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FUTURE AGENDA FOR *MUCUNA* RESEARCH AND PROMOTION

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SUMMARY

This chapter discusses the highlights of the recent research on *Mucuna* as a food and feed crop and outlines the future agenda based on the products of the working groups that met at the end of the workshop. Good progress has been made in recent research. The promising results in ruminant animal studies mean that efforts to promote *Mucuna* as a ruminant feed should be initiated. Processing studies have identified effective ways to reduce L-Dopa and other anti-nutritional factors and have led to improved understanding of the cooking and eating quality of *Mucuna* beans. In non-ruminant studies, wet heating of *Mucuna* beans results in good poultry growth but further work is needed to confirm its suitability to feed processing. Histological and anatomical investigations have improved our understanding of the factors that underlie some of the negative impacts seen in non-ruminants. The toxicological studies have determined that the amounts of previously reported alkaloids in *Mucuna* are not expected to pose risk to human health; in addition, no mutagenic activity was found in raw and gently roasted *Mucuna* beans. Finally, we now have a much better knowledge base in *Mucuna* taxonomy/breeding, including the impact of genotype and environment on L-Dopa production.

Much research remains to be done, however. Areas of priority work in ruminant feeding include studies that would eliminate any possibility of negative impacts of *Mucuna* consumption, even at high levels of intake; these efforts should include process-oriented work to understand the fate of L-Dopa in the ruminant system and especially accumulation in products consumed by people (i.e., milk and meat). In studies oriented towards food utilization and processing of *Mucuna*, priorities include further work on issues related to L-Dopa (including optimum particle size, solubility limits of L-Dopa at higher temperatures and in acid and alkaline media, and the possible presence and impacts of melanin in the processed *Mucuna* beans) and the bioavailability of nutrients in processed *Mucuna* beans.

Similarly, in studies oriented towards non-ruminant feeding, a priority topic would be the impact of various treatments on protein quality of the feed. In future work on the toxicity of *Mucuna* for humans, priority areas include possible allergenic impacts of *Mucuna* and the development of a definitive inventory of antinutrients in *Mucuna*. In taxonomy and genetic improvement of *Mucuna*, future work should first focus on the evaluation of a large number of *Mucuna* accessions for characteristics that have been found important in the studies to date, and on the assessment of potential breeding methods. In the future, collaborative mechanisms that enable exchange of information and experiences and the support of researchers working in different regions should be continued.

INTRODUCTION

The workshop in Mombasa demonstrated that in the past two years, good progress has been made in research aimed at increasing the potential of *Mucuna* as a food and/or feed crop. This, we believe, will help future efforts to introduce it for soil improvement in the tropical regions (Eilittä and Carsky, this volume). The results of animal studies on ruminants are so promising that the scientists present agreed that efforts to promote *Mucuna* as a ruminant feed can and should be initiated. Processing studies have found ways to reduce L-Dopa and other anti-nutritional factors and have increased understanding of the cooking and eating quality of *Mucuna* beans. Methods utilizing wet heating of *Mucuna* beans have resulted in poultry feeds that have been successfully used to replace some of the conventional protein source in poultry ratios, whereas the recent histological and anatomical investigations have improved our knowledge base of the factors that underlie some of the negative impacts seen in non-ruminants. Work on important aspects of *Mucuna* taxonomy/breeding, including determining the impact of genotype and environment on L-Dopa production, has progressed greatly.

Much research remains to be done, however. This chapter discusses both the highlights of the recent research and the future agenda, based on the products of the three working groups that met at the end of the workshop. In this chapter, recommendations are presented under the section headings: *Mucuna* as a ruminant feed, as a food, as a non-ruminant feed, genetic improvement and taxonomy of *Mucuna*, and toxicity of *Mucuna*.[1] Within each section, topics of high priority are listed first. At the end, the project's experience with collaborative modalities is reviewed and discussed. Since the state of knowledge prior to the current project has been reviewed and summarized (Flores *et al*., 2002; Eilittä *et al*., 2002), emphasis here is given on the new findings of the period 2001-2002.

MUCUNA AS A RUMINANT FEED

Overview

The nutritional analyses presented in this volume clearly indicate that *Mucuna* is a high-quality feed for ruminants. Animal studies with goats, sheep and dairy cows confirm this. Such studies include both detailed on-station work examining physiological responses as well as more adaptive on-farm efforts. Seed, husk, and foliage were found to promote weight gain and sustain milk production without the ill effects commonly seen in monogastric animals and humans. Workshop participants concluded that future work should therefore focus on development efforts, research to verify these results and to support the development efforts at a local level, and research to fill in knowledge gaps.

Research findings

Research work during 2001-2002 progressed on several fronts (discussed below): 1. Improved understanding of the feed potential of *Mucuna* foliage, grain, and husks; 2. Improved understanding of ensiling; 3. Improved understanding of ruminant feed parameters, such as fermentation kinetics, digestibility and acid detergent fiber (ADF); and 4. Increased certainty that *Mucuna* does not negatively affect the health and well-being of ruminant animals when fed to them in the long term.

1. Improved understanding of the feed potential of foliage, grain, and husks

Almost all previous research has focused on *Mucuna* seed, ignoring two other plant parts that could be used as ruminant feed, the foliage and the husk (i.e., pod pericarp). The husk constitutes 40% of the pod weight but becomes a by-product when the seed is used for food or feed. In contrast, utilization of *Mucuna* foliage is a more complex issue. Usually the foliage is incorporated into the soil or left as mulch to improve soil fertility; most studies to date have found reduced or no positive impacts on soil or main crop yield if a portion or all of the foliage is removed (Carsky *et al*., 1998). At the farm level, the farmer may, however, find the highest benefit if a portion of the foliage is used as a feed with the remaining foliage left for soil fertility improvement. Location-specific research is needed to establish such trade-offs.

Three research projects (Nyambati and Sollenberger; Muinga *et al*.; Mbuthia and Gachuiri) focused on foliage utilization. A goal of the Ph.D. work by Nyambati (Nyambati, 2002; Nyambati and Sollenberger) was to explore whether *Mucuna* could be intercropped with a maize crop towards the latter part of the rainy season and then harvested for feed during the dry season. It was evident that the quantity of *Mucuna* feed obtained was greatly reduced under these conditions. However, the upper-canopy *Mucuna* foliage produced (and available for farmers to utilize in a cut-and-carry system) was of good quality. Similarly, foliage in the studies by Muinga *et al*. and Mbuthia and Gachuiri was of good quality, with a crude protein (CP) of 16-17%. Ayala-Burgos *et al*. and Sandoval *et al*. analyzed husk and reported that its digestibility was higher than that of most tropical grasses, indicating that husks only could be fed to ruminant livestock in situations where beans are used for other purposes. Due to the low protein content of the husk, ideally a nitrogen source should be included in the feed. In situations of feed scarcity, however, it might act as a source of dry matter since its quality seems to be better than most tropical hays and straws.

2. Improved understanding of ensiling

Interestingly, two of the research projects reported here focused on ensiling *Mucuna*. Matenga *et al*. ensiled *Mucuna* seeds in plastic bags with molasses (10% v/v) and water (1 L to 10 kg). Different levels of

[1] A number of processing issues are shared between the work on food and non-ruminant feed utilization; those issues are mainly presented in the section on food.

Mucuna were included in the silage (30, 50, 70, and 100%); all mixtures ensiled well, but the 50:50 mixture had a stronger typical silage smell. Mbuthia and Gachuiri ensiled wilted and chopped *Mucuna* foliage harvested at 17 weeks. When included at 20% of total weight and with 3% w/w of molasses, the crude protein content improved from 9.7% for Napier grass only to 11.3% for Napier-*Mucuna* silage.

3. Improved understanding of ruminant feeding parameters

While prior studies had mainly focused on general parameters of interest (such as content of crude protein and various anti-nutritional factors), the research conducted during the past two years provides more detailed information on parameters of interest for *Mucuna's* utilization as a ruminant feed. As such parameters are highly dependent on the age of harvesting and the environment, as well as other factors, variability within any plant part is expected for different studies. For the foliage, neutral detergent fiber (NDF) content was 53.0, 49.7, and 32.5-37.4% in the studies by Muinga *et al*., Mbuthia and Gachuiri, and Nyambati and Sollenberger, respectively. For the seed, it was 29.6 and 40.8 in the studies by Ayala-Burgos *et al*., and Sandoval *et al*., respectively, and 59.8 and 59.9% in the husk (Ayala-Burgos *et al*. and Sandoval *et al*., respectively). The content of acid detergent fiber (ADF) in the foliage was 38.9% (Mbuthia and Gachuiri) and in the seed 8.8% (Ayala-Burgos *et al*.), and, in the husk, 37.0 and 37.5% (Ayala-Burgos *et al*. and Sandoval *et al*., respectively). *In vitro* dry matter digestibility (IVDMD) of all fractions measured was quite high, from 61.7-65.9% in foliage (Nyambati and Sollenberger), to 79.0% in husk (Sandoval *et al*.), to 98.0% in seed (Sandoval *et al*.).

4. Beneficial impacts on ruminant animals

The animal studies confirmed the high nutritional potential of *Mucuna*. In the study by Muinga *et al*., dairy cows were supplemented with 8 kg of either fresh *Mucuna* forage or *Gliricidia sepium*, a recommended supplement for the region. The total dry matter intake (7.7-8.0 kg cow^{-1} d^{-1}) and milk yield (5.2-5.5 kg cow^{-1} d^{-1}) were similar for the two groups, although *Gliricidia* intake was higher than that of *Mucuna*. Over the six-month experimental period (24 wk), no negative impacts on milk production were found. Mbuthia and Gachuiri, in feeding *Mucuna* foliage-Napier grass silage to sheep, found only trends, but no significant differences, in improved dry matter and organic matter intake with the inclusion of *Mucuna* in the silage, but clearly observed improved nitrogen balance and acceptability. Matenga *et al.* found that ensiling *Mucuna* and maize grain at a 1:1 ratio increased feed intake and decreased doe weight loss, but it did not result in more milk production. In this experiment, sheep preferred commercial dairy meal over the *Mucuna* mixtures (a finding not necessarily surprising given the high acceptability of the commercial feeds); does on this diet produced more milk (957, 614 and 624 mL day^{-1} for dairy meal and unensiled and ensiled *Mucuna*-maize diets, respectively) and lost less weight than does given the *Mucuna*-maize mixture that had not been ensiled. Kid growth was similar across treatment diets. *Mucuna*-maize supplements resulted in slightly lower milk lactose, but otherwise had no effect on milk composition. Utilization of nitrogen (N) was satisfactory in all diets.

The most extensive and diverse animal studies were conducted at the Autonomous University of the Yucatan, Mexico. At the more strategic end of research, studies by Ayala-Burgos *et al*. indicated high potential degradability of both grain (100%) and husks (94%) when fed to rumen-cannulated cows with a basal diet of forage. Similarly, Sandoval *et al*. found that *in vitro* dry matter digestibility of *Mucuna* beans (97.9%) and husks (78.96%) and total gas production were similar to those achieved with commercial feed mixtures. In the more adaptive on-farm studies, Castillo-Caamal *et al*. (a) found that growing sheep consuming *Mucuna* at 10 and 15 g kg^{-1} live weight (LW) had dry matter intake that equaled to or exceeded those consuming 300 g a^{-1} d^{-1} of commercial feed. The average daily weight gain at the highest substitution rate (60 g) was greater than that of animals on the commercial feed (44 g), and the behaviour of animals consuming *Mucuna* was similar to those consuming the concentrate. Mendoza-Castillo *et al*. also found no negative impacts and similar milk production and feed intake for goats whose Napier grass diet was supplemented with *Mucuna* or ramon (*Brosimum alicastrum*), a traditional supplement in the region. In a farmer-managed on-farm trial with sheep and goats, favourable trends were found in animal production, although variability in farmer management resulted in no significant differences (Castillo-Caamal *et al*., b). Importantly, in all the ruminant studies, the negative impacts typically seen in animal behaviour in poultry and pigs were not observed.

Future work on *Mucuna* as a ruminant feed

Diffusion efforts and supportive location-specific research

Based on the above research results, the workshop participants agreed that diffusion efforts to incorporate *Mucuna* as a ruminant feed in farming systems of the tropics should begin. Caution and a systems approach to research were recommended to avoid a reliance on uniform solutions to diverse situations. First, the results from Mexico should be confirmed in and adapted to target African countries. A number of management options may be suitable for a given location, depending on the social, physical, and economic environment and on farmer objectives, among other considerations. Location-specific solutions can be sought through adaptive research efforts. During this process, the value of *Mucuna* for soil improvement ought to be kept in mind. The following issues need to be addressed:

1. High nutrient content of seeds and husks

As described earlier, both seeds and husks can be considered good ruminant feeds and have been fed, after coarse grinding, to different ruminants without a negative impact. Harvesting of these plant parts does not sacrifice any of *Mucuna's* beneficial soil fertility impacts; instead, harvest of seed is necessary in many cropping systems to prevent *Mucuna* from becoming a weed through self re-seeding.

2. Economics of *Mucuna* feeds

Almost all studies to date have considered only biophysical and agronomic factors at the expense of economic factors. Future development efforts need to be initiated with explicit consideration of the economic factors. For example, the shadow price of *Mucuna*, i.e., its price in comparison to other alternatives, needs to be assessed to determine its potential in commercial feeds.

3. Incorporation in commercial feeds to stimulate production for sale by farmers

While many of the studies conducted to date have focused on *Mucuna's* use as a home-produced feed, the most economically attractive solution may be its utilization in commercial mixtures. Special attention needs to be paid to preventing spoilage of ground *Mucuna* seeds (frequently a concern for early 20th century workers; Lamaster and Jones, 1923) and to incorporate the husk or pericarp in these mixtures.

4. Direct grazing vs. cut-and-carry systems

While direct grazing of *Mucuna* foliage and pods may be suitable in some situations, in others, cut-and-carry systems may be more desirable. In certain environments, silage of either pods or foliage may be the recommended way to preserve the nutritional value of *Mucuna*.

5. Optimum age to harvest *Mucuna*

For any given accession, there is some variability in *Mucuna's* time to maturity due to the environment in which it is grown, including photoperiod. Varying harvest times result not only in feeds of differing quality, but affect the ability of *Mucuna* to regrow and generate additional biomass for soil fertility or feed purposes. Straightforward and simple trials focusing on different harvest dates would enable researchers and development specialists to design productive systems for a specific location.

Research to fill gaps in knowledge

Although the future research emphasis will be on diffusion, certain important lines of research that are not location-specific and diffusion-oriented should also be undertaken, to eliminate the possibility of negative impacts and to improve the efficiency and effectiveness of *Mucuna's* food utilization.

The promising research results on *Mucuna* as a ruminant feed need to be confirmed through the following studies:

1. Long-term impacts of *Mucuna* feed on ruminant performance, including neurological symptoms, and on reproduction at high levels of intake

Most studies thus far were conducted at ordinary levels of supplement intake. An exception to this has been the preliminary study of Perez-Hernandez *et al.* where four sheep were fed only with *Mucuna* for 10 days. With an average intake of 680 g d^{-1}, no negative impacts or neurological symptoms were observed. Future studies should take this work further, by feeding a larger group of animals a diet consisting of *Mucuna* only for a longer duration. Particular attention should be paid to any pathological changes in the kidney or liver, as well as in those tissues consumed by humans.

2. The fate of L-Dopa in the ruminant system

While ruminants seem to consume feed containing high levels of L-Dopa without problems, the mechanisms that enable this consumption are not understood. How much L-Dopa reaches the intestines and what is the reaction of the ruminants to this L-Dopa? This area of research is a priority since it helps to eliminate the existence of any possible negative impacts on ruminants. These studies could utilize digestibility tests done with an "artificial rumen" technique. A major constraint to studies focusing on L-Dopa in the ruminant system has been the inability to carry out L-Dopa analyses in several research locations in the African continent, and an inadequate knowledge about L-Dopa analysis in samples of animal origin. Two research projects proposed to analyze L-Dopa in milk products (Muinga *et al*., Matenga *et al*.), but at the time of writing, these analyses had not yet been realized. Without such studies, it will be impossible to confidently explain the apparent tolerance of ruminants to L-Dopa.

Research efforts will also need to focus on improving the effectiveness and efficiency of utilizing *Mucuna* as feed:

1. Best use of the pericarp/husk as a fiber source

As described by Ayala-Burgos *et al*. and Sandoval *et al*., the husk itself is a good feed. If seed is to be used for food or for other purposes, efficient means for processing and integrating the husk into ruminant ratios need to be investigated.

2. Improved efficiency of grain use

Mucuna grain is clearly a good source of protein, and could be used as a by-pass source of protein for the ruminants. Studies are needed to obtain information on the by-pass protein available from *Mucuna*. In addition, the possibility of using dry heat processing of the seed utilized for ruminant feeding should be explored, as it would decrease nitrogen solubility, allowing for an improvement in by-pass protein.

3. Particle size for cattle

Much of the recent research efforts have ground *Mucuna* beans or pods for ruminant consumption. This is in contrast to the early 20th century USA practices in which whole pods were often consumed, either in stalls or by field grazing of *Mucuna* (Eilittä and Sollenberger, 2002). It is not known whether the smaller pod size of the varieties utilized at that time was a factor that made this possible. Utilizing whole pods or only slightly crushed pods is obviously less labor- and energy-intensive. To assess its feasibility, animal acceptability and intake at various particle sizes need to be determined.

4. Efficiency of N-capture (energy-N ratios) and N-recycling

Much work remains to be done on the biological value of *Mucuna* protein and on the efficiency of bacteria to capture N under diverse energy supply scenarios. Further N-balance studies are needed to determine N in urine and faeces. To explore the issues of trade-offs among different ways of utilizing *Mucuna* foliage, further agronomic studies are needed to determine whether it is more effective to recycle N through the livestock (i.e., feeding *Mucuna* foliage and applying the manure on crops) or to apply the foliage in the soils.

5. Replacement value

When ideal N-energy ratios have been established, replacement value (i.e., equivalency) of *Mucuna* grain can be estimated either in terms of standard grains (e.g., maize or sorghum), or in terms of local grains. This would allow farmers to determine the trade-offs between *Mucuna* and other grains.

6. Incorporation of chemical data on *Mucuna* in nutritional models

During the past years, data on *Mucuna's* nutritional constitution has greatly increased and we are now in a position to initiate efforts to incorporate *Mucuna* in currently utilized nutritional models.

MUCUNA AS A FOOD

Overview

Due to the research efforts of the past two years, we now have an improved knowledge base on issues affecting *Mucuna's* potential as a food source for humans. First, the studies recounted in this volume have continued to increase our knowledge on ways to decrease L-Dopa content in beans to a level that would be acceptable for human consumption (including, e.g., fermentation and soaking). We also have an improved understanding of the factors impacting cooking and eating quality of *Mucuna* beans, including cooking time, water absorption, protein quality, and consumer acceptability. Additionally, the processing studies - whether conducted as a part of the food or non-ruminant feed studies have revealed promising processing options on which future efforts should be based. Finally, recent work has resulted in improved understanding of the traditional utilization of *Mucuna* as a food. Future work should focus on improving the understanding of the processes involved in the development of *Mucuna* foods, on the bioavailability of its nutritional components, on product development, and on furthering our understanding of traditional uses of *Mucuna* as a food.

Research findings

Review of processing methods

The many studies in this volume have greatly improved our understanding of different processing methods to effectively reduce the content of L-Dopa and other anti-nutrients, which would also be economical, and result in products that are acceptable to consumers. As discussed earlier, there is little information on the acceptability of *Mucuna* to consumers. A brief synopsis of these findings:

1. Wet heating

As shown by chemical analysis and the studies on poultry and rats, wet heating continues to be the most effective way to process *Mucuna* for human consumption (Bressani *et al.*; Wanjekeche *et al.*, Janardhanan *et al.*; Egounlety *et al.*). Although cooking times are relatively long, they can often be somewhat reduced by various additives. The accepta-bility and logistical difficulties (e.g., storage) of beans cooked by wet heating for feeds need to be examined.

2. Fermentation

In the work by Egounlety, L-Dopa content decreased during fermentation, suggesting the presence of an L-Dopa degrading enzyme. In Java, Indonesia, a common way to process *Mucuna* has been through fermentation into *tempe* (K. Hairiah, personal communication). Although minimally studied, this processing method seems promising.

3. Dry heating

Previous research (Flores *et al*., 2002; Myhrman, 2002) and studies reported in this volume (Bressani *et al*.) clearly confirm that dry heating (i.e., roasting or toasting) is not an efficient way to remove L-Dopa. However, roasting is an efficient way to reduce trypsin inhibitors. Work by Bressani *et al*. shows that even with a roasting time of 10 min, trypsin inhibitor content was reduced from 18.9 to 4.7 TUI mg^{-1}. Additional roasting up to 20 and 30 min brought levels further down to 2.6 and 1.6 TUI mg^{-1}, respectively. More-over, the net protein ratio (NPR) of beans roasted for longer times was better than that of beans that had been cooked under atmospheric pressure; however, the latter resulted in clearly better rat performance in the biological studies.

4. Germination

Germination is seemingly only of limited utility in decreasing L-Dopa, but even short, 2-day germination times decrease trypsin inhibitors significantly (Bressani *et al*.). The protein quality of the resulting beans was poor, and rats fed with them failed to gain weight.

5. Crude $\propto$-galactosidase enzyme

Janardhanan *et al.* demonstrate that crude $\propto$-galactosidase enzyme from *Cassia sericea*, a common weed, can be used to substantially reduce the quantity of oligosaccharides in *Mucuna*. *C. sericea* is a promising source of $\propto$-galactosidase due to its high content of the substance and its relatively lower cost in comparison to sources of fungal origin.

Traditional food use patterns

Work by Onweluzo and Eilittä provides us a detailed example of traditional food use patterns of *Mucuna* in southeastern Nigeria. In that region, it is clear that commonly employed processing methods are typically not sufficient to eliminate *Mucuna* of L-Dopa. Therefore other factors, such as limiting the amount of *Mucuna* consumed during a meal and the development of certain human tolerance to L-Dopa, may explain the continued use of *Mucuna* as a food. At least in this region, *Mucuna* beans are considered to be "poor people's food" and their current consumption is decreasing. These factors may indicate that *Mucuna* is less palatable than alternative foods (and, indeed, its alternative, the seed of the *egusi* melon, was preferred in these locations).

Reducing L-Dopa content

Recent work has made good progress on principles that govern effective L-Dopa extraction from *Mucuna* beans. Highlights include:

1. Importance of small particle size

Research by Teixeira, Bressani *et al*., Diallo and Berhe, and Nyirenda *et al*. has confirmed earlier studies and given more detailed data on the importance of particle size as a factor determining the rate of L-Dopa extraction in water. Teixeira *et al*. concluded that L-Dopa extraction was feasible if very small particles - 1 mm - were soaked even in room tempera-ture water. At room temperature, the target level considered safe by Teixeira *et al*. (0.1%) was reached in approximately 55 h (2.5 d) in ample water (40:1 water:bean ratio; i.e., exceeding the solubility limit of L-Dopa in water). When water temperature was increased, extraction rates increased dramatically and at 66 ^{o}C, only 3 h were required to attain the target level. For boiling water, extrapolation indicated that only 40 min would be needed. Bressani *et al*., working with beans that had been previously roasted (for 10, 20, and 30 min) and ground into flour, found a complete or almost complete (95-100%) extraction of L-Dopa with boiling water. In other components of the study by Bressani *et al*. where whole beans were used, L-Dopa extraction was 28.0-67.6%, even at long cooking times. In the work by Diallo and Berhe, *Mucuna* beans were placed in a porous bag and soaked in running water from a faucet or in a river. Rate of removal of L-Dopa from cracked seeds was faster under the faucet than in the river. The cracked seeds reached an L-Dopa level below 1.0% within 36 h in running water from a faucet and within 48 h in a flowing river. L-Dopa extraction from whole beans was much slower than that from cracked seeds, yielding final L-Dopa content of 4.2 and 3.6%, respectively, at 12 h, and of 1.60 and 0.1% at 48 h. In the study by Nyirenda *et al*., cracking seeds allowed for a faster L-Dopa extraction through different combinations of soaking and boiling. Utilizing small particle size may also prove to be less resource intensive (in water usage), as well as less labor and energy intensive. Long processing times may have been an important factor preventing larger-scale utilization of *Mucuna* as a food in some locations, as discussed by Nyirenda *et al*. for Zambia.

2. Improved understanding of the extraction process

Until now, the process of L-Dopa extraction has not been well understood, but the studies by Teixeira *et al*. and Diallo and Berhe shed light on it. Both confirm that the rate of extraction is fast at the beginning of the extraction process and then slowly diminishes. Knowledge of the extraction process will help future efforts on reducing L-Dopa content.

3. Role of additives

A number of researchers used additives in attempting to reduce cooking time and improve L-Dopa extraction. In the study by Teixeira *et al*., acidification of room temperature water (pH 3) allowed a 0.1% L-Dopa level to be attained within 8 h (instead of 55 h with plain water). Alkaline pH (pH 11) was equally effective, but the extraction water turned black, raising concerns about the presence of melanin. Wanjekeche *et al*. found only a limited positive impact of additives in their study where seeds chopped in three parts and boiled were used. Cooking in water, with Magadi soda (0.5%), citric acid (0.5%), maize cob ash, and bean stover ash solution resulted in L-Dopa levels of 4.6, 3.4, 3.9, 3.5, and 4.2%, respectively. Ukachukwu and Szabo boiled *Mucuna* beans in ordinary water or in water with additives of $Ca(OH)_2$, *trona* (a traditional lake salt in the region) or wood ash for 30-45 min, which resulted in a 64.1-66.0% reduction of L-Dopa (to 2.2-2.3%). Wood ash was judged to have the most beneficial impact on anti-nutrients and on the weight gain, feed conversion ratio (FCR) and protein efficiency ratio (PER) of broilers. Nyirenda *et al*. found that bicarbonate of soda (at 0.25 and 0.50%) did not have a constant effect on reducing L-Dopa levels.

Cooking and eating quality of Mucuna beans

An important limitation in our understanding of *Mucuna* as a food has been the almost complete lack of knowledge on the basic factors that constitute the cooking and eating quality of its beans (Bressani, 2002). Good progress has been made towards correcting this deficit, especially through the studies of Bressani *et al*. and Wanjekeche *et al*.:

1. Water absorption and cooking time

Bressani *et al*. found that the seeds had the low water absorption, 51.8-61.0%, common for beans. Absorption increased when seeds were soaked prior to cooking. Cooking times were reduced by pre-soaking from 5.8-6.1 h to 5.1-5.8 h (Bressani *et al*.) and from 6.0 h to 2.3-5.7 h (Wanjekeche *et al*.).

2. Pre-soaking

Bressani *et al*. found that the impact of pre-soaking in water was not significant and that the addition of sodium bicarbonate to the soaking solution was minimal. In contrast, in the study by Wanjekeche *et al*., soaking in Magadi soda reduced cooking time considerably. Pre-soaking also enabled the removal of seed coats relatively early in the cooking process, a beneficial effect due to the high content of fiber and phenolic compounds in the seed coats.

3. Special cooking characteristics

In studies involving boiling, Bressani *et al*. noted that cooked *Mucuna* beans lack the characteristic pastiness of cooked common beans. Instead, they break easily. Furthermore, both studies by Bressani *et al*. and Wanjekeche *et al*. identified an important drawback in *Mucuna* beans: the common occurrence of hard seeds that do not cook easily.

4. Protein quality

While previous studies (Bressani, 2002; Flores *et al*., 2002) and many studies in this volume have clearly shown that the amount of protein in *Mucuna* beans is equal to that in commonly eaten beans, the quality of the raw beans and the impact of processing on the content and quality of that protein has been insufficiently assessed (Bressani, 2002). Interestingly, in the study of Teixeira *et al*., no significant loss of protein was found in any samples assayed, even in the smallest 1-mm particle size. As a first step towards understanding and improving *Mucuna's* protein quality (a factor that would be particularly important for *Mucuna* to become a food eaten on a daily basis), Bressani *et al*. conducted a 16-d study with 22-d old rats. The results of the study showed relatively poor weight gains and net protein ratios (NPR). This happened despite relatively good true protein digestibility - often better than that of the common bean (*Phaseolus vulgaris*). Of all treatments, 48-h soaking followed by 3-h cooking gave the best results. NPR of roasted samples was good, but resulted somewhat inexplicably in poor rat performance. Germination of seeds, with or without malting, resulted in weight loss in the rats, as well as in low NPR and protein digestibility.

5. Consumer acceptability

Wanjekeche *et al*. conducted limited testing of the consumer acceptability of beans cooked in the local Magadi soda, citric acid, and maize cob ash. The samples cooked in Magadi soda were rated as high as common beans with respect to texture, and those cooked in citric acid rated high with respect to color, but in taste, all samples were clearly not as acceptable as common beans.

Future work on *Mucuna* as a food

There are some encouraging results from the processing studies, which should lay the foundation for future work in the following research areas:

1. Process development

We know that at a very small particle size and in the presence of ample water, L-Dopa can be extracted at room temperature, and that this process is far more rapid if the temperature is elevated only slightly. The following work should therefore be undertaken to understand and develop these processes further:

- **Optimum particle size:** While the finest particles allow the easiest passage of L-Dopa from the bean into the water, they may introduce negative impacts on the nutritional quality. Further nutritional evaluation and L-Dopa determination of processed beans at various particle sizes and soaking times should therefore be done.
- **Solubility limits:** The solubility of L-Dopa in water at 25 ^{o}C is known and is quite low (66 mg per 40 mL of water). If seeds containing 6% L-Dopa are to be extracted, these numbers translate into a requirement of 40 liters of water for each kilogram of seed. Thus, the extraction requires great amounts of a commodity that is scarce, especially in many areas of the tropics. Little is known about the solubility of L-Dopa in solvents at high temperatures or at different ranges of acidity and alkalinity; these issues should be explored in the future.
- **Impact of melanin:** Several studies have shown the beneficial impact of alkaline additives on L-Dopa extraction (Teixeira *et al*.; Diallo and Berhe; Ukachukwu and Szabo). This impact has not been consistent (Nyirenda *et al*.), which may partly be due to the amount of additive used. Consistently, the cooking or soaking water has darkened when bicarbonate has been added, which is presumably an indication that some L-Dopa has been converted into melanin. Melanin is a worrisome product with potentially negative health impacts (Pfutzner and Przybilla, 1997; Dollery, 1999; Hegedus, 2001; Pawalek and Murray, 1986) and its presence in the processed foods should not be taken lightly. Future work involving alkaline additives should also evaluate melanin levels both in the processed beans and in the soaking or cooking water to better understand the extent to which L-Dopa converts into melanin inside and outside of the plant tissue.
- **Possibilities of using fungi (enzymes) to reduce L-Dopa during fermentation:** Egounlety's study indicated the presence of an L-Dopa degrading enzyme during the fermentation process. Fermentation techniques are a relatively cheap and familiar processing method in many developing countries and could provide an efficient way to reduce L-Dopa content.

2. Bioavailability of nutritional components

In this project, Bressani *et al*. conducted one such evaluation. Further studies on bioavailability will need to be conducted, and should focus on the most promising processing methods. In fact, at this stage, bioavailability studies should be an integral component of future work on processing. Further studies should focus on exploring the possible negative impact of the low amount of sulfur-containing amino acids on bioavailability, and on the assessment of L-Dopa in inducing the poor weight gains observed with roasted *Mucuna*. To explore the latter, a study comparing ordinary roasted flour (with L-Dopa) and L-Dopa-free roasted flour should be conducted.

3. Product development studies

We have advanced towards formulating the principles on which the future processing of *Mucuna* beans for food should be based, e.g., utilization of small particle size and slightly elevated temperature, together with cooking methods that require little labor (such as roasting) and energy. These principles need to be incorporated in practically-oriented product development studies that utilize standardized methods.

4. Further evaluation of current food use patterns

Despite the relatively common use of *Mucuna* as a minor food crop, most knowledge of such food use patterns has been incidental. Future efforts should include further surveying of food use patterns, and should focus on elucidating the factors that seem to enable the utilization of *Mucuna* as a food in these locations. Importantly, the local processing methods should be replicated and studied to determine their impact on the food quality.

5. Economic feasibility of processing by small-scale farmer and commercial processors

Most studies to date have been conducted with home-processing of *Mucuna* beans in mind. It may be, however, that the most economical means of processing the beans is on an industrial scale. Future efforts should take into account industrial options.

6. Standardization of processing techniques

The methods used to process *Mucuna*, particularly heating techniques, need to be standardized using common equipment. In some of the studies, very different results were obtained with techniques that were seemingly similar (Ferriera *et al*., Carew *et al*.). Only through standardization, can experiments be reproduced in different laboratories.

MUCUNA AS A FEED FOR NON-RUMINANTS

Overview

Studies with poultry (Camara *et al.*; Carew *et al.*; Ferriera *et al.;* Iyayi and Taiwo; Nyirenda *et al.*; Ukachukwu and Szabo) and pigs (Boahen *et al.*) gave less encouraging results than those with ruminants. The workshop participants were able to establish a target level of L-Dopa that can be considered safe based on the studies to date. Roasted *Mucuna* beans can be included at low levels in the poultry feeds and do not result in many of the negative impacts seen in the blood chemistry and anatomy of the birds fed raw beans. The most promising results were obtained with cooked beans, but the appropriateness of this technique for feed processing needs to be assessed. Future work should focus on determining the impact of processing on the protein quality and on exploring the shelf life of the feeds.

Research findings

Highlights of the recent research include:

1. Determination of safe L-Dopa level in poultry feed

The workshop participants felt comfortable in placing a value for a safe L-Dopa level for poultry feed at 0.3-0.4%, based on animal studies conducted to date (Ferriera *et al*.; Iyayi and Taiwo; Carew *et al*.; Ukachukwu and Szabo). Future studies on poultry feed should therefore aim at achieving L-Dopa concentrations at or below this level.

2. Wet heating of *Mucuna* beans renders better poultry performance

Nyirenda *et al*. and Ukachukwu and Szabo both used wet heating of beans, with and without additives. Nyirenda *et al*. provided 50% of the crude protein of a broiler ration with *Mucuna* and found that if beans were boiled (with or without soaking, with or without presence of sodium bicarbonate) the ration induced similar weight gains, feed intake, and feed conversion ratios as the control diet. Ukachukwu and Szabo found that *Mucuna* boiled in water containing wood ash for 90 min could be included at levels of up to 30% (but levels of 7.5 and 15% gave better broiler weight gain).

3. Many, but not all, negative impacts on blood chemistry and anatomy in poultry are reversed with heating

In an earlier study, Carew *et al*. (2002) showed extensive impacts on blood chemistry and anatomy when raw *Mucuna* beans were fed to broilers. The present study, which was conducted through the pair-feeding technique that allowed the elimination of the impacts of reduced feed consumption, shows that most of these changes were either partially or wholly reversed by dry heating of the beans. However, negative impacts were found on plasma creatinine and alanine aminotransferase, as well as on the kidney and intestinal musculature.

4. Low levels of roasted *Mucuna* beans can be incorporated in broiler diets without negative impacts on performance

In the study by Iyayi and Taiwo, replacement of soybean with *Mucuna* for broilers at a 33% level (which constituted 6 and 5% of the diet in the starter and finisher phases, respectively) did not induce any negative impacts on weight gain or feed intake. Histologically, however, harmful effects on kidneys were seen even at this level ($P<0.05$). Similarly, in a study of layers, roasted beans seemed not to induce changes in the egg quality in the short term. It is possible, however, that in the long term such changes could be seen since intake of feed containing *Mucuna* was lower, which would most likely eventually cause reduced egg quality.

Future work on *Mucuna* as a non-ruminant feed

Participants recommended that future work should focus on:

1. The impact of the various treatments on protein quality in the feed

A number of additives and processing methods were used in the poultry feeding studies with varying results. As no formal assessment of the quality of the protein in these feeds was conducted, this should be the focus of future efforts. These studies should be conducted eliminating each of the limiting factors in a step-wise fashion and by utilizing cooking techniques that do not affect the amino acid balance.

2. Shelf life

Mucuna beans are typically available only at certain time of the year and the effects of storage have not been studied at all. Two studies reported in this volume mention concerns about *Mucuna* storage (Castillo-Caamal *et al*., b; Onweluzo and Eilittä). Storage problems may particularly affect processed products that have undergone wet heating and may be an issue with non-ruminant feeds due to the need to process large quantities of beans at one time. Future studies should explore the issues of shelf life of *Mucuna* pods and non-processed and processed *Mucuna* beans.

TAXONOMY AND GENETIC IMPROVEMENT OF *MUCUNA*

Overview

In the past years, knowledge of *Mucuna's* taxonomy has increased considerably, largely due to the Ph.D. project of L. Capo-chichi at Auburn University (Capo-chichi, 2002; Capo-chichi *et al*., 2001); a part of which was conducted within the currently discussed project (Capo-chichi *et al*., this volume a). This project has greatly increased our understanding of the contribution of genotype and environment to L-Dopa levels in *Mucuna* (Capo-chichi *et al*., b). The results of these studies have provided novel insights into the potential of genetic improvement to reduce *Mucuna's* antinutritional factors. Future work should both continue the evaluation of current *Mucuna* materials and initiate breeding, whether through conventional or new methods.

Research findings

Highlights of the recent research findings include:

1. Improved understanding of the taxonomy of the *Mucuna* genus

There has been a great deal of confusion regarding the status of the *Mucuna* types within the genus. Some authors have classified them as separate species within the genus, while others have considered them as subspecies or varieties of the species *Mucuna pruriens*. A major finding of the work by Capo-chichi is that the evaluated accessions (many of which are commonly utilized) can all be considered as mere varieties of the species *Mucuna pruriens* (Capo-chichi, 2002). Additionally, the agronomic evaluation in his work yields baseline performance and phenology data on these varieties. The work on molecular markers that was conducted as a part of this project yielded further information on the relatedness among *Mucuna* varieties (Capo-chichi *et al.*).

2. Improved understanding of the level of genetic diversity among *Mucuna* accessions

The study conducted by Capo-chichi (Capo-chichi *et al*., 2001), assessed the level of genetic variability, an important parameter for the success of any breeding program. In the collection analyzed, the authors found that *Mucuna* accessions exhibited an appreciable level of genetic diversity that needs to be assessed in further work. Future work should therefore encompass a larger number of accessions than efforts to date.

3. Improved understanding of the variability in nutritional characteristics among different accessions of *Mucuna pruriens*

In the current project, a number of accessions were evaluated for nutritional quality, either as the main focus of work (Janardhanan *et al*.; Gurumoorthi *et al*.; Ezeagu *et al*.) or as a part of a processing study.

4. Assessment of the role of genotype and environment on the production of L-Dopa in *Mucuna*

Prior to this project, only two limited studies had assessed the role of environment and genotype on L-Dopa production in the *Mucuna* plant. The main purpose in each case was the screening of a large number of *Mucuna* plants, with each considering genotype as equivalent to seed color, and environmental factors as the latitude where the seed was grown (due to the impact of illumination on the production of L-Dopa). Both also concluded that environment and genotype affected L-Dopa content in *Mucuna* seeds. Due to the central role of L-Dopa in limiting utilization of *Mucuna* as a food and feed, it was considered necessary to design a formal genotype-by-environment study in which all varieties were grown at all sites. Four to eight accessions were grown in numerous sites in latitudes ranging from 18°S to 30°N (Capo-chichi *et al*., this volume b). The results showed that out of the total variance, 42.6-49.1% was attributed to genotype effect, and that environment and genotype by environment effects were either small or not highly significant. Interestingly, the two accessions which consistently had the lowest L-Dopa content (Rajada and Ghana) are also early-maturing, providing a hypothesis for future work to deepen understanding of this issue.

Future work on taxonomy and genetic improvement of *Mucuna*

Prior to the initiation of breeding - whether conventional or utilizing novel methods - further evaluation of the available *Mucuna* varieties should be conducted. This work can build on the previous work conducted within this project and by Capo-chichi (2001), but should not greatly delay the efforts to genetically improve the potential of *Mucuna* as a food and feed crop. The following areas merit future work:

1. Evaluation of available *Mucuna* cultivars for multiple characteristics

Important issues include:

- **Hardness and size of the seed:** A large number of hard seeds, a negative cooking characteristic, were noticed by Bressani *et al*. and Wanjekeche *et al*. The prevalence and factors affecting this characteristic should be ascertained. Moreover, due to the importance of particle size in facilitating L-Dopa extraction, future efforts should consider favoring smaller seed size (with, e.g., higher seed number per pod so that yield would not be reduced). Smaller size seed may also be advantageous in animal feeding.
- **Yield and yield components:** Yield enhancement would undoubtedly vary widely with the morphological characteristics or the genotype, and light environment. Greater light interception during the reproductive period may be a major factor contributing to higher seed yield and should be considered in future efforts
- **Nutritional quality:** Several research projects of the past two years involved efforts to quantify the nutritional quality of different *Mucuna* cultivars (Janardhanan *et al*.; Gurumoorthi *et al*.; Bressani *et al*.; Ezeagu *et al*.). A large-scale evaluation, including all cultivated types, should take place.
- **Growth habit (dwarf versus vining type)**: This is particularly important in fitting *Mucuna* in cropping systems. While certain crops, such as maize, are well suited to intercropping with vining types, for others, the dwarf type that does not produce vines is the only suitable option.
- **Characteristics important for use as a green manure/cover crop:** Although food and feed production is the goal of the current efforts, sight should not be lost of the characteristics that are important for green manuring or cover cropping, such as biomass yield and resistance to pests and diseases. Information is already available on these characteristics from the work by Capo-chichi (2002), by IITA (Carsky *et al*., 1998; Chikoye and Ekeleme, 2001; Hauser and Nolte, 2002) and from various other agronomic trials focusing on green manure/cover crop qualities of *Mucuna*.

2. Evaluation of conventional and novel methods for improving *Mucuna* through breeding

Improved *Mucuna* varieties should have the following characteristics:

- **Lower L-Dopa in beans, similar L-Dopa in foliage and roots:** Note that current concentrations in foliage and roots are already low, and pose no problems in its utilization, and, in fact, may have a positive role by decreasing its susceptibility to pests and disease. The focus of the work should therefore be on decreasing L-Dopa content in *Mucuna* beans to a level that would not sacrifice their relative resistance against insects. Possibilities for selecting early-maturing lines (which were shown to have low L-Dopa) as a basis for future work need to be considered.
- **High production of beans:** In certain environments - or with certain genotypes - high seed yields of *Mucuna* have been reached (Gilbert, 2002). Bean yield should of course be taken into account when lines with greatest potential for breeding are chosen.
- **Good nutritional quality:** This characteristic in *Mucuna* consists at least of low content of trypsin inhibitors, phenolics, and other anti-nutrients, as well as high content of protein, fat, and minerals. Since utilized varieties of *Mucuna* seem to have a beneficial nutrient content, focus of the future work should be on decreasing content of trypsin inhibitors.
- **High production of foliage:** *Mucuna's* superiorrity as a cover crop is based on its ability to produce large amounts of foliage in diverse environments. Favoring bean production greatly at the expense of foliage production in the improvement efforts would sacrifice *Mucuna's* soil-improving abilities.
- **High biological nitrogen fixation:** There have been relatively few efforts to evaluate *Mucuna's* potential for nitrogen fixation in both on-station and on-farm conditions. Like high foliage production, this attribute is key to *Mucuna's* productivity and ability to improve soils.

3. Initiation of breeding efforts

It is clear that breeding *Mucuna* for improved nutritional quality, productivity, and acceptability can be initiated in the short term as an effort to improve its potential as a food and feed.

TOXICITY OF *MUCUNA*

Overview

Two limited studies on the topic were conducted in the past two years, and results from them were encouraging for future utilization of *Mucuna* as a food and feed. Future work should continue on a limited scale, testing the processed products for mutagenic effects and for other potentially harmful substances.

Research findings

As is frequently the case with *Mucuna*, knowledge on the content of alkaloids and other potentially harmful compounds has been limited and is often contradictory. The project included two activities - both of them small-scale - to attempt to clarify the issues. Highlights include:

1. No mutagenic activity found in *Mucuna* beans

Using Ames test, Burgess *et al*. examined ground samples from raw and gently roasted *Mucuna* seeds for the presence of mutagens. None were found to exhibit mutagens. Additionally, by gas chromatography in tandem with mass spectrometry they found no benzo[a]pyrene, a representative of the class of potentially carcinogenic compounds known as polycyclic aromatic hydrocarbons. The authors suggest further work on eliminating the possibility that in the presence of intense heat, such compounds may be formed.

2. Alkaloids found only at low levels

Szabo, building on an earlier alkaloid screening study, has quantified the levels of five related alkaloids (tryptamine, serotonin, *N,N*-dimethyltryptamine, 5-methoxy-dimethyl-tryptamine, and bufotenine) in different plant parts of *Mucuna*. Neither tryptamine nor *N,N*-dimethyltryptamine were detected (<0.50 µg g^{-1}) in any sample, but bufotenine, 5-methoxy-dimethyltryptamine, and an unknown compound close to the weight of serotonin (possibly *N*-methyltryptamine), were quantified in almost all samples. Due to their presence at very low levels (0.0001%) and their typically poor gastro-intestinal absorption, and the probability that they will extract alongside L-Dopa during processing, these compounds are not expected to pose any problem in foods and feeds made with *Mucuna*.

Future work on the toxicity of *Mucuna*

Despite these encouraging results, additional work should be conducted in the future to ensure *Mucuna's* safety as a food and feed. The following topics should be addressed:

1. Other negative health impacts

As discussed earlier, it is imperative to understand the formation and quantify the levels of melanin in processed *Mucuna* foods. Furthermore, no studies on potential allergenic impacts of *Mucuna* have been conducted and will need to be part of the future efforts.

2. Definitive inventory of antinutrients, toxic levels and appropriate remedies

Recent efforts have yielded a great deal more knowledge on the presence and levels of anti-nutrients in the *Mucuna* beans. This information should be reviewed, with a particular attention to the impact of various processing methods.

3. Mutagenic activity and levels of melanin in beans that have been roasted for long periods

The work to date included beans that had been gently roasted. A future study should assess beans with longer roasting times and possibly other processing methods that may be of concern.

4. Levels of tannins and polyphenolic compounds and impact of processing on them

Further evaluation of the content of tannins and polyphenolic compounds is needed.

MODALITIES FOR COLLABORATIVE WORK

Experiences to date

After the workshop, the participants evaluated the *Mucuna* Project as a modality of work. As discussed earlier in this volume (Eilittä and Carsky), the participants fell into two categories: those formally part of the project (i.e., project participants) and those who had received *Mucuna News* and had exchanged information with the project and its participants (i.e., project collaborators). On the whole, both groups reported very positive experiences with the project that mainly revolved around two aspects:

1. The ability to communicate with and learn from others working on similar themes, wherever they were located

Throughout the project, the online bulletin, *Mucuna News,* allowed the researchers to share their experiences and to inform each other of the work they were doing. Individual contacts among the researchers were also quite common. In fact, the project participants and collaborators felt that they were working within a "*Mucuna* Community" or "*Mucuna* Family" of researchers that was willing to share knowledge and resources.

2. The information, contacts, and assistance given by the project coordinators

The project coordinators took a hands-on approach to ensure that researchers had access to all relevant literature, had contact with other researchers who could assist them, and also to assist in solving some bottlenecks in their research projects. Particularly challenging were the efforts to try to analyze L-Dopa in several African countries.

Future work

The project participants recommended that future work would be most effective in improving *Mucuna's* utilization as a food and feed if it is continued in a coordinated fashion. The exact nature of the future project is not clear, but due to the positive experiences with the past project, it is clear that many of its elements should be incorporated in it. These efforts should include simpler methods and technical backstopping for L-Dopa testing. Analyzing for L-Dopa proved to be very difficult to do in most of the study localities despite a great deal of backstopping by R. Myhrman and N. Szabo, the two experienced chemists participating in the project, as well as facilitation work by the project coordinators. It is probably unrealistic and clearly not cost-effective to attempt to build capabilities for L-Dopa analysis in all institutions; instead, future arrangements should build on collaborative work with regional laboratories that could conduct analyses for the other researchers.

CONCLUSIONS

The investigations presented in this volume emerged from the disappointing adoption of *Mucuna* as a green manure/cover crop in tropical smallholder farms in the 1980s and 1990s. It became evident that to be adopted, *Mucuna* needs to have utilization beyond soil fertility improvement. Past successful experiences worldwide indicate that *Mucuna* has potential as a food and feed crop and have lent both inspiration and information on ways to utilize it to those involved in the recent efforts. Much progress in diverse disciplines - food and animal sciences, taxonomy and toxicology - has been made in the past two years in research aimed at making *Mucuna* a food and feed crop, some of it within the formal *Mucuna* Project, some of it by researchers outside of it. This volume summarizes the state of knowledge on these efforts as of September 2002. Clearly, more work on the subject is needed and future research efforts can build on the knowledge and recommendations presented in this volume.

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