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**Physiological and Performance Effects on Rats Fed Detoxified *Mucuna pruriens***

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**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 *Mucuna*-based 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 cereal-based 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 55°C to 97% DM.

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

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

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

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

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

### Chemical analysis

Ensiled and solvent-extracted residues were dried at 55 °C 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 60°C for 72 hours and ash was measured by combustion in a muffle furnace at 550°C overnight. Gross energy levels were determined by an adiabatic bomb calorimeter (1261 isoperibol bomb calorimeter, Parr Instrument Company, Moline, Illinois, USA), using benzoic acid as a standard. 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.

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

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

Mean ± standard error; within a row, means without a common superscript letter differ ( $P < 0.05$ ); <sup>a</sup> control diet, standard rat chow without *Mucuna*; <sup>b</sup> untreated *Mucuna* diet; <sup>c</sup> *Mucuna* beans detoxified by acetic acid extraction; <sup>d</sup> *Mucuna* beans detoxified by sodium hydroxide extraction; <sup>e</sup> *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 <sup>A</sup>	MUC <sup>B</sup>	ACD <sup>C</sup>	BAS <sup>D</sup>	SIL <sup>E</sup>
Heart, % BW	3.84 ± 0.04	3.76 ± 0.05	3.88 ± 0.06	3.68 ± 0.05	3.71 ± 0.07
Liver, % BW	45.2 ± 0.6	44.8 ± 1.6	45.1 ± 1.1	44.2 ± 1.0	44.6 ± 0.9
Kidney, % BW	7.7 ± 0.4	7.6 ± 0.3	7.6 ± 0.2	7.2 ± 0.1	7.6 ± 0.2
Testicles, % BW	12.4 ± 0.7	13.6 ± 0.3	13.2 ± 0.2	12.9 ± 0.3	12.8 ± 0.3
Spleen, % BW	2.48 <sup>b</sup> ± 0.05	2.79 <sup>a</sup> ± 0.08	2.67 <sup>ab</sup> ± 0.05	2.54 <sup>b</sup> ± 0.06	2.64 <sup>ab</sup> ± 0.06
Red blood cells, x10 <sup>6</sup> /ul	7.4 ± 0.1	7.3 ± 0.1	7.4 ± 0.1	7.3 ± 0.1	7.1 ± 0.1

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

## DISCUSSION

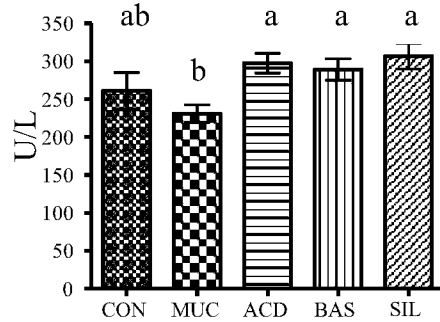
*Mucuna*-based diets have reportedly been associated with a decrease in acceptability and intake compared to soybean based diets (Del Carmen et al., 1999; Flores et al., 2002). Feed intake and weight gain did not differ among treatments suggesting that acceptability and nutrient bioavailability of control 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.

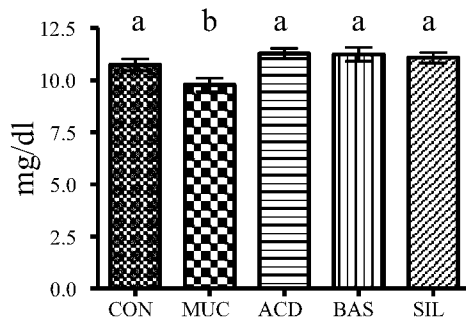
Relative to CON, the dietary inclusion of undetoxified *Mucuna* bean (MUC) also caused monocytosis, a state of excess monocytes in the peripheral blood indicative of

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



### C. Monocytes

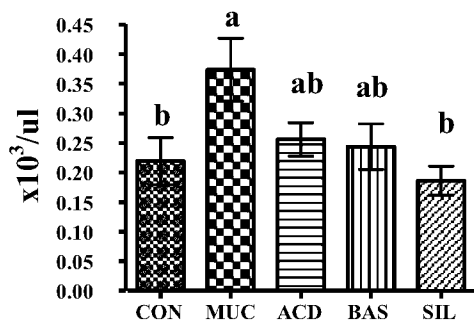


Figure 1. Effects of feeding detoxified *Mucuna pruriens* on blood levels of A) alkaline phosphatase; B) phosphorus; and C) monocytes. CON = control diet; MUC = untreated *Mucuna* diet; ACD = *Mucuna* beans detoxified by acetic acid extraction; BAS = *Mucuna* beans detoxified by sodium hydroxide extraction;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

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