Scientific Paper

Influence of three Morus alba *L. varieties on the growth of* Bombyx mori *L.*

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ABSTRACT: Three *Morus alba* varieties (tigreada, universidad and yu-62) were studied in order to determine their proximal composition and their incidence on the growth of *Bombyx mori* larvae, polyhybrid Chul Tai-6. For the analysis and feeding mature leaves were used, and the dry matter, moisture, crude fiber (CF), calcium (Ca), phosphorus (P), potassium (K) and ash were determined. The design was completely randomized (three replications of 100 silkworms per variety). The weight of the larva on the first and fifth days of the fifth instar and the average growth of a worm per day were measured. The moisture and CP percentages were higher for var. yu-62 (75,9 and 20,10 %, respectively); there were no significant differences among the CF percentages. Var. tigreada showed significantly high values of ash. The larvae fed yu-62 showed the highest relative growth rate –RGR– (638,95^{a} ± 114,2), while the lowest RGR was obtained with tigreada (466,59^{c} ± 87,97). It is concluded that variety yu-62 showed the best bromatological indicators and a higher RGR, for which it is recommended to be used in Cuban sericulture.

Keywords: silkworm, leaves, minerals

INTRODUCTION

Mulberry (*Morus* sp.) leaves constitute the traditional and almost exclusive feed source of the silkworm (*Bombyx mori* L.), from which they obtain the necessary nutrients and water to grow and develop. The larval growth is closely related to the nutritional quality of the mulberry variety (Cifuentes and Sohn, 1998).

Silkworms prefer the leaves with higher CP and moisture content. Minerals also play an important role in the growth and development of this lepidopteran (Pescio *et al*., 2008). The lack of Ca influences palatability, and regarding K, although its functions are not well defined yet, it influences the ionic balance of the hemolymph and improves the silk cortex content of the cocoon, for which the continuous contribution of these chemical elements is required (Cifuentes and Sohn, 1998).

The contents of protein, lipids, carbohydrates, vitamins, minerals and water in the mulberry leaves depend on the variety, soil fertility, climate, season, and leaf age and position. It is recommended to feed the adult larvae (fourth and fifth instar) with mulberry branches in vegetative growth, between 75 and 100 days, period during which they consume more than 90 % of the food ingested during the rearing stage (Porto and Okamoto, 2013).

Studies have been conducted on the varietal response and the aspects related to the biochemical composition of the mulberry leaves (Jyothi *et al*., 2014), in which the importance of their quality for feeding the silkworm has been discussed. In this regard, Pescio *et al*. (2008) stated that silkworms must "like" the leaves, for which this is one of the first requisites that are taken into consideration to feed *B. mori*. Although all the leaves are consumed, frequently those of one variety are preferred with regards to another.

The objective of this research was to determine the proximal and mineral composition of the leaves of three *Morus alba* varieties (tigreada, universidad and yu-62), as well as to evaluate its effect on the growth of the silkworm larvae.

MATERIALS AND METHODS

Climate and soil. The *M. alba* varieties (tigreada, universidad and yu-62) were cultivated in February, 2014, in the experimental area Norte,

field No. 4, in the Havana province. The soils are classified as Ferralitic Red, with an adequate content of organic matter (3,39 %), and are fertilized three times per year with doses of 300-120-300 kg N-P-K/ha/year (Instituto de Suelos, 2013).

Experimental procedure. The plants used had been established for two years, in a planting frame of 1,0 x 0,5 m (2 000 plants/ha); the pruning frequency was 90 days, at a cutting height of 0,5 m; the net area had 200 plants and 10 % was sampled. The biomass in the three varieties, at the moment of study, was higher than 300 g of fresh leaves per plant, the leaves were selected taking into consideration their size, texture, color and health.

Proximal and mineral analysis. Three samples of mature and fresh leaves of the different varieties were randomly collected, at a rate of 100 g from each. The fresh mulberry leaves were weighed and dehydrated in a forced-air stove –Memmert model–, at 60-80 ºC, to determine DM and moisture. The indicators crude fiber (CF), crude protein (CP), calcium (Ca), phosphorus (P), potassium (K) and ash were determined, following the regulations of the AOAC (1995).

Rearing of silkworm larvae. The silkworm rearing was carried out in January and February, 2014. The polyhybrid Chul Thai-6, which had already been evaluated for two years in Cuba, was used. The rearing room and the tools were previously disinfected with bactericide and fungicide agents. The floors of the beds were dressed with Kraft paper and changed at the end of each instar of the larval stage. During the rearing controlled environmental conditions were maintained: 23 ± 2 ºC, 72 ± 3 % of relative humidity, with photoperiod of 16 hours light and 8 hours darkness.

Sampling and evaluation. Rearing units of 100 worms were established, in a completely randomized design, with three replications for each mulberry variety. The feed supply to the larvae started from the third instar. A feeding regime of three meals per day was carried out (8:00 a.m., 1:00 p.m. and 7:00 p.m.) with fresh and mature leaves. In the moult days, which marked the end of each stage during the larval phase, no feed was provided to them.

Larval weight. The individual weight of the larvae was measured in the three replicas used for each variety, on the first and fifth day of the fifth instar, according to the studies conducted by Meneguim *et al*. (2007) and Munhoz *et al*. (2009).

Determination of the growth rate (RGR). The average growth of one silkworm in one day (mg/silkworm/day) was determined, according to the equation proposed by Meneguim *et al*. (2010).

$$RGR = \frac{G}{T}$$

G: weight gain of the larvae between the first and fifth day of the fifth instar (mg)

T: Duration of the feeding period (days).

Statistical analysis. The average values of the different variables were compared through a simple ANOVA and Tukey's test was applied, with 0,05 % of significance. The statistical package GraphPad Prism 5.01 was used. The statistical processing was made according to the reports by different authors who work the topic of sericulture (Meneguim *et al*., 2007; Munhoz *et al*., 2009; Rodríguez-Ortega *et al*., 2012, 2013).

RESULTS AND DISCUSSION

Dry matter and moisture content. The highest DM value of the leaves (31 %) was shown in var. tigreada, which significantly differed with regards to vars. universidad and yu-62 (table 1).

González *et al*. (2011) reported 28,13 % DM for var. tigreada under similar cultivation conditions to the ones in this study, but in a different region of the country. Such differences could be the consequence of the genotype-environment interaction, due to the use of different soil types and to the variation of their organic matter content.

Table 1. Dry matter and moisture in the mulberry leaves (%).

Variety	DM	Moisture
Tigreada	$31,00^{a} \pm 0,30$	$69,00^{c} \pm 0,30$
Universidad	$27,50^{b} \pm 1,30$	$72,50^{b} \pm 1,30$
Yu-62	$24,10^{c} \pm 1,00$	$75,90^{a} \pm 1,00$

a, b, c: values with different superscripts in each row differ at $p \leq 0,05$ (Tukey's test). The average value is shown with the standard deviation, from three randomly taken leaf samples.

The moisture in the leaves of var. yu-62 was higher and significantly differed from the others. In this sense, Porto (2000) referred that the larvae in the fifth instar showed better performance when they were fed with leaves that contained between 75 and 90 % of moisture, and that when the water percentage was insufficient, the silkworm did not use the feed adequately because it showed direct affectations on the digestive process.

According to the DM and moisture percentages shown by the varieties, which coincide with the recommendations made by Porto and Okamoto (2013), they can be qualified as adequate to feed the larvae in the fifth instar.

Knowing the CP content is important, because proteins and their aminoacids play a fundamental role in the growth and development of the silkworm (Porto, 2002). Table 2 shows the CP percentage in the DM of the leaves of the studied varieties. The highest CP value was found in var. yu-62 followed by universidad and tigreada.

The management of the mulberry crop remarkably influences the CP content of the leaves. The chemical or organic fertilization (Benavides, 2002), the season (González and Cáceres, 2002), the cutting height (Martín *et al*., 2002), the planting density (Boschini *et al*., 1998) and the variety (Huo, 2002) are some of the aspects of the agronomic management that have incidence on this indicator.

Studies conducted by Singh and Makkar (2002) proved that in India, to feed the silkworm larvae, the mulberry leaves must have between 15,0 and 27,6 % CP; from this criterion it was estimated that the CP values of this research are adequate.

The highest CF percentage (19,25 %) was found in var. universidad, which did not differ from the others (table 2). Although there were no significant differences in the CF percentage of the varieties, it is important to state that the CF content in the leaves of yu-62 is in the upper limit of the range proposed by Singh and Makkar (2002). These authors recommend values between 9,1 and 15,3 % CF to feed silkworms in India. However, the leaves of vars. tigreada and universidad did not fulfill this criterion, because they exceeded it.

The CF content has direct influence on digestibility; in this sense with the increase of fiber there is lower digestibility of the feedstuff, along with lower intake. The chemical components present in the CF hinder the digestibility of proteins and their bioavailability (Meneguim *et al*., 2010).

Regarding minerals, tigreada showed high values of ash, P and Ca. In these three indicators it significantly exceeded universidad and yu-62, but showed lower percentage of K (table 3).

The ash content in vars. tigreada and universidad was higher than the one reported by Martín *et al*. (2002), while yu-62 showed the lowest percentage. Meneguim *et al*. (2010), when evaluating seven *M. alba* cultivars in Brazil found that the ash varied between 11,6 and 12,0 %; while Rodríguez-Ortega *et al*. (2013) obtained higher values (16,2 %) than the ones in this research.

Singh and Makkar (2002) suggested that the optimum percentages to feed the silkworm are between 0,23-0,97 for P and 1,66-3,25 for K. In this sense, the P values in vars. yu-62 and universidad showed favorable values for larval development;

Table 2. Crude protein and crude fiber in the DM of the mulberry leaves (%).

Variety	CP	CF
Tigreada	$17,85^{c} \pm 0,01$	$17,78^{a} \pm 2,27$
Universidad	$18,29^{b} \pm 0,03$	$19,25^{a} \pm 1,36$
Yu-62	$20,10^{a} \pm 0,03$	$15,33^{a} \pm 1,30$

a, b, c: values with different superscripts in each row differ at $p \leq 0,05$ (Tukey's test). The average value is shown with the standard deviation.

Table 3. Content of ash, P, K and Ca in *M. alba* leaves (%).

Variety	Ash	P	K	Ca
Tigreada	$14,40^{a} \pm 0,20$	$1,64^{a} \pm 0,04$	$3,92^{c} \pm 0,10$	$2,65^{a} \pm 0,10$
Universidad	$13,10^{b} \pm 0,30$	$0,66^{b} \pm 0,01$	$4,44^{a} \pm 0,10$	$2,24^{b} \pm 0,10$
Yu-62	$10,00^{c} \pm 0,20$	$0,58^{c} \pm 0,01$	$4,18^{b} \pm 0,10$	$2,17^{c} \pm 0,10$

a, b, c: values with different superscripts in each row differ at $p \leq 0,05$ (Tukey's test). The average value is shown with the standard deviation.

nevertheless, tigreada had a significantly high value (1,64 %), and according to Cifuentes and Sohn (1998) a high P content causes the hardening of the leaves and the decrease of palatability.

With regards to the K content, the three varieties showed higher values than the suggested standards. Universidad showed the highest percentage and differed from the others.

Minerals play different functions associated to cell metabolism in the silkworm. Insects need considerable quantities of different minerals (potassium, phosphorus, magnesium, calcium, sodium and chlorine), and their importance lies on the fact that they are protagonists in the ionic balance and the permeability of the cell membrane, besides acting as activators of the enzymes and being part of respiratory pigments (Porto, 2002).

Regarding the silkworm growth on the fifth day of the fifth instar, the larvae fed var. yu-62 showed the highest average weight, followed by those that received the universidad and tigreada leaves (figs. 1 and 2).

The growth of silkworm larvae is closely related to the nutritional quality of the *M. alba* variety they consume (Porto, 2002; Meneguim *et al*., 2007).

Generally, the *B. mori* larvae with high body weight have more developed silk glands. This gland

Figure 1. Silkworm larvae in the fifth instar.

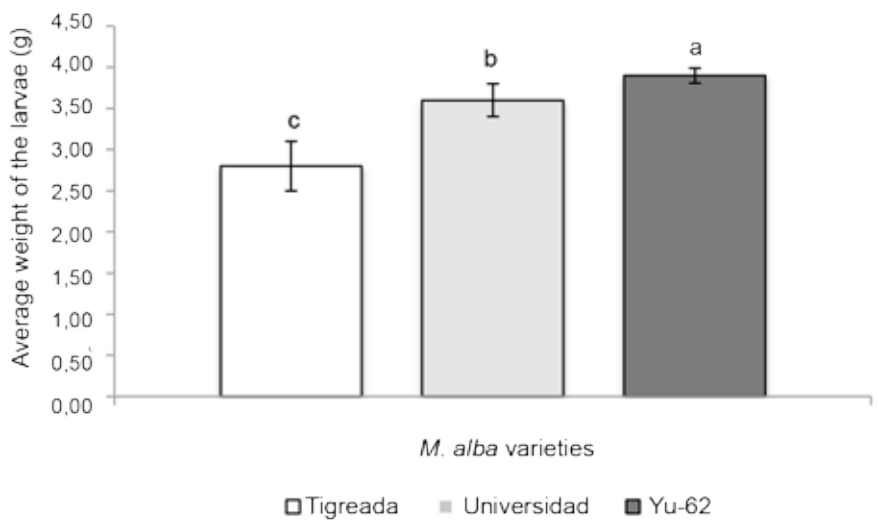

Figure 2. Average weight of the B. mori larvae on the fifth day of the fifth instar. (Superscripts a, b and c statistically differ according to Tukey's test, $p \leq 0,05$).

grows fast in the fifth instar and occupies up to 40 % of the insect's body, for which the quantity and quality of the mulberry it consumes in this phase are very important for cocoon production (Cifuentes and Sohn, 1998).

The larvae fed yu-62 showed a higher RGR than the ones which ingested vars. universidad and tigreada (table 4).

The growth of *B. mori* varies according to the breed, rearing season, temperature and mulberry quality (Meneguim *et al*., 2010). Approximately 80-90 % of the total weight of the silkworm's body is constituted by water, but during the fifth instar its elimination is activated as cocooning approaches, until representing 75 % of the live weight (Cifuentes and Sohn, 1998).

The biomolecules that accumulate the most in the silkworm are fats and glycogen, which reach up to 33 and 50 % of the total dry weight of the larvae, respectively (Cifuentes and Sohn, 1998). Although proteins are less accumulated than carbohydrates and lipids in the body of the silkworm, they are much more determining for the growth and development of these insects, because they regulate cell growth from their functions as enzymes, ionic channels and cell structural elements.

Protein is considered the most required nutrients by phytophagous insects, and generally, the highest limitation for optimum growth. In this study the highest nutritional quality was shown by var. yu-62. It is also necessary to emphasize that yu-62 behaved in correspondence with the reports by other authors (.Porto, 2002; Meneguim *et al*., 2007; Munhoz *et al.,* 2009; Kalaivani *et al.,* 2013) regarding the positive correlation that is achieved when feeding the larvae with mulberry leaves that show higher CP and moisture percentage, lower CF percentage and better balance among the minerals.

It is concluded that there was a relation between the nutritional quality of the mulberry leaves and larval growth. Var. yu-62 showed the best bromatological traits and the highest RGR, for which it is recommended to be used in Cuban sericulture.

Table 4. Growth rate of the *B. mori* larvae (mg/ silkworm/day).

Variety	RGR
Tigreada	$466,59^{c} \pm 87,97$
Universidad	$586,38^{b} \pm 144,30$
Yu-62	$638,95^{a} \pm 114,20$

a,b,c: values with different superscripts differ at $p \leq 0,05$ (Tukey's test). The average value is shown with the standard deviation.

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