

***Mucuna pruriens* seeds given in broiler diets on growth performance and carcass yield**

Semillas tratada de *Mucuna pruriens* en dietas para pollos de engorda sobre el comportamiento productivo y rendimiento en canal

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ABSTRACT. The effects of *Mucuna pruriens* (MP) seeds soaked in water (WMP), acetic acid (AAMP) or calcium hydroxide (CHMP) solution + boiling on both its L-Dopa content and the productive performance of broiler chickens were evaluated. The experiment included a control without MP (C). The CHMP treatment reduced 71% of L-Dopa in comparison to AAMP (53%) or WMP (41%). In the starter phase, the AAMP group had the lowest live weight gain ($p < 0.05$) and the highest feed conversion ratio than C ($p < 0.05$). Broilers fed with CHMP and the C did not differ ($p > 0.05$). In the finisher phase, there was not statistical difference in any of the variables evaluated ($p > 0.05$). Differences were not observed in carcass yield ($p > 0.05$), but the broilers fed with WMP or AAMP had a heavier liver than birds on C group ($p < 0.05$). Results showed that 25% of CHMP can be used as ingredient in broiler diets.

Key words: Feed intake, L-Dopa, poultry, velvet bean, weight gain.

RESUMEN. Se evaluó el efecto de remojar semillas de *Mucuna pruriens* (MP) en soluciones con agua (AMP), ácido acético (AAMP) o hidróxido de calcio (HCMP) + hervido sobre su contenido de L-Dopa y el rendimiento productivo de pollos de engorda. El experimento incluyó un control sin MP (C). El tratamiento HCMP disminuyó 71% la L-Dopa comparado con AAMP (53%) o AMP (41%). En la fase inicial, el grupo AAMP tuvo menos ganancia de peso ($p < 0.05$) y mayor conversión alimenticia que el C ($p < 0.05$). El rendimiento productivo de los pollos alimentados con HCMP y C fueron similares ($p > 0.05$). En la fase final, el rendimiento productivo fue igual ($p > 0.05$). El rendimiento de canal fue similar ($p > 0.05$), pero los pollos alimentadas con AMP o AAMP presentaron un hígado más pesado que los pollos del grupo C ($p < 0.05$). Los resultados indicaron que 25% de HCMP podría incluirse como ingrediente en el alimento para pollos de engorda.

Palabras clave: Aves de corral, L-Dopa, frijol terciopelo, ganancia de peso.

INTRODUCTION

In developing countries, there is a large dependence on grains and oilseeds as the main source of protein for livestock. The high demand for these energy and protein sources causes a constant increment of grain prices. This situation has led to the search for the use of under-utilized tropical legume seeds in animal feeding. The ecological conditions prevailing in the tropical regions allow the growth of a large variety of species with nutritional potential. Legumes are a good source of crude protein (CP,

Siddhuraju and Becker 2005), amino acids (Mugendi *et al.* 2010), and minerals (Arrivalagan *et al.* 2014). *Mucuna pruriens* (L) DC. Var (Wight) Burck called Velvet bean is originally from tropical Asia (Huisden *et al.* 2014) and has high CP content, carbohydrates and acceptable levels of fiber for animal feeding (Safwat *et al.* 2015). This legume can be an alternative to animal nutrition. However, it contains a variety of toxic secondary metabolites such as phytochemicals, trypsin inhibitors, L-3,4-Dihydroxyphenylalanine (L-Dopa), cyanogenic glycosides, tannins, oxalates, saponins and lectins have been identified in *M.*

pruriens (Iyayi *et al.* 2008), which reduce digestibility of the diet (Bath and Karin 2009).

The heat labile characteristic of some antinutritional factors reported for *M. pruriens*, such as cyanogenic glycosides (Acamovic and Brooker 2005), trypsin inhibitors, tannins (Siddhuraju and Becker 2001) and phenols (Preet and Punia 2000), allows their elimination with thermic treatments (Del Carmen *et al.* 1999, Iyayi *et al.* 2008). L-Dopa in *M. pruriens* is not heat labile and solubilization is one way to eliminate it (Josephineand and Janardhanan 1992). Thus, soaking of this seed with calcium hydroxide (Safwat *et al.* 2015), water (Tuleun and Igba 2008) sodium bicarbonate, citric acid and tamarind extract reduces this metabolite (Siddhuraju and Becker 2001). Inclusion of 40% of MP in the starter phase and 60% in finishing phase have been reported as an adequate percentage for poultry diets (Vadivel *et al.* 2011). In the same way, the inclusion of boiled MP is viable up to 60% resulting in an improvement in productive performance (Tuleun *et al.* 2009), however when raw MP is toxic to birds (Tuleun and Igba 2008). Thus, although MP contains several secondary metabolites, some methods have been reported to be effective to eliminate most L-Dopa and other toxic secondary metabolites from the seeds, which allows its utilization in broiler diets without compromising their productive performance. Therefore, the objective of this study was to evaluate the effect of soaked *Mucuna pruriens* in tap water, acid or alkaline solution + boiling on its L-Dopa content and on the growth performance of broilers and carcass yield.

MATERIALS AND METHODS

Study area

The study was carried out at the poultry production facility of the Faculty of Veterinary Medicine and Animal Science, University of Yucatan, Merida, Yucatan, Mexico. Animals were kept in a poultry house with an ambient temperature between 22 - 24 °C (CONAGUA 2016).

Processing methods and experimental diets

The treatments used to eliminate L-Dopa from

MP seeds were: a) soaked seeds in water; b) soaked seeds in acetic acid solution (200 ml L⁻¹; pH = 3.0) and c) soaked seeds in calcium hydroxide solution (40 g L⁻¹, pH = 12.3). Each batch was soaked during 24 h in plastic pots, after the seeds were rinsed with tap water until the solution residues were eliminated. The soaked seeds were boiled for 1 h in a metal buckets in a gas stove, and were rinsed again with water, finally the boiled seeds were dried in an electric oven for 48 h. Then, seeds were ground in a hammer mill (Azteca®) (particle size between one to two mm) and stored at room temperature (22 - 25 °C) in a plastic container until diets elaboration. Four diets were formulated: A) Control without MP (C); B) 25% inclusion of soaked MP in tap water + boiling (WMP); C) 25% inclusion of soaked MP in acetic acid solution + boiling (AAMP); D) 25% inclusion of soaked MP in calcium hydroxide solution + boiling (CHMP).

Mucuna pruriens and diets composition

Chemical composition of treated MP seeds and experimental diets were determined according to AOAC (2000). The composition of treated MP seeds are shown in Table 1, and the diets used during starter and finishing phases are shown in Table 2 and 3. To determine L-Dopa content the treated MP seeds were extracted with a solvent mixture made up with acetonitrile, water and formic acid (50:50:1, respectively), the extracts obtained were analyzed using a Smartline UV Detector 2500 Knauer HPLC with a Hypersil Gold HILIC column according to Takashi *et al* (2011).

Experimental animals

During the experiment, 96 sexed 21 days-old Hubbard FLEX® chicks were used. The birds were divided into two blocks with 48 birds each (first block between August - September and the second block between September - October). There were 24 replicates per treatment. Similar number of males and females were assigned to each treatment. The animals were handled individually in metal cages (40 x 40 x 40 cm), when starting the trial the broiler chickens were identified and weighed. The starter phase was between week four and five of age and the finishing phase comprised week six to seven. The animals had

Table 1. Chemical composition (%) of treated *Mucuna pruriens* seeds.

Treatment	Dry matter	Crude protein	Ether extract	Crude fiber	Ash
Soaked in Water	92.01	21.54	2.75	7.99	2.15
Soaked in Acid	91.76	22.80	2.90	7.91	2.19
Soaked in Alkali	92.99	22.48	1.88	7.92	5.38

Table 2. Composition (%) and proximal analyses of experimental diets containing treated *Mucuna pruriens* seeds during the starter phase.

Ingredient	Control	WMP	AAMP	CHMP
Maize	61.0	46.5	47.7	47.4
<i>Mucuna pruriens</i> seeds	-	25.0	25.0	25.0
Soybean meal	34.6	23.4	22.3	22.6
Calcium carbonate	1.5	1.6	1.6	1.6
Calcium orthophosphate	1.1	1.3	1.3	1.3
Vegetable oil	1.2	1.3	1.2	1.2
Methionine 99%	0.1	0.3	0.3	0.3
Lysine 99%	-	0.2	0.3	0.3
NaCl	0.3	0.3	0.3	0.3
Minerals premix ¹	0.1	0.1	0.1	0.1
Vitamins premix ²	0.1	0.1	0.1	0.1
Total	100.0	100.0	100.0	100.0
Determined analysis				
Dry matter	85.3	89.4	89.0	89.0
Crude protein	21.4	21.4	21.4	21.4
Crude fiber	0.9	2.1	2.7	2.4
Ether extract	1.6	2.4	3.3	3.0
Ash	6.1	4.7	3.5	4.4
Calculated analysis				
Metabolizable energy	3.0	3.0	3.0	3.0
Calcium	0.8	0.8	0.8	0.8
Available phosphorus	0.4	0.4	0.4	0.4
Sodium	0.1	0.1	0.1	0.1
Arginine	0.9	1.0	0.9	0.9
Lysine	1.0	1.0	1.0	1.0
Methionine	0.5	0.5	0.5	0.5
Meth+Cys	0.8	0.8	0.8	0.8
Threonine	0.6	0.6	0.6	0.6
Tryptophan	0.2	0.2	0.2	0.2

¹Minerals premix: manganese, 65 mg; iodine, 1 mg; iron, 55 mg, copper, 6 mg; zinc, 55 mg; selenium, 0.3 mg. ²Vitamins premix: vitamin A, 8,000 UI; vitamin D, 2,500 UI; vitamin E, 8 UI; vitamin K, 2 mg; vitamin B12, 0.002 mg; riboflavin, 5.5 mg; pantothenate of calcium, 13 mg; niacin, 36 mg; choline, 500 mg; folic acid, 0.5 mg; thiamine, 1 mg; pyridoxine, 2.2 mg; biotin, 0.05 mg. WMP: seeds soaked in water + boiling, AAMP: seeds soaked in acid solution + boiling and CHMP: seeds soaked in alkaline solution + boiling.

free access to water and were fed *ad libitum*.

Growth performance

Live weight gain (LWG) and feed intake (FI) were registered weekly. The FI was determined by difference between the offered and the consumed food, the feed conversion ratio (FCR) was calculated as the ratio between feed intake and live weight gain. At the end of the rearing periods, all birds were slaughtered

Table 3. Composition (%) and proximal analyses of experimental diets containing treated *Mucuna pruriens* seeds during the finishing phase.

Ingredient	Control	Water	Acid	Alkaline
Corn grain	69.0	54.6	55.8	55.5
<i>Mucuna pruriens</i> seeds	-	25.0	25.0	25.0
Soybean meal	26.9	15.5	14.4	14.7
Calcium carbonate	1.4	1.5	1.5	1.5
Calcium orthophosphate	1.1	1.2	1.2	1.2
Vegetable oil	1.0	1.1	0.9	1.0
Methionine 99%	0.2	0.3	0.3	0.3
Lysine 99%	0.1	0.4	0.4	0.4
Minerals premix ¹	0.1	0.1	0.1	0.1
Vitamins premix ²	0.1	0.1	0.1	0.1
NaCl	0.3	0.3	0.3	0.3
Total	100.0	100.0	100.0	100.0
Determined analysis				
Dry matter	87.8	89.0	89.1	89.0
Crude protein	19.6	19.6	19.6	19.6
Crude fiber	1.2	2.6	2.8	2.5
Ether extract	2.2	2.8	2.8	2.4
Ash	4.9	4.7	4.2	5.2
Calculated analysis				
Metabolizable energy	3.1	3.1	3.1	3.1
Calcium	0.8	0.8	0.8	0.8
Available phosphorus	0.4	0.4	0.4	0.4
Sodium	0.1	0.1	0.1	0.1
Arginine	0.7	0.7	0.7	0.7
Lysine	1.0	1.0	1.0	1.0
Methionine	0.5	0.5	0.5	0.5
Meth+Cys	0.7	0.7	0.7	0.7
Threonine	0.5	0.5	0.5	0.5
Tryptophan	0.2	0.2	0.2	0.2

¹Minerals premix: manganese, 65 mg; iodine, 1 mg; iron, 55 mg, copper, 6 mg; zinc, 55 mg; selenium, 0.3 mg. ²Vitamins premix: vitamin A, 8,000 UI; vitamin D, 2,500 UI; vitamin E, 8 UI; vitamin K, 2 mg; vitamin B12, 0.002 mg; riboflavin, 5.5 mg; pantothenate of calcium, 13 mg; niacin, 36 mg; choline, 500 mg; folic acid, 0.5 mg; thiamine, 1 mg; pyridoxine, 2.2 mg; biotin, 0.05 mg.

to determine carcass yield (CY) (carcass without drumsticks and head), in addition the liver weight (LW) was recorded.

Statistical analysis

The collected data were analyzed using the General Linear Model (GLM of SAS) using a randomized complete block design (SAS 2010). The sex of the broilers chickens was included as fixed

effect in the statistical model. Least-Squares Means were compared using the PDIF option of SAS when significant effects ($p < 0.05$) between treatments were detected.

RESULTS AND DISCUSSION

In this study it was found that raw seeds contained 2.0% of L-Dopa (Table 4). This value was lower than that reported by others studies (3.5 - 5.7%) (Dahouda *et al.* 2009, Cassani *et al.* 2016). The L-Dopa content in MP has been associated to seed colors, black varieties have a higher amount of this secondary compound, whereas white varieties contain a lower percentage and mottled varieties have intermediate values (Gurumoorthi *et al.* 2003). Mixed seeds were used in the current study, with predominance of white seeds. Probably, for this reason L Dopa content was lower in the current study compared to earlier studies. CHMP treatment reduced 71.0% of L-Dopa content (Table 4). This result is similar to previous reports where soaked MP in sodium bicarbonate (pH = 8.5) reduced L-Dopa 68.0% (Gurumoorthi *et al.* 2003). Similarly, Ukachukwu and Szabo (2003) when soaked MP in calcium hydroxide achieved 63.0% elimination of L-Dopa relative to raw seeds.

Table 4. Quantification of L-Dopa in treated *Mucuna pruriens* seeds.

Sample	Content (g kg ⁻¹)
Raw seeds	20.0
Seeds soaked in water + boiling	11.8
Seeds soaked in acid solution + boiling	9.3
Seeds soaked in alkaline solution + boiling	5.8

The reduction of L-Dopa content was lower in AAMP and WMP treatment (Table 4). These values are comparable to earlier reports. Siddhuraju and Becker (2001) found a reduction of 54.0% with tamarind extract and 40.0% when citric acid was used, while in other reports a reduction of only 27.0% was reached when MP was soaked in water (Diallo and Berhe 2003). The chemical structure of L-Dopa allows its elimination easier in an alkaline medium (Siddhuraju and Becker 2001). In the current study, CHMP treatment (pH = 12.3) eliminated L-Dopa in a greater proportion than the other treatments and

would be the recommended treatment for MP for inclusion in broiler diets.

The inclusion of 25.0% of treated MP in diets for broiler chickens did not affect FI ($p > 0.05$). This inclusion level is higher than those recommended in other studies, where MP was processed with different treatments. Tuleun and Igba (2008) recommend an inclusion of up to 20.0% of soaked seeds + boiling. Iyayi *et al.* (2008) suggest 15.0% with MP soaked in water and Akinmutimi and Okwu (2006) suggested up to 10.0% inclusion of heated MP. Young birds are more susceptible to L-Dopa because the ingestion of this compound causes a reduction of animal growth (Del carmen *et al.* 1999, Gurumoorthi *et al.* 2008). In the current study, broilers in the AAMP group had the lowest LWG and higher FCR compared to C ($p < 0.05$). Nevertheless, although the broilers from AAMP treatment had the lowest daily live weight gain, this was superior to the 41.8 g day⁻¹ reported by Tuleun *et al.* (2011).

When the three groups offered MP are compared, no significant differences were found for LWG and FCR ($p > 0.05$), however broilers growth in the CHMP treatment was similar to C ($p > 0.05$). These results suggest that CHMP treatment improved MP nutritive value. According to Tuleun and Patrick (2007), soaking and boiling MP seeds increases the digestibility of protein and carbohydrates compared to raw seeds and when soaking is realized in an alkaline solution it is further improves due to higher elimination of L-Dopa, thus reducing its negative effect on birds (Siddhuraju and Becker 2001).

During the finishing phase, there were not statistical differences ($p > 0.05$) in FI, LWG and FCR between treatments (Table 6). These results indicate that up to 25.0% of treated MP could be used in broiler diets without affecting the performance. This level of inclusion is higher to that recommended by Vadivel and Pugalenti (2010) with soaked MP in a sodium bicarbonate solution + autoclaving (11%). Others have suggested up to 10.0% of heated MP without negative effects (Del Carmen *et al.* 1999) or up to 6.0% of roasted MP (Emenalom and Udedibie 1998). Other authors have suggested levels not higher than 20.0% when using soaked MP in water + boiling,

Table 5. Productive performance of broiler chicks fed diets containing *Mucuna pruriens* seeds treated with water, an acid solution and an alkaline solution during the starter phase. Values are least square means values \pm standard deviation.

Treatments	Initial live weight (kg)	Feed intake (kg)	Live weight gain (kg)	Feed conversion ratio
Control	0.71 \pm 0.080 ^a	1.82 \pm 0.215 ^a	1.03 \pm 0.129 ^a	1.78 \pm 0.143 ^b
WMP	0.73 \pm 0.088 ^a	1.76 \pm 0.170 ^a	0.95 \pm 0.095 ^{ab}	1.86 \pm 0.148 ^{ab}
AAMP	0.70 \pm 0.089 ^a	1.76 \pm 0.249 ^a	0.94 \pm 0.149 ^b	1.90 \pm 0.289 ^a
CHMP	0.75 \pm 0.060 ^a	1.80 \pm 0.222 ^a	0.98 \pm 0.132 ^{ab}	1.83 \pm 0.101 ^{ab}

Means within the same column with different superscript differ ($p < 0.05$). WMP: seeds soaked in water + boiling, AAMP: seeds soaked in acid solution + boiling and CHMP: seeds soaked in alkaline solution + boiling.

Table 6. Productive performance during the finishing phase and carcass yield of broiler chicks fed diets containing *Mucuna pruriens* seeds treated with water, an acid solution and an alkaline solution. (Least square means values \pm standard deviation).

Treatments	Feed intake (kg)	Liveweight gain (kg)	Feed conversion Ratio	Carcass yield (%)	Liver weight (g)	Liver (g) / carcass (kg)
Control	2.3 \pm 0.3 ^a	1.0 \pm 0.1 ^a	2.3 \pm 0.2 ^a	77.1 \pm 3.5 ^a	47.7 \pm 7.2 ^a	22.4 \pm 3.4 ^b
WMP	2.4 \pm 0.4 ^a	0.9 \pm 0.2 ^a	2.4 \pm 0.2 ^a	76.3 \pm 2.9 ^a	49.3 \pm 10.1 ^a	24.6 \pm 4.5 ^a
AAMP	2.4 \pm 0.3 ^a	1.0 \pm 0.2 ^a	2.4 \pm 0.3 ^a	75.6 \pm 3.3 ^a	50.1 \pm 10.5 ^a	25.1 \pm 3.9 ^a
CHMP	2.4 \pm 0.3 ^a	1.0 \pm 0.2 ^a	2.4 \pm 0.2 ^a	76.4 \pm 3.2 ^a	50.9 \pm 6.4 ^a	24.4 \pm 3.2 ^{ab}

Means within the same column with different superscripts differ ($p < 0.05$).

or fermented (Tuleun *et al.* 2009, Tuleun *et al.* 2011). The results obtained during this growing phase suggest that WMP, AAMP or CHMP treatments were sufficient to reduce L-Dopa present in MP, and the broiler performance was not affected.

Table 6 shows the data regarding the carcass yield. Broilers had an average carcass yield of 76.0 % without statistical differences between treatments ($p > 0.05$) However, birds fed with WMP or AAMP had heavier livers than the C group ($p < 0.05$). There were not differences in liver weight between broilers fed CHMP in comparison to those in the C group ($p > 0.05$). According to Emenalom and Udedibie (1998) and Emenalom and Nwachukwu (2006) toxic compounds in the diet causes an increase in liver size. However, no effect on growth performance was observed in broilers fed MP in the present experiment. Due to the higher growth rates of broiler in a short period of time the MP content after treatment possibly did not affect the broiler performance in the finishing phase, but a long term effect might be observed if

broilers are fed MP for longer periods of time. These aspects warrant further research to assess the effect of feeding MP in laying hens which are fed during longer periods.

The alkaline solution was the most effective treatment to reduce L-Dopa from *M. pruriens* seeds. Broilers fed MP soaked seeds in acetic acid solution had lower growth rate during the starter phase. Nevertheless, the presence of L-Dopa in the diet did not affect negatively the performance of broilers during the finishing phase; therefore 25.0% of *M. pruriens* seeds soaked in alkaline solution and boiled could be included in broiler diets without unfavorable effects on feed intake, live weight gain and feed conversion ratio.

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