Available online at www.ijpab.com

ISSN: 2320 – 7051
Int. J. Pure App. Biosci. **2 (5):** 196-202 (2014)

Research Article

INTERNATIONAL JOURNAL OF PURE & APPLIED BIOSCIENCE

Total Carbohydrates and Economic Parameters of *Bombyx mori* under the Influence of Zinc Chloride-Enriched Mulberry Diet

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ABSTRACT

The study traces the correlation between tissue- based carbohydrate levels and economic traits of the silkworm, Bombyx mori, both under normal and zinc-fed conditions during fifth instar development. The growth trends of carbohydrates in silk gland (SG), fat body (FB), haemolymph (HL) and segmental muscle (SM) indicate that the total carbohydrates tend to accumulate in remarkable proportions (~814%) in SG and maintain a constant rate of influx in FB, HL and SM. In terms of their rate of accumulation, the SG recorded a higher compound periodical growth rate (CPGR) of 41.82%, followed by SM (40.54%), FB (27.24%) and HL (9.17%). When, the silkworm larvae were fed with zinc chloride-enriched mulberry leaves, the rate of accumulation of carbohydrates increased remarkably (CPGR:46.61%) in SG, but a constant steady trend, as observed in the control batches, was maintained in the other three tissues. While carbohydrate levels constitute the chief energy reserves for metabolism and silk production, the economic parameters of both cocoon and silk (silk yield, shell weight, floss weight, floss-shell ratio, floss-silk ratio, raw silk weight, renditta and denier) were significantly modulated under the influence of zinc salt.

***Key words**: Bombyx mori, Carbohydrates, Economic parameters, Zinc chloride.*

INTRODUCTION

Bombyx mori is a monophagous insect that feeds on mulberry leaves. It obtains all the essential minerals (eg. sodium, potassium, calcium, magnesium etc) and other nutrients (eg. proteins, carbohydrates, lipids, vitamins etc) from the mulberry diet. Zinc is an essential trace element well known for its beneficial effects. It acts as a cofactor of many enzymes including alkaline phosphatase, carbonic anhydrase, glutamate dehydrogenase, alcohol dehydrogenase and pancreatic carboxypeptidase[1]. In insects, the larval growth and development are regulated essentially by stimulating metabolism through enhanced enzyme activity, hormonal mediation, replication, transcription and neuronal activity[2]. Carbohydrates are the major sources of metabolic energy for plant feeding insects like *Bombyx mori*. The ability of a plant feeding insect to use a particular substance largely depends upon the type of digestive enzymes it produces. The nutritive effects of carbohydrates and selected nutrients are studied either by determining their effects on weight gain and survival or by assaying the rate of conversion to blood trehalose or fat body glycogen[3]. In addition, the impact of nutrients on *Bombyx mori* is assessed with reference to economic traits of sericulture. It has been reported that zinc chloride yielded significant gains in economic parameters such as cocoon weight, pupal weight, raw silk weight, renditta and denier [4]. However, no sincere attempt has been made to correlate the impact of zinc chloride on the levels of total carbohydrates vis-à-vis the economic parameters of sericulture. The present study attempts to realize this objective.

MATERIALS AND METHODS

Broadly, the experimental design included the following aspects

Test species : Silkworm, *Bombyx mori*
Mulberry leaves : M_5 variety
Larval Instar : Fifth instar
Test chemical : Zinc chloride ($ZnCl_2$), obtained from Merck Limited, Mumbai. India.
Dose of $ZnCl_2$: 1 µg /100ml
Tissues Selected : Silk gland, haemolymph, fat body and segmental muscle

Since the experiments required continuous maintenance of the test species, the silkworms were reared in the laboratory as given by Krishnaswami[5]. The methodology was divided into three phases namely feeding pattern, assay of total carbohydrates and analysis of economic parameters.

Feeding pattern: After the third moult, the fourth instar larvae were divided into two batches of 100 worms each. The first batch was given normal feedings 5 times a day and treated as the control. The second batch was considered as the experimental batch and fed with the mulberry leaves fortified with 1 µg of zinc chloride in 100ml of distilled water. The enriched mulberry leaves, so obtained, were dried in shade and fed to the fourth and fifth instar larvae, once in a day at 6PM, while continuing normal feeding pattern during the rest of the day.

Assay of total carbohydrates: Total carbohydrate content was estimated in 5% homogenate of silk gland and 2% homogenate of muscle and the fat body and 1:9 diluted haemolymph (1:9 haemolymph and water) of the control and experimental batches by the method of Caroll[6]. Before the assay the desired tissues (silk gland, fat body and muscle) were isolated from the fifth instar larval body by making mid-dorsal dissection in the Silkworm Ringer[7], while the haemolymph was extracted by cutting the telson and pro-legs of the larval body. The total amount of carbohydrates present in these samples were computed using a standard prepared from the glucose and the values were expressed as mg/g wet weight of tissue or mg/ml of haemolymph.

Analysis of economic parameters: Economic parameters of the sericulture, such as the shell yield, cocoon weight, shell weight, floss weight, cocoon-shell weight, floss-shell ratio, floss-silk ratio, raw silk weight, renditta, denier (filament size) were analyzed as per the established standard methods [8-11].

Statistical analysis of data: The data obtained from the present investigation were analyzed by appropriate statistical tools such as the mean, standard deviation (SD), percent changes and test of significance. While the mean and SD were computed using M.S Excel, the test of significance and percent changes was calculated online by using the Graph Pad (*www.graphpad.com/quick calcs/ index cfm/)* and Percent Change (*www.percent–change.com/index php/*) software. The growth trends in the levels of total carbohydrates were interpreted in terms of an innovative statistical parameter called compound periodical growth rate (CPGR) as suggested by Sivaprasad[12].

RESULTS

The changes in the levels of total carbohydrates were analyzed in terms of day-to-day percent changes, overall percent changes (OPC) and compound periodical growth rates (CPGR). The impact of the zinc salt on total carbohydrates varied from tissue to tissue and from control to experimental batches as detailed below.

Silk gland carbohydrates (SGC): The silk gland, which is the prime site of silk protein synthesis recorded significant gains in total carbohydrate levels under the impact of $ZnCl_2$ during the fifth instar development. The day-to-day trends in their levels reflected ~45% increase on day-5, ~55% increase on day-3, ~19.0% increase on day-5 and ~95% elevation on day-7 of fifth instar. Further, while the control batch showed an OPC of ~814% during the entire period of fifth instar, the zinc-treated batch (experimental batch) recorded a higher OPC of ~ 993% during the same period. At the same time, the SGC levels showed a CPGR of 41.82% in the control batch and 46.61% in the experimental batch (Table 1; Fig 1A).

Haemolymph carbohydrates (HLC): The haemolymph is the chief circulating fluid in the body. It projected a different pattern of growth trends in HLC levels. Under the impact of the zinc salt, their levels declined slightly on day-1 and day-3 by ~3% and 5% respectively, but elevated by~ 57% on day 5 and by just 1.5% on day-7 during the fifth instars development. The trends in OPC indicate that the HLC profiles rose almost equally in both the control (~169%) and experimental (~172%) batches. However, the CPGR showed marginal improvement in HLC levels of the experimental batch (9.45%), compared to the control batch (9.17%) during the fifth instar development (Table 1; Fig 1B).

Fat body carbohydrates (FBC): The fat body is the chief metabolic organ in *Bombyx mori*. The FBC levels showed interesting growth trends in the control and experimental batches. While, the effect of zinc salt is the positive on day-1 (~135%), day-3 (~55%) and day-5(~83%), the final impact is not recognizable. Surprisingly, the OPCs (~424%) and CPGRs (27.24%) remained unchanged in both the control and experimental batches (Table 1; Fig 1C).

Segmental muscle carbohydrates (SMC): The SMC levels showed interesting growth trends in the control and experimental batches. While, the effect of zinc is the positive on day-1 (~132%), day-3 (~107.2%) and day-5(~42.6%), the final impact is not recognizable as in the fat body. Surprisingly, the OPCs (~770.4%) and CPGRs (40.54%) remained unchanged in both the control and experimental batches (Table 1; Fig 1D).

Economic parameters: As shown in Table 1, the impact of $ZnCl_2$ on the economic traits of sericulture is positive. The cocoon yield (no. of cocoons in one Kg) declined from 86.5 to 80 showing ~8% yield in cocoon production. While the raw silk weight and raw silk percentage and renditta increased by ~5%, ~3% and ~29% respectively under the influence of zinc salt. At the same time the renditta value recorded a 4% decline, reflecting a positive yield in silk production to that extent.

Table 1: Total Carbohydrate levels in different tissues of *Bombyx mori* during fifth instar development under the impact of $ZnCl_2$-enriched mulberry diet

Day of fifth instar	Statistical parameters	Silk gland		Haemolymph		Fat body		Muscle	
		Control	$ZnCl_2$ treated	Control	$ZnCl_2$ treated	Control	$ZnCl_2$ treated	Control	$ZnCl_2$ treated
1	Mean	8.85	12.8	23.1	22.5	46.9	110.0	25.7	59.5
	P.C	-	+44.6	-	-2.6	-	+135.2	-	+131.8
	S.D	±1.3	±1.5*	±0.4	±0.1*	±1.8	±1.2*	±1.2	±1.8*
3	Mean	25.3	39.3	35.3	33.7	77.0	119.0	41.8	86.6
	P.C	-	+55.3	-	-4.5	-	+54.5	-	+107.2
	S.D	±1.2*	±0.8*	±0.2*	±1.0*	±2.2**	±2.3*	±0.6*	±1.3*
5	Mean	64.7	77.0	24.0	33.7	66.7	122.0	65.5	93.4
	P.C	-	+19.01	-	+57.1	-	+82.9	-	+42.6
	S.D	±3.4*	±2.8*	±1.2*	±0.2*	±3.1*	±3.0*	±2.3*	±3.1*
7	Mean	72.0	87.9	39.1	39.7	199.0	199.0	198.0	198.0
	P.C	-	+94.5	-	+1.5	-	+0.0	-	+0.0
	S.D	±2.1*	±3.6*	±0.6*	±0.3**	±0.6*	±0.5**	±1.3*	±0.5**
OPC (%)		813.6	993.2	169.3	71.9	424.0	424.3	770.4	770.4
CPGR (%)		41.82	46.61	9.17	9.45	27.24	27.24	40.54	40.54

* Statistically significant: **statistically not significant

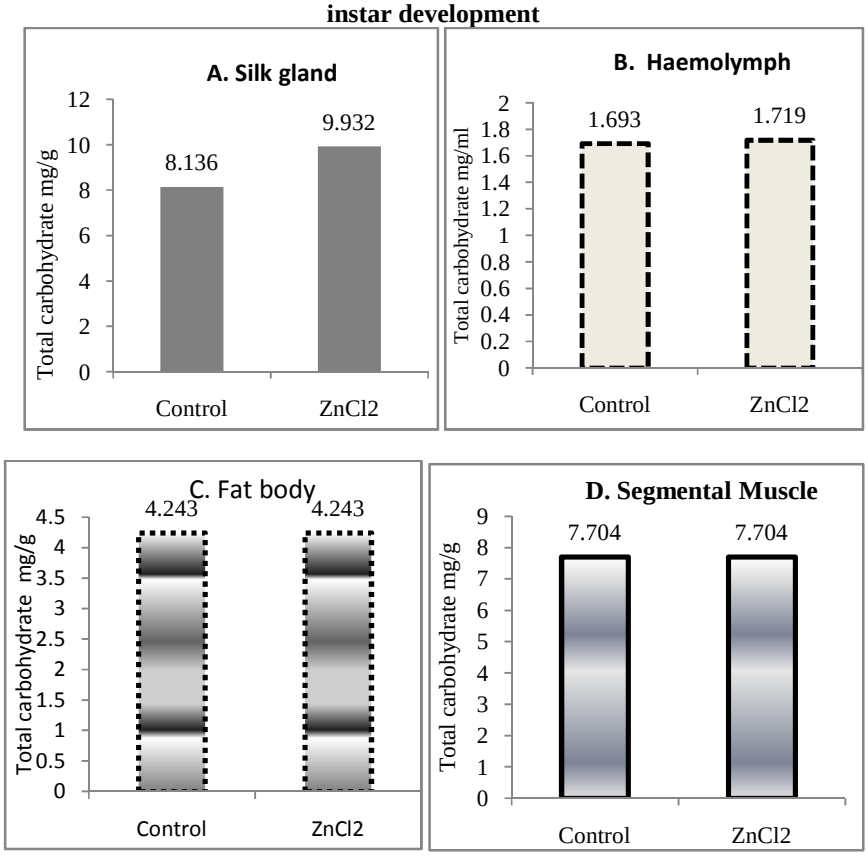
Each value expressed in mg/g in silk gland, fat body and muscle and mg/ml in haemolymph is a mean, ± standard deviation of four individual observations.(P value < 0.001). The day-to-day percent changes were calculated taking the control as the base value while, the overall percent change (OPC) is calculated taking the day 1 values as the control and day 7 values as the experimental. The compound periodical growth rates (CPGR) were computed on the basis of initial (day-1) and final (day-7) values obtained during fifth instar development.

Table 2: Economic parameters of *Bombyx mori*, under the impact of $ZnCl_2$-enriched mulberry diet

Parameter		Control	$ZnCl_2$ treated
Cocoon yield (No./Kg)	Mean	86.5	80.0
	P.C	-	-7.5
	S.D	±1.3	±0.8*
Shell weight (g)	Mean	0.2	0.3
	P.C	-	+50.0
	S.D	±0.01	±0.01*
Foss weight (g)	Mean	0.02	0.03
	P.C	-	+50.0
	S.D	±0.00	±0.01*
Floss- shell ratio	Mean	8.7	10.1
	P.C	-	+16.0
	S.D	0.3	1.6**
Floss-Silk ratio	Mean	20.5	18.6
	P.C	-	-9.3
	S.D	±0.05	±0.05*
Raw silk weight (g)	Mean	18.7	19.7
	P.C	-	+5.3
	S.D	±0.01	±0.01*
Renditta	Mean	7.9	7.6
	P.C	-	-3.8
	S.D	±0.01	±0.01*
Denier (d)	Mean	14.0	18.1
	P.C	-	+29.3
	S.D	±0.5	±0.9*

* Statistically significant.
** Statistically not significant.
Each value is a mean, ± standard deviation of four individual observations (P<0.001). The percent changes of the experimental values were calculated taking the corresponding control value as base the value.

Fig-1: Growth rates in the levels of total carbohydrate levels in silk gland (A), haemolymph (B), fat body (C), and segmental muscle (D) in *Bombyx mori*, under the impact of $ZnCl_2$-enriched mulberry diet during fifth instar development



(Source Table)
Each value represents overall percent change in carbohydrate levels from the first day to second day of fifth instar. Note significant elevation in the levels of carbohydrates in the silk gland, while a constant trend is maintained in other tissues.

DISCUSSION

Zinc modulates metabolism of organisms and ensures their growth and development[2]. The present study demonstrates the positive impact of zinc chloride on the carbohydrate levels of *B. mori* vis-à-vis the economic traits of sericulture. It has been demonstrated that carbohydrates are utilized by the silkworm as energy sources for the synthesis of lipids and amino acids and that the energy requirement of the larvae serves as a determinant factor of normal growth and development, which ultimately determines the quality of silk produced. The present study highlights two points; firstly the carbohydrate profile accumulates in the silkworm body in a tissue-dependant fashion and secondly, their levels are reinforced by the zinc diet, with appropriate improvements in economic parameters of sericulture.

Accumulation of carbohydrates in tissues: The carbohydrates reserves tend to accumulate in tissues as per their energy requirements during fifth instar development. The fat body, being the major site of metabolism, builds-up its energy reserves in significant proportions and maintains higher levels of carbohydrates (~47 to 199 mg/g). This is followed by the segmental muscle (~26 to 198 mg/g), haemolymph (~23 to 39 mg/g) and silk gland (9 to 72 mg/g). Quite interestingly the rate of their accumulation is higher in silk gland and muscle, moderate in fat body and lower in haemolymph. The rate of accumulation of carbohydrates reflects the increasing demand for energy reserves in different tissues of *B. mori*. The silk gland being the prime site of silk production, demands more and more energy reserves and hence, its carbohydrate profiles recorded an elevation of ~814%, with a CPGR of 41.82% during the 7-day period of fifth instar. The segmental muscle, which is the contractile machinery of cocoon spinning, has recorded a 8-fold rise (~770% increase) in its energy reserves with a CPGR of 40.54%, while the fat body showed a 4-fold rise (~424% increase) in its carbohydrate levels with a CPGR of 27.24%[13,14]. The haemolymph, which is the chief circulating and transport medium in the body, has registered ~170% rise in its carbohydrate levels with a low CPGR of just 9.17% (Table 1; Fig 1). The growth trends in carbohydrate levels indicate that their turnover during silkworm metamorphosis is coordinated by the fat body, from which the energy reserves are transported to the silk gland and segmental muscle through the medium of haemolymph. The higher CPGRs in the carbohydrate levels of silk gland and muscle, and their lower turnover in the fat body and haemolymph, amply demonstrate the prevalence of such a transport mechanism in *B. mori*. Needles to say, the study further substantiates the earlier findings that the glycogen reserves of the fat body are metabolized to glucose and trehalose and mobilized to the tissues through the blood in insects[15, 16].

Effect of Zinc diet: The zinc salt seems to play a catalytic role in metabolism and silk production in *B. mori*. This, obviously, it does so, by boosting the mobilization of carbohydrate energy reserves in the body as demonstrated in the present investigation (Table 1,2 and Fig 1).When the silkworm larvae were fed with $ZnCl_2$-enriched mulberry leaves, both carbohydrate levels and economic parameters of sericulture recorded significant improvements. Under its influence, the SGC levels grew remarkably by 179.6 percentile points in OPC (893.2-713.6%) and by 4.79 percentile points in CPGR (46.61-41.82%), while those of the haemolymph, fat body and muscle remained unchanged both under normal and zinc-fed conditions (Table 1;Fig.1). Thus, the impact of zinc salt in specific and directed towards the silk gland. Further, the constant growth rates in three tissues, viz., haemolymph, fat body and silk gland coupled with lop- sided growth trends in the silk gland emphasize the role of zinc in diverting the energy reserves from different tissues to silk gland, while at the same time sustaining their energy levels and metabolism at a constant rate during metamorphosis.

Because of diversion of energy resources to silk gland, it grow remarkably during the fifth instar larval stage, with the result, the gland- body ratio reaches higher levels by accumulating silk proteins that are required for spinning the cocoon[17]. Obviously, the zinc salt enhances the rate of silk protein synthesis in the silk gland by meeting its increasing energy requirements by mobilizing carbohydrate reserves on the basis of equity, if not on equality basis. Presumably, most of the trace elements like cobalt, magnesium, iron, potassium, nickel etc, facilitate such mobilization of energy reserves to the silk gland that showed positive improvement in cocoon production and the quality of silk obtained from such cocoons[18-24].

Such an effect of zinc chloride on economic parameters of sericulture manifests in two-fold improvement. Firstly, the profit- making parameters such as cocoon yield, shell weight, floss-silk ratio, raw silk weight, and the denier. Secondly the loss making economic parameters such as the floss-silk ratio, renditta, declined significantly not withstanding considerable elevation in floss weight (50%) and floss-shell ratio (16%). Despite increase in the levels of sericultural wastage (eg. floss weight and floss-silk ratio) profitable gains were recorded in the cocoon yield, shell weight, raw silk weight, denier (thickness and texture of silk fiber) and the renditta (the no. of kilogram of cocoons required for production of 1 Kg raw silk) (Table 2). Thus, the enrichment of mulberry leaves with $ZnCl_2$ results in boosting the production of both silk (fibroin) and floss (sericin) proteins, while at the same time, it fine-tunes the quality parameters of the silk. More elaborate field trials are needed before applying zinc salts in sericulture.

Acknowledgement

The authors, Prof. K. Yellamma and Dr. S. Sivaprasad are grateful to the University Grants Commission, New Delhi for financial assistance in the form of a major research project.

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