX procedure from SAS was used to analyze counts of fecal eggs. Significance was declared at $P < 0.05$.

Results

Clinical Measurements

All lambs developed mature egg-laying worms (Figure 6-4). The ANT treatment decreased FEC and AWC by 87 and 83% respectively, relative to the CON treatment. The MUC treatment did not statistically affect FEC or AWC though a numerical reduction ($P > 0.1$) was evident. As illustrated in Figure 6-5, neither ANT nor MUC treatment affected packed cell volume (32.4%), blood protein concentration (6 g/dL), or average FAMACHA score (2) and sheep weights were not affected by treatment (Figure 6-6).

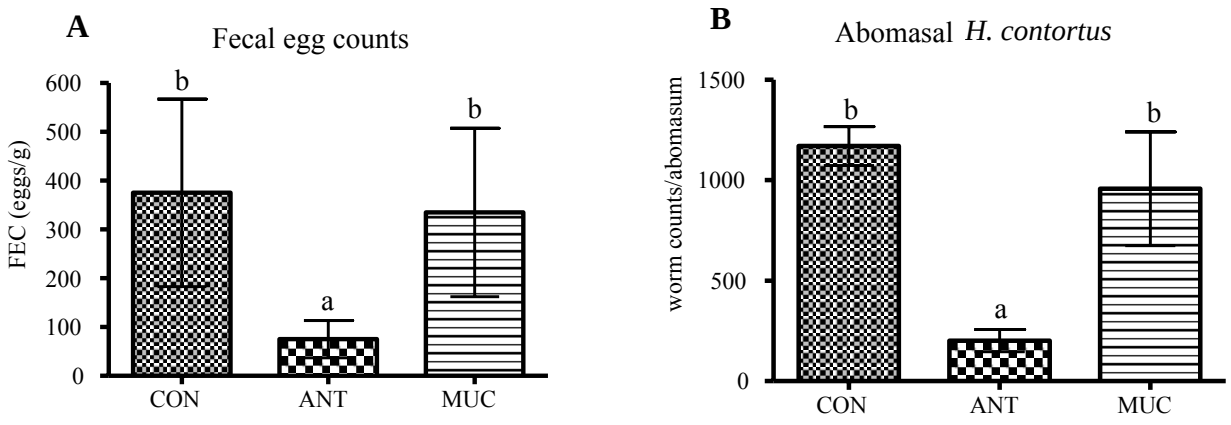


Figure 6-4. Effect of feeding *Mucuna* (MUC) versus feeding a control diet without (CON) or with subcutaneous levamisole anthelmintic (ANT) treatment on A) fecal egg counts and B) *H. contortus* counts in the abomasums. Bars without a common superscript letter differ ($P < 0.05$); error bars denote the standard error.

Performance Measurements

Daily feed intake, final body weight, average daily gain, and dressing were not different across treatments (Table 6-1). On average, body weight was higher for ANT (41.5 $\pm$ 1.1 kg) than for MUC (37.6 $\pm$ 1.1 kg) treatment groups, but MUC and CON (39.4 $\pm$ 1.1 kg) had similar body weights and body weights did not differ between treatments during successive weeks (Figure 6-6).

Table 6-1. Effect of feeding *Mucuna* versus Levamisole injection on performance of lambs

	Control	Levamisole	*Mucuna*	SEM
Initial weight, kg	28.7	28.7	28.8	1.5
Intake, %BW	5.0	5.2	5.6	0.4
Intake per pen, kg/d	5.8	6.3	6.5	0.4
Final weight, kg	43.1	44.9	41.5	1.5
Total weight gain, kg	14.5	16.2	12.7	1.2
Daily gain/lamb, kg	0.3	0.4	0.3	0.1
Dressing 1[A], %	49.1	48.6	49.6	1.8
Dressing 2[B], %	47.0	50.3	45.8	1.9

Means within each row with different superscripts are different, $P < 0.05$; SEM = standard error of mean. [A] Dressing of lambs fed the total mixed ration alone for 5 weeks. [B] Dressing of lambs grazed on pasture after the 5-week total mixed ration feeding regime.

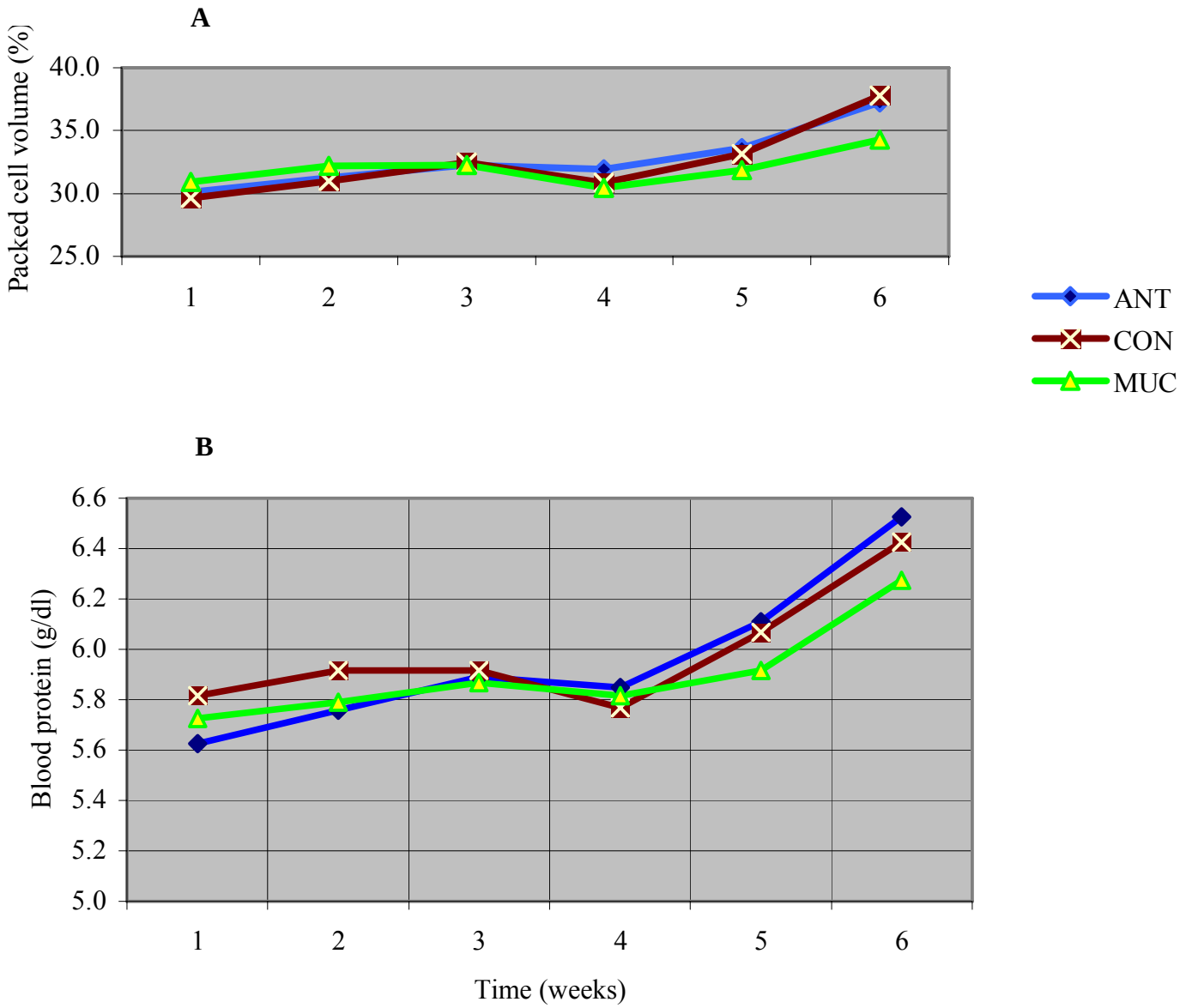


Figure 6-5. Effect of feeding *Mucuna* (MUC) versus feeding a control diet without (CON) or with (ANT) subcutaneous levamisole anthelmintic treatment on A) packed cell volume (pooled SEM = 0.5%) and B) blood protein (pooled SEM = 0.1 g/dl) measurements at various time points; NS = not significant ($P > 0.05$). Treatment and treatment x time effects were not significant, $P > 0.05$; time effect was significant, $P < 0.05$.

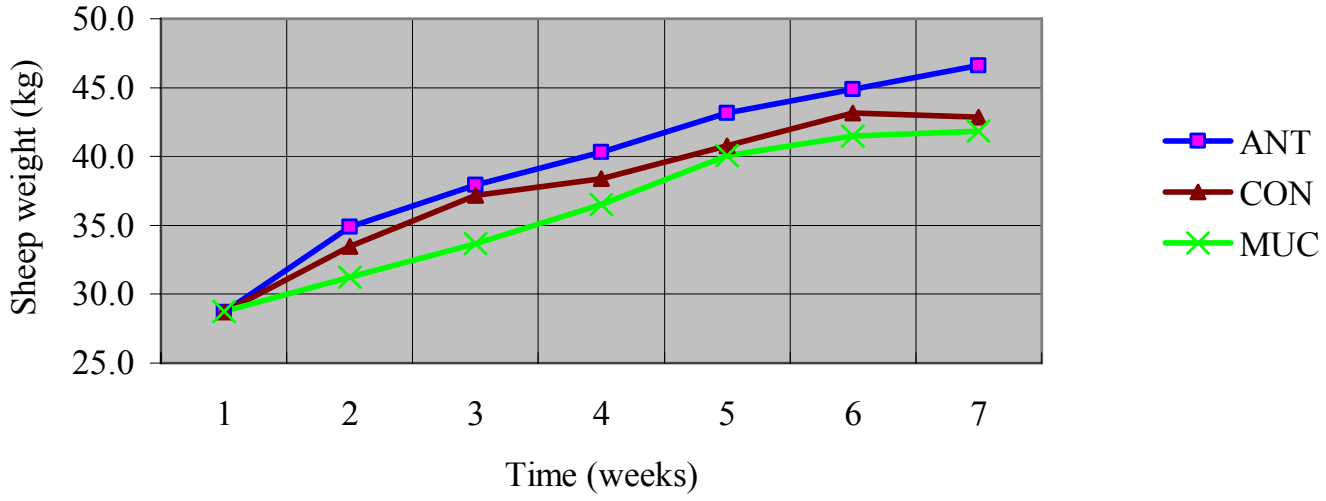


Figure 6-6. Effect of feeding *Mucuna* (MUC) versus feeding a control diet without (CON) or with (ANT) subcutaneous levamisole anthelmintic (ANT) treatment on sheep weights (pooled SEM = 1.1 kg) at various time points. NS = not significant ($P > 0.05$); treatment and treatment x time effects were not significant, $P > 0.05$; time effect was significant, $P < 0.05$.

Discussion

Neither levamisole treatment nor feeding *Mucuna* affected anemia indicators. Even with the experimentally induced abomasal worm burden, which resulted in up to 1400 adult worms per lamb, no signs of anemia or adverse affects on animal productivity performance were observed. Roberts and Swan (1982) investigated the correlation between worm burden and anemia. Their findings suggest that the number of worms associated with low hemoglobin (Hb) levels varied with the bodyweight of the sheep. For sheep up to 20 kg, 10.5 g% Hb was associated with 112 worms and 8 g% with 355 worms. However, 355 worms caused only moderate depression of Hb levels in sheep over 50 kg, and 1259 worms were required to cause severe depression (< 8.0 g%) in sheep over 50 kg. In the current study average animal weights were less than 50 kg suggesting that the worm burden of up to 1400 worms would have been sufficient to cause severe Hb depression and anemia. Some sheep in poor condition and kept

under poor grazing conditions can be severely anemic in the presence of less than 100 worms (Roberts and Swan, 1982).

This suggests that the clinical signs of infection were likely obscured by a combination of factors such as a high plane of nutrition and utilization of lambs of breeding known to be somewhat resistant/resilient to this parasite. The Katahdin is a hair-type sheep developed in the United States from West African hair sheep and wooled British sheep while the Dorper was developed in South Africa from the Dorset Horn and Blackheaded Persian for use in arid areas. Dorper sheep have low resistance to parasites, but are able to cope with infection by maintaining their PCV levels and body condition; in contrast, Katahdins are more resistant, showing lower FEC than Dorpers (Vanimisetti et al., 2004). Breed differences are more apparent when infection levels are higher and animals are less affected when on a better plane of nutrition. Dietary CP and metabolizable protein (MP) especially impact susceptibility to *H. contortus*. Sheep fed moderate (10.2% CP, 75 g MP) or high (17.2% CP, 129 g MP) levels of dietary protein responded differently to helminth infection (Bricarello et al., 2005). The higher MP supply resulted in higher body weight gains and PCVs ($P < 0.05$) in addition to an ability to withstand the pathophysiological effects of *H. contortus*. Optimal nutrition thus increases resilience to *H. contortus*-induced pathophysiology (Bricarello et al., 2005). In the current study the dietary CP concentration was about 14% of DM and this high CP supply may have conferred some resistance to haemonchosis. Kahn et al. (2003a, b) noted that increased immunity and resistance as a result of protein supplementation is dependent on the prevailing supply and demand for scarce nutrients such as protein. Knox and Steel (1999) suggest that even urea supplementation can increase resilience to parasitism, thereby improving performance and enhancing resistance mechanisms against worms in sheep on low quality diets. According to Datta et al. (1999),

animals fed high protein diets showed higher performance, higher antibody responses to *H. contortus* antigens, and reduced fecal egg counts. In future research, *Mucuna's* anthelmintic potential will need further evaluation under less optimal nutritional conditions.

In the current study, the lack of pathologic responses in spite of the presence of considerable numbers of adult abomasal worms and fecal egg output as well as evidence of abomasal hemorrhage (Figure 6-7), indicates that the usefulness of anemia detection methods, such as PCV, blood protein, and FAMACHA scoring to monitor internal parasite burdens is limited to situations with low levels of nutrition and poorer body conditions in less resistant/resilient breeds.

Mucuna treatment had no clinically significant anthelmintic effect compared to the controls. Levamisole is a broad-spectrum anthelmintic drug widely used to reduce parasitic nematode burdens in livestock, but the formerly high efficacy of levamisole against *H. contortus* in sheep and goats has been compromised by the development of resistance in field populations; this resistance occurs at a slower pace than with other classes of anthelmintic drugs (Conder et al., 1991). However, the fact that levamisole treatment did not reduce FEC or AWC by more than 87% and 83% respectively, suggests that the worms in this study were partially resistant to levamisole. *Haemonchus contortus* strains that are considered resistant provide levamisole efficacy rates as high as 77% as opposed to 100% efficacy with drugs that are considered effective (Uppal et al., 1993).

Some literature suggests that the anecdotal anthelmintic properties of *Mucuna* are in the hairs on the pods (Conroy and Thakur, 2005). In some varieties, these hairs or trichomes cause a burning/itching sensation upon touch, which can be attributable to serotonin, mucunain or some other compound (Szabo and Tebbett, 2002). Szabo and Tebbett (2002) screened for toxic

compounds, but only reported serotonin to be present in the trichomes. Other literature suggests the L-Dopa concentration to be responsible for possible anthelmintic effects (Faridah Hanum and van der Maesen, 1996). Chikagwa-Malunga et al. (2008a) report the L-Dopa concentration of the *Mucuna* plant to be mostly concentrated in the beans and the pods and these authors showed that replacing soybean meal with *Mucuna* reduced coccidia scores significantly and numerically reduced FEC. Therefore more studies on the anthelmintic effects of *Mucuna* trichomes versus beans are needed.

Although the main focus of this experiment was to investigate the anecdotal claims about anthelmintic properties of *Mucuna*, another very important consideration is the potential for adverse effects of *Mucuna* ingestion on animal health and production. According to Githiori et al. (2006), Ketzis et al. (2006) and Athanasiadou et al. (2001), dose-dependent anti-parasitic properties and cost effectiveness need to be carefully determined, because some of the active compounds of plants with anti-parasitic properties may also have antinutritional effects which may lead to reduced feed intake and poor performance. Several adverse effects have been associated with *Mucuna* intake in monogastrics (Del Carmen et al., 1999; Carew et al., 2002; Del Carmen et al., 2002; Flores et al., 2002), most of which are attributable to L-Dopa intake including reduced feed intake, weight loss, and feed conversion efficiency, diarrhea, vomiting, and skin lesions (Del Carmen et al., 2002; Flores et al., 2002). In contrast, ruminants are seemingly not adversely affected by intake of *Mucuna* L-Dopa. This is partly because up to 53% of dietary L-Dopa can be digested in ruminal fluid (Chikagwa-Malunga et al., 2008c). Other studies showed that feeding *Mucuna* improved DM intake, weight gain and milk production in ruminants without detrimental effects (Burgos et al., 2002; Muinga et al., 2003; Eilitta et al., 2003; Matenga et al., 2003; Mendoza-Castillo et al., 2003; Nyambati and Sollenberger, 2003;

Perez-Hernandez, 2003). In the present study the mean daily feed intake (2.5 kg), final body weight (37.9 kg), average daily gain (0.31 kg/d), dressing (48.8%) were not different across treatments. This indicates that inclusion of 36% *Mucuna* in the diets did not adversely affect performance of the lambs, and suggests that in future research, the level of *Mucuna* in the diet could be increased to further reveal anti-parasitic properties.

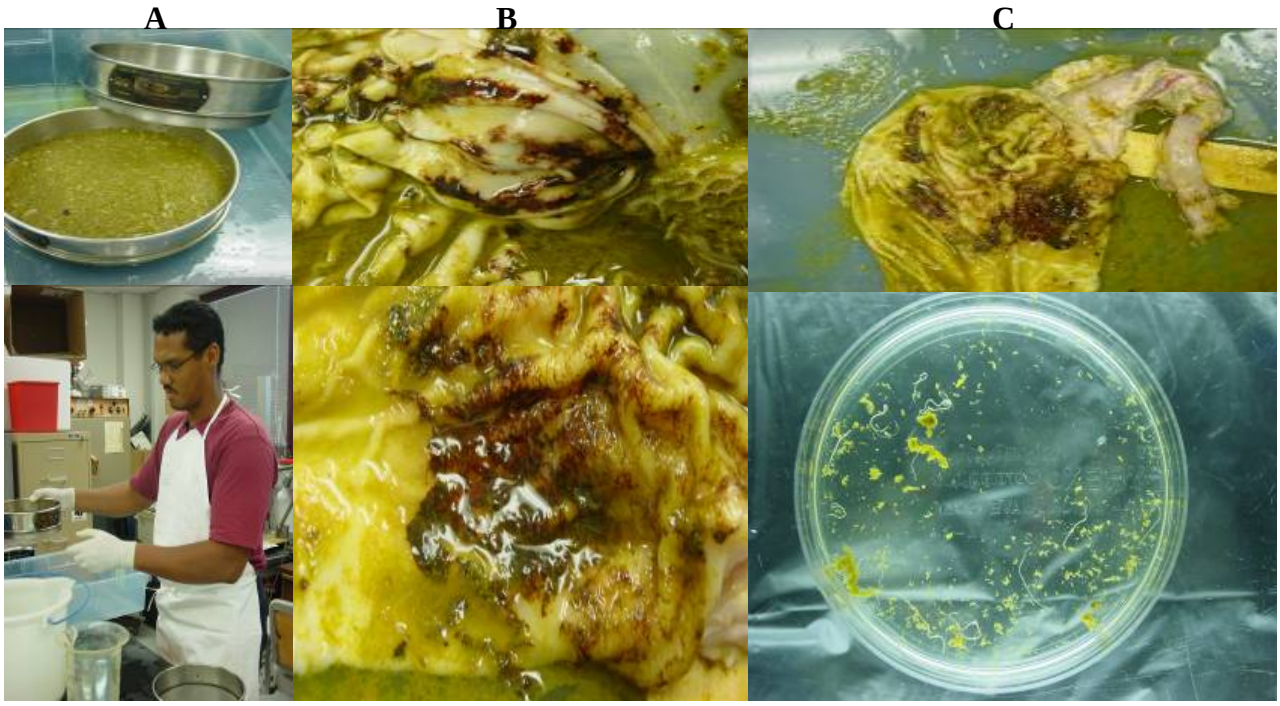


Figure 6-7. Upon necropsy *H. contortus* worms were harvested from the abomasums and quantified: A) Abomasal content was screened for worms and representatively subsampled; B) Necropsy revealed abomasal hemorrhage and internal bleeding; C) Subsamples from the abomasal content were collected in petri dishes and worms counted on a light box.

Conclusion

It can be concluded that *Mucuna* bean intake did not reduce *H. contortus* infection in lambs fed at a high plane of nutrition. In the current study, feeding *Mucuna* did not affect fecal egg counts or abomasal worm counts, though a numerical ($P > 0.10$) reduction was evident at the 36% *Mucuna* inclusion rate. Future studies should investigate whether higher levels of dietary

Mucuna inclusion result in anthelmintic responses and determine if the 36% dietary *Mucuna* inclusion level reduces helminth infection in lambs fed poorer quality diets.

CHAPTER 7
GENERAL SUMMARY, CONCLUSIONS AND RECOMMENDATIONS

Mucuna pruriens is a tropical legume, a versatile cover crop attractive as a green manure for sustainable farming systems, a promising protein and starch supplement for the food and feed industry, as well as a potential source of nutraceutical compounds. However, *Mucuna* also contains toxic secondary compounds, particularly L-Dopa, which reduce its appeal as a dietary ingredient for humans and monogastric livestock. Interestingly, these toxic compounds reportedly contribute to *Mucuna's* effectiveness as a nutraceutical such as in the treatment of Parkinson's disease. Several additional anecdotal claims about *Mucuna's* healing properties exist, but many of these require further scientific validation.

In ruminant feeding practice *Mucuna's* toxicity has not posed a problem. Rather, *Mucuna* supplementation to ruminants increased nitrogen intake and retention, weight gain, and milk production in ruminants (Eilitta et al., 2003). Until recently, the reason why ruminants are less susceptible to the negative impact of *Mucuna's* high L-Dopa concentration was not known. Recent evidence indicates this is because the L-Dopa is degraded in the rumen (Chikagwa-Malunga et al., 2008b), therefore, it is subsequently not present in the muscle tissue or blood of animals fed *Mucuna*-based diets at high concentrations. Conversely, studies have shown that humans who consume high levels of undetoxified *Mucuna* may experience anorexia, confusion, nausea, diarrhea, and vomiting. Research has also shown that feeding undetoxified *Mucuna* to monogastric livestock reduces intake, body weight and feed conversion efficiency, and increases mortality.

Some studies indicate that various processing techniques can reduce the L-Dopa concentration in *Mucuna* beans to safe levels (< 0.4%), but these techniques are often costly, time consuming, or require scarce resources. Few studies have examined the effects of feeding

detoxified *Mucuna* to monogastrics and little is known about their impact on the nutritional value of the bean. Four studies were conducted to address some of these unknowns. The first study examined the effect of ensiling duration on the fermentation of *Mucuna* and how ensiling affects the L-Dopa content and nutritional value of the bean. Subsequent detoxification experiments removed L-Dopa through sonication, or acid or alkaline solvent extraction, and investigated how these methods affect its nutritive value. Another experiment examined the performance, physiological and behavioral changes of Sprague-Dawley rats fed undetoxified or detoxified beans. The last experiment investigated how ingestion of *Mucuna* beans affected helminth parasite infection in lambs. Further details on each of these experiments are summarized below.

The objective of Experiment 1a) was to examine how long it takes to decrease the pH of ensiled *Mucuna* to 4.5. Crushed beans (6 mm) were ensiled in the dark at room-temperature (18 to 25^{o}C) for 0, 3, 7, 21, and 28 days. A pH of 4.5 and an L-Dopa concentration of 1.3% (54% reduction) were recorded after 28 days of ensiling. The objective of Experiment 1b) was to study the effect of particle size of ensiled *Mucuna* on L-Dopa concentration and on fermentation and nutritional characteristics. *Mucuna* beans were ground to pass through 2, 4, and 6 mm screens and ensiled for 28 days. Ensiling decreased the L-Dopa concentration of 2, 4 and 6 mm particles from 2.8% to 1.2, 1.6, and 1.1%, respectively. Ensiling also reduced the WSC concentration and pH and increased NH_3N concentration. Neither ensiling nor particle size affected concentrations of fat (5%), crude protein (23-25%), starch (38-40%), or neutral detergent fiber (17-20%). Dry matter losses (< 1%) and mold or yeast counts were unaffected by particle size. Aerobic stability was maintained beyond 657 hours in all treatments. The total-acid concentration of 2, 4, and 6 mm particles contained 54, 58, and 46% lactate, respectively. Therefore the lactate:acetate ratio of all samples exceeded 3.0. In conclusion, ensiling *Mucuna* bean for 28 days reduced the L-

Dopa concentration by 43 to 61% while preserving most nutrients. Coarse and fine particles had similar effects on nutritional composition, fermentation indices and extent of L-Dopa removal, indicating that grinding is unnecessary for ensiling to be effective. Further research should examine the L-Dopa concentration and nutritive value of cracked (by blunt force) versus coarsely crushed beans, as cracking requires less energy. The ensiling experiments were terminated after 28 days due to achievement of the typical minimum pH for legume silages. Future research should examine whether ensiling *Mucuna* beans for periods in excess of 28 days will further reduce the pH and the L-Dopa concentration. During the current process, an automated vacuum-sealer was used to ensure an anaerobic environment in the bags. Future research should investigate if similar results are obtained when *Mucuna* is ensiled in conventional plastic bags without the vacuum air exclusion step.

In Experiment 2, the goal was to study the effects of three extraction methods on L-Dopa concentration and nutritional composition of finely- (1 mm) or coarsely- (6 mm) ground *Mucuna* beans. Methods included extraction in solutions of acetic acid (ACD, pH 3) or sodium hydroxide (ALK, pH 11) for 8 hours or sonication (SON) for 5 minutes. All three methods reduced L-Dopa concentrations of fine *Mucuna* particles from 2.8 to < 0.2% and increased NDF and starch concentrations by at least 62 and 14%, respectively. Fat concentration of fine particles was reduced from 5.5% to 4.2% by SON but not by ACD and ALK. The methods also reduced CP and WSC concentrations of fine particles by 24-31% and 78-81%, respectively. Sonication and ACD did not reduce L-Dopa concentration of coarsely ground beans but ALK reduced it from 2.8 to 2.0%. Sonication reduced CP, WSC, and fat concentration of coarse particles by 6, 17, and 27%, respectively and ALK increased their starch concentration by 17%. The ACD treatment increased the NDF concentration of coarse particles by 35% but ALK and SON did not; ACD

and ALK reduced fat concentration by 31 and 35%. It was concluded that the extraction methods reduced the L-Dopa concentration of fine *Mucuna* particles to safe levels but increased their NDF and starch concentrations at the expense of their WSC and CP concentrations. Extraction methods were less effective at reducing the L-Dopa in coarse particles and had fewer, less consistent effects on their nutritional composition. The melanin concentrations in the detoxified bean particles need to be measured in future research because of concerns that they could predispose to melanoma. The maximum solubility of L-Dopa in acidic and alkaline solutions at pH of 3 and 11 needs to be determined in order to reduce the amount of solvent necessary to detoxify the bean particles. In addition, sonication in solutions other than water should also be investigated.

Experiment 3 examined the effect of feeding detoxified *Mucuna* bean on the performance, behavior, and health of rats. Sixty Sprague-Dawley rats were randomly assigned to five treatments (n=12). Dietary treatments consisted of a commercial rat chow (CON) or diets in which 10% of a customized rat chow was replaced with either untreated (undetoxified) *Mucuna* (MUC), or *Mucuna* detoxified by acetic acid extraction (pH 3), sodium hydroxide extraction (pH 11), or ensiling for 28 days (SIL). During the course of the 14-day trial, behavior, physiological development, and signs of clinical pathology were evaluated. Animals were necropsied afterwards. Feeding MUC caused splenomegaly and monocytosis, and reduced blood phosphorus concentrations relative to CON, but these effects were not observed in rats fed detoxified diets. Feeding detoxified diets increased alkaline phosphatase concentration by 11-17% compared to MUC, but not CON. No differences in performance or physiology were observed in any of the rats on the detoxified diets. Compared to CON, *Mucuna*-based diets gave similar feed intake and weight gain. When behavior was examined in the open field on days 3 and 10 individually, no

abnormalities were observed; however, when the total distance traveled and total line crossings over the two days were taken into account, rats on all *Mucuna*-based diets showed decreased activity, yet this response tended to be less severe in rats fed ACD and SIL treatments but not in MUC and BAS. It can be concluded that at the 10% level of dietary inclusion, there were fewer measurable adverse effects due to feeding the detoxified *Mucuna* bean compared to untreated *Mucuna* bean. The current research suggests that the preferred detoxification method was ensiling for 28 days at 2 mm particle size and 70% moisture. This method of ensiling was reasonably successful at reducing L-Dopa levels (57%), it allowed a shelf life of at least 657 hours and preserved nutrients such as crude protein, starch, fat, and fiber (Chapter 3). This suggests superior nutritive value relative to acid or alkali solvent extraction methods, which were more effective at reducing L-Dopa concentration but also decreased concentrations of key nutrients. Due to the equipment that malfunctioned in the current study, the open field test should be repeated to better assess effects of the treatments on behavior. Future studies should repeat this study on other monogastric species and compare effects of greater dietary inclusion levels of detoxified and undetoxified *Mucuna.*

The aim of Experiment 4 was to determine if ingestion of *Mucuna* beans reduces helminth parasite infestation in lambs. Thirty-six Dorper x Katahdin ram lambs (six months old, 28.8 + 5 kg body weight) were dewormed subcutaneously with levamisole (2 ml/45.4 kg), balanced for fecal egg counts and body weight, and randomly allocated to three treatment groups. The 12 lambs in each treatment group were randomly assigned to four pens, each containing three lambs. All lambs were fed *ad libitum* amounts of an isonitrogenous (14% CP), isocaloric (64% total digestible nutrients) total mixed ration in which the main protein supplement was cottonseed meal or *Mucuna*. Treatments consisted of a control diet, a diet in

which *Mucuna* replaced cottonseed meal and a further treatment that involved administering levamisole (2 ml/45.4 kg) to lambs fed the control diet. Lambs were adapted to diets for 2 weeks and trickle infected 3 times per week by gavage with infectious Haemonchus contortus larvae (2000 larvae/lamb) for 3 weeks. Subsequently, two lambs per pen were necropsied and the third lamb was grazed on bahiagrass pasture for 14 d and then necropsied. All animals developed mature worms. Levamisole treatment decreased fecal egg counts by 87% (445 vs. 58 eggs/g) and abomasal worm counts by 83% (1170 vs. 202 worms/lamb). *Mucuna* intake did not affect fecal egg counts (445 vs. 412 eggs/g) or abomasal worm counts (1170 vs. 958 total worms), though a numerical ($P > 0.10$) reduction was evident. Neither levamisole nor *Mucuna* treatment affected anemia indicators [FAMACHA score (2), packed cell volume (32.4%) and blood protein concentration (6 g/dL)], daily feed intake (2.5 kg), final body weight (37.9 kg), average daily gain (0.31 kg/d) and dressing (48.8%). *Mucuna* intake did not reduce infection in lambs fed the high quality diet. Pathological signs of infection were obscured, most likely by a combination of the high nutritional plane and lambs of breeding known to be at least somewhat inherently resistant to this parasite as compared to highly improved breeds. Future studies should examine if *Mucuna* exhibits anthelmintic properties in more susceptible lambs fed poorer quality diets.

This series of experiments show that *Mucuna pruriens* can be detoxified by means of solvent extraction in both acidic (pH 3) and alkaline (pH 11) solutions. Both extraction methods are equally successful provided the bean particles are finely ground (1 mm) as this increases the surface interaction with the solvent. The acidic extraction method is somewhat favorable over the alkaline method due to lesser discoloration of the bean. Sonication at the 1-mm particle size level is, however, somewhat preferable to the solvent extraction methods because it resulted in similar L-Dopa removal from fine particles without discoloration. Although successful in detoxifying

the bean, these methods also reduced the CP and WSC concentration of the bean. In contrast, ensiling *Mucuna* beans for 28 days reduced the L-Dopa concentration to a lower extent, but did not affect the CP concentration of the bean, which is the primary reason for using *Mucuna* as a feed or food source. The ensiled bean had a long shelf life and ensiling did not cause discoloration of the bean. Furthermore, course and fine particles had similar L-Dopa concentrations and nutritive value. Ensiling is also less labor intensive and more practical because grinding, washing, sieving and drying are not required. Therefore, ensiling is a more promising *Mucuna* detoxification method for resource-limited smallholders. Nevertheless, detoxifying *Mucuna* beans by acid or alkali solvent extraction or ensiling is recommended for monogastric diets because all of these methods prevented negative effects of feeding the undetoxified bean such as monocytosis and splenomegaly when the beans accounted for 10% of the diet of rats. The anthelmintic properties of *Mucuna* require further investigation under less optimal feeding conditions since the high nutritional plane used in the anthelmintic study typically increases resilience to helminths and obscures pathological signs of *H. contortus* infection.

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BIOGRAPHICAL SKETCH

Christiaan Max Huisden ("Max") was born on August 9, 1966 in the Dutch Caribbean (Curacao); he is a citizen of Suriname, South America. His late parents, Max Franklin Huisden and Amoi Trude Leonie Huisden-Lie A Kwie, both citizens of Suriname, lived in Curacao for 24 years and all of their five children were born there.

In 1989, Max attended the Agricultural Production program of the Faculty of Technology at the Anton de Kom (ADEK) Universiteit van Suriname, and graduated in 1994 with his Bachelor of Science degree. After graduation he was employed as Department Head at the Palm-oil and Animal Production company (GPOV) and as a Researcher by the Center for Agricultural Research (CELOS) in Suriname.

In 1996 Max was awarded a fellowship by the Organization of American States (OAS) for a graduate program at the University of Florida's Animal Sciences Department. Max received his Master of Science and Master of Agriculture degrees in Animal Science working respectively with Dr. Roger West in Meat Science and with Dr. Frank Simmen in Reproductive Physiology/Molecular and Cell Biology. From 2001 through 2004 he was employed by the Center for Environmental and Human Toxicology at the University of Florida as a Biological Scientist in Molecular Toxicology with Dr. Evan Gallagher. During this time Max earned his certification as a Toxicologist and pursued a dual Doctorate in Holistic Nutrition (Ph.D.) and Naturopathic Medicine (ND) from Clayton College (Birmingham); he graduated with High Honors in 2006.

In 2004 Max joined the Nutrition laboratories of Dr. Adegbola Adesogan at the University of Florida's Animal Sciences Department as a Biological Scientist. Currently Max is working

toward certification in Drug Chemistry at the College of Pharmacy and the completion of a second Ph.D. degree, specializing in Nutrition and Pharmaceutics.

As the point person for collaboration between the University of Florida and the ADEK Universiteit van Suriname, Max is currently spearheading an effort to catalog and verify the medicinal properties of a myriad of functional foods and naturally-occurring plant species in the Amazon rainforest.

Max married his precious wife, Andrea Feaster Huisden, in 2001 and they are blessed with four wonderful sons: Raoul Max Franklin Huisden (12), Christiaan Henry Huisden (5), John Franklin “Sjaak” Huisden (2), and Carlo Dennis Huisden (1).