CHAPTER 3

Nutritional Studies
INTRODUCTION

Nutrition comprises various chemical, physiological and biochemical activities, which transform food elements into body elements. Nutrients are the main components of the food that leads to weight gain and increase in body volume. Hence food plays major role in growth, fattening, silk secretion, or other reproductive functions, however, a substantial part of its food is used for supporting body processes, which must go on whether or not any new tissue or product is being formed.

This demand for food is referred to as the maintenance requirement, as it comprises the amount needed to keep intact the tissues of an animal, which is not growing, working, or yielding any product. If this need is not met, which is commonly revealed by a loss in weight and which leads to various undesirable consequences. This destruction of body tissue is referred to as the fastening metabolism, and it can be measured in terms of the waste product eliminated through the various parts of excretion. Most of the breakdown is for energy, which occurs in response to the demand of the fasting
organism. An animal, which is receiving sufficient protein and energy to permit growth of its tissues and organs, show an increase in size and weight.

The growth of the body as a whole is most commonly measured as an increase in weight. An animal may increase in weight through the deposition of fat without any increase in the structural tissues and organs, which characterize growth. An animal, which receives insufficient protein and energy to permit growth of its organs and tissues, may still show an increase in size due to skeletal growth. In nutritional studies normal growth is referred to as the state of nutrition and health and in descending growth and reproductive performance. Growth retardation is reduced by malnutrition, either in calories or in some specific essential nutrients. The nature and extent of the effect on growth are dependent upon the character and severity of the deficiency and upon the period involved. A deficiency of energy, for example, will immediately check growth in mass while lack of calcium may not, as it's primary effect is upon bone structure rather than its size. A deficiency of certain other nutrients such as phosphorus or Vitamin-B exerts an indirect influence on increase in size by decreasing appetite, as well as causing direct physiological effects. Restriction in diet upto 800 days of age in rats resulted in much leaner animals with somewhat less skeletal size, but improved health, female fertility and longevity and delayed the onset of degenerative disease (Benjamin, 1960; Benjamin and Simms, 1960).
Tonge and McCance (1965) exhibited that growth retardation is due to food restriction in pigs.

Study of various nutritional parameters such as food consumption, excretion, food assimilation and oxidization give an idea regarding growth and energy. The Silkworm, Bombyx mori L. feeds on the food Mulberry leaves from which it ingests various nutrients to support physiological activities. The nutrients include protein, carbohydrate, fat, vitamin, inorganic salt and water. Food preference of Silkworm largely depends upon the physical and chemical components of the food. It was reported that the appetizing factors, biting factors, swallowing factors and repellent substances play a key role in accepting the diet (Hanamura et al., 1962). The appetizing factors include many volatile substances such as alcohol, citric aldehyde and linalol influence the larval appetite and feeding reaction. Whereas biting factors like beta sito sterol, isoquercetrin and flavinin cause the larval biting motion. Continuous feeding of the larvae is kept by the swallowing factors such as cellulose, sucrose, inositol, phosphate, silicate, Vitamin-c and sulphur amino acids etc.

Food ingestion by the larvae of Bombyx mori L. varies during its different instars. The intake of mulberry leaves into oral cavity is “Ingestion”. Active feeding occurs only during IV and V instars and 97% accounts for of total ingestion. The larval feeding is discontinuous, and initial feeding time is different among various
instars. This quiescent rest before feeding helps the newly moulted larvae in hardening of new cuticle and the continuous developing of internal organs (Naik 1985; Radhakrishna 1989). The duration of feeding is only about 27 percents of the larval feeding period. Each time of feeding lasts for 12-16 minutes. Delvi (1972) described the cessation of appetite considerably 20-30 hrs prior to moult in many insects as pre-moult starvation period. The complex organic nutrients in mulberry leaves are covered by cell wall of cellulose hence are insoluble, impermeable and are not utilized by the silkworm directly and silkworm needs to convert the macro, complex insoluble and impermeable substances into simple, permeable products, those actions are called “Digestion”.

Food in the buccal cavity, is digested, primarily by the saliva and lubricated and expelled to midgut, which is the main region for digestion and assimilation. The goblet cells of midgut play a key role in digestion by secreting digestive juices and absorb nutrients into the haemolymph through cylindrical cells of midgut. The remains are combined with the secretions by the Malpighian tubules, the mixtures are pressed by the colon into the hexagonal excrements and then expelled into rectum. Water in the excrements is reabsorbed and the faecal matter is pressed further and excreted the undigested matter as solid faecal pellets.
Eventhough, the literature available on selenium nutritional aspects of vertebrates is abundant, a little is available in lower organisms such as arthropods. Hence the author is made an attempt to study various nutritional parameters such as food consumption, excretion, assimilation, food combustion and total food converted under lethal and sub lethal doses of selenium at 3, 4, 5, and 6 days of V instar silkworm of PM X NB₄D₂ parentage.

**RESULTS**

The data on the various nutritional parameters such as food consumption, faecal excretion, assimilation, food conversion and oxidation of V instar silkworm *Bombyx mori* *L* (groups 2, 5, 8, and 11) exposed to lethal and groups 3, 6, 9, and 12 to sub lethal doses of selenium at 3, 4, 5 and 6 days of exposure period besides controls (groups 1, 4, 7, and 10) are presented in table 4. For comparative assessment, the differences obtained in relation to controls in nutritional parameter at the said exposure periods of lethal and sub lethal doses were converted as percentage of the corresponding controls and these percent change values were also given in the table 4 and plotted against exposure periods in figure 4.

**Food consumption**

From the data presented in the table 4 and figure 4, it is observed that, relative to controls, the total amount of food consumed at 3, 4, 5 and 6 days exposure of V instar silkworms (groups 2, 5, 8
and 11) to lethal dose of selenium was significantly (P<0.05) decreased. Based on percent values, the percent decrease in the food consumption in the lethal dose was progressed gradually from the 3 day to 6 days of exposure period studied and was in the order 3< 4< 5< 6 days. In the sub lethal dose (groups 3, 6, 9, and 12), however, the amount of food consumed gradually increased in all days of exposure periods studied and this increase was significant (P< 0.05). The percent increase also found high at 6 day when compared to that of 3 day.

**Fecal output**

Corresponding to the decrease in food consumption in lethal dose of selenium (groups 2, 5, 8 and 11), the excretion of faecal matter also decreased significantly (P<0.05) in relation to controls (groups 1, 4, 7, and 10) on all exposure periods studied and followed the trend 3<4< 5< 6. Based on percent change values, it is seen that the percent decrease in faecal output of silkworm exposed to lethal dose progressed gradually from 3 day to 6 day and was in the order 3< 4< 5< 6.

Even in sub lethal dose (groups 3, 6, 9, and 12), a significant decrease in faecal output was observed at 3, 4, 5 and 6 days of exposure periods studied indicating the enhanced food assimilation due to increased oxidation.
**Food assimilation, oxidation and conversion**

Corresponding to the decrease in food consumption and faecal output of the V instar silkworm exposed to lethal dose of selenium (groups 2, 5, 8 and 11), the other nutritional parameters such as food assimilation, conversion and oxidation registered a significant ($P<0.05$) decrease at 3, 4, 5 and 6 days of exposure periods studied. Based on percent values, the percent decrease in these parameters is less at 3 day of exposure and this decrease progressed gradually and was in order $3<4<5<6$. In sub lethal dose of selenium (groups 3, 6, 9, and 12), however, all the above nutritional parameters registered an elevation at 3, 4, 5 and 6 days of exposure.

**DISCUSSION**

The Parameters like food consumption, digestion and utilization in insects are of immense importance to understand the nutritional aspects in insects (Waldbauer 1964). It is well known that fluctuations in food intake in the silkworm *Bombyx mori* L. were clearly evident during its different instars. The results presented on food consumption, digestion, utilization and excretion bring out many significant and interesting aspects with regard to the lethal and sub lethal doses of selenium at different days. The literature on food utilization budgets related to selenium effects on animals is very scanty (Harrison and Conrad, 1984). The food intake in selenium treated silkworms was significantly reduced at lethal intoxication, whereas sub lethal dose enhanced the food intake significantly when
compared to the controls. Here the lethal dose acted as inhibitor and sub lethal dose acted as attractant. However these changes in the food intake may be attributed to food assimilation efficiency when fed with selenium may not be entirely due to the decrease in the consumption, but it may also be due to the action of selenium, which enhances the digestibility by increase in enzyme secretion rate/ activity at least in the sub lethal selenium exposed silkworm. It has been suggested that selenium acts as an oxidant functioning as the metal co-factor for important enzymatic activity requiring glutathione peroxidase (Mayland, 1994). Lethal dose of an insecticide when fed to Philosamia ricini resulted in inhibition of Acetyl Choline activity and the inhibited esterase, may induce the toxicity that results in the lack of appetite in Silkworm (Pant et al., 1982). The decrease in food assimilation at lethal intoxication is reportedly due to lack of enzymatic mechanism, due to breakdown of proteins into amino acids and peptones and also may be due to the malfunction of mid intestine for transport of amino acids and peptones to respective organs. The results coincide with the reports of Lemly (1998), who observed that high concentration of selenium that substituted for sulphur in sulphur-to-sulphur linkages of proteins. This results in inability to form a helical structure leading to non-functioning of malformed proteins. Such an inhibitory activity in the digestive process might also have brought in the silkworm of the present study during the lethal dose exposure of selenium.
The total food assimilation and oxidation in silkworm treated with lethal selenium dose are suppressed and silkworm treated with sub lethal dose of selenium, are enhanced. The results are in agreement with Deka et al, (1999) as in non-mulberry silkworms. The enhanced assimilation and oxidation of food is probably due to the better consumption of food and enhanced transaminases activity of the intestine and the haemolymph of the silkworm as suggested by Shyamala and Bhatt, (1956), with reference to toxicity of chloromycetin. The lepidopteran larvae are able to adopt and express in different ways in various environmental conditions by altering one or more parameters of food utilization. The efficiency increases during toxicant supplement is in agreement with the homolygosis hypothesis which credits that sub lethal does of any stressing agent may be stimulatory to the organism by providing it increased sensitivity or respond to change in its environment and increased efficiency for coping with sub optimum environment (Lucky 1968). Cholinesterase inhibition may induce the toxicity by killing the larva reflecting on their lack of appetite and under nourishment. This further leads to the lysis of all nutrients such as carbohydrates, glycogen, proteins and lipids and also an increase in lipolytic, proteolytic phosphorylase and amino transferase activities (Pant et al, 1982; Pant and Katiyar, 1983). From the present study it is evident that the findings of the present are in agreement with earlier reports.
In the present study, the selenium at lethal doses could decrease the parameters like food consumption assimilation efficiencies and food conversion rate. However, the sub-lethal dose of selenium particularly at 6 days of exposure, silkworm could exhibit significant increased levels of the above parameters leading to better survival and normal life by suppressing toxic action by detoxification mechanisms.
Table 4: Estimation of Nutritional Parameters (mg/Kg. body weight/day/ larva) at 3, 4, 5, & 6 days in V instar of Silkworm fed on Mulberry leaves treated with Lethal and Sub lethal doses of selenium

<table>
<thead>
<tr>
<th>Days exposure</th>
<th>Dosage</th>
<th>Consumption</th>
<th>Excretion</th>
<th>Assimilation</th>
<th>Conversion</th>
<th>Oxidization</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>510 b</td>
<td>254 c</td>
<td>256 b</td>
<td>21.24 b</td>
<td>234.76 b</td>
</tr>
<tr>
<td>3 days</td>
<td>Lethal</td>
<td>400 a (-21.56)</td>
<td>216 a (-14.9)</td>
<td>184 a (-28.1)</td>
<td>16.0 a (-24.6)</td>
<td>168 a (-28.4)</td>
</tr>
<tr>
<td></td>
<td>Sub lethal</td>
<td>560 c (+9.8)</td>
<td>221.2 b (-12.9)</td>
<td>338.8 c (+32.3)</td>
<td>29.47 c (+37.8)</td>
<td>309.33 c (+31.7)</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>605 b</td>
<td>302 c</td>
<td>303 b</td>
<td>24.24 b</td>
<td>278.75 b</td>
</tr>
<tr>
<td>4 days</td>
<td>Lethal</td>
<td>470 a (-22.31)</td>
<td>253.8 a (-16.2)</td>
<td>216.2 a (-28.7)</td>
<td>18.7 a (-22.8)</td>
<td>197.5 a (-29.14)</td>
</tr>
<tr>
<td></td>
<td>Sub lethal</td>
<td>685 c (+13.2)</td>
<td>270.5 b (-10.4)</td>
<td>414.5 c (+36.6)</td>
<td>36.0 c (+48.5)</td>
<td>378.5 c (+35.7)</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>720 b</td>
<td>359 c</td>
<td>361 b</td>
<td>29.7 b</td>
<td>331 b</td>
</tr>
<tr>
<td>5 days</td>
<td>Lethal</td>
<td>515 a (-28.47)</td>
<td>278 a (-22.5)</td>
<td>237 a (-34.3)</td>
<td>20.61 a (-30.6)</td>
<td>216.3 a (-34.6)</td>
</tr>
<tr>
<td></td>
<td>Sub lethal</td>
<td>810 c (+12.5)</td>
<td>319.95 b (-10.8)</td>
<td>490.05 c (+35.7)</td>
<td>42.6 c (+43.4)</td>
<td>447.45 c (+35.1)</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>940 b</td>
<td>469 c</td>
<td>471 b</td>
<td>38.8 b</td>
<td>432.18 b</td>
</tr>
<tr>
<td>6 days</td>
<td>Lethal</td>
<td>600 a (-36.17)</td>
<td>324 a (-30.9)</td>
<td>276 a (-41)</td>
<td>24.01 a (-38.14)</td>
<td>251.9 a (-41.7)</td>
</tr>
<tr>
<td></td>
<td>Sub lethal</td>
<td>1115.0 c (+18.6)</td>
<td>440 b (-6.2)</td>
<td>675 c (+43.3)</td>
<td>58.7 e (+51.2)</td>
<td>616.3 e (+42.6)</td>
</tr>
</tbody>
</table>

* Each value is a mean of eight estimates
** Percent decrease over control is given in parenthesis
*** Mean with in a column followed by the same method are not significantly different (P > 0.05) from each other according to Duncan's Multiple Range Test.
Fig. 4: Estimation of Nutritional Parameters (mg /Kg. body weight/day/ larva) at 3, 4, 5, & 6 days in V instar of Silkworm fed on Mulberry leaves treated with Lethal and Sub lethal doses of selenium.