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COCOON PARAMETERS IN THE SILKWORM, *BOMBYX MORI* ON EXPOSURE TO TRACE ELEMENT AND NUTRIENTS

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ABSTRACT

The present study has been aimed at investigating various Economic Parameters of the silkworm cocoon, when fed on mulberry leaves fortified with selected trace element Zinc, vitamin, Pyridoxine and hormone, Methoprene. The experimental worms were divided in to four groups and fed with mulberry leaves soaked in the selected compounds i.e. Zinc chloride, Pyridoxine, Methoprene and with Mixed dose (Zn+B6+H). The Control group of silkworm larvae was fed with normal mulberry leaves. Cumulatively, the findings of the present study were observed to evaluate the modulatory role of Zinc, Pyridoxine and Methoprene with particular reference to the quality and quantity of the silk. The Mixed dose of Zinc, Pyridoxine and Methoprene on selected days significantly elevated all the selected Economic Parameters of the cocoons.

Keywords: Cocoons, Economic Parameters, Fortified, Methoprene, Pyridoxine

Number of Tables : 1 **Number of Figures**: 6 **Number of References**: 33

INTRODUCTION

All insects require a variety of minerals and trace elements as micronutrients. Mineral nutrition (Locke and Nichol, 1992) has been neglected compared with other nutrient requirements, and the quantitative requirements for insects are largely unknown. However, caterpillars are known to require appreciable amounts of potassium (8000-9000 ppm DW), phosphate (2000-6000 ppm), and magnesium (1000 ppm) (Muniandy, 2001). Because many basic biological functions depend on sodium, calcium, and chloride ions, these also are believed to be essential. Saha and Khan (1996) described the extensive effects of multi-vitamin compounds as diet factors on growth interruption and the decrease of cocoon economical characteristics. It is showed that multi-vitamin and mineral compounds could increase the food intake, growth and conversion efficiency of silkworm (Muniandy *et al.*, 2001). Evangelista *et al.* (1997) reported that the larval and cocoon weight increased under multi-vitamin compound treatment, but did not have any positive effects on cocoon shell weight. The role of mineral nutrition, more particularly that of Zinc, needs to be ascertained as it is known to play a vital role in the synthesis of lipids, proteins and carbohydrates and in reducing the duration of larval and pupal stages (Bhattacharya and Kaliwal, 2005). Such studies provide substantial evidences for practical application of Zinc and other microelements for qualitative and quantitative improvements in silk production. The cocoon spinning activity is an important phase in the silkworm which produces the cocoons, the final product of the animal. This activity which lasts for 5 to 6 days requires continuous function of the nervous system and the muscular system where the Cholinergic and Glutametargic neurotransmitters play key roles. Since, the larval stage is the only feeding stage in silkworm development, intake of balanced diet is very essential for silk production. By supplementing the diet with minerals, vitamins and trace elements, the various functions of the hormonal system, neuromuscular system, reproductive system etc., can be modulated effectively. Application of multi-vitamins as supplementary nutrients on biological and economical characteristics of silk worm *Bombyx mori* L. (by Kayvan Etebari and Leila Matindoost, 2005), had radically changed the results based on the concentration they were administered at.

Chang and Li (2004) reported that nutritional interactions exist between vitamin B3 and other groups of vitamin B. The juvenile hormone analogue (JHA) products, when applied in di minute and appropriate rates, promoted the extension of the larval period, when the insect feeds. Use of JHA in sericulture practices has been shown to boost good cocoon yield.In recent years, many attempts have been made to improve the quality and quantity of silk (Hiware, 2005), through enhancing the leaves with nutrients, spraying with antibiotics, juvenile hormone, plant products, with JH-mimic principles or using extracts of plants. The supplementation and fortification of mulberry leaves is a recent technique in sericulture research (Murugan et al., 1998). Etebari et al., (2004) reported the yield decrease, when ascorbic acid concentration is enhanced in silk worm diet. Chang and Li (2004) reported that nutritional interactions exist between vitamin B3 and other groups of vitamin B.

MATERIALS AND METHODS:

Test species : **Silkworm,** *Bombyx mori* (Disease-free larvae from local grainages)

Mulberry : M_5 Variety

Larval Instar : 5^{th} Instar

Test chemicals : 1. Zinc chloride (Fisher Inorganics & Aromatics Ltd,

2. Pyrol / Pyridoxine hydrochloride (vitamin B6) (FI & AL)

3. Methoprene Hormone (Seri-Agro market: Bangalore)

Duration of treatment : 7 Days

Dose Selected

Zinc chloride : 2 µg/ml

Pyridoxine hydrochloride : 2 µg/ml

Methoprene Hormone : 2 µg/ml

Economic parameters : Cocoons characters and quality and quantity of silk

Test species:

The present investigation was carried out on the Pure Mysore x CSR2 hybrid variety of the silkworm, *Bombyx mori*. Since the experiments required continuous maintenance of the test species, silkworms were reared in the laboratory itself in accordance with the procedure (Krishnaswami, 1978).

Treatment of fifth instar larvae with Zinc, Pyridoxine and Methoprene:

Required concentration (2 µg/ml) of Zinc chloride, Pyridoxine and Methoprene solutions were prepared in distilled water as shown below.

Preparation of standard stock solutions

For the preparation of standard stock solution 1, 1g of zinc chloride was dissolved in 100ml of distilled water (1000 mg x 1000 µg /100 ml) which is equivalent to 10000 µg/ml. From this solution 1ml was taken and added to 99ml of distilled water (10000 µg /100 ml) which is equivalent to 100 µg/ml, known as standard stock solution 2. The same procedure was followed in the case of Pyridoxine , Methoprene and Mixed dose also.

For the preparation of 2 µg/ml concentration, 2ml of standard stock solution 2 was added to 98ml of distilled water. But in the preparation of Mixed dose, the above prepared standard stock solution 2, each 2ml i.e. Zinc +Pyridoxine + Methoprene (2ml+2ml+2ml) was added to 94ml of distilled water. 100 ml of each of these concentrations were prepared as per the above table and 25-75 mulberry leaves were soaked in these solutions, dried at room temperature till the wetness is removed & were used to feed four groups of experiment larvae of the 5th instar stage for 7 days.

Economic parameters of cocoons:

By the end of seventh day of 5th instar, mounting of the silk worm larvae on the chakra was started. After the cocoons were completely formed (in 7-10 days), the below given economic parameters were analyzed in control and the experimental groups of silk worms.

1. Weight of cocoon: Cocoon is a protective case of silk or similar fibrous material spun by the larvae of silk worm. After the completion of cocoon development, 25 cocoons were taken and the weights were recorded and the single cocoon weight was calculated in grams.

2. Weight of shell with floss: 25 shells were taken and the weights were recorded and the single shell weight was calculated in grams.

3. Weight of shell without floss: 25 cocoons were taken and floss layer was removed for these 25 cocoons. Then the weight of these de flossed shells was calculated.

4. Weight of the floss: The floss layer of 25 shells was removed and weighed accurately. The weight of the floss of a single shell was calculated in grams.

5. Floss – shell ratio:

The floss-shell ratio can be calculated by using the following formula:

Floss- Shell ratio = {[Weight of the floss (25 cocoons)]/ [Weight of the shell (25 cocoons)]} x 100

6. Total proteins in shell: After removing the floss layer of the cocoons, 5 to 6 shells were collected. From these shells the pupae were removed by cutting the shell and the shell was made into small pieces with the help of a scissors.

7. Pupal weight: 25 pupae were taken, and weighed their weight accurately in grams.

8. Filament length: The total filament length was measured in meters.

Filament length = {Length of raw silk(m) x 1.125 (circumference)} / No of reeling cocoons = **……..mt.**

9. Filament weight: The total filament was weighed accurately. The weight of the total filament was calculated in grams.

10. Denier: The denier can be calculated by using the following formula:

Denier = {Filament Weight in grams / Filament length in meters} x 9000 = **…….. gm.**

11. Cocoon yield: Cocoon yield per 100 dfls were calculated per kg. It can be calculated by using the following formula:

Cocooning percentage = {Total no. of cocoons / Total no of matured larvae} x 100

12. Reeling breaks (RB): The reeling breaks were calculated at the time of reeling, which are significant in accessing filament quality.

Preparation of Homogenate: For preparation of Homogenate of shell, to 0.05gm of Shell pieces one or two drops of NaOH (1 ml of distilled water in a test tube + 1 – 2 pellets of NaOH) was added and a fine homogenate was prepared in 5 ml of distilled water and used for estimation of total proteins.

STATIATICAL TREATMENT OF DATA:

Values of the measured parameters were expressed as Mean ± SEM. Repeated Measures of ANOVA was used to test the significance of difference among four different groups followed by Dunnet's Multiple Range Test (DMRT). Statistical analysis was performed by using Statistical Program of Social Sciences (SPSS) for windows (Version 19; SPSS Inc., Chicago, 1L, USA). The results were presented with the F-value and p-value. In all cases F-value was found to be significant with p-value less than 0.01**. This indicates that the effects of factors are significant.

RESULTS AND DISCUSSION: (Table: 1 and Figs: 1 to 6)

In this section, the effect of some selected nutritive compounds such as Zinc, Pyridoxine and Methoprene as already done in the case of protein profiles was assessed on various economic parameters of the cocoons and the quality and quantity of the silk was determined with the help of the appropriate techniques. After the cocoons were completely formed (in 7-10 days), the following economic parameters were analyzed:

1. Weight of cocoon	7. Pupal weight
2. Weight of shell with floss	8. Filament length
3. Weight of shell without floss	9. Filament weight
4. Weight of the floss	10. Denier
5. Floss-shell ratio	11. Cocoon yield
6. Total proteins in shell	12. Realing breaks

The results of the present study revealed that the mixed dose on any selected day significantly elevated all the above mentioned parameters related to the economic variables of the cocoons. Of all four experimental groups studied, the most effective group was found to be the Mixed dose (E4). At this dose (Zn+B6+H), the following changes in the economic parameters were observed.

EXP. GROUP	WC	WSF	WS	WF	FSR	TPS
Control	0.92g	0.17g	0.14g	0.02g	10.7	30.0mg
E1 (Zinc)	1.05g	0.20g	0.18g	0.05g	15.5	46.9mg
E2 (Pyridoxine)	1.08g	0.23g	0.21g	0.07g	17.5	48.5mg
E3 (Methoprene)	1.36g	0.28g	0.25g	0.09g	20.2	51.3mg
E4 (Zn+B6+H)	**1.82g**	**0.35g**	**0.32g**	**0.11g**	**22.3**	**53.5mg**

From the above results, it was obvious that both the weight of the total cocoon, the shell and the floss recorded significant increase after treatment of the silkworms with different compounds such as Zinc, Pyridoxine and Methoprene. As a corollary to these parameters, the total proteins in the shell were also elevated in the same trend as in the case of the other economic parameters. However, the mixed dose was found to be 2 times more effective than the individual doses. From the above results it was inferred that at lower concentrations, the mixed dose has a positive effect on all the parameters.

EXP. GROUP	PW	FL	FW	DNR	CY	RB
Control	1.30g	1036m	0.286g	2.67	72.04	2.32
E1 (Zinc)	1.52g	1065m	0.289g	2.68	74.33	1.55
E2 (Pyridoxine)	1.58g	1068m	0.292g	2.69	75.61	1.52
E3 (Methoprene)	1.62g	1072m	0.295g	2.72	77.3	1.49
E4 (Zn+B6+H)	**1.68g**	**1078m**	**0.298g**	**2.75**	**83.13**	**1.43**

From the above results, it was obvious that the pupal weight of the silk worm was relatively high in E4 group than the other groups. Similarly, the filament length and weight recorded significant increase after treatment of the silkworms with different compounds. The increased cocoon shell weight was understood to have been converted to the end product, the reelable silk filament. In this way the denier and cocoon yield per 100 dfls were also elevated in the same trend as in the case of the other economic parameters. The number of reeling breaks was reduced in all experimental groups indicating the improvement in the quality of silk by Zinc, Pyridoxine and Methoprene. Among these compound Methoprene had more positive effect followed by Pyridoxine and Zinc. Finally, the Mixed dose was found to be more effective than the other doses.

In the present investigation, various economic parameters of the cocoon of control and experimental silkworms treated with some selected compounds such as were observed to evaluate the modulatory role of Zinc, Pyridoxine and Methoprene with particular reference to the quality and quantity of silk. The results of the present study demonstrated that the Mixed dose of

Zinc, Pyridoxine and Methoprene on selected days significantly elevated all the above mentioned parameters related to the economic variables of the cocoons. When compared to the individual doses, the most effective dose was found to be the Mixed dose (E4). At this dose (Zn+B6+H), the following changes in the economic parameters were observed. It was obvious that both the weight of the total cocoon, the shell and the floss recorded significant increase after treatment of the silkworms with different compounds. As a corollary to these parameters, the total proteins in the shell were also elevated in the same trend as in the case of the other economic parameters.

The stimulatory capacity of the mixed dose on various cocoon characters contributing to silk yield may be attributed to the synthesis of proteins and nucleic acids in the silkworm. Further, the mixed dose may affect body cells or regulation of the organs viz. Neurosecretary glands, or both. Increases in biochemical constituents corresponded to increases in cocoon weight and silk output. Increase in total proteins and free amino acid implies increased metabolic activities. Because amino acids are the basic units of silk, increased amino acid content suggests activation of silk production and increased free amino acids associated with increased amino acid catabolism. The other reason for increased levels of silk proteins may be due to increase in Glutamate dehydrogenase, an important enzyme for transformation of glutamic acid, the main amino acid in silk protein and filament. Further, increase in cocoon weight and biosynthesis of silk protein (fibroin and sericin) concentration, which in turn is related to superior silk quality can be attributed to the increased synthesis of RNA in silkworm.

The results of the present study clearly demonstrated that the Mixed dose significantly elevated all the parameters related to the economic traits of the cocoons such as the total weight of cocoon, weight of shell without floss, weight of the floss, floss – shell ratio. However, the mixed dose was found to be more effective than the other experimental groups. From the above results it was inferred that at lower doses, the nutrients such as minerals and vitamins has a positive effect on all the parameters but at higher doses it has a negative effect. This observation derives support from the research findings of Etebari and Matindoost (2005) who reported that feeding of silk worm on mulberry leaves enriched with multi vitamins from fourth instar increased female cocoon shell weight at 2.5% concentration, while pupal weight got increased at 1% concentration. Further Experimental findings suggest that cofactor functions of iron (Nichol et al., 2002) and Zinc, for example are considered of almost universal importance to organisms and trace elements can be essential for insects in general. Intracellular homeostasis of zinc (McFarlane 1976) is believed to be critical because of different biological roles that zinc performs. In the present context, it is necessary to mention that Silk protein from silk glands of the silkworm, *Bombyx mori* is a 100% natural protein that is mainly made of 25-30% sericin and 70—75% fibroin proteins.

The silk threads of the cocoons of the silkworm *Bombyx mori* are composed of two major proteins (fibroin and sericin), produced by secretions of the silk glands. The silk gland is divided into three regions: anterior, central and posterior. The posterior silk gland secretes fibroin, while sericin, a glycoprotein which coats fibroin, is secreted by the central silk gland. The fibroin protein is transferred by peristalsis into the central silk gland where it is stored until required for spinning (Shimura, 1993). Mulberry leaves are rich in protein and amino acids, and there is a high correlation between leaf protein levels and the production efficiency of the cocoon shell, i.e. the cocoon shell weight relative to the total amount of mulberry leaves consumed by the silkworm (Machii & Katagiri, 1991). It is therefore possible that an increase in the protein level of mulberry leaves may lead to improvements in cocoon productivity.

Mulberry varieties with nutritional supplements, exerted more variations, when the leaves dipped in different concentrations of thiamin solutions were supplied to silkworms, and resulted in increased protein content of the fat body and of the haemolymph, but only at low concentrations: at high concentrations the protein content of the fat body was almost unchanged (Nirwani & Kaliwal 1998). L-ascorbic acid (vitamin C) is an important vitamin and is abundant in plant tissues: green leaves have the same amount of ascorbate as chlorophyll (Foyer, 1993). Zang & Ma, (1991) studied the haemolymph vitamins of fifth-instar silkworm larvae, finding that vitamin concentrations of healthy larvae were greater than that of unhealthy larvae (viral-infected or fluoride-poisoned). Vitamin C also aids in detoxification of various metabolic or tissue toxins and acts as a strong antioxidant, increasing protein synthesis. Egg production increases on rearing on mulberry leaves supplemented with thiamine (vitamin B) (Nirwani & Kaliwal, 1998). A second hypothesis is the action of vitamin B as a coenzyme in amino-acid and nucleic-acid metabolism. Activity levels of enzymes in the mid gut, fat body tissues and silk glands increase as the levels of dietary vitamin B6 increase (Horie & Nakamura, 1986).

In recent years, *Bombyx mori* nutrition research has finally started to yield decent results with regards to at least partial replacement of feeding material with alternates for fresh mulberry leaves (which can be difficult to obtain depending on season) and with regards to dietary supplements which could improve both the quality and quantity of the silk thread as well as the nutritional value of the chrysalis With additional research in the field, in order to better determine the optimal food preservation methods and beneficial additives that need to be used, silkworms could be grown all around the year as opposed to only seasonally, which could have strong positive economic implications in case the desired yields (silk and/or biomass) are not significantly negatively affected by the alternate nutrition.

The silkworm, *Bombyx mori* L. is a typical monophagous insect and mulberry (Morus spp.) leaf is its sole food. Man has immensely benefited from the silk produced by silkworms and subsequently researchers have always been trying to unveil the factors that can be manipulated to the benefit of the silkworm rearers (Nair and Kumar, 2004). Sericulture is an age-old land-based practice in India with high employment potential and economic benefits to agrarian families. No doubt, India is the second largest producer of mulberry silk next only to China (Vijayaprakash and Dandin, 2005). Plants are the richest source of organic chemicals on earth and phytochemicals have been reported to influence the life and behavior of different insects (Rajasekaragouda et al., 1997). Various extracts of medicinal plants have been tested by supplementation in the silkworm *Bombyx mori* and were seen to influence the body weight, silk gland weight and the silk thread length in *Bombyx mori* (Murugan et al., 1998). Dietary supplementation of the leaf, flower and pod extracts of *Moringa oleifera* (Rajeswari and Isaiarasu, 2004) and chitosan solution (Bin Li et al., 2010) elicited varied responses in the final instar larvae of *Bombyx mori*. Nutrition plays an important role in improving the growth and development of *B. mori* (Kanafi et al., 2007). Alagumalai et al. (1991) observed fortification of mulberry leaves with the flour of black gram and red gram to improve the larval growth and cocoon characteristics in *B.mori*. Similarly, the growth of silkworm larvae improved significantly upon feeding them with mulberry leaves supplemented with different nutrients (Sarker, 1993). Sundaram-ahalingam et al., (1998) noticed that the growth rate and protein utilization of silkworm are high as a result of the supplementation of Miraculan, a plant growth regulator. Murugan et al., (1998) noticed a strong correlation between the growth of silkworm and the silk production in the silkworm after the treatment with plant extracts and attributed the growth promoting effect of the plant extracts to the stimulation of biochemical processes leading to protein synthesis. The economic characters of the silk cocoon were reported to improve by feeding the silkworm with mulberry leaves treated with amino acids (Sridhar and Radha, 1986). Subburathinam et al., (1990) observed the enrichment of mulberry leaves with calcium chloride to increase the cocoon characters like cocoon weight, shell weight cocoon /shell ratio and silk proteins. Chamudeswari and Radhakrishnaiah, (1994) reported the increase of cocoon weight, when the silkworm larvae were fed with zinc and nickel fortified mulberry leaves. Majumdar and Medda, (1995) reported the supplementation of tyrosine to enhance the cocoon weight due to the increased synthesis of DNA, RNA and proteins in silk gland. The weight and the size of cocoon shell ratio and fibroin content of the shell increased with the supplementation of the amino acid, glycine (Isaiarasu and Ganga, 2000) reported that administration of JH analogue, Methoprene, to fifth in star larvae of *B. mori* through hypodermic injection increased the shell weight by 16 percent over the control. Improvement in economic characters of silkworm was also noticed with folic acid administration. The silkworm larvae fed on mulberry leaves treated with Coffea arabica leaf extracts at 1:25 concentration recorded significantly higher.

The economic parameters were measured by the procedures given by Sonwalkar, (1993). Parameters such as raw silk, filament length, reliability, denier and shell ratio were found to be good considerably in the case of cocoons reared by feeding mulberry leaves treated with Zinc, Pyridoxine and Methoprene and with mixed dose. In the Zinc treated group the economic traits elevated significantly. In the case of Pyridoxine treated group, elevation was slightly enhanced. The Methoprene treated group, all the economic parameters were elevated more significantly. In the Mixed dose treated group, economic traits were more pronounced, when compared to other experimental groups.

It could be suggested that the higher concentration applied to the larvae, may not have any effect on the hormonal level, juvenile hormone and moulting hormone which control moulting and metamorphosis in insects. Similar results have been reported in the polyvoltine breed of the silkworm, *B. mori* where larval duration did not show any significant change (Magadum et al., 1993). It has been shown that ion concentration mechanism has stimulatory effect on the nervous system in the moth, *Menduca sexta* (Rubin et al., 1988). It is likely that the decreased larval duration might possibly be due to the increased synthesis and release of moulting hormone and / or decreased level of juvenile hormone, which might have resulted in early pupation. It has also been reported that the supplementation with potassium sulphate significant increase caused in the survival percentage in silkworm, *B. mori*. The results of the present study showed that dietary supplementation with Pyridoxine, Methoprene and Mixed dose significantly increased the cocoon weight, cocoon shell weight and its ratio significantly increased in all selected experimental groups. The significant increase in the cocoon weights and their shell weights are dose dependent. The significant increase in cocoon weights and their shell weights are preceded by a significant increase in silk gland weights. Similar results have been reported with the dietary supplementation with potassium iodide (Majumder, 1982) and potassium sulphate in the silkworm, *B. mori* (Kochi et al., 2005) have reported that there was a significant increase in cocoon weight, cocoon shell weight and cocoon shell ratio in CSR2, CSR4 and CSR2 x CSR4 crossbreed races of silkworm treated with mineral mixture of potassium bromide and nickel sulphate. The results of the present study, therefore, seem to suggest that the supplementation with Zinc, Pyridoxine, Methoprene and Mixed dose are more effective in increasing the cocoon weight when compared to control group.

The present results showed that there was a significant increase in the filament length and its weight in all the experimental groups. However, the denier was significantly increased in all the Zinc, Pyridoxine, Methoprene and their mixture treated groups. Similar results have been reported in the filament length and its weight after supplementing the feed with zinc and nickel chloride in the silkworm, *B. mori* (Hugar et al., 1997). It has also been reported that there was a significant increase in filament length, weigh and denier supplemented with mineral mixture of potassium bromide and nickel sulphate of the silkworm, *B. mori* (Kochi et al., 2005). The present results, suggest that the filament length and its weight can be increased after supplementing the food with Zinc, Pyridoxine, Methoprene and their mixture which might possibly be due to the stimulatory effect of these chemicals on the silk gland activity. The selected experimental groups such as Zinc, Pyridoxine, Methoprene and Mixed dose resulting high yield and enhance the nutrients in plants leaves which in turn influence the better growth of silk worms containing higher proportion of silk proteins yields spinning of long silk threads in cocoons resulting in increased weight of cocoons, minimizes the cost of cultivation, and increase the parameter values of cocoons resulting in high silk production, this elevates the economy of the farmers, since cultivation of mulberry is made without using fertilizer.

In the literature, much was emphasized in relation to the impact of JHA's on economic profiles of various breeds of silkworms; practically there is little or no literature as to how various JHA's alter the key enzyme activities of *B.mori* during its growth period. Silk production basically depends on the *B.mori* larval protein metabolism which in turn needs more energy generating events, spinning requires more muscular activity and silk is being produced by the silk gland. On these lines, the selection of enzymes involved in protein and energy metabolism as well as tissues like muscle and silk gland in the present study is justifiable.

In the present investigation, various economic parameters of the cocoons of control and experimental silkworms treated with some selected experimental groups such as Zinc, Pyridoxine, Methoprene and Mixed dose were observed to evaluate the modulatory role of these selected experimental groups with particular reference to the quality and quantity of silk. The results of the present study clearly demonstrated that Zinc chloride significantly elevated all the parameters related to the economic traits of the cocoons such as the total weight of cocoon, weight of shell without floss, weight of the floss, floss – shell ratio. However, Pyridoxine and Methoprene also exhibited the same trend i.e. to be effective in the elevation of economic traits. The Mixed dose significantly elevated all the economic traits. When compared to these groups Mixed dose elevated more significantly in all the economic parameters. The reeling breaks were reduced in all the selected experimental groups when compared to control, indicating the quality of silk thread. This observation derives support from the research findings of Etebari and Matindoost, (2005) who reported that feeding of silk worm on mulberry leaves enriched with multi vitamins from fourth instar increased female cocoon shell weight in 2.5% concentration, while female pupal weight get increased in 1% concentration. Further Experimental findings suggest that cofactor functions of iron (Nichol et al., 2002) and Zinc, for example are considered of almost universal importance to organisms and trace elements can be essential for insects in general. The mineral availabilities in insects were reviewed by Muniandy et al., (2001). In *Bombyx mori*, the assimilation efficiencies ranged from negative values to 51% (Zn), with that for P at 27% (Horie et al., 1985). Studies of Valle, (1976) suggest that proteins involved in controlling such processes would be regulated directly by zinc. The nutrition of silkworm should be incorporated with only required amounts of trace elements like Zinc and anything excess can be detrimental instead of increased productivities. At higher concentration, Zinc exerted inhibitory effect on protein turnover as well as the economic parameters.

CONCLUSION

The present study substantiates the positive role of Zinc, Pyridoxine, Methoprene and Mixed dose on economic traits of sericulture. Further, it demonstrates that the positive impact of all the selected experimental groups reflects at two levels. Firstly, it stimulates silk protein synthesis in the silk gland and enhances silk output, as reflected in higher shell-cocoon ratios, silk-body ratio, raw silk percentage, denier and filament length, weight and secondly and it lowers the floss-shell ratio by decreasing the floss protein synthesis as well as reeling breaks, which are removed as wastage at the time of silk reeling.

In the present context, it is necessary to mention that Silk protein from silk glands of the silkworm, *Bombyx mori* is a 100% natural protein that is mainly made of 25-30% sericin and 70—75% fibroin proteins. Silk filaments, Fibroin, are extruded in pairs and are cemented together with silk gum, Sericin. Silk protein is widely used in cosmetics, health food, textiles and medical fields as it provides normal 19 amino acids which are fundamental to the human body to construct proteins.

Tier's silk protein products, made of very good quality silk protein are now a biggest silk farm in Thailand. They have 40 years of working experience with silk industry and thus, they can control the production of the best quality of the whole silk's life cycle, from silk egg to silkworm to silk cocoon to silk yarn and the silk protein.

In view of the increasing application of the silk proteins in cosmetic industry, research work to improve the quality and also the quantity of silk proteins has become the fastest emerging area in the countries which are associated with the sericulture industry worldwide. It is the concern of every researcher because the best product has to use the best ingredients to launch the premium silk products in the market for the consumers. The present investigation is one such attempt in the direction of improving the economic parameters of the silk. However, at this stage, it is too early to extrapolate the concept of application of Zinc, Pyridoxine and Methoprene in the combined form, as a promoting agent for improving the quality and also the quantity of silk proteins for their inclusion in cosmetology.

Cumulatively, the findings of the present study finally suggest that zinc + vitamin B6 + hormone induced active turnover of all protein profiles and the metabolic events in the posterior silk gland, creating congenial metabolic conditions for growth and silk production in experimental larvae treated with Mixed dose. Based on above results, the application of lower doses of nutrients such as trace elements and vitamins and juvenile hormone analogues can be recommended for application in sericulture for the improvement of the quality and also the quantity of silk yield.

TABLE-1: Changes in Economic parameters 0f the cocoons from Control and different Experimental groups of silkworms

ECONOMIC PARAMETERS		CONTROL	E1 (Zn)	E2 (Vit.B6)	E3 (H)	E4 (Zn + E6 +H)
1. Weight of Single Cocoon (g)	Mean	0.92	1.05	1.08	1.36	1.82
	PC	-	14.1	17.40	47.83	97.83
	SD	±0.02	±0.01*	±0.01*	±0.01*	±0.01*
2. Weight of the Single shell with floss (g)	Mean	0.17	0.20	0.23	0.28	0.35
	PC	-	17.6	35.30	64.71	105.9
	SD	±0.001	±0.001*	±0.001*	±0.001*	±0.001*
3. Weight of the single shell with out floss (g)	Mean	0.14	0.18	0.21	0.25	0.32
	PC	-	28.6	50.0	78.6	128.6
	SD	±0.001	±0.001*	±0.001*	±0.001*	±0.001*
4. Weight of the floss of a single shell (g)	Mean	0.02	0.05	0.07	0.09	0.11
	PC	-	150.0	250.0	350.0	450.0
	SD	±0.001	±0.001*	±0.001*	±0.001*	±0.001*
5.Floss – Shell ratio	Mean	10.7	15.5	17.5	20.2	22.3
	PC	-	44.9	63.6	88.8	108.4
	SD	±0.001	±0.001*	±0.001*	±0.001*	±0.001*

6. Total proteins in single shell (mg)	**Mean**	30.0	46.9	48.5	51.3	53.5
	PC	-	56.3	61.7	71.0	78.3
	SD	±0.05	±0.26*	±0.05*	±0.05*	±0.05*
7. Pupal weight (g)	**Mean**	1.30	1.52	1.58	1.62	1.68
	PC	-	16.92	21.54	24.62	29.23
	SD	±0.211	±0.105*	±0.01*	±0.01*	±0.01*
8.Filament length (m)	**Mean**	1036	1065	1068	1072	1078
	PC	-	2.80	3.1	3.5	4.1
	SD	±0.5	±0.5*	±0.5*	±0.5*	±0.5*
9.Filament Weight (g)	**Mean**	0.286	0.289	0.292	0.295	0.298
	PC	-	1.05	2.1	3.1	4.2
	SD	±0.01	±0.001*	±0.001*	±0.001*	±0.001*
10. Denier	**Mean**	2.67	2.68	2.69	2.72	2.75
	PC	-	0.4	0.74	1.9	3.0
	SD	±0.01	±0.01*	±0.01*	±0.01*	±0.01*
11.Cocoon Yield per 100 dfls (kg)	**Mean**	72.04	74.33	75.61	77.30	83.13
	PC	-	3.2	5.1	7.3	15.4
	SD	±1.041	±2.206*	±0.164*	±0.853*	±0.873*
12.Reeling	**Mean**	2.32	1.55	1.52	1.49	1.43

breaks (RB)	**PC**	-	-33.2	-34.5	-35.8	-38.4
	SD	±0.01	±0.01*	±0.01*	±0.01*	±0.01*

Values are Mean ± SEM of four observations each from tissues pooled from 4 silkworms

Values in parentheses are percent change from control

Values are significantly different from control at $p < 0.01$

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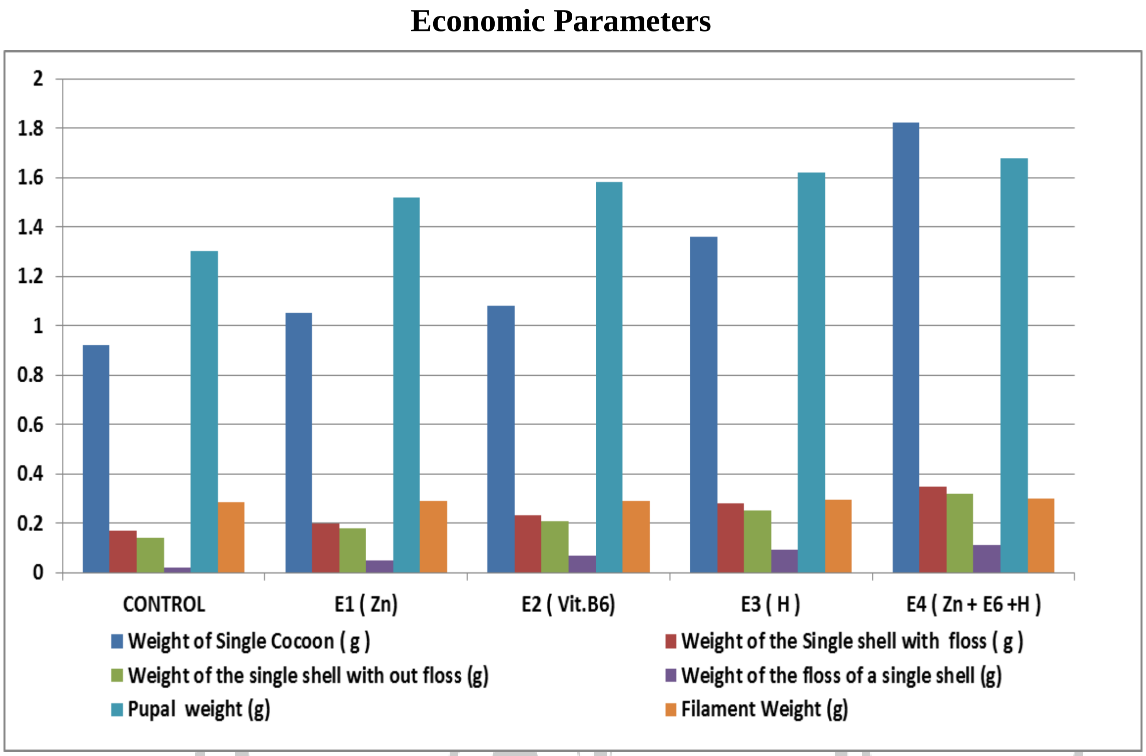
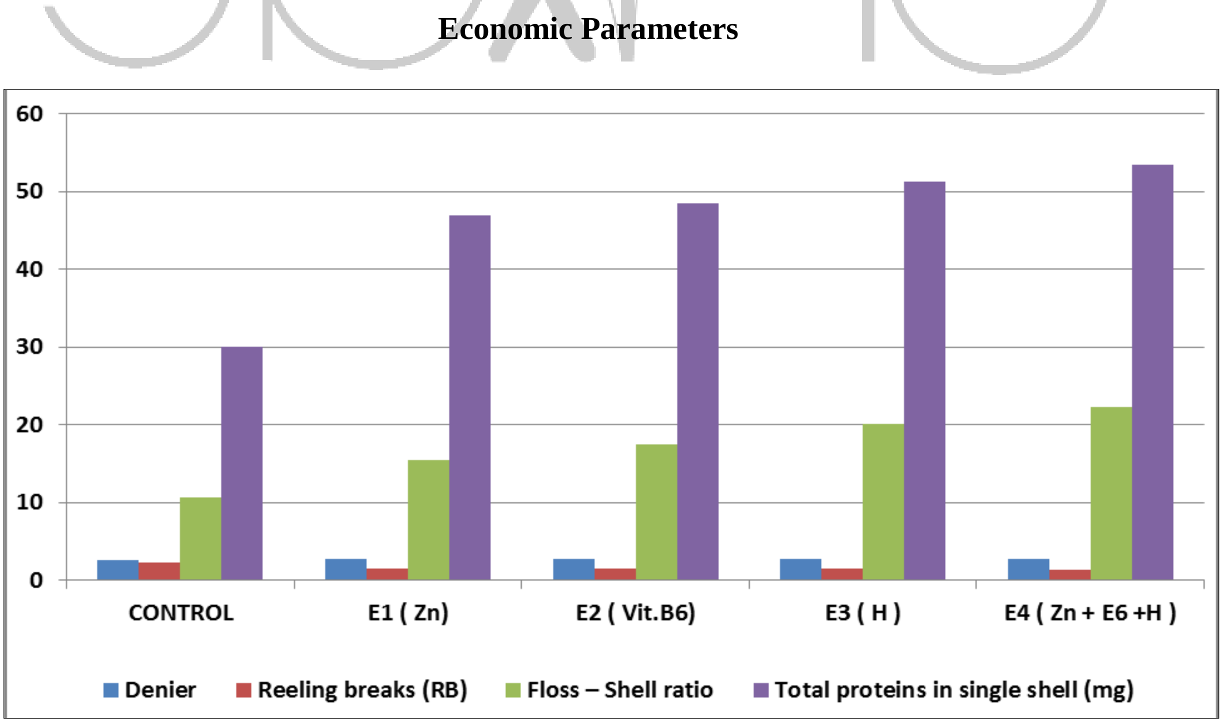
Fig 1-4: *Changes in the qualitative and quantitative Economic parameters in the cocoons of Control and different Experimental groups of silkworms*





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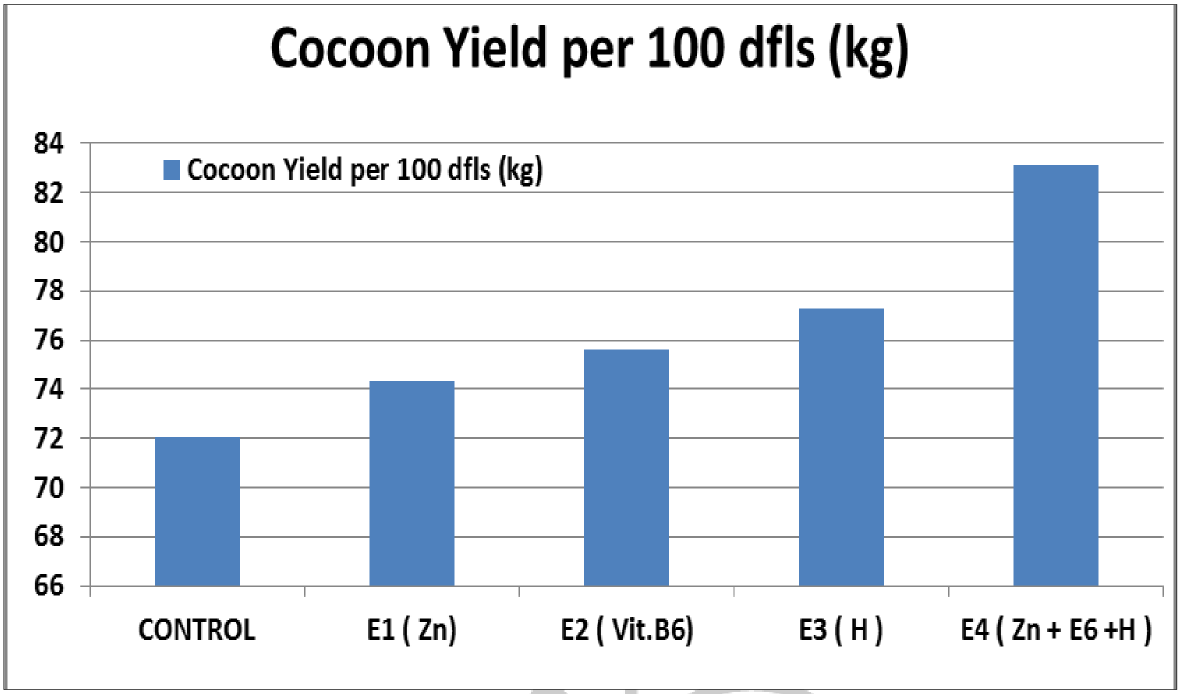

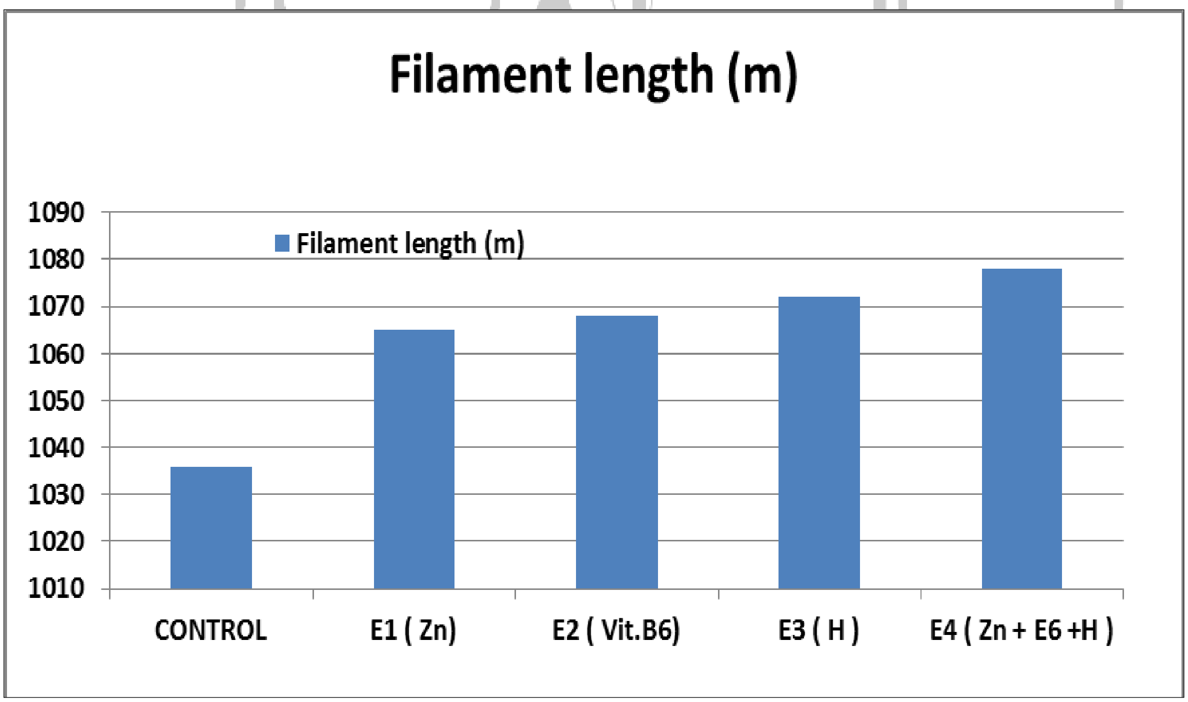

Fig 5: *Size variations in Cocoons produced by different experimental groups of silkworms*

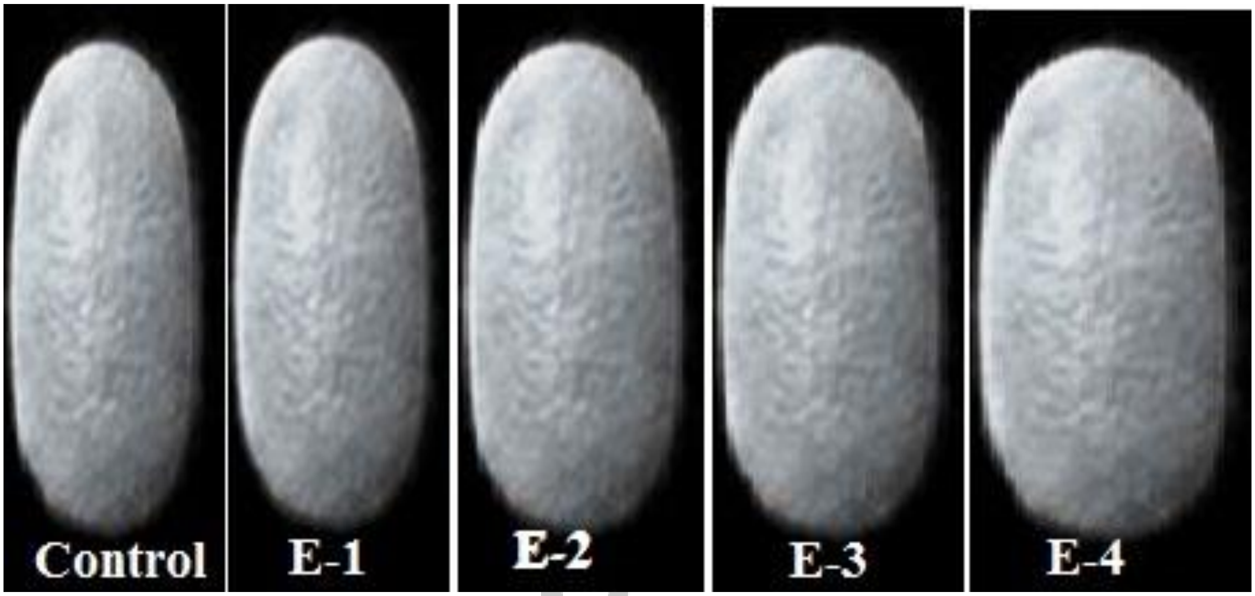

Fig 6: *Variations in the silk-yarn obtained from different experimental groups of silkworms*

E1 – Zinc

E2 – Pyridoxine

E3 – Methoprene

E4 – Mixed Dose

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