

FEEDING RABBITS (*ORYCTOLAGUS CUNICULUS*) WITH TREE FRUITS FROM TROPICAL DECIDUOUS FOREST

HERNÁNDEZ-HERNÁNDEZ E.* , LÓPEZ-ORTIZ S.* , VILLARRUEL-FUENTES M.† ,
PÉREZ-HERNÁNDEZ P.* , VELASCO-VELASCO J.‡ , SALINAS-RUIZ J. ‡

*Colegio de Postgraduados, Campus Veracruz, Carretera Federal Xalapa-Veracruz, km. 88.5, Predio Tepetates, MUNICIPIO MANLIO FABIO ALTAMIRANO, Veracruz, México. C.P. 91690.

†Instituto Tecnológico de Úrsulo Galván, Carretera Cardel-Chachalacas, km. 4.5, Colonia Campestre, C.P. 91667, ÚRSULO GALVÁN, Veracruz, México.

‡Colegio de Postgraduados, Campus Córdoba, Carretera Federal Córdoba-Veracruz, km. 348, Congregación Manuel León, MUNICIPIO AMATLÁN DE LOS REYES, Veracruz, México, C.P. 94946.

Abstract: The dietary preference and voluntary intake of fruits from *Acacia cochliacantha*, *Caesalpinia cacalaco*, *Vachellia pennatula*, *Chloroleucon mangense*, *Senna atomaria* and *Guazuma ulmifolia* [containing 8.5-13.6% crude protein (CP) and 25.9-72.9% neutral detergent fibre (NDF) on dry matter (DM) basis], and the partial replacement of a commercial diet with 0, 15, 30 and 45% *G. ulmifolia* fruit were studied in rabbits. Rabbits fed a standard diet (17.9% CP and 28.4% NDF) were used to study their preference (during 20-min periods) for 6 ground fruits (Experiment 1: 12 rabbits, from 45 to 56 d of age and 1180±114 g live weight [LW]), 6 pelleted fruits (Experiment 2: same rabbits from 57 to 68 d of age), and the voluntary intake of the 5 most preferred pelleted fruits (Experiment 3: 18 rabbits from 70 to 81 d of age and 2200±200 g LW). Then, we examined the inclusion of *G. ulmifolia* fruit up to 45% in the diet (Experiment 4: 12 rabbits/diet from 25 to 66 d of age, and 419±80 g initial LW). When ground fruits were offered, rabbits tended to prefer *C. mangense* (0.83±0.12 g DM; $P<0.05$), and when offered as pellets they preferred *G. ulmifolia* (4.50±0.47 g DM; $P<0.05$), as well as in Experiment 3 (36.9±3.11 g DM/d; $P<0.05$). Substitution of a commercial diet with 15, 30 and 45% *G. ulmifolia* fruits in Experiment 4 resulted in higher DM intake when fruits were included in the diet, across all experimental periods (25-38, 39-66 and 25-66 d; $P<0.001$). Feeding up to 30% of fruits improved growth rate from 25 to 38 d of age ($P<0.001$) and produced similar growth rates to the control treatment from 25 to 66 d of age. Feed efficiency was impaired above 15% substitution level during the 25-38 d period ($P>0.05$) and in all groups fed *G. ulmifolia* in the 25-66 d period ($P<0.001$). *Guazuma ulmifolia* appears to have the greatest potential as a supplementary feed for rabbits in the pelleted form. Its inclusion up to 15% in the diet might render a similar performance to that of rabbits fed commercial diets.

Key Words: preference, voluntary intake, rabbit performance, diet, tropical trees.

INTRODUCTION

In animal feed production, local products and by-products from agro-industry are always evaluated in order to supply conventional ingredients for feed formulations (Fernández-Carmona *et al.*, 2001; Nieves and Terán, 2006; Safwat *et al.*, 2014). In tropical countries, scientific research has focused on reducing production costs by replacing conventional ingredients with local resources and relying less on commercial feeds. This, in turn, has led to the testing of partial diet replacement vs. total replacement using grasses, herbaceous legumes and leguminous forage trees (Nieves *et al.*, 2001; Montejo *et al.*, 2010). The physical form of diets varies from fresh to dried, ground, pelleted and nutritional blocks (Nouel *et al.*, 2003).

Correspondence: S. López-Ortiz, silvalopez@colpos.mx. Received May 2015 - Accepted March 2017.
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Rabbits require fibrous feeds (40 to 50% fibre in the total diet) to stimulate passage through the gut (Gidenne *et al.*, 2010a). An excess of highly digestible ingredients in feeds can increase the likelihood of death due to enteritis (Fernández-Carmona *et al.*, 1998; Gidenne *et al.*, 2010b). Tree fruits have been proposed as forage (Olivares *et al.*, 2012; Cervantes-Marín *et al.*, 2015) and offered to cattle, goats and sheep, demonstrating that these tree resources have acceptable chemical and nutritional characteristics for use in animal feeds as sources of fibre, protein and energy. The fibre and energy content of some fruits could make them good feeds for fattening rabbits.

In the present research, fruits from the trees *Acacia cochliacantha* (Humb. & Bonpl.) ex Willd., *Caesalpinia cacalaco* Bonpl., *Vachellia pennatula* (Schlttdl. & Cham.) Seigler & Ebinger, *Chloroleucon mangense* (Jacq.) Britton & Rose, *Senna atomaria* (L.) H.S. Irwin & Barneby and *Guazuma ulmifolia* Lam. were used to evaluate dietary preference, voluntary intake and productivity of domestic rabbits by partially replacing a commercial diet with preferred fruits. The trees used were native tropical deciduous forest species that grow spontaneously and abundantly in soils used for crops, animal grazing and soils in disuse, and have been identified as feeds for cattle (Medina *et al.*, 2008; Villa-Herrera *et al.*, 2009; Olivares *et al.*, 2012; Cervantes-Marín *et al.*, 2015). The hypothesis was that ripe fruits from these trees can be integrated into the diets of domestic rabbits without affecting their growth traits.

MATERIALS AND METHODS

Investigation location

The research was conducted in the central region of the state of Veracruz, Mexico, where the mean annual temperature is 27°C. Minimal and maximal temperatures inside the rabbit housing facility during the experiments were 24.9±0.9 and 34.5±1.8°C, respectively.

Research protocol

Between February and July, 2014, 4 experiments were conducted to test rabbit dietary preferences, voluntary intake and performance. The experiments were performed in a 30 m² (5×6 m) barn with walls (3 sides), a concrete floor (well ventilated) and a sheet-metal roof. Rabbits were caged in suspended wooden crates (40×93×40 cm wide×long×high) lined with wire mesh and equipped with automatic water dispensers and plastic and metal feed containers. White New Zealand rabbits [*Oryctolagus cuniculus* (L.)] were acquired at weaning (25 d) from a farm located in a temperate climate area. Rabbits were provided a commercial diet that exceeded recommended levels for crude protein (CP), gross energy (GE) and digestible energy (DE) (Table 1) for rabbit fattening (Lebas, 1989; Maertens, 1992), while crude fibre (CF) and neutral detergent fibre (NDF) content were lower than that proposed by De Blas and Mateos (2010).

Fruits of *A. cochliacantha*, *C. cacalaco*, *V. pennatula*, *C. mangense*, *S. atomaria* and *G. ulmifolia* were collected after the trees had dropped their fruit (February to May, 2013) along living fences, secondary vegetation allotments and in pastures in the region. The fruits were then dehydrated by exposing them to the sun for 1 d and immediately grinding them in a hammer mill using a 5 mm screen. The ground material was sealed hermetically in 200 L plastic drums and stored in a cool, dry place.

We performed chemical analyses of the commercial diet and tree fruits. Dry matter content (DM) was quantified following Mexican standard NMX-Y-098-SCFI-2001 (Diario Oficial de la Federación, 2012). Procedures of AOAC were used to determine CP (method 990.03, AOAC, 2005) and ash (method 942.05; AOAC, 1995). Lipids (LIP) were quantified using the submersion method (Thiex *et al.*, 2003) and GE according to the crude combustion bomb calorimeter system (IKA Calorimeter System C 2000 Basic; IKA®, Werke Staufen, Germany). CF, acid detergent fibre (ADF), NDF, and lignin (LIG) were quantified following the filter-bag technique for A200 and A200I (methods 5 for ADF, 6 for NDF, and 9 for LIG) in ANKOM (2011; ANKOM Technology; New York, USA); NDF, ADF, and LIG were performed sequentially and were not corrected for ash. The samples for NDF were incubated in pancreatin (38°C for 4 h) instead of amylase prior to digestion. Chemical analyses of the commercial diet and tree fruits were performed in the Laboratory of Animal Nutrition at the Universidad Autónoma de Yucatán (Table 1).

Table 1: Chemical composition of the commercial diet and fruits.

Species	HUM %	ASH %DM	EE %DM	CP %DM	NDF %DM	ADF %DM	LIG %DM	CF %DM	DE ¹ MJ/kg DM
Commercial diet	91.3	11.0	4.6	17.9	28.4	19.5	5.0	15.1	11.11
<i>Caesalpinia cacalaco</i>	‡	4.3	‡	8.5	25.9	‡	‡	21.7	‡
<i>Senna atomaria</i>	94.5	5.0	0.7	9.7	63.0	40.9	‡	39.4	4.72
<i>Vachellia pennatula</i>	93.5	4.0	0.4	9.8	72.8	49.5	‡	40.9	4.32
<i>Guazuma ulmifolia</i>	93.9	4.7	1.5	13.6	56.0	41.1	15	36.0	6.08
<i>Acacia cochliacantha</i>	92.5	4.2	0.7	12.1	72.9	45.5	‡	29.8	7.23
<i>Chloroleucon mangense</i>	90.5	4.2	0.4	20.7	45.4	31.0	‡	25.9	8.95

DM: dry matter; HUM: humidity; ASH: ash; EE: ether extract; CP: crude protein; NDF: neutral detergent fibre; ADF: acid detergent fibre; LIG: lignin; CF: crude fibre; DE: Digestible energy.

‡ no chemical analyses were performed.

¹ Estimated according to De Blas *et al.* (1992) for the commercial diet and Wiseman *et al.* (1992) for the fruits.

The chemical composition of the fruits selected showed a wide variation in CP [ranged from 8.5 (*C. cacalaco*) to 20.7% (*C. mangense*)] and NDF [ranged from 25.9 (*C. cacalaco*) to 72.9% (*A. cochliacantha*)]. DE of the fruits was estimated using the equation from Wiseman *et al.* (1992), which considers the content of CF and CP in the feed. DE in all fruits was below 9.0 MJ/kg (Table 1), lower than the recommended levels for fattening rabbits. Even though the nutritive composition of the fruits varied and were mostly below the requirements for fattening rabbits, they were considered acceptable for mixing with other ingredients to meet the main fibre requirements of growing rabbits.

Secondary metabolites were determined for all fruits. Saponins were quantified according to Lee *et al.* (2011). Total phenolic compounds were quantified using Folin and Ciocalteu's phenol reagent (Sigma Aldrich, Saint Louis, MO, USA) and Lee *et al.* (2011). Then, total tannin content was determined following Makkar (2003), and flavonoid compounds were quantified following Chang *et al.* (2002) and Meneses *et al.* (2008). All fruits contained flavonoids, total phenolic compounds and tannins, but only *V. pennatula* and *A. cochliacantha* had saponins in high amounts (Table 2).

Experiments 1 and 2: Dietary preferences

These tests were performed to determine which of the assessed fruits were preferred by rabbits. Two preference experiments were performed using a cafeteria protocol where tested feedstuffs were offered at the same time for short periods to single rabbits (Van Soest, 1994; Nieves *et al.*, 2005; Olivares *et al.*, 2012).

Experiment 1. Twelve rabbits of 45 d of age and 1180±114 g of live weight (mean±standard deviation), with no experience in the consumption of fruits, were housed individually in the wooden crates and offered the 6 tested fruits simultaneously. The test was conducted for 12 d, of which the first 5 were used to adapt rabbits to the consumption of fruits, and the last 7 d to assess their preferences. During the adaptation period, 4.0 g of each fruit (ground to 1-3 mm particle size and non-pelleted) were offered to each rabbit for 20 min (07:40 to 08:00 h). The fruits were

Table 2: Secondary compounds in the fruits (on mg/g DM) used to feed white New Zealand rabbits during the experiments.

Fruit species	Saponins	Flavonoids	Total phenolics	Tannins
<i>Caesalpinia cacalaco</i>	nd	1.26	162.7	121.2
<i>Senna atomaria</i>	nd	3.65	41.5	2.0
<i>Vachellia pennatula</i>	252.6	1.69	30.0	10.5
<i>Guazuma ulmifolia</i>	nd	1.35	18.9	3.2
<i>Acacia cochliacantha</i>	333.1	2.20	35.2	12.8
<i>Chloroleucon mangense</i>	nd	1.56	16.2	0.8

DM: dry matter; nd: compound not detected.

placed simultaneously in feeders equipped with 6 individual spaces, one space for each fruit. Prior to these tests, close observations were made on the behaviour of the rabbits offered fruits at different times of the day, using protocols previously applied to other livestock species such as cattle (Sandoval-Castro *et al.*, 2005; Cervantes-Marín *et al.*, 2015), sheep (Borman *et al.*, 1991; Degen *et al.*, 2010) and goats (Alonso-Díaz *et al.*, 2008; Degen *et al.*, 2010). We observed that rabbits showed a better ingestive response during the morning, so we concluded that the most adequate time for testing preference was at 08:00 h, for 20-min periods.

Each animal received 120 g DM/d of a commercial diet (17.9% CP and 11.11 kcal/kg DE, on a DM basis), in 2 portions: the first (60 g) was offered in the morning immediately after removing the fruit (08:00 h) and the second at 19:00 h. Throughout the experimental period, the same protocol was followed; only the quantity of each ground fruit was increased up to 10 g.

Experiment 2. The same rabbits were used in a second preference test, 1 d after completing the first test (from 57 to 68 d of age), and using the same protocol as in the previous test, where all rabbits were offered the 6 fruits simultaneously. In this experiment, the presentation of the fruits was as pellets. Due to the greater age and greater consumption by rabbits during this period, if a fruit was completely consumed during the 20-min period, 5 g more were added to maintain the offer. Preference was based on dry matter intake of each fruit during each 20-min offering period.

The pellets were prepared manually, hydrating the coarsely ground fruits and passing the paste formed through a manual meat grinder to obtain the pellets. Then, they were dehydrated under the sun for five hours and stored in plastic buckets without lids. Pellet preparation was performed weekly.

Experiment 3. Voluntary intake

Based on results obtained from the first stage, voluntary intake of the 5 fruits having greater acceptance was measured. At this stage, *C. cacalaco* was removed because it was the fruit least preferred by rabbits. Thus, only *S. atomaria*, *V. pennatula*, *G. ulmifolia*, *A. cochliacantha* and *C. mangense* were examined. A total of 18 rabbits of 70 d of age, an average weight of 2200 ± 200 g and experienced in the consumption of fruits were used.

The experiment lasted for 12 d, of which the first 5 d were taken as an adaptation period and the remaining 7 for the experiment. The rabbits were randomly assigned to the 6 treatments (3 rabbits/treatment). Treatments consisted of offering free access to commercial diet and fruits of *V. pennatula*, *C. mangense*, *G. ulmifolia*, *A. cochliacantha* or *S. atomaria*.

During the adaptation period and experimentation, daily offerings were 80 g of each fruit type (pelleted) and 180 g of commercial diet (used in previous tests). These amounts were calculated to maintain the offer and test for voluntary intake. Fruits and commercial diet were provided daily at 08:00 h simultaneously to all rabbits after removing orts. Daily voluntary intake of fruits was evaluated by the difference between what was offered and what was refused on a dry weight basis.

Experiment 4. Productive performance of fattening rabbits

This experiment was performed to analyse the production efficiency of rabbits fed a commercial diet and *G. ulmifolia* fruit in various proportions. This fruit was chosen for the experiment because it was the most preferred and most consumed in previous tests.

Commercial diet and *G. ulmifolia* fruit were used as pellets and mixed in proportions that corresponded to each treatment. Fruit pellets were prepared weekly and stored in plastic buckets without lids for this period. The DM for the pellets made from the fruits as well as the commercial diet was determined each week in order to adjust consumption based on dry weight.

Forty-eight white New Zealand rabbits at 25 d of age, weighing 419.2 ± 80.7 g, and naïve to the experimental fruit were randomised to each of 4 treatments, which consisted of substituting the daily ration of commercial diet pellets with those of *G. ulmifolia* using the following arrangement: 0 (T0), 15 (T15), 30 (T30) and 45% (T45). Each treatment had 6 replicates and the experimental unit was 2 rabbits housed together in the same cages as in previous stages.

Rabbits were offered the corresponding experimental diets from day 1 of the experiment and the response was evaluated over a period of 6 wk from 25 to 66 d of age. During this time, diets and water were offered *ad libitum*, and feed offered and refused was weighed.

In the first week, 60 g/d of each treatment was offered to each rabbit and the amount was increased over time according to daily consumption, ensuring that rabbits would not be without feed at any time. From the first to the fourth week, the daily ration of feed was divided into 2 portions, the first offered at 08:00 h and the second at 20:00 h. During the last 2 wk, the daily rations for treatments T30 and T45 were divided into 4 portions to prevent selection of the most preferred component (commercial diet or fruit) and to ensure a more homogeneous consumption over time, with offerings at 08:00, 12:00, 20:00 and 24:00 h.

We measured the feed and fruit dry matter intake, which was determined daily by the difference between the quantity of feed offered and that rejected, with the result adjusted to dry weight. To assess weight gain, rabbits were weighed without fasting on the first day of the experiment, and then every seven days at 20:00 h using the same scale as in the previous tests.

Statistical analyses

Consumption data from Experiments 1 to 3 were analysed using a mixed model for repeated measurements, considering a compound symmetry covariance structure:

$$y_{ijk} = \mu + \tau_i + a_{k(i)} + d_j + (\tau \times d)_{ij} + e_{ijk},$$

where y_{ijk} is the consumption response variable, μ is the overall mean, τ_i is the effect of preferring the i th fruit species, $a_{k(i)}$ is the effect of animal within treatment, d_j is the effect of time (day), $(\tau \times d)_{ij}$ is the interaction effect between fruit species and time, and e_{ijk} is the experimental error assuming $e_{ijk} \sim N(0, \sigma^2)$. The random effect was rabbit nested in fruit species with $a_{k(i)} \sim N(0, \sigma_{a_{k(i)}}^2)$, and the comparison of means for all variables was performed using the LSMEANS test.

Consumption, growth rate and feed efficiency from Experiment 4 were calculated for 3 periods: day 1 to 14 (25-38 d), day 15 to the end of the experiment (39-66 d), and day 1 to 42 of the experiment (25-66 d). Analyses were performed using a generalised linear mixed model (GLIMMIX Procedure). The model for each period included treatment as the fixed effect, and replicate nested in treatment as the random effect, and considering the autoregressive AR(1) covariance structure. The model was:

$$y_{ij} = \mu + \tau_i + a_{j(i)} + e_{ij},$$

where y_{ij} is the response variable (consumption, weight gain or feed conversion), μ is the overall mean, τ_i is the effect of consuming the i th treatment diet, $a_{j(i)}$ is the random effect of the animal within treatment with $a_{j(i)} \sim N(0, \sigma_{a_{j(i)}}^2)$, and e_{ij} is the experimental error assuming $e_{ij} \sim N(0, \sigma^2)$. When F-tests were significant ($P < 0.05$), orthogonal contrasts were tested to describe performance of rabbits during the 3 calculated periods, except for mortality, which was not analysed (25-66 d). A correlation analysis associating total DM intake and maximum temperatures during Experiment 4 was performed. All analyses were performed using the Statistical Analysis System (SAS, 2012; SAS Institute Inc., Version 9.4, Cary, NC, USA).

RESULTS AND DISCUSSION

Preference for ground fruits

The most preferred ground fruit was *C. mangense* (0.84±0.12 g; Experiment 1), whereas *G. ulmifolia* and *C. cacalaco* were the least preferred (Table 3). Fruit intake fluctuated over days differently for each fruit treatment due to a day by fruit interaction ($P < 0.001$). Although rabbits expressed an overall higher intake of *C. mangense* during the 20 minutes of supply, intakes were low and overall preference was unclear.

Some rabbits showed resistance to consumption and other feeding behaviours such as sneezing and increased chewing time. These responses could occur because rabbits are not strongly capable of biting and processing highly fragmented feeds (Gidenne *et al.*, 2010c); when given a choice, rabbits prefer pelleted feed to meal or raw feedstuff (Harris *et al.*, 1983; Maertens, 2010). Consumption of the paired feedstuffs depends on their palatability, a behaviour

Table 3: Ground fruit intake by 45-d old rabbits for periods of 20 min, over a period of 7 d (n=12). Average standard feed intake: 0.39±0.63 g DM (Experiment 1).

Fruit	Fruit intake (g DM)*
<i>Caesalpinia cacalaco</i>	0.16±0.12 ^a
<i>Senna atomaria</i>	0.52±0.12 ^{bc}
<i>Vachellia pennatula</i>	0.26±0.12 ^{ab}
<i>Guazuma ulmifolia</i>	0.13±0.12 ^a
<i>Acacia cochliacantha</i>	0.40±0.12 ^{ab}
<i>Chloroleucon mangense</i>	0.84±0.12 ^c
MSE	0.213
P-value	≤0.003

*Different letters represent significant differences at α=0.05; DM: dry matter; MSE: mean square error.

Preference for pelleted fruits

In this second experiment, consumption of the most preferred fruits was higher than in the previous test (Table 4), but cannot be attributed only to the fact that fruits were pelleted. At this point, *G. ulmifolia* fruits were the most accepted (4.50±0.47 g), followed by *C. mangense*, which in the first test was the most preferred. In this experiment, *C. cacalaco* fruits were the least consumed (0.26±0.47 g). Even though intakes varied over days depending on the fruit treatment ($P<0.001$), *G. ulmifolia* was the most ingested and, hence, the preferred fruit.

In this research, the preference for fruits was observed again after changing presentation to a pelleted form, which permitted the choice of fruits more palatable for rabbits. Pelleted feed is known to favour DM intake and reduce consumption time compared to ground feeds (Fernández-Carmona *et al.*, 1998; Nieves *et al.*, 2005).

The preference among fruits could be due to their hedonic value sensed through taste and odour (Provenza, 1995). For example, *G. ulmifolia* granules have a sweet taste and a smell of molasses (an ingredient known to increase feed palatability; Blas *et al.*, 1995), while the other fruits have different flavours and aromas which can promote the sensation of saturation in an animal more rapidly compared to *G. ulmifolia* (Villalba *et al.*, 2004). It is known that rabbits search for energy-rich foods because energy is the dominant regulator of feed intake (Gidenne *et al.*, 2010c); in other species, individuals tend to show preference for foods having more sugar, leading to greater intake (Burrit and Provenza, 1992).

Table 4: Pelleted fruit intake by 57-d old rabbits for periods of 20 minutes, over a period of seven days (n=12). Average standard feed intake: 2.16±2.46 g DM (Experiment 2).

Fruit	Fruit intake (g DM)*
<i>Caesalpinia cacalaco</i>	0.26±0.47 ^a
<i>Senna atomaria</i>	2.18±0.47 ^b
<i>Vachellia pennatula</i>	1.75±0.47 ^b
<i>Guazuma ulmifolia</i>	4.50±0.47 ^c
<i>Acacia cochliacantha</i>	1.49±0.47 ^{ab}
<i>Chloroleucon mangense</i>	2.79±0.47 ^b
MSE	2.41
P-value	≤0.0001

*Different letters represent significant differences at α=0.05; DM: dry matter; MSE: mean square error.

attributed to the fact that small particle sizes increase chewing and feeding time, reducing DM intake (Maertens, 2010). However, it could also be a reaction to secondary plant metabolites, as the tested fruits contain saponins, flavonoids and/or polyphenols (Table 2). For example, *C. cacalaco*, one of the least preferred fruits, contained the highest amounts of phenolic compounds (162.7 mg/g DM) and tannins (121.2 mg/g DM), while *C. mangense*, the most preferred fruit in this experiment, contained only 16.2 and 0.8 mg/g DM, respectively. At high amounts in the diet, these secondary compounds are known to limit intake by herbivores (Provenza *et al.*, 2007). However, we determined that improving the physical presentation of fruits was a priority for testing preference through the elimination of non-acceptance as a limitation for rabbits consuming feeds with small particle sizes.

Preference for the fruits tested in this study are known to exist in ruminants (Medina *et al.*, 2008; Olivares *et al.*, 2012; Cervantes-Marín *et al.*, 2015), but not in rabbits. The present research indicates that tree fodder fruits can be included in rabbit diets and that *G. ulmifolia* fruit has the greatest potential.

Voluntary fruit intake

Even though interaction of fruit×day existed ($P=0.04$), the magnitude of the difference between the most consumed fruit and the others enabled us to discern a higher intake of *G. ulmifolia* ($P<0.001$; Table 5), as it was 3 times higher (36.9±3.11 g/d) than the consumption of *V. pennatula* (the next highly ingested fruit). This finding was consistent with the results of the previous preference test with pelleted fruits, in the sense that the most preferred fruit

Table 5: Voluntary intake of commercial diet and fruit by 70-d old rabbits, over a period of 7 d (n=18). Average standard fruit intake: 12.69±15.57 g/d (Experiment 3).

Fruit	Total consumption (g DM/d)	Commercial diet (g DM/d)	Fruit (g DM/d) [†]	Fruit (%)*
Commercial feed	127.5±10.8	127.5±12.7	‡	‡
<i>Senna atomaria</i>	128.1±10.8	125.8±24.2	1.9±3.1 ^a	1.5
<i>Vachellia pennatula</i>	140.5±10.8	128.0±18.4	12.4±3.1 ^c	8.8
<i>Guazuma ulmifolia</i>	147.3±10.8	110.4±22.9	36.9±3.1 ^d	25.0
<i>Acacia cochliacantha</i>	127.9±10.8	123.7±16.9	4.3±3.1 ^a	3.4
<i>Chloroleucon mangense</i>	139.8±10.8	131.9±18.8	7.9±3.1 ^b	5.7
MSE	197.07	‡	52.55	‡
P-value	0.70	‡	≤0.001	‡

[†]Different letters represent significant differences at α=0.05; *Values represent the consumption of fruit (g) expressed as a percentage of total consumption; ‡No data.

DM: dry matter; MSE: mean square error.

(*G. ulmifolia*) was consistently the most ingested when it was offered on a daily basis. The least ingested fruits were *A. cochliacantha* and *S. atomaria*. Despite the differences in fruit consumption expressed by the rabbits, daily DM feed intake was similar in all treatments (on av. 127 g/d; P=0.69; Table 5).

Consumption of fruits by rabbits differed over the 7 d of the experiment due to fruit×day interaction (P=0.04). Most ingestion occurred during the first 2 d for most of the fruits, followed by a decline on days 3 and 4, and finally an increase during the last 3 d (Figure 1). The consumption pattern over time was considered normal for individuals given the opportunity to choose among feeds (commercial diet and one fruit) with different chemical characteristics. Animals having the capacity to detect flavour and nutrients among feeds can temporarily set aside food for later consumption, thus alternating their intake (Provenza, 1995; Villalba *et al.*, 2004).

Rabbit performance

The DE estimated for commercial diet (11.11 MJ/kg) was higher than the recommended amount for growing rabbits (De Blas and Mateos, 2010), and also higher than for *G. ulmifolia* fruit (Table 1). Consequently, DE content in the offered diets in Experiment 4 decreased when *G. ulmifolia* fruit was included (9.34, 8.34 and 6.56 MJ/kg in T15, T30 and T45, respectively).

Based on the experimental treatments, 0, 15, 30 and 45% of pelleted *G. ulmifolia* were used as a substitute for commercial diet (offered simultaneously in each treatment). However, the true intake was 0, 16, 25 and 41% for each treatment. This discrepancy occurred because rabbits had the opportunity to choose between fruits and commercial diet, even when they were mixed. At low substitution levels, the fruit was completely consumed first, and at high substitution levels the first to be completely consumed was the commercial diet. Percentages of true daily consumption had a similar tendency to those observed in the previous voluntary intake test where rabbits voluntarily included up to 25% of *G. ulmifolia* fruit in their daily diet (Experiment 3, Table 5), and some included up to 41%.

Substitution of the commercial diet with *G. ulmifolia* fruit resulted in greater intake by rabbits (P<0.001) during the 25-38 d period (Table 6), whereas DM intakes at 15,

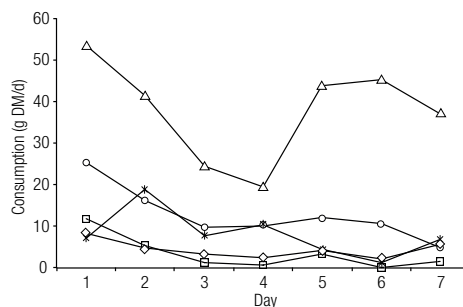


Figure 1: Voluntary daily intake of fruits by 70-d old rabbits over a period of 7 d (n=18; P_{fruit×day}=0.041; Experiment 3). DM: dry matter. ◇ *Acacia cochliacantha*; △ *Guazuma ulmifolia*; ✱ *Chloroleucon mangense*; ◻ *Senna atomaria*; ○ *Vachellia pennatula*.

30 and 45% levels of fruit inclusion were similar ($P>0.05$). *G. ulmifolia* fruits in the diets led to an increase in total DM intake. This could be explained by the fact that the NDF content in the commercial diet was below the recommended content for fattening rabbits, thus maintaining lower intakes by rabbits in T0, but compelling rabbits in the other treatments to increase intake to meet their energy needs and to maintain caecal fermentation activity (de Blas and Mateos, 2010). In fact, digestible energy offered in the daily diet in our experiment decreased from 11.11 to 6.56 MJ/kg DM, from T0 to T45. While the content of this component in T0 was about adequate for fattening rabbits, it was deficient in the treatments that included *G. ulmifolia* fruits. Even though fibre fractions and digestible energy content in the diets might explain increased daily intakes, there is the possibility that the pleasant flavour (sweet) of the fruits had also contributed to increased consumption (Atwood *et al.*, 2006; Al-Dobaib *et al.*, 2007). During this period, feeding *G. ulmifolia* fruits also improved weight gain in rabbits ($P=0.0007$). Although gains declined when 45% of fruit was included in the diet, they still remained higher or equal to gains in T0 (Table 6). Consequently, feed efficiency during this period was similar among treatments including *G. ulmifolia* and T0 ($P>0.05$; Table 6), although the efficiency declined in T45.

During the 39-66 d period, substitution of commercial diet by *G. ulmifolia* resulted in a greater intake ($P<0.001$; Table 6), and as in the first complementary period, DM intake at 15, 30 and 45% levels of fruit inclusion remained similar ($P>0.05$). There was an effect of treatment on daily weight gain during this period ($P<0.001$), feeding *G. ulmifolia* fruits (average of T15, T30 and T45) reduced by 13% the growth rate compared to T0 ($P=0.004$), and decreased successively with each level of inclusion. Feed conversion also differed among treatments during this period ($P<0.001$) and, consistently with the decline in daily weight gains, it increased in treatments that included fruits and was also higher in T30 and T45. Thus, feed efficiency decreased from 4 to 30% when *G. ulmifolia* fruits were added to the diets (see higher feed conversion values for T15, T30 and T45 in Table 6), with feed efficiency declining at higher levels of fruit inclusion (up to 26% in T45 relative to T15).

Substitution of commercial diet by *G. ulmifolia* fruit also resulted in a higher intake by rabbits over the whole experiment (25-66 d period; $P<0.001$) with rabbits in treatments including the fruit having greater intake (Table 6). Conversely, feeding *G. ulmifolia* maintained similar growth rates to those in the control treatment ($P=0.073$), and growth declined as the amount of fruit in the diet increased, as during the second period (39-66 d; Table 6). Rabbits fed 45% fruits had the lowest growth rate. Overall, feed conversion was impaired when fruits were included in the diet ($P<0.001$), reducing feed efficiency from 13 to 31% (from T15 to T45) relative to T0, and clearly increasing the amounts of fruits in the diet beyond 30 and 45% reduced efficiency by 8 to 20%, relative to T15.

Table 6: Effect of substituting commercial diet with *Guazuma ulmifolia* fruits on the performance of fattening rabbits over a 42-d period. The treatments contained 0 (T0), 15 (T15), 30 (T30) and 45% (T45) fruit in the diet (Experiment 4).

Variable	Experimental diets				SEM ¹	Contrasts ²		
	T0	T15	T30	T45		T0 vs. T15+T30+T45	T15 vs. T30+T45	T30 vs. T45
25-38 (d)								
Feed intake (g/d)	52.4±6.5	64.3±8.6	67.5±5.8	67.7±5.4	1.0	-42.316***	-6.591 NS	-0.279 NS
Growth rate (g/d)	32.1±9.2	40.2±2.6	39.2±3.3	32.5±4.8	48.8	-17.057***	9.486**	6.201**
FCR (g/d)	1.60±0.22	1.61±0.20	1.71±0.25	2.08±0.12	0.04	-0.594 NS	-0.574**	-0.370**
39-66 (d)								
Feed intake (g/d)	82.9±9.9	89.9±2.3	94.8±2.6	94.4±1.2	1.0	-30.323***	-9.348 NS	0.431 NS
Growth rate (g/d)	35.9±4.0	33.9±2.6	31.6±1.3	28.4±2.7	8.1	13.868***	7.930**	3.141**
FCR (g/d)	2.26±0.25	2.63±0.15	2.98±0.09	3.33±0.22	0.03	-2.178 ***	-1.048 ***	-0.345**
25-66 (d)								
Feed intake (g/d)	72.8±8.3	81.4±3.2	85.7±2.3	85.5±2.6	1.0	-34.107***	-8.432 NS	0.195 NS
Growth rate (g/d)	35.3±3.9	36.0±1.6	34.1±1.8	29.8±2.3	7.3	5.391 NS	8.249 **	4.241**
FCR (g/d)	2.00±0.11	2.25±0.12	2.49±0.15	2.87±0.15	0.01	-1.619***	-0.859***	-0.377***
Mortality (%)	25.0	8.0	25.0	0				

¹ n=12 rabbits/diet; ²NS: not significant; ** $P<0.01$; *** $P<0.001$. FCR: feed conversion ratio. SEM: standard error of mean.

Intake was higher during the 39-66 d period, when rabbits were older, but the tendency to increase intake with fruit inclusion was similar throughout the experimental periods. Nevertheless, because this experiment was performed during the hottest period of the year (April to May), when maximum daily temperatures ranged between 30.4 and 38.0°C, we observed that DM consumption over days was negatively correlated with maximum temperatures (-0.453 ; $P < 0.001$). High temperatures are known to negatively influence DM intake (Eberhart, 1980; Gidenne *et al.*, 2010c), and thus it is possible that temperatures negatively influenced consumption in this experiment.

The higher growth rates of rabbits consuming *G. ulmifolia* in the first period (25-38 d) helped to maintain similar growth rates throughout the overall experiment (25-66 d period) between T0 and all treatments containing fruits. It seemed that the increased daily DM intake when adding fruits to the diet may have compensated for the deficient protein and energy in the treatments including fruits. However, increasing fruit content beyond 25-30% in the diet impaired growth. This can be attributed to a lower amount of protein and energy in the diet, which resulted in a lower nutrient and energy intake in rabbits from T15, T30 and T45 groups, as *G. ulmifolia* fruits contained less than 14% CP and 6.1 MJ DE/kg, which is lower than the content of commercial diets. Moreover, *G. ulmifolia* contains secondary metabolites such as phenolic compounds, tannins and flavonoids that obviously did not affect intake of rabbits in our experiment, but could have affected nutrient digestibility and daily weight gain. Saponins do not affect DM intake by rabbits (Gidenne *et al.*, 2010c), and moderate amounts of tannins in the diet of ruminants are known to benefit digestibility and true protein uptake (Min *et al.*, 2003), although high concentrations in the diet might negatively affect intake and performance in ruminants. Nonetheless, it is not possible to know to what extent tannins or other compounds in *G. ulmifolia* fruits could have affected daily weight gain in these rabbits, but it remains as a possibility.

Growth rate during the 25-38 d period was higher in the treatments including fruits than in T0; yet growth declined as dietary fruit content increased, until the mean for T45 was similar to that for T0 (Table 6). At that point, feed efficiency was not negatively affected by including fruits in the diet, but it changed during the second complementary period, when rabbits in T15, T30 and T45 had higher feed conversions, which could be a result of undergoing a longer exposure to energy and nutrient imbalances (Gidenne *et al.*, 2010c), or to an accumulative adverse effect of plant secondary metabolites in the diet. Overall, these results showed that *G. ulmifolia* fruits could be included up to 15% in the diet.

CONCLUSIONS

Guazuma ulmifolia and *C. mangense* were the 2 preferred fruits by rabbits irrespective of the fruit presentation. Rabbits with free access to pelleted *G. ulmifolia* fruits voluntarily included up to 30% in their diet, but during fattening their performance was limited when fruits exceeded 15% in the daily DM intake. Although *G. ulmifolia* fruits were the most preferred and further evaluated in this research, the other evaluated fruits also might have the potential for inclusion in rabbit diets, but under other feeding protocols that need to be investigated.

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