Impact of Nutritional Composition of Wild and Cultivated Mulberry Varieties on Productivity and some Biochemical Parameters of Silkworm Bombyx mori L. (Bombycidae: Lepidoptera)

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Received: 5/3/2017

Abstract: Two mulberry varieties, the cultivated variety, Morus alba var. Morittiana and the wild variety, M. laevigata, grafted on M. alba branches were investigated to assess the effect of their nutritional components on larval haemolymph biochemical constituents and economic traits of silkworm, Bombyx mori L. Results indicated that there were significant differences (p ≤ 0.05) between the two tested mulberry varieties in their nutritional composition. Protein content, free amino acids and nitrogen were significantly higher in the cultivated variety M. alba var. Morittiana, while reducing sugars content was significantly higher in M. laevigata with no significant differences between both varieties. Interestingly, total proteins and total carbohydrates were significantly high in haemolymph of larvae fed on M. laevigata leaves, while free amino acids were significantly high in haemolymph of larvae fed on M. alba var. Morittiana leaves, no significant difference was registered between total lipids content in haemolymph of larvae fed on both mulberry varieties. There were no significant differences between male full grown larvae weights were significantly high in larvae fed on M. laevigata leaves. No significant differences were found between cocoon incidences produced from larvae fed on the both mulberry varieties.

Keywords: Bombyx mori, mulberry varieties, Morus alba. Morus laevigata, biochemical study.

INTRODUCTION

In sericulture, the mulberry variety plays an important role and has long been cultivated for silkworm rearing. Their leaves are the exclusive source of nutrition for growth and development of silkworms (Tang et al., 2006). Mulberry leaves contain a rich source of protein and amino acids. However, the nutritional composition of mulberry leaves are ascertained by their biochemical constituents and vary with different cultivars that influences growth and cocoon production (Subhan et al., 2013; Babu et al., 2014 ; Manjula and Vijaya Kumar, 2015 ; Prieto-Abreu et al., 2016).

Silk production in Egypt should have special attention because the local production does not meet the domestic consumption requirements although dominant climate conditions are proper (Diab et al., 2009). Mulberry production alone need more than 60% of the total cost of sericulture and cocoon production, so maximum attention has been given for the improvement of mulberry in terms of both quality and quantity (Bongale and Chaluvachari, 1995). In India, Morus is represented by four species i.e., M. indica L., M. alba L., M. laevigata Wall. and M. serrata Roxb. (Tikader and Dandin, 2005). Japan is the only sericulturally advanced country where the wild mulberry, M. laevigata genetic resources have been exploited for developing new improved mulberry varieties (Naik et al., 2015).

The performance of the Bombyx mori L. was evaluated on different mulberry cultivars based on economic, biological characters and productivity in silkworm rearing (El-Banna et al., 2013; Subhan et al., 2013; Babu et al., 2014; Kumar et al., 2014; Prieto-Abreu et al., 2016). However the biochemical contents were studied in different varieties of the cultivated mulberry leaves and silkworm haemolymph in relation to silk production (Zannoon et al., 2008; Yoganananda Murthy et al., 2013), meanwhile the effects of the wild mulberry variety on silk production in comparison to the cultivated varieties was studied by Mahmoud Soad et al. (2013).

Therefore, the present study aims to evaluate two mulberry varieties, the cultivated one Morus alba var. Morittiana and the grafted wild one M. laevigata to assess their feeding effect on some biochemical and economical traits of the silkworm, Bombyx mori L.

MATERIALS AND METHODS

Silkworm rearing

Experimental silkworm eggs, the four-way hybrid (KK × Hesa 1) × (Vesletz 2 × Gergana 2), were imported from Sericulture and Agriculture Experiment Station, Vratza, Bulgaria, reared in the laboratory of Plant Protection Department, Faculty of Agriculture, Suez Canal University at 25 ±1°C and 75 ± 5% RH from hatching till cocoon formation according to Mohamed (2000). Rearing was carried out during two successive spring seasons of 2015 and 2016. Removal of fecal matter, diseased larvae and bed cleaning was done regularly. Larvae of each two groups were fed on one of two chosen mulberry variety leaves four times a day, each group containing 3 replicates.

Mulberry varieties

Mulberry leaves were collected from healthy plants at the Faculty of Agriculture Farm, Suez Canal University, Ismailia Egypt, Morus alba var. Morittiana and Morus laevigata which was grafted on M. alba var. Morittiana branches.
Biochemical analysis

Mulberry leaves preparation and biochemical analysis

Leaves samples were collected from medium portion of mulberry branches as pooling sample with three replicates, weighed (0.1-0.4 g) and stored at -20°C, then processed as described by Ni et al. (2001). Enzymes were extracted from the frozen plant samples using cold potassium phosphate buffer. An aliquot (1.5 ml) of the extract was centrifuged for 10 min at 4°C.

Total Protein content of leaves was determined using the method of Bradford (1976) while total lipids and free amino acids were colorimetrically assayed by ninhydrin reagent according to the method described by Lee and Takabashi (1966). Reducing sugars were extracted by weighed 100 mg of the sample then adding hot 80% ethanol twice (5 ml each). Carbohydrates were determined by estimating the reducing sugars content, while nitrogen in protein is converted to ammonium sulphate by H2SO4 during digestion (Sadasivam and Manickam, 1992).

Larval haemolymph preparation and biochemical analysis

Haemolymph of larvae fed on the two selected mulberry leaves were collected separately in glass tubes by punctured pro-abdominal leg of larvae at the 7th day of the fifth instar, each tube contains haemolymph of 10 larvae, as pooling samples, with three replicates for each mulberry variety then deep freezeed at -20°C. Haemolymph samples were centrifuged (2500 rpm for 6 minutes).

Total proteins were determined using the method of Bradford (1976) while total lipids were estimated according to the method of Knight et al. (1972). Total carbohydrates were extracted and prepared for assay according to Crompton and Birt (1967). Assessment was conducted in acid extract of each sample by the phenol-sulphuric acid reaction (Dubois et al., 1956). A modified ninhydrin reagent for the photometric determination of free amino acids was used according to Moore and Stein (1968).

Economic traits of silkworm

At the end of the 5th instars, the weights of 30 individuals’ male and female full grown larvae, cocoons, pupae and cocoons shell from three replicates of each group were recorded separately, the mean weights and cocoons cortex percentages were calculated.

Statistical analysis

Data collected from the experimental observations were statistically analyzed using Independent Samples t-test to compare means of both treatments in relation to the recorded traits using the statistical software SPSS. Statistical significance was assigned as (p ≤ 0.05). In all the cases experimental work was carried out for three replicates.

RESULTS

Biochemical analysis

Mulberry leaves

Nutritional analyses of mulberry leaves are presented in Table (1). Results indicated a high level contents of total proteins in M. alba var. Morittiana (19.83 mg/gdw) while it was (15.43 mg/gdw) in M. laevigata leaves. Reducing sugars were 103.03 mg/gdw and 115.83 mg/gdw. Free amino acids registered 3.63 mg/gdw and 1.72 mg/gdw, while nitrogen were 3.16 mg/gdw and 2.69 mg/gdw in M. alba var. Morittiana and M. laevigata leaves, respectively.

Larval haemolymph

The influence of the mulberry varieties on the biochemical constituents in the haemolymph of larvae was shown in Table (2). The total protein content, total carbohydrates, total lipids and free amino acids were 24.10, 4.35, 1536.70 and 13.17 mg/ml in comparison to 30.21, 6.71, 1563.30 and 10.30 mg/ml in haemolymph of larvae fed on M. alba var. Morittiana and M. laevigata leaves, respectively.

Interestingly, the present larvae biochemical haemolymph analysis showed that total proteins and total carbohydrates were significantly higher in haemolymph of larvae fed on M. laevigata leaves. On the other hand, haemolymph of larvae fed on M. alba var. Morittiana exhibited highly significant differences in their content of free amino acids, while no significant difference was recorded between total lipids content in haemolymph of larvae fed on both mulberry varieties.

Table (1): Nutritional analysis of M. alba var. Morittiana and M. laevigata mulberry varieties leaves

<table>
<thead>
<tr>
<th>Mulberry varieties</th>
<th>Total proteins</th>
<th>Reducing sugars</th>
<th>Free amino acids</th>
<th>Nitrogen</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>mg/gdw</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>M. alba var. Morittiana</td>
<td>19.83± 0.55</td>
<td>103.03± 2.58</td>
<td>3.63±0.12</td>
<td>3.16± 0.09</td>
</tr>
<tr>
<td>M. laevigata</td>
<td>15.43± 0.34</td>
<td>115.83±4.69</td>
<td>1.72±0.12</td>
<td>2.69±0.11</td>
</tr>
</tbody>
</table>

Sig. * ns

Each value represents the mean ± SEM, n=3.

* Significant differences between means in the same column (p≤ 0.05).

ns (P > 0.05) no significant between means in the same column.
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Table (2): Mean of biochemical constitutes in haemolymph of larvae fed on M. alba var. Morittiana and M. laevigata mulberry leaves

<table>
<thead>
<tr>
<th>Mulberry varieties</th>
<th>Total proteins</th>
<th>Total carbohydrates</th>
<th>Total lipids</th>
<th>Free amino acids</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>mg/ml</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>M. alba var. Morittiana</td>
<td>24.10± 1.05</td>
<td>4.35± 0.45</td>
<td>1536.70±156</td>
<td>13.17± 0.50</td>
</tr>
<tr>
<td>M. laevigata</td>
<td>30.21± 0.90</td>
<td>6.71±0.48</td>
<td>1563.30±163</td>
<td>10.30±0.67</td>
</tr>
</tbody>
</table>

Sig. * * ns *

| Each value represents the mean ± SEM, n=3. |
| * Significant differences between the same column (p< 0.05). |
| ns ( P > 0.05) no significant between the same column. |

Silkworm economic traits

Mean weights of male full grown larvae fed on the grafted wild and the cultivated mulberry leaves and cocoon incidences are shown in Table (3). Full grown larvae weights registered 1921.2 and 1913.9 mg. Weights of pupae, cocoons and cocoons cortex that produced from larvae fed on M. alba var. Morittiana and M. laevigata leaves were 705 & 716 mg, 913 & 945 mg and 208 & 230 mg, respectively. Cocoons cortex percentages registered 22.71% and 24.35% in cocoons produced from larvae fed on M. alba var. Morittiana and M. laevigata leaves, respectively.

Data in Table (4) shown that the females full grown larvae weights were 2288.3 and 2418.5 mg, while pupal weights were 850 and 883 mg. Cocoon weights were 1065 and 1113 mg and cocoon cortex weights were 209 and 230 mg, while cocoon percentage registered 19.50% and 20.71% in cocoons produced from larvae fed on M. alba var. Morittiana and M. laevigata leaves, respectively.

Data in (Tables 3 and 4) showed that there were no significant differences (p > 0.05) between full grown larvae of males fed on both mulberry varieties, while there were significant differences between full grown larvae of females. No significant differences were recorded between weights of cocoons, cocoons cortex and pupae produced from larvae fed on both mulberry varieties in both sexes.

Table (3): Mean of male larvae, pupae and cocoon, weights which fed on M. alba var. Morittiana and M. laevigata mulberry varieties

<table>
<thead>
<tr>
<th>Mulberry varieties</th>
<th>Full Grown Larvae</th>
<th>Pupae</th>
<th>Cocoon</th>
<th>Cortex</th>
<th>Cortex %</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Weights (mg)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>M. alba var. Morittiana</td>
<td>1921.2±17.91</td>
<td>705±17.80</td>
<td>913±23.16</td>
<td>208±7.14</td>
<td>22.71%</td>
</tr>
<tr>
<td>M. laevigata</td>
<td>1913.9±32.41</td>
<td>716±24.79</td>
<td>945±22.05</td>
<td>230±15.30</td>
<td>24.35%</td>
</tr>
</tbody>
</table>

Sig. ns ns ns ns -

| Each value represents the mean ± SEM. |
| ns (P > 0.05) no significant between treatments. |
Table (4): Mean single weight of female larvae, pupae and cocoon traits which fed on M. alba var. Morittiana and M. laevigata mulberry varieties

<table>
<thead>
<tr>
<th>Mulberry varieties</th>
<th>Full Grown Larvae</th>
<th>Pupae Weights</th>
<th>Cocoon Weights</th>
<th>Cortex</th>
<th>Cortex (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>M. alba var. Morittiana</td>
<td>2288.3± 36.42</td>
<td>850±25.47</td>
<td>1065±33.80</td>
<td>209±10.05</td>
<td>19.5%</td>
</tr>
<tr>
<td>M. laevigata</td>
<td>2418.5± 51.48</td>
<td>883±23.10</td>
<td>1113±26.95</td>
<td>230±7.58</td>
<td>20.71%</td>
</tr>
</tbody>
</table>

Sig. * ns ns ns -

Each value represents the mean ± SEM. * Significant differences between the same column (p ≤ 0.05). ns (p > 0.05) no significant between treatments.

DISCUSSION

Silk cocoon yield mainly depends on the quality of mulberry leaves, which differ considerably from variety to variety in their chemical composition and nutritive value, like protein and carbohydrate contents (Jyothi et al., 2014; Manjula and Vijaya Kumari, 2015).

The carbohydrates, proteins and lipids play an important role in the biochemical process underlying growth and development of insects (Murthy, 2015), thus changes in the composition of haemolymph reflect the physiological and biochemical transformations taking place in the insect tissues (Brennan and Anderson, 2004).

Present results concluded that M. alba var. Morittiana leaves had a significantly high content of total proteins, free amino acids and nitrogen, while M. laevigata had high content of reducing sugars only. On the contrary, haemolymph biochemical analysis indicated that larvae fed on M. laevigata leaves recorded more total proteins, carbohydrates and lipids. However, larvae fed on M. laevigata leaves have unexpectedly gained more full grown larvae weights and cocoon incidences. Present results are in agreement with Subhan et al. (2013) Manjula and Vijaya Kumari (2015) who stated that carbohydrates particularly the sugar content in mulberry leaves is closely related to the health of the silkworm. Likewise, Mahmoud et al. (1987) who indicated that larvae fed on M. laevigata leaves gained more body weight in all the instars and gave better cocoon yield compared to which larvae fed on M. alba variety leaves.

On the other hand, these results disagreed with Subhan et al. (2013), Kumar et al. (2014), Babu et al. (2014) and Bhojne et al. (2014) who found that mulberry leaves are rich in protein content, total carbohydrates, nitrogen and amino acids, and also had a highly significant positive association with production efficiency of the cocoons, cocoon weight, shell weight and shell ratio. Also the 5th larval weight was the lowest in larvae fed with M. alba var. Laevigata comparing to the other four studied varieties (El-Banna et al., 2013).

The present results open a new venue of studies that focus on the nutritional value of mulberry leaves which may not depends only on the biochemical composition, but also on physiological profile of silkworm. Hence while M. laevigata leaves had the lowest nutritional contents, larvae fed on its leaves converted the low obtained proteins and carbohydrates to high value in its haemolymph which was considerably converted to larval weights and high cocoon incidences. Such process that is due to other larval endogenous factors which may need more studies like haemolymph enzymes.

Amino acids are the building blocks of the proteins. Obviously, the amino acid pool in silkworm is derived both from proteins through histolysis and from non-protein sources like carbohydrates and lipids through de novo synthesis. Continuous increase in the levels of free amino acids is attributable to the synthesis of amino acids from non-protein sources like glucose and fatty acids (Bose et al., 1989). Transamination reactions play a leading role in the synthesis of silk amino acids. Meanwhile elevations in activities of Glutamate Oxaloacetate Transaminase and Glutamate Pyruvate Transaminase in the haemolymph of silkworm are sound indicators of the active transportation of amino acids, which increases synthesis of silk fibers (Saravanan et al., 2011).

Transaminases enzymes were high in larvae fed on M. alba var. Laevigata leaves compared to other mulberry varieties leaves as reported by Mahmoud Souad et al. (2013). This may explain that larvae produced more proteins in haemolymph and cocoons while obtained low protein content in mulberry leaves due to transaminases enzymes activity which play a vital role in the synthesis of silk protein and they are concerned with amino acids group transfer.
REFERENCES


