Effect of Insulin on Food Utilization And Growth of The Mulberry Silkworm Bombyx Mori L

Vinotha M[1], Karthikeyan A[2]
[1, 2] PG and Research Department of Zoology
[1, 2] Government Arts College (Autonomous), Karur, Tamil Nadu, India

Abstract- *Silkworm rearers often face shortage of mulberry leaves when the worms are in the fifth instar stage. In this work insulin fortification in different concentrations reduced the consumption of mulberry leaves significantly. This might be attributed to the prothoracicotropic activity of the insulin. Inspite of the reduction in consumption the rates of assimilation and metabolism, Approximate digestibility (AD), Efficiencies of conversion of digested food and ingested (ECD & ECI) increased. The effective utilization of mulberry leaves led to the increase in growth and quality of cocoon. About 24% increase in cocoon weight and 76% increase in shell weight were noticed. So insulin can be used as a fortification agent after studying its effect at molecular level.*

Keywords- Consumption, Assimilation, Metabolism, ECD, ECI.

I. INTRODUCTION

Silk, ‟ the queen of textiles”plays an important role as foreign exchange earner for many of the silk producing countries of the world (Krishnaswamiet al 1988). Yet even in this age of high technology, to produce silk we must rely on a carefully coddled caterpillar and therein lies the miracle of silk (Ninahyde, 1986). The most common source of silk, the mulberry silkworm Bombyx mori L is raised domestically but only where there are abundant mulberry leaves that satisfy its finky appetiteB.mori is an oligophagous insect that feeds mainly on mulberry leaves, which is a major drawback in sericulture (Narasimhamoorthy, et al 1986). High economic importance, sericulture industry requires active research for suggesting the appropriate measures for reducing the amount of mulberry leaves consumed as well as for the improvement of crop yield quantitatively and qualitatively (Narasimhamoorthy, et al 1986).

Thus, the study of nutrition and food utilization is of paramount importance in sericulture (Yokayama, 1963; Benchamin and Jolly, 1986). Even though the early works on food utilization and growth are abundant, studies on food utilization using vertebrate hormones as fortification agents are scanty.

The insect hormones, like Juvenile Hormone Analogue (Hiware, 2006), Methoprene (Lakshmi Devi et al 2013) have been shown to boost cocoon yield. Only a few vertebrate hormones like testosterone and Thyroxine have been proved to improve the silk yield (Sudhakaret al 2008; Magadum and Hooli, 1988). In the present study, the insulin has been chosen as a fortification agent because of the following reasons: Firstly, insulin is found to contain several aminoacids (Thompson, 1955) such as essential, non-essential, semi essential and acidic aminoacids which are required for the growth of the larvae and silk protein synthesis; Secondly B.mori produces a 5KDa peptide hormone called ‘Bombyxin’ consisting of two chains whose aminoacid sequences show considerable homology with vertebrate hormone insulin (Nagasawa, et al 1984) and also it is reported to manifest the prothoracicotropic activity (Ishizaki et al 1983). Thirdly, fortification of vertebrate hormone such as insulin could increase the utilization of glucose, the rate of glycogenesis and protein synthesis, etc in insects (Kramer, 1983).

II. MATERIALS AND METHODS

Insects

In the present study the commercially exploited multivoltine cross breed Bombyx mori L race Bivoltine cross breed (CSR2 x CSR4) was selected. The eggs of this race were procured from Silkworm Rearing Department, Rayanur, Karur district, Tamil nadu, India. They were kept under lab conditions and allowed to hatch. The emerged first instar larvae were fed with young leaves of mulberry variety MR2. The larvae were acclimatised to the lab conditions by rearing them till fifth instar with the relative humidity of 80 - 90 % and the temperature of 27 - 30 ˚C according to the conventional method in trays and provided with suitable amounts of fresh mulberry leaves. All larvae which molted to the last instar at the same time were grouped and used in the experiments.

Preparation of Fortification Agents

A synthetic vertebrate peptide hormone, insulin, manufactured by the Torrent Pharmaceuticals Limited, Indrad

was used as a fortification agent along with the mulberry leaves. Solutions of different concentrations of insulin such as 10 µg/100ml, 20 µg/100ml and 30 µg/100ml were prepared.

Control and Experimental Sets

In this study two sets of control each with fifty larval were maintained. Larvae in the control were fed with mulberry leaves only whereas these in the second control were fed with mulberry leaves smeared with 2% sucrose solution. Three sets of experimental larvae each with fifty larvae were named as 10, 20 and 30 µg Insulin and they were fed with mulberry leaves smeared with a mixture of 2% sucrose solution and insulin of respective concentration.

Food Utilization Parameters

The food utilization parameters, such as consumption (C), assimilation (A), production (P), metabolism (M); their rates approximate digestibility (AD), efficiency of conversion of digested food (ECD) and efficiency of conversion of ingested food (ECI) and precocooning characters have been evaluated by adopting the formulae of Petrusewicz and MacFadyan, (1970).

Growth

In the present work, the larval duration and larval weight were studied in the fifth instar larvae of B.mori L fed with mulberry leaves smeared with insulin in three different concentrations 10, 20 and 30 µg/ml.

Statistical Analysis

All the date were subjected to the statistical tool Standard Error. The experimental data were compared with the control data statistically to know the level of significance in differences by Student 't' test.

III. RESULTS AND DISCUSSION

Food utilization

In the present work, the feeding budget, rates and efficiencies of food utilization of the final instar larvae of Bombyx mori L reared on mulberry leaves fortificated with 10, 20 and 30 µg/ml of insulin were studied.

Consumption rate (Cr) was found to be decreased significantly over control with the increasing concentrations of insulin. Comparatively, consumption was found to be less in the case of larvae supplemented with all concentrations of insulin. Consumption rate decreased over control by 17%, 19% and 25% in the case of 10, 20 and 30 µg/ml insulin treated larvae respectively (Table 1 & Fig 1). The acceptability and quality of the food often act as limiting factors in determining the growth of an insect (Periyasamy et al 1984). The probable reason for the significant reduction in consumption might be the slightly unpalatable nature of the fortification agent or insulin, since the taste of food is more important than odour for silkworm (Ito1980).

Silkworm rearers often face the shortage of mulberry leaves when the worms are in the fifth instar stage and thus they are unable to adhere to the normal feeding schedule (Radhakrishnan et al 1985). Under such circumstances, the use of Insulin as a fortification agent will reduce the consumption of mulberry leaves. Thus less consumption is an advantageous factor for silkworm rearers.

Eventhough the rate of consumption decreased, the assimilation rate (Ar) was found to be increased with the increasing all concentrations of insulin. Similarly, significant increase of 3%, 7% and 10% in assimilation rate was observed in 10, 20 and 30 µg/ml insulin, treated larvae respectively (Table 1 & Fig 1). This finding is in line with the view of SooHoo and Fraenkal (1966) that the decreased consumption can be compensated by increased assimilation. Further, the increase in assimilation of the insulin treated larvae might also be due to the glycine content of the insulin molecule since the glycine is reported to promote the assimilation in animals (Shimura, 1978).

Production rate (Pr) was observed to be increased with the increasing concentrations of insulin. Similarly, significant increase in production rate by 6% and 8% and 15% was observed in 10, 20 and 30 µg/ml insulin treated larvae respectively. Similarly, metabolic rate was also found to be increased significantly by 10% and 22% and 33% over the control with the increasing concentrations of insulin 10, 20, and 30µg/ml respectively (Table 1 & Fig 1). Significant increase in production and metabolic rates in insulin treated larvae might be due to the nutritive effect of the non-essential aminoacids, such as alanine, tyrosine, cystine, glycine and serine that are present in the insulin molecule and also its stimulatory effect on protein metabolism and growth (Ito and Arai, 1966), synthesis of protein from aminoacids, fat synthesis (Satoskar and Bhandarkar, 1988) and activity of TCA cycle enzymes and glutamate dehydrogenase (Dhanajaya Naidu, 1983).

The utilization of food is expressed in terms of AD (Walbauer, 1968) AD is an index of the proportion of ingested food that is transferred from the gut lumen into the body of the

animal (Pandiyan and Marian, 1986). AD by leaf-eating insects is commonly poor. In lepidopterous larvae, it ranges from 25 to 40% (Chapman ,1982). However, in the present work , Shoot up in the values of approximate digestibility (AD) was observed in the case of experimental larvae over control. Significant increase ($P > 0.05$) in AD 4.0%, 4.2% and 4.9% treated larvae respectively. The shoot up in AD values may be attributed to the increase in the level of digestive enzymes (Sharma and Tara1988; Radhakrishnan and Delvi 1987) under the influence of insulin. The increase in AD values reflect in the enhanced accumulation of reserve food energy during the last larval instar to tide over the subsequent spinning stage (Delvi and Pandiyan 1971;1972, Pandiyan 1973). Such accumulation of reserve food energy results in high calorific value of the larvae (Moon and Carefoot, 1972). The increase in the AD may also be due to the reduction in food consumption (Pandiyan 1973).

Significant elevation in the efficiency of conversion of ingested food (ECI) over control was noticed in all experimental sets. Maximum increase in ECI 30%, 38% and 40% was observed in 10, 20 and 30µg/ml insulin treated larvae respectively. Similarly 25%, 29% and 31% of increase in the efficiency of conversion of digested food (ECD) over control was noticed in 10, 20 and 30µg/ml insulin treated larvae respectively (Table2 & Fig 2). This may possibly due to the acceleration of enzyme activity (Dhananjaya Naidu,1988) under the influence of insulin and their anabolic nature (Turner, 1966).

The larval duration of the fifth instar larvae of B.mori was found to be decreased by a day in the case of 20 and 30 µg/ml insulin treated larvae (6 days) over control and 10 µg/ml insulin treated larvae (7 days) (Table 3 & Fig 3). Rapid or accelerated growth was observed in the case of 20 and 30 µg/ml insulin treated larvae. The decrease in larval duration of the insulin treated larvae may be due to the presence of phenylalanine and tyrosine in the insulin molecule which play a key role in the sclerotization of the cuticle as suggested by (Shymala and Gowda, 1980) which might have accelerated moulting process resulting in decreased larval duration. Further, insulin might have acted as ʻBombyxinʼa 5KDa brain secretory peptide of the silkworm B.morihaving structural homology with vertebrate insulin (Nagasawaet al 1986; Ishizaki and Suzuki 1988; Suzuki et al 1989) which can stimulate the prothoracic glands to synthesise and release ecdysone necessary for moulting and metamorphosis (Ishizaki and Ichikawa 1967; Nagasawa et al 1984). The shortening of larval period has also been reported by the early workers Magadum and Hooli (1989) in insulin treated larvae.

Growth

The daily increment in weight of the larvae reared on mulberry leaves smeared with insulin indicates that the growth was found to be maximum on the first four days and minimum on the later days.The wet weight of the whole body, increased with the increasing concentrations of the fortification agent. In the present work, significant increase was recorded in the case of 10, 20 and 30 µg/ml insulin fortified larvae (Table 3 & Fig 3). The increased growth of the insulin treated larvae is in line with the report that the vertebrate insulin may stimulate growth, lipid mobilization in insects (Kramer1983). Magadum and Hooli (1989) have also reported the increase in larval weight in insulin treated larvae. This might be due to its growth stimulating effect and stimulatory influence on protein synthesis (Turner 1966). The increased growth may also be attributed to the presence of essential and semi-essential aminoacids, in the insulin molecule, that are required for the better growth and development (Ito and Arai 1966; Bose et al 1989). Further, it has been reported that insulin, when applied to insects, exerts a stimulatory effect on cell growth and metabolism (Kramer 1983). Insulin stimulates carrier mediated transport of glucose and aminoacids into the cell (Turner 1966). The increased larval growth has already been reported by Znamenskaya (1956) in glucose supplemented larvae. Insulin stimulates the transfer of aminoacids into the cell and their incorporation into proteins (Froeschet al1985) resulting in improved growth of the larvae of B.mori.

Cocoon Parameters

The data on cocoon parameters indicate that the insulin has significant influence on improving the silk yield. The most important commercial parameters are the cocoon weight and shell weight as the price of the cocoon is fixed on the basis of these parameters. The weights of the cocoon and shell were found to be increasing significantly with the increasing concentration of insulin over the control (Table 4 & Fig 4). About 10 %, 16%, 24% of increases in cocoon weight were observed in 10, 20 and 30 µg/ml insulin fortified larvae respectively. The larger the weight of the shell and its ratio greater will be the silk yield from it (Magadum and Hooli, 1989). Therefore it is surmised that the insulin has a greater effect on the improvement of silk yield. This might be due to the stimulatory effect of insulin on the replication of DNA, accumulation of RNA and protein synthesis in the silk gland as reported by Magadum and Hooli (1989). The direct incorporation of the aminoacids in the insulin may also be responsible for the greater silk production as suggested by (Bose, et al 1989). The improvement of the commercial characters of the cocoon can be positively correlated with the enhanced growth observed in insulin fortified larvae.

The profitability of sericulture depends on the production of the mulberry leaf at economic cost and so as to ensure greater cocoon yield with less investment (Jolly, 1986). From the present investigation it is known that insulin has a greater potential in reducing the consumption and improving the silk yield. Insulin is available commercially and its price is also cheap. Hence, insulin can be recommended an economically feasible agent to silkworm rearers for the improvement of silk yield after studying the physiological mechanism of its improving the growth and silk yield.

IV. CONCLUSION

Use of insulin as a fortification agent reduced the consumption of mulberry leaves by the silkworm. Whereas it in increased the production, metabolism, digestibility and conversion efficiencies. Besides, it also enhanced the quantity and quality of cocoon. Hence with less investment economic traits of cocoon can be increased using insulin as a fortification agent.

Table 1. Effect of insulin on the rates of consumption (Cr), assimilation (Ar), production (Pr) and metabolism (Mr) of the fifth instar larvae of the mulberry silkworm Bombyx mori L.

S. No.	PARAMETERS	CONTROL LARVAE FED WITH		EXPERIMENTAL LARVAE FED WITH MULBERRY LEAVES SMEARED WITH 2% SUCROSE +		
		ONLY MUBERRY LEAVES	MULBERRY LEAVES + 2 % SUCROSE	10 µg INSULIN	20 µg INSULIN	30 µg INSULIN
1.	Consumption (Cr) (mg/g live wt of larvae/day)	142±1.6	129±1.6 t = -11.9* (9.1%)	117±1.7 t = -23* (17.6 %)	114±1.5 t = -27.3* (19.7 %)	106±1.6 t =-34.8* (25.3 %)
2.	Assimilation (Ar) (mg/g live wt of larvae/day)	110±1.7	111±1.5 t = -1.3** (0.9%)	114±1.6 t = -3.6* (3.6 %)	118±1.7 t = -7.1* (7.2%)	121±1.5 t = -10.8* (10%)
3.	Production (Pr) (mg/g live wt of larvae/day)	60±1.7	62±1.5 t = -1.9* (3.3%)	64±1.5 t = -4.2* (6.6 %)	65±1.6 t = -4.8* (8.3%)	69±1.6 t = -8.6* (15%)
4.	Metabolism (Mr) (mg/g live wt of larvae/day)	59±1.6	61±1.5 t = -1.9* (3.3%)	65±1.7 t = -5.9* (10.6 %)	72±1.6 t = -12.6* (22%)	78±1.6 t = -18.4* (33.2 %)

Note: Values inside the parentheses indicate the percentage of change over the control.

Note: t = 't' test value; *significant, ** Not Significant at the level of $p < 0.05$.

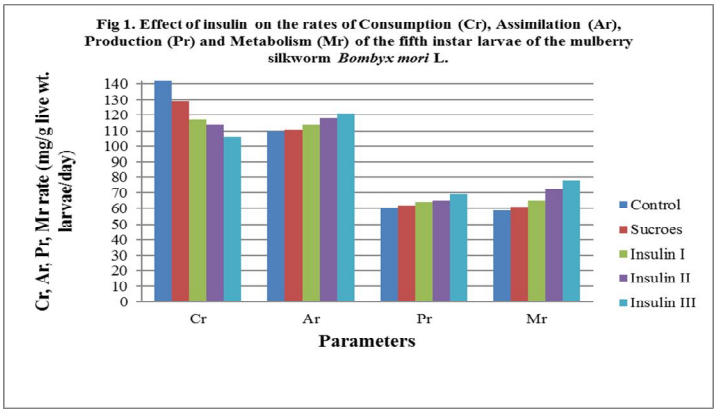

Figure 1.

Table 2. Effect of Insulin on the approximate digestibility (AD), efficiencies of conversion of digested food (ECD), efficiencies of conversion ingested food (ECI) of fifth instar larvae of the mulberry silkworm, Bombyx mori L.

S.No.	PARAMETERS	CONTROL LARVAE FED WITH		EXPERIMENTAL LARVAE FED WITH MULBERRY LEAVES SMEARED WITH 2% SUCROSE +		
		ONLY MUBERRY LEAVES	MULBERRY LEAVES + 2 % SUCROSE	10 µg INSULIN	20 µg INSULIN	30 µg INSULIN
1.	Approximate Digestibility(AD) %	77±1.5	79±1.4 t = -1.9* (2.5%)	80.1±1.5 t = -2.5* (4%)	80.4±1.7 t = -2.6* (4.4%)	80.8±.6 t = -2.6* (4.9%)
2.	Efficiency of Conversion of Digested food (ECD) %	54±1.7	60±1.6 t = -5.4* (11.1%)	68±1.4 t = -13.4* (25.9%)	70±1.6 t = -14.6* (29.6%)	71±1.7 t = -15.1* (31.4%)
3.	Efficiency of Conversion of Ingested food (ECI) %	42±1.5	48±1.5 t = -5.6* (14.2%)	55±1.7 t = -12.33* (30.9%)	58±1.6 t = -15.9* (38%)	59±1.4 t = -17.6* (40.4%)

Note: Values inside the parentheses indicate the percentage of change over the control.
Note: 't' test value; *significant at the level of $p < 0.05$.

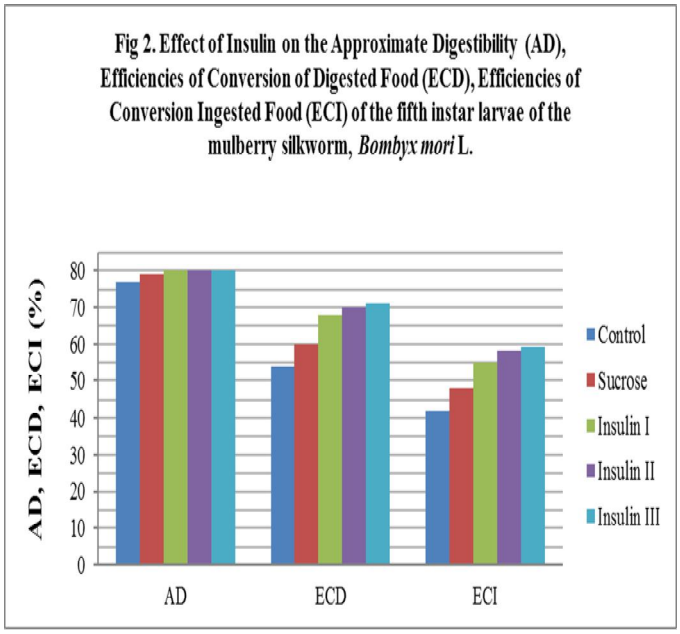

Figure 2.

Table 3. Effect of Insulin on the Larval duration and daily increment in weight of fifth instar larvae of the mulberry silkworm Bombyx mori L.

DAYS	CONTROL LARVAE FED WITH		EXPERIMENTAL LARVAE FED WITH MULBERRY LEAVES SMEARED WITH 2% SUCROSE +		
	ONLY MUBERRY LEAVES	MULBERRY LEAVES + 2 % SUCROSE	10 µg INSULIN	20 µg INSULIN	30 µg INSULIN
Larval Duration (h)	**168**	**168**	**168**	**144**	**144**
1.	24.879±1.61	29.091±1.58 t = -4.2* (16.9%)	30.615±1.67 t = -5.5 * (23.05%)	38.093±1.58 t = -13.1 * (53.1%)	42.429±1.74 t = -16.6 * (70.5%)
2.	31.380±1.70 [26.1%]	33.875±1.58 t = -2.4* (7.9%) [16.4%]	37.450±1.54 t = -5.8 * (19.3%) [22.3%]	42.100±1.50 t = -10.5* (34.1%) [10.5%]	46.243±1.67 t = -15.2 * (47.3%) [8.9%]
3.	38.475±1.39 [22.6%]	40.660±1.63 t = -2.2* (-5.6%) [20.02 %]	44.020±1.54 t = -5.9 * (14.4%) [17.5%]	46.243±1.53 t = -7.9 * (20.1%) [9.8%]	50.607±1.49 t = -13.2 * (31.5%) [9.4%]
4.	41.735±1.55 [8.4%]	44.229±1.80 t = -2.3* (5.9%) [8.7%]	48.900±1.58 t = -7.2 * (17.1%) [11.08%]	51.200±1.59 t = -9.5 * (22.6%) [10.7%]	57.824±1.48 t = -16.7 * (38.9%) [14.2%]
5.	45.280±1.70 [8.49%]	48.540±1.57 t = -2.9* (7.1%) [9.7%]	50.400±1.42 t = -5 * (11.3%) [3.06%]	63.154±1.60 t = -12.1 * (24.9%) [15.6%]	68.669±1.72 t = -16.7 * (35.91%) [14.3%]
6.	50.524±1.69 [11.5%]	52.910±1.58 t = -2.3* (4.7%) [9%]	59.800±1.58 t = -8.9 * (18.3%) [18.6%]	66.323±1.68 t = -12.6* (26.8%) [5.01%]	70.500±1.61 T = -17 * (34.8%) [2.6%]
7.	52.269±1.59 [3.4%]	58.217±1.64 t = -4.8* (11.3%) [10.03%]	63.900±1.59 t = -10.6 * (22.2%) [6.8%]	———	———

Note: Values inside the ( ) brackets indicate the percentage of change over the control.
Note: Values inside the [ ] brackets indicate the percentage of change over the previous day.
Note: t = ' t ' test value; *significant at the level of $p < 0.05$.

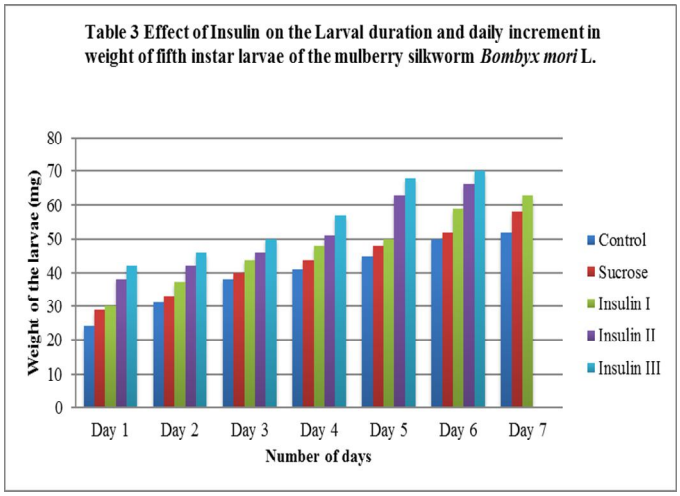

Figure 3.

Table 4. Effect of Insulin on the Cocoon Parameters of the mulberry silkworm Bombyx mori L

S. No.	PARAMETERS	CONTROL LARVAE FED WITH		EXPERIMENTAL LARVAE FED WITH MULBERRY LEAVES SMEARED WITH 2% SUCROSE +		
		ONLY MUBERRY LEAVES	MULBERRY LEAVES + 2 % SUCROSE	10 µg INSULIN	20 µg INSULIN	30 µg INSULIN
1.	Cocoon Weight (mg wet wt. / cocoon)	0.955	0.985 (3%)	1.055 (10%)	1.115 (16%)	1.190 (24%)
2.	Shell Weight (mg wet wt. / shell)	0.116	0.184 (58%)	0.190 (63%)	0.199 (71%)	0.205 (76%)
3.	Shell Ratio (%)	12.146	18.680	18.009	17.847	17.226
4.	Pupal Weight (mg wet wt. / pupa)	0.639	0.690 (7%)	0.708 (10%)	0.792 (23%)	0.914 (43%)

Note: Values inside the parentheses indicate the percentage of change over the control.

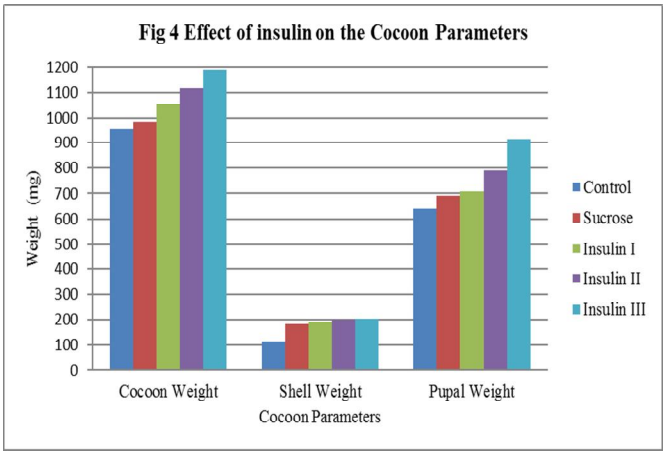

Figure 4.

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