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Nota técnica

Digestibility of *Acacia macracantha* attenuated in secondary compounds and *Acacia polyphylla* in rabbit diets

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ABSTRACT

This study, conducted in Lara State, Venezuela, aimed to evaluate the effects of dolomitic lime treatment on Úveda pods to reduce secondary compounds (CSAs) and its impact on nutrient intake and digestibility in rabbit diets. The trial utilized iso-protein diets with varying inclusion levels of *Acacia macracantha* pods (Pam) and *Acacia polyphylla* foliage (Fap), alongside different levels of lime treatment (0.5% and 1.0%) on Pam. The experimental design was completely randomized with 5 treatments: T0 (commercial balanced feed); T1 (30.0% Cf, 2.0% vitamins and minerals (vit), 7.5% M, 45.0% Fap, and 15.5% Pam); T2 (30.0% Cf, 2.0% vit, 7.0% M, 45.0% Fap, and 16.0% Pam); T3 (30.0% Cf, 2.0% vit, 7.5% M, 30.0% Fap, and 30.5% Pam) and T4 (30.0% Cf, 2.0% vit, 7.0% M, 30.0% Fap, and 31.0% Pam). In this way, 5 repetitions per treatment with one rabbit/cage/experimental unit (Californian rabbit, 1.286 ± 0.045 kg of initial live weight). The trial lasted 12 days: 7 of habituation and 5 of collection. The study focused on evaluating the intake and digestibility of several nutrients: dry matter (DM), organic matter (OM), cell wall content (neutral detergent insoluble fiber, NDF), and crude protein (CP). These variables were examined to assess the effectiveness of different dietary treatments in rabbits. Significant differences were found regarding DM intake. The highest was for

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T0 (141.0 g/animal/d), followed by T3 and T4, with 84.8 g/animal/d and 85.5 g/animal/d, respectively. The digestibility of DM and NDF had the highest values in T0 (69.3% and 51.8% in that order), followed by T3 (52.0% and 39.7%). Regarding CP, the highest digestibility was for T0 (81.8%), secondly by T3 (41.3%). This allows to conclude that lime treatment did not completely neutralize the enzymatic inhibitor of protein digestibility present in Pam, being the best treatment T0, and then processed food T3 and T4. Further studies are recommended to assess long-term impacts on growth and nutrient utilization.

Keywords: Rabbits, *Acacia macracantha* pods, *Acacia polyphylla* leaves, dolomitic lime, intake, digestibility, secondary compounds.

RESUMEN

Digestibilidad de *Acacia macracantha* atenuada en los compuestos secundarios y *Acacia polyphylla* en las dietas de conejos. Este estudio, realizado en el Estado Lara, Venezuela, tuvo como objetivo evaluar los efectos del tratamiento de los frutos de úveda con cal dolomítica para reducir los compuestos secundarios (CSAs) y su impacto en la ingestión y digestibilidad de nutrientes en dietas para conejos. El ensayo utilizó dietas iso-proteicas con diferentes niveles de inclusión de vainas de *Acacia macracantha* (Vam) y follaje de *Acacia polyphylla* (Fap), junto con distintos niveles de tratamiento con cal (0,5% y 1,0%) sobre Vam. El diseño experimental fue completamente aleatorizado con 5 tratamientos: T0 (alimento balanceado comercial); T1 (30,0% Cf, 2,0% vitaminas y minerales (vit), 7,5% M, 45,0% Fap y 15,5% Vam); T2 (30,0% Cf, 2,0% vit, 7,0% M, 45,0% Fap y 16,0% Vam); T3 (30,0% Cf, 2,0% vit, 7,5% M, 30,0% Fap y 30,5% Vam) y T4 (30,0% Cf, 2,0% vit, 7,0% M, 30,0% Fap y 31,0% Vam). Se realizaron 5 repeticiones por tratamiento con un conejo/jaula/unidad experimental (conejo californiano, 1,286 ± 0,045 kg de peso vivo inicial). El ensayo duró 12 días: 7 de habituación y 5 de recolección. El estudio se centró en evaluar la ingestión y digestibilidad de varios nutrientes: materia seca (MS), materia orgánica (MO), contenido de pared celular (fibra insoluble en detergente neutro, FDN) y proteína cruda (PC). Se encontraron diferencias significativas en cuanto a la ingestión de MS, siendo la más alta para T0 (141,0 g/animal/d), seguido de T3 y T4, con 84,8 g/animal/d y 85,5 g/animal/d, respectivamente. Los valores más altos de digestibilidad de MS y FDN se registraron en T0 (69,3% y 51,8%, en ese orden), asimismo, le siguió T3 (52,0% y 39,7%). En cuanto a la PC, la digestibilidad más alta fue para T0 (81,8%), seguida por T3 (41,3%). Esto nos permite concluir que el tratamiento con cal no neutralizó completamente el inhibidor enzimático de la digestibilidad de proteínas presente en Vam, el mejor tratamiento T0, procedido de los alimentos procesados T3 y T4. Se recomiendan más estudios para evaluar los impactos a largo plazo sobre el crecimiento y la utilización de nutrientes.

Palabras clave: Conejos, vainas de *Acacia macracantha*, hojas de *Acacia polyphylla*, cal dolomítica, consumo, digestibilidad, compuestos secundarios.

INTRODUCTION

Rabbits (*Oryctolagus cuniculus*) can receive high amounts of fibrous material in their diets, from 24.5% to 44.3% of neutral detergent insoluble fiber (NDF), so that they can take advantage of energy, protein, and vitamins from fermentative processes carried out by microorganisms in the cecum and the rest of the large intestine. This is how they manage to digest or ferment from 3% to 70% of the NDF, 0% to 82% of the hemicellulose, and 1% to 59% of the cellulose (De Blas and Wiseman, 2010). Intensively managed rabbit diets are based on balanced mixtures of cereals, legume grains, oilseed cakes, cereal by-products, oils, alfalfa, mineral supplements, and vitamins from a relatively small group of raw materials (NRC, 1977). They can also take advantage of various fresh or preserved fibrous vegetables (hay or silage), crop residues, and forage (grasses, legumes, and other dicotyledonous species). Also, rejected post-harvest fruits can provide them with easily digestible energy, proteins, and microelements (De Blas and Wiseman, 2010; Bonilla-Vivas et al., 2016; Jaramillo, 2019; Sánchez et al., 2012).

Hassan et al. (2020) conclude that alternative ingredients often have a double-edged sword effect, in that, they can supply animals with the necessary nutrients although they contain anti-nutritional factors such as tannins; which are complex secondary metabolites commonly present in the plant kingdom, known to bind with protein and make it unavailable. However, recently they have been proven to have the potential to replace conventional ingredients, in addition to their health benefits, like controlling zoonotic pathogens such as *Salmonella* (Wink, 2013).

Among forages, legumes are abundant in the tropics, either growing naturally or being cultivated in dry and semi-arid regions of Venezuela, representing potential sources of energy and protein for rabbits (Nouel and Rincón, 2005; Espejo-Díaz and Nouel-Borges, 2014; Espejo-Díaz and Nouel-Borges, 2020). However, there are limitations due to the presence of secondary compounds that reduce the use of these nutrients (Kurman, 1991; Makkar, 2000; Romero et al., 2010; Romero-Cáceres et al., 2008; Salas-Araujo et al., 2008; Espejo-Díaz and Nouel-Borges, 2014; Espejo-Díaz and Nouel-Borges, 2020), specifically decreasing protein digestion and fiber fermentation due to the action of polyphenolic compounds (Champagne et al., 2020; Romero et al., 2010; Romero-Cáceres et al., 2008; Salas-Araujo et al., 2008).

This negative effect can be reduced or eliminated through chemical methods (such as soaking or the use of alkalis) and/or physical methods (such as heat) (Hagerman et al., 1998; Kok and Delgado, 2004; Calderón, 2007; De Nobrega and Pérez, 2004). These methods enable greater incorporation of these plants into rabbit diets, promoting adequate intake, digestion, and growth (Espejo-Díaz and Nouel-Borges, 2014; Espejo-Díaz and Nouel-Borges, 2020). In times of climate change and desertification, taking advantage of leguminous tree species can be an alternative to help mitigate these issues.

Alternatives to this are *Acacia macracantha* and *Acacia polyphylla* DC. *Acacia macracantha* [commonly known as úveda, cují negro, cují yaque, cují hediondo, tusca (Argentina, Bolivia); espino, faique, taque, and guarango (Ecuador, Peru); wild tamarind, stink casa (Virgin Islands); cambrón, aroma, carambomba (Dominican Republic)] is distributed across western and northern South

America, from Guyana and Venezuela to Peru and Chile. It is also found in subtropical regions of North and Central America, as well as the Caribbean Islands (Nouel-Borges, 2015).

This species blooms from May to January and thrives in dry valleys, particularly in the Andean region from Venezuela to Bolivia. However, it is better adapted to hot and arid environments, withstanding temperatures of up to 25 ºC. It produces leaves and pods that are of interest in herbivore diets (Nouel-Borges, 2015).

Similarly, *Acacia polyphylla* DC [also known as tiamo, tiamo guire, tiamo arrow, small leaf, cari-cari (Bolivia); maricá, monjolera, monjoleiro, espinheiro preto (Brazil); espinito blanco (Colombia); rabo de iguana (Mexico); jukeri guasu (Paraguay); pashaco negro (Peru)] is distributed from Mexico to Paraguay, thriving in dry and semi-arid regions (Nouel-Borges, 2015).

Barbehenn and Constabel (2011) conclude that tannins are especially prone to oxidize in animals with high pH guts, forming semiquinone radicals and quinones, as well as other reactive oxygen species; tannin structure has an important effect on biochemical activity; ellagitannins (ellagic acid attached to a central carbohydrate unit) oxidize much more readily than do gallotannins (gallate esters attached to a central carbohydrate unit) (Olivas-Aguirre et al., 2015), which are more oxidatively active than most condensed tannins. Unlike insects, which can tolerate ingested tannins due to various biochemical and physical defenses in their guts (such as surfactants, high pH, antioxidants, and a protective peritrophic envelope lining the midgut). These findings suggest that alkaline materials, such as clays, may help mitigate the negative effects of these polyphenolic compounds on digestion (Barbehenn and Constabel, 2011).

For these reasons, this study aimed to evaluate the effect of attenuating secondary compounds (CSAs) by treating *Úveda* pods with dolomitic lime suspensions and its impact on nutrient intake and digestion when incorporated into rabbit diets along with *Tiamo* leaves (a protein source), corn flour, sugarcane molasses, and microelements.

MATERIALS AND METHODS

Location

The experiment was conducted from June 2022 to December 2022 at the Animal Production Research Unit (UIPA), under the Dean of Agronomy at Lisandro Alvarado Central Western University, Lara State, Venezuela. The experimental analysis took place in the Jesús Rojas Castellanos Research and Development Laboratory at UIPA.

This location falls within the "Tropical Dry Forest" biome, a sub-humid region. The area's average annual climate data includes a temperature of 25 ºC, precipitation of 812.6 mm, relative humidity of 74.6%, solar radiation of 371 cal/cm², evaporation of 2,084.9 mm, and an elevation of 550 m a.s.l. (Ortiz et al., 2015).

Experiment design

A completely randomized design was carried out with four treatments and one control (commercial feed), using 5 replicates per treatment. Each experimental unit (EU) consisted of a Californian rabbit (1.286 ± 0.045 kg initial live weight), for a total of 25 EUs. The trial lasted 12 days, 7 for acclimatization and 5 for intake and digestibility measurements. The diet rations contained varying levels of Úveda pods and Tiamo leaves, while maintaining constant levels of molasses, cornmeal, and minerals (Table 1).

Table 1. Amount on a dry basis of ingredients used in the preparation of the food for each treatment.

Ingredient (g/kg)	T1	T2	T3	T4
Corn flour	300	300	300	300
Vitamins and Minerals	20	20	20	20
Molasses	75	70	75	70
Tiamo leaves	450	450	300	300
Úveda pods	155	160	305	310

Isoproteic diets were formulated by varying the inclusion levels of leaves and pods, along with 2 lime levels (0.5% and 1.0%) to neutralize secondary compounds in Úveda pods (Table 2). This process resulted in 4 experimental rations for growing rabbits.

Table 2. Analysis of polyphenolic compounds (total polyphenols, simple polyphenols, condensed tannins, protein precipitating tannins, and total tannins) in *Acacia macracantha* pods.

Vegetable part	Dolomitic lime Level (%)	*TP %	*SP %	*CT %	*TTPP %	*TT %
Pods	0.0	0.229	0.087	2.524	0.001	0.141
Pods	0.5	0.168	0.076	0.391	0.00225	0.092
Pods	1.0	0.102	0.081	1.937	0.00195	0.027

*TP: total polyphenols. SP: simple polyphenols. CT: condensable tannins. TTPP: tannins that precipitate proteins. TT: total tannins.

The trial aimed to evaluate the effect of the rations on the intake and digestibility of dry matter (DM), organic matter (OM), cell wall components (neutral detergent fiber, NDF), and crude protein (CP). Each rabbit was housed in a galvanized steel cage measuring 25 cm in width, 30 cm in height, and 40 cm in depth. The cages were equipped with galvanized iron hoppers with a 1.5 kg food capacity, dripper-type drinkers installed in polyvinyl chloride (PVC) pipes, and a tray at the bottom for feces collection, designed with holes to separate feces from urine. At the start of the trial, rabbits were weighed using an electronic scale with a 4 kg (±1 g) capacity to determine their initial weight. Measurements were taken on the 4th, 8th, and 12th days of the trial. Weight differences were used to calculate weight gain and daily weight increase. Additionally, rejected feed was weighed daily for each treatment to assess intake throughout the trial. Feces were collected daily during the final 5 days of the experiment.

To determine intake and digestibility, the standard method described by Pérez et al. (1995) was used. Feces were weighed, dehydrated at 60 °C in a forced-air oven (Binder FD model) for 48 hours, and then stored for later digestibility analysis.

The animals were managed in accordance with the ethics and laboratory management standards proposed by Aller et al. (2000), ensuring the highest possible level of animal welfare throughout the experiment. Additionally, the data were analyzed using analysis of variance (ANOVA), and differences between means were determined using the Tukey Honestly Significant Difference (HSD) test, performed with Statistix for Windows software, version 8.0 (Analytical Software, 2007).

Collection of vegetable material

The harvest and processing of Úveda pods and Tiamo leaves were conducted at the Héctor Ochoa Zuleta Nucleus of the Dean of Agronomy. Úveda pods were harvested at the field ripeness stage, identified by their dark brown color, while Tiamo leaves were collected during branch pruning from selected trees. The collected samples were spread out on trays and dried in a forced-air oven at 55 °C for 48 hours. After drying, they were ground using a hammer mill, passed through a 2 mm sieve, and stored in plastic bags for future use.

Total polyphenols, simple polyphenols, total tannins, condensed tannins, and protein-precipitating tannins were quantified using the methodologies described by Makkar (2000), as well as those developed by Porter et al. (1986), Makkar et al. (1988), Makkar et al. (1993), and Hagerman et al. (1998). Treatment of *Acacia macracantha* pods with 0.5% and 1.0% dolomitic lime followed the procedure described by Romero-Cáceres (2006).

Diets preparation

The four iso-protein and iso-calorie experimental rations were prepared at the Jesús Rojas Castellanos Research and Development Laboratory of the UIPA, while the balanced feed control (T0) was sourced from the local market in Cabudare, Lara State.

The 4 experimental rations were manually prepared. To facilitate the production of granulated feed for each treatment, an electric butcher's mill (Boia brand, 1.5 HP) was used.

The process was carried out through the steps described below:

1. Each of the raw materials was weighed based on the proportions of the treatments.
2. Úveda pods and Tiamo leaves were mixed, trying to make the mixture homogeneous.
3. Added 30% water, diluting the molasses and vitamins in the mixture.
4. Corn flour and minerals were added to the mixture and homogeneously united.
5. The wet mix was passed through the mill.
6. The granules (4 mm diameter) were placed in trays and placed in the oven at 55 ºC for 48 h.

At the end of the process, the food obtained was ready to be supplied to the rabbits. Samples from each of the rations and feces were analyzed for moisture content (AOAC 977.11). DM content was determined using the proximal method as outlined in the AOAC (1997) guidelines. Total OM was calculated by determining ash content (method AOAC 942.05). CP levels were evaluated using the method proposed by Bilbao et al. (1999). Gross energy was measured using a calorimetric pump (Parr-1261 model). The levels of NDF and acid detergent insoluble fiber (ADF) were determined following the method described by Van Soest et al. (1991).

RESULTS AND DISCUSSION

Table 2 shows that the percentages of TP, SP, CT, and TT in treated Úveda pods tend to decrease compared to untreated pods. However, this trend is not observed in TTPP values. Liu et al. (2012) and Bhat et al. (2013) confirm that condensed tannins inhibit several digestive enzymes, including amylases, cellulases, pectinases, lipases, and proteases. These tannins have a significant antinutritive effect, negatively impacting the digestibility of lipids, starch, and amino acids.

Additionally, secondary compounds in herbivores such as ruminants and lagomorphs can limit the consumption of legume foliage and pods due to their bitter taste (polyphenols/alkaloids), repellent odors (simple phenols), or polyphenolic compounds that inactivate enzymes (proteins), thereby reducing digestion (Bhat et al., 2013; Wink, 2013; Liu et al., 2012).

The presence of condensable tannins (1% chestnut tannins), on the same basal diet, affected weight gain by decreasing it and the conversion of feed into live weight by increasing it compared to the control. In this study, the levels of polyphenols and tannins in the Úveda pods (Table 2) are consistent with those reported by Pizzani et al. (2006) for Úveda pods from the Central Llanos in Venezuela (2.40 ± 0.05). This similarity suggests that environmental and genetic conditions in these regions may favor comparable tannin profiles.

It is well established that condensed tannins, particularly proanthocyanidin polymers from *Acacia* spp., have the ability to adsorb proteins and deactivate enzymes, as highlighted by Ohara et al. (1994) and Kusano et al. (2011). This property could explain the inhibitory effects observed in the evaluated diets. Furthermore, the attenuation values achieved in this study align with those reported by Espejo-Díaz and Nouel-Borges (2020), where tannin concentrations were reduced after soaking the pods under controlled conditions. This effect is attributed to the rupture of polyphenol rings in tannins during alkaline treatments, as proposed by Cilliers et al. (1990).

Regarding the bromatological composition (Table 3), the diets formulated in this experiment achieved crude protein (CP) levels of 15%-16%, meeting the requirements for growing rabbits as recommended by the NRC (1977). However, some deviations from the typical ingredients suggested by De Blas and Wiseman (2010) were introduced. For instance, alfalfa hay was entirely replaced with *Acacia polyphylla* (Tiamo) leaves, and *Acacia macracantha* (Úveda) pods were incorporated at levels of up to 30%, as proposed by Espejo-Díaz and Nouel-Borges (2020). These modifications reflect an emphasis on utilizing local resources, though they may impact diet quality and digestibility due to tannin content.

The levels of neutral detergent fiber (NDF) and acid detergent fiber (ADF) exceeded the ranges recommended by De Blas and Wiseman (2010) and Trocino et al. (2013), which are 30%-37% for NDF and 18%-19% for ADF. These differences may be attributed to the inclusion of *Acacia polyphylla* (Tiamo) leaves and *Acacia macracantha* (Úveda) pods, both of which are rich in cell wall components. Nevertheless, hemicellulose values remained within acceptable ranges, suggesting that the diets provided an adequate balance of fermentable fiber, which is essential for digestive health in rabbits (De Blas and Wiseman, 2010).

The gross energy (GE) values obtained (3.85 to 3.96 Mcal/kg) fall within the expected range for pelleted rabbit diets (Carmona et al., 2004), reinforcing the viability of the evaluated treatments. Meanwhile, variations in ash and organic matter (OM) levels among treatments are likely attributed to differences in the composition of ingredients, such as leaves and pods, which may contain varying proportions of minerals and organic compounds.

Table 3. Bromatological analysis of the evaluated diets (granulated food).

Component	T0	T1	T2	T3	T4
*DM (g/kg)	908	940	948	943	950
*CP (g/kg)	159	164	159	151	153
*NDF (g/kg)	378	441	437	455	376
*ADF (g/kg)	189	323	301	258	271
Hemicellulose (g/kg)	189	118	136	197	105
Ash (g/kg)	129	87	92	87	92
*OM (g/kg)	871	913	908	913	908
*GE (Mcal/kg)	3.96	3.87	3.85	3.88	3.87

*DM: dry matter. CP: crude protein. NDF: fiber insoluble in neutral detergent. ADF: fiber insoluble in acid detergent. OM: organic matter. GE: Gross energy.

Table 4 presents the intake and apparent digestibility values for the different treatments. The highest intake of DM, OM, and CP was recorded in T0 (control), nearly doubling the values observed in T1 to T4. No significant differences were found among T1 to T4, indicating that despite the attenuation of secondary compounds, the inclusion of Úveda pods still limited feed intake compared to the control. According to De Blas and Wiseman (2010), an adequate DM intake for rabbits from post-weaning to 10 weeks of age ranges between 100 g/d and 170 g/d, a level reached only by T0.

The lower intake observed in T1 to T4 can be attributed to several factors such as higher fiber content, reduced CP intake, as well as CP digestibility, and lower levels of digestible energy in these diets. These factors are consistent with findings from Romero-Cáceres et al. (2008), who reported an intake of 55.2 g/d using diets with 30.0% untreated Úveda pods, 23.5% Cassava root meal (*Manihot esculenta*), and 20.0% Úveda leaves. This suggests that treating Úveda pods effectively mitigates the impact of secondary compounds, improving intake, but it does not fully eliminate the associated limitations.

Similarly, Salas-Araujo et al. (2008) observed an intake of 56.0 g/d with diets containing 37.5% untreated Úveda pod flour, 25.0% of *Mimosa grit* leaves, and 12.5% of cassava root flour. The findings in this study corroborate their observations, emphasizing that treating Úveda pods enhances intake compared to untreated pods but does not match the intake levels achieved with conventional feed ingredients. In comparison, Legendre et al. (2018) provided granulated feed with 16.5% CP, 37.5% NDF, and 21.2% ADF at a rate of 51.9 g/d DM, supplemented with fresh legumes. Total intake ranged from 54.7 g/d to 114.1 g/d of DM and 18.1 g/d to 32.0 g/d of CP. Well-preserved fresh and preserved legumes comprised 65.0% to 75.0% of the total ration. The DM and CP intake observed in this experiment aligns with these values, particularly in treatments T1 to T4, where preserved ingredients such as Úveda pods and Tiamo leaves played a key role. This supports the findings of Martin et al. (2016), who emphasized the importance of balanced feed and forage legumes in maintaining post-weaning rabbit nutrition.

Table 4. Intake and digestibility of the evaluated rations compared to the control.

Variable	T0	T1	T2	T3	T4
DM intake (g/animal/d)	141.60 ± 7.67^{a}	69.50 ± 6.86^{b}	76.20 ± 6.86^{b}	84.80 ± 6.86^{b}	85.50 ± 6.86^{b}
OM intake (g/animal/d)	123.40 ± 6.99^{a}	63.40 ± 6.26^{b}	69.30 ± 6.26^{b}	77.40 ± 6.26^{b}	77.70 ± 6.26^{b}
CP intake (g/animal/d)	22.50 ± 1.25^{a}	11.40 ± 1.12^{b}	12.10 ± 1.12^{b}	12.80 ± 1.12^{b}	13.10 ± 1.12^{b}
*ADDM (%)	69.30 ± 2.60^{a}	40.00 ± 2.68^{b}	41.60 ± 2.40^{b}	52.00 ± 2.40^{b}	48.60 ± 2.50^{b}
*ADOM (%)	70.40 ± 2.64^{a}	40.40 ± 2.64^{b}	41.30 ± 2.36^{b}	52.10 ± 2.36^{b}	48.40 ± 2.36^{b}
*ADNDF (%)	51.80 ± 3.90^{a}	19.20 ± 3.90^{b}	14.70 ± 3.50^{b}	39.70 ± 3.50^{a}	33.30 ± 3.50^{ab}
*ADCP (%)	81.80 ± 2.77^{a}	33.40 ± 2.77^{bc}	20.60 ± 2.47^{c}	41.30 ± 2.47^{b}	39.20 ± 2.47^{b}
*ADE (Mcal/kg)	2.77 ± 0.05^{a}	1.39 ± 0.07^{c}	1.19 ± 0.08^{d}	1.79 ± 0.06^{b}	1.68 ± 0.07^{b}

*ADDM: Apparent Digestibility of dry matter. ADOM: Apparent Digestibility of organic matter. ADNDF: Apparent Digestibility of Insoluble Fiber in Neutral Detergent. ADCP: Apparent Digestibility of Crude Protein. ADE: Apparent Digestibility of Energy. Different letters in the same row indicate significant differences ($p < 0.05$).

Joly et al. (2018) predicted a dry matter intake (DMI) of 46.5 g DM/rabbit/day for rabbits under organic management in a legume grazing model. Upon validation, the actual intake was 42.9 g DM/rabbit/day with supplementary feed, and weight gain remained unaffected. These findings are consistent with the DMI values of 42.0 g/d and 51.3 g/d observed for legumes in the current study, reinforcing the idea that these intake levels are in line with both predicted and observed values for rabbits grazing legumes and receiving supplementary concentrate. Importantly, this consistency indicates that the DMI levels for legumes in the present study are well-tolerated, without adverse effects on digestive health, which is crucial for maintaining growth and overall performance in rabbits (FEDIAF, 2024).

The apparent digestibility results (Table 4) revealed significant differences between T0 and the other treatments, with T0 exhibiting superior values for most variables, except for ADNDF, where similar values were observed in T3 and T4. When examining only the proposed treatments (Table 5), excluding T0 to focus on the effects of legume levels and tannin attenuation, distinct differences emerged. For example, treatments T3 and T4 exhibited higher apparent digestibility values for DM, OM, NDF, and CP, with T3 showing slightly better digestibility across most parameters.

This trend suggests that the inclusion levels of attenuated Úveda pods (30.5% and 31.0% of total DM in T3 and T4, respectively) positively influenced digestibility. However, the higher proportion of Tiamo leaves (45.0%) in T4 may have counteracted some of these benefits, potentially reducing apparent digestibility compared to T3. These findings highlight the importance of balancing the inclusion levels of fibrous legumes and attenuated pods to optimize nutrient utilization. Although lime treatment did not enhance the total tannin-protein precipitating capacity (TTPP, Table 2), it may have indirectly influenced protein digestibility. For instance, the lime treatment could act as a trypsin inhibitor, thereby reducing ADCP. This effect aligns with the findings of Romero-Cáceres (2006), who reported that diets containing 30% untreated Úveda pods and 20% of their leaves resulted in a DMI of 55.1 g/day, with apparent digestibility values of 65.3% for OM, 24.8% for NDF, and 36.3% for CP.

Notably, while DMI and OM digestibility in the present study were lower than those reported by Romero-Cáceres (2006), the ADNDF and ADCP values in the current experiment were higher. These results emphasize the potential benefits of pod treatment in enhancing fiber and protein digestibility. This improvement may be attributed to the partial attenuation of tannins and other secondary compounds through lime treatment, as observed in previous studies. Overall, the findings suggest that the inclusion of attenuated Úveda pods in rabbit diets, when combined with other feed components, can enhance nutrient utilization compared to untreated pods. However, achieving the optimal balance between pod and legume leaf inclusion levels is essential to maximize digestibility and maintain feed intake (Ngwa et al., 2000).

Table 5. Intake and digestibility of the evaluated rations compared to each other, excluding the control group.

Variable	T1	T2	T3	T4	P-value
DM intake (g/animal/d)	69.50 ± 13.80[a]	76.20 ± 5.80[a]	84.80 ± 5.80[a]	85.50 ± 5.80[a]	0.4011
OM intake (g/animal/d)	63.40 ± 12.60[a]	69.30 ± 5.30[a]	77.40 ± 5.30[a]	77.70 ± 5.30[a]	0.4085
CP intake (g/animal/d)	11.40 ± 2.25[a]	12.10 ± 0.93[a]	12.80 ± 0.93[a]	13.10 ± 0.93[a]	0.7558
*ADDM (%)	40.00 ± 2.27[b]	41.60 ± 2.03[ab]	52.00 ± 2.03[a]	48.60 ± 2.03[ab]	0.0029
*ADOM (%)	40.40 ± 2.27[b]	41.30 ± 2.03[b]	52.10 ± 2.03[a]	48.40 ± 2.03[ab]	0.0031
*ADNDF (%)	19.20 ± 3.11[bc]	14.70 ± 2.78[c]	39.70 ± 2.78[a]	33.30 ± 2.78[ab]	0.0000
*ADCP (%)	33.40 ± 2.82[ab]	20.60 ± 2.52[b]	41.30 ± 2.52[a]	39.20 ± 2.52[a]	0.0001
*ADE (Mcal/kg)	1.390 ± 0.075[b]	1.190 ± 0.082[c]	1.790 ± 0.064[a]	1.680 ± 0.074[a]	0.0024

*ADDM: Apparent Digestibility of dry matter. ADOM: Apparent Digestibility of organic matter. ADNDF: Apparent Digestibility of Insoluble Fiber in Neutral Detergent. ADCP: Apparent Digestibility of Crude Protein. ADE: Apparent Digestibility of Energy.
Different letters in the same row indicate significant differences ($p < 0.05$).

Espejo-Díaz and Nouel-Borges (2020) reported a DMI of 128.0 g/d, with apparent digestibility values of 54.8% for DM, 55.1% for OM, 66.9% for CP, and 40.0% for NDF in diets containing 30.0% Úveda pods that were attenuated through soaking and temperature treatment. These values exceed the DMI and apparent CP digestibility observed in this study and are comparable to the ADDM, ADOM, and ADNDF values achieved in T3. This side-by-side comparison underscores the beneficial effects of including a higher proportion of attenuated Úveda pods, combined with a reduced amount of non-attenuated Tiamo leaves. It supports the hypothesis that optimized combinations of these ingredients can improve nutrient utilization.

Table 6 summarizes the weight gain and feed conversion efficiency into live weight across treatments. Although the dataset is preliminary and does not meet the recommended 30 replicates proposed by Fernández-Carmona et al. (2005), the coefficients of variation (ranging from 14.9% to 16.7%) fall within an acceptable range, particularly in instances where significant differences between treatments were observed. Notably, the T0 treatment exhibited superior performance in terms of both daily weight gain and feed-to-live-weight conversion, followed by T3 and T4. From an economic perspective, a cost analysis of one kilogram of live weight gain, including conversion and opportunity costs of the diets, revealed that the experimental treatments had a significantly lower cost compared to the control feed (T0). This reduced cost was mainly due to the inclusion of opportunity costs for raw materials supplied by the Agronomy Deanship, as well as labor costs for harvesting, processing, drying, grinding, mixing, and forming the diets at local rates. Among the experimental treatments, T4 demonstrated the lowest cost per kilogram of live weight gain, followed closely by T3, while T0 showed the highest cost.

These findings suggest that the artisanal feed formulations incorporating attenuated Úveda pods can provide a cost-effective alternative to conventional feeds, with the potential to reduce production costs while maintaining adequate growth and feed efficiency. However, further studies are necessary to rigorously evaluate the scalability, long-term impacts, and practical applications of such formulations under diverse production conditions.

Table 6. Weights, live weight gain, and feed conversion (live weight basis) of evaluated rations.

Variable	T0	T1	T2	T3	T4	P-value
Initial weight (kg)	1.28 ± 0.12[a]	1.16 ± 0.16[a]	1.32 ± 0.11[a]	1.19 ± 0.11[a]	1.35 ± 0.11[a]	0.8482
Final weight (kg)	1.68 ± 0.18[a]	1.30 ± 0.11[a]	1.37 ± 0.16[a]	1.38 ± 0.16[a]	1.54 ± 0.16[a]	0.2980
Live weight gain (g/animal/day)	39.95 ± 3.64[a]	12.38 ± 3.26[bc]	4.38 ± 3.26[c]	19.9 ± 3.26[b]	19.24 ± 3.26[b]	0.0000
Conversion (g DM* Food/g LWG*)	3.65 ± 0.93[b]	5.52 ± 0.84[ab]	7.97 ± 1.32[a]	5.38 ± 0.84[ab]	4.56 ± 0.84[ab]	0.0313
Opportunity cost ** (food USD/kg DM)	0.433	0.183	0.181	0.180	0.178	NA
Food cost (USD/kg LW*)	1.58 ± 0.17[a]	1.01 ± 0.15[ab]	1.44 ± 0.17[ab]	0.97 ± 0.15[ab]	0.81 ± 0.15[b]	0.0145

*DM: dry matter. LWG: live weight gain. LW: live weight.
**In the metropolitan area of Cabudare, Lara State, Venezuela, includes collection, transfers and/or processing of fresh materials, raw materials, and/or balanced feed.
Different letters in the same row indicate significant HDS differences ($p < 0.05$).

Abdu et al (2011) concluded that including charcoal (2.5%) in a diet with *Acacia nilotica* pod meal (20.0% of total DM) improved the performance of growing rabbits, achieving a DMI of 56.60 g/d and a live weight gain of 16.52 g/d. However, both feed intake and daily weight gain reported by these authors were lower than those observed in the best treatments (T3 and T4) in this study, which incorporated pod levels 50.0% higher than those used in the referenced experiment.

One possible explanation for the reduced performance in weight gain in rabbits fed diets containing *Acacia* pods is the inhibitory activity of condensable tannins on α-amylase (Kusano et al., 2011), which limits starch utilization. Additionally, the diets in this study were at the upper limit of acceptable cell wall content (De Blas and Wiseman, 2010), potentially restricting the availability of easily digestible energy.

The daily weight gains and feed conversion efficiency observed in T0, which used commercially balanced feed, were consistent with findings from Romero-Cáceres et al. (2008) and Salas-Araujo (2008), who reported similar results under comparable experimental conditions for rabbits fed conventional diets. In contrast, the weight gains and feed conversions observed in T3 and T4 were comparable to the results of Legendre et al. (2018), Martin et al. (2016), Bonilla-Vivas et al. (2016), and Jaramillo (2019), where tropical legumes, whether fresh or dehydrated, were included in growing rabbit diets, yielding similar improvements in growth performance and feed efficiency.

Additionally, the inclusion of tropical forage legumes, such as Tiamo leaves and attenuated Úveda pods, resulted in a reduction in feeding opportunity costs. This observation is consistent with the findings of Sánchez et al. (2012), Bonilla-Vivas et al. (2016), and Jaramillo (2019), who demonstrated the cost-effectiveness of using locally available, non-conventional feed ingredients to reduce feed costs without compromising the nutritional quality and growth of rabbits.

Although the use of Tiamo leaves and attenuated Úveda pods did not achieve the same weight gain efficiency as a standard control diet, the economic benefits and potential for artisanal feed production offer a viable and sustainable alternative. These findings highlight the need for further research to optimize feed formulations and assess their long-term effects on rabbit production systems in various conditions.

CONCLUSIONS

The incorporation of attenuated Úveda pods into rabbit diets has shown some drawbacks in terms of dry matter intake (DMI) and digestibility of dry and organic matter, crude protein, and cell wall content compared to the control (T0). However, treatments with higher levels of attenuated Úveda pods, especially T3 and T4, demonstrated improved digestibility of these components, suggesting that the rabbits might adapt to the diet over time.

In terms of weight gain and feed conversion, T0 outperformed the experimental treatments in absolute terms, but T3 and T4 still showed comparable trends, indicating that these treatments could partially replace conventional feed without a significant compromise in growth performance. When considering the opportunity cost of using these ingredients, T3 and T4 offered more cost-effective options, largely due to the use of locally sourced, attenuated ingredients. This suggests that artisanal feed formulations using Úveda pods could be a promising sustainable alternative for rabbit production systems, especially in resource-limited settings.

Despite these encouraging results, further research is essential to validate these findings. Future trials should involve a larger sample size and explore additional variables such as long-term health effects, reproductive performance, and carcass quality. Such studies will provide a stronger foundation for incorporating attenuated Úveda pods into rabbit feeding strategies, potentially offering both economic and nutritional benefits.

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