

The World's Largest Open Access Agricultural & Applied Economics Digital Library

This document is discoverable and free to researchers across the globe due to the work of AgEcon Search.

Help ensure our sustainability.

Give to AgEcon Search

AgEcon Search http://ageconsearch.umn.edu aesearch@umn.edu

Papers downloaded from **AgEcon Search** may be used for non-commercial purposes and personal study only. No other use, including posting to another Internet site, is permitted without permission from the copyright owner (not AgEcon Search), or as allowed under the provisions of Fair Use, U.S. Copyright Act, Title 17 U.S.C.



CARIBBEAN FOOD CROPS SOCIETY

44

Forty Fourth Annual Meeting 2008

Miami, Florida, USA

Vol. XLIV – Number 2 Plenary Session and Oral Presentations



2008 Proceedings of the Caribbean Food Crops Society. 44(2):123-131. 2008

Physiological and Performance Effects on Rats Fed Detoxified Mucuna pruriens

Christiaan M. Huisden¹, Adegbola T. Adesogan¹, Veronika Butterweck², Nancy J. Szabo* ³, Jack M. Gaskin⁴, Ademola Raji¹, Lv Yongning² and Elizabeth Maxwell¹.

¹Department of Animal Sciences, IFAS, University of Florida, USA. ²Department of Pharmaceutics, University of Florida, USA. ³Center for Environmental and Human Toxicology, University of Florida, USA. ⁴Department of Infectious Diseases and Pathology, University of Florida, USA.

*Corresponding Author: Email: adesogan@ufl.edu; Fax: 1 352 392 7652

ABSTRACT.

L-Dopa (3, 4 dihydroxy-L-phenylalanine), a toxic compound in Mucuna pruriens, causes reduced feed intake, anorexia, diarrhea, vomiting, skin lesions and potential mortality when consumed by humans and monogastric livestock. Processing the bean can reduce L-Dopa in *Mucuna* beans to safe levels (< 0.4%), but few studies have examined the effects of feeding detoxified Mucuna to monogastrics. The objective of this study was to examine the effect of feeding detoxified Mucuna bean on the performance, behavior, and health of rats. Sixty Sprague-Dawley rats were randomly assigned to five treatments (n=12). Dietary treatments consisted of a commercial rat chow (CON) or diets in which 10% of a customized rat chow was replaced with either undetoxified Mucuna (MUC), or Mucuna detoxified by acetic acid extraction (pH 3), sodium hydroxide extraction (pH 11), or ensiling for 28 days (SIL). During the 14-day trial, behavior, physiological development, and signs of clinical pathology were evaluated. Necropsy revealed that MUC caused splenomegaly and monocytosis, and reduced phosphorus concentrations relative to CON. These effects were not observed in rats fed detoxified diets, which alkaline phosphatase concentrations 11-17% greater than those of MUC, but similar to those of CON. No abnormalities in behavior, performance, or physiology were observed in any of the rats on the detoxified diets. Compared to those fed CON, rats fed Mucunabased diets had similar feed intake, weight gain, and behavioral results in the open field. It can be concluded that at the 10% level of dietary inclusion, there were fewer measurable adverse effects due to feeding the detoxified Mucuna bean compared to untreated Mucuna bean.

KEYWORDS: Mucuna pruriens, detoxification, monogastric

INTRODUCTION

The major problem that has compromised the usefulness of *Mucuna pruriens* as a food source is its concentration of antinutrients. According to Szabo and Tebbett (2002) and Ukachukwu et al. (2002), 3,4-dihydroxy-L-phenylalanine (L-Dopa) is the most potent toxic compound in the Mucuna bean, which contains between 3.1 and 6.7% L-Dopa (Daxenbichler et al., 1972). Consequences of excessive L-dopa intake in humans can include nausea, vomiting, and anorexia, orthostatic hypotension resulting in dizziness, staggering, increased heart rate, and psychiatric disturbances such as nervousness, anxiety and agitation, insomnia, vivid dreams, confusion, delirium,

depression and psychotic reactions with hallucinations (Szabo and Tebbett, 2002). Symptoms of Mucuna intake in broilers and pigs include weight loss, reduced feed intake, and feed conversion efficiency (Flores et al., 2002; Del Carmen et al., 2002).

Despite the health hazards caused by L-Dopa, the Mucuna bean's high protein concentration makes it an important part of the diet in Asia, Africa, and South/mid-America. According to Ezeagu et al. (2003), Mucuna beans are not only high in protein (25-30%), but also in starch (39-41%). Adebowale et al. (2007) showed that except for methionine and cysteine, concentrations of bioavailable amino acids in Mucuna protein isolates exceeded the values for human diets recommended by the Food and Agricultural Organization (FAO), World Health Organization (WHO), United Nations (UN/ONU). Mucuna's high lysine concentration makes it a suitable supplementary protein to cerealbased diets, which are known to be lysine deficient (Adebowale, 2007). Therefore, Mucuna could be used to alleviate malnutrition in developing countries, if its L-Dopa concentration is effectively reduced (Bressani, 2002; Teixeira et al., 2003). The safety threshold is a bean L-Dopa concentration of less than 0.4% (Eilitta et al., 2003; Carew et al., 2003; Ferriera et al., 2003; Iyayi and Taiwo, 2003; Ukachukwu and Szabo, 2003). Processing techniques have been evaluated that reduce the Mucuna L-Dopa concentration to safe levels (Bressani, 2002), but few studies have examined the residual nutritional value of detoxified Mucuna bean and the effects of feeding it to monogastrics.

The objective of this study was to evaluate the effect of feeding Mucuna beans detoxified by acid or alkali extraction or ensiling on the performance, physiology and behavior of Sprague-Dawley rats.

MATERIALS AND METHODS

Mucuna Detoxification

Mucuna pruriens cv. Georgia bush, were obtained from Dr. Sharad Phatak at the University of Georgia, Tifton, GA, USA and detoxified using the following methods.

Detoxification through acid or alkali solvent extraction: Mucuna beans were ground in a Wiley mill to pass through a 1-mm screen (Arthur H. Thomas Company, Philadelphia, PA, USA). An acidic solution was brought to pH 3 by diluting 0.8 ml of a 25% (v/v) acetic acid solution in 2 L of distilled water. The alkali solution was brought to pH 11 by dissolving 0.1 g of sodium hydroxide in 2 L of distilled water. Suspensions (25 g/l) of Mucuna in the acid and alkaline solutions were shaken (Eberbach shaker, Michigan, USA) at room temperature for 8 hours, then filtered through four layers of cheesecloth and a Whatman #1 filter paper (1001-240, Fisher Scientific, Pittsburgh, PA, USA). The residue was subsequently rinsed with 1 L of distilled-deionized water and dried at 55oC to 97% DM.

Detoxification through ensiling: Mucuna beans were ground in a Wiley mill to pass through a 6-mm screen (Arthur H. Thomas Company, Philadelphia, PA, USA). Ground beans were weighed (1500 g) into individual vacuum bags (26.5 x 38.5 cm, VacLoc Vacuum Packaging Rolls, FoodSaver, Neosho, MO, USA) and 900 ml of double distilled water were added to provide sufficient moisture for fermentation. A vacuum sealer (V2220, FoodSaver, Neosho, MO, USA) was used to remove residual air from the bags and to heat seal them. Bags were placed in brown paper bags and kept in the dark at room temperature (18 to 25oC) for 28 days. The bags were inspected daily and manually

vented by pricking with a pin to remove excessive gas accumulation when necessary. Pin holes were immediately sealed with silo-tape after venting. After ensiling, the concentrations of each bag were dried at 55oC to 97% DM. All procedures were performed under conditions of limited lighting since L-Dopa is light sensitive. Upon detoxification, representative samples were analyzed for L-Dopa and nutritional value (Table 1).

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

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

WSC = water-soluble carbohydrate; NDF = neutral detergent fiber.

Chemical analysis

Ensiled and solvent-extracted residues were dried at 55 oC to 97% DM and ground to pass through a 1-mm screen in a Wiley mill (Arthur H. Thomas Company, Philadelphia, PA). Dry matter concentration was determined after drying at 60oC for 72 hours and ash was measured by combustion in a muffle furnace at 550oC overnight. Gross energy levels were determined by an adiabatic bomb calorimeter (1261 isoperibol bomb calorimeter, Parr Instrument Company, Moline, Illinois, USA), using benzoic acid as a standard. The following analyses were also conducted: NDF (Van Soest et al., 1991), EE (AOAC, 1984), WSC (MAFF, 1986), L-Dopa (Siddhuraju and Becker, 2001), CP (Noel and Hambleton, 1976), and starch (Hall, 2001).

Rat feeding study

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

Animals and Measurements: Sixty 6- to 8-week-old male Sprague-Dawley rats with an initial body weight of 200 grams were purchased from Harlan (Indianapolis, IN, USA). Rats were individually-housed in 40 x 30 x 20 cm cages and randomly assigned to the five treatments (n=12). All animals had an ad libitum water and food supply.

Performance and physiological analysis: Feed intake during the first 10 days of the trial was calculated on a daily basis. Animals were also weighed daily during the first 12 days of the trial and growth records used to determine average daily gain and total weight gain. After 14 days the rats were necropsied and the heart, liver, kidneys, spleen, and gonads were weighed. Organ weights were normalized to reflect percent of body weight.

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

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

RESULTS

Performance: Table 2 shows that dietary treatments did not affect DM intake or weight gain. However, rats fed MUC had numerically (P > 0.1) lower values than those fed other diets.

Physiology: Necropsy revealed that in all treatments the heart, liver, kidney and testicular weights remained unchanged relative to CON (Table 3). Levels of red blood cells were not different among treatments. Feeding MUC increased spleen weight (splenomegaly) and monocyte occurrence (monocytosis) relative to CON, but feeding the detoxified beans did not (Figure 1). Concentrations of alkaline phosphatase were increased by 11-17% due to feeding detoxified beans instead of MUC, but all Mucuna treatments resulted in similar alkaline phosphatase concentrations as CON. Blood phosphorus concentration was decreased by feeding MUC relative to CON (9.78 vs 10.74 mg/dl) but it was similar in rats fed CON and detoxified diets.

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

Table 2. Effects of feeding unprocessed or detoxified *Mucuna pruriens* on dry matter intake and growth of rats

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

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

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

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

DISCUSSION

Mucuna-based diets have reportedly been associated with a decrease in acceptability and intake compared to soybean based diets (Del Carmen et al., 1999; Flores et al., 2002). Feed intake and weight gain did not differ among treatments suggesting that acceptability and nutrient bioavailability of control and Mucuna-based diets were similar, though the relatively low Mucuna inclusion rate (10% of diet DM) may also be implicated. Although the detoxification methods resulted in different L-Dopa and CP concentrations, similar performance and clinical data suggest the CP bioavailability and food safety were comparable among detoxified treatment diets. Solvent extraction typically disrupts the protein structure and degrades AA in Mucuna (Adebowale et al., 2007), nevertheless, feeding BAS and ACD did not adversely affect growth and performance.

Adverse effects due to MUC consumption evidenced by splenomegaly and monocytosis were not evident when detoxified diets were fed. The splenomegaly caused by MUC agrees with studies where spleen enlargement occurred when poultry were fed Mucuna beans (Iyayi and Taiwo, 2003; Iyayi et al., 2005; Pugalenthi et al., 2005; Carew and Gernat, 2006). The spleen is the largest collection of lymphoid tissue in the body and splenomegaly resulting from feeding MUC probably reflects increased workload or hyper-function of the organ. Splenomegaly is associated with red blood cell destruction in the spleen, congestion due to portal hypertension and infiltration by leukemias and lymphomas, obstruction of blood flow or antigenic stimulation, and infection (Grover et al., 1993). Carew et al. (2003) observed lymphoid necrosis, macrophage proliferation and lympho-phagocytosis of the spleen at a 12% Mucuna inclusion in the diet of broilers. Iyayi et al. (2005) reported that lymphoid depopulation in the spleen is indicative of the degenerative effects associated with feeding raw Mucuna beans.

Relative to CON, the dietary inclusion of undetoxified Mucuna bean (MUC) also caused monocytosis, a state of excess monocytes in the peripheral blood indicative of

various disease states. Monocytes are leukocytes that replenish macrophages and dendritic cells and elicit an immune response at infection sites.

A. Alkaline phosphatase



B. Phosphorus







Figure 1. Effects of feeding detoxified *Mucuna pruriens* on blood levels of A) alkaline phosphatase; B) phosphorus; and C) monocytes. CON = control diet; MUC = untreated *Mucuna* diet; ACD = Mucuna beans detoxified by acetic acid extraction; BAS = Mucuna beans detoxified by sodium hydroxide extraction; SIL = Mucuna beans

detoxified by ensiling. Means without a common superscript letter differ (P < 0.05); error bars denote standard errors.

In the tissues, monocytes mature into different types of macrophages that are responsible for phagocytosis of foreign substances in the body. Monocytosis can indicate inflammation, stress due to disease, hyperadrenocorticism, immune-mediated disease, and malignant tumors (Meuten, 2008).

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

Alkaline phosphatases remove phosphate groups by dephosphorylation, and they are most effective in an alkaline environment (Coleman, 1992). Phosphatases are involved in signal transduction because they regulate the action of proteins to which they are attached (Steelman et al., 2008; Yi and Lindner, 2008). Feeding undetoxified Mucuna resulted in lower plasma alkaline phosphatase (hypophosphatasemia) and phosphorus concentrations relative to detoxified Mucuna treatments partly suggesting that detoxification reduced adverse effects of L-dopa on signaling.

CONCLUSION

Dietary inclusion of detoxified or undetoxified Mucuna at 10% of diet DM did not affect any performance measure. Compared to feeding CON, feeding MUC decreased blood phosphorus concentration and caused splenomegaly and monocytosis but feeding detoxified Mucuna-based diets did not have these effects. Feeding MUC also decreased alkaline phosphatase levels relative to feeding detoxified Mucuna diets. Therefore, the detoxification processes improved the safety of Mucuna. Follow up research should also focus on long term effects of feeding the detoxified diets to multiple monogastric species.

REFERENCES

- Adebowale, Y.A., Adeyemi, I.A., Oshodi, A.A., and Niranjan, K., 2007. Isolation, fractionation and characterisation of proteins from Mucuna bean. Food Chemistry 104, 287–299.
- Association of Official Analytical Chemists (AOAC), 1984. Official methods of Analysis, Fourteenth edition, Washington DC, Procedure 24.005.
- Bressani, R., 2002. Factors influencing nutritive value in food grain legumes: Mucuna compared to other grain legumes. In: B. M. Flores, M. Eilitta, R. Myhrman, L.B. Carew, R. J. Carsky (Eds), Food and Feed from Mucuna: Current Uses and the Way Forward. Proceedings of the Centro Internacional de Informacion sobre Cultivos de Cobertura (CIDICCO), Tegucigalpa, Honduras, p. 164-188.
- Carew, L.B., Hardy, D., Weis, J., Alster, F., Mischler, S.A., Gernat, A., Zakrzewska, E.I., 2003. Heating raw velvetbeans (Mucuna pruriens) reverses some antinutritional effects on organ growth, blood chemistry, and organ histology in growing chickens. Tropical and Subtropical Agroecosystems 1, 267-276.

- Carew, L.B. and Gernat, A.G., 2006. Use of velvetbeans, Mucuna spp. as a feed ingredient for poultry: a review. World's Poultry Science Journal 62,131-143.
- Coleman, J.E., 1992. Structure and mechanism of alkaline phosphatase. Annual Review of Biophysics and Biomolecular Structure 21, 441-483.
- Daxenbichler, M.E., VanEtten, C.H., Earle, F.R., Tallent, W.H., 1972. L-Dopa recovery from Mucuna seed. Journal of Agriculture and Food Chemistry, 20, 1046-1048.
- Del Carmen, J., Gernat, A.G., Myhrman, R., and Carew, L.B., 1999. Evaluation of raw and heated velvetbeans (Mucuna pruriens) as feed ingredients for broilers. Poultry Science 78, 866-872.
- Del Carmen, J., Gernat, A.G., Myhrman, R., Carew, L.B., 2002. Evaluation of raw and heated Velvetbeans (Mucuna pruriens) as feed ingredients for broilers. In: B. M. Flores, M. Eilitta, R. Myhrman, L.B. Carew and R. J. Carsky (Eds), Food and Feed from Mucuna: Current Uses and the Way Forward. Proceedings of the Centro Internacional de Informacion sobre Cultivos de Cobertura (CIDICCO), Tegucigalpa, Honduras, p. 258-271.
- Eilitta, M., Carsky, R.J., Mureithi, J., Szabo, N., Bressani, R., Myhrman, R., Sandoval, C., Muinga., R., Carew, L.B., Capo-chichi, L.J.A., Teixeira. A., 2003. Future agenda for research and promotion of Mucuna. Tropical and Subtropical Agroecosystems 1, 329-343.
- Ezeagu, I.E., Maziya-Dixon, B., Tarawali, G., 2003. Seed characteristics and nutrient and antinutrient composition of 12 Mucuna accessions from Nigeria. In Eilittä, M., Mureithi, J., Muinga, R., Sandoval, C., & Szabo, N. (Eds.) Increasing Mucuna's Potential as a Food and Feed Crop [Proceedings of an international workshop held September 23-26, 2002, in Mombasa, Kenya]. Tropical and Subtropical Agroecosystems 1, 129-140.
- Ferriera, H.A., Peňa, B.K., Gernat, A.G., Carew, L.B., Matamoros, I.A., 2003. Studies of the effect of heated, water extracted and extruded velvetbeans (Mucuna pruriens) and of methionine and lysine supplementation in diets for broilers. Tropical and Subtropical Agroecosystems 1, 277-286.
- Flores, L., Esnaola, M.A., Myhrman, R., 2002. Growth of pigs fed diets with Mucuna bean flour (Mucuna pruriens) compared to soybean meal. In: B. M. Flores, M. Eilitta, R. Myhrman, L.B. Carew and R. J. Carsky (Eds), Food and Feed from Mucuna: Current Uses and the Way Forward. Proceedings of the Centro Internacional de Informacion sobre Cultivos de Cobertura (CIDICCO), April 26-29, 2000, Tegucigalpa, Honduras, pp. 288-305.
- Grover, S.A., Barkun, A.N., Sackett, D.L., 1993. The rational clinical examination. Does this patient have splenomegaly? Journal of the American Medical Association 270, 2218-2221.
- Hall, M.B., 2001. Starch gelatinization and hydrolysis method. In: Neutral Detergent-Soluble Carbohydrates. Nutritional Relevance and Analysis, A Laboratory Manual. Bulletin 339, University of Florida, Department of Animal Sciences, Florida.
- Iyayi E.A., Taiwo V.O., and Fagbohun, A.O., 2005. Performance, carcass characteristics, aemotological and histopathological studies of broilers fed Mucuna (Mucuna utilis) bean meal based diets. Israel Journal of Veterinary Medicine 60, 2.

- Iyayi, E.A. and Taiwo, V.O., 2003. The effect of diets incorporating Mucuna (Mucuna pruriens) seed meal on the performance of laying hens and broilers. In Eilittä, M., Mureithi, J., Muinga, R., Sandoval, C., & Szabo, N. (Eds.) Increasing Mucuna's Potential as a Food and Feed Crop [Proceedings of an international workshop held September 23-26, 2002, in Mombasa, Kenya]. Tropical and Subtropical Agroecosystems 1, 239-246.
- Ministry of Agriculture, Fisheries and Food (MAFF), 1986. The analysis of agricultural materials. Reference book 427, HMSO, London.
- Meuten, D.J. 2008. Monocytosis. https://www.vetconnect.com.au/5min/data/02500251.htm# BASICS. Accessed June 2008.
- Noel, R.J. and Hambleton, L.G., 1976. Collaborative study of a semi automated method for determination of crude protein in animal feeds. Journal of the Association of Official Analytical Chemists 59, 134-140.
- Pugalenthi, M., Vadivel, V., and Siddhuraju, P., 2005. Alternative food/feed perspectives of an underutilized legume Mucuna pruriens var. utilis—a review. Journal of Plant Foods for Human Nutrition 60, 201-218.
- Siddhuraju, P. and Becker, K., 2001. Rapid reversed-phase high performance liquid chromatographic method for the quantification of L-Dopa (3,4-dihydroxyphenylalanine), non-methylated and methylated tetrahydroisoquinoline compounds from Mucuna beans. Food Chemistry 72, 389-394.
- Steelman, L.S., Abrams, S.L., Whelan, J., Bertrand, F.E., Ludwig, D.E., Bäsecke, J., Libra, M., Stivala, F., Milella, M., Tafuri, A., Lunghi, P., Bonati, A., Martelli, A.M., McCubrey, J.A., 2008. Contributions of the Raf/MEK/ERK, PI3K/PTEN/Akt/mTOR and Jak/STAT pathways to leukemia. Leukemia 22, 686– 707.
- Szabo, N.J. and Tebbett, I.R., 2002. The chemistry and toxicity of Mucuna species. In: B. M. Flores, M. Eilitta, R. Myhrman, L.B. Carew and R. J. Carsky (Eds), Food and Feed from Mucuna: Current Uses and the Way Forward. Proceedings of the Centro Internacional de Informacion sobre Cultivos de Cobertura (CIDICCO) Workshop, Tegucigalpa, Honduras, p. 120-141.
- Teixeira, A.A., Rich, E.C. and Szabo, N.J., 2003. Water extraction of L-Dopa from Mucuna bean. Tropical and Subtropical Agroecosystems 1, 159-171.
- Ukachukwu, S.N., Ezeagu, I.E., Tarawali, G., and Ikeorgu, J.E.G., 2002. Utilization of Mucuna as food and feed in West Africa. In: Food and Feed from Mucuna: Current Issues and the Way Forward. International Cover Crops Clearinghouse, Honduras, p. 189-217.
- Ukachukwu, S.N. and Szabo, N.J., 2003. Effect of processing, additives and vitamin B6 supplementation of *Mucuna pruriens* var conchinensis on broilers. Tropical and Subtropical Agroecosystems 1, 227 237.
- Van Soest, P.J., Robertson, J.B., Lewis, B.A., 1991. Methods for dietary fiber, neutral detergent fiber and non-starch polysaccharides in relation to animal nutrition. Journal of Dairy Science 74, 3568-3597.
- Yi, T. and Lindner, D., 2008. The role and target potential of protein tyrosine phosphatases in cancer. Current Oncology Reports 10, 114-21.