The Effect of Zinc Supplementation on the Production Parameters of *Bombyx mori* L. species

Mihai Benţea[1], Aurel Şara[1], Liviu Al. Mărghitaş[1], Erol Gabor[1], Daniel Dezmirean[1], Bogdan Vlaic[1], Călina Creţa[2]

[1]*University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca*
Faculty of Animal Science and Biotechnologies, 400372-Cluj-Napoca, Mănăştur, 3-5, Romania
[2]*National Institute of Public Health, Teritorial Center of Public Health Cluj,*
400349-Cluj-Napoca, Pasteur, 6, Romania

Abstract
This research aimed to evaluate the influence of Zinc, administered as food supplement for silkworm (*Bombyx mori* L.) on the larvae weight, the weight of the serigene gland, cocoon weight, cocoon shell. The research has been carried out on 5 groups of silkworms one control group (Gr.1) and 4 experimental groups (4 groups receiving zinc), consisting of 50 larvae/group. Zinc was administered in doses of 17, 34, 68 and 136 mg kg^{-1} larvae - Gr.2-Gr.5. The use of Zinc determined a very significant increase of the larvae weight, to group Gr.5 followed by groups Gr.4, Gr.3 and Gr.2 compared to the control group. The use of Zinc positively influenced the mass of the serigene glands, the cocoon weight, cocoon shell. The Zn content of the pupae from the experimental groups was higher compared to the one recorded in the control group.

Keywords: pupae, serigene glands, silkworm, zinc

1. Introduction

Minerals are major constituents of the silkworm's diets, playing a crucial role in osmotic pressure regulation of the intra- and extra-cellular liquids and participating as co-factors in different enzyme systems.
The production parameters of silkworms depend on the larval nutrition and health status. In order to improve these production parameters, a large number of minerals have been used, many studies focusing on the effect of these minerals on silkworms [1-4].
Zinc is an essential mineral and has an important role in RNA and DNA metabolism, in gene expression and is found in many classes of enzymes. Although is an "ubiquitous" element in organism, if in excess, Zn can accumulate in cells causing toxicity and metabolism disorders [5, 6]. On silkworm, Zn increases the weight of the larvae and serigene gland and reduced the mortality rate and the larval duration [7, 8].
The goal of this research was to determine the effect of Zinc, administered in the food of silkworms, on the larvae growth, the serigene glands, the cocoon weight, cocoon shell, and the Zn content of the pupae.

*Corresponding author: Mihai Benţea, Tel: 745909526
Email: mihai.bentea@usamvcluj.ro

2. Materials and methods

The experiment was carried out in the Sericulture Laboratory of the Department of Beekeeping and Sericulture, Laboratory of Animal Nutrition and the mulberry plantation of the University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca.
The biologic material was represented by the Baneasa White variety. The larvae have been reared in the same conditions of climate and

density, the only variable being the administered food. The silkworm larvae were randomly distributed in 5 groups: one control group and 4experimental groups, each group consisting of 50 larvae.

The measurements were carried out at the end of 5th larval age and after cocoon making.

The larvae were fed to fresh mulberry leaf, untreated for the control group and treated by pulverizing the mineral solutions: Zinc - 17, 34, 68, 136 mg kg^{-1} larvae. Zinc was procured from Walmark Czech Republic.

The maximal doses were chosen with regard to the maximal doses administered by other authors: for Zinc, Ashfaq [8], reported a maximum quantity of 136 mg kg^{-1} larvae deposited in larvae and feces. This dose was chosen as the maximum administered dose. The other doses were obtained by repeatedly halving the maximum administered dose.

The Zn was determined using Atomic Absorption Spectroscopy and the method of Flame Atomizer.

The statistical analysis of data and the differences significance between the variants was carried out with the Student-Newman-Keuls multiple comparison test, using the GraphPad InStat software version 3.10.

3. Results and Discussions

Data regarding the evolution of body mass of larvae from the experimental groups (treated with Zn), at the 5th age, are presented in Table 1.

Analyzing the data recorded, it can be seen that all the experimental groups had superior performances compared to the control group, the differences observed being very significant. The Zn treated groups shower superior values compared to control group, as reported by Chamundeswari and Radhakrishnaiah [9] and Mărghitaş [10]. The best results were recorded in the L5 group treated with Zinc in a dose of 136 mg Zn kg^{-1} larvae. We can say that Zinc does not have inhibitory effect in high doses, in contrary having a strong bio-stimulating effect.

Table 1. Body weight from the Zn treated larvae

Issue	UM	Gr.1 (C)	Gr.2 Zn 17 mg kg^{-1}	Gr.3 Zn 34 mg kg^{-1}	Gr.4 Zn 68 mg kg^{-1}	Gr.5 Zn 136 mg kg^{-1}
A5D7	X± $\bar{S}x$ (g)	3.930± 0.103	4.456 ± 0.082***	4.866 ± 0.083***	4.898 ± 0.087***	5.092 ± 0.075***
	Minim/ Maxim	2.824/ 5.058	3.806/ 5.265	4.137/ 5.791	4.083/ 5.747	4.529/ 6.178
	Relative value (%)	100.00	113.38	123.82	124.63	129.57

*** - p< 0.001 very significant differences (Student test); A5D7- 5th age, day 7

Significant increases of the serigene gland masses have been observed in the Zn treated groups, compared to the control group. This can be explained by the bio-stimulating effect of Zinc on the silkworm [9]. The Zn treated groups presented the highest values compared to control group (Table 2).

Table 2. The serigene gland weight extracted form the Zn treated larvae

Issue	UM	Gr.1 (C)	Gr.2 Zn 17 mg kg^{-1}	Gr.3 Zn 34 mg kg^{-1}	Gr.4 Zn 68 mg kg^{-1}	Gr.5 Zn 136 mg kg^{-1}
A5D7	X± $\bar{S}x$ (g)	1.072±0.012	1.173±0.016	1.206±0.083	1.168±0.038	1.289±0.057*
	Minim/ Maxim	1.026/1.106	1.097/1.220	0.948/1.558	1.045/1.304	1.155/1.563
	Relative value (%)	100.00	109.42	112.50	108.96	120.24

* – p < 0.05 significant differences (Student test); A5D7 - 5th age, day 7

Regarding the cocoon weight and the cocoon shell, all the experimental groups showed superior values compared to the control group (Table 3). The results are similar to those reported by Balamani [11] and Mărghitaş [10].

Table 3. Cocoon weight and the silk shell of the Zn treated groups

Issue	UM	Gr.1 (C)	Gr.2 Zn 17 mg kg^{-1}	Gr.3 Zn 34 mg kg^{-1}	Gr.4 Zn 68 mg kg^{-1}	Gr.5 Zn 136 mg kg^{-1}
Cocoon weight	X± $S\bar{x}$ (g)	1.877±0.048	2.020±0.048	2.042±0.073	2.053±0.070	2.124±0.060
	Minim/ Maxim	1.466/2.335	1.642/2.438	1.488/2.639	1.550/2.790	1.643/2.555
	Relative value (%)	100.00	107.62	108.79	109.38	113.16
Cocoon shell weight	X± $S\bar{x}$ (g)	0.392±0.030	0.438±0.011	0.447±0.018	0.465±0.007	0.466±0.009
	Minim/ Maxim	0.338/0.499	0.397/0.463	0.381/0.481	0.453/0.488	0.441/0.495
	Relative value (%)	100.00	111.73	114.03	118.62	118.88

The Zn content of the pupae was higher in all experimental groups. The differences recorded were very significant for groups 4 and 5 (68 mg kg^{-1} and 136 mg kg^{-1}) and distinctly significant and non significant in groups 3 and 2 (34 mg kg^{-1} and 17 mg kg^{-1}) (Table 4).

Table 4. Pupae Zn content

Issue	UM	Gr. 1 (C)	Gr.2 Zn 17 mg kg^{-1}	Gr.3 Zn 34 mg kg^{-1}	Gr.4 Zn 68 mg kg^{-1}	Gr.5 Zn 136 mg kg^{-1}
Dry pupae	X± $S\bar{x}$ (mg/kg)	102.83±12.75	123.80±0.38	146.23±5.43**	194.45±5.03***	282.57±6.79***
	Minim/ Maxim	80.75/124.91	123.13/124.46	136.82/146.23	185.73/203.17	270.81/294.33
	Relative value (%)	100.00	120.39	142.21	189.10	274.79

** – $p<0.01$ distinctly significant differences (Student test);
*** - $p<0.001$ very significant differences (Student test)

These high values of Zn deposited in the body, determine the high productivity of the larvae. Because Zn is a component of different enzymes [12] it determines cellular proliferation.

4. Conclusions

1. The use of Zinc in silkworms, lead to an improvement of the final body weight of the larvae, the differences observed being very significant.
2. The weight of the serigene glands was higher in the Zinc treated groups compared to the control group. The differences observed being significant for Gr. 5 (136 mg kg^{-1}).
3. The use of Zn enhanced the cocoon weight and the cocoon shell.
4. The maximum dose of Zn administered did not have any negative effects. In order to determine the toxicity threshold of Zn for silkworms, further studies are necessary.

Acknowledgements

This work has benefited from financial support through the 2010 POSDRU/89/1.5/S/52432 project, „ORGANIZING THE NATIONAL INTEREST POSTDOCTORAL SCHOOL OF „APPLIED BIOTECHNOLOGIES" WITH IMPACT ON ROMANIAN BIOECONOMY", project co-financed by the European Social Fund through the Sectorial Operational Program Human Resources Development 2007-2013.

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