

Behavior of mulberry (*Morus alba* L.) and its impact on the animal production and the breeding of silkworms in Cuba

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Mulberry has excellent nutritional qualities, like its high content of proteins and energy, so it has been offered as food to many species of animals since the 90's. The "Estación Experimental Indio Hatuey" has a gene bank of 20 varieties from Costa Rica, Brazil, South Korea, China and Spain. Studies have been directed to evaluate the behavior of this arboreous plant under different edaphoclimatic conditions, to meet the effect of the factors of the agronomic management in order to determine the potential of the total and edible biomass production, to characterize the bromatological and phytochemical composition of the varieties, determine the intake, digestibility, the animal response and its medical use, as well as to develop the breeding of silkworms. Results state that this plant has a great ability of adapting to different edaphoclimatic conditions. It can produce between 10 and 12 t of DM/ha/year (edible biomass), from 20 to 25 % of CP and the DM digestibility surpasses the 80%. Indicators of liveweight gain and milk production show similar levels to those obtained with the use of imported concentrates. The anti-inflammatory, cicatrizing, antimicrobial and anthelmintic properties of this plant have been demonstrated. Besides, with a proper management of the specie, it is possible to develop the breeding of silkworms for commercial purposes, without the influence of the climate as a limitation for the development of this insect. This known specie is used all over the country by companies, cooperatives and producers, and it has a great acceptance, mainly for feeding smaller species from different cattle subprograms of the urban agriculture.

Key words: *gene bank, feeding, medicinal, sericulture.*

Introduction

The search for local alternatives for animal health and feeding, the generation of exportable products, the import reduction, the development of traditional and natural medicine, and the generation of employment at the level of communities and territories, are among the socioeconomic and political priorities of the country.

The "Estación Experimental de Pastos y Forrajes Indio Hatuey" has the objective, among other goals, of searching for new forage sources for the animal feeding and validate the promising results reached in other countries of the hemisphere using the *Morus alba* L. specie.

Therefore, researches are developed in order to evaluate the behavior of this shrub-like forage plant under different edaphoclimatic conditions, to meet the effect of the agronomical management, to determine the intake, the digestibility, the animal response in terms of meat and milk production and their medicinal potential, and to value its use for breeding silkworms.

Development

Gene bank of Morus alba L. in Cuba. The first varieties of *Morus alba* L. (Indonesia, Tigriada, Acorazonada, Criolla or Doña Betty var.) were taken from Costa Rica since 1994. At the same time, the Cuban variety, which was introduced from Ethiopia during the 80's, was naturalized.

Two selections (IZ-40 and IZ-64) and three hybrids (IZ-15/7, IZ-13/6, and IZ-56/4) from Brazil were introduced in 2000. In 2005, the Ichinose, Super

mulberry, Cheongol and Ppong varieties from South Korea were included.

In 2011, the Estación Experimental "Indio Hatuey" widened the gene bank with four varieties: Universidad, Universidad Mejorada, Yu-12 and Yu-62 from China, and Murcia variety from Spain.

In order to study the behavior of the Indonesia, Tigreada, Acorazonada and Criolla varieties, the IZ-13/6, IZ-15/7 and IZ-56/4 hybrids, and the IZ-40 and IZ-64 selections, various tests were carried out, which provided the following results.

During the nursery phase, Indonesia was significantly different from the rest of the varieties regarding survival (98 % at 21 days after planted), number of sprouts/stems (3.1 at 49 days), length of the branches (32.02 cm at 49 days) and amount of leaves developed per plant (3.1 sprouts/plant at 42 and 49 days). There results can be associated with the least distance between the nodes, which is a characteristic of this variety and, therefore, with the highest production of buds. For the studied indicators, there were no significant differences among the hybrids. The survival percentage was not so high as the results found in the varieties from Costa Rica, which showed a proper growth, significantly superior at 66 d of the pruned seedlings without leaves, previous to the transplant.

It was demonstrated that the initial growth of mulberry (Acorazonada, Indonesia, Cuban and Tigriada varieties) is a process highly dependent on the reserves accumulated in the stem and the roots. It is characterized by three well defined phases: slow growth (between 0

and 21 days), gradual increase of the growth (between 21 and 53 days) and intense growth (between 53 and 77 days) (Pentón *et al.* 2006 and Pentón *et al.* 2007).

Researchers from the IBP achieved the macro-spreading of the Cuban variety in green houses, using the cuttings technique from the re-invigorated sprouts of stem donor plants and of plants cultivated *in vitro*. A methodology was defined for the massive spreading with the use of the cuttings as a sustainable method, regarding the *in vitro* spreading technology and the conventional stems method. Different physiological variables related to growth were determined. The field evaluation of the forage biomass yield of the plant from the cuttings technique was similar to those obtained by the method of *in vitro* cultivation, and superior to the plants obtained by means of the sticks. It was demonstrated that the cuttings technique within the green house is more effective than the *in vitro* cultivation and guarantees its economical sustainability (Salas Barboza *et al.* 2004, Salas Barboza *et al.* 2005, Salas Barboza *et al.* 2006 and Salas Barboza *et al.* 2010).

The morphological and agronomical evaluations with the Universidad, Yu-12 and Yu-62 varieties showed a significant response to the interactions between the varieties and the sowing distance in all the studied variables. The Universidad variety, planted at 0.50 m between plants, had the highest agricultural yield (table 1).

Agronomical studies. The experimental results with the varieties from Costa Rica and the naturalized in Cuba demonstrated that the yield of edible biomass

was influenced, mainly, by the cut frequency (C) and by the season of the year (S). The Acorazonada variety had the best behavior during the rainy season, in a 60 days frequency (6.35 t of DM/ha). It was very similar to the Indonesia variety during this season, but in a 90 days frequency (6.45 t of DM/ha). The Tigreada variety had very similar results during the rainy season, with the frequencies of 60 (6.02 t of DM/ha) and 90 days (6.19 t of DM/ha).

The increase of the fertilization with nitrogen stimulated the biomass production (table 2), but with a decrease in the efficiency of the nitrogen utilization within the poultry litter (organic fertilization) (Martín 2004).

Regarding the percentage of crude fiber of leaves, there were significant differences among the factors of variety (V), cut frequency (C) and season (S), but not among the fertilization levels (F). The contents of calcium (between 1.21 and 2.73), potassium (between 1.49 and 2.59) and ash (between 10.41 and 12.43), found within the edible biomass, were high and superior to the traditional forages in the tropical areas. This means that this plant has nutritional advantages regarding others, used with the same objective.

The effect of the interaction between the factors of height (H) and frequency (C) was very different. The highest values were found when cutting the plant every 90 days at 50 cm high, for the leaf yield during the rainy season (91.75 g of DM/plant) and the total biomass yield (134.72 and 183.25 g of DM/plant, respectively) (Martín 2004).

Table 1. Effect of the variety and distance between plants on the yield of total dry matter, edible dry matter, leaves, young stems, woody stems (kg/ha/year) and number of sticks per plants

		RMSBT	RMSBC	RMSBH	RMSTT	RMSTL	No. stems
Universidad	0.50	3.49 ^a	2.29 ^a	2.07 ^a	0.22 ^a	1.20 ^a	
	0.20	0.91 ^{def}	0.56 ^{cdef}	0.50 ^{cdefg}	0.07 ^{bc}	0.34 ^{def}	5.70 ^c
	0.40	0.89 ^{def}	0.40 ^{def}	0.34 ^{defg}	0.05 ^c	0.49 ^{de}	4.50 ^{cd}
	0.60	2.03 ^b	0.78 ^{bc}	0.68 ^{bc}	0.10 ^{bc}	1.25 ^a	11.60 ^a
Yu-62	0.50	1.00 ^{def}	0.44 ^{cdef}	0.38 ^{cdefg}	0.06 ^c	0.56 ^{cd}	4.50 ^{cd}
	0.20	0.67 ^{efg}	0.34 ^{ef}	0.30 ^{efg}	0.04 ^c	0.33 ^{def}	4.50 ^{cd}
	0.40	1.22 ^{cd}	0.71 ^{cd}	0.62 ^{cde}	0.09 ^{bc}	0.51 ^d	6.10 ^c
	0.60	1.51 ^c	0.65 ^{cde}	0.57 ^{cdef}	0.08 ^{bc}	0.86 ^b	9.10 ^b
Yu-12 plantlet from the root ball	0.20	0.39 ^g	0.21 ^f	0.19 ^g	0.02 ^c	0.18 ^f	3.00 ^{de}
	0.40	0.53 ^{fg}	0.30 ^{ef}	0.27 ^{fg}	0.03 ^c	0.23 ^{ef}	3.10 ^{de}
	0.60	0.99 ^{def}	0.47 ^{cdef}	0.43 ^{cdefg}	0.04 ^c	0.52 ^d	2.90 ^{de}
Yu-12 plantlet from Cells	0.20	0.68 ^{efg}	0.24 ^f	0.22 ^g	0.02 ^c	0.44 ^{def}	2.10 ^e
	0.40	1.17 ^{cde}	0.56 ^{cdef}	0.53 ^{cdefg}	0.03 ^c	0.61 ^{bcd}	3.30 ^{de}
	0.60	2.34 ^b	1.08 ^b	0.94 ^b	0.14 ^b	1.26 ^a	8.10 ^b
SE (±)		0.25	0.12	0.11	0.006	0.08	3.79

^{a,b,c,d,e,f,g} values with different superscripts between the lines differ at P<0,05 (Duncan, 1955)

RMSH- yield in leaf dry mass,

RMSBC- yield of edible biomass,

RMSBT- yield of total biomass

Table 2. Effect of different levels of fertilization (kg/ha/year) on the dry matter yield (t/ha/year) of leaves, edible biomass, and total biomass of mulberry

Fertilization	RMSH	Kg DM/kg N	RMSBC	Kg DM/kg N	RMSBT	kg DM/kg N
100	6.1 ^c	61.0	7.5 ^c	73.9	13.48 ^c	134.80
300	6.8 ^b	22.7	8.2 ^b	27.4	15.58 ^b	51.93
500	7.3 ^a	14.6	8.8 ^a	17.5	16.84 ^a	33.68
SE, ±	0.06***		0.07***		0.14***	

^{a,b,c} values with different superscripts between in columns differ at $P < 0,05$ (Duncan 1955)

*** $P < 0.001$

RMSH- yield of leaf dry matter

RMSBC- yield of edible biomass

RMSBT- yield of total biomass

According to the bromatological composition of the leaves, the H x F interaction was significant in the contents of CP during the rainy period (20.27 %, with the lowest cuts and a frequency of 45 days) and the contents of calcium and potassium were higher with the cuts at 50 cm and 90 days (Noda *et al.* 2007 y Noda *et al.* 2008).

In other studies of height (50 and 100 cm) and sowing density, there was a high production (6.98 g of DM/plant) when pruning at 50 cm, regarding the plants pruned at 100 cm (5.3 g of DM/plant).

When using superior sowing densities up to 25,000 plants/ha, the agronomical response of the crop was significantly better. The lowest results were obtained in the treatment of 12,500 plants/ha (Noda *et al.* 2007 and Noda and Martín 2008).

In more specific experiments with the Tigreada variety, about the use of alternatives of organic fertilization and the use of bio-products for increasing the yield and quality of forage, a significant multifactorial effect was obtained. The intercropping of the sword bean as green manure, inoculated with HMA, determined a better productive response during the rainy season, without affecting the forage exploitation as edible biomass (6 250 kg of leaves/ha and 18 194.4 kg of EB during the rainy period).

It was demonstrated that the best management alternatives of *Canavalia ensiformis*, integrated as green manure in plantations of *Morus alba* L., are related to the application of effective strains of Rhizobium, co-inoculated with HMA, the sword bean sowing, at a distance of 20 cm between plants, the harvest and concentrate of green manure with 60 days, and the use of a mineral fertilizer over 200-100-100kg N-P-K/ha/season. The great effectiveness of the Rhizobium EMBRAPA strain, introduced from Brazil, and followed by the CAN3 and CAN5 isolates from national production, was confirmed. The advantages of the co-inoculation with HMA were also demonstrated. The production of sword bean seeds, sowed as a border of the mulberry plantation, turned to be advantageous, which were co-inoculated with rhizobium and mycorrhizic-arbuscular fungi. However, the intercropping for producing seeds within the mulberry plantations was not positive. The

high speed of decomposition of the green manure of sword bean, located over the ground as concentrate for the mulberry, was confirmed.

As the *C. ensiforme*, *L. leucocephala*, *A. lebeck*, *A. procera* and *G. sepium* were intercropped as green manure in the mulberry plantation (var. Tigreada), there was a positive response in the colonization of the ground by invertebrates. Out of the present groups, the mollusks occupied the highest density and biomass at the beginning, together with diplopods and isopods.

When evaluating the effect of the EcoMic® product on the establishment of *Morus alba* L. (Tigreada and Acorazonada varieties) and its behavior, undergoing different alternatives of organic fertilization, it was observed that after 90 days the green weight of little roots and roots of the plants inoculated with HMA tripled the results of the control. After 240 days, the inoculated plants doubled the yield of the control. In the establishment cut, the mulberry fertilized with 15 t of organic matter reached a yield significantly superior to the association mulberry-*Albizia lebeck* as green manure and the control without fertilization (72.4; 45.0 and 41.6 g of foliar dry biomass per individual, respectively). However, after two years of exploitation, during the rainy season, the accumulated production did not show variations between the association and the fertilized mulberry (88.8 and 86.7 g of foliar dry biomass per individual), although it had variation for the control, which only produced 40.7 g/plant (Pentón 2007).

A significant effect of the interaction of EcoMic + Biocep-6 on all the studied agronomical variables was obtained in another essay on the Tigreada variety, as well as the interaction of 150 kg of N/ha/year + EcoMic + Biocep-6. Both treatments were not different, for any of the variables, and the means marked were higher than the control. Besides the biomass production was higher ($P < 0.001$) in the treatments with humus or biogas effluents (35.8 or 40.0 g DM/tree) regarding the control (13.7 g DM/tree).

Animal feed production. The experiments carried out to determine the best drying technologies for producing mulberry meal have allowed to meet the dehydration curve of the edible biomass at the sun light or under the

shadow of an open sided building with air circulation.

Out of the evaluated methods of drying, the dehydration of the entire plant under the sun light was determined to be the most appropriate because the mulberry shows, under these conditions, low resistance to dehydration. Besides, the meal of its leaves has nutritional indicators (91.2 % of dry matter (DM), 26.4 % of crude protein (CP), 10.1 % of crude fiber (CF), 90.7 % of organic matter (OM), 2.1 % of Ca and 0.15 % of P) which allow it to compete, from the bromatological point of view, with the conventional concentrates and with some meals of oleaginous plants.

In terms of ensilability, the digestibility of organic matter (DOM) ($x = 77.1\%$) indicated that they are good energy carriers. The inclusion of mulberry favored the digestibility of crude protein (DCP) (78.1 %), because it presented superior values to the rest of silages ($x = 72.0\%$). The lowest digestibility of crude fiber (DCF) was found in the silages with sugarcane bagasse (63.9 %), because of its low nutritional value. The feasibility of achieving quality silages was demonstrated, in which the husk of citric is the main component, as well as the importance of including absorbent materials in them (Ojeda *et al.* 2006).

Animal response. The main results on the animal response with the use of mulberry foliage focus on rabbits, pigs, sheep, and bovines. The results on the productive behavior of the crossbred female rabbits showed that it is convenient to use mulberry forage in the diet. In a first experiment, the average of young rabbits born alive per parturition was 7, while the average of weaned young rabbits was 5.2 with a weight of 874 g/animal. In a second experiment, the mean gain during the lactation period was 18 g/day. As average, there were 6.4 young rabbits born alive per parturition, with 0,054 kg of liveweight at birth. There were 5.4 weaned young rabbits, of 45 days old, with 694 g of weight. Besides, there was an 84.4% of survival during the lactation period. The mean daily gain during the lactation was of 14 g/animal/day (López *et al.* 2011).

According to the response of Large White crossbred pigs when using foliage of fresh mulberry (leaves and young stems), there was an increase of the mean daily gain with the increase of the live weight and a better efficiency of the total diet due to the usage of mulberry. There were no differences in the intake indicators among the different reproductive categories. There was an increase in the amount of born young (27.8 %), young pigs alive at 48 h (66.7 %), average weight of the litter, weight at the weaning moment (31.5 %) and viability percent of 86.7 % regarding the control without mulberry (66,7 %). Diarrheas suffered by the young had a significant decrease. Due to the inclusion of mulberry in the diets, there was a better morphometric, histological and hematological behavior in the reproducers (Contino *et al.* 2008a and Contino *et al.* 2008b).

There has been confirmed that the use of mulberry can

mean a substantial substitution of imported concentrates for feeding ruminants.

In experiments with growing ovine, there was a higher weight gain with the supply of mulberry forage + 700 g of citric husk silage. The offspring raised the intake of nutrients as the level of inclusion of mulberry forage increased. The productive results ranged between 1.5 and 2.5 L/animal/day, and between 80 and 90 g/animal/day of weaned young goats.

The food balance showed an excess of protein in the diets, although the energy/protein balance improved with the supplementation, which manifested in the achieved results. The preliminary results of the forage potential of *Morus alba* for feeding crossbred dairy cows from the Holstein x Zebu crossing, with 54 days of lactation, showed that it is possible to obtain productions of 10 L/animal/day, using the mulberry forage in addition to the pasture of improved species, without the supplementation of concentrates. The mulberry forage had high values of protein and low contents of fiber. The intakes during the first period reached 2.7 % of the liveweight. The average milk production was 10.6 kg/cow/day during the 140 days of evaluation, and the maximum production was registered during the first 53 days. The growing bovine produced between 700 and 750 g/animal/day.

In the test with crossbred males from the Holstein x Zebu crossing, which were eight months old and an average weight of 108 kg, the evaluation of the forage of *M. alba* lasted 140 days. During the first stage (53 days), the mulberry forage was given *ad libitum*, without being cut into pieces, and the animals grazed for four hours during the morning. The rest of the time they remained into the stable. During the second stage (58 days), these animals were given chopped mulberry forage and remained in the stables from 11:00 a.m. to 5:00 p.m. The rest of the time they were grazing. The availability of the grass was of 3.5 and 2.8 t of DM/ha/rotation for the first and second stages, respectively. The mulberry forage showed high protein values (20.5-24.6 %) and low fiber values (16.6-20.3 %). The intakes were of 3.93 and 3.12 kg/animal/day in each stage, respectively. The gains were of 0.759 and 0.405 kg/animal/day in the first and second stages, respectively.

It can be concluded that mulberry has a high quality, when the edible biomass is used as forage and a better exploitation was observed when delivering the chopped forage. Besides, the developing bovine can have gains superior to 0.400 kg/animal/day as average, without concentrate, with the use of mulberry (Milera *et al.* 2010).

Sericulture. In 2001, the encouragement of sericulture started in the “Estación de Pastos y Forrajes “Indio Hatuey”. The breeding started in 2006 and, according to the observations carried out, everything seems to indicate that temperatures and relative humidity registered in Cuba do not limit the breeding of silkworms, which can

be carried out safely during the whole year, with better indicators during the second semester.

The first breeding of *Bombyx mori* started in the "Estación Experimental "Indio Hatuey" on January, 2006, with an F2 line and varieties from Korea, which had gone to a process of quarantine in the INISAV. The results were encouraging, with an average weight of the cocoon of 1.71 g. The construction of the building that is used nowadays for its breeding finished in 2010.

Between 2006 and 2011, 32 raisings of silkworms have been carried out within the facilities of the "Estación Experimental "Indio Hatuey". The behavior of the worm was evaluated at an experimental scale in different seasons of the year. The experimental groups registered percentages of eclosion were 94.3 %, 95 %, 96.6 %, 96.1 % and 95.3 % during the periods of January-February, March-April, June-July, September-October and November-December. The average weight of the cocoon (g) and the percentage of crude silk (%) in four periods, 2005-2009, were of 1.66 and 20.58, respectively. The behavior of the duration of the larval stage was 25 days. During March and April, the lowest indicators in the cocoon weight (1.41g) and the percentage of crude silk (19.17%) were registered. At the end of the experimentation, there was a pupation rate of 91.2, 89.1, 93.2, 92.6 and 92.7 % in each period, respectively (Fiallo and Marlene 2007).

Medicinal uses. The studies carried out in Cuba with medicinal purposes have demonstrated that mulberry has significant differences in the specific activity of the catalase and peroxidase antioxidant enzymes of different organs of the plant and among the varieties and hybrids of *Morus alba*. The highest values were reported on leaves, stems and roots in decreasing order (Díaz *et al.* 2010). In the toxicity essay, it was observed that the fresh concentrate of mulberry can inhibit the gastric injuries provoked by the ethanol supplementation.

Another result showed the presence of the anti-inflammatory activity of concentrates of leaves and roots, as well as the healing activity. The antimicrobial activity was verified with the leaf concentrates of varieties and hybrids of *Morus alba* using five bacterial pathogens (Díaz *et al.* 2011). Besides, the Cuban variety of *M. alba* has anthelmintic properties in front of L3 larvae of gastrointestinal nematodes under lab conditions.

Conclusions

- In Cuba, there are currently 26 varieties of *Morus alba* L. introduced or naturalized, evaluated under the edaphoclimatic conditions of the country.

- Nowadays, there is a methodology for spreading the mulberry by *in vitro* cultivation from the axillar buds of plants selected from the field.

- In a cutting and carrying system, the right moment for starting the exploitation is 270 days after the plantation, being the cut frequency and the fertilization

the factors of higher impact on the biomass production.

- The low contents of crude fiber and the high contents of crude protein, calcium, potassium and ashes found in the edible biomass of the studied varieties of mulberry allow to make good use of the crop for feeding animals, as a meal or green forage supplement.

-The isolation of secondary metabolites, essential amino acids, fatty acids and other active substances show the medicinal potential of mulberry.

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