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Original Article

Research Concerningthe Influence of some Supplements (Spirulina and Spirulina with Sea Buckthorn extract) on Larval Weight of Silkworm (*Bombyx mori* L.)

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Abstract

The goal of our research is to organize an experiment with 3 variants of silkworms. The biological material was represented by a single silkworm breed: Baneasa 75 (B75), in order to obtain the best genetic uniformity. Only environmental conditions (feeding) were different. This research present the influence of 2 supplements on serigene glands weight at Vth age of larva.Spirulina and spirulina with sea buckthorn extract capsules provided frome S.C Hofigal Export-Import S.A. Spirulina is a rich source of proteins, contains a wide range of aminoacids, fatty acids, vitamins (biotin, tocopherol, thiamine, riboflavin, niacin, folic acid, pyrodozoic acid, beta-carotene and vitamin B12), vegetal hormones, enzymes, microelements, biological pigments. Sea Buckthorn extract is a very rich source of vitamins (vitamins A, E, C, P, carotenoids and B complex), microelemnts (phosporus, calcium, magnesium, potasium) and fatty acids. Regarding larval weight at the begining of Vth age we can observe that the mean values of the variant treated with spirulina (S) and the variant treated with spirulina with sea buckthorn extract (S+SB) are higher than the control. The mean values of both treatments in the middle of Vth age and the end of Vth age are much lower than the control.

Keywords: silkworm, spirulina, sea buckthorn, mulberry leaf, genotype x environment interaction

1. Introduction

The purpose of this paper is to study the influence of some supplements used in larva feed (aqueous extract of Spirulina 500 ppm and aqueous extract of Spirulina with Sea Buckthorn extract 500 ppm) on sericigene glands weight as aspects of the interaction genotype x environment (reaction of the same genotype - individuals of the same breed and different environment conditions - feed).

Mulberry (*Morus* species) leaf is the solo food and source of nutrition for the silkworm, Bombyx mori L. due to the presence of morin [6]. The growth and development of larva, and subsequent cocoon production are greatly influenced by nutritional quality of mulberry leaves. Supplement in silkworm nutrition like protein substitute fortified with food stuff are needed for nutritional requirement among several insects [7].

Mulberry leaf supplemented with spirulina as a feed to *Bombyx mori* L. oraly found to be effective in enhancing the larval and cocoon characters [5].

In 2007 Venkatesh Kumar et al. used in larva feeding aqueous extract of Spirulina in various

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concentrations (100 ppm, 200ppm şi 300ppm). Differences between the treatments were found significant in all the quantitative cocoon characters except shell percentage. Single cocoon weight, single shell weight, pupal weight and silk filament length are significantly higher at 300 ppm concentration compared to control [2, 5, 9].

Etebari et al. în 2004 suplimented larva feed with vitamins (ascorbic acid, thiamine, niacin and folic acid) obtaining very good results [8].

Nicula A. et al. in 2007 fed the larvae on mulberry leaves, sprayed with different biostimulatory extracts: Netin, Sea buckthorn juice, Vitis, Citriseed, and Yeast hidrolysate solutions. They report changes in larva weights, sericigene glands protein content and water retention during the IV and V age. The results showed that some biostimulatory extracts, especially Yeast hydrolisates induced the increase of larva and sericigene glands weight [1, 3].

1.Material and Method

The experiment was developed within the Laboratory of Sericulture of the Department of the 107 mulberry trees very well adapted to the pedoclimatic conditions from Transylvania.

The first silkworm group is the control where the larva were fed only with mulberry leaves (**CONTROL**). The second group was fed with mulberry leaves treated with Spirulina aqueous solution 500 ppm (**S**).

Technologies of Apicultural and Sericulture Products of the Faculty of Animal Science, USAMV Cluj-Napoca, in 2011.

The 3 experimental silkworm groups (variants) were feed with mulberry leaves from the intensive mulberry tree plantation from USAMV Cluj-Napoca. The plantation is made up of Ukraine

The third group was fed with mulberry leaves treated with Spirulina with Sea Buckthorn aqueous solution 500 ppm (**S+SB**). Supplements administration started on V^{th} age (during 8 days) 3 - 4 times/day. Larval weight was recorded at the begining, middle and the end of V^{th} age.

Fresh mulberry leaves were soaked with aqueous extract of S and S+SB , and then leaves were dried under fan before feeding to the silkworms till end of the fifth instar.

Statistical processing of data and the signification of differences between variants mean values was performed by multiple comparisons test Student-Newman-Keuls.

20 larvae of each variant were weighted to record the body weight at beginning, middle and the end of V^{th} age.

3. Results and Discussions

Because the variance (mean square) between treatments is very significant (table 1) we proceed to the multiple comparison test Student-Newman-Keuls for testing the signification of differences between means value.

Table 1. Analysis of variance regarding larval weight in the begining of V^{th} age

Source of variation	Degrees of freedom	Sum of squares	Mean square	F	Signification
Treatment	2	1.286	0.6429	25.519	***
Individual	9	1.040	0.05475	2.173	*
Error	38	0.9574	0.02519	-	-
Total	59	-	-	-	-

In the beginning of the V^{th} age (table 2) we can observe that the biggest value of maximal weight is recorded on the variants treated with S and S+SB (2.800 g and 2.831 g), those weights are higher than the control (2.344 g). Regarding the mean values, the highest value (2.270 g) was recorded in that variants fed with mulberry leaves treated with S. The mean values recorded on treatments with S+SB (2.144 g) were higher than control (1.916 g) but lower than the treatment with S (2.270 g). Variability coefficient is higher at variants treated with S (10.02) and S+SB (8.72). Confidence interval signifies the limits of theoretical mean between the ranges with a 95% probability. Table 3 show the multiple comparisons test between variants. Differences between the variant treated with S and S+SB and control are very significat.

Between those variants treated with S and S+SB diferences are significant (0.125 g) in the favor of variant treated with S.

Regarding larval weight in the middle of V^{th} age because the variance (mean square) between treatments is significant (table 4) we proceed to the multiple comparison test Student-Newman-Keuls for testing the signification of differences between means value.

Table 2. Mean values and variability estimates of larval weight in the begining of Vth age

Variant/Parameters	S	S+SB	Control
n	20	20	20
Minimal weight	1.874	1.957	1.711
Maximal weight	2.800	2.831	2.344
$x \pm s_x$	2.270 ± 0.050	2.1446 ± 0.041	1.916 ± 0.030
Standard deviation (s)	0.227	0.04185	0.135
Variability coefficient (V%)	10.02	8.72	7.05
Relative value (%)	118.475	111.899	100
Confidence interval (95%)	2.164 - 2.377	2.057 - 2.232	1.853 - 1.980

Table 3. Multiple comparisons test of larval weight in the begining of Vth age

Compared variants	Difference (g)	q	p value
S VS. CONTROL	0.353	9.965	p < 0.0001***
S+SB VS. CONTROL	0.228	6.426	p < 0.001***
S VS. S+SB	0.125	3.539	p < 0.05*

Table 4. Analysis of variance regarding larval weight in the middle of Vth age

Source of variation	Degrees of freedom	Sum of squares	Mean square	F	Significance
Treatment	2	0.5316	0.2658	3.798	*
Individual	19	1.760	0.09266	1.324	ns
Error	38	2.659	0.06998	-	-
Total	59	4.951	-	-	-

In the middle of the Vth age (table 5) mean values recorded by variants treated with S (3.187 g) and S+SB (3.364 g) are lower compared with the control (3.403 g). The variability coefficient (V%) is much higher than control at the variants treated with S (9.51) and S+SB (7.23).

Significant differences are shown between variant treated with S (-0.216) and control and also between the variant treated with S+SB (-0.039) and control (table 6). Between the variants treated with S and S+SB we can observe non significant differences.

Regarding larval weight to the end of Vth age, because the variance (mean square) between treatments is very significant (table 7) we proceed to the multiple comparison test Student-Newman-Keuls for testing the signification of differences between means value.

The mean values presented in table 8 show lower values compared to control (4.044 g) respectively 3,558 g in the variants feded with mulberry leaves treated with S and 3.869 g in the variants feded with mulberry leaves treated with S+SB.

Table 5.Mean values and variability estimates on larval weight in the middle of Vth age

Variant/Parameters	S	S+SB	Control
n	20	20	20
Minimal weight	2.720	2.765	2.868
Maximal weight	3.801	4.108	3.814
$x \pm s_x$	3.187 ± 0.058	3.364 ± 0.071	3.403 ± 0.055
Standard deviation (s)	0.263	0.320	0.246
Variability coefficient (V%)	8.25	9.51	7.23
Relative value (%)	93.652	98.853	100
Confidence interval (95%)	3.064 - 3.311	3.214 - 3.514	3.289 - 3.519

Table 6.Multiple comparisons test of larval weight in the middle of Vth age

Compared variants	Difference (g)	q	p value
S VS. CONTROL	- 0.216	3.660	p <0.05*
S+SB VS. CONTROL	- 0.039	0.6693	p < 0.05*
S VS. S+SB	- 0.176	2.991	p > 0.05^{ns}

Table 7.Analysis of variance regarding larval weight in the end of V^{th} age

Source of variation	Degrees of freedom	Sum of squares	Mean square	F	Signification
Treatment	2	2.426	1.213	20.303	***
Individual	9	1.821	0.09584	1.604	ns
Error	38	2.270	0.05975	-	-
Total	59	6.517	-	-	-

Table 8.Mean values and variability estimates on larval weight in the end of V^{th} age

Variant/Parameters	S	S+SB	Control
n	20	20	20
Minimal weight	3.026	3.525	3.662
Maximal weight	4.225	4.808	4.396
$x \pm s_x$	3.558 ± 0.068	3.869 ± 0.067	4.044 ± 0.037
Standard deviation (s)	0.308	0.302	0.168
Variability coefficient (V%)	8.66	7.82	4.17
Relative value (%)	87.982	95.672	100
Confidence interval (95%)	3.414 - 3.703	3.728 - 4.011	3.966 - 4.124

The variability coefficient (V%) is much higher at the variants treated with S (8.66) and S+SB (7.82) than control (4.17). Multiple comparisons test (table 9) show very significant differences between control and the variants treated with S (-0.486 g) and between the control and variants treated with S+SB (-0.175 g).Between variants treated with S and S+SB the differences are signifcant statisticaly (-0.311 g) for the variant treated with S+SB.

Table 9.Multiple comparisons test of larval weight in the end of V^{th} age

Compared variants	Difference (g)	q	p value
S VS. CONTROL	-0.486	8.897	$p < 0.001$***
S+SB VS. CONTROL	-0.175	3.206	$p < 0.001$***
S VS. S+SB	-0.311	5.691	$p < 0.05$*

4. Conclusions

In the V^{th} age the best effect concerning the larva weight is on mulberry leaves treated with Spiruina and Spirulina with Sea Buckthorn in the beginning of the V^{th} age where the mean values was 2.270 g and 2.144 g compared with the control (1.196g).

On the middle of the V^{th} age the mean values was close to the control regarding the treatment with Spirulina with Sea Buckthorn.

Very significant differences are shown between the control and the two treatments (S and S+SB) at the beginning and the end of Vth age.

We can observe that in the middle and the end of the V^{th} age the larval weight of treated variants with S and S+SB are lower compared with larval weight from beginning of V^{th} age.

This is due the refuse of larva to consume the treated leafs with age.

Another reason may be that leafs are in a higher concentration in aqueous solutions (500 ppm).

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