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Degradability of the dry matter and crude protein of fruits of *Chloroleucon mangense* and *Acacia cochliacantha* in sheep

Abstract – The objective of this work was to determine the nutritional content and the degradability of the dry matter and protein of fruits of *Chloroleucon mangense* and *Acacia cochliacantha* and of a 1:1 mixture of both, offered as supplements to Rambouillet sheep. In situ ruminal degradation was evaluated in three adult rams, fitted with a rumen cannula, with different incubation times of 0, 6, 12, 24, 48, and 72 hours. Protein intestinal degradability was quantified with a three-step procedure: in situ ruminal incubation, in vitro enzymatic digestion, and abomasal-intestinal digestion. The fruits of *C. mangense and A. cochliacantha* contain 21 and 12% crude protein, 47 and 56% neutral detergent fiber, 31 and 43% acid detergent fiber, and 0.9 and 6.0% condensed tannins, respectively. The fruits of *C. mangense* showed a higher nutritional value and a higher dry matter and crude protein degradability ($p<0.05$) than those of *A. cochliacantha* and the 1:1 mixture. The amount of protein that reaches the small intestine is higher for the 1:1, which is an indicative that its tannin concentration is enough to increase the bypass protein that can be absorbed in the small intestine.

Index terms: enzymatic digestion, feeding, legumes, ruminants, tannin.

Degradabilidade da matéria seca e da proteína bruta de frutos de *Cloroleucon mangense* e *Acacia cochliacantha* em carneiros

Resumo – O objetivo deste trabalho foi determinar o conteúdo nutricional e a degradabilidade da matéria seca e da proteína de frutos de *Cloroleucon mangense* e *Acacia cochliacantha* e de uma mistura 1:1 de ambos, oferecidos como suplementos para carneiros Rambouillet. Foi avaliada a degradação ruminal in situ em três carneiros adultos, com colocação de uma cânula ruminal, com diferentes tempos de incubação de 0, 6, 12, 24, 48 e 72 horas. A degradação intestinal da proteína foi quantificada por meio de procedimento com três etapas: incubação ruminal in situ, digestão enzimática in vitro e digestão abomasal intestinal. Os frutos de *C. mangense* e *A. cochliacantha* contêm 21% e 12% de proteína bruta, 47% e 56% de fibra em detergente neutro, 31 e 43% de fibra em detergente ácido e 0,9 e 6,0% de taninos condensados, respectivamente. Os frutos de *C. mangense* apresentaram valor nutricional edegradabilidade da matéria seca e da proteína bruta ($p<0,05$) maiores que os de *A. cochliacantha* e da mistura 1:1. A quantidade de proteína que chega ao intestino delgado é maior para a mistura 1:1, o que é indicativo de que sua concentração de tanino é suficiente para aumentar a proteína não degradável no rúmen absorvida no intestino delgado.

Termos para indexação: digestão enzimática, alimentação, legumes, ruminantes, tanino.

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Introduction

The cost of livestock feed supplementation with protein concentrates is high, which has motivated the search for alternative feed sources with a good nutritional quality, accessibility, and low costs (Pérez-Gil Romo et al., 2014). Tree fruits are resources with little-explored potential that can be used as alternative feed supplements for animal feed. Although their chemical composition varies, several fruits have high protein and low fiber contents, which promote a higher voluntary consumption and digestibility of dry matter (Clavero, 2013). Fruits may also contain secondary compounds (Hernández-Morales et al., 2018) whose concentrations vary depending on climatic factors over time (Herrera et al., 2017), including condensed tannins, which, at high concentrations, can alter ruminant consumption, digestibility, and nitrogen use.

In adequate quantities (<4%), tannins function as natural protectors of proteins (Bunglavan & Dutta, 2013), preventing their degradation in the rumen by forming stable complexes that are dissociated in the abomasum, leading to protein absorption in the lower digestive tract (Min et al., 2012; Sosa-Pérez et al., 2017). Therefore, forage plants with high tannin levels, mixed with species with high soluble nitrogen, are an alternative for optimizing nitrogen use by ruminants and improving the productive behavior of these animals (Kelln et al., 2021).

Fruits of *Chloroleucon mangense* Britton & Rose and *Acacia cochliacantha* Humb. & Bonpl. ex Willd., which are native arboreal species from low deciduous forests, can be used to supplement livestock feed due to their nutritional characteristics, acceptability, and tannin content (Cervantes-Marín et al., 2015), mainly during the dry season when the production of these fruits coincides with a lower grass yield. Fruits of *C. mangense* and *A. cochliacantha* contain 20 and 12% crude protein, 45 and 72% neutral detergent fiber, 31 and 45% acid detergent fiber, and 0.08 and 1.20% condensed tannins, respectively (Hernández-Hernández et al., 2017).

However, in addition the nutritional content of the fruits, it is also important to know their nutritional value, measured through their digestibility, which determines if they can be absorbed and, consequently, used efficiently in sheep diets with a reduced dependence on external inputs, such as fertilizers and commercial concentrate feeds.

The objective of this work was to determine the nutritional content and the degradability of the dry matter and protein of fruits of *Chloroleucon mangense* and *Acacia cochliacantha* and a 1:1 mixture of both offered as supplements to Rambouillet sheep.

Materials and Methods

The fruits of *C. mangense* and *A. cochliacantha* were harvested manually during the fruiting season between January and March, 2018, in pastures in the community of Angostillo, in the municipality of Paso de Ovejas, in Veracruz, Mexico (96°54'19''W, 19°21'80''N, at 260 m of altitude). After harvested, the fruits were sundried for approximately two days, milled using a 3 mm diameter sieve in a hammer mill, and stored in closed containers under 19°C and 33% relative humidity. A 1:1 (m/m) mixture with the milled fruits of both species was prepared to be used in the experiments. Samples from each crushed fruit and from the 1:1 mixture were ground in a Wiley mill, with a 1 mm sieve for the chemical compositions analysis and ruminal incubation to determine in situ degradability.

The proximate chemical analysis of the ground fruits was performed using the methods described by Association of Official Analytical Chemists (AOAC International) (Latimer Jr., 2016). Neutral detergent fiber (NDF) and acid detergent fiber (ADF) were obtained using the detergent method described in the Agricultural Handbook of United States Department of Agriculture (Goering & Van Soest, 1970). These analyses were carried out at the Animal Nutrition Laboratory of Colegio de Postgraduados, located in Montecillo, Mexico.

Total tannins concentration in the fruits was determined using the total phenol content calculated by the Folin-Ciocalteu method (Makkar, 2003). The concentration of tannins associated with proteins was obtained following the technique proposed by Barahona et al. (2003). All analyses were conducted at the Phytochemical Laboratory of Colegio de Postgraduados, also in Montecillo, Mexico.

To estimate the in situ degradability of dry matter and protein, three adult Rambouillet rams, with 65.06 ± 1.75 kg live weight, 2.50 ± 0.04 of age, were used. A permanent cannulae, with 50 mm in diameter, was fitted in the rumen of the animals, which were housed

in individual cages and fed with 2 kg dry matter per animal per day of a comprehensive diet composed of 30% commercial Borrega Plus concentrate (Alimentos Unión Tepexpan, Tepexpan, Mexico) and 70% oat hay. The entire ration contained 12.0% crude protein, 40.0% ADF, 51% NDF, 2.3% ether extract, and 9.0% ash. Water was available ad libitum throughout the experiment.

Fruit degradability was evaluated using a simple crossover design in three experimental periods of 72 hours. Prior to each evaluation period, there was an adaptation and a rest period of seven days to stabilize the ruminal environment of the animals. Throughout the adaptation period, 500 g per animal per day of fruits of *C. mangense* and *A. cochliacantha*, individually or together in the 1:1 mixture were offered, representing the different treatments assigned to each ram in each evaluation period.

Ruminal incubation was performed using 5.0x5.5 cm multilayer polyester F57 filter bags (Ankom Technology, Macedon, NY, USA). The bags were filled with 0.5 g of the fruits used in each experimental period, which were placed, together with a metal weight, inside a net tied to a 70 cm nylon rope to guarantee that the samples remained immersed in the ventral sac of the rumen. A total of four bags of each fruit species and of the 1:1 mixture were incubated at 0, 6, 12, 24, 48 and 72 hours, being. introduced into the rumen in a reverse order to incubation time, so that all of them could be removed the same time. Once removed, the bags were washed with low-pressure tap water until the water was clear. All samples were dried in a forced-air oven at 65°C for 24 hours and then weighed.

To calculate the crude protein in situ degradability, the contents of dry matter and crude protein were determined from the residues of ruminal incubation using methods 954.01 and 925.09 of AOAC International (Latimer Jr., 2016). Dry matter losses were calculated as the weight difference in the food sample in the bags before and after ruminal incubation, according to the protocol of Orskov & McDonald (1979).

To determine intestinal protein degradability, a three-step procedure developed by Calsamiglia & Stern (1995) was followed, consisting of an in situ ruminal incubation (step 1) and a subsequent in vitro digestion (steps 2 and 3).

In step 1, for ruminal incubation, eight F57 filter bags, with 1 mm particle size, were filled with 1.5 g of samples of both fruits and the 1:1 mixture, being then, incubated in the rumen of the three rams. A crossover design was used with three experimental periods of 16 hours of incubation. Prior to each period, there was a rest and an adaptation period of seven days for each treatment before incubation. After the incubation period, the bags were removed from the rumen, washed with tap water until the water was clear, and dried in an oven at 65°C for 24 hours. The contents of each bag were removed and divided into two portions: one to quantify crude protein using the micro-Kjeldahl method (AOAC Internacional) (Latimer Jr., 2016) and the other to be used for enzymatic digestion.

In step 2, to determine abomasal enzymatic digestion, a sample of the residue from ruminal incubation was taken from each bag, which was estimated to contain, at least, 15 mg of residual nitrogen, and was placed in a 50 mL centrifuge tube. Afterwords, 10 mL of 0.1 N HCl solution at pH 1.9, containing 1.0 g L^{-1} P7012 pepsin (Merck & Co. Inc., Rahway, NJ, USA) were added to the tube, which was mixed for 10 s in the 37600 vortex (Barnstead Thermolyne Corporation, Thermo Fisher Scientific Waltham, MA, USA). The sample was incubated for 1 hour at 38°C in the BD-100 water bath (RIOSSA, Monterrey, Mexico) under constant shaking. After incubation, 0.5 mL of 1.0 N NaOH was added. The product obtained in this step was reserved to be used in step 3.

In step 3, to determine intestinal enzymatic digestion, 13.5 mL pancreatin buffer, with 0.5 mol L^{-1} KH_2PO_4 of standardized buffer at pH 7.8, containing 50 ppm thymol and 3.0 g L^{-1} of P7545 pancreatin (Merck & Co. Inc., Rahway, NJ, USA) were added to each one of the tubes with remaining sample from step 2. The mixture was incubated at 38°C for 24 hours in the BD-100 water bath (RIOSSA, Monterrey, Mexico), with manual shaking every 8 hours. After incubation, to precipitate proteins, 15 mL of 10% trichloroacetic acid were added to the mixture that was gently shaken manually to mix its contents. The mixture was incubated for 15 min at room temperature (25°C), then it was centrifuged using a J2-HS centrifuge (Beckman Coulter Inc., Brea, CA, USA) at 7,500 rpm for 15 min and the supernatant was used to determine crude protein content through the micro-Kjeldahl method (AOAC International) (Latimer Jr., 2016).

Given the results of the three-step in vitro and in situ procedures described previously(Calsamiglia & Stern, 1995), the percentages of initial crude protein, ruminal undegraded protein, digested protein in the small intestine, and digested protein were calculated in relation to the initial content in the small intestine. Specifically, the respective values obtained were: percentage of initial crude protein in foods not subjected to ruminal incubation; proportion of initial crude protein undegraded in the rumen (RUP), showing the relationship between the amount of protein obtained after ruminal incubation and before incubation; protein fraction digested in the intestine considering the value of the protein that remained from ruminal incubation (IDP), representing the ratio between the amount of protein after abomasal enzymatic action and that after ruminal incubation; and proportion of initial crude protein digested in the small intestine in relation to the initial protein content (IADP), that is, the ratio the ratio between the proportion of initial crude protein that was not degraded in the rumen and the protein fraction that was digested in the intestine.

The percentages obtained for each bag were used to project the quantity, in grams, of: crude protein that an animal can absorb from 500 g of ingested fruits, crude protein that the animals received during the adaptation period for the analysis of in situ degradability initial crude protein offered, rumen undegraded protein, and small-intestine digested protein that the animal can assimilate.

The chemical-nutritional quality of the fruits, the presence of total tannins, and the tannins bound to proteins were analyzed using descriptive statistics. The ruminal degradability (D) values of dry matter and crude protein were adjusted with the model (equation) proposed by Orskov & McDonald (1979), using a non-linear regression within the NLIN procedure of the SAS software (SAS Institute Inc., Cary, NC, USA). The parameters (a, b, c, and a + b) for each fruit analyzed were compared with the 95% asymptotic confidence interval: $D = a + b \times (1 - e^{-c \times t})$, where a is the soluble fraction of the substrate, b is the insoluble but potentially degradable fraction, c is the degradation rate of the potentially degradable fraction, and t is incubation time.

To estimate the effective degradability (ED) of dry matter and crude protein, the previously estimated values of a, b, and c were applied in another equation proposed by Orskov & McDonald (1979), including the addition of a passage rate constant (k), whose value was 2% per hour: $ED = a + (b \times c)/(c + k)$.

The data obtained from the intestinal degradability of protein (percentages of crude protein, RUP, IDP, and IADP, as well as grams of crude protein, RUP, and IDP) were analyzed in a completely randomized design using the GLM procedure of the SAS software (SAS, Institute Inc., Cary, NC, USA). Differences between means were estimated using Tukey's test, at 5% probability.

Results and Discussion

The fruits of *C. mangense* contained high percentages of crude protein and low ones of NDF, ADF, and tannins, whereas those of *A. cochliacantha* had low percentages of crude protein, but higher ones of NDF, ADF, and tannins (Table 1). The 1:1 mixture had intermediate concentrations compared with both fruit species. The nutritional composition of the two fruits and the 1:1 mixture sufficiently covers the nutritional requirements for sheep maintenance recommended by National Research Council (NRC, 2007). Compared with tropical grasses, which can contain 4–8% crude protein, 70–75% NDF, and 48–52% ADF (Rivas-Martínez et al., 2023), the fruits of *C. mangense* and *A. cochliacantha* and the 1:1 mixture have relatively higher crude protein concentrations and lower NDF contents. Therefore, the nutritional content of the fruits and the 1:1 mixture can increase the amount of nitrogen consumed by grazing animals,

Table 1. Chemical composition of fruits of *Chloroleucon mangense* and *Acacia cochliacantha* and of a 1:1 mixture of both, collected from trees dispersed in pastures in the municipality of Paso de Ovejas, in Veracruz, Mexico.

Fruit composition	*C. mangense* (%)	*A. cochliacantha* (%)	1:1 mixture (%)
Humidity	7.2	6.3	6.8
Dry matter	92.8	93.6	93.1
Crude protein	21.7	12.1	18.4
Ash	4.3	3.7	4.0
Neutral detergent fiber	47.4	56.8	51.1
Acid detergent fiber	31.6	43.5	37.4
Ether extract	1.0	0.4	0.8
Condensed tannins	0.9	6.2	2.9
Tannins/protein	0.4	3.8	1.9

causing the microbial flora in the rumen to function adequately, consequently, improving the dry matter intake and digestibility of small ruminants (Quiroz-Cardoso et al., 2015).

The concentrations of condensed tannins in the fruits of *C. mangense* and *A. cochliacantha* differ from that reported by Hernández-Hernández et al. (2017) for fruits collected from the same region but in different years, which is an indicative that different environmental conditions affect the concentration of secondary compounds in plants (Herrera et al., 2017). The concentrations of condensed tannins in *C. mangense* and in the 1:1 mixture are less than 4%, which is a percentage when they are expected to form stable complexes with proteins in the ruminal environment, limiting ruminal degradation (Min et al., 2002). In the abomasum, where the pH is lower, this complex dissociates to permit the digestion of proteins in the lower parts of the digestive tract, enhancing ruminant performance (Min et al., 2012; Sosa-Pérez et al., 2017). Cervantes-Marín et al. (2015) added that *C. mangense* and *A. cochliacantha* showed considerable acceptability and did not affect small-ruminant consumption as long as their tannin contents did not exceed 75 and 52 g dry matter per animal and other foods or forages were also offered as feed.

The degradation kinetics for the dry matter of both fruits and the 1:1 mixture differed (Table 2). The easily soluble degradable fraction of *C. mangense* and the mixture was higher ($p<0.05$) than that of *A. cochliacantha*. However, the potentially degradable fraction, potential degradability (soluble fraction + degradable fraction), and effective degradability were higher only in *C. mangense* ($p<0.05$). The greater in situ ruminal degradability of the dry matter of *C. mangense* and the 1:1 mixture is attributed to the higher soluble fraction of both that results in a rapid fermentation of the dry matter consumed and in a shorter retention time of the digesta in the gastrointestinal tract. In addition, the higher degradable fractions and potentially degradable fractions in *C. mangense* may be explained by its higher cellular contents, lower fiber fractions, and low total tannin concentrations in the bonds with other nutritive compounds, whose concentrations are lower than in *A. cochliacantha* and in the 1:1 mixture (Hernández-Morales et al., 2018). Moreover, the effective degradability of the dry matter of *A. cochliacantha* was lower due to the presence of tannins and the higher content of ADF in its composition, affecting dry matter digestibility, important both for fiber composition and quantity, due to the negative correlation between ADF content and degradability (Gerude Neto et al., 2016; Ma et al., 2019).

The 1:1 mixture showed a better and effective dry matter degradability in the rumen because of the combination of the nutritional characteristics of both fruits, allowing to obtain a considerable proportion of soluble nutrients, with a value intermediate to those of *C. mangense* and *A. cochliacantha*, but similar to that of the latter. The dry matter degradability of the mixture was also favored by its lower tannin concentration and biological activity (Pinto Trinidad et al., 2019). This confirms that the ruminal degradability of dry matter is determined by the interactions between nutrients and secondary compounds in food, which regulate the consumption of foods rich in secondary compounds with nutritional benefits (Egea et al., 2016).

According to Barahona et al. (1997), decreasing tannin concentrations in forage of legume species, the degradation of the protein in the rumen increases and, consequently, less nitrogen reaches the duodenum.

Table 2. Parameters of ruminal and effective degradability of fruits of *Chloroleucon mangense* and *Acacia cochliacantha* and of a 1:1 mixture of both offered as supplements to sheep[(1)].

Parameter	*C. mangense*	*A. cochliacantha*	1:1 mixture
	Ruminal degradability of dry matter		
Soluble fraction (a) (%)	27.6 ±0.7a	14.0±1.5b	25.6±0.6a
Degradable fraction (b) (%)	34.8±0.9a	33.5±1.9ab	30.6±0.9b
Degradation rate (% per hour)	5.9±0.4a	5.9±0.9a	5.03±0.41a
Potentially degradable fraction (a+b) (%)	62.4±1.5a	47.5±3.1b	56.2±1.3b
Dry matter effective degradability (%)	53.7±1.1a	39.1±2.3c	47.5±1.0b
	Ruminal degradability of crude protein		
Soluble fraction (a) (%)	37.6±1.7a	18.1±3.0b	37.8±1.8a
Degradable fraction (b) (%)	45.7±3.0a	36.9±4.0a	37.4±3.8a
Degradation rate (% per hour)	3.7±0.6a	5.1±1.5a	3.2±0.8a
Potentially degradable fraction (a+b) (%)	83.3±3.8a	55.1±6.1b	75.2±4.3ab
Crude protein effective degradability (%)	67.3±3.4a	44.7±5.0c	60.8±3.9b

[(1)]Means followed by equal letters do not differ by Tukey's test, at 5% probability.

Combining forage plants with high levels of tannins with species containing high soluble nitrogen in the ruminant diet improves nitrogen use by decreasing the soluble protein degradability in the rumen. In the 1:1 mixture, this occured for crude protein degradability since condensed tannins inhibited the activity of rumen bacteria, indirectly limiting proteolysis, and a the stable union was formed between tannins and food proteins (Rodríguez et al., 2014).

The initial crude protein content in before digestion was lower in *A. cochliacantha* (Table 3), but the percentages of RUP, IDP, and IADP were higher than those of *C. mangense* and the 1:1 mixture ($p<0.05$). The greater amount of protein that bypasses ruminal degradability and the degradable protein in the intestinedue to the nutritional content of *A. cochliacantha* are attributed to the 11 and 18% higher NDF in these fruits than in the 1:1 mixture and *C. mangense*, respectively, which is enough to modify the activity of rumen microorganisms on crude protein. Furthermore, fiber acts as a protective layer for crude protein, preventing its rapid colonization by rumen microorganisms, causing a decrease in its degradability (Slanac et al., 2013). The tannin concentration (6.2% of dry matter) of this species by combines with proteins, modifying their degradability, which affect their digestibility between 3 and 15%, especially at a concentration equal to or above 5% (Bunglavan & Dutta, 2013). This effect occurs because tannins influence the processes of ruminal protein metabolism, mainly in proteolysis, as well as the degradation of peptides and the deamination of amino acids in food, as observed in the kinetics of protein degradation (Huyen et al., 2016). Bunglavan & Dutta (2013) concluded that this species showed a lower rate of degradation and a reduction in the immediately degradable fraction of protein due to the formation of insoluble tannin-protein complexes.

Considering the initial crude protein content and RUP and IDP percentages, supplementation with 500 g per animal of the 1:1 misture resulted in a higher amount of degraded protein absorbed by the intestine, which was 20 and 25% higher than that when *C. mangense* and *A. cochliacantha* were offered individually ($p<0.05$), respectively (Table 4). The higher percentages found for the 1:1 mixture can be attributed to its crude protein content and to the percentage of this protein that escapes ruminal degradability. The combination of the soluble nitrogen concentration of *C. mangense* with the tannin content of *A. cochliacantha* compromises the protein, which can be used only partially by rumen bacteria, acting through bypass proteins or protected proteins. According to Huyen et al. (2016), tannins are metabolites that are highly resistant to enzymatic action, consequently decreasing protein digestibility by inhibiting the hydrolysis of peptide bonds and reducing the bioavailability of amino acids which causes the protein to escape ruminal degradation and increases the flux and absorption of ammoniacal nitrogen and essential amino acids in the small intestine.

The obtained results are an indicative that both *C. mangense* and *A. cochliacantha* fruits and the 1:1 mixture are viable alternatives to be used as a supplement to improve the diet of small ruminants in the dry season in systems based on cattle feeding on grasses, whose availability and nutritional quality

Table 3. Intestinal degradability of the crude protein of fruits of *Chloroleucon mangense* and *Acacia cochliacantha* and of a 1:1 mixture of both offered as supplements to sheep[(1)].

Intestinal degradability	*C. mangense* (%)	*A. cochliacantha* (%)	1:1 mixture (%)
Initial crude protein	21.7±0.5a	12.1±0.6c	18.4±0.6b
Ruminal undegraded protein	65.9±0.4c	82.1±0.4a	76.2±0.5b
Small-intestine digested protein	40.8±5.0c	60.9±6.5a	52.1±6.3b
Small-intestine initial content of crude protein	26.9±3.3c	50.0±6.4a	39.7±3.5b

[(1)]Means followed by equal letters do not differ by Tukey's test, at 5% probability.

Table 4. Degradable crude protein in the rumen and small intestine based on a simulated supplementation with 500 g per animal per day of fruits of *Chloroleucon mangense* and *Acacia cochliacantha* and of a 1:1 mixture of both, offered as supplements to sheep[(1)].

Degradable crude protein	*C. mangense* (g)	*A. cochliacantha* (g)	1:1 mixture (g)
Initial crude protein	108.89±2.9a	61.2±3.1c	93.04±3.1b
Ruminal undegraded protein	71.9±2.5a	50.3±2.9b	71.2±2.7a
Small-intestine digested protein	29.4±4.0b	30.6±3.5b	36.9±4.4a

[(1)]Means followed by equal letters do not differ by Tukey's test, at 5% probability.

fluctuates during the year, negatively affecting animal production and reproduction. The combination of these or other fruits with different nutritional quality and tannin content also allows of making better use of dietary nitrogen.

Conclusions

1. *Chloroleucon mangense* and *Acacia cochliacantha* fruits contain 21 and 12% crude protein, 47 and 56% neutral detergent fiber, 31 and 43% acid detergent fiber, and 0.9 and 6.0% condensed tannins, respectively, but *C. mangense* fruits have a higher nutritional value than those of *A. cochliacantha* and the 1:1 mixture of both.

2. The degradability of dry matter and crude protein is higher for *C. mangense* than for *A. cochliacantha* and the 1:1 mixture.

3. The amount of protein that reaches the small intestine of Rambouillet rams is higher for the 1:1 mixture than for *C. mangense* and *A. cochliacantha* fruits individually, meaning that tannin concentration in the 1:1 mixture is enough to increase the bypass protein that can be absorbed by the small intestine of the animals.

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