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Mucuna pruriens detoxification: Effects of ensiling duration and particle size

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ABSTRACT

Mucuna pruriens is grown for food and feed despite its L-3,4-dihydroxyphenylalanine (L-dopa) concentration (30–70 g/kg), which is toxic to non-ruminants. This study determined if ensiling could reduce the L-dopa concentration of *Mucuna*. Experiment 1 examined the effect of ensiling duration on the fermentation characteristics and L-dopa concentration of *Mucuna*. Crushed (6 mm) *Mucuna* beans (912 g DM/kg) were ensiled in triplicate for 0, 3, 7, 14, 21, and 28 days in vacuum-sealed plastic bags. During the fermentation, pH decreased, whereas concentrations of ammonia-nitrogen, lactate, isobutyrate, and isovalerate increased ($P<0.05$) non-linearly. The ammonia-nitrogen concentration remained below 100 g/kg of total N throughout the ensiling period but lactate concentration was 25.7 g/kg DM by d 28. A pH of 4.5 and an L-dopa concentration of 13 g/kg (54% reduction) were achieved after 28 days of ensiling. Experiment 2 examined effects of particle size of *Mucuna* on the L-dopa concentration and nutritional value after ensiling. Crushed *Mucuna* beans (6 mm, Coarse) were used intact or ground in a Wiley mill to pass through a 4-mm (Medium) or 2-mm (Fine) screen. Samples (1500 g) of each particle size were weighed into vacuum plastic bags in quadruplicate. Double-distilled water (900 ml) was added to each bag and the bags were sealed and ensiled for 28 days. Ensiling *Mucuna* with particle sizes of 2, 4 and 6 mm for 28 days decreased the L-dopa concentration from 28 to 12, 16, and 11 g/kg, representing decreases of 57, 42 and 64%, respectively. Ensiling also reduced ($P<0.05$) the water-soluble carbohydrate, lysine and arginine concentrations and increased ($P<0.05$) concentrations of ammonia-nitrogen and most amino acids. Coarse particles had greater gross energy values than Medium or Fine particles and the greatest or among the greatest ($P<0.05$) concentrations of all amino acids. Concentrations of amylase-neutral detergent fiber, starch, crude protein and fat were unaffected by treatment ($P>0.05$). Therefore, ensiling coarse *Mucuna* particles for 28 days is recommended to reduce the L-dopa concentration and preserve the nutritional value.

Abbreviations: aNDF, amylase neutral-detergent fiber; CP, crude protein; DM, dry matter; EE, ether extract; L-Dopa, L-3,4-dihydroxyphenylalanine; NH_3N, ammonia-nitrogen; WSC, water-soluble carbohydrates; VFA, volatile fatty acids; mm, millimeter.

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1. Introduction

Mucuna pruriens is a legume indigenous to Asia that grows in many tropical regions. The beans have high concentrations of crude protein (CP; 240–380 g/kg) and starch (390–410 g/kg) (Ezeagu et al., 2003; Adebowale et al., 2005), consequently various species are grown as a food or feed crop despite the presence of the toxic component, L-3,4-dihydroxyphenylalanine (L-dopa) in the bean. Szabo and Tebbett (2002) reported L-dopa concentrations ranging from 44.7 to 53.9 g/kg in the bean, but wider ranges have been reported (31–67 g/kg; Daxenbichler et al., 1972). Although ruminants are not adversely affected by ingestion of *Mucuna* (Eilitta et al., 2003; Matenga et al., 2003; Chikagwa-Malunga et al., 2008) numerous publications report its toxic effects on non-ruminants (Carew et al., 2002; Flores et al., 2002). Some consequences of excess L-dopa ingestion or increased peripheral dopamine in humans include orthostatic hypotension resulting in dizziness and in some cases staggering, increased heart rate, nausea, vomiting, and anorexia (Szabo and Tebbett, 2002). Safe levels of L-dopa in non-ruminant livestock diets are considered to be 4 g/kg or less (Carew et al., 2002; Eilitta et al., 2002; Ferriera et al., 2003; Ukachukwu and Szabo, 2003).

Processing techniques can reduce the L-dopa concentration of *Mucuna* beans to a safe level (Huisden, 2008; Mugendi et al., 2010). Processing methods that utilize heat decrease the L-dopa concentration to or near 10%; permeation of the bean with hot water removes over 75% of the water-soluble L-dopa, and boiling eliminates almost all (>99%) of the L-dopa (Szabo and Tebbett, 2002). Extraction rates increase with increasing water temperature, allowing safe levels to be reached within 13 h at 40 °C, 3 h at 66 °C, and 40 min in boiling water (Teixeira and Rich, 2003). However, these methods are inappropriate for many developing countries (Gilbert, 2002) because heating fuel is expensive and copious amounts of water are required. Acid-solvent extraction can be just as effective as boiling water extraction (Myhrman, 2002). For instance, Teixeira et al. (2003) used a concentrated (17.4 N) acetic acid solution at pH 3 to obtain successful L-dopa extraction. However, such methods depend on availability of corrosive and harmful acids.

Little is known about strategic use of ensiling or fermenting as an L-dopa detoxification method. *Mucuna* has been boiled and fermented to produce food products such as tempe (Egounlety, 2003), and mixtures of *Mucuna* and corn have also been ensiled (Matenga et al., 2003). In both instances, the digestibility of the bean increased after fermentation but effects on L-dopa concentration were not measured. Matenga et al. (2003) ensiled various mixtures of *Mucuna* and corn grain for 21 days and reported that the L-dopa concentration was reduced by 10% for a *Mucuna* sample and by 47% for a 0.3:0.7 *Mucuna*–corn mixture. The decrease in L-dopa concentration due to *Mucuna* fermentation might be a useful strategy for making *Mucuna* safe for feeding to non-ruminants. However, the ensiling duration that is required for fermentation of *Mucuna* alone has not been determined and little is known about the ideal particle size for ensiling and preserving the nutritive value of ensiled *Mucuna*.

This study had two objectives. The first one was to determine the effect of ensiling duration on the fermentation characteristics and L-dopa concentration of *Mucuna*. Previous studies showed that removal of L-dopa from *Mucuna* beans depends on the particle size (Teixeira et al., 2003), therefore, a second objective was to study the effect of particle size on L-dopa concentration, nutritive value and fermentation characteristics of ensiled *Mucuna*. This study is the first in a series of studies (Huisden, 2008) aimed at evaluating the efficacy of *Mucuna* detoxification methods and their effects on the nutritive value of the detoxified bean and the performance of rats fed undetoxified and detoxified beans.

2. Materials and methods

About 370 kg of *M. pruriens*, cv. preto beans were harvested from a 10 ha field in Sao Paolo, Brazil, air-dried, bagged, and stored in an enclosed barn prior to use for this study and the sheep deworming study of Huisden et al. (2010). The beans contained 250 g/kg crude protein (CP), 46 g/kg ether extract (EE), 173 g/kg amylase-neutral detergent fiber (aNDF), 181 g/kg water-soluble carbohydrates (WSC), 382 g/kg starch, and 28 g/kg L-dopa.

2.1. Effects of ensiling duration (Experiment 1)

In the first of two experiments, air-dry *Mucuna* beans that had been stored in a paper bag were crushed in a roller mill (model 10004; Peerless International, Joplin, MO, USA), placed in plastic bags within a brown paper bag and mixed thoroughly. Approximately 1500-g sub-samples were weighed into individual vacuum mini silo bags (26.5 × 38.5 cm; VacLoc Vacuum Packaging Rolls, FoodSaver, Neosho, MO, USA) in quadruplicate. To provide sufficient moisture for the fermentation, 900 ml of double-distilled water were added to the beans in each bag. A vacuum sealer (V2220, FoodSaver, Neosho, MO, USA) was used to remove residual air from the bags. Individual mini-silos were placed in brown paper bags and kept in the dark at room temperature (18–25 °C) for up to 28 days. The dark ensiling conditions were used to prevent degradation of L-dopa. The mini-silos were inspected daily and manually vented by pricking with a pin to remove excessive gas accumulation when necessary to prevent rupture of the bags. Pin holes were immediately sealed with silo-tape after venting. Four bags containing *Mucuna* were opened after 0, 3, 7, 21, and 28 days. The unensiled and ensiled samples were analyzed for dry matter (DM), pH, and concentrations of volatile fatty acids (VFA), lactic acid, CP, and NH_3-N. A pH of 4.6 or lower was taken to indicate adequate fermentation because this pH represents the typical minimum value for ensiled forage (Heinrichs and Ishler, 2000) and grain (González et al., 2012) legumes. Samples with a pH of 4.6 or lower were also tested for L-dopa.