VOL. 7, NO. 11, NOVEMBER 2012 ISSN 1990-6145
ARPN Journal of Agricultural and Biological Science


www.arpnjournals.com

EFFECT OF SELENIUM AND ZINC SUPPLEMENTATION ON PRODUCTION PARAMETERS AND DNA OF THE MULBERRY SILKWORM (*Bombyx mori* L.), BANEASA WHITE VARIETY

M. Bențea[1], A. Şara[1], L. Al. Mărghitaş[1], D. Dezmirean[1], E. Gabor[1], B. Vlaic[1] and Călina Creța[2]
[1]Faculty of Animal Science and Biotechnologies, University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca, Mănăştur Cluj-Napoca, Romania
[2]National Institute of Public Health, Teritorial Center of Public Health Cluj, Pasteur Cluj-Napoca, Romania
E-Mail: mihaib78@yahoo.com

ABSTRACT

This research aimed to evaluate the influence of Selenium (Se) and Zinc (Zn), administered as food supplements for silkworm (*Bombyx mori* L.) on the larvae weight, the weight of the serigene gland, cocoon weight, cocoon shell, DNA concentration and purity. The research has been carried out on 9 groups of silkworms one control group (Gr.1) and 8 experimental groups (4 groups receiving zinc and 4 groups receiving selenium), consisting of 50 larvae/group. Zn was administered in doses of 17, 34, 68 and 136 mg kg^{-1} larvae - Gr.2-Gr.5 and Se was administered in doses of 4, 8, 16 ad 32 mg kg^{-1} body weight - Gr.6-Gr.9. The use of Zn 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 Zn positively influenced the mass of the serigene glands, the cocoon weight, cocoon shell, length of the silk thread and the concentration and purity of DNA extracted from the serigene glands. The use of organic selenium determined an increased larvae weight, the differences being very significant compared to the control group. Se influenced the weight of the serigene gland, the cocoon weight, cocoon shell and the concentration and purity of DNA extracted from the serigene glands. The Zn and Se content of the pupae from the experimental groups was higher compared to the one recorded in the control group.

Keywords: silkworm, DNA,selenium, zinc, serigene glands, minerals.

INTRODUCTION

Although most of the insect species have similar nutritional needs due to the similarities between their metabolisms (Etebari *et al*., 2004), the silkworm's nutritive requirements are very different and they are almost exclusively covered by the use of mulberry leaves, due to the presence of morin (Vlaic *et al*., 2004). Mulberry leafs contain minerals up to 10%; 28% of the larval structure of the silkworm, in different ages, is represented by the absorbed minerals (Ito, 1978). 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, both in quantity and quality, a large number of minerals have been used, many studies focusing on the effect of these minerals on silkworms (Dasmahapatra, 1989; Qader *et al*., 1993; Sarker *et al*, 1995; Zaman *et al*, 1995; Nirwani and Kaliwal, 1996b; Hugar *et al*., 1998; Rajashekhargouda *et al*., 1998; Etebari and Fazilati, 2003; Goudar *et al*., 2000c, 2001b; Bhattacharya and Kaliwal, 2003; Islam *et al*., 2004; Khan *et al*., 2010).

Selenium is an essential micronutrient (Fleet, 1997) for both humans and animals. Se is found in glutathione peroxidase, an antioxidant enzyme that also maintains the integrity of the cellular membranes. Although it plays an important role in organism, Se can be toxic in higher doses, especially when administered in inorganic form, as selenites (Jensen, 1975; Gary *et al*., 1988). Although much information is available regarding the effects of Se on mammals, little is known on the effect of Se on insects (Simmons *et al*., 1988; Deka *et al*., 1999; Smitha and A. Vijaya Braskara Rao, 2010). On silkworm, Se improves the larvae body weight, the weight of the cocoon, silk gland and the cocoon shell (Smitha, 2001, 2006; Mărghitaş *et al*., 2009).

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 (Albergoni *et al*., 1980; Spurgeon *et al*., 2000; Kizilkaa, 2005). On silkworm, Zn increases the weight of the larvae and serigene gland and reduced the mortality rate and the larval duration (Balamani *et al*., 1995; Hugar and Kaliwal, 1999, 2002; Ashfaq *et al*., 2010).

The goal of this research was to determine the effect of Se and Zn, administered in the food of silkworms, on the larvae growth, the serigene glands, the cocoon weight, cocoon shell, length of the silk thread, concentration and purity of DNA extracted from the serigene glands and the Zn and Se content of the pupae.

MATERIAL 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

mulberry trees belong to the Ukraine 107 variety, well adapted to the Transylvanian region.

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 9 groups: one control group and 8 experimental groups, each group consisting of 50 larvae.

The larvae were fed to fresh mulberry leaf, untreated for the control group and treated by pulverizing the mineral solutions: Zn - 17, 34, 68, 136 mg kg^{-1} larvae, Gr.2-Gr.5 and Se - 4, 8, 16, 32 mg kg^{-1} larvae, Gr.6-Gr.9.

Zinc was procured from Walmark Czech Republic, as 15 mg tablets, and the Selenium was administered in form of Sel-Plex, aquired from Alltech USA, calculated at an average dose of 2200 mg Se kg^{-1} commercial product (EFSA, 2011).

The maximal doses were chosen with regard to the maximal doses administered by other authors: for Se, Smitha S. (2001) found the LD50 to be of 32.39 mg Se kg^{-1} body weight, in our experiment the highest dose being 32 mg Se. Regarding Zn, Ashfaq *et al*. (2010), 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.

In the 7^{th} day of the V instar, the larvae were weighted and the serigene gland of 15 worms in each group was extracted for weighting and DNA extraction. After spinning, the cocoon weight, silk incartment weight and the length of the silk thread were measured in all remaining individuals.

DNA was extracted from the posterior region of the serigene gland using the Wizard Genomic DNA Purification Kit, purchased from Promega. The concentration and purity of the DNA were determined using the method described by Suzuki *et al*., (1972), using a NanoDrop ND-2000 spectrophotometer.

The Zn and Se content were determined on 10 worms in each group, using Atomic Absorption Spectroscopy, according to the method described by Walsh and Willis (1966), using a Shimadzu AA-6300 Atomic Absorption Spectrometer.

Data was expressed as mean ± standard error of mean. The statistical analysis was carried out using one-way ANOVA an a comparison of the mean values was done by using Student-Newman-Keuls multiple comparison test, using the GraphPad InStat software version 3.10.

RESULTS AND DISCUSSIONS

Data regarding the evolution of body weight and serigene gland of larvae from the experimental groups (treated with Zn and Se), at the V instar, are presented in Table-1.

Table-1. Weight of the body and serigene gland from the Zn and Se treated groups.

Issue	**Body weight** (g)	**Serigene gland weight** (g)
Gr.1 (C)	3.930 ± 0.103	1.072 ± 0.012
Gr.2 (Zn 17 mg kg^{-1})	4.456 ± 0.082***	1.173 ± 0.016
Gr.3 (Zn 34 mg kg^{-1})	4.866 ± 0.083***	1.206 ± 0.083
Gr.4 (Zn 68 mg kg^{-1})	4.898 ± 0.087***	1.168 ± 0.038
Gr.5 (Zn 136 mg kg^{-1})	5.092 ± 0.075***	1.289 ± 0.057*
Gr.6 (Se 4 mg kg^{-1})	4.456 ± 0.082***	1.092 ± 0.024
Gr.7 (Se 8 mg kg^{-1})	4.511 ± 0.083***	1.123 ± 0.064
Gr.8 (Se 16 mg kg^{-1})	4.589 ± 0.074***	1.148 ± 0.051
Gr.9 (Se 32 mg kg^{-1})	4.442 ± 0.084***	1.060 ± 0.069

*** - $p < 0.001$ very significant differences (Student test); * - $p < 0.05$ significant differences (Student test)

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 results obtained confirm the data observed by Mărghitaş *et al*. (2009). The Zn treated groups shower superior values compared to the Se treated groups and the control group, as reported by Chamundeswari and Radhakrishnaiah (1994) and Mărghitaş *et al*. (2009). 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.

The Se treated groups presented values superior to the control group, but lower compared to the Zn treated group, the differences observed being very significant for all the experimental groups. The maximum administered dose (32 mg Se kg^{-1} larvae - Gr9) did not have a lethal effect, reported as DL50 by Smitha (2001). This is due to

the fact that the organic combinations of Selenium have a lower toxicity than the sodium selenite.

Dasmahapatra *et al*. (1989); Etebari and M. Fazilati (2003); Goudar K.S. and B.B. Kaliwal (2000c); Hugar I. and B.B.Kaliwal (1999) and Islam *et al*. (2004), confirm that mineral supplementation of mulberry leafs enhances the final body weight of the silkworm larvae.

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 Chamundeswari and Radhakrishnaiah, (1994). Similar results have been reported by Mărghitaş *et al*. (2009). The Zn treated groups presented the highest values compared to all groups, including the control group (Table-1).

In Se treated groups, the weight of the serigene gland increases directly proportionally to the Se dose. The maximum administered dose (32 mg Se kg^{-1} larvae) had an inhibitory effect, the group receiving this dose (Gr.9) presenting lower performances, even compared to the control group. Although the body weight recorded in this group were superior to the ones recorded in the control group, the serigene gland masses were lower compared to the control group, the high dose of selenium having a negative influence on the serigene glands mass (Table-1). This effect was reported by Mărghitaş *et al*., (2009) after administering Selenium (SelPlex) in doses of 400 ppm compared to 100 and 200 ppm. The results are confirmed by Smitha and Rao (2010).

Regarding the cocoon weight and the cocoon shell, all the experimental groups showed superior values compared to the control group (Table-2). The results are similar to those reported by Balamani *et al*. (1995) and Mărghitaş *et al*. (2009).

Table-2. Cocoon weight, silk shell and silk thread length of the Zn and Se treated groups.

Issue	**Cocoon weight** (g)	**Cocoon shell weight** (g)	**Length of the silk thread** (m)
Gr.1 (C)	1.877 ± 0.048	0.392 ± 0.030	981,5 ± 7,89
Gr.2 (Zn 17 mg kg^{-1})	2.020 ± 0.048	0.438 ± 0.011	1075,5 ± 9,59***
Gr.3 (Zn 34 mg kg^{-1})	2.042 ± 0.073	0.447 ± 0.018	1173,7 ± 6,47***
Gr.4 (Zn 68 mg kg^{-1})	2.053 ± 0.070	0.465 ± 0.007	1259,2 ± 9,18***
Gr.5 (Zn 136 mg kg^{-1})	2.124 ± 0.060	0.466 ± 0.009	1264,9 ± 7,58***
Gr.6 (Se 4 mg kg^{-1})	1.930 ± 0.056	0.419 ± 0.025	1042,3 ± 8,01***
Gr.7 (Se 8 mg kg^{-1})	1.985 ± 0.053	0.434 ± 0.013	1101,1 ± 7,79***
Gr.8 (Se 16 mg kg^{-1})	2.042 ± 0.073	0.438 ± 0.019	1132,8 ± 12,65***
Gr.9 (Se 32 mg kg^{-1})	1.888 ± 0.058	0.401 ± 0.080	986,8 ± 9,76

*** - $p < 0.001$ very significant differences (Student test)

As with larvae weight and serigene glands weight, maximum administered dose (32 mg Se kg^{-1} larvae) had an inhibitory effect on the weight of the cocoon and the cocoon shell, the group receiving this dose (Gr.9) presenting lower performances compared to all experimental groups (Table-2). Result are similar to those reported by Mărghitaş *et al*. (2009), who reported that the cocoon shell weight was lower in groups receiving 400 ppm Se compared to groups receiving 100 and 200 ppm.

The length of the silk thread was significantly higher in all the Zn treated groups compared to the control group. The length of the silk thread increased as the Zn dose increased, the highest value being recorded in group 5 (136 mg Zn/kg larvae); the average length of the silk thread was 283.4 meters longer in this group compared to the control group.

The inhibitory effect of the high Se dose could be observed in the length of the silk thread. At a dose of 32 mg Se/kg larvae the length of the silk thread was higher compared to the control group but lower compared to the other experimental groups (Table-2). The results are similar to those recorded by Mărghitaş *et al*. (2009) on Romanian silkworm hybrids, the performances having a similar ascending trend in the Zn and Se treated groups.

DNA has the outmost importance in the synthesis of specific silk proteins (fibroin and sericins), a higher concentration and purity of DNA leading to higher silk quantity and quality. In the Zn treated groups, the DNA concentration and purity were superior to those of the control group, the highest concentration being recorded in group 3 (34 mg Zn kg^{-1}) (Table-3).

Table-3. DNA concentration and purity in the Zn and Se treated groups.

Issue	**DNA concentration** (ng/µl)	**DNA purity** (260/280)
Gr.1 (C)	234.72 ± 18.87	1.106 ± 0.040
Gr.2 (Zn 17 mg kg^{-1})	245.26 ± 103.83	1.300 ± 0.101
Gr.3 (Zn 34 mg kg^{-1})	771.42 ± 223.29	1.574 ± 0.113
Gr.4 (Zn 68 mg kg^{-1})	295.2 ± 106.35	1.480 ± 0.136
Gr.5 (Zn 136 mg kg^{-1})	311.54 ± 98.45	1.182 ± 0.024
Gr.6 (Se 4 mg kg^{-1})	295.2 ± 106.35	1.48 ± 0.136
Gr.7 (Se 8 mg kg^{-1})	301.92 ± 114.07	1.366 ± 0.032
Gr.8 (Se 16 mg kg^{-1})	395.12 ± 62.20	1.686 ± 0.086*
Gr.9 (Se 32 mg kg^{-1})	254.12 ± 47.28	1.278 ± 0.088

* - p < 0, 05 significant differences (Student test)

The Se treated groups showed superior values compared to the control group, excepting group 9 (32 mg Se kg^{-1}), were Se had an inhibitory effect. The highest values were recorded in group 8 (16 mg Se kg^{-1}), were significant differenced were recorded compared to the control group (Table-3). The results are comparable to those reported by Furdui Emilia (2011) who recorded values of DNA concentrations, in different varieties, varying between 217.09 ng μl^{-1} and 565.19 ng μl^{-1}. Also the DNA purity varied between 1.27-1.58 (Furdui Emilia, 2011).

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). The values recorded are close to those reported by Ashfaq *et al*. (2010) who found maximum accumulated Zn in quantities of 91.375±0.019 mg kg^{-1} in larvae fed with mulberry leaf harvested from mulberry trees sprinkled with residual water containing Zn.

Table-4. Zn and Se content of the pupae.

Issue	**Zn** (mg/kg)	**Se** (µg/ kg)
Gr.1 (C)	102.83 ± 12.75	33.66 ± 1.50
Gr.2 (Zn 17 mg kg^{-1})	123.80 ± 0.38	nd
Gr.3 (Zn 34 mg kg^{-1})	146.23 ±5.43**	nd
Gr.4 (Zn 68 mg kg^{-1})	194.45±5.03***	nd
Gr.5 (Zn 136 mg kg^{-1})	282.57 ± 6.79***	nd
Gr.6 (Se 4 mg kg^{-1})	nd	55.67 ± 2.61***
Gr.7 (Se 8 mg kg^{-1})	nd	58.10 ± 3.68***
Gr.8 (Se 16 mg kg^{-1})	nd	73.26 ± 2.13***
Gr.9 (Se 32 mg kg^{-1})	nd	99.38 ± 2.58***

*** - p < 0.001 very significant differences (Student test);** - p < 0.01 distinctly significant differences (Student test); nd- not determined

The Se Content of the experimental groups was very high, with values over 3 times the ones recorded in the control group. The differences recorded are very significant for all the experimental groups (Table-4).

These high values of Zn and Se deposited in the body, determine the high productivity of the larvae. Because Zn is a component of different enzymes and insuline (Şara A., 2006) it determines cellular proliferation and a an increase in DNA concentration and purity.

Also Se is an essential part of the glutathione-peroxidase enzyme family (GSX-Px), having a positive effect on cell proliferation and on growth. Dasmahapatra *et al*. (1989) reported that minerals (K, Co and Ca) had a positive influence on the DNA content of the serigene glands; in this experiment the influence of Zn and Se on DNA concentration and purity has been observed.

CONCLUSIONS

The use of Zinc and Selenium in silkworms, lead to an improvement of the final body weight of the larvae.

The weight of the serigene glands was higher in the Zinc treated groups compared to the control group. In the selenium treated groups, the weight of the serigene glands directly proportional with the increase of the Selenium dose; the results recorded being lower than those recorded in the control group, the highest administered dose of selenium (32mg kg^{1}) having an inhibitory effect.

The use of Zn and Se enhanced the cocoon weight, the cocoon shell and the length of the silk thread.

Regarding the use of Zn and Se, the 34 mg Zn kg^{-1} dose and the 16 mg Se kg^{-1} dose are recommended in order to obtain a highly concentrated and pure genetic material; the 32 mg Se kg^{-1} is not indicated.

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.

REFERENCES

Albergoni V., E. Piccinni and O. Coppellotti. 1980. Response to heavy metals in organisms-I. Excretion and accumulation of physiological and non physiological metals in Euglena glacilis. Comp. Biochem. Physiol C. 67: 121-127.

Ashfaq M., W. Afzal and M. Asif Hanif. 2010. Effect of Zn (II) deposition in soil on mulberry-silk worm food chain. African Journal of Biotechnology. 9(11): 1665-1672.

Balamani R., S.P. Prince, W. Subburam and V Subburam. 1995. Effect of zinc on the nutritional indices, economic characters of cocoon and quality of silk ob *Bombyx mori* L. Indian J., Seric. 34: 69-71.

Bhattacharya A. and B.B. Kaliwal. 2003. Influence on mineral potassium permanganate on the economic parameters of the silkworm, *Bombyx mori* L. Proceeding of National Conference on Tropical Sericulture for Global Competitiveness. p. 91.

Chamundeshwari P. and K. K. Radhakrishnaih. 1994. Effect of zinc and nickel on the larval and cocoon characters of silkworms, *Bombyx mori*. L. Sericologia. 34(2): 327-330.

Dasmahapatra A.K., M.K. Chakrabarti and A.K. Medda. 1989. Effect of potassium iodide, cobalt chloride, calcium chloride and potassium nitrate on protein, RNA and DNA content of silkgland of silkworm nistari race, Sericologia. 29: 355-359.

Deka J, M.R.H. Azad, S. Saharia, D.K. Sharma and A. Borkotoki. 1999. Effect of sodium selenite on nutritional indices, cocoon characters, silk gland, protein and productivity of silk yarn of ERI silk worm, (*Samia cynthia* ricini). Proceedings of NSTS, 3, Department of Sericulture, University of Agric. Sciences, GKVK, Bangalore, India.

EFSA Panel on Additives and Products or Substances used in Animal Feed (FEEDAP) European Food Safety (EFSA), Parma, Italy. 2011. Scientific Opinion on Safety and efficacy of Sel-Plex (organic form of selenium produced by *Saccharomyces cerevisiae* CNCM I-3060) for all species. 9(4): 2110.

Etebari K. and M. Fazilati. 2003. Effect of feeding on mulberry's supplementary leaves with N, P and K in some biological and biochemical characteristics of silkworm. Journal of Science Technology Agriculture and Natural Resources. 7: 233- 244.

Etebari K., R. Ebadi and L. Matindoost. 2004. Effect of feeding mulberrys enriched leaves with ascorbic acid some biological, biochemical and economical characteristics of silkworm *Bombyx mori* L. J. Indust. Entomol. 7: 81-87.

Fleet J.C. 1997. Dietary selenium repletion may reduce cancer incidence in people at high risk who live in areas with low soil selenium. Nutr. Rev. 55: 29-48.

Furdui E. 2011. Characterization of some breeds and hybrids of *Bombyx mori* L. silkworms through molecular techniques, Ph.D. Thesis. Cluj-Napoca, Romania . p. 124.

Gary H.H., David J. Hoffman and Lyn G. Gold. 1988. Toxicity of organic and inorganic selenium to Mallard Ducklings. Arch. Environ. Contam. Toxicol. 77: 561-568.

Goudar K.S. and B.B. Kaliwal. 2000c. Effect of potassium nitrate supplementation on economic parameters of silkworm, *Bombyx mori* L., Int. J. Indust. Entamol. 1: 47-52.

Goudar K.S. and B.B. Kaliwal. 2001b. The effect of mineral salt, magnesium sulphate and potassium nitrate on the economic parameters of the silkworm. *Bombyx mori* L., Int. J. Indust. Entomol. 3: 69-73.

Hugar I. and B.B. Kaliwal. 1999. Effect of zinc chloride on some economic parameters of the bivoltine silkworm. *Bombyx mori* L. Bull. Sericult. Res. 10: 35-42.

Hugar I. and B.B. Kaliwal. 2002. Effect of zinc chloride economical traits of the bivoltine silkworm. *Bombyx mori* L. Int. J. Indust. Entomol. 5: 75-79.

Hugar I.I., R.B. Nirwani and B.B. Kaliwal. 1998. Effect of zinc chloride on the bio-chemical changes in the fat body and haemolymph of the bivoltine silkworm. *Bombyx mori* L. Sericologia. 38: 299-303.

Islam R., A. Abdul, P. Dipak, S. Shaheen, B. Nilufa and R. Islam. 2004. Effect of salt, nickel cloride supplimentation on the growth of silkworm *Bombyx mori* L. Journal of Biological Science. 4: 170-172.

Ito T. 1978. Silkworm Nutrition; in the Silkworm an Important Laboratory Tool. Tazima, Y (ed). Kodansha Ltd., Tokyo, Japan. pp. 121-157.

Jensen L.S. 1975. Modification of selenium toxicity in chicks by dietary silver and copper. J. Nutr. 105: 769-775.

Khan M. A., W. Akram, M. Ashfaq, H.A.A. Khan, Y. K. Kim and J.-J. Lee. 2010. Effects of optimum doses of nitrogen, phosphorus, potassium and calcium on silkworm. *Bombyx mori* L., growth and yield. Entomological Research. 40: 285-289.

Kizilkaa R. 2005. The role of different organic wastes on zinc bioaccumulation by earthworm *Lumbricus terrestris* L. (oligochaeta) in successive Zn (II) added soil. Ecol. Eng. 25: 322-331.

Mărghitaş L., D. Dezmirean, Cristina Bojan, I. Paşca and G. Lenhel. 2009. Hormonal supplements and nutrition for silkworm. (In Romanian) Ed. Academic Press Cluj-Napoca, Romania. pp. 130-136, 150-157.

Nirwani R. B. and B.B. Kaliwal. 1996b. Increase of silk production and quantitative changes of carbohydrate and protein in the fat body and haemolymph after feeding potassium sulphate to bivoltine *Bombyx mori* L. Sericologia. 36: 523-530.

Quader M. A., A. Sarker, M.A. Rab and A.C. Barman. 1993. Effects of potassium iodide and iodised salt on the larval growth and cocoon characters of the silkworm, *Bombyx mori* L. Race BSR-83. Sericologia. 33: 595-598.

Rajashekhargouda R., M. Gopalan, S. Jayaraj and N. Natarajan. 1998. Influence of thyroxine sodium tablets on mulberry silkworm *Bombyx mori* L. Sericologia. 38: 651-655.

Sarker A., M. Haque, M. Rab and N. Absar. 1995. Effects of feeding mulberry (Morus sp.) supplemented with different nutrients to silkworm. *Bombyx mori* L., Curr. Sci. 69: 185-188.

Simmons T.W., I.S. Jamall and R.A. Lockshin. 1988. Accumulation distribution and toxicity of selenium in the adult house fly Musca domestrica. Comp. Biochem. Psysiol. Pharmacol. Toxicol. Endocrinol. 91: 559- 564.

Smitha S. 2001. Impact of selenium on some physiological and cocoon commercial characters of silkworm *Bombyx mori* L. M Phil. Dissertation, Sri Krishnadevaraya University, Anantapur, India.

Smitha S. 2006. Impact of Selenium on some physiological and cocoon commercial characters of silkworm *Bombyx mori* L. M. Phil. Dissertation, S.K. University, Anantapur, India.

Smitha S. and A. Vijaya Braskara Rao. 2010. Effects of Selenium on the Physiology of Heart Beat, Oxygen Consumption and Growth in Silkworm *Bombyx mori* L. American- Eurasian Journal of Toxicological Sciences. 2(4): 215-219.

Spurgeon D.J., Svendsen C., Rimmer V.R., Hopkin S.P. and Weeks J.M. 2000. Relative sensitivity of life cycle and biomarker responses in four earthworm species exposed to zinc. Environ. Toxicol. Chem. 19: 1800-1808.

Suzuki Y., L. Gage and D.D. Brown. 1972. The genes for silk fibroin in *Bombyx mori* L. J. Mol. biol. 70: 637-649.

Şara A. 2006. Fodder and feed use in animal nutrition (in Romanian). Risoprint Cluj-Napoca, Romania. p. 14.

Vlaic B, L. Al. Mărghitaş, D. Dezmirean and M. Bențea. 2004. Research concerning the influence of biostimulating apicultural products administered in silkworm feeding on body weight gain during V-th age, Buletin UASVM-Animal Science and Biotechnologie, 60/2004, ISSN 1454-2382: 274-277.

Walsh A and Willis JB. 1966. Atomic absorption spectrometry. In: Standard Methods of Chemical Analysis, Part A, Instrumental Methods. Welcher FJ (ed). Van Nostrand, Princeton, NJ. 3: 105-117.

Zaman K. and M. Ashfaq şi W. Akram. 1995. Effect of feeding Mg and N treated mulberry leaves on larval development of silkworm *Bombyx mori* L. and silk yield. Pak. Entomol. 18: 78-79.