



EFFECTS OF DETOXIFICATION OF *Mucuna pruriens* ON THE FEED INTAKE, BEHAVIOR, ORGAN WEIGHTS, BLOOD CELL COUNTS AND METABOLITES OF RATS[†]

[EFECTOS DE LA DESINTOXICACIÓN DE LA *Mucuna pruriens* EN EL CONSUMO, COMPORTAMIENTO, PESO DE LOS ÓRGANOS, RECUENTO DE CÉLULAS SANGUÍNEAS Y LOS METABOLITOS DE LAS RATAS]

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SUMMARY

Mucuna pruriens has been underexploited as a food source and nutraceutical due to its L-Dopa content, which is toxic. We examined the effect of detoxification methods on feed intake, behavior, and physiological parameters. Sixty Sprague-Dawley rats were randomly assigned to five treatments consisting of commercial chow or diets with undetoxified or detoxified *Mucuna*. Rats were housed in metabolic cages and feed intake was monitored. Animal behavior was video recorded using the Open field test. Upon necropsy, organ weights and blood were analyzed. Feed intake was not affected by dietary treatment. The total distance travelled in the Open field was reduced by *Mucuna* diets. Treatments did not affect most organ weights. Relative to control, feeding undetoxified *Mucuna*, suggests that detoxifying *Mucuna* prevented splenomegaly and monocytosis. Rats fed undetoxified *Mucuna* had less blood phosphorus than control, but those fed detoxified diets did not. Rats fed undetoxified *Mucuna* had less alkaline phosphatase than those fed detoxified diets and tended to be anemic. Feeding detoxified *Mucuna* prevented the splenomegaly and monocytosis associated with feeding undetoxified *Mucuna*. Therefore, detoxification improved the food safety of *Mucuna*, making it more widely usable as a source of nutrients and a nutraceutical.

Keywords: *Mucuna pruriens*; L-Dopa; Detoxification; Monogastric; Nutrition; Nutraceutical; Ethnopharmacology; Metabolism; Parkinson.

RESUMEN

Mucuna pruriens ha sido subexplotado como fuente de alimento y nutraceutico debido a su contenido de L-Dopa, que es tóxico. Se examinó el efecto de los métodos de desintoxicación sobre la ingesta de alimento, el comportamiento y los parámetros fisiológicos. Se asignaron aleatoriamente sesenta ratas Sprague-Dawley a cinco tratamientos que consistían en chow o dietas comerciales con *Mucuna* no desintoxicada o desintoxicada. Las ratas se alojaron en jaulas metabólicas y se monitorizó el consumo de alimento. Tras la necropsia, se analizaron los pesos de los órganos y la sangre. En relación con el control, la alimentación con *Mucuna* no desintoxicada sugiere que la desintoxicación de *Mucuna* previno la esplenomegalia y la monocitosis. Las ratas alimentadas con *Mucuna* sin desintoxicar tenían menos fósforo en la sangre que el control, pero las alimentadas con dietas desintoxicadas no. Las ratas alimentadas con mucuna sin desintoxicar tenían menos fosfatasa alcalina que las alimentadas con dietas desintoxicadas y tendían a ser anémicas. La alimentación detoxificada de *Mucuna* previno la esplenomegalia y la monocitosis asociadas con la

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alimentación de *Mucuna* no desintoxicada. Por lo tanto, la desintoxicación mejoró la seguridad alimentaria de *Mucuna*, haciéndola más ampliamente utilizable como fuente de nutrientes y un nutraceutico.

Palabras claves: *Mucuna pruriens*; L-Dopa; Desintoxicación; Monogástrico; Nutrición; Nutraceutico; Etnofarmacología; Metabolismo; Parkinson.

INTRODUCTION

Mucuna pruriens seeds are known for their nutritional value, and also useful in drought stricken areas (Bressani, 2002; Teixeira *et al.*, 2003); in the Ayurveda they are known as an ethnopharmaceutical and its applications in the treatment of Parkinson's disease are well known (Rai *et al.*, 2017). Huisden *et al.* (2010) reported the anthelmintic properties of the seeds. The high protein content of the seeds contributes to their nutraceutical and nutritional value (Huisden *et al.*, 2012), however, some main antinutrients in *Mucuna pruriens* have compromised its use as a food and ethnopharmaceutical source. Szabo and Tebbett (2002) and Ukachukwu *et al.* (2002) noted that 3,4-dihydroxy-L-phenylalanine (L-Dopa) is the most potent toxic compound in the *Mucuna* bean and the concentrations range between 3 and 7 g/100g in the bean (Daxenbichler *et al.*, 1972). Consequences of increased peripheral dopamine in humans can include nausea, vomiting, and anorexia, orthostatic hypotension, which results in dizziness, staggering, and increased heart rate and Dopamine Dysregulation Syndrome, which causes disabling motor and behavioral features such as pathological gambling, hypersexuality, and mood swings (Grosset, 2008). Tavares *et al.* (2017) suggest an impact on androgenic and anabolic systems. Psychiatric disturbances have also been reported in patients receiving high doses of L-Dopa in a dose-dependent manner. These can include nervousness, anxiety, agitation, insomnia, vivid dreams, confusion, delirium, depression, and psychotic reactions with hallucinations (Szabo and Tebbett, 2002).

Cotzias *et al.* (1974) reported that feeding rodents high levels of L-Dopa caused motor hyperactivity, muscle jerks, stereotyped movements, jumping, gnawing, corkscrew tails, ataxia, salivation, piloerection, red muzzle, and convulsions. Most side effects arise directly from dopamine's activity as a neurotransmitter involved in the regulation of the heart, vascular system, digestive tract, and excretory system, rather than from its well-known effect on receptors in the brain (Szabo and Tebbett, 2002). Symptoms of *Mucuna* intake in broilers and pigs include weight loss, reduced feed intake, and reduced feed conversion efficiency (Del Carmen *et al.*, 2002; Flores *et al.*, 2002). Despite the health hazards caused by L-Dopa, the *Mucuna* bean's high protein (25-30 g/100g) and starch (39-41 g/100g) concentrations (Ezeagu *et al.*, 2003) make it an important part of the diet in Asia, Africa, and South and Central America. Adebowale *et al.* (2007) showed that except for methionine and cysteine, concentrations of bioavailable

amino acids in *Mucuna* protein isolates exceeded the recommended levels for human diets by the Food and Agricultural Organization, World Health Organization, and United Nations. The high lysine concentration of *Mucuna* makes it a suitable supplementary amino acid source to cereal-based diets of livestock that are lysine deficient (Adebowale *et al.*, 2007). Therefore, *Mucuna* could be used to alleviate malnutrition in developing countries provided its L-Dopa concentration is effectively reduced (Bressani, 2002; Teixeira *et al.*, 2003).

The safety threshold is a bean concentration of less than 0.4 g/100g (Eilitta and Carsky, 2003; Ferreira *et al.*, 2003; Ukachukwu and Szabo, 2003). Processing techniques have been evaluated that reduce the *Mucuna* L-Dopa concentration to safe levels (Bressani, 2002; Mugendi *et al.*, 2010), but many of these are costly as they rely on fuel to generate heat or require copious amounts of water or are labor intensive and time consuming. Few studies have examined the residual nutritional value of detoxified *Mucuna* bean and the effects of feeding it to monogastrics. A few promising detoxification methods were identified in preliminary studies (Huisden *et al.*, 2014). Ensiling the *Mucuna* bean for 28 d reduced the L-Dopa concentration by 54%, preserved the starch and protein concentrations, and resulted in a shelf life (aerobic stability) that exceeded 657 h (Huisden *et al.*, 2014). Acid and alkali extractions for 8 h at pH 3 or 11, respectively almost eliminated (> 90% removal) *Mucuna* L-Dopa but also reduced the protein concentration by 24-31% (Huisden *et al.*, 2012). The objective of this study was to evaluate the effect of feeding detoxified *Mucuna* beans on the feed intake, behavior and blood metabolites of rats.

MATERIALS AND METHODS

Mucuna Detoxification

About 370 kg of *Mucuna pruriens*, cv. preto beans were harvested from a 10-ha field in Sao Paulo, Brazil and used for this study as well as those of Huisden *et al.* (2010; 2014; 2019). The beans contained 25 g/100g CP, 4.6 g/100g EE, 17.3 g/100g aNDF, 18.1 g/100g WSC, 38.2 g/100g starch, and 2.8 g/100g L-Dopa. The beans were ground in a Wiley mill to pass through a 1-mm screen (Arthur H. Thomas Company, Philadelphia, PA, USA) and subjected to detoxification by acid or alkaline extraction and ensiling as described by Huisden *et al.* (2014) and Huisden *et al.* (2012), respectively. Briefly, for the detoxification by ensiling, air-dry *Mucuna* beans were crushed in a roller mill (model 10004; Peerless

International, Joplin, MO, USA) and weighed into vacuum mini silo bags (26.5 × 38.5 cm; VacLoc Vacuum Packaging Rolls, FoodSaver, Neosho, MO, USA) in quadruplicate. Exactly 900 ml of double-distilled water were added to the beans in each bag to provide sufficient moisture for the fermentation. A vacuum sealer (V2220, FoodSaver, Neosho, MO, USA) was used to remove residual air and bags were sealed and kept in the dark at room temperature (18–25 °C) for 28 days.

Analysis of L-Dopa

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

Chemical component analysis

Dried samples of *Mucuna* were ground to pass through a 1-mm screen in the Wiley mill, and ash was measured by combustion in a muffle furnace at 550°C overnight. Total N was determined by rapid combustion using a macro elemental N analyzer (Elementar, vario MAX CN, Elementar Americas, Mount Laurel, NJ) and used to calculate CP (CP = N × 6.25). Neutral Detergent Fiber concentration was measured using the method of Van Soest *et al.* (1991) in an ANKOM 200 Fiber Analyzer (ANKOM Technologies, Macedon, NY). Amylase was used in the analysis and the results were expressed exclusive of residual ash. The anthrone method described by Ministry of Agriculture, Fisheries and Food (1986) was used to quantify water-soluble carbohydrates (WSC). Starch was measured by a modification (Hall, 2001) of the glucose-oxidase-peroxidase method of Holm *et al.* (1986). Ether extract (EE) was determined using the soxhlet procedure (AOAC, 1984; Method 24.005).

Gross energy was determined with an adiabatic bomb calorimeter (1261 isoperibol bomb calorimeter, Parr Instrument Company, Moline, Illinois, USA), using benzoic acid as a standard. *Mucuna* silage was made as described by Huisden *et al.* (2014) and the silage extract was obtained by blending 20 g of the ensiled bean with 200 ml of distilled water for 30s at high speed in a commercial blender (Waring model 31BL91, Dynamics Corporation of America, New Hartford, Connecticut, USA).

The mixture was filtered through two layers of cheesecloth and the pH measured with an electrode

(Accumet pH meter, model HP-71, Fischer Scientific, Pittsburg, PA, USA).

Dietary Treatments

The treatments consisted of a control diet (CON) consisting of a commercial rat chow (TD 8604 rodent diet, Harlan Teklad, Madison, WI, USA) and four *Mucuna*-based diets in which 10 g/100g of the commercial rat chow was replaced with either undetoxified *Mucuna* (MUC), or *Mucuna* beans detoxified (Huisden *et al.*, 2019) by acetic acid extraction (ACD), sodium hydroxide extraction (BAS), or ensiling (SIL). The inclusion level of the detoxified and undetoxified *Mucuna* in the diets was chosen because it had caused hepatotoxic effects on rats in a previous study (Ezeja and Omeh, 2010). The chemical composition of the rat chow and the detoxified and undetoxified *Mucuna* are shown in Table 1.

Animals and housing

Sixty 6- to 8-week-old male Sprague-Dawley rats with an initial body weight of 203±9g 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 rats were housed for 14 d at 25 ± 1°C in a 12-h light/dark cycle with *ad libitum* water and feed supply. The rats were weighed daily as change in bodyweight is a sensitive criterion for detecting toxicity in short-term rodent assays (Lyon *et al.*, 1988; Smith *et al.*, 2003). Experiments were performed according to the policies and guidelines of the Institutional Animal Care and Use Committee (IACUC) of the University of Florida, Gainesville, USA.

Open field behavior analysis

Animal behavior was evaluated on d 3 and d 10 using the open field test (Carlini *et al.*, 1986), which consisted of a round grey plastic arena measuring 70 cm in diameter surrounded by a grey plastic wall 34 cm high, lit with three 40W bulbs. The floor of the arena was divided into several concentric units with black lines dividing the arena into 19 fields. Open field activity helps determine locomotion and motor activity as well as speed (Vogel, 2002). On d 3 and 10 the 60 rats were placed one at a time at the center of the arena for 5 minutes. The open field test was videotaped using a high-resolution video camera WV-CP244 (Panasonic, Secaucus, NJ, USA). Video analysis was performed using TopScan, Top View Animal Behavior Analyzing System (version 1.00, Clever Sys Inc., Preston, VA, USA) by an unbiased and treatment-blinded technician. Due to a malfunction of the Top View analyzing system only data from six of the ten and three of the ten rats per treatment were recorded on d 3 and 10, respectively.

Table 1. Chemical composition (g/100g DM) and energy value of the Teklaad 8604 rat chow and the undetoxified (Control) and detoxified *Mucuna* beans.

Item	Rat chow	Undetoxified <i>Mucuna</i>	<i>Mucuna</i> detoxification method		
			Ensiling	Acid extraction	Alkaline extraction
Crude protein	27.0	25.4	23.28	19.3	18.1
Ash	7.4	6.0	13.4	9.0	11.3
Gross energy, MJ/kg DM	16.1	17.1	16.0	15.1	15.1
Starch	NM	38.2	38.0	45.9	46.2
Water-soluble carbohydrates	NM	18.1	4.8	3.8	3.9
Ether extract	4.6	5.5	4.8	5.9	5.6
aNDF	13.8	17.3	19.9	32.0	38.0
Lysine	1.6	1.12	1.01	1.20	1.12
Methionine	1.1	0.22	0.24	0.25	0.23
pH	NM	6.2	4.5	3.0	11
L-Dopa,	NM	2.8	1.2	0.1	0.03

CP, fat, aNDF, ash, Ca, and P concentrations (g/100g DM) of 27.0, 4.6, 13.8, 7.4, 1.6, and 1.1, respectively, metabolizable energy value of 14.0 MJ/kg DM, and lysine and methionine, concentrations of 1.4 and 0.4 g/100g CP).

Feed intake and organ weights

Rats were individually caged in a laminar flow hood. Feed intake during the first 12 d of the trial was calculated as the difference between the dry weights of the feed offered on one day and the orts remaining the following day. Rats were also weighed daily during the first 12 d of the trial and growth records used to determine total and average daily gain. On d 14 rats were necropsied and the heart, liver, kidneys, spleen, and gonads were weighed. Organ weights were normalized to reflect percent of body weight.

Blood cell counts and metabolite

At the end of the trial (d 14), blood was collected post-anesthesia through cardiac puncture and stored in vacutainer tubes containing EDTA anticoagulant (Vacurette, Greiner Bio-One NA, Inc, Monroe, NC, USA). Blood samples were analyzed at the University of Florida veterinary clinical pathology laboratory for complete blood counts (CBC) using a Bayer Advia 120 hematology analyzer (Bayer Diagnostics, Tarrytown, NY, USA) and blood chemistry profile using a Roche/Hitachi 912 Clinical Chemistry System (Roche Diagnostics, Indianapolis, IN).

Statistical analysis

Data were analyzed by one-way analysis of variance (ANOVA) with GraphPad Prism software (version 4.00,

GraphPad Software Inc., San Diego, CA, USA). The model included treatment effects. When the treatment effect was significant, ($P < 0.05$), the Student–Newman–Keul’s multiple comparison test was used to separate means. Tendencies were declared if $P > 0.05 < 0.10$.

RESULTS

Table 1 shows that ensiling, acid and alkali extraction reduced ($P < 0.05$) the L-Dopa content of the *Mucuna* bean from 2.8 g/100g in the Control to 1.2, 0.1, and 0.0 g/100g, respectively. Concomitantly, the gross energy value and crude protein and water-soluble carbohydrate concentrations decreased ($P < 0.05$) while the starch and aNDF contents increased ($P < 0.05$). Total DM intake, average daily DM intake, total weight gain, initial and final body weights, feed conversion ratio and average daily weight gain were not different among treatments ($P > 0.05$; Table 2). Dietary treatments did not affect ($P > 0.05$) distance traveled or number of line crossings in the open field test during d 3 or 10 (Figure 1). However, compared to CON, feeding *Mucuna* diets reduced ($P < 0.05$) the sum of total distance traveled and total number of line crossings on d 3 and 10. The reduction was more statistically significant ($P < 0.01$) in rats fed MUC versus those fed detoxified *Mucuna* diets ($P < 0.05$). Necropsy revealed that treatments did not affect ($P > 0.05$) liver, kidney, and testicular weights (Table 3). However, rats fed ACD tended to have greater ($P = 0.07$) heart weight (g/100g BW) than other treatments.

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

	CON ^a	MUC ^b	ACD ^c	BAS ^d	SIL ^e	SEM	P value
DM intake, g/d	20.7	19.2	20.3	20.9	20.3	0.5	0.24
Initial BW, g	206	201	204	204	205	2.8	0.74
Final BW, g	263	256	263	266	270	3.6	0.14
Total weight gain, g	57.3	55.5	59.1	62.1	64.2	4.1	0.57
Average daily gain, g/d	4.78	4.62	4.93	5.18	5.35	0.34	0.57
Feed conversion ratio, g/g	4.75	5.01	4.24	4.05	3.84	0.48	0.41

Mucuna; ^b 10 g/100g of Control diet replaced with untreated *Mucuna*; ^c 10 g/100g of Control diet replaced with *Mucuna* beans detoxified by acetic acid extraction; ^d 10 g/100g of Control diet replaced with *Mucuna* beans detoxified by sodium hydroxide extraction; ^e 10 g/100g of Control diet replaced with *Mucuna* beans detoxified by ensiling. SEM – Standard error of the mean

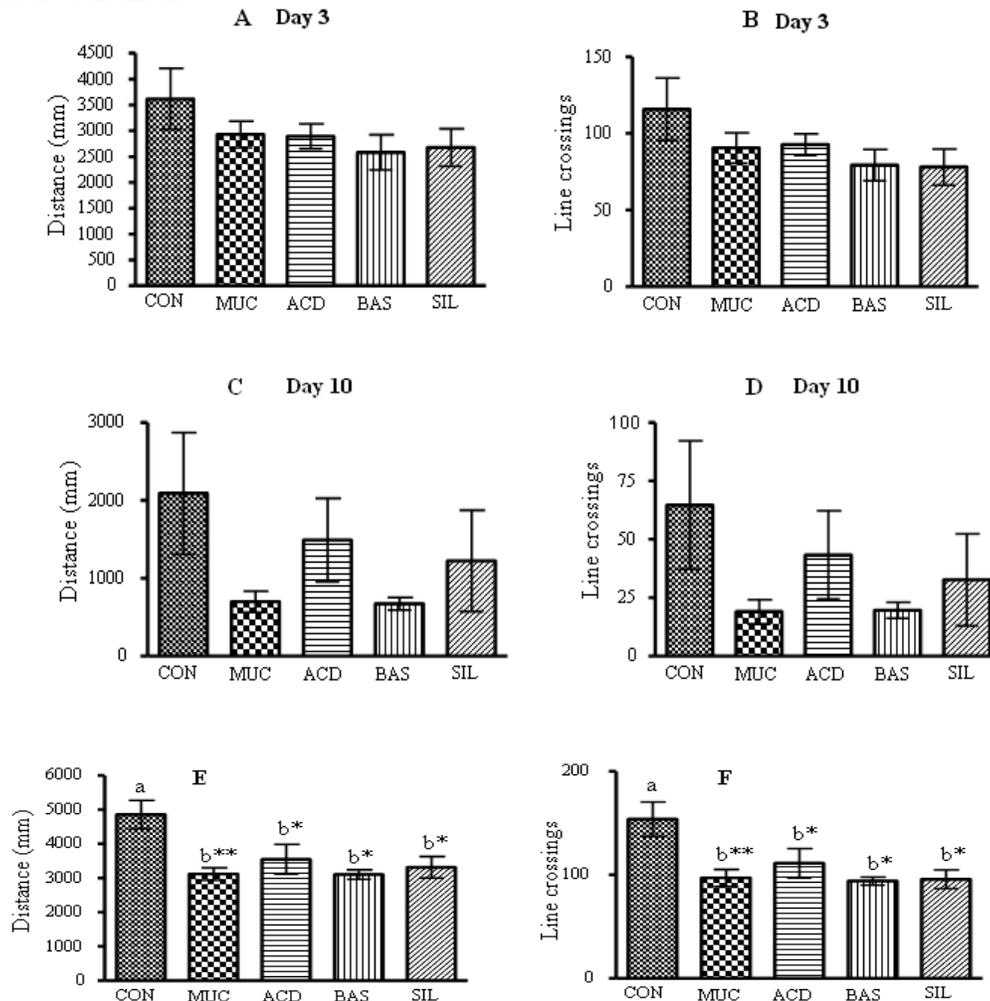


Figure 1. Effect of feeding unprocessed or detoxified *Mucuna pruriens* on A) distance traveled on d 3; B) open field line crossings on d 3; C) distance traveled on d 10; D) open field line crossings on d 10; E) total distance traveled on both days; and F) total line crossings on both days. Means without a common superscript letter differ ($P < 0.05$ *, $P < 0.01$ **). CON = control diet; MUC = untreated *Mucuna* diet; ACD = *Mucuna* beans detoxified by acetic acid extraction; BAS = *Mucuna* beans detoxified by sodium hydroxide extraction; SIL = *Mucuna* beans detoxified by ensiling. Error bars denote standard error.

Further feeding MUC increased ($P=0.01$) spleen weight (g/100g BW; splenomegaly) by 12.5% and increased ($P=0.01$) monocyte counts (monocytosis) by 68% relative to CON (Figure 2) but feeding the detoxified beans did not ($P>0.05$). Concentrations of alkaline phosphatase were increased ($P<0.05$) by 11-17% due to feeding detoxified beans instead of MUC, but all *Mucuna* treatments resulted in similar ($P>0.05$) alkaline phosphatase concentrations to CON. Blood phosphorus concentration was decreased ($P<0.05$) by feeding MUC relative to CON (9.78 vs. 10.74 mg/dl) but it was similar in rats fed CON and detoxified diets. Dietary treatments

did not affect ($P>0.05$) concentrations of total bilirubin, total protein, albumin, globulin, albumin to globulin ratio and Ca in the blood. Feeding MUC tended ($P=0.06$) to produce the greatest concentrations of mean corpuscular hemoglobin concentration (MCHC) and cellular hemoglobin concentration mean (CHCM; $P = 0.08$; Table 4). Feeding MUC, CON or BAS also produced greater ($P<0.05$) hemoglobin concentration distribution width (HDW) than feeding SIL or ACD. Counts of eosinophils tended ($P=0.07$) to be greatest in rats fed BAS followed by CON and SIL, and least in those fed ACD.

Table 3. Effects of feeding diets containing unprocessed or detoxified *Mucuna pruriens* on organ weights (g/100 g of body weight) of rats.

	CON ^a	MUC ^b	ACD ^c	BAS ^d	SIL ^e	SEM	P value
Heart	3.84	3.76	3.88	3.68	3.71	0.05	0.07
Liver	45.2	44.8	45.1	44.2	44.6	1.14	0.96
Kidney	7.7	7.6	7.6	7.2	7.6	0.30	0.79
Testicles	12.4	13.6	13.2	12.9	12.8	0.41	0.30
Spleen	2.49 ^c	2.79 ^a	2.67 ^{abc}	2.54 ^{bc}	2.64 ^{abc}	0.06	0.01

Means within a row, means without a common superscripts differ ($P<0.05$) significantly; ^a Control diet, standard rat chow without *Mucuna*; ^b 10 g/100g of Control diet replaced with untreated *Mucuna*; ^c 10 g/100g of Control diet replaced with *Mucuna* beans detoxified by acetic acid extraction; ^d 10 g/100g of Control diet replaced with *Mucuna* beans detoxified by sodium hydroxide extraction; ^e 10 g/100g of Control diet replaced with *Mucuna* beans detoxified by ensiling. SEM= Standard error of the mean

Table 4. Effects of feeding diets containing unprocessed or detoxified *Mucuna pruriens* on complete blood count data.

	CON ^a	MUC ^b	ACD ^c	BAS ^d	SIL ^e	SEM	P value
Red blood cells, $\times 10^6$ cells/ μ L	7.4	7.3	7.4	7.3	7.1	0.12	0.49
White blood cells, $\times 10^3$ cells/ μ L	7.0	9.7	7.9	7.8	7.1	0.79	0.15
Hemoglobin, Hb, g/dL	14.8	14.7	14.8	14.6	14.4	0.21	0.56
Hb distribution width, HDW, g/dL	2.5 ^a	2.5 ^a	2.4 ^b	2.5 ^a	2.4 ^b	0.03	0.04
Hematocrit, ml/100ml	42.7	41.3	42.2	42.2	41.1	0.67	0.42
Red cell volume distribution width, RDW, ml/100ml	11.6	11.5	11.4	11.6	11.5	0.1	0.45
Mean corpuscular volume, MCV, fL	57.78	56.94	56.93	57.87	57.65	0.37	0.21
Mean corpuscular hemoglobin, MCH, pg/cell	20.26	20.22	19.96	20.04	20.15	0.16	0.66
Mean corpuscular hemoglobin concentration, MCHC, g/dL	34.76	35.5	35.04	34.65	34.97	0.21	0.06
Cellular hemoglobin concentration mean, CHCM, g/dL	33.47	34.3	33.88	33.59	33.68	0.22	0.08
Cellular hemoglobin content, CH, pg	19.31	19.5	19.29	19.4	19.39	0.15	0.87
Platelet counts, $\times 10^3$ cells/ μ L	1247	1234	1235	1262	1212	57.3	0.98
Mean platelet volume, fL	8.0	7.6	7.7	7.7	8.0	0.17	0.33
Leucocytes $\times 10^3$ cells/ μ L	0.48	0.69	0.54	0.63	0.50	0.07	0.16
Neutrophils $\times 10^3$ cells/ μ L	12.2	12.8	13.0	12.3	12.9	1.28	0.99
Lymphocytes $\times 10^3$ cells/ μ L	75.5	81.5	81.9	82.3	82.2	3.35	0.57
Eosinophils $\times 10^3$ cells/ μ L	1.3	1.0	0.9	1.5	1.3	0.16	0.07
Basophils $\times 10^3$ cells/ μ L	0.3	0.2	0.3	0.3	0.3	0.04	0.61

Means within a row without a common superscripts differ ($P<0.05$); ^a Control diet, standard rat chow without *Mucuna*; ^b 10 g/100g of Control diet replaced with untreated *Mucuna*; ^c 10 g/100g of Control diet replaced with *Mucuna* beans detoxified by acetic acid extraction; ^d 10 g/100g of Control diet replaced with *Mucuna* beans detoxified by sodium hydroxide extraction; ^e 10 g/100g of Control diet replaced with *Mucuna* beans detoxified by ensiling. SEM= Standard error of the mean

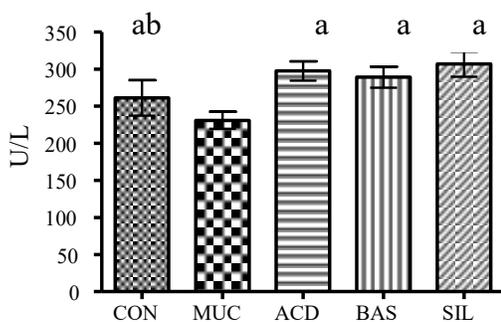
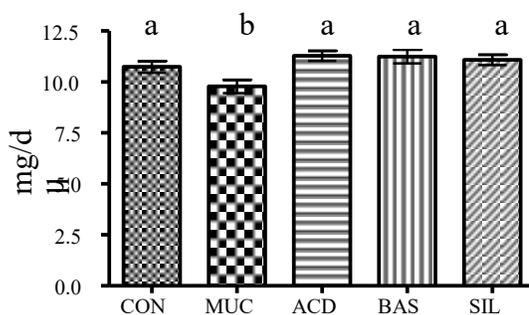
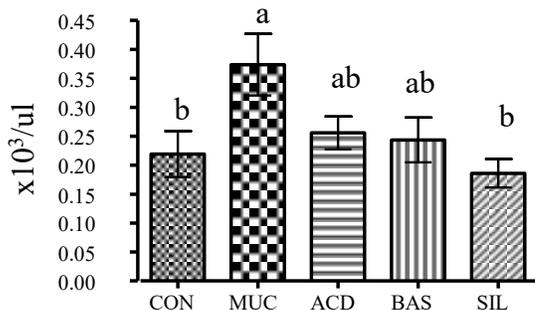
A: Alkaline phosphatase**B: Phosphorus****C: Monocytes**

Figure 2. 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.

DISCUSSION

At the 10 g/100g inclusion rate, feeding detoxified or undetoxified *Mucuna* did not affect rat behavior in the

open field on d 3 or 10. The open field is an unfamiliar environment to rats, which naturally tend to explore novel situations. A rat that is sedated or ill will travel less when exposed to a new environment such as the open field, which allows for rat locomotion and motor activity (Vogel, 2002). In contrast, an animal that is stimulated and healthy will spend more time exploring the center of the open field and will travel further. During the open field test on d 10, a software problem prevented computer analysis of data from nine out of twelve animals per treatment. Greater treatment replication may have increased the numerical trends for *Mucuna* ingestion to reduce line crossings to significant levels and for the latter effect to be less pronounced when *Mucuna* was detoxified. The fact that detoxification of *Mucuna* (Huisden *et al.*, 2019) did not affect open field behavior on d 3 or 10 may have been because of the relatively low *Mucuna* inclusion rate. Nevertheless, the sum of the total distance traveled and total line crossings on d 3 and 10 were reduced by feeding *Mucuna* diets, indicating increased abnormal behavior relative to CON, which may be related to side effects caused by *Mucuna*-derived hallucinogens (Szabo and Tebbett, 2002) or hormonal imbalance (Tavares *et al.*, 2017). Cotzias *et al.* (1974) reported that feeding rodents high levels of L-Dopa adversely affected behavior and caused motor hyperactivity, muscle jerks, and stereotyped movements. Intake of L-Dopa in humans is also associated with psychiatric disturbances, which can lead to nervousness, anxiety, agitation, insomnia, confusion, delirium, depression, and psychotic reactions with hallucinations and anorexia (Szabo and Tebbett, 2002). The greater statistical significance of the reductions in total line crossing and distance on d 3 and 10 in MUC versus detoxified diets suggests the latter may have reduced adverse effects of MUC on behavior in the open field.

The fact that feed intake, weight gain, and feed efficiency did not differ among treatments, indicates that acceptability and nutrient bioavailability of all diets were relatively similar. *Mucuna*-based diets have reportedly been associated with a decrease in acceptability and intake compared to soybean-based diets (Del Carmen *et al.*, 1999; Flores *et al.*, 2002). The relatively low inclusion rate (10 g/100g of diet DM) in this study may explain why intake and performance was not adversely affected by feeding *Mucuna*.

Although the detoxification methods resulted in different L-Dopa and CP concentrations, similar performance and clinical data in rats fed all detoxified diets suggest the CP bioavailability and food safety were comparable among detoxified *Mucuna* diets. Solvent extraction typically disrupts the protein structure and degrades amino acids in *Mucuna* (Adebowale *et al.*, 2007), yet, feeding BAS and ACD did not adversely affect performance, indicating the promise of these

detoxification strategies. However, longer term feeding trials are needed to validate their effects on the performance of rats and non-ruminant livestock.

It is interesting to note that unlike MUC, feeding detoxified diets did not result in splenomegaly and monocytosis. The splenomegaly caused by MUC agrees with studies where spleen enlargement occurred when poultry were fed *Mucuna* beans (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 hyperfunction of the organ. Splenomegaly is associated with red blood cell destruction in the spleen, congestion due to portal hypertension, 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 when *Mucuna* was fed at a 12 g/100g inclusion rate in the diet of broilers (Iyayi *et al.* 2005; Pugalenthi *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, dietary inclusion of MUC also increased the potential for monocytosis, a state of excess monocytes in the peripheral blood indicative of various disease states (Meuten, 2002). Monocytes are leukocytes that replenish macrophages and dendritic cells and elicit an immune response at infection sites. In the tissues, monocytes mature into different types of macrophages that are responsible for phagocytosis of foreign substances in the body. Monocytosis can indicate inflammation, stress due to disease, hyperadrenocorticism, immune-mediated disease, and malignant tumors (Meuten, 2002).

Interestingly, the splenomegaly, monocytosis and reduced P concentrations due to feeding MUC did not occur when detoxified diets were fed. 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 the adverse clinical conditions in rats fed MUC.

Alkaline phosphatases remove phosphate groups by dephosphorylation, and they are most effective in an alkaline environment (Coleman, 1992). Feeding MUC resulted in lower plasma alkaline phosphatase (hypophosphatasemia) and lower phosphorus concentrations relative to detoxified *Mucuna* treatments. Phosphatases are involved in signal transduction because they regulate the action of proteins to which they are attached. Therefore, detoxification of *Mucuna* may have reduced adverse effects of the bean on signal transduction; the modulating effect on transcription

factors is in agreement with the findings Ulu *et al.* (2018).

Elevated MCHC and CHCM are often associated with sickle cell anemia and other diseases (Clark, 1989). Therefore, the higher, MCHC and CHCM in rats fed MUC suggests that they were more anemic and may have had greater red blood cell deformation than Control rats. Whereas, rats fed detoxified diets had similar CHCM and MCHC to Control rats. Hemoglobin distribution width is a measure of the heterogeneity of the red cell hemoglobin concentration and abnormally higher values have been associated with iron deficiency anemia (Lee *et al.*, 1994) and steady state sickle cell anemia (Billet *et al.*, 1988). The HDW values were only reduced by SIL and ACD, suggesting that these treatments were more effective at preventing anemia and red blood cell deformities; this agrees with findings of Mohamed *et al.* (2018).

CONCLUSIONS

Dietary inclusion of detoxified or undetoxified *Mucuna* at 10 g/100g 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* diets did not have these effects. Feeding MUC also decreased alkaline phosphatase levels relative to feeding detoxified *Mucuna* diets. The behavior of rats fed *Mucuna* diets instead of CON was characterized by decreased total distance travelled and decreased total line crossings in the open field, but the effect was more statistically significant for the undetoxified *Mucuna* diet. This suggests that detoxifying *Mucuna* reduced the severity of the abnormal behavior caused by feeding *Mucuna*. Therefore, the detoxification processes improved the safety of *Mucuna* by reducing the toxic effects of the L-Dopa it contained, making it more widely usable as a safe food source and a nutraceutical.

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