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Effect of Chromium on Hemolymph Catalase Activity and Cocoon Quality of Two Mulberry Silkworm (*Bombyx mori* L.) Races

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Even though there are strict government policies regarding the establishment of mulberry plantations near industrial processes and irrigating plantations with the waste from these industrial processes, most rural industrial sources and farmers in China, but more particularly in India evade these policies (Patil 1999, Nath and Kumar 1999). The interest in chromium (Cr) speciation originates from the widespread use of this metal in many industrial processes such as metallurgy (alloys, corrosion inhibitors), electroplating, painting pigments and chemical compounds, and leather tanning (Nusko and Heumann 1994, Kotas and Stasicka 2000). As a result of these industrial processes, large quantities of chromium compounds are discharged as liquid, solid and gaseous wastes into the environment and can cause significant adverse biological and ecological effects.
In this study, the commercial silkworm (*Bombyx mori* L.) was used as a bio-indicator species. According to our preliminary investigations, *B. mori* is highly sensitive to hexavalent chromium. Other attributes of this insect as a bio-indicator organism include: ease of collection and sampling, excellent survival in the laboratory and feces readily collected for analysis. Above all, it is an economically important insect in the Asian continent and in most parts of Europe and Africa as a mainstay of the sericulture industry. Our study aims to determine the effects of form and quantity of chromium on enzyme activities in functionally important tissues and on cocoon quality in two mulberry silkworm races.

MATERIALS AND METHODS

All test chemicals and reagents used were of analytical grade purchased from Zhejiang University Chemical Store, Huajiachi campus, Hangzhou, P.R.China. Chemicals used were Cr (VI) (supplied as Potassium Dichromate ($K_2Cr_2O_7$)) and Cr (III) (supplied as chromium sulphate, $Cr_2(SO_4)_3 \cdot 6H_2O$).

The two silkworm races namely, Qiufeng X Baiyu and Qingsong X Haoyue, were kindly provided by Zhejiang Silk Research Center of China Sericulture Info Center and the Sericulture Laboratory of College of Animal Sciences, Zhejiang University, Huajiachi campus, Hangzhou, P.R.China.

Two sets of experiments were carried out simultaneously. To investigate

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hemolymph catalase activity, 135 5th instar larvae of either race were placed on control or test chemicals and held through the onset of spinning. Each treatment containing 10-15 silkworm larvae was replicated three times. Fresh mulberry leaves were sprayed with concentrations of 400 mg/L of either Cr (III) or Cr (VI) ions, removed immediately and allowed a few minutes for water evaporation before being fed to silkworm larvae. Later the silkworm larvae were separately fed three times a day up to spinning on (i) fresh mulberry leaves treated with distilled water (controls), (ii) fresh leaves treated with 400 mg/L of Cr (III) and (iii) fresh leaves treated with 400 mg/L of Cr (VI) species. Silkworm hemolymph (0.6 mL) for each treatment was collected for a period of eight consecutive days and analyzed to determine the hemolymph catalase activity using the procedures and calculations of Lu and Du (2000). The specific activity of catalase was expressed as decomposed milligram of H_2O_2 per minute per milliliter of silkworm hemolymph (mg/min/mL).

Determination of cocoon quality for both silkworm races was initiated immediately after molt IV of silkworm larvae. Each experimental tray containing 10-15 silkworm larvae of comparable size and age was replicated three times. Fresh mulberry leaves were sprayed with concentrations of Cr (III) and Cr (VI) ions containing 400, 500, 600, 700 and 800 mg/L. After spraying, the leaves were held in the air a few minutes for water evaporation before being fed to silkworms. Later the silkworms were separately fed three times a day up to spinning on (i) fresh leaves treated with distilled water (controls), (ii) leaves treated with concentrations of trivalent chromium ions and (iii) fresh leaves treated with hexavalent chromium species. Ten cocoons were harvested on the fifth day of spinning and were deflossed. The deflossed cocoons were weighed. Each weighed, deflossed cocoon was slightly split open and the pupa removed and weighed. The cocoon shell rate (%) was determined as:

$$\text{Cocoon shell rate (\%)} = (\text{cocoon shell weight} / \text{cocoon weight}) \times 100$$

This rate is one of the parameters used to describe the quality of the cocoon. A cocoon shell rate ≥20% indicates good silk. The statistical significance of differences between treatments or races was analyzed with Tukey's HSD test at a significance level of 0.05.

RESULTS AND DISCUSSION

Table 1 shows the decomposition of hydrogen peroxide (H_2O_2) (a measure of hemolymph catalase activity) of 5th instar larvae of chromium-treated silkworm larvae. ANOVA showed that means for treatment groups did not differ significantly according to Tukey's test when 400 mg/L of either Cr (III) ions or Cr (VI) species was used compared to the control.

Observable trends were seen in hemolymph catalase activity over time. There was a progressive increase in hemolymph catalase activity peaking on day 6, then declining on day 7 and 8 for both silkworm races. Similar results were obtained by Lü and Du (2000) in which day 6 showed the highest hemolymph catalase activity of 5th instar larvae treated with fluoride.

Table 1. Hemolymph catalase activity (mg/min/mL) of 5th instar larvae of chromium-treated silkworms.

Race	Days	Control	Treatment 400mg/L Cr(III)	400 mg/L Cr (VI)
Qiufeng X Baiyu				
	1	0.97	1.01	1.06
	2	1.01	1.09	1.30
	3	1.09	1.13	1.37
	4	1.11	1.17	1.49
	5	1.07	1.20	1.63
	6	2.73	2.94	3.01
	7	1.07	2.77	2.81
	8	1.26	1.03	1.06
	Mean	1.26	1.54	1.72
Qingsong X Haoyue				
	1	1.16	1.23	1.31
	2	1.17	1.30	2.30
	3	1.25	1.39	2.73
	4	1.32	1.78	2.95
	5	1.27	1.90	3.50
	6	2.90	3.75	3.91
	7	2.78	3.66	3.80
	8	1.11	1.21	1.30
	Mean	1.62	2.03	2.81

Based on Tukey's test, there were no significant differences for means between treatments or races.

Table 2 shows the effects of chromium species on cocoon quality. As the concentrations of either chromium ion increased, reductions in cocoon weights and cocoon shell weights were observed in both silkworm races. The lowest cocoon weight and cocoon shell weight in both silkworm races occurred when treated with 800 mg/ L of either Cr (III) or Cr (VI) ions. Higher cocoon weights and cocoon shell weights were seen in the Qiufeng X Baiyu race compared to the Qingsong X Haoyue race. The lowest cocoon weight and cocoon shell weight of 1.30 g and 0.12 g respectively was seen in Qingsong X Haoyue silkworm races when 800 mg/L of Cr (VI) ions was used.

Significant differences were observed in cocoon shell rate for both races irrespective of the form and quantity of chromium used. The linear regressions of chromium species versus cocoon shell rate of each silkworm race produced strong negative correlation indicating that increasing the chromium contents significantly reduced the cocoon shell rate. As the concentrations of either chromium ion increased, the cocoon shell rate (%) of both silkworm races was significantly reduced. Cr (VI) at 800 mg/L drastically reduced the cocoon shell rate in both silkworm races. Overall, the higher cocoon shell rate shown in Qiufeng X Baiyu

Table 2. Comparison of cocoon qualities due to different chromium ions concentrations.

Treatment		Qiufeng X Baiyu			Qingsong X Haoyue		
Compound	Conc. (mg/L)	Cocoon Wt.(g)	Cocoon Shell Wt.(g)	Cocoon Shell Rate (%)	Cocoon Wt.(g)	Cocoon Shell Wt.(g)	Cocoon Shell Rate (%)
Control		2.03 NS	0.43 NS	21.2	2.01 NS	0.42 NS	20.9
Cr (III)	400	2.03	0.42	20.7	2.01	0.40	19.9
	500	1.99	0.41	20.6	1.99	0.38	19.1
	600	1.99	0.39	19.6	1.98	0.37	18.7
	700	1.81	0.28	15.5	1.80	0.27	15.0
	800	1.75	0.26	14.9	1.70	0.22	12.9
r		-0.934*	-0.937*	-0.932*	-0.926*	-0.946*	-0.948*
Cr (VI)	400	2.02	0.42	20.8	2.01	0.39	19.4
	500	1.99	0.41	20.6	1.99	0.37	18.6
	600	1.98	0.35	17.7	1.97	0.33	16.8
	700	1.37	0.17	12.4	1.36	0.14	10.3
	800	1.33	0.15	11.3	1.30	0.12	9.23
r		-0.891*	-0.943*	-0.958*	-0.894*	-0.938*	-0.950*

NS: No significant difference among treatments. r: Correlation coefficient. Values marked with * are significant at 0.05 significance level (2-tailed).

in Cr (III) and Cr (VI) treatments indicated better cocoon quality than in the Qingsong X Haoyue race.

Chromium-fed larvae had increased hemolymph catalase activity, possibly due to changes in oxygen consumption in treated larvae. Hydrogen peroxide (H_2O_2), due to its small molecular mass, can act on cells, pass cell membranes and enter into the nuclei of cells. In these cells, H_2O_2 reacts with metal ions producing hydroxyl radicals which cause DNA oxidation and damage (Sagripanti and Kraemer 1989, Fernandes et al. 2000). Catalase enzymes produced by organisms can impede the production of H_2O_2, indicating that these enzymes act as a defensive mechanism. The increased hemolymph catalase activity observed in larvae of both silkworm races may thus represent a defensive mechanism to protect cells against the poisonous effects of chromium ions taken up by the larvae. The high hemolymph catalase activity on day 6 in all treatment groups may be due to changes in the oxygen consumption pattern of silkworm larvae as a result of histolysis and histogenesis taking place within the silkworm body fluid prior to pupation.

Cocoon spinning and pupation are instinctive physiological traits of silkworms. However, some silkworm larvae fail to spin and pupate and in certain cases, naked pupae are formed. Some 5^{th} instar silkworm larvae fall to the ground and die. As reported by Maribashetty et al. (1999), silkworm larvae are sensitive to certain chemicals and pesticides. Dietary contact with these chemicals or pesticides at the early stages of an instar can lead to abnormal growth of the silk gland and production of non-cocooning silk. We found significant differences in the quality of cocoons for both silkworm races when exposed to chromium ions in their diet. Cocoon shell rates below 20% indicated poor quality cocoons, possibly, due to the abnormal growth of the silk gland and production of non-cocooning silk. We therefore conclude that the high hemolymph catalase activity coupled with the poor cocoon quality as seen in both silkworm races indicate that mulberry leaves exposed to supernormal levels of chromium ions, especially hexavalent chromium ions, can be deleterious to silkworm larvae.

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