

# Evaluation of nutritional and ruminal degradability potential of sandbox (*Hura crepitans* L.) seeds in stabled Blackbelly sheep

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**ABSTRACT** - This study evaluated chemical composition, *in situ* dry matter degradability (DMD), energy utilization, and amino acid profile of the sandbox seed meal (SSM) obtained from ground seeds of *Hura crepitans* trees. Two cannulated male Blackbelly sheep (initial weight of 40 kg) were fed a balanced feed *ad libitum* for 21 days; rumen samples of animals were collected for seven days using the nylon bag technique for degradability. The results were interpreted in reference to incubation times from 0 to 72 h, evaluating degradation kinetics with an exponential model. The SSM showed crude protein and dry matter contents of 251.1 and 931.7 g/kg, respectively. The highest DMD value was recorded from 0 to 3 h with a change rate of 41%, and the energy contained in SSM had a gradual ruminal disappearance with a maximum value of energy utilization of 14.6% after 72 h post-incubation. The most representative amino acids of SSM were glutamic (16.9%), arginine (13.0%), and aspartic (9.7%) acids. The results suggest that SSM has adequate nutritional quality and ruminal DMD for ovine feeding systems.

**Keywords:** feed efficiency utilization, non-conventional ingredients, ovine feeding systems, sandbox seed meal

## 1. Introduction

The use of non-conventional ingredients of plant origin in functional feed development for ovine feeding systems in the Latin American region is relevant due to economic, environmental, and social limitations (Theodorou et al., 1994). This research provides a frame of reference to improve feedstuff use of sandbox seeds in a sustainable way, in relation to ingredients of wide abundance that are not used in a conventional way in ovine feeding systems. The sandbox tree (*Hura crepitans* L.) is part of the endemic native flora representative of the medium sub-deciduous forests of Latin America from Mexico to the Brazilian Amazonia (Marinho et al., 2010) and valued for its ecological, economic, and cultural attributes (Pineda-Herrera et al., 2015).

Sandbox seeds have been evaluated in terms of their proximal composition and their content of minerals, vitamins, fatty acids, antinutritional factors, amino acid profile, and energy content (Fowomola and Akindahunsi, 2007; Ajani et al., 2019). However, the whole seed degradability and potential energy utilization in ovine is unknown according to the background described in the literature using different models with animals and microorganisms (Abdulkadir et al., 2013;

Velázquez-González, 2019; Inegbenose et al., 2021). From these perspectives, the objective of this research is to evaluate chemical composition, *in situ* dry matter degradability, energy utilization, and amino acid profile of the sandbox seed meal, as well as determine its potential use as a non-conventional ingredient for ovine feeding systems.

## 2. Material and Methods

### 2.1. Ethics statement

The experimental design was performed with the principle of small groups of animals suggested by Kramer and Font (2017). Research on animals was conducted according to the Ethics Committee on Animal Research (case no. OBA-UAMVZ-01-2016).

### 2.2. Experimental site

The experimental research was carried out in Compostela, Nayarit, Mexico. The livestock production system is located between 21°17'46" N latitude and 104°54' W longitude, at 880 m a.s.l. (INEGI, 2015).

### 2.3. Obtaining and processing sandbox seeds

One hundred and fifty brown-colored fruits were collected at the end of spring 2018 from different *H. crepitans* trees; subsequently, seeds with a diameter of 2.6±0.1 cm were divided into four parts of 200 g and ground in a high-speed blender machine (Oster®, John Oster Manufacturing Co. Milwaukee, WI, USA). The final product, whole sandbox seed meal (SSM), was dried in a forced-air oven at 65 °C for 48 h and then stored at 5±1 °C until use.

### 2.4. Chemical composition analysis

A representative portion of SSM (100 g) was analyzed in triplicate for its proximal chemical composition. Dry matter was determined by gravimetric analysis, for which the samples were put into an oven at 105 °C for 16 h (method 967.03; AOAC, 1990), and the calculation was defined as:

$$\text{Dry matter \%} = 100 \times \frac{W_f}{W_i} \quad (1)$$

in which  $W_i$  is the initial weight of the seeds (g) and  $W_f$  is the final dry matter of seed content (g).

Moisture was determined based on dry matter calculations, applying the next equation:

$$\text{Moisture \%} = (100 - \% \text{ dry matter}) \quad (2)$$

Crude protein was determined by the micro-Kjeldahl method according to protocols of the method number 955.04 (AOAC, 1990), to convert total nitrogen into protein using factor  $f$  as described in the following calculations:

$$\text{Crude protein \%} = [(V_2 - V_1 \times N)/P] \times 1.4 \times f \quad (3)$$

in which  $V_2$  is the volume in mL of the hydrochloric acid (HCl) solution (Sigma-Aldrich, St. Louis, MO, USA) required for the test sample,  $V_1$  is the volume in mL of the HCl solution required for the blank test,  $N$  is the normality of HCl solution (0.1),  $P$  is the sample weight in g, and  $f$  is the standard value of 6.25.

Ash was determined by calcination of the sample in a muffle at 550 °C for 3 h, applying the following mathematical equation (method number 923.03; AOAC, 1990):

$$\text{Ash \%} = 100 \times \left[ \frac{\text{final sample weight (g)} - \text{crucible weight (g)}}{\text{sample weight (g)}} \right] \quad (4)$$

Organic matter was determined based on ash determination utilizing the following calculation criteria based on the methodology of Martínez-González et al. (2015):

$$\text{Organic matter \%} = (\text{dry matter \%} - \text{ash \%}) \quad (5)$$

## 2.5. Experimental feed design and elaboration

The ingredient composition of the experimental feed (Table 1) was designed using local ingredients from Mexico, and the value of metabolizable energy requirement for Blackbelly sheep breed proposed by the National Research Council (NRC, 2007). Experimental feed was elaborated at a pilot plant scale; the micro-ingredients were weighed and mixed in a plastic container and then added to the macro-ingredients to form a homogeneous mixture. The resulting experimental feed was stored at room temperature (27 °C) until use.

**Table 1** - Ingredients and chemical composition of the experimental feed

Ingredient (as-fed basis)	g/kg
Alfalfa hay <sup>1</sup>	170.0
Ground corn stubble (complete plant) <sup>1</sup>	370.0
White corn, grain, ground <sup>1</sup>	180.0
Canola meal <sup>1</sup>	80.0
Soybean meal <sup>1</sup>	170.0
Mineral and vitamin premix <sup>2</sup>	30.0
Calculated chemical composition (in dry basis)	
Dry matter	871.5
Crude protein	179.0
Neutral detergent fiber	380.0
Ethereal extract	28.9
Total digestible nutrients	630.0
Ash	90.0
Calcium	11.7
Phosphorus	8.2
Metabolizable energy (MJ/kg) <sup>3</sup>	10.8

<sup>1</sup> Forrajera Barajas, Tepic, Nayarit, México (commercial ingredients).

<sup>2</sup> GRUPO PROMIN S.P.R. DE R.L. Tepatitlán de Morelos, Jalisco, México. Content per kilogram of mineral and vitamin premix<sup>®</sup>: P, 20 g; Ca, 270 g; Na, 76 g; Co, 9 mg; Se, 1,440 mg; Cu, 2.6 mg; Zn, 2,550 mg; Cl, 11.7 mg; Mn, 47 mg; Mg, 16 g; vitamin A, 116,000 IU; vitamin D, 967 IU; vitamin E, 976 IU.

<sup>3</sup> Value calculated with the ingredient composition reported by the NRC (2007).

## 2.6. *In situ* degradability procedures

*In situ* dry matter degradability (DMD) of SSM was determined by conventional ruminal cannulation method of two Blackbelly sheep males of 40±1 kg body weight with fixed Bar Diamond<sup>®</sup> cannulas (Bar Diamond, Inc. Parma, ID, USA). The animals were fed the experimental diet *ad libitum* for 21 days with an adaptation period of 14 days, and seven days for collecting samples according to the nylon bag technique described by Ørskov et al. (1980) with the following procedure: samples of 5 g of SSM were incubated in the ovine rumen for 0, 9, 24, 30, 48, 56, and 72 h. Determination of degradation kinetics was conducted using nylon bags with an average porosity of 1400 holes/cm<sup>2</sup> with a rectangular size

of 12×8 cm for both sides (Mertens, 1977). After removing bags from the rumen for each incubation time, they were washed five times with distilled water, taking the crystalline color of the wash water as the final reference point, and then dried at 65 °C for 48 h.

### 2.7. Energy utilization efficiency and amino acid analysis

Energy utilization efficiency by sheep was determined with the following procedure: before and after incubation periods (0-72 h), gross energy content of SSM was obtained using an isoperibolic calorimeter model IKA C6000 (Ika Works, Inc., Wilmington, NC, USA). Amino acid profile of SSM was calculated by high-performance liquid chromatography according to the official method 982.30E described by the Association of Official Analytical Chemists (AOAC, 2006).

### 2.8. Descriptive analyses and calculations

Descriptive analyses of chemical composition, dry matter degradability, energy utilization efficiency, and amino acid content of SSM parameters were carried out using Microsoft Excel™ spreadsheet (Microsoft Corporation, Redmond, WA, USA).

Degradation of dry matter was calculated using the exponential model defined by Ørskov and McDonald (1979) as follows:

$$p = a + b (1 - e^{-ct}) \quad (6)$$

in which  $p$  is the dry matter disappearance (%) at time  $t$ ,  $a$  is the intercept of the degradation curve at time zero,  $b$  is the potential degradability of the dry matter component (fraction degraded by microorganisms as percentages),  $e$  is the natural logarithm ( $a + b \leq 100$ ), and  $c$  is the rate constant for the degradation of ' $b$ ' (%/h).

## 3. Results

### 3.1. Chemical composition and energy content

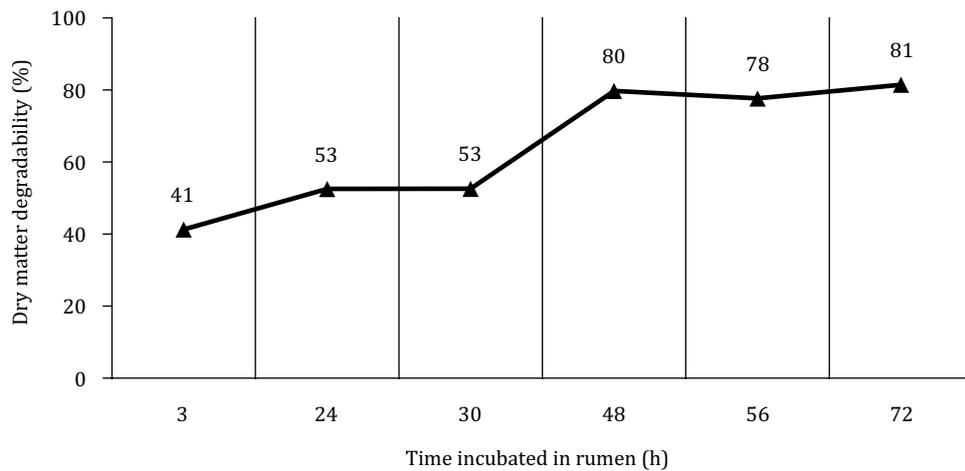
The analyzed SSM shows an important protein and energy content (Table 2). On the other hand, organic matter and ash contents indicate an ingredient with a low contribution of mineral salts and other inorganic residues (Table 2).

**Table 2** - Nutritional and energetic composition of the sandbox seed meal (mean ± standard deviation, n = 3)

Proximal composition in dry basis	g/kg
Dry matter	931.7±1.2
Moisture	68.1±1.2
Crude protein	251.1±8.2
Organic matter	890.3±1.4
Gross energy (MJ/kg)	25.3±0.46

### 3.2. Dry matter degradability

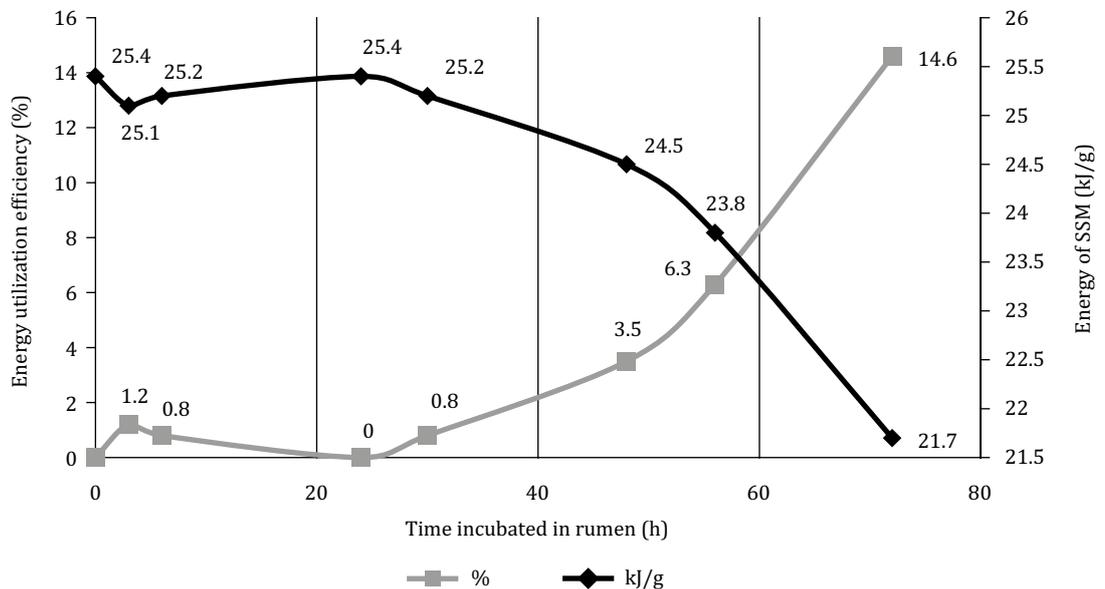
The DMD results of SSM can be described as follows: the highest increment occurred between 0 and 3 h (41%), the second one between 30 and 48 h (27%), and the maximum sustained value at 72 h of 81% (Figure 1). Values of DMD did not show relevant variation after 48 h, and the current results of  $a + b$  showed that dry matter degradation of SSM in sheep rumen potentially reached approximate values of 81% in terms of their total nutrient availability (Figure 1).



**Figure 1** - Dry matter degradability of sandbox seed meal (SSM) evaluated in sheep rumen.

### 3.3. Energy utilization efficiency

Energy utilization efficiency by sheep was specifically defined in percentages. In general, ruminal utilization of the energy contained in SSM had a gradual increase with a maximum value of energy utilization of 14.6% after 72 h post-incubation by reducing the caloric content from 25.4 to 21.7 KJ/g (Figure 2).



**Figure 2** - Study on energy utilization efficiency of sandbox seed meal (SSM).

### 3.4. Comparative analysis of amino acid content

The most relevant amino acid profile of SSM in terms of their content were glutamic (16.9%), arginine (13.0%), aspartic (9.7%), leucine (6.2%), valine (5.5%), and serine (5.0%); on the contrary, cysteine and methionine had the lowest percentages (1.8 and 1.7%, respectively) (Table 3).

**Table 3** - Comparative amino acid profile and content of sandbox seed meal (in crude protein percentages)

Amino acid	Present study	Udoh et al. (2019)
Methionine	1.7	1.2
Cysteine	1.8	0.9
Lysine	2.8	4.6
Methionine + Lysine	4.5	5.8
Threonine	3.3	3.1
Arginine	13.0	5.3
Isoleucine	3.6	3.4
Leucine	6.2	6.5
Valine	5.5	4.3
Histidine	2.5	2.2
Phenylalanine	3.9	5.1
Glycine	4.0	4.5
Serine	5.0	3.9
Proline	3.4	4.9
Tryptophan	Not detected	0.9
Tyrosine	Not detected	2.4
Norleucine	Not detected	3.7
Alanine	3.9	3.9
Aspartic acid	9.7	8.9
Glutamic acid	16.9	11.9
Total dry matter content	87.4	81.6

#### 4. Discussion

Sandbox seed nutritional assessment reveals higher protein content than edible seeds, such as chickpea (*Cicer arietinum*) and black bean (*Phaseolus vulgaris*) (19.93% and 21.70%, respectively), according to Nosworthy et al. (2020) and Trugo et al. (1999), as well as regarding cereals, such as wheat, sorghum, corn, and rice (12.39, 10.13, 8.58, and 10.49%, respectively) in accordance with data reported by Abdulrahman and Omoniyi (2016). The SSM protein profile results of this study indicate minimal and maximal differences compared with the investigations of Oderinde et al. (2009) and Abdulkadir et al. (2013), who found values of 22.20 and 37.64, respectively. Values of DMD showed a slow degradation during the first 30 h; this result is probably due to the fact that the ruminal degradability of SSM is more susceptible after a long time because of the presence of important fractions of neutral (NDF) and acid (ADF) detergent fibers, as reported by Olaleru et al. (2018) for *H. crepitans* seeds. By comparison, research conducted evaluating alfalfa silo showed that feeds with high levels of NDF and ADF (>27%) have significant effects on DMD (Michalski et al., 2020). On the other hand, cellulose, hemicellulose, and lignin compounds that decrease feed degradability should be determined (Chesson et al., 1983). In turn, a high disappearance of the degradable fraction in the rumen is favorable because it represents an increase in energy for ruminal microorganisms (Chaves et al., 2006). Regarding degradability, Olivares-Palma et al. (2013) observed a decrease in the disappearance of the soluble DM fraction when oilseed cakes contained high levels of NDF and lignin. Gross energy content of SSM shows that seed has a high energy value (25.3 MJ/kg) compared with other seeds widely used in ruminant feedings, such as corn and sorghum (16.3 and 16.1 MJ/kg, respectively) in reference to the work of Pan and An (2020). The SSM energy level post-incubation for 72 h decreased by 14.6% (Figure 2), which probably indicated a moderate use of energy from the total digestible fractions of the seeds that apparently are not degraded efficiently at ruminal level. In

this regard, Milis and Liamadis (2008) reported digestibility interactions among protein level of the diet, primary protein source (differing in rumen-undegradable protein), and non-forage fiber source when sheep (59–63 kg live weight) are fed high and low protein-level (179–180 g/kg DM vs 142–145 g/kg DM) feeds, and in turn made with different portions of corn grain, alfalfa hay, wheat straw, cotton seed cake, corn gluten meal, wheat bran, and corn gluten feed. Therefore, future evaluations of *in situ* degradability of lipids, nitrogen, crude fiber, carbohydrates, NDF, and ADF are suggested to elucidate which seed fractions are less digestible. Higher degradability after 3 h may be explained by the rumen pH increase since Osungbade et al. (2016) found that protein solubility increases in alkaline pH. The pH declines 3 h post-feeding because of rumen fermentation (Loya-Olguin et al., 2020). Therefore, degradability may be explained by solubility during the first hours after feeding and later by digestible insoluble fraction, which depends on NDF content. Methionine + lysine amino acid score reported was lower than that reported by Udoh et al. (2019), in which SSM samples were analyzed with a total dry matter content greater than 80% (Table 3). These differences could have been attributed to seasonal and geographical changes to seed origin, as reported for nutritional composition by the samples analyzed from different regions, mainly from countries such as Nigeria (Fowomola and Akindahunsi, 2007; Udoh et al., 2019). The amino acid profile of SSM (Table 3) could have been one of the factors responsible for increasing the level of protein utilization and growth performance in the animal models as rats and chickens as described by Fowomola and Akindahunsi (2005) and Ozeudu et al. (2015), respectively.

## 5. Conclusions

The results suggest that sandbox seed meal has adequate composition of amino acids and ruminal dry matter degradability for Blackbelly sheep; therefore, it can be considered as non-conventional ingredient for further research and application in ovine feeding systems, such as those that affect well-being and growth performance.

## Conflict of Interest

The authors declare no conflict of interest.

## Author Contributions

Conceptualization: F. Escalera-Valente. Data curation: J.L. Loya-Olguín, P.U. Bautista-Rosales and R. Gutiérrez-Leyva. Formal analysis: C.A. Carmona-Gasca and R. Gutiérrez-Leyva. Funding acquisition: F. Escalera-Valente. Investigation: R. Gutiérrez-Leyva. Methodology: J.L. Loya-Olguín. Project administration: F. Escalera-Valente. Resources: F. Escalera-Valente and R. Gutiérrez-Leyva. Software: S. Martínez-González. Supervision: R. Gutiérrez-Leyva. Validation: F. Escalera-Valente, P.U. Bautista-Rosales and R. Gutiérrez-Leyva. Visualization: J.L. Loya-Olguín, S. Martínez-González, P.U. Bautista-Rosales and R. Gutiérrez-Leyva. Writing – original draft: F. Escalera-Valente, J.L. Loya-Olguín, S. Martínez-González, C.A. Carmona-Gasca, P.U. Bautista-Rosales and R. Gutiérrez-Leyva. Writing – review & editing: F. Escalera-Valente, J.L. Loya-Olguín, S. Martínez-González, C.A. Carmona-Gasca, P.U. Bautista-Rosales and R. Gutiérrez-Leyva.

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