

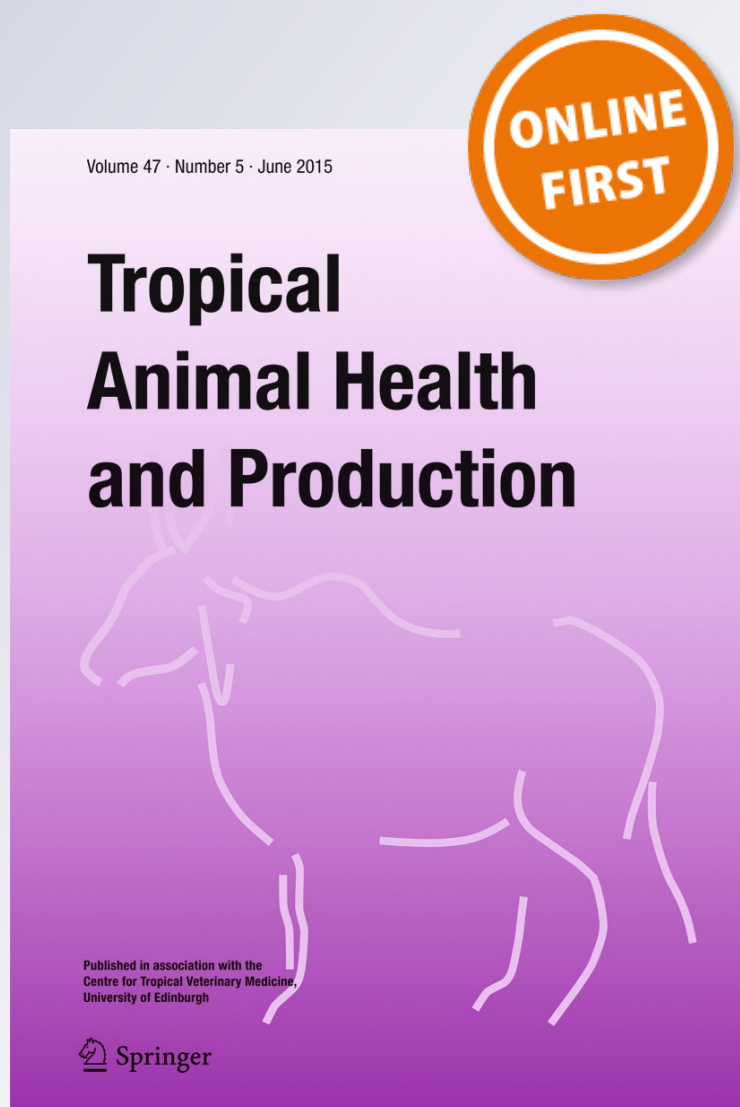
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Productive response of lambs fed *Crescentia alata* and *Guazuma ulmifolia* fruits in a tropical region of Mexico

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Abstract In vitro gas production with and without polyethylene glycol (PEG) of the fruits of *Crescentia alata* and *Guazuma ulmifolia* was evaluated, the degradation kinetics of lamb diets with added fruit of the tree was determined, and the ration intake and growth rate of lambs fed these diets were measured. Twenty-five entire male lambs of 23.5±0.44 kg body weight were used and distributed in treatments: T0 (control without fruit); T1 and T2, 15 and 30 % of the fruit of *C. alata*; and T3 and T4, 15 and 30 % of the fruit of *G. ulmifolia*. Data variables chemical composition, fermentation kinetic, and digestibility in vitro were analyzed by a completely randomized design and data production response factorials design of five treatments by three evaluation periods. The total phenolic content (TP) (23.0 g/kg DM) was higher ($P<0.01$) in the fruits of *G. ulmifolia*. The addition of PEG increased ($P<0.05$) in vitro gas production (156.6 mL/g DM) in fruits of *G. ulmifolia*. In the fermentation kinetics, the total gas volume was higher ($P<0.01$) at T0 ($b=293$ mL/g DM), and the rate of degradation (c) but Lag time (t_{lag}) was not different. In animal response, total dry matter intake was higher in lambs that received T4 (1.35 kg), and the daily weight gain and feed conversion did not differ ($P>0.05$) among lambs receiving the treatments. Thirty percent

G. ulmifolia fruit added in the diet increased dry matter intake and improved feed conversion but did not increase weight gain.

Keywords *G. ulmifolia* · *C. alata* · Consumption · Weight gain · Fermentation kinetics

Introduction

In Mexico, there are native trees that keep their foliage and fruit during the dry season and can be used for animal feed (Olivares et al. 2013; Rojas et al. 2013). *Crescentia alata* and *Guazuma ulmifolia* have high density in the area (6.0 trees/ha) with fruit production in the dry season when there is low feed availability; however, the large size and hardness of the fruit limit the intake of the small ruminants in the field, which places the mature fruit collection and grinding to feed the sheep into a viable practice in production units to provide fiber, energy, and protein in diet (Rojas et al. 2013). Mlambo et al. (2008) suggested the need to know the nutritional value of all food for proper use in animal feed. Tropical fruit tree species may contain tannins in substantial concentrations that can reduce the digestibility and voluntary intake and therefore affect animal productivity (Dey et al. 2008). However, moderate amounts of tannin may be beneficial because it can bind with the dietary protein and form complexes that prevent their degradation in the rumen and thus increase the amount of amino acids absorbed in small intestine (Waghorn 2008).

Polyethylene glycol (PEG-4000) is a nonnutritive synthetic polymer having a high affinity for forming complexes, especially with the condensed tannins (CT) (Singh et al. 2005). It has been used to mitigate the adverse effects of the secondary compounds in rumen fermentation, causing an increase in the in vitro gas production and food degradation parameters when

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PEG is added, indicating the biological activity of tannins (Getachew et al. 2000). Studies have been conducted using the fruits of trees as a source of alternative food in animal production, with attention to the nutritional and productive response of animals (Rojas et al. 2013). In this study, the in vitro gas production was determined with polyethylene glycol (PEG) of *Crescentia alata* and *Guazuma ulmifolia* fruits. Also, the kinetics of degradation of diets, with fruit added at 15 and 30 % and the productive response of lambs fed diets, were evaluated.

Materials and methods

The study was conducted in the Limones community, Pungarabato Municipality, Guerrero Mexico (18° 20' 30" NL and 100° 39' 18" WL). The area has a climate Aw0 (the driest of the subhumid), annual minimum, and maximum temperature of 28 °C to 46 °C, 250 m above sea level, and an annual rainfall of 750 mm (Fragoso 1990).

Fruits and animals used in the study

The ripe fruit of the tree was collected manually and dried by exposure to the sun at an average ambient temperature of 40 °C (with a variation of 35 to 45 °C) for 5 days until constant weight. The fruits, ground to particle size of 2 mm, were added to the diets used in the feeding experiment of growing lambs. Twenty-five entire male (Dorper X Pelibuey) lambs were used in the experiment. They weighed 23.5±0.44 kg and were 7 months old. They were dewormed with Closantel orally at doses of 10 mg/kg body weight, at the beginning of the adaptation period.

Chemical composition of fruits and treatment diets

Triplicate samples were collected randomly (i.e., 500 g of ripe fruit of *C. alata* and *G. ulmifolia* manually collected of three trees, and 250 g of the treatment diets were collected directly from the storage bags). The dried samples were placed in forced air oven at 40 °C for 72 h and were ground in a Willey mill with sieve number 1. Each sample was analyzed for crude protein (CP) (AOAC 2000), neutral detergent fiber (NDF), and acid detergent fiber (ADF) (Van Soest et al. 1991). The content of total phenols (TP) (Folin Ciocalteu method) and condensed tannins (CT) (butanol-HCl) in fruits were determined (Waterman and Mole 1994).

In vitro digestibility and fermentation kinetics of fruits and treatment diets

One gram of each sample was digested in triplicate in 160-mL bottles for estimating the in vitro gas production (IVGP) and

in vitro digestibility of dry and organic matter (IVDMD, IVOMD), by the gas production technique, proposed by Theodorou et al. (1994). The ruminal fluid collectors were placed orally through a tube in three adult sheep (Katahdin× Dorper) fed a standard diet 70:30 forage and concentrate for 30 days.

Approximately 1 g of each sample (fruit and diet treatment) was incubated at 39 °C (Incubator—Binder Company, Germany) in bottles with a capacity of 160 mL added to 90 mL of reducing solution buffered and 10 mL of ruminal fluid (previously filtered through four layers of gauze) for digestion in an anaerobic environment by adding CO₂, but only the fruits were incubated in replicate samples with and without PEG, 4000 Sigma[®] 2:1 proportion, that determined the biological activity of the tannins and total phenols (Waghorn 2008).

The gas volume (mL/g DM sample) was registered every hour during the first 8 h, then every 4 to 60 h and subsequently at 72, 84, and 96 h of incubation, using the pressure reading technique (PRT; DELTA OHM, Italy; Theodorou et al. 1994).

At the end of the incubation period, the contents of each bottle were filtered using Gooch's crucible vacuum (1 μ pore size, Pyrex, Stone, UK), and the residue was dried at 105 °C for 12 h to estimate the potential degradation of dry matter (DM). The residue was later incinerated at 550 °C for 6 h, and weight loss after incineration was used as a measure of non-degradable organic matter (OM).

The kinetics of in vitro fermentation on diets (treatment) was assessed using the model of France et al. (2000) $A = b \times (1 - e^{-c(t-L)})$, where A is the gas volume production at time t , b is the asymptotic gas production milliliter per gram DM, c is the speed the gas produced (h) of fraction b of slowly fermentable food, and L is the starting time of the fermentation of NDF (Grafi 1992).

Animal experiment

The twenty-five entire male lambs used in the experiment were distributed randomly into five groups. The animals received treatments where they were housed, in individual pens 2 m² with free access to feeders and waterers.

The ground fruits were integrated into isonitrogenous (180.0 g CP) and isoenergetic (10.0 MJ kg DM) diets, balanced according to the nutritional requirements of the lambs (NRC 2007). Fifteen and 30 % of each fruit were added to experimental diets *C. alata* (T1 and T2), *G. ulmifolia* (T3 and T4), and T0 diet without fruit served as control (Table 2). To prepare diets, regional ingredients (ground corn, ground cob, soybean meal, and mineral premix) were used.

The experiment was carried out during the dry season (March–May 2012) for a period of 65 days (20 days adaptation to diets and confinement, then 45 days of the trial). The change in body weight (g/day) was

measured at 0, 15, 30, and 45 days, using an electronic scale (TOR-KING Mod. CRS 500/1.000). The dry matter intake (g/day), the daily weight gain (DWG), and feed conversion (FC) were measured in the animals. The cost per kilogram of live weight gain was estimated by quantifying the total dry matter intake by animal, multiplied by the cost of a kilogram of ration (\$0.33, \$0.30, \$0.29, \$0.32, and \$0.30 USD, for T0, T1, T2, T3, and T4, respectively), divided between the kilograms gained during the experimental phase.

Data analysis

The data were analyzed by general linear model procedures in SAS (2002) by the Tukey's test at a significance of $P < 0.05$.

The chemical composition, IVGP, IVOMD, and IVDMD were analyzed in a completely randomized statistical design model: $Y_{ij} = \mu + T_i + \xi_{ij}$, where Y_{ij} is the response to the i th treatment in the j th measurement, μ is the overall mean, T_i is the effect of i th treatment, ξ_{ij} is the random error and a mean of zero, and variance σ^2 is assumed.

The animal response analysis design was completely randomized 5×3 factorial arrangements (five treatments for three periods of evaluation, days 15, 30, and 45). The statistical model was $Y_{ijk} = \mu + T_i + B_k + T_i \times B_k + E_{ijk}$, where Y_{ijk} is the response variable, μ is the general mean, T_i is the treatment effect ($i = T0, T1, T2, T3, \text{ and } T4$ treatments), B_k is the effect of period ($k = 1, 2, \text{ and } 3$ evaluation periods), $T_i \times B_k$ is the interaction of treatment (i) for the period (k), and E_{ijk} is the random error treatment (i) in repetition (j) in the period (k), term: $n - 1$ ($0, \sigma^2$).

Results

Chemical composition of fruits

The protein content of the fruit is relatively low compared to the minimum required (80 g/kg DM) by ruminal microflora, and the content of acid and neutral detergent fibers is relatively high, limiting its digestibility (Table 1). The TP and CT of *G. ulmifolia* fruits had higher biological activity to bonding the fiber and protein. It was demonstrated that the observed gas production increased ($P < 0.05$) when the fruits were subjected to digestion with added PEG-4000 (Table 1).

Chemical composition of treatment diets

The protein and energy content of the diets were similar and sufficient for the nutritional requirements of the lambs according to their body weight and growth stage. T0 diet was lower in neutral and acid detergent fiber and had higher digestibility of dry and organic matter, compared to diets of treatments 2, 1, and 4 that had more acid detergent fiber and less digestibility of the dry and organic matter (Table 2). The total gas volume (b) produced in the diets of groups was higher ($P < 0.05$) in T0 treatment (Table 2). The degradation rate and Lag time were not different ($P > 0.05$) between groups.

Productive response of the lambs

The total dry matter intake (TDMI) was higher ($P < 0.01$) in lambs of T0 compared to T4 animals (Table 3). The animals of the different groups did not differ ($P > 0.05$) in DWG and FC;

Table 1 Chemical composition (g/kg DM) of the *C. alata* and *G. ulmifolia* fruits

	<i>C.alata</i>	<i>G.ulmifolia</i>	SEM/SD	Significance ^a
Crude protein	71.0	82.0	—	—
Neutral detergent fiber	489.0	441.0	—	—
Acid detergent fiber	376.0	341.0	—	—
In vitro dry matter digestibility	375.0	404.0	—	—
Total phenols	14.0	23.0	0.76	**
Condensed tannins	11.9	12.0	0.38	ns
In vitro gas production (mL/g DM)				
−PEG ^b	152.2	136.2	10.4	Ns
+PEG ^b	161.7	156.6	4.8	Ns
SEM/SD	8.3	7.9		
Significance ^b	ns	*		

−PEG without polyethylene glycol, +PEG with polyethylene glycol, SEM/SD standard error of means/standard deviation, ns not significant

^a Values in the same line with different subscripts indicate significant difference

^b Values in the same column with different subscripts indicate significant difference by effect of PEG

* $P < 0.05$; ** $P < 0.01$ (Tukey)

Table 2 Chemical composition of diets with *C. alata* and *G. ulmifolia* fruits added

Ingredient (kg)	T0	T1	T2	T3	T4	SEM/SD	Significance
Ground cob	38.5	32.4	15.0	29.5	12.0	–	–
Ground corn	34.0	25.2	28.0	27.0	29.0	–	–
Soybean meal	25.5	25.4	25.0	26.5	27.0	–	–
<i>C. alata</i> fruit	0.0	15.0	30.0	0.0	0.0	–	–
<i>G. ulmifolia</i> fruit	0.0	0.0	0.0	15.0	30.0	–	–
Minerals premix ^a	2.0	2.0	2.0	2.0	2.0	–	–
Total (dry matter basis)	100	100	100	100	100		
Chemical composition per kilogram dry matter basis							
Crude protein (g)	180.0	179.0	175.0	180.0	177.0	–	–
Neutral detergent fiber (g)	178.0 ^d	247.0 ^b	289.0 ^a	218.0 ^c	258.0 ^b	0.717	**
Acid detergent fiber (g)	79.0 ^d	113.0 ^c	155.0 ^a	96.0 ^{dc}	134.0 ^b	0.737	**
Ash (%)	4.7	3.8	4.9	5.2	5.9	–	–
In vitro dry matter digestibility (%)	76.2 ^a	65.8 ^{bc}	61.0 ^d	69.1 ^b	63.4 ^{dc}	1.25	**
In vitro OM digestibility (%)	73.0 ^a	63.4 ^b	58.3 ^c	65.5 ^b	59.7 ^c	1.17	**
Metabolizable energy (MJ)	11.5	10.0	9.15	10.5	9.37	–	–
Fermentation kinetic							
<i>b</i> (mL/g DM)	293.0 ^a	239.0 ^b	229.0 ^b	239.0 ^b	220.0 ^b	13.1	*
<i>c</i> (%/h)	0.069	0.069	0.063	0.072	0.061	0.005	Ns
<i>t</i> _{Lag} (h)	4.0	4.0	3.6	3.6	4.6	0.428	Ns

Values in the same line with different subscripts indicate significant difference

T0 control without fruit, T1 15 % *C. alata*, T2 30 % *C. alata*, T3 15 % *G. ulmifolia*, T4 30 % *G. ulmifolia*, OM organic matter, *b* total volume of gas, *c* degradation rate % in hours, *t*_{Lag} colonization time, SEM/SD standard error of means/standard deviation, ns not significant ($P > 0.05$)

* $P < 0.05$; ** $P < 0.01$ (Tukey)

^aMineral premix: dry matter (99 %), phosphorous (0 %), calcium (24 %), sodium (4 %), chlorine (10 %), magnesium (1.8 %), sulfur (2.1 %), iron (1.170 ppm), manganese (670 ppm), copper (335 ppm), zinc (1000 ppm), selenium (7 ppm), cobalt (3 ppm), iodine (17 ppm), vitamin A (73 KUI/kg), vitamin D (9 KUI/kg), and vitamin E (1335 UI/kg).

therefore, the lambs fed diets with fruits added (T1, T2, T3, and T4) maintained a growth performance similar to control animals (T0) (Table 3). All lambs showed a significant increase in the total dry matter intake ($P < 0.001$) and daily

Table 3 Productive response of lambs fed with diets with *C. alata* and *G. ulmifolia* fruits added

Variable	T0	T1	T2	T3	T4	SEM/SD	Significance
Initial weight (kg)	22.7	23.8	23.6	23.6	23.7	–	–
Final weight (kg)	33.4	34.6	35.1	35.6	35.8	–	–
Total dry matter intake (kg)	1.04 ^b	1.15 ^{ab}	1.20 ^{ab}	1.25 ^{ab}	1.35 ^a	0.72	**
Daily weight gain (g)	236.4	238.2	255.1	267.1	268.2	32.2	Ns
Feed conversion (kg)	4.9	5.1	5.1	4.9	5.2	0.93	Ns
Kilograms live weight gain (\$USD)	1.5*	1.5	1.4	1.5	1.6	0.011	Ns
Periods	1		2	3			
Total dry matter intake (kg)	1.12 ^b		1.21 ^{ab}	1.27 ^a		0.14	**
Daily weight gain (g)	233.6 ^b		247.7 ^{ab}	277.9 ^a		53.1	*
Total dry matter intake (g) Period×treatment						95.0	Ns
Daily weight gain (g) Period×treatment						50.6	Ns

Values in the same line with different subscripts indicate significant difference

T0 control without fruit, T1 15 % *C. alata*, T2 30 % *C. alata*, T3 15 % *G. ulmifolia*, T4 30 % *G. ulmifolia*, SEM/SD standard error of means/standard deviation, ns not significant ($P > 0.05$)

* $P < 0.01$; ** $P < 0.001$ (Tukey)

weight gain ($P < 0.01$) from the first to the third evaluation period, respectively. There was no significant interaction effect of period \times treatment ($P > 0.05$) in the TDMI and DWG ($P > 0.10$). In the economic evaluation, the cost per kilogram of live weight gain was similar in lambs receiving the different treatments ($P > 0.05$) (Table 3).

Discussion

Chemical composition of the fruit

The contents of neutral and acid detergent fibers indicate that the two fruits can be an important source of fiber in the diet of lambs and thus promote good digestion of the diet (Arroquy et al. 2005). On the other hand, the low crude protein content of the fruit can be a limiting factor; however, the contributions in this nutrient are comparable to most feed and forage energy prevalent in tropical regions of Mexico.

The addition of PEG-4000 to *G. ulmifolia* fruits increased *in vitro* gas production during digestion, demonstrating that the TP and CT content in the fruits has biological activity for binding to the fiber and protein and inhibiting their digestion. Studies have shown that the addition of PEG forms tannin complexes, reversing the astringent effects that limit the digestibility of dietary nutrients (Olivares et al. 2013; Waghorn 2008). The tannin-PEG reaction is determined by the chemical composition and structure of the tannins in each food (Torres et al. 2008).

Chemical composition of treatment diets

The T0 diet yielded a higher gas volume (293 mL/g DM) and was attributed to the greater IVDMD and lower NDF due to increased quantity of grains (Table 2). Haddad et al. (2007) observed that diets with considerable quantities of grains increased the rate of digestion and prolonged digestion time.

Productive response of lambs

The low TDMI observed in lambs that received the control diet (T0) was attributed to the lower content of NDF (178 g) and higher IVDMD (76.2 %) (Table 2) that could have an effect on the rate of digestion by an imbalance in the ruminal microflora. Russell et al. (1992) suggest that diets with less than 200 g NDF/kg DM reduce the synthesis of microbial protein by as much as 2.5 % and lower the concentration of rumen microorganisms.

Moreover, the TDMI observed in lambs that received treatments with added fruits was similar to results reported by other researchers using fruits in small ruminants (Rojas et al. 2013).

In addition, the fruits integrated to the diets favored DWG in lambs higher than 230 g, similar to animals of the control treatment (T0), which indicates that the fruits of *C. alata* and *G. ulmifolia*, can be used up in the 30 % inclusion in diets of growing lambs without risk of decreasing their production parameters. Several studies have demonstrated the usefulness of the fruit from the trees as a supplement in feeding small ruminants in the dry season to overcome the nutrition deficit and increase and/or maintain production levels (Rojas et al. 2013).

The increase in DWG of lambs from first to third evaluation period was directly attributable to the increase in dry matter intake of animals and therefore the increased nutrient intake to the digestive tract functions for growth and development.

Equality in economic benefit per kilogram of live weight gain was attributed to the similarity observed between animals of different treatments in TDMI, the cost of feed ingredients that constituted the treatments, and the DWG and FC, which were key variables for estimating the cost per kilograms of live weight gain. Anbarasu et al. (2004) reported that the cost of feeding depends on dry matter intake, ingredient costs, and efficiency in the use of diets.

Conclusions

The fruits contributed significant amounts of detergent fibers in the diets (30 to 40 % of NDF and 18 to 50 % of ADF) that affected the IVDMD and IVOMD. The lambs did not reject diets containing *G. ulmifolia* and *C. alata* fruits, i.e., the addition of fruits increased linearly dry matter intake during the evaluation period. The fruit of *G. ulmifolia*, when comprising 30 % of the diet, increased the TDMI but was not reflected in higher DWG, FC, or higher benefit-cost ratio. The fruits of *G. ulmifolia* and *C. alata* can substitute approximately 24.0 % of the ground corn without diminishing the productive response of growing male lambs.

Conflict of interest The authors declare that they have no conflict of interest.

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