Mucuna News

Developing Multiple Uses for a Proven Green Manure/Cover Crop

Update on the Progress of the Project "Increasing Mucuna's Potential as a Food and Feed Crop"

CENTER FOR COVER CROPS INFORMATION AND SEED EXCHANGE IN AFRICA (CIEPCA)

Second issue, April 2001

Progress of the Project

By R.J. Carsky and M. Eilittä

Since the publication of the first *Mucuna News*, the project "Increasing *Mucuna*'s Potential as a Food and Feed Crop" has gotten off to a good start. CIEPCA-IITA has already sent funds to most investigators. They now have approximately 6 to 9 months to complete their research activities, and another few months (until March 2002) to draft technical reports and take care of their financial accounting with us.

During these upcoming months, we will continue to support the investigators with any needs for information, technical assistance, or literature they may have. We also hope to continue our efforts to locate investigators who are working on related issues outside of this particular project, both to share information regarding their activities with the participants of our project and to establish contacts among those involved with research on *Mucuna*'s food and feed uses. We will publish information and insights gained from such contacts in this newsletter. We would also like to publish updates from individual researchers of the project. Please share with us your news and preliminary findings.

We would not like to see information flowing only through us. We encourage the investigators to continue communicating with each other throughout the process. We already have examples of investigators commenting on each others' plans and contacting each other when they have had needs for information or literature. We expect that such exchanges will continue and contribute not only towards making this project a success, but also towards initiating longer-lasting collaborations among those working on *Mucuna*'s food and feed uses, thereby constituting one long-term contribution of this project.

In fact, with the various activities firmly in place, it may also be time both for the investigators involved in this project and those outside but interested in continuing *Mucuna*-related research to begin turning their attention to the future, to plan for the continuation of the ongoing activities. Are there funding sources that can be approached for continued funding of *Mucuna*-related work? Please consider this and share information you may have with your colleagues and with us.

This issue of the bulletin updates you on two new project activities in Kenya and on plans for repeating the trial to examine genotype-by-environment effects on L-dopa in *Mucuna* in 2001-2002. It also describes *Mucuna* accessions in India and feed work conducted in the Yucatan Peninsula, Mexico. The issue also initiates a multi-part article presenting various methods for L-dopa analysis. We are grateful to the articles supplied by K. Janardhanan and N. Szabo, and the materials sent by J. Castillo which enabled us to write the synopsis of the past research projects in Yucatan. Finally, our thanks to the MOIST-CIIFAD of Cornell University for posting our newsletter to the Internet.

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If you are interested in posting news or inquiries in this bulletin, please contact Marjatta Eilittä.

Project Update

Two New Activities from Kenya

As in many other parts of the tropics, in Kenya *Mucuna* has been promoted for soil fertility improvement. Among other Kenyan efforts, two projects, the Legume Research Network Project and the Soil Management Project, both based at the Kenya Agricultural Research Institute (KARI) and funded by the Rockefeller Foundation, have been working on *Mucuna* as well as on other cover crops.

Two new activities from Kenya have recently been incorporated in the *Mucuna* Project. These activities will be carried out by members of the two networks. The research project by Elizabeth Wanjekeche from KARI-Kitale focuses on potential ways to process *Mucuna* for food from both immature and mature beans. She will use *Mucuna* with white seed color in her study. Various treatments such as of boiling in locally available alkaline media (i.e., a form of sodium carbonate, Magadi soda, maize cob and bean stover ash) and acid media (i.e., citric acid), pressure and extrusion cooking, and fermentation and germination will be applied. Processed and unprocessed samples will be analyzed for both anti-nutritional factors (i.e., L-dopa, trypsin inhibitors, tannins, and polyphenols) and nutritional factors (i.e., crude protein, ash, fat, carbohydrates, fiber, sugars and amino acids). The bio-availability of nutrients and protein quality will be determined through rat feeding studies. Processed products with very low (<0.1%) L-dopa content will be subjected to sensory evaluation. E. Wanjekeche's collaborators include J. Mureithi, B. Kirungu, and B. Omamo.

The project by Rahab Muinga from KARI-Mombasa originates from previous work with dairy cows at the station. In that research, the overall effect of fresh *Mucuna* forage on milk production was found to be similar to that of other legume forages like *Gliricidia sepium, Clitoria ternatea* and *Lablab purpureus.* However, during the 12th week of experimentation, cows fed with *Mucuna* forage produced one liter of milk less than those fed with the other legumes. Levels of L-dopa in the forage and in the cows' blood were not determined in the previous study.

The planned study therefore proposes to assess the long-term effect (6-9 months) of feeding *Mucuna* forage on ruminal dry matter degradation, feed intake, milk production and the relationship between animal performance and L-dopa level in the blood. In addition, the fate of L-dopa in the gastrointestinal tract will be assessed through *in vivo* forage degradability studies and by determining the L-dopa concentration in the feces, urine, milk and blood. Tannin levels in *Mucuna* forage will also be determined. R. Muinga's collaborators include H. Saha and J. Mureithi.

For further information, please contact E. Wanjekeche (email: smpktl@africaonline.co.ke), R. Muinga (email: rwmuinga@africaonline.co.ke) or J. Mureithi (jmureithi@net2000ke.com).

Plans for the 2001-2002 Genotype by Environment Trial

As a part of the *Mucuna* Project, in 2000-2001, the genotype by environment trial on *Mucuna*'s L-dopa production was conducted on four different *Mucuna* accessions in five locations in latitudes ranging from 18º S to 38º N. *Mucuna* seed from these locations is currently being sent to Rolf Myhrman, Judson College, for the analysis of L-dopa.

Although the trial was initially planned for one year only, it has become evident that it would be valuable to conduct the trial during another year. First, this would add certainty to the results and yield data that would enable statistically valid conclusions. Second, due to budgetary and labor constraints, the first year's work mainly focused on L-dopa content of the seed. Other plant parts of *Mucuna*, such as leaves and stems, have been shown to contain L-dopa, which may have important implications to the plant's disease and pest resistance, as well as to its utilization as a forage. Finally, the first year's trial focused on only four accessions, but it would be valuable to gain information on a larger number of accessions that are genetically as diverse as possible.

The project therefore plans to continue the trial during the year 2001-2002 and at the moment, additional funds are being sought for this activity. Eight *Mucuna* accessions available from CIEPCA-IITA will be grown in 9 locations, in latitudes ranging between 18º S and 38º N. These eight accessions are: Rajada, Ghana, IRZ, Jaspeada, Cochinchinensis, Preta, Deeringiana, and Utilis. The accessions vary in regard to time to maturity (early-intermediate-late) and seed color (black-mottled-white). The nine locations and interested collaborators are presented in Table 1.

The experimental design is a randomized complete block with two replicates. Plots will consist of a single plant, typically supported by an A-frame (see Photo 1, page 9). L-dopa analyses for the trial will be conducted by R. Myhrman, Judson College.

Table 1. Locations and collaborating institutions of the proposed G X E trial in 2001-2002.

Country	Latitude	Collaborating institution
Zimbabwe	18° S	University of Zimbabwe
Colombia	3° N	CIAT
Benin	6° N	CIEPCA
Honduras	14° N	CIDICCO
Mexico (Chiapas)	16° N	Univ. Autonoma de Chiapas
Mexico (Yucatan)	20° N	Univ. Autonoma de Yucatan
USA (Florida)	26° N	University of Florida
USA (Alabama)	33° N	Auburn University
USA (California)	38° N	University of California-Davis

Other News

Mucuna Accessions in India

By K. Janardhanan, Bharathiar University, India

Out of the six species/cultivars of the genus *Mucuna* that have been recorded in India, the seeds of only four species/cultivars are known to be consumed by various ethnic groups. Below is a brief account of the distribution, species status and mode of consumption of these edible *Mucuna* beans.

Mucuna gigantea is believed to be distributed in coastal areas of Tamil Nadu, Kerala and in the Andaman Islands. One accession of this bean was collected at Chiriatapu, South Andaman Islands, by the author's team of researchers; the seeds were deposited in the National Bureau for Plant Genetic Resources (NBPGR), New Delhi, in 1989 (IC No. 83295). This species is endangered, and it was not seen along the entire coastal areas of Tamil Nadu and Kerala that were surveyed by the author and his team during the period 1988-1995. Though the seeds obtained from the herbarium of the Government of India (Botanical Survey of India) germinated and produced plants in experimental plots, the plants failed to flower and set seed. In view of this, urgent and extensive conservation strategies are recommended to prevent genetic erosion of this plant species. Boiled seeds are eaten by the oceanic ethnic groups of Onges, and by Great Andamanese and Sompens.

Mucuna monosperma is distributed in the states of Tamil Nadu, Kerala, Andhra Pradesh, Karnataka, Bihar, West Bengal, Assam, Chittagong, and Pegu, in the hills of Western Peninsula and in the Andaman Islands. Eight accessions of this bean were collected from the Western Ghats and Eastern Ghats during the period 1988-1995. Out of the eight accessions collected only one accession from Wynaad district in Kerala was deposited in NBPGR in 1989 (IC No. 83.196). This species is included under the category rare, which is also recommended for both *in situ* and *ex situ* conservation. Boiled seeds are consumed by the members of Northeastern India and the Oceanic groups.

Mucuna pruriens var. *pruriens* (L.) DC. (itching bean) is widely distributed in India. It is found in Tamil Nadu, Kerala, Andhra Pradesh, Dehra Dun, Siwalikrange, sub-Himalayan tracts, Bundel Khand, Merwara, Punjab plains, Madhya Pradesh, Gujarat, Orissa, Gorakhpur and Lucknow in Uttar Pradesh. As many as 14 germplasm accessions were collected mostly from the Western Ghats and Eastern Ghats during the period 1988-1995. Only three collected germplasm seed samples were deposited in NBPGR. They are: 1) Begur Reserve Forest, Wynaad district, Kerala accession (IC No. 83193), 2) Mukkali (Silent Valley), Palkkad, Kerala accession (IC No. 83 194), and 3) Mothimahal campus, Lucknow district (U.P.) accession (IC No. 83 209). Mature seeds, seeds from unripe pods and young pods are soaked in water followed by boiling or roasting and consumed as such or mixed with salt by the Northeast groups (such as Khasi, Naga, Kuki, Jainitia, Chakma and Mizo), groups of Northwestern part of Madhya Pradesh (such as Abujh - Maria, Maria, Muria, Gond and Halbs) and South Indian groups (such as Mundari, Dravidian, Kani, Kader, Muthuwan, Savera, Jatapu, Gadebe and Kondadora).

Mucuna pruriens (L.) DC. var. *utilis* (Wall ex Wight) Bak. Ex Burck. (velvetbean) is distributed mostly in the Western Ghats in wild condition. From 1990's onward, Dravidian groups in Tirunelveli district in Tamil Nadu started cultivating velvetbeans (which had previously grown wild in the area) in their habitations. As many as seven germplasm seed samples were collected or procured mostly from Western Ghats by the author's team during 1981-1995. The Kanikars, a hill tribe of Kerala and other Dravidian groups in Tamil Nadu consume the seeds after boiling and decanting the water for seven times. Besides the author's team of researchers, Dr. P. Rajyalakshmi and Dr. P. Geervani from the Department of Foods and Nutrition, Postgraduate and Research Centre, at the Andhra Pradesh Agricultural University

(Rajendranagar, Hyderabad - 500 030, India) have recorded that the seeds of *Mucuna pruriens* are consumed by the groups Savera, Jatapu, Gadabe and Kondadora, of Vizianagaram district, Andhra Pradesh, South India. The mature dry seeds of this pulse are eaten following a special processing method of continuous boiling and draining for about eight hours till the boiled water changes from black to milky white (Plant Foods for Human Nutrition 46: 53 - 61, 1994).

For further information, please contact K. Janardhanan (email: karnam-janardhanan@usa.net).

Research on Mucuna as a Feed in Yucatan

In Mexico, a number of research projects have investigated the potential of *Mucuna* as a feed. A large part of these projects have taken place in the Yucatan Peninsula where *Mucuna* has been promoted for soil fertility improvement by several organizations. There are also a number of ongoing studies, both on monogastrics and ruminants. Three of the studies reported here are M.Sc. theses, one, a report of a development-oriented on-farm study. The M.Sc. investigations utilized diets that were balanced for nutrients and energy according to the National Research Council recommendations. L-dopa analyses were conducted in two studies only (Trejo Lizama, 1998; Ruíz Sesma, 1999) in a semi-quantitative manner, utilizing the chromatography technique of Chattopadhyay and Datta (1985).

Interesting issues are apparent from these studies:

- Some of the results are more promising than those reported on monogastrics in the April 2000 workshop. Such difference may be partly attributable to *Mucuna* pre-treatment (Duque Díaz, 1993) and the age of animals (see below). Other factors may also be at work. Research by Ruíz Sesma (1999) also sheds more light on the use $CaOH_2$ as an additive to *Mucuna*, an issue discussed in the first issue of *Mucuna News*.
- Studies by Duque Díaz (1993) and Castillo (1996) indicate that negative impacts of *Mucuna* feeds on chickens may be less if they are introduced at a later stage. This may confirm the hypothesis of N. Szabo on the severity of impacts of L-dopa on young monogastric animals, which she discussed during the April 2000 workshop. These studies should be further confirmed and the exact time determined from which chickens can feed on *Mucuna*-based feeds.

Research on Poultry

Duque Díaz (1993) compared the impact of processed *Mucuna* flour (12 hr. soaking followed by 2 hr. boiling, seed coat removal, sun drying, and grinding) incorporated at different levels in the feed of Hubbard chickens (240-300 in number). He conducted two experiments, one using chickens at the finalization phase (36-55 days) and another through the full cycle (8-55 days). During the finalization phase, there was no difference in daily weight gain or in feed intake between chickens eating feed containing 0, 10, and 20% *Mucuna*. During a complete cycle, 0, 12.5, and 25% levels were tested. The daily weight gain of chickens eating feed containing 25% *Mucuna* was 88% of those eating the control diet ($p<0.05$). At the 12.5% level, the gain was intermediate and not different from the two other treatments. Feed intake was lowest at the highest level of *Mucuna*. No differences were found among the three treatments in mortality, weight of liver or pancreas, nor in abdominal fat. These results therefore indicate that *Mucuna* processed by boiling in water may, in fact, provide a feasible feed for chickens, at least in the finalization phase.

Utilizing similar *Mucuna* processing methods, Castillo (1996) conducted three on-farm trials in 1995-96 in collaboration with rural Mayan women living in the community of Xmaben in the state of Campeche. The trials focused on substituting a part of commercial chicken feed with *Mucuna* flour for commercial Ross chickens. In addition to using different levels (11-33%) of *Mucuna*, he compared the growth response when *Mucuna* was offered at early growth phase (10 days-5 weeks, Experiment 2) in comparison to final phase (3-7 weeks, Experiment 1 or 5-7 weeks, Experiment 3). Depending on the trial, Mucuna seed was peeled either before or after cooking. The scale of the trial was limited, as each replication consisted of only one chicken; typically, there were eight replications and the trials lasted 2-4 weeks.

Also in this study, the age of chickens eating *Mucuna* flour seemingly had an impact on their weight gain. While inclusion of the processed *Mucuna* flour in the diet even at 11% was deleterious to young chickens, chickens at 5-7 weeks could seemingly tolerate a diet containing 20% (Experiment 1, seeds peeled after cooking) or 22-33% (Experiment 3, seeds peeled before cooking) of *Mucuna* flour with no negative impacts on weight gain. The weight gains of younger *Mucuna*-fed chickens were

poorest in comparison to the control group in the beginning of the experiment (Week 1) and improved later in the experiment. The report does not include feed intakes and it is not clear whether low weight gains are related to poor intake or to other factors.

Trejo Lizama (1998) conducted four experiments with commercial Hubbard chickens. Two studies were three-day acceptance studies (days 1 and 3: no *Mucuna*, day 2: *Mucuna*) of feeds containing either processed (cracked seed soaked 24 hrs and dried at 60ºC, ground with 3mm blade) or raw *Mucuna*. The experimental unit was a pen containing two chickens; this was replicated 15-16 times. Two other experiments lasted 14 d and compared the impact of feeds containing raw and processed *Mucuna* on animal performance (feed consumption, weight gain, and feed conversion).

During the acceptance trial, the higher the content of raw *Mucuna* in the feed, the lower was the intake of the feed by the chickens; chickens consumed more of the feed containing treated *Mucuna* than raw beans, but consumption of control feed was highest. During one of the 14-day feeding trials, chickens ate more feed when it included processed rather than raw *Mucuna*. Increasing the percentage of raw *Mucuna* (0, 14, 28, 42%) brought a progressively lower daily weight gain (from 60 to 28g) and feed consumption (from 100 to 79g). In another, factorial trial, there was no interaction between *Mucuna* treatment and percentage of *Mucuna* in the feed. Daily weight gain was only slightly lower (at 34.9g) with unprocessed *Mucuna* flour than with processed flour (38.6 g) but chickens consuming feed containing *Mucuna* at 42%-level had clearly lower daily weight gain (29.8 g) than those at 28% level (43.7 g). Feed consumption followed similar patterns for *Mucuna* treatments. Although chickens fed with control diet had a clearly higher daily weight gain (59.4 g), their feed consumption was only 0-22% higher than those fed feeds containing *Mucuna*, suggesting a poor feed conversion for *Mucuna*-fed chickens.

Research on Pigs

Ruíz Sesma (1999) compared the impacts of soybean-sorghum based pig feed (control) to three feeds that contained 25% *Mucuna*: 1) untreated *Mucuna* flour, 2) *Mucuna* flour produced by crushing seed, 24 hr. soaking in water, drying at 60ºC, and grinding, and 3) *Mucuna* flour derived as in 2, but the soaking was done in water containing 4% $CaOH_2$. Each of the five blocks consisted of 4 pigs with an adaptation period of 8 days before the experiment. Feeds were given *ad libitum* throughout the experimental period of 28 days.

L-dopa and total phenol content were substantially lower in the *Mucuna* flour produced after $CaOH_2$ soaking than after water-soaking or after no treatment of *Mucuna*. There was no difference in weight gain between animals eating control diet and feeds containing treated *Mucuna* flour. Feed intake was higher in animals eating treated *Mucuna* flour than animals eating the control feed; with untreated *Mucuna* flour, there was a clear depression in intake and weight gain. Feed conversion was lowest (i.e., most desirable) in the control group. Pigs fed untreated *Mucuna* had the darkest urine, followed by those fed with water-soaked *Mucuna*. Urine of pigs fed with $CaOH_2$-soaked *Mucuna* was very similar to those with control diet. Total phenol concentrations in urine were ten times higher in group fed with $CaOH_2$-soaked *Mucuna* (3.57g l^{-1}) than in the control group (0.35 g l^{-1}); in the other groups, the value was over twenty times as high.

Table 2. Performance of pigs with feeds containing *Mucuna*.

	Control	Un-treated	Water-soaked	$CaOH_2$-soaked
Intake*	2.22b (100%)	1.47c (66%)	2.43a (109%)	2.48a (112%)
Weight gain*	0.72a (100%)	0.31b (43%)	0.65a (90%)	0.62a (86%)
Feed conv.	3.06a (100%)	4.69b (153%)	3.72a (122%)	3.97ab (130%)

* kg animal^{-1} day^{-1}

Adapted from: Ruíz Sesma (1999). Means followed by different letters $p<0.05$.

Works Reviewed:

Castillo C., J.B. 1996. Uso del frijol terciopelo (*Mucuna* sp.) en la alimentacion de aves en el contexto del sistema agricola de pequeños productores (Use of velvetbean in the feeding of poultry in the context of smallholder producers' agricultural systems). Paper presented at the Fourth Meeting of the RED of the Rockefeller Foundation. Universidad Autónoma de Yucatán, Facultad de Medicina Veterinaria y Zootecnia, Unidad de Posgrado e Investigación.

Duque Díaz, A. 1993. Evaluacion del frijol terciopelo (*Stizolobium deeringianum*) en el control de malezas en citricos y como fuente de proteina en la racion de pollos de engorda. (Evaluation of velvetbean (*Stizolobium deeringianum*) in weed control in citrus and as a protein source in the fattening of chickens). M.Sc. thesis. Instituto Tecnológico Agropecuario No. 2, Conkal, Yucatan.

Ruíz Sesma, B. 1999. Evaluación de frijol terciopelo (*Stizolobium deeringianum*) sin tratar y tratado como ingrediente en dietas de cerdos (Evaluation of velvetbean (*Stizolobium deeringianum*) without and with processing as an ingredient in the diet of pigs). M.Sc. thesis. Universidad Autónoma de Yucatán, Facultad de Medicina Veterinaria y Zootecnia, Unidad de Posgrado e Investigación.

Trejo Lizama, W. 1998. Evaluación nutricional del frijol terciopelo (*Stizolobium deeringianum*) en la alimentación de pollos de engorda (Nutritional evaluation of velvetbean (*Stizolobium deeringianum*) in the fattening of chickens). M.Sc. thesis. Universidad Autónoma de Yucatán, Facultad de Medicina Veterinaria y Zootecnia, Unidad de Posgrado e Investigación.

Analyzing for L-dopa: Introduction

By Marjatta Eilittä

For investigators working on *Mucuna*'s food and feed utilization in the past decade, L-dopa analysis could have been a great obstacle, were it not for the support services of Rolf Myhrman, Judson College, who throughout the years has provided a service analyzing L-dopa for a number of investigators based both in Africa and Latin America. He converted the relatively standard facilities of a chemistry laboratory in the USA into a resource for the scientists of developing countries who otherwise would have been left without recourse to such analysis.

In the current *Mucuna* project, we are fortunate to have two scientists (R. Myhrman and N. Szabo, see *Mucuna News* no. 1), who have experience in L-dopa analysis and have committed to act as resource persons to any researchers interested in analyzing *Mucuna* samples in their own country. In addition to providing information directly to researchers, they will co-author a multi-part article describing various methods for analyzing L-dopa. In the following, N. Szabo introduces some general issues and then describes one method of extraction and one method of detection/quantitation.

Analyzing for L-dopa. Part I. General Considerations, Acidic Extraction and LC-MS/HPLC-MS

By N. Szabo, University of Florida, USA

As with any analysis, the analysis of L-dopa consists of two main steps: (1) sample preparation, including sample extraction and clean up and (2) detection for identification and/or quantitation. For sample preparation of L-dopa containing materials, special attention should be given to two issues: (1) spoilage, especially of leaf samples (and animal tissues), which can affect chemical composition, and (2) degradation of L-dopa with temperature (decomposition has been noted near its melting point of 260° C) and light (which induces oxidation to melanin). For leaf, stem, and root samples, fresh samples are preferable if they can be either analyzed immediately or stored in a freezer. Otherwise, such samples should be air-dried in a dry, clean place, or oven-dried at 60º C. For seed samples, if analysis cannot be performed relatively rapidly, we suggest air- or oven-drying.

For both extraction and detection, a number of methods exist. In the following, one method of extraction and one of analysis are presented.

Acidic Extraction Method (Brain, 1976)

The extraction method by K.R Brain is suitable to pair with most analysis methods. It is a simple technique that requires only basic skills in using a balance and in the transferal and measurement of liquids. It also has few equipment requirements, namely a balance accurate to tenths or hundredths of a milligram and a centrifuge; most other steps utilizing equipment (such as a water bath) can be easily modified to available resources. Although numerous samples may be prepared at one time, the method is somewhat labor intensive and does require several hours to complete. Also, access to suitable glassware and to solvents may be a drawback in some cases. If the essential pieces of equipment are available, cost will be dependent on technical assistance and solvent-glassware supplies in the area. This method has been successfully used for a number of years and is considered to be direct, rugged, and exhaustive.

Procedure

A. Sample Preparation

1) Seed samples:

a. Grind to pass through a 1-mm mesh (Wiley mill if available).

b. Weigh 0.5 g of ground sample into a culture tube.

2) Leaf and stem samples (dried and fresh):

a. Chop finely by hand (dried samples can be chopped or ground).

b. Weigh 0.5 g of dried sample into a culture tube or weigh 1 g of fresh sample into a large culture tube. (Adjust all solvent volumes proportionally.)

B. Extraction (Note: If available use a fume hood for the extraction process. Do not cap tubes tightly during heating and cooling steps.)

1) Add 3 mL of 0.1 N HCl to the dried samples and mix thoroughly.
2) Heat in a boiling water bath for 5 min.
3) Allow the sample to cool to room temperature.
4) Add 3 mL of ethanol to the cooled sample.
5) Shake the sample for 10 min.
6) Centrifuge the sample at 2000 rpm for 10 min.
7) Transfer the supernatant to a clean graduated vial.
8) Re-extract the residue, collecting the supernatants together.
9) Take the combined extracts to a known volume in ethanol, usually 10 or 15 mL.

Special considerations

Necessary supplies and considerations include:

1. A means of grinding or chopping the samples into small, similarly sized pieces. During an extraction of this nature, sample particles must be sufficiently small for penetration by the solvents. Particles in a narrow size range also tend to yield more reproducible results.

2. A balance accurate to tenths or hundredths of a milligram, as solvent volumes are dependent on sample weight.

3. Access to hydrochloric acid (HCl), distilled water, ethanol, appropriate glassware for transfer and measurement, a water bath, a centrifuge, and, if at all possible, a fume hood. If a fume hood is unavailable, the extraction should be carried out in a well-ventilated room with hot sample vials always pointed away from any personnel present. Protective eye ware should be worn. Heating and cooling sample vials should be loosely covered (covered to avoid contamination, loosely to avoid breaking).

4. Because L-dopa concentrations are generally reported on a dry weight basis for plant and animal tissues, % moisture of the sample will also need to be determined. Taking several grams of chopped/ground sample to constant weight in an oven at 60-80° C is sufficient to obtain this information.

Analysis by LC-MS/HPLC-MS

One of the most intensive methods available for analyzing L-dopa in complex matrices (plant or animal tissues) is High-Performance Liquid Chromatography with Mass Spectrometric detection (LC-MS, also called HPLC-MS). To my knowledge, thus far, we are the only researchers to have utilized LC-MS for the analysis of L-dopa (Szabo and Tebbett, forthcoming). The selection was based partly on a need to both confirm and quantify L-dopa and partly to answer some relatively serious questions regarding the alleged presence of other alkaloids (i.e. serotonin, bufotenine). Overall, we found LC-MS entirely suitable for our objectives.

As a method for analyzing L-dopa, LC-MS is attractive because it, unlike other analytical methods, gives both quantitative information and positive confirmation of the analyte identity. Positive confirmation is based on the retention time (essentially, the time L-dopa is retained in the instrument before reaching the detector) and on the ion fragmentation pattern generated by the mass spectrometer. The fragmentation pattern is based on the idea that all L-dopa molecules are identical in structure and that when struck with identical amounts of high energy, the molecules will shatter into identical fragments. It is these charged fragments that are detected and indicate both identity and quantity of L-dopa. Due to its selective nature, this method is also the least susceptible to background (i.e., matrix) interferences.

Unfortunately, LC-MS is also the most expensive of the current methods. A complete instrumentation system typically costs $150,000 - 200,000 US dollars with a per sample cost of $50-100 in an independent laboratory. These sample charges support sample extraction (supplies and labor), analysis, generation of results from the data, maintenance and parts for the instrument, and the salary of an appropriately trained technician/chemist, along with general overhead. Per sample charges tend to be less in a research lab receiving financial support from a university or similar institution, especially if a client is not seen as an outsider to the academic system.

Although LC-MS is an excellent technique for analysis of L-dopa from any matrix, it is certainly not the only suitable means for such assays. The next issue will cover one or two less expensive and more generally applicable methods. For those who work with such less expensive methods, LC-MS could be thought of as a recourse if they have some problem (with, for example, background interference) that does not sort out easily with their systems.

Procedure

In preparation for analysis by HPLC-MS, filter ~1 mL of extract through glass wool and a 0.45 mm PTFE syringe filter and place in an amber HPLC vial. Depending on the concentration of L-dopa in the sample, the filtered extract may require dilution in 50:50 ethanol:water prior to the addition of an internal standard (usually caffeine at 20 mg/mL). At this point, the sample is ready for analysis. Standards may range from 1 to 100 mg/mL. Instrumentation and analytical parameters used on one system in the US are given below:

HPLC System:

Instrument:	Hewlett Packard HP1100 system with autosampler, degasser, binary pump modules, and variable wavelength UV detector, Gateway 2000 desktop computer for data acquisition
Column:	Adsorbosil C18 10 µm (4.6 mm ID, 350 mm length; Serial #99121046.1; Alltech)
Column Temp:	25 °C
Mobile Phase:	15% acetonitrile in water
Flow Rate:	1 mL/min
Injection Volume:	50 µL
UV Detection:	280 nm

Mass spectrometer:

Instrument:	Finnigan LCQ Ion Trap Mass Spectrometer
Range:	100-2000 m/z full scan mode
Source:	Atmospheric Pressure Chemical Ionization (APCI)
Vaporiz. Temp:	375 °C
Discharge Curr:	5 µA
Capillary Temp:	150 °C
Acquisition Time:	15 min

Special considerations

1. The major considerations for this analytical technique are access to the equipment and the availability of skilled personnel for sample analysis, interpretation of data, and instrument maintenance/repair.
2. Assuming the above are available, this analysis method may be coupled with any exhaustive extraction, as long as the samples have been filtered prior to analysis and are in a pH range suitable for the analytical column, usually pH 4-9. (Adequate filtration of samples is necessary to avoid clogging the narrow lines and separation column of the instrument, either of which can result in expensive down time and the need for repairs. The given pH range is generally acceptable; the pH range suited for a specific analytical column will be found in the insert material from the manufacturer that accompanies the column.)

For further information, please contact N. Szabo (email: szabon@mail.vetmed.ufl.edu).

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Selected Bibliography of Mucuna

Introduction

There is relatively little literature on issues related to *Mucuna*'s food and feed uses. For many of our colleagues based in developing countries it may be hard to learn about available sources, let alone obtain literature. This is the first in a series of reference lists to be published in the *Mucuna News*. The purpose of the series is to inform researchers of available materials on the species *M. pruriens* (velvetbean); other *Mucuna* species will be not covered. In order to alert readers to new materials we will list the most recent articles first.

In this issue, we will present articles whose focus is nutritional characterization of *Mucuna* beans. Interestingly, some quite detailed work took place in the USA in the 1920's. Only a couple of these articles are listed below; if you are interested in other references from that time period, please contact M. Eilittä. Nutritional characterization is also routine in articles describing research results on *Mucuna* as a feed; those articles will be presented later when feed-related articles will be covered.

In the next issue, we will present articles that specifically focus on L-dopa and alkaloids.

If you are unable to locate any of these materials please contact M. Eilittä (meilitta@gru.net). Additionally, please inform her of any significant materials not listed here.

Part I. Nutritional Characterization of Mucuna Beans

Flores, M., M. Eilittä, R. Carsky, R. Myhrman, L. Carew, and J. Rojas (Eds.). Forthcoming. Food and Feed From *Mucuna*: Current Uses and the Way Forward. Proceedings of a workshop held in Tegucigalpa, Honduras, April 26-29, 2000. CIDICCO, Honduras. (Please watch future issues of this bulletin for further information.)

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Photo 1. A-frame utilized by L. Capo-chichi in his experiments at Auburn University, Alabama, USA. Similar frames will be utilized in the G by E study during 2001-2002. Photo *by* L. Capo-chichi.