VOL. 7, NO. 3, MARCH 2012 ISSN 1990-6145
ARPN Journal of Agricultural and Biological Science


www.arpnjournals.com

RESEARCH CONCERNING THE INFLUENCE OF SOME SUPPLEMENTS (SPIRULINA AND SPIRULINA WITH SEA BUCKTHORN EXTRACT) ON QUANTITATIVE PARAMETERS OF COCOON IN SILKWORM (*Bombyx mori* L.)

B. Vlaic[1], L. Al. Mărghitaş[1], D. Dezmirean[1], A. Vlaic[1], M. Bentea[1] and Alexandra Matei[2]
[1]University of Agricultural Sciences and Veterinary Medicine, Manaştur Street, Cluj-Napoca, Romania
[2]Sericarom Company, Bucureşti-Ploieşti Avenue No. 69, Bucuresti, Romania
E-Mail: bogdan.vlaic@yahoo.com

ABSTRACT

The goal of our research is to organize an experiment on silkworms, with 3 variants. 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 presents the influence of 2 supplements on quantitative cocoon traits. Spirulina (S) and spirulina with sea buckthorn extract (S+SB) capsules provided from S.C Hofigal Export-Import S.A. Spirulina (S) 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 (SB) 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 raw cocoon weight we can observe that the mean values of the variant treated with spirulina (S) (1.836 g) are higher compared to control (1. 685 g). The mean values of shell weight (0. 395 g) are higher compared to control (0.387) in the variants fed with mulberry leaves treated with S. Regarding pupal weight, the highest average was obtained in variants fed with mulberry leaves treated with S.

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

INTRODUCTION

The aim of the present work 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 the larval weight of the V th age 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 (Tribhuwan, *et al*., 1989). 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 (House, 1996).

Mulberry leaf supplemented with spirulina used as orally administered feed to *Bombyx mori* L. found to be effective in enhancing the larval and cocoon traits (Venkataramana, 2003).

In 2007 Venkatesh Kumar *et al*., used in larva feeding aqueous extract of Spirulina in various concentrations (100 ppm, 200ppm şi 300 ppm). Differences between the treatments were found significant in all the quantitative cocoon traits except shell percentage. Single cocoon weight, single shell weight, pupal weight and silk filament length are significantly higher when 300 ppm concentration was administered, compared to control (Venkatesh K. *et al*., 2007).

Etebari *et al*., in 2004 supplemented larva feed with vitamins (ascorbic acid, thiamine, niacin and folic acid) obtaining very good results.

Our research reveals the influence of two supplements (S and S+SB) on larva weight in the Vth age, sericigen glands weight and quantitative parameters of cocoon (raw cocoon weight, shell weight and pupal weight), respectively.

In this paper we present the influence of supplements (S and S+SB) in quantitative parameters of cocoon.

MATERIALS AND METHODS

The experiment was developed within the Laboratory of Sericulture of the Department of the Technologies of Apicultural and Sericulture Products of the Faculty of Animal Science, USAMV Cluj-Napoca, in May - June, 2011.

The three experimental silkworm groups (variants) were fed with mulberry leaves from the intensive mulberry tree plantation from USAMV Cluj-Napoca. The plantation is made up of Ukraine 107 mulberry trees very well adapted to the pedoclimatic conditions from Transylvania.

The first silkworm group is the control where the larva was fed only with mulberry leaves (CONTROL).

The second group was fed with mulberry leaves treated with Spirulina aqueous solution 500 ppm (S).

The third group was fed with mulberry leaves treated with Spirulina with Sea Buckthorn aqueous solution 500 ppm (S+SB).

Supplements administration started on Vth age (during 8 days) 3-4 times/day.

Fresh mulberry leaves were soaked with aqueous extract of S and S+SB, and then they 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. Fifteen (15) cocoon of each variant were weighted.

RESULTS AND DISCUSSIONS

Because the variance (mean square) between treatments is non 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 raw cocoon weight.

Source of variation	Degrees of freedom	Sum of squares	Mean square	ˆF	Signification
Treatment	2	0.304	0.152	2.155	ns
Individual	14	0.636	0.045	0.6433	ns
Error	28	1.980	0.070	-	-
Total	44	2.921	-	-	-

The biggest values of maximal weight were recorded in variants treated with S and S + SB (2.133g and 2.331 g); these weights are bigger than control (2.129 g).

Regarding the mean values, the highest value (1.836 g) was recorded in variant fed with mulberry leaves treated with S. The mean values recorded in treatments with S+SB (1.645 g) were lower than control (1.685 g).

Compared to control (14.78%), variability coefficient is bigger in variants fed with S+SB treated leaves (16.62%) and smaller in variants fed with S treated leaves (12.19%).

Confidence interval signifies the limits of theoretical mean between the ranges with a 95% probability.

Table-2. Mean values and variability estimates of raw cocoon weight.

Variant / Parameters	S	S+SB	Control
n	15	15	15
Minimal weight	1.484	1.289	1.363
Maximal weight	2.133	2.331	2.129
$x \pm s_x$	1. 836±0.057	1. 645±0.070	1. 685±0.064
Standard deviation (s)	0.2238	0.2734	0.2491
Variability coefficient (V%)	12.19	16.62	14.78
Relative value (%)	108.96	97. 62	100
Confidence interval (95%)	1.712-1.960	1.493-1.796	1.574-1.823

Table-3 show the multiple comparisons test between variants. Differences between all comapred variants are statisticaly not significant.

Table-3. Multiple comparisons test of raw cocoon weight.

Compared variants	Difference (g)	q	P value
S vs. Control	- 0.151	-	p>0.05 ns
S+SB vs. Control	- 0.040	-	p>0.05 ns
S vs. S+SB	- 0.191	2.784	p>0.05 ns

Because the variance (mean square) between treatments is non significant (Table-4), we proceed to the multiple comparison test Student-Newman-Keuls for testing the signification of differences between means value.

Table-4. Analysis of variance regarding shell weight.

Source of variation	Degrees of freedom	Sum of squares	Mean square	ˆF	Signification
Treatment	2	0.004	0.0023	1.043	ns
Individual	14	0.034	0.0024	1.091	ns
Error	28	0.062	0.0022	-	-
Total	44	0.101	-	-	-

The mean values from Table-5 show that the biggest value (0.395 g) was recorded in variants fed with mulberry leaves treated with S.

The mean values recorded in treatments with S+SB (0.371 g) were smaller than control (0.387 g).

Compared to control (11.88%), variability coefficient is bigger in variants fed with S+SB treated leaves (17.52%) and smaller in variants fed with S treated leaves (5.06%). Confidence interval signifies the limits of theoretical mean between the ranges with a 95% probability.

Table-5. Mean values and variability estimates of shell weight.

Variant / Parameters	S	S+SB	Control
n	15	15	15
Minimal weight	0.359	0.184	0.244
Maximal weight	0.431	0.445	0.439
$x \pm s_x$	0.395±0.005	0.371±0.016	0.387±0.012
Standard deviation (s)	0.020	0.065	0.046
Variability coefficient (V%)	5.06	17.52	11.88
Relative value (%)	102.06	95.86	100
Confidence interval (95%)	0.384-0.406	0.334-0.407	0.360-0.413

Table-6 present the multiple comparisons test between variants. Differences between all comapred variants are statisticaly not significant.

Table-6. Multiple comparisons test of shell weight.

Compared variants	Difference (g)	q	P value
S vs. Control	-0.008	-	p>0.05 ns
S+SB vs. Control	-0.015	-	p>0.05 ns
S vs. S+SB	-0.024	2.014	p>0.05 ns

Because the variance (mean square) between treatments is non significant (Table-7), we proceed to the multiple comparison test Student-Newman-Keuls for testing the signification of differences between means value.

Table-7. Analysis of variance regarding pupal weight.

Source of variation	Degrees of freedom	Sum of squares	Mean square	ˆF	Signification
Treatment	2	0.236	0.118	1.948	ns
Individual	14	0.647	0.046	0.762	ns
Error	28	1.697	0.060	-	-
Total	44	2.581	-	-	-

The mean values from Table-8 show that the biggest value (1.437 g) was recorded in that variants fed with mulberry leaves treated with S and it is higher than control (1.274 g). In variant fed with S+SB mulberry leaves (1.296 g) the average values are bigger than control but smaller than variants fed with mulberry leaves treated with S.

Variability coefficient is bigger in variants fed with S+SB treated leaves (18.91%) and in control (18.75%), than in variants fed with S treated leaves (15.51%).

Confidence interval signifies the limits of theoretical mean between the ranges with a 95% probability.

Table-8. Mean values and variability estimates of pupal weight.

Variant / Parameters	S	S+SB	Control
n	15	15	15
Minimal weight	1.067	1.004	1.005
Maximal weight	1.724	1.923	1.755
$x \pm s_x$	1.437±0.057	1.274±0.062	1.296±0.062
Standard deviation (s)	0.223	0.241	0.243
Variability coefficient (V%)	15.51	18.91	18.75
Relative value (%)	110.87	98.30	100
Confidence interval (95%)	1. 314-1.561	1. 140-1.407	1.161-1.431

Table-9 present the multiple comparisons test between variants. Differences between all comapred variants are statisticaly not significant.

Table-9. Multiple comparisons test of pupal weight.

Compared variants	Difference (g)	q	P value
S vs. Control	-0. 141	-	p>0. 05 ns
S+SB vs. Control	-0. 022	-	p>0.05 ns
S vs. S+SB	-0. 163	2. 573	p>0. 05 ns

CONCLUSIONS

The best effect concerning the weight of raw cocoon was recorded in mulberry leaves treated with spirulina. The mean values were 1.836 g compared with the control (1.685 g).

Regarding the shell weight, the biggest mean value was recorded in variants fed with spirulina treated leaves (0.395 g).

Concerning pupal weight, we can observe that the highest mean value was recorded in the variants fed with spirulina treated leaves (1.437 g) compared to the control (1.296 g).

Regarding larval weight and serigene glands weight, the treatment of leaves with supplements have a positive influence.

As general conclusion, the treatment of mulberry leaves with Spirulina aqueous solution 500 ppm plays an essential role in development of cocoon quantitative parameters (raw cocoon weight, shell weight and pupal weight).

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