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RESEARCH ARTICLE

The Effect of Calcium Glucono-Galacto-Gluconate on Certain Developmental and Parameters in Silkworm, *Bombyx mori* (L).

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ABSTRACT

Silkworm, *Bombyx mori* race cross-breed PM X NB4D2, has been chosen for the present study. With the intention of understanding the role of calcium on growth and yield performance this, work was designed and carried out. Four different concentrations of calcium gluconate (CG) in four different categories of worms of different larval stages were treated for the performance study. Fairly, a dose-dependent positive response was evident. Among all the schedules, 6% (CG) with reference to III and IV instar combined treatment exhibit vast response over the other groups. As CG is found to be costly for sericulture farmers, a plant source of calcium may be selected for further nutritive and fortification study to get a similar type of response is suggested.

Keywords: Silk, Gluconate, Hormone, Parathyroid

INTRODUCTION

Increased production of quality leaves for feeding silkworm through improved cultural practices and nutrient application play key role in silk worms growth, development, and cocoon yield.[1] It has been reported that injection, dietary supplementation or topical application of plant hormone, gibberllic acid and Indole acetic acid, affect development, longevity, fertility, egg productivity, egg viability, and emergence percentage in a few species of insects.[2] In recent years, attempts have been made to study the effect of vertebrate hormones in the economic parameters of the silkworm *Bombyx mori*. The treatment of Cyclic adenosine 3′,5′-monophosphate and PGN-E to V instars larvae enhanced the pupal weight and cocoon shell weight. It has also been reported that dietary supplementation or injection of thyroxin increased the larval growth and body weight, gonad weight, fecundity protein, and RNA content of the silk gland and silk production. Amino acids too form important constituents of the mulberry leaf going for the nutrition of the silkworm. The mulberry leaves are quite rich in amino acid content and satisfy the requirements of amino acids of silkworm.[3]

Silkworm being a monophagous insect almost all the nutrients required for its growth from the mulberry leaf itself. Increased production of quality leaves for feeding silkworms through mineral supplementation play key role in the larval development and cocoon characters. Magnesium, calcium, phosphorous, potassium, iron, manganese, and zinc were essential salt elements required by *B. mori*.[4,5]

The influence of micronutrients on larval development and cocoon characters of silkworm was also studied making use of bivoltine race Kalimpong-A. The results on feeding trails indicated that the mulberry leaves sprayed with micronutrients did not have any detrimental effect on larval development and cocoon characters.

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However, silkworm larvae fed with magnesium sprayed leaves tended to give better cocoon yield and cocoon weight as compared to control.[6]

Furthermore, to increase the silk yield, efforts were made to study the effects of temperature, light, photoperiod, X-rays, gamma rays, and artificial diets and amino acids on the development of the silkworm *B. mori*. Calcium, in general, is reported to have role in body homeostasis, cellular function, control of sodium permeability, and heart functioning. It is also evident that calcium level changes have impact on lethargy, anorexia, weakness, muscular hypotonia, nausea, vomiting, and constipation of animals at large.[7]

In insects, the process of growth and development is regulated by circulating hormones, namely, prothoracicotropic hormone juvenile hormone and ecdysone which greatly and directly, manifest the phenomenon of molting and metamorphosis. The pattern of insect development can be regulated artificially to a certain extent by exogenous administration of some mimics and analogues.[8] Several researchers have contributed much informations on fortification, through artificial diet and disclosed the positive response with good correlation.

Adoption of new technologies in sericulture happens to be inevitable as to make the enterprise more productive. This long run objective of making the enterprise promising calls for concern of various agencies such as researchers and farm management specialists extension personnel for their contribution at full strength. Technology in sericulture is changing at an increasing rate which is being noticed through the improvement in the levels of productivity. However, there still exists a notable gap in their diffusion at the field level. This emanates as a result of various factors such as physical, social, economical, psychological, and biological. Over the period, diffusion of sericulture technologies at the farmer level has been a great challenge for the extension personnel, who are working on it constantly.

Keeping this in view, an attempt was made to screen some of the pharmaceutical compounds, to check their effects on silkworm growth, development, and silk yield. The calcium gluconate (CG) was selected out of such compounds screened for the purpose.

MATERIALS AND METHODS

The diseases free laying (NB4D2X PM and PMXNB4D2) were obtained from NSSP through state sericulture department. The polyvoltine silkworms, reared in the laboratory following the improved methods of silkworm rearing,[9] were used in the present work. The worms were maintained on fresh Kanva-2 (K2) Mulberry leaves. Worms were fed 4 time a day at 26 ± 1°C, 12L:12D photoperiod, and a suitable humidity (RH75%) for each instars, respectively. The second, third, fourth, and fifth instars larvae were grouped into four experimental groups and one control group; each consisting of 50 worms of three replicates.

CG, a mxture of calcium salt, and D-Gluconic acid (2,3,4,5,6- pentahydroxy caproic acid) (calium glucono-galacto gluconate) obtained from Novartis were first dissolved separately in a double distilled water and then diluted to 1%, 2% 3% and 6%. The dilution was done in distilled water keeping in view of its easy and practical use in sericulture. Freshly, collected leaves were soaked in the respective dilutions of CG and air dried. Each time after soaking the leaves, the used dilutions were disposed off. Distilled water treated control was maintained for each experimental group and for all the experimental groups, a common untreated control group was also maintained.

After treatment, the pre-cocooning parameters such as the larval duration and larval weight and post-cocooning parameter such as cocoon weight and cocooning percentage were studied. The observations on larval and cocoon characters were recorded as per the procedure adopted in CSRTI, Mysore. Mean values of the results are given. Each mean value is the average reading from 10 worms. The larval duration and larval weight were taken at the end of the last larval phase. The experiments were designed by randomized block design. The data collected were subjected to one-way analysis of variance test to find out the significance between the corresponding parameters of the treated and untreated groups.

The percent index value was calculated for each parameter of the experimental groups over the corresponding parameter of the untreated controls.

RESULTS

The influence of amalgamation of calcium salt and D-Gluconate application on mulberry leaf while feeding silkworm, *B. mori* L in different instars phases, had been studied here and the results with regard to alone are given here in this text for analysis. (A) Larval duration (hours)-Table 1; (b) larval weight (V Instar) – Table 2; (c) cocoon weight (post-harvest pre marketing) – Table 3; and (d) cocooning percentage –Table 4.

Larval duration

Over the entire study, the larval duration in hours was ranging from 610 ± 3 (minimum) to 648 ± 3 (treated) and 646 ± 11 (Control). Universally in all cases, in all the treated groups, the larval duration was found to exhibit an oscillating trend. Out of four groups and four treatments III and IV instar (Combined) treated, silkworms groups alone reveal a rapid growth rate (610 ± 3 h) over the control (646 ± 11 h).

Larval weight (V INSTAR)

Out of the observations made in four different schedules on four different groups 2%, 3%, and 6% subjected worms of III and IV instar combined treatment exhibit an encouraging trend. Larval weight is one of the positive economic parameter that has a say on all other follow-up economic characteristic. Here, in the total study, the larval weight ranges from 1.463 ± 0.096 g (minimum) to 3.982 ± 0.081 g (maximum). The control group worms show only 1.596 ± 0.099 g. However, in all the treatments, a correlatory increases depending on the concentration of CG was evident. Out of various instars treated, III and IV combined treatment groups reveal a highest response over other best (IV and V combined).

Cocoon weight

Amongst the various concentrations on various developmental phases, the CG response on the weight of cocoon (post-harvest and pre-market phase) is crystal clear with respect to all the 6% treatment worms. Similar is to the 2% and 3% treatment groups in III and IV instar combined treated worms. However, the range of cocoon weight is 1.028 ± 0.199 g (minimum) and 1.984 ± 0.112 g (control) is 1.192 ± 0.084 g. The dose response of the given treatment over the cocoon weight is strongly evident from the data obtained.

Table 1: Impact of calcium gluconate on the larval duration (h) under different treatment schedules

Groups/ Treatment	Treatment given at instars			
	II alone	III and IV	IV and V	V alone
1%	630±12	624±9	629±6	648±3
2%	632±8	620±6	628±4	644±6
3%	627±5	618±3	624±4	640±7
6%	621±4	610±3	620±6	632±5
Control	646±11	646±11	646±11	646±11

The values are the mean±SD of 5 observations

Table 2: Impact of calcium gluconate on larval weight (pre-mounting) under different treatment schedules

Groups/ treatment	Treatment given at instars			
	II alone	III and IV	IV and V	V alone
1%	1.463±0.096	1.476±0.132	1.657±0.201	1.481±0.162
2%	1.631±0.121	3.502±0.142	2.063±0.192	1.811±0.201
3%	1.639±0.108	3.704±0.124	2.671±0.134	1.863±0.191
6%	1.845±0.092	3.982±0.081	2.777±0.99	1.889±0.084
Control	1.596±0.099	1.596±0.099	1.596±0.099	1.596±0.099

The values are the mean±SD of five observations

Table 3: Impact of calcium gluconate on cocoon weight (5th day) under different treatment schedules

Groups/ treatment	Treatment given at instars			
	II alone	III and IV	IV and V	V alone
1%	1.042±0.194	1.128±0.181	1.303±0.148	1.028±0.199
2%	1.271±0.089	1.886±0.121	1.532±0.181	1.161±0.136
3%	1.434±0.146	1.941±0.133	1.723±0.102	1.593±0.164
6%	1.689±0.126	1.984±0.012	1.811±0.098	1.791±0.13
Control	1.192±0.084	1.192±0.084	1.192±0.084	1.192±0.084

The values are the mean±SD of five observations

Table 4: Impact of calcium gluconate on cocooning percentage under different treatment schedules

Groups/ treatment	Treatment given at instars			
	II alone	III and IV	IV and V	V alone
1%	88±2	84±6	88±2	86±4
2%	85±2	85±5	89±1	88±1
3%	88±2	92±2	90±2	89±1
6%	89±3	96±2	91±1	89±2
Control	81±9	81±9	81±9	81±9

The values are the mean±SD of five observations

Cocooning percentage

Leaving alone the other parameters, the cocooning percentage is a paramount factor and is being a primary concern for sericulture farmer; this feature has been studied with utmost care. In spite of very many efforts put forth by R and D ventures, the sericulture performers still at times could not achieve cent percent cocoon from all the worms reared. Hence, with the possible hope of getting high cocooning percentage, this calcium-glucono-galato-gluconate had been used. In this treatment, a correlatory results are evident, in all the groups and treatments. However, the cocooning percentage ranges from 81 ±9 (Control minimum) to 96 ± 2 (treated maximum). It is similar to earlier other parameters, studied that only in III and IV instar combined treatment, the response is done dependent.

DISCUSSION

Calcium like many other inorganic elements in biological systems has during the last decade become the subject of much attention both by scientists and by the general public. Calcium ions are central to a complex intracellular messenger system that is mediating a wide range of biological processes; muscle contraction, secretion, glycolysis, and gluconeogenesis, ion transport, cell division, and growth. As on today, with wide variety of role of calcium, it is considered as "Biomineral". All living organisms need calcium which must be taken up from the environment. Thus, calcium ions have to be distributed throughout.[10] With this in view, calcium role in the development of silkworm has been chosen for study. The uptake of calcium from food has mostly been studied in typical laboratory animals such as rats, hamsters, chickens, and humans. As it is lacking in the insect group, especially in silkworm, the present approach has been chosen for the study. The uptake of calcium occurs in the intestine and transport is regulated by a metabolite of Vitamin D and calcitriol (1,2,5 – dinydroxy Vitamin D3). To maintain homeostasis and keep, the calcium level in blood plasma constant excess calcium is excreted. The main factor controlling this phenomenon (invertebrates) is the levels of the parathyroid hormone that acts on kidney and bone and indirectly through stimulated production of calcitriol, on the intestinal tract, calcium is almost an universal regulator of cellular function in stimulating the silk gland ells that duplicate fastlly at the later part of the larval phase. In the present work, when calcium is given at various stages (individually and combined), the III and IV instar treatments have tremendous effect on the chosen parameters. To influence the cellular machinery, the calcium ion must interact with different proteins, intracellular calcium receptors, and calmodulin. Calmodulin is a small acidic protein. The binding of calcium to calmodulin is quite likely cooperative denote that the switch from inactive to active confirmation over a much more narrow calcium concentration. Calcium, along with iron, silicon, and the alkaline earth metals, is an important constituent of mineralized biological tissues. The formation of calcified biominerals is a highly regulated process.

Although the number of instars in many species of insects is generally fixed, larval growth and development are modified by environmental and certain physic-chemical inductions leading either to precocious metamorphosis or to super numeracy larval molts. In the silkworm, *B. mori* precocious metamorphosis could by induced by manipulating external factors such as temperature and moisture.[11] During the growth of the silk glands in the fifth instar larvae, the principal sugar trehalose accumulates and attains its maximum level just before spinning of the cocoon.[12]

Food is a factor paramount importance which regulates growth reproduction and diversity, of animals in nature. The quantity, rate, and quality of food consumed by insect larvae have great bearing on survival, growth rate, developmental duration, and final body weight. The quantity of food consumed directly or indirectly, which influences the digestibility and conversion efficiency. Food ingestion, digestibility, and growth in the larval stage are interrelated. Characters such as health, survival, and cocoon quality are influenced by the quantum of mulberry fed to the silkworm larvae. The correlations of ingest to body weight gain against cocoon weight, shell weight, and fecundity

are highly significant and positive.[13] Similar is the trend exhibited in the present work in all parameters studied. The quantum of leaf fed to V instar larvae has direct relationship on growth and development and also on cocoon characters. However, this feature with the application of CG is not evident in any of the parameters.

Larval duration and larval weight

Duration of larval phase depends on many factors (both extrinsic and intrinsic). One among the intrinsic factors is food and its constituents. The calcium plays predominant role in the metabolism of most biological systems. The reduced larval phase may be due to hastened activity of calcium and the hormones responsible for molting of the inset. The possibility exist is the strong interaction of calcium with the metabolic products and enzyme machinery which helps in early conclusion of larval phase. In the third and fourth instar combined treatment group (at 6% concentration). Further, in this group, a dose-dependent deviation was observed. However, it is not that much reduction, in larval duration. The reason attributed with available review of the literature is that both the earlier and late feeding of calcium have less impact when compared to III and IV combined. However, in IV and V combined treatment, the value is close to III and IV combined. The value attributed to it is that perhaps IV instar may be an appropriate respondent phase to calcium to act.

Besides the calcium, the D-Gluconic acid is found to have strong impact on metabolic system, especially in carbohydrate metabolism in to with the intermediary metabolic tool the ingested food might have metabolized and diverted to large scale assimilation, which might be the reason for gorgeous weight increase with reference to III and IV instar combined treatment group. A correlatory response with increase in dose of CG a similar type of enhancement has been reported.[14]

The increment in weight of larva is attributed both to tissue gorgeousness as well as silk gland enlargement. However, with the observed data on weight of IV instar too (not projected), it is evident that the body tissue weight increment is found to be more as the silk gland gorgeousness which is predominant at final instar only. From the observed data on larval weight, the II and V instar treated independent groups did not exhibit any significant increase in larval weight i.e. 10845 (II instar) and 1.889 (V instar) against 3.982 g (III and IV combined trated). There is also close correlation exist between larval duration and larval weight, with regard to enhanced stimulus of calcium over duration and weight of larva. With decreased larval phase, if there is increased larval weight, it indicates the metabolic trigger with reference to carbohydrates, protein, and lipid metabolism as a whole toward tissue development and animal weight: Caused by calcium and D. gluconic acid. Similar type of response was reported with regard to mobilization of metabolites.

Cocoon weight and cocooning percentage

India being the second largest producer of silk in the world contributes to 19% of global silk production. More than 90% of silk produced in India is by multivoltine X bivoltine hybrid, popularly known as cross breed. One of the goals of silkworm breeding is to produce superior hybrid progeny. As a result, it is pertinent to raise methods of promoting cocoon weight. One such measure by R and D is by fortification and amendment studies to see, the possible impact on cocoon weight. Herein, the cocoon weight is found to be influenced by CG application while feeding silk worms. It is nature by rule or law that the weight increases in larval phase at V instar denote the gorgeousness of silkworm silk gland weight. Especially in the V instar, its index is evident in 6% treatment with reference to III and IV combined treated groups, it was 1.984 ±0.012 g. Similar to cocoon weight percentage of worms reaching, the successful harvestable cocoon is one of the paramount feature, and a factor to be considered primarily by sericulture farmers. With the sufficient positive response of calcium glucono galacto gluconates in the previous parameters, the achievements of all larva to become cocoon are also found to have correlatory results. The sericulture farmer cannot afford to lose his worms to be away from cocoon nesting. Due to circumstantial factors,

the cocooning percentage is found to be largely affected, few to mention are the silk secretion and ejection, muscle tone capacity, conversion and assimilation efficiency, and general activity of the silk worm. Herein, the work carried out a strong note is observed with reference to 6% treatment groups over the others (i.e., 96 ± 2). Moreover, CG being a component of having composite activity in controlling metabolism in a broad spectrum must be responsible for this higher percentage. A similar type of report is available for review to show the cocooning percentage.[1]

It is concluded that the rearing of silkworm with calcium supplementation along with D-gluconic acid found is to have a positive response ion the economic parameters of *B. mori*.

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