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Chemical Composition, *In vitro* Digestibility of Foliage *Guazuma ulmifolia* and *Crescentia alata* and its Use in Feeding Lambs

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Abstract: Chemical composition, Gas Production *in vitro* (IVGP), *in vitro* Dry Matter (IVDMD) and Organic Matter (IVOMD) digestibility, was determined in foliages of *C. alata* and *G. ulmifolia*; it was also determined the growth performance of 25 lambs (body weight: 22.9±0.8kg), assigned to the following diets: T0 = control diet without foliage, T1 and T2 = 15 and 30% of *C. alata* and T3 and T4 = 15 and 30% of *G. ulmifolia* respectively. The animal measured response variables were: Dry Matter Intake (DMI), Total Weight Gain (TWG), Daily Weight Gain (DWG) and Feed Conversion (FC). Crude Protein (CP), Total Phenolics (PT), Condensed Tannins (CT) and IVDMD were higher ($p<0.01$) in *G. ulmifolia*, 167.0, 38.2, 41.5 and 590.0 g/kg DM respectively. Neutral Detergent Fiber (NDF), Acid Detergent Fiber (ADF) and Metabolizable Energy (ME) was higher ($p<0.001$) in *C. alata*, 502.0, 315.0 g/kg DM and 4.7 MJ/kg DM respectively; the IVGP at 24, 48 and 96 h were higher ($p<0.001$) in the foliage of *C. alata* with 66.1, 170.9 and 210.6 ml/g DM respectively. The DMI was higher ($p<0.05$) in lambs of T1 (1339.8 g/animal/day) and T2 (1381.4 g/animal/day), the TWG and DWG was higher in lambs of T1 (13.2 kg/animal and 295.6 g/animal, respectively). T0 lambs had more FC (4.4 kg DM) ($p<0.01$). We conclude that foliages *G. ulmifolia* and *C. alata* are important source of CP and fiber in the diet of lambs and their low content of TP and CT did not affect the growth performance of animals.

Key words: *Guazuma ulmifolia*, *Crescentia alata*, lambs, productive response, kinetics of degradation

INTRODUCTION

Sheep production in the tropics is based in the use of native or induced forage as a major source of nutrients, however fluctuations in the availability and low nutritional value of forages mainly during the dry season pastures, makes present low protein and high fiber proportions, with a low digestibility and intake which determines that animal productivity is limited (Rodriguez *et al*., 2009; Ruiz *et al*., 2006).

Studies on the integration of trees and shrubs to ruminant production systems have allowed a preliminary estimate of species that currently have no use. With the integration of trees, shrubs and grasses can integrate a sustainable system of production, higher productivity and lower cost; the latter related to the high cost of raw materials for food processing, prevents the productive potential of improving ruminants (Bolio *et al*., 2006).

There is a variety of species of trees and shrubs with potential to be incorporated into sheep production systems in tropical regions. Among them are included: *Gliricidia sepium*, *Leucaena leucocephala*, *G. ulmifolia*, *Hibiscus rosa-sinensis* and *Morus alba*, mainly for their versatility of use, rapid growth and recovery after cutting and good protein content and digestibility (Garcia *et al*., 2006; Mata *et al*., 2006; Sosa *et al*., 2004). In addition, they are characterized by having an important source of protein and a good dry matter digestibility (Narvaez and Lascano, 2004; Palma, 2005). However using the foliage of these species in the diet of ruminants is a common practice by livestock farmers, their use is limited because it is ignored what is the use in animal feed and what its limitations are. Therefore, the objective was to determine in foliages of *C. alata* and *G. ulmifolia*: chemical composition, Gas Production *in vitro* (IVGP), *in vitro* Dry Matter (IVDMD) and Organic Matter (IVOMD) digestibility and the growth performance of lambs feeding diets that included tree foliages.

MATERIALS AND METHODS

The study was conducted in the community of “Los Limones”, Mpio. Pungarabato Gro., Mexico, located in the Tierra Caliente region at 18° 20' 30" north latitude and 100° 39' 18" west longitude. With Aw0 prevailing climate, annual minimum and maximum temperature of 28°C and 46°C, height above sea level 250 meters and annual rainfall of 750 mm (Fragoso, 1990).

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Table 1: Ingredients of the treatments diets, chemical composition, digestibility *in vitro* and kinetics of degradation

	Treatments						
	T0	T1	T2	T3	T4	SEM	p-value
Ingredients including (%)							
Ground cob	38.5	28.5	21.0	35.0	22.0	-	-
Ground corn	34.0	31.5	26.0	26.0	28.0	-	-
Soybean	25.5	23.0	21.0	22.0	18.0	-	-
Foliage *C. alata*	0.0	15.0	30.0	0.0	0.0	-	-
Foliage *G. ulmifolia*	0.0	0.0	0.0	15.0	30.0	-	-
Mineral salts*	2.0	2.0	2.0	2.0	2.0	-	-
Total	100.0	100.0	100.0	100.0	100.0		
Chemical composition (g/kg DM)							
CP (g/kg DM)	183.0	179.0	177.0	180.0	175.0	-	-
NDF (g/kg DM)	178.0^{c}	244.0^{b}	277.0^{a}	244.0^{b}	277.0^{a}	0.442	***
ADF (g/kg DM)	79.0^{c}	106.0^{b}	137.0^{a}	97.0^{b}	137.0^{a}	0.472	***
Ash (g/kg DM)	4.7	4.5	5.4	4.7	69.0	-	-
ME (MJ/kg DM)	12.0	12.0	10.5	11.8	10.4	-	-
***In vitro* digestibility**							
IVDMD (g/kg DM)	670.0^{a}	605.0^{b}	584.0^{bc}	637.0^{ab}	560.0^{c}	1.53	***
IVOMD (g/kg DM)	762.0	724.0	684.0	736.0	690.0	5.73	ns
Kinetic of degradation							
b (ml/g DM)	293.0^{a}	252.0^{c}	233.0^{d}	276.0^{b}	246.0^{cd}	5.7	***
c (%/h)	0.069^{a}	0.060^{bc}	0.055^{c}	0.066^{ab}	0.066^{ab}	0.003	***
t_{Lag} (h)	4.0^{ab}	3.8^{ab}	3.6^{b}	4.3^{a}	4.2^{ab}	0.233	*

T0: Control without foliage; T1: 15% foliage of *C. Alata*; T2: 30% foliage of *C. Alata*; T3: 15% foliage *G. Ulmifolia*; T4: 30% foliage *G. Ulmifolia*; CP: Crude Protein; NDF: Neutral Detergent Fiber; ADF: Acid Detergent Fiber; IVDMD: *In vitro* Dry Matter Digestibility; IVOMD: *In vitro* Organic Matter Digestibility; ME: Metabolizable Energy; b: Asymptotic gas production; c: The rate of the gas produced (h) of section b of the food slowly fermentable; t_{Lag}: Time when initiating the fiber degradation; SEM: Standard Error Means.

[abc]Means with different literals in the same row are statistically different, Tukey (ns: not significant, *p<0.05, ***p<0.001).

*Dry matter (99%), phosphorus (0%), Calcium (24%), sodium (4%), chlorine (10%), magnesium (1.8%), Sulphur (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 IU/kg)

Foliage collection: The leaves of *G. ulmifolia* and *C. alata* were collected during the months from October to February 2009, in the town of Vines, State of Mexico and the municipality of Pungarabato, Gro. There are sun exposure and ground in a hammer mill for flour and thus became part of the diet treatments.

Treatments: Diets treatments had isonitrogenous (180.0 g/kg DM of CP) and isoenergetic (11.7 Mj/kg DM of ME) according to the requirements of the lambs (NRC, 1985): T0 = control diet without foliage, T1 = diet with 15% foliage *C. alata* and T2 = diet with 30% foliage of *C. alata*. T3 = diet with 15% foliage *G. ulmifolia* and T4 = Diet with 30% foliage *G. ulmifolia*. The inclusion of ingredients in the treatments is displayed in Table 1.

Chemical composition of foliages and treatments diets: Samples of foliages and treatments diets were analyzed for Dry Matter (DM) by drying them at 105°C for 24 hrs in a forced air oven. Ash content was measured after igniting samples in a muffle furnace; at 550°C for 12 hrs and Organic Matter (OM) by a difference between DM and ash content (AOAC, 1990). The CP was determined by the Kjeldahl method (AOAC, 1990; ID 954.01). Neutral Detergent Fiber (NDF) and Acid Detergent Fiber (ADF) were determined by the method of Van Soest and Wayne (Van Soest *et al*., 1991). Total Phenolic Content (TP), (Folin Ciocalteu) and Condensed Tannins (CT) (butanol-HCl) according to the method described by Waterman and Mole (1994).

***In vitro* gas production of foliages and treatments diets:** *In vitro* Gas Production (IVGP) and *in vitro* Digestibility of Dry and Organic Matter (IVDMD and IVOMD) were determined by the gas production technique, proposed by Theodorou *et al.* (1994), based on the methodology of Menke and Steingass (1988), modified by Herrero and Jessop (1996). Rumen fluid was collected from three F1 sheep (Katahdin x Dorper), in the morning (07:00) before feeding with a standardized diet (70:30 ratio) of forage and concentrate. Rumen digest was collected directly from rumen of each animal in pre-warmed thermos flasks and hand-squeezed with the assistance of a portable bomb (BARNANT COMPANY, USA) and transported immediately to the laboratory. Rumen contents was strained through four layers of cheesecloth and kept at 39°C under a CO_2 atmosphere.

Approximately one gram of foliage and diets sample was weighed, into 160 ml bottles. Using an automatic dispenser (Jencons, Hemel Hemstead, England) 90 ml reduced reading pressure technique buffer, containing micro- and macro-elements; a reducing agent and a reduction indicator of resazurin were transferred to each serum bottle. Serum bottles without samples (i.e., blanks) were also included to allow a correction of 96 hrs in degradability values for residual feed from rumen fluid and three bottles of blank (containing only rumen fluid inoculum were incubated as blanks and used to compensate for gas production in the absence of substrate) and three samples of alfalfa as slandered were incubated in each run.

After the addition of buffer, the flasks were sealed and stored at 39°C, 8 hrs before inoculation with rumen fluid before, then they were transferred into the incubators (Binder-Incubator, Binder Company, Germany) to confirm the color that assures the suitable anaerobic conditions. Ten ml of rumen fluid were added to each serum bottle (previously prepared to contain 90 ml of buffer and one gram of browse sample) and incubated at 39°C for the 96 hrs of experiment. Once they were filled up, all the bottles were closed with rubber stoppers, crimped with aluminum seals, shaken and placed in the incubator at 39°C. The volume of gas produced was recorded at several incubation times (1, 2, 3, 4, 5, 6, 7, 8, 12, 16, 20, 24, 28, 32, 36, 40, 44, 48, 52, 56, 60, 72, 84 and 96 hrs after inoculation time), using the reading pressure technique (RPT; DELTA OHM, Italy) of Theodorou *et al.* (1994).

Estimation of truly degraded substrate in foliage and treatment diets: At the end of incubation (i.e., 96 hrs), the contents of each serum bottle were filtered using centered glass crucibles (coarse porosity no. 1, pore size µm porosity, Pyrex, Stone, UK) under vacuum. Fermentation residues were dried at 105°C overnight to estimate the potential DM disappearance (IVDMD) and then incinerated in a muffle furnace at 550°C for 12 hrs. Loss in weight after incineration was used as a measure of undegradable OM. The IVOMD at 96 hrs of incubation (g/kg DM) was calculated as the difference between OM content of substrate and its undegradable OM.

Calculations

Metabolizable Energy (ME): Metabolizable energy (ME, Mj/kg DM) in foliages was estimated according to Menke *et al.* (1979) as:

$$ME\ (MJ/kg\ DM) = 2.20 + 0.136\ GP_{24}\ (ml/0.2\ g\ DM) + 0.0057\ CP \quad (1)$$

Where GP_{24} was 24 hrs gas volume and CP (% DM) was that of the tree foliage; the content of metabolizable energy (ME MJ/kg DM) in the diets was calculated using the equation proposed by AFRC (1993) to concentrate. Equation:

$$ME\ (MJ\ .\ kg\ DM) = 0.15 * DOMDM\ (\%) \quad (2)$$

Where, DOMDM = Digestibility of Organic Matter in Dry Matter.

Kinetics of degradation: The *in vitro* degradation kinetics to 96 hrs of the diets, were calculated with the equation of France *et al.* (2000):

$$A = b \times (1 - e^{-c(t - L)}) \quad (3)$$

Where, a = volume of gas produced at time t, b = asymptotic gas production ml/g DM; c = The produced gas velocity (% h) of the fraction b of slowly fermentable food and Lag = time which is initiated the degradation of fiber

Productive response in lambs

Animal management: We used 25 lambs Dorper x Pelibuey, live weight 22.9±0.8 kg were randomly assigned to five treatments; they were housed in pens of 1 m^2 equipped with feeding and drinking. The lambs were dewormed at doses of 10 mg/kg body weight with closantel by oral application.

Measured variables: Treatments were given *ad libitum* and the study lasted 55 days which included 10 days of adaptation and 45 day trial, during which there was weight change and DMI. BW was measured at 0, 15, 30 and 45 days with an electronic scale with a capacity of 500 kg and graduated in grams; DMI was measured by subtracting the amount offered the rejected daily. Total Weight Gain (TWG) was calculated by subtracting the final weight initial weight from each lamb, the DWG was determined by subtracting the final weight, initial weight and divided by the number of days of the study. The Feed Conversion (FC) was calculated by dividing the feed intake between the DWG.

Experimental design and statistical analysis: The Data variables were analyzed by GLM, using a completely randomized design, Means were compared using Tukey test (p<0.005), procedure in SAS (2001); Statistical model:

$$Y_{ij} = \mu + T_i + \xi_{ij}$$

Where: Y_{ij} = variable response to the (I) treatment in the (j) repeated; µ = general mean; T_i = effect of (j) treatment; ξ_{ij} = the error random in the treatment (i) in the repeated (j), term n-1(0, σ^2).

RESULTS

Chemical composition, gas production and *in vitro* digestibility of the foliage: The chemical composition of the foliage was different. The foliage of *G. ulmifolia* was higher (p<0.001) in CP content (167.2 g/kg DM), MO (944.0 g/kg DM), PT (38.2 g/kg DM) and CT (41.5 g/kg DM g/kg DM); on the other side the foliage of *C. alata*

Table 2: Chemical composition, gas production and *in vitro* digestibility of the foliage of *C. alata* and *G. ulmifolia* (g/kg DM)

Variable	*C. alata*	*G. ulmifolia*	SEM	p-value[1]
CP	132.0	167.0	1.4	***
NDF	502.0	392.0	29.2	***
ADF	315.0	238.0	25.1	***
OM	935.0	944.0	2.3	***
IVDMD	470.0	491.0	1.0	ns
IVOMD	558.0	590.0	1.1	**
ME (Mj/kg DM)	4.7	4.4	0.1	***
TP	21.0	38.2	1.2	***
CT	9.8	41.5	0.5	***
***In vitro* gas production**				
24 hrs	66.1	44.1	2.7	***
48 hrs	170.9	88.1	6.4	***
96 hrs	210.6	117	7.5	***

CP: Crude Protein; OM: Organic Matter; NDF: Neutral Detergent Fiber; ADF: Acid Detergent Fiber; IVDMD: *In vitro* Dry Matter Digestibility; IVOMD: *In vitro* Organic Matter Digestibility; ME: Metabolizable Energy; TP: Total Phenols (tannic acid equivalent), TC: Condensed Tannins (equivalent leucocyanidin). [1]Statistical significance of means of variables between foliage on rows, Tukey (ns: not significant, **p<0.01, ***p<0.001)

was higher (p<0.001) in NDF (502.0 g/kg DM), ADF (315.0 g/kg DM) and ME (4.7 Mj/kg DM). The IVOMD was higher (p<0.01) in the foliage of *G. ulmifolia* with 590.0 g/kg, DM and IVDMD did not differ between foliage (Table 2). The IVGP at 24, 48 and 96 hrs of incubation was higher (p<0.001) in the foliage of *C. alata* with volumes of 66.1, 170.9 and 210.6 ml/g DM, respectively (Table 2).

Chemical composition and *in vitro* digestibility of the treatments diet: The treatment diets were isonitrogenous (between 175 to 183 g/kg DM) and isoenergetic (from 10.4 to 12.0 Mj/kg DM), the ADF and NDF content was higher (P<0.001) in the treatments diets 2 and 4, which included 30% of foliage of *C. alata* and *G. ulmifolia*. The IVOMD was higher (p<0.001) in the diet of T0 (670.0 g/kg DM) compared to the treatment diets 1, 2 and 4 with digestibilities 605.0, 584.0 and 560.0 g/kg DM respectively; the IVDMD was not different between dietary treatments (Table 1).

Kinetics of degradation of treatments diets: Table 1 indicates that the asymptotic gas production (b) and the rate of gas produced per hour (C) was higher (p<0.001) in the diet of T0 with b = 293.0 ml/g DM and c = 0.069%/h respectively. The lag time was higher (p<0.05) in T3 with 4.3 hrs and lower in the T2 with 3.6 hrs, which means that the fiber in the diet of T2 begins to degrade more quickly than the one of T3.

Productive response in lambs: The Intake of Dry Matter (DMI) differed (p = 0.025) among the lambs of the different treatments (Table 3). The DMI was higher in lambs of T1 and T2 with 1339.8 and 1381.4 g/animal/day respectively and lowest in the T0 with 1061.4 g/animal/day. The TWG and DWG was higher (p<0.05) in lambs of T1 with 13.2 kg/animal and 295.6 g/animal respectively, in relation to the lambs of T4: 10.3 kg/animal and 227.8 g/animal respectively (Table 3). The FC was more (p = 0.016) in lambs from T0 with 4.4 kg DM/kg BW, which was related in part to lower DMI based on body weight (3.5% BW, p = 0.044) observed in these lambs (Table 3).

DISCUSSION

Chemical composition, gas production and *in vitro* digestibility of the foliage: The CP content of the foliage of *C. alata* and *G. ulmifolia* exceeded in most grasses and so did the minimum (80 g/kg DM of CP), which is considered critical to affect the growth of bacteria in the rumen (Lopez *et al*., 2008; Jimenez *et al*., 2008). This demonstrates the potential of the foliage of *G. ulmifolia* and *C. alata* to be used as contributions of N to the rumen for the synthesis of protein and microbial growth. By its content of NDF and ADF (Table 2), the leaves of *C. alata* and *G. ulmifolia*, can be used as a source of fiber in the diet of animals, especially when you consider that observed levels can be comparable to those reported in other species distributed in the tropics and have been used as food in grazing conditions (Garcia *et al*., 2008; Jimenez *et al*., 2008; Narvaez and Lascano, 2004).

The contents of TP and CT observed in the leaves of *C. alata* and *G. ulmifolia* (Table 2) are higher than those reported by Gonzalez *et al*. (2006) and lower than those reported by Garcia *et al*. (2008) and Moforte *et al*. (2005), in the same tree species in tropical conditions, but is not a limitation to use them as ingredients in feed for

Table 3: Growth performance of lambs Dorper x Pelibuey feed foliage of *C. alata* and *G. ulmifolia*

Variable	T0	T1	T2	T3	T4	SEM	p-value
Initial weight (kg)	22.0	24.0	23.7	22.6	22.3	-	-
Final weight (kg)	33.6	37.2	36.6	34.6	32.6	-	-
DMI (g DM/animal/day)	1061.4[b]	1339.8[a]	1381.4[a]	1239.3[ab]	1210.0[ab]	94.70	*
DMI (% body weight)	3.5[b]	4.1[a]	4.3[a]	4.1[a]	4.2[a]	0.26	*
TWG (kg/animal)	11.6[ab]	13.2[a]	12.9[ab]	12.0[ab]	10.3[b]	30.10	*
DWG (g/animal)	257.8[ab]	295.6[a]	286.7[ab]	267.8[ab]	227.8[b]	32.60	*
FC (kg DM)	4.4[b]	4.6[ab]	5.0[ab]	4.7[ab]	6.7[a]	1.13	**

T0: Control without foliage; T1: 15% foliage of *C. Alata*; T2: 30% foliage of *C. Alata*; T3: 15% foliage *G. Ulmifolia*; T4: 30% foliage *G. Ulmifolia*; TWG: Total Weight Gain; DWG: Daily Weight Gain; FC: Feed Conversion; DMI: Dry Matter Intake.
[abc]Means with different literals in the same row are statistically different, Tukey (*p<0.05, **p<0.01)

ruminants. In addition, Dey *et al*. (2008) report that levels between 1-4% of phenolic compounds in the foliage of trees do not cause negative effects on dry matter intake and digestion of the substrate to ruminal level.

The higher IVDMD observed foliage *G. ulmifolia* is attributed to the higher CP and lower NDF and ADF, the foliage of *C. alata*; this because it has been reported that the highest CP content favors substrate digestion, in contrast to the high content of detergent fibers, can have a negative effect (Tefera *et al*., 2008; Getachew *et al*., 2004; Das and Ghosh, 2007; Mlambo *et al*., 2008). The highest ME content in the foliage of *C. alata* (Table 2) is directly related to the higher IVGP at 24, 48 and 96 hrs of incubation of foliage; which is consistent with reports by Salem *et al*. (2006); Makkar (2005) indicating that IVGP has positive correlation with the ME containing foods.

Chemical composition and *in vitro* digestibility of the treatments diets: It is noted that the inclusion of 30% of the foliage of *C. alata* and *G. ulmifolia* in the diets of treatments 1, 2 and 4 lower IVDMD, due to an increase in NDF and ADF in the final mix (Table 1), it occurred that the foliage of the trees constitute an source important fiber (Table 2). There are reports that the inclusion of fibrous ingredients in high proportions in the diet of ruminants, may decrease the digestibility of the final mix because a negative relationship between the increase in NDF and ADF with the *in vitro* digestibility (Al-Soqeer, 2008).

Kinetics of degradation of treatments diets: The largest fraction by rate of c in the diet of T0 is because of the higher content of easily fermented carbohydrates from corn and cob ground; however the lag time was similar in treatments 1, 2, 3 and 4 with T0. Reported similar results Haddad and Nasr (2007), Moujahed *et al*. (2007); Moreno *et al*. (2007), which mention that diets with grain, increase the concentration of nonstructural carbohydrates easily fermented, which favors digestion rate.

Productive response in lambs: The lower DMI observed in the T0 lambs (Table 3) was associated with higher grain content and lower NDF and ADF (Table 1), which could cause subclinical acidosis that decreased DMI without affecting other parameters production in lambs (Haddad and Nasr, 2007); also Russell *et al*. (1992) note that in rations containing less than 200 g of NDF from forage per kg DM, microbial protein synthesis is reduced. Carro *et al*. (2000) reported that diets containing ingredients high in starch and low in fiber, decrease rumen pH and affect the activity of the rumen flora cellulolytic. In general it is observed that the addition of foliage of *G. ulmifolia* and *C. alata* to the diets of treatments 1, 2, 3 and 4, balanced the CP, NDF and ADF, which favored the DMI, reported similar results (Mata *et al*., 2006; Phengvichith and Ledin, 2007; Hue *et al*., 2008) were supplemented with tree foliage sheep.

The difference in TWG and DWG from lambs of T1 and T4 was related with NDF and ADF was higher in T4, which affected the IVDMD, Mata *et al*. (2006), reported that lambs supplemented with *Hibiscus rosa-sinensis*, DWG improved by an increase in IVDMD. Importantly, the TWG and DWG between lambs from treatments 1, 2, 3 and 4 with those of T0 and the FC between lambs of T1, T2 and T3 with the T0, were similar indicating that inclusion the foliage of *G. ulmifolia* and *C. alata* can be used in the feeding of lambs without affecting their growth performance, which coincides with that reported by Moforte *et al*. (2005); Dey *et al*. (2008); Hue *et al*. (2008); Tufarelli *et al*. (2009) that have successfully tested the use of trees for feeding ruminants.

Conclusion: The foliage of *G. ulmifolia* and *C. alata* are an important source of protein and fiber in the diet of lambs; in addition, the low TP and CT in the foliage of these trees did not affect productive response lambs fed diets supplemented with dried leaves; therefore, the foliage of the two species have potential use for animal feed in tropical regions.

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