Available online at www.worldscientificnews.com

World Scientific News

WSN 42 (2016) 73-86 EISSN 2392-2192

Influence of magnetic energy on protein contents in the fifth instar larvae of silkworm, *Bombyx mori* (L) (Race: PM x CSR2)

Madhuri Anil Shivpuje[1], Hanumant V. Wanve[2], Sadashiv N. Belpatre[3], Vitthalrao B. Khyade[4,*]

[1]Student (T. Y. B. Sci. Physics), Zulal Bhilajirao Patil College, Z. B. Patil Road, Deopur, Dhule – 424002, MS, India

[2-4]Research Group and Science Association, Shardabai Pawar Mahila Mahavidyalaya, Hardanagar, Tal. Baramati, Dist. Pune – 413115, India

*E-mail address: vitthalrao.khyade@gmail.com

ABSTRACT

The fifth instar larvae of multivoltine crossbreed race (PM x CSR2) of silkworm, *Bombyx mori* (L) were exposed to the magnetic energy of various strengths (1000, 2000, 3000 and 4000 Gauss magnetic field). The magnetization of fifth instar larvae was carried out on the first four days, for half an hour for each day before first feeding. Bioassay of total proteins was carried out on the fifth day of fifth instars. The attempt reveals influence of magnetization of *Bombyx mori* larvae on the total protein content level in the silk glands, fat bodies and haemolymph. The total protein content was increased with increase in the strength of magnetic field from 1000 to 4000 Gauss magnetic field. The larvae magnetized with 4000 Gauss magnetic field were found with sustained or decreased in total protein contents. Silk gland total proteins were increased from 5.901 to 17.481 percent. Total proteins of fat bodies were increased from 18 to 46.517 percent. And the total proteins of haemolymph were increased from 16.606 to 33.588 percent. Magnetization may have had influence on the increase in the levels of amino acids followed by accelerated rate of protein synthesis in the fifth instar larvae of silkworm, *Bombyx mori* (L) (Race: PM x CSR2). Magnetic energy should be utilized as efficiently as possible for the progression of growth of larval instars of silkworm, *Bombyx mori* (L).

***Keywords*:** Magnetic exposure; Protein content; Silk Glands; Fat Bodies; Haemolymph; PM x CSR2

1. INTRODUCTION

The nervous system of the animal beings is a highly computerised organ. It is a greet intelligent, active and natural centre that controls the body working through the motor nerves, sensory nerves and mixed nerves. In human being, he brain acting through nerves, controls the mussels all over the body including face, neck and joints as well as the vagus nerves which goes into the heart, lungs, intestines, kidneys, liver and spleen. The brain consists of approximately 10 billion nerve cells called neuron. Each neuron has a thread like fibre extending from either end and each such fibre interconnects many of fibres of one or more other nerve cells. Along with the tiny nerve fibres sensation are conveyed to the brain, and after it reaches the brain, certain electrical impulses are caused by means of which messages are sent to the limbs and other mussels (52).

The brain gives the meaning to one's experiences initiates and regulates thoughts, emotions and actions. The brain machinery also hold mind, which is responsible for the joys, laugh, smile, love, hate, smile, dancing, singing, sorrows, grief, fear, terrors etc. Human brain not only shows evidence of electrical activity within, but also generates small currents which cause *brain waves*. The frequency of such wave is approximately10Hz (10 per second), but often vary. This variation differs from person to person, and it may be say that every individual differs in type of his brain waves. In a man whether asleep or awake, insane or insane an adult size brain generates operates 20 to 24 watts of electrical energy. The individual nerve cells are the source of the electricity, each of which is in effect of a tiny dynamo. The electrical charge generates within the cell from a chemical fuel of glucose and hydrogen and when charge exceeds certain level the cells go through discharge (53). As a rule when the stimulation is greeter (by danger, anger, emotion etc.) the greeter the rate of charging and discharging.

The nervous system, through which outward sensation carried to the brain or central nerve and conducts the response to the effectors mussels or glands is a highly equipped and compact mechanism (54). So it may clear that human body and magnets has some co-relation. Scientists and the specialists in medical science have already proved that the human body is a source of magnetic field. Different organs of the human body, such as heart, brain, nerves, muscles and tissues produces different value of magnetic fields and they differ in some of the diseases. Attempts have been made to measure the frequency of magnetic field of the different organs of the human body in different condition of physical state of body and mind. It has been observes that the magnetic fields, produced by all the organ of the body that consist or contain muscles and nerves, is of different fluctuating nature. It has found that the peak value of the fluctuating magnetic field produced by heart is greater than 106 Gauss. Some muscles when flexed produce quite high frequency magnetic fields whose peak value may be 107 Gauss. It has also been found that the strongest magnetic field from the nerve tissue is from brain which produces its largest fields of amplitude of 3 x 108 Gauss. In certain diseases (e.g. epilepsy) larger magnetic fields can be produced (54).

The presence of Sodium, Potassium, Chlorine ions that in the nerves and muscles generate in the process of contraction or signal transmission is the source of the magnetic field in human body (55). Measurement of the Magneto-cardiogram and Magneto-encephalogram for measuring the ion currents of the heart and brain respectively has led by the process of magnetic field measurement of body parts. The presence of magnetic materials in the body is another possible source of magnetic properties in the body.

There are steady magnetic fields in the body besides the fluctuating fields produces by the different organs. We know the earth's steady magnetic field is about 0.5 Gauss, so the steady fields from the body are to be measured in a steady background that is greater by five or more order of magnetic amplitude (56). The advantages of measurement of magnetic fields of heart, brain, lungs and other organs may be calculate the condition of an organ and to know any approaching malfunction of that organ. For example the magneto-cardiogram may be used to investigate heart when it is injured by shortening of blood supply of the condition like, Ischemia, Angina or Infraction.

Anything which affects the blood, either favorably or adversely is sure to have an effect on human health, either good or bad. Magnetism is a physical phenomenon as well as a phenomenon related to electricity. So it has some biological effect on human beings. The clinical studies conducted by many medical institutions have established that the magnetic flux promotes health and provides energy by eliminating disorders in the various systems of the body by stimulation of blood circulation and building new cells to rejuvenate the tissues of the body. The magnetic flux affects magnetic substances like iron and oxygen with the result that the hemoglobin in the blood vessels moves actively to effect the activated circulation (57). The treatment with magnets increases the number of new blood corpuscles in the body. As magnetic power promotes better breathing action also, it results in prevention and cure of the diseases which are connected very much with circulatory system such as bronchitis, asthma, blood pressure etc. Internal Secretion of hormones and its activity is remarkably improved by the joint effect of internal heat of the body and the external heat caused by the magnet.

The hormone secretion glands get properly warm and their function becomes active by supply of excess oxygen. So, all diseases caused by the lack of hormone secretion are favorably affected and as well as improved by the Constant use of magnets. The magnetic flux penetrates the tissues and it works to regulate hormone secretion which provides energy to normalize the functions of the internal organs. In the matter of reformation, resuscitation and promotion of the growth cells, the power of magnet observed is quite remarkable. The magnetic flux generates some comfortable warm feeling in the body which strengthens the function of cells and cures inflammation and spasms. When the magnetic flux passes through the tissue, a secondary current is created around the lines of force in the tissue cells which ionized the protoplasm and rejuvenates the tissues by activating and vitalizing the metabolism (58).

Sericulture, or silk farming, is the rearing of silkworms for the production of silk. Although there are several commercial species of silkworms, *Bombyx mori* (L) is the most widely used and intensively studied silkworm. Silk was first produced in China as early as the Neolithic period. Sericulture has become an important cottage industry in countries such as Brazil, China, France, India, Italy, Japan, Korea, and Russia. Today, China and India are the two main producers, with more than 60% of the world's annual production. The sericulture is both an art and science of raising silkworms for commercial silk. Silkworm larvae are fed with mulberry leaves, and, after the fourth moult, climb a twig placed near them and spin their silken cocoons. This process is achieved by the worm through a dense fluid secreted from its structural glands, resulting in the fiber of the cocoon (44). The silk is a continuous filament comprising fibroin protein, secreted from two salivary glands in the head of each larva, and a gum called sericin, which cements the filaments. The sericin is removed by placing the cocoons in hot water, which frees the silk filaments and readies them for reeling.

This is known as the degumming process. The immersion in hot water also kills the silkworm pupae. Single filaments are combined to form thread, which is drawn under tension through several guides and wound onto reels. The threads may be plied to form yarn. After drying, the raw silk is packed according to quality.

In order to increase in the production of quality raw silk, efforts have been made to investigate the effect of temperature [1], relative humidity [2], photoperiod [3], X-rays [4] on the silk producing potential of *B. mori*. The effect of magnetism on biological system has been the subject of world wide interest. Magnetic field influences morphological, physiological and biochemical characteristic of biological system [5]. Magnetic field affects larval behaviors of silkworm [6], hormonal level [7] and acid phosphatase activities [8] in mouse and germination of seed [9]. Its positive effects include cell viability [10], nerve regeneration [11] and bone healing in guinea pig [12]. Magnetization of eggs influences incubation period [13], silk producing potential [14] and amino acids content in the larvae of *B. mori* [15]. The present attempt has been planned to study, the influence of magnetic energy on the total protein content in the silk glands, fat bodies and haemlymph of fifth instar larvae of multivoltine crossbreed race (PM x CSR2) of silkworm, *Bombyx mori* (L).

2. MATERIAL AND METHODS

Silkworm rearing is an extensive month-long exercise starting from egg stage and terminating in adults laying eggs and dying their natural death. During this course, they pass through five larval instars intervened by four moults, cocoon and pupal stage. Silkworm rearing effectively means the culturing of five larval instars as other stages like egg, pupa and adults are non-feeding stages. Whole life cycle spans through 45-55 days with 10-12 days of egg stage, 25-30 days of larval stage, 2-3 cocoon spinning days, 5-7 days as pupal duration and 4-5 days in adult stage. Prevailing environmental conditions especially, temperature and relative humidity conditions are vital in determining silkworm physiology as it is a cold-blooded organism. Hence, maintenance of recommended temperature, relative humidity (RH), light and ventilation conditions for every stage of rearing are of utmost importance for successful silkworm rearing. Dark condition, room temperature and 65% RH is required for incubation of silkworm eggs. 27-28 °C and 80-85% RH is required for first and second instar larvae (Chawki silkworm rearing), while 24-25 °C and 60-65% RH is required for third, fourth and fifth instar larvae (late-age silkworm rearing).

During the intervening moulting stage of 24 hour each between two instars, temperature of 25-26 °C and RH 60% is recommended for the smooth integument change over. Room temperature and 65% RH is required during spinning, cocoon preservation, moth emergence, coupling, decoupling processes. Dark conditions with room temperature and 75-80% RH is required for oviposition process. A day in silkworm culture consists of various activities like harvesting of mulberry leaves, food preparation, feeding, bed cleaning etc. Silkworms are fed four times in a day – morning (9-10 A.M.), afternoon (1-2 P.M.), evening (4-5 P.M.) and night (9-10 P.M.). Leaves after harvesting from plantations are first washed with plain running water and then treated with mild $KMnO_4$ for general disinfection. After adequate drying they are fed to silkworms. During first and second instars, silkworms are fed with chopped tender and succulent mulberry leaves with high moisture content from apical portions of the plant.

During third instar, 3-4 pieces of medium sized leaves are given to the silkworms. Later on, the entire leaf and complete shoot is given during fourth and fifth instar after required treatment. Bed cleaning is an important process to ensure the hygiene in the immediate vicinity of silkworms in order to protect them from infection. Four different mesh-sized bed cleaning nets are used for cleaning the rearing beds. Bed cleaning is done once in first instar, twice in second instar and preferably daily in third, fourth and fifth instar. Bed cleaning nets are spread just before the morning feed. Before the afternoon feed, nets with the silkworms are shifted to new beds and feeding is then resumed.

The litter, leftover food and dead silkworm, if any, are removed carefully and disposed of away from the rearing house. Moulting is the process when silkworms seized feeding, becomes immobile and prepare themselves for shedding their old skin to accommodate the fast growth. Four moults takes place during the entire larval period. During this period, special care is required for the moulting worms. Lime powder is dusted in the rearing bed to reduce the humidity to 60-65% RH to facilitate the moulting process. Moulting period lasts for about 24 hrs and care should be taken not to disturb silkworms during this period. During late fifth instar, after completing the feeding silkworms reaches the ripened stage (ready – to-spin silk). Ripened silkworms are identified by their characteristics movement to the corners of the rearing treys, reduction in size by one-third and transparent yellow appearance. These ripened silkworms are transferred to the mountages (equipment to provide support for cocoon formation) for spinning cocoons.

Plastic collapsible mountage, bamboo-brush mountage and mountage made out of locally available materials (dried leaves and branches of different plants arranged in a zig-zag manner in card-board boxes). After two-three days of spinning, cocoons are harvested from the mountages. Cocoons can be used for either propagating the generation or extraction of silk fibre. For propagating generation, cocoons are left at the room temperature and 65-70 % RH for moths to emerge from cocoons after passing through intermediate pupal stage after 6-7 days. After emergence, males and females are coupled for four-five hours, decoupled, and females are kept for oviposition process. Males can be used for second coupling after short-term refrigeration at 5 °C for 1-2 days. After this, the adult approaches their natural deaths in 1-2 days. The silk worm larvae hatched out of oviposited eggs in 10-12 days after completing embryonic growth. For extracting silk, the cocoons are subjected to the stifling process in which, the pupa inside the cocoons are killed by subjecting them to high temperature treatment via. sun drying, steam or hot air in order to maintain the continuity of silk filament making up the cocoon. Then, cocoon is boiled or cooked for 3-4 mins at 95-96 °C to make the serecin soft to dissolve upto 25-26%. Then, silk filament can be extracted out easily on suitable reeling apparatus by finding the true end in brushing process in which the coarser floss layer is removed.

The Disease Free Laying (DFLs) of multivoltine crossbreed race (PM x CSR2) of silkworm, *Bombyx mori* (L) were procured from government office of Sericulture at Malegaon Sheti Farm of Agricultural Development Trust, Baramati (India). They were processed for incubation at 26±1°C temperature and 75±5% relative humidity in plywood trays (23X20X5 cm) in BOD incubator under the ideal rearing conditions [16] in the Dr. APIS laboratory, at Shrikrupa Residence, Teacher Society, Malegaon colony, Baramati (India). The hatched larvae were reared through the standard methods through the use of fresh leaves of mulberry, *Morus alba* (L). Soon after the fourth moult, the fifth instar larvae were grouped into one control group and four experimental groups (1000, 2000, 3000 and 4000 Gauss

magnetic field), each with hundred individuals. The fifth instar larvae were magnetized in bulk, by keeping them in plastic container (perforated) and suspending in between two pole of an axial electromagnet. The desired field strength was developed by adjustment of electric power supply, which is regulated by electric power regulator. Magnetic field strength was measured by a digital gausso meter. The magnetic exposure was carried for the first four days of fifth instar larvae, daily before the first feeding. The larvae of first (I) experimental group were magnetized in 1000 Gauss magnetic field for half an hour. The larvae of second (II) experimental group were magnetized in 2000 Gauss magnetic field for half an hour. The larvae of third (III) experimental group were magnetized in 3000 Gauss magnetic field for half an hour. And the larvae of fourth (V) experimental group were magnetized in 4000 Gauss magnetic field for half an hour. After magnetization, the larvae were reared in BOD incubator maintain at optimum rearing conditions as control group study. The bioassay of total proteins was carried out on fifth day.

Bioassay of Total Proteins: The total protein content in the silk glands, fat bodies and haemolymph of larvae was studied according to Lowry *et al.* [17] and modified by Singh and Agarwal [18]. For estimation of protein content 1.0 gm silk gland, 0.1 gm fat body and 0.5 ml hamolymph from fifth instar larvae were taken out. The tissues were homogenated separately in 4.0 ml of 10% TCA and centrifuged at 20,000 rpm for 10 minutes. The supernatant was discarded and precipitate was washed with 5% TCA and centrifuged for 10 minutes. It was rewashed with 10% TCA, centrifuged and supernatant discarded. The precipitate was dissolve in 4.0 ml of 10N NaOH. In 1.0 ml diluted solution, 0.5 ml freshly

prepared alkaline copper solution was added. The reaction mixture was kept for 10 minutes at room temperature, then 0.5 ml of folin ciocalteu reagent was added and mixed thoroughly. After 30 minutes blue colour developed which was measured at 600 nm. Six replicates of each experiment were made. Standard curve was prepared with different concentration of Bovine Serum Albumen (BSA).

For the purpose to obtain consistency in the results, whole experimentation was repeated for three times. Data obtained was subjected for statistical analysis. To test significance of data, analysis of variance [19] was performed. The regression and correlation coefficient of data was also determined for interrelationship [20].

3. RESULTS AND DISCUSSION

The results of the attempt are summarized in table-1. Changes in the strength of magnetic field from 1000 to 4000 Gauss exerted considerable change to the total protein content in the silk glands of fifth instar larvae of silkworm, *Bombyx mori* (L) (Race: PM x CSR2). The total protein content in the silk glands was found increased with increase in the strength of magnetic field from 1000 to 3000. Total protein content of silk glands untreated control larvae was 22.807 units. Magnetization of the larvae with 1000; 2000; 3000 Gauss was found resulted into 24.153 (±0.697); 25.213 (±0.637) and 26.794 (±0.716) units respectively. The larvae of 4000 Gauss magnetic field group enrolled 26.786 (±0.629) units of silk glands proteins. The maximum level of total protein content in the silk gland of *B. mori* was recorded (26.794 ±0.716 μg/mg) in case of 3000 Gauss magnetic exposure of larvae, while minimum level of protein content (24.153 ±0.697μg/mg) in silk gland was recorded for control study.

Table 1. Influence of magnetic energy on total protein contents in the fifth instar larvae of silkworm, *Bombyx mori* (L) (Race: PM x CSR2).

Group Tissue	Control Magnetic Energy (00 Guass)	(I) Magnetic Energy (1000 Guass)	(II) Magnetic Energy (2000 Guass)	(III) Magnetic Energy (3000 Guass)	(IV) Magnetic Energy (4000 Guass)
Silk Glands	22.807 (±0.443)	24.153 (±0.697) 5.901	25.213 (±0.637) 10.549	26.794 (±0.716) 17.481	26.786 (±0.629) 17.446
Fat Bodies	14.945 (±0.783)	17.635 (±0.921) 17.999	20.512 (±0.926) 37.249	21.897 (±0.953) 46.517	21.891 (±0.971) 46.477
Haemolymph	13.561 (±0.684)	15.813 (±0.595) 16.606	17.371 (±0.784) 28.095	18.041 (±0.826) 33.035	18.116 (±0.789) 33.588

F2-ratio = 11.43* n2 = 2 *P1 < 0.01 *P2 < 0.01
-Each Figure is the mean of three replications
-Figures in parentheses with ± signs are standard deviations

The two way ANOVA shows that variation in the strength of magnetic field significantly ($P < 0.01$) influenced to the total protein content in the silk gland of fifth instar larvae of *B. mori*. The regression in between independent variable [X] (magnetic power) and dependent variable [Y] (total protein content in the silk gland) shows positive correlation with mathematical equation: $Y1 = 25.936+0.000286X$ and $r1 = 0.30871$. In statistics, linear regression is an approach for modeling the relationship between a scalar dependent variable y and one or more explanatory variables (or independent variables) denoted X. The case of one explanatory variable is called simple linear regression. For more than one explanatory variable, the process is called multiple linear regression.[1] (This term should be distinguished from multivariate linear regression, where multiple correlated dependent variables are predicted, rather than a single scalar variable.) [2]. In linear regression, the relationships are modeled using linear predictor functions whose unknown model parameters are estimated from the data. Such models are called linear models [3]. Most commonly, the conditional mean of y given the value of X is assumed to be an affine function of X; less commonly, the median or some other quantile of the conditional distribution of y given X is expressed as a linear function of X. Like all forms of regression analysis, linear regression focuses on the conditional probability distribution of given X, rather than on the joint probability distribution of y and X, which is the domain of multivariate analysis.

The contents of total proteins of fat bodies of fifth instar larvae of untreated group were found measured 14.945 (±0.783) units. Treating the larvae with magnetic exposure of 1000; 2000 and 3000 units was found effected into the fat bodies total proteins of 17.635 (±0.921); 20.512 (±0.926) and 21.897 (±0.953) units. The 4000 Gauss magnetic field have had

sustained (21.891 ±0.971) influence on the fat body total proteins. Regression in between independent variable [X] i.e. strength of magnetic power and dependent variable [Y] i.e. total protein content in the fat body of fifth instar larvae shows positive correlation i.e. Y2 = 17.2452 + 0.0005578X and r2 = 0.4186226.

The total protein contents of the haemolymph of fifth instar larvae was effected due to the variation in the strength of magnetic field for exposure of fifth instar *B. mori* larvae (Table 1). The total protein content was increased slowly from 1000 to 4000 Gauss of magnetic energy exposure in comparision with untreated control group larvae. The increase in the total protein contents of haemolymph was from 16.606 to 33.588 percent. Regression in between independent variable [X] (magnetic power) and dependent variable [Y] (total protein content) in the haemolymph fifth instar larvae shows positive correlation i.e. Y3 = 16.482 + 0.0019558X and r3 = 0.275391. Linear regression finds application in a wide range of biological studies.

The total protein contents in the silk glands of fifth instar *B. mori* larvae in the present attempt exhibited increasing trend due to increase in the strength of magnetic field for magnetization of first instar larvae from 1000 Gauss to 3000 Gauss whereas, in 4000 Gauss magnetic field, the total protein content in the silk gland is decreased. The most rapid protein metabolism was earlier reported in the silk glands of silkworm [21]. Thirty per cent of the silk protein was derived from the free amino acids and protein of the haemolymph while the rest was synthesized by the salivary gland during the spinning process [22]. T

he starvation caused inhibition of protein synthesis in *B. mori* [23] whereas, silk glands start synthesis of silk protein at 10 days of embryonic life which continue till the beginning of the spinning [23]. The exposure of biological system to different magnetic field is known to induce the biochemical changes [24], with an increase in the protein metabolism and utilization of mulberry leaves [25], 2-3% in forth and 97-98% in fifth instar larvae assimilated protein was utilized in silk protein synthesis [26].

Magnetization of larvae in 3500 Gauss caused an increase in protein content in the tissues [27] and magnetization of eggs influenced to the amino acids content in the silk gland of *B. mori* [15]. The total protein content in the fat body of fifth instar *B. mori* larvae is increased up to 3000 Gauss magnetic exposure of first instar larvae but in 4000 Gauss, the protein content was decreased. The fat body plays important role in the synthesis of protein [28-30]. Some of the protein in the fat body originally synthesizes their fat body of fifth instar larvae of *B. mori* [31,32] while, proteinous sphere were reported in the fat body of honey bee larvae [33].

In pupal stage of grain moth, *Sitotroga cerealella*, four major proteins were lost from the haemolymph and sequenced by the fat body [34,35] and magnetization of eggs influenced to the amino acids content in the fat body of *B. mori* [15]. In the haemolymph of fifth instar larvae of *B. mori* the level of total protein content is influenced due to the variation of the magnetic field and it is maximum in 3000 Gauss magnetic field. A decreased protein level in the haemolymph is recorded in *Rhodnius prolixus* at high temperature regime [36] and the level of protein content in the haemolymph at the end of the last instar was attributed to the increase in protein biosynthetic rate in the fat body of *Galleria mellonella* [37].

The variation of protein and related component in the haemolymph during insect development is directly related to spinning process [38, 39] and the synthesis of protein and kinetics of certain enzyme are known to associate with the synthesis and degradation of amino acids and protein [40].

The application of magnetic field caused increase in enzyme activities in the biological system [41] whereas, magnetization of eggs influenced to the amino acids content in the haemolymph of *B. mori* [15]. How exactly magnetic field influences to biological system is not cleared and efforts are being made in the direction but it may concluded on the basis of literatures available that magnetization of larvae in the low magnetic field may caused increase in metabolic activities due to activation of some enzymes and cytochrome as a result more and more food is consumed by larvae, resulting increased cellular activity in the silk gland, fat body and haemolymph thus, protein content in the tissues increased whereas, high strength of magnetic field caused stress responses.

4. CONCLUSIONS

The exposure of fifth instar larvae of multivoltine crossbreed race (PM x CSR2) of silkworm, *Bombyx mori* (L) to the magnetic energy of various strengths (1000, 2000, 3000 and 4000 Gauss magnetic field) exert increasing influence on the contents of total proteins of silk glands, fat bodies and haemolymph. The total protein content was increased with increase in the strength of magnetic field from 1000 to 4000 Gauss magnetic field. The larvae magnetized with 4000 Gauss magnetic field were found with sustained or slightly decreased in total protein contents. Silk gland total proteins were increased from 5.901 to 17.481 percent. Total proteins of fat bodies were increased from 18 to 46.517 percent. And the total proteins of haemolymph were increased from 16.606 to 33.588 percent. Magnetization may have had influence on the increase in the levels of amino acids followed by accelerated rate of protein synthesis in the fifth instar larvae of silkworm, *Bombyx mori* (L) (Race: PM x CSR2). Magnetic energy should be utilized as efficiently as possible for the progression of growth of larval instars of silkworm, *Bombyx mori* (L).

The variation of protein and related component in the haemolymph during insect development is directly related to spinning process and the synthesis of protein and kinetics of certain enzyme are known to associate with the synthesis and degradation of amino acids and protein. The application of magnetic field caused increase in enzyme activities in the silkworm, *Bombyx mori* (L).

ACKNOWLEDGEMENTS

The academic support and inspiration received from Dr. Tomasz Borowski (Editor-in-Chief *World Scientific News*) deserve appreciations & exert salutary influence. The work had been supported by Agricultural Development Trust, Baramati (India); International Foundation for Environment and Ecology; 42, Station Road, Rahara, Kolkata - 700118, West Bengal, INDIA and International Science Congress Association, Indore (India) through Hon. Dr. Deepak A. Sharma and it's dignitaries.

References

[1] Verma, A.N. and A.S. Atwal, 1968. Effect of temperature and food on the development and silk production of *Bombyx mori*. L. *Beitr. Entomol.*, 18(1&2): 249-258.

[2] Upadhyay, V.B. and A.B. Mishra, 2002. Influence of relative humidity on the nutritive potential of mulberry silkworm *Bombyx mori* L. larvae. J. *Adv. Zool.*, 23(1): 54-58.

[3] Jolly, M.S., S.S. Sinha and J.L. Razdan, 1971. Influence of temperature and photoperiod on the termination of pupal diapouse in taser silkworm, *Anthearea mylita*. *Indian J. Insect Physiol*., 17: 743-760.

[4] Kanarev, G. and G.T. Cham, 1985. Effect of laser irradiation of silkworm eggs on development and productivities. *Zhiotnov and Nauki*, 229: 47-53.

[5] Patnev, T.P. and M.I. Mankova, 1986. Direct and indirect effect of a constant magnetic field on biological objects. *Kosm. Biol. Aviakosm. Med.*, 20: 73-76.

[6] Chaugale, A.K., 1993. Effect of magnetic energy on silkworm development and silk production. Ph.D. Thesis, Shivaji University, Kohlapur, India.

[7] Udinsteve, N.A. and V.A. Moraz, 1982. Function of hypophysial adrenal system under the effect of power frequency variable magnetic field of different recime. *Gig. Tr. Prof. Zobl.*, 12: 54-66.

[8] Conely, C.C., W.J. Mills and A.G. Patricia, 1966. Enzymes activities in macrophages from animals exposed to a very low magnetic field. III International Biomagnetic Symposium, University of Illinosis, Chicago, pp: 13-15.

[9] Pittman, U.J., 1965. Magnetism and plant growth III. Effect on germination and early growth of corns and beans. *Canadian Journal of Plant Sci.*, 45: 549-555.

[10] Ferment, J., 1994. Effect of high magnetic field on biological reactions. *Bioeng.*, 77: 453-456.

[11] Byers, G.M., K.F. Clark and G.C. Thompson, 1998. Effect of pulsed electro magnetic stimulation on the facial nerve regeneration. *Archive of Otolaryngology Head and Neck Surgery*, 124(4): 383-389.

[12] Darendetiler, M.A., A. Darendetiler and P.M. Sinclair, 1997. Effect of static and pulsed magnetic field on bone healing. *International J. Orthodont, Orthognath Surgery,* 12(1): 43-53.

[13] Tripathi, S.K. and V.B. Upadhyay, 2005. Magnetization of eggs influences the incubation period of multivoltine mulberry silkworm (*Bombyx mori* Linn.) Eggs. *J. Adv. Zool.*, 26(1): 24-28.

[14] Upadhyay, V.B. and S.K. Tripathi, 2006. Effect of the magnetization of eggs on the silk producing potential of multivoltine mulberry silkworm (*Bombyx mori* Linn.). *Sericologia*, 46(3): 269-278.

[15] Tripathi, S.K., S.K. Shukla and V.B. Upadhyay, 2012. Impact of magnetization of eggs on the free amino acids content in the silk gland, fat body and haemolymph of *Bombyx mori* var. nistary larvae. *World J. Zool.*, 7(1): 47-54.

[16] Krishnaswami, S., M.N. Narsimhanna, S.K. Suryanarayan and R. Kumar, 1973. Sericulture manual-2. Silkworm rearing. F.A.O. Agric. Seroes. *Bull Rome.*, 15(2): 1-131.

[17] Lowry, O.H., N.J. Rosenbrought, A.L. Farr and R.J. Randall, 1951. Protein measurement with the folin phenol reagent. *J. Biol. Chem.,* 193: 265-275.

[18] Singh, D.K. and R.A. Agarwal, 1989. Toxicity of piperanyl butaxide carbonyl synergism on the snail *Lymnaea accuminata, International Review Dergesamtem Hydrobiologic,* 74: 689-699.

[19] Sokal R.R. and F.J. Rohlf, 1973. Introduction to Biostatistics. W.H. Freeman and co, San Franscisco CA, USA, pp. 185-207.

[20] Arora, P.N. and P.K. Malhan, 2004. Biostatistics. Himalaya Publishing House, Girgaon, Mumbai, pp: 137-166.

[21] Fllipovich, Y.B. and S.M. Klunova, 1990. Relationship between the dynamic of free amino acids and the biosynthesis of silk protein in the silk gland of *Bombyx mori* L. *Obsch. Biol.,* 28(6): 715-723.

[22] Terra, W.R., A.G. De Blanchi, A.G. Gambrini and F.J.S. Larra, 1973. Haemolymph amino acids and related compounds during cocoon production by the larvae of fly, *Rhynchosciara americana*. *J. Insect Physiol.,* 19(11): 2097-2106.

[23] Prudhomme, J.C., P. Cauble, J.P. Garel and J. Dallie, 1985. Silk synthesis. In comparative *Insect Physiology, Biochemistry and Pharmacol.,* pp. 571-594.

[24] Pittman, U.J. and D.P. Ormord, 1970. Physiological and chemical features of magnetically treated winter seeds and resulting seedling. *Can. J. Plant Sci.,* 50: 211-217.

[25] Ishaaya, I., I. Moore and D. Joseph, 1971. Protease and amylase in larvae of the Egyptian cotton worm, *Spodoptera litoralis*. *J. Insect Physiol.,* 17: 945-953.

[26] Suzuki, Y., 1977. Differentiation of silk gland: A model system for the differential gene action. In: Result and problems in cell differentiation. W.B. Beermann, (Ed.), Springer-Verlag, Berlin.

[27] Chaugale, A.K. and N.K. More, 1992. Effect of magnetization on the developmental period and cocoon characters of the silkworm, *Bombyx mori*. *Indian J. Seric.,* 31(2): 115-122.

[28] Coupland, R.E., 1957. Observation on the normal histology and histochemistry of the fat body of locust (*Schistocerca gregaria*). *J. Exp. Biol.,* 34: 206-290.

[29] Wigglesworth, V.B., 1965. The principle of Insect Physiology, Chapman and Hall. Londan., pp. 663-690.

[30] Chen, P.S., 1972. Advances in Insect Physiology. Academic Press, Londan, 3: 53.

[31] Shigematsu, H., 1960. Protein metabolism in fat body of silkworm *Bombyx mori* L. *Bull. Seric. Exp. Sta. Japan,* 16: 141-170.

[32] Price, G.M., 1969. Protein synthesis and nucleic acid metabolism in the fat body of larvae blowfly. *J. Insect Physiol.,* 15: 931-944.

[33] Octel, R., 1930. The synthesis of different constituents in Cercopia silkworm. *J. Insect Physiol.,* 17: 677-689.

[34] Chippendale, G.M., 1971. Metamorphic changes in fat body protein of Southwestern corn borer, *Diatraea grandiosella*. *J. Insect Physiol.,* 16: 1057-1068.

[35] Patel, H., 1972. Concept of temperature adaptation of unchanging reaction of cold blooded animal. In Procer, C.L. ed. Physiological adaptation. *Amer. Phsiol. Soc.*, pp. 50-78.

[36] Okasha, A.Y.K., 1964. Effect of high temperature on the *Rhodinus prolixus* (Stad.). Nature London, 204: 1221-1222.

[37] Collins, J.V. and A.E.R. Downe, 1970. Selective accumulation of haemolymph protein by the fat body of *Galleria mellonella*. *J. Insect Physiol.,* 16(9): 1697-1708.

[38] Beament, J.W.L, J.E. Trehern and V.B. Wigglesworth, 1995. Amino acid and protein metabolism. In *Advances in Insect Physiol.,* 3: 84-86.

[39] Terra, W.R., C. Ferreora and A.G. De Balanchi, 1975. Distribution of nutrients reserves during spinning of larvae of fly *Rhynchosciara americana*. *J. Insect Physiol.,* 21(8): 1501-1510.

[40] Digley, F. and J.M. Smith, 1969. Temperature acclimation in *Drosophila melanogaster*. *J. Insect Physiol.,* 14: 1185-1194.

[41] Young, W., 1969. Magnetic field and in-situ acetycholine esterase in the vagal heart system, 79-102. In Biological effect of magnetic field (Ed. Barnothyet) and Plenum Press.

[42] Vitthalrao B. Khyade; Patil, S.B; Khyade,, S.V and Bhavane, G.P (2002). Influence of acetone maceratives of Vitisvinifera on the larval parameters of silkworm *Bombyxmori* (L). *Indian journal of comparative animal physiology* 20: 14-18.

[43] Vitthalrao B. Khyade; Patil, S.B; Khyade, S.V and Bhavane, G.P (2003). Influence of acetone macerative of *Vitisvinifera* on the economic parameters of *silkworm Bombyx mori* (L). *Indian journal of comparative animal physiology.* 21: 28-32.

[44] Vitthalrao B. Khyade and Jiwan P. Sarwade (2013). Utilization of Retinol through the topical application to the fifth instar larvae of the silkworm, Bombyx mori (L) (Race : PM x CSR2) for qualitative improvement of the economic parameters. International *Journal of Advanced Life Sciences* 6 (5): 532-537.

[45] Vitthalrao B. Khyade; Karel Slama; Rajendra D. Pawar and Sanjay V. Deshmukh (2015). Influence of Various Concentrations of Acetone Solution of Retinol on Pattern of Chitin Deposition in the Integument of Fifth Instar Larvae of Silkworm, Bombyx Mori (L) (Pm X Csr2). *Journal of Applicable Chemistry* (4)15: 1434-1445. www.joac.info

[46] Gajanan B. Zore; Archana D. Thakre; Sitaram Jadhavand S. Mohan Karuppayil (2011). Terpenoids inhibit Candida albicans (L) growth by affecting membrane integrity and arrest cell cycle. *Phytomedicine* 18: 1181-1190.

[47] Vitthalrao B. Khyade and Anil N. Shendge (2012). Influence of *Aloe vera* (L) herbal formulation on the larval characters and economic parameters of silkworm, *Bombyx mori* (L) (Race : PM x CSR2). The Ecoscan Special Issue, 1: 321-326. http://theecoscan.in/journalpdf/spl2012_v1-55%20b.%20vitthalrao%20khyade.pdf

[48] Sucheta S. Doshi; Anil N. Shendage and Vitthalrao B. Khyade (2016). The monoterpene compounds for juvenile hormone activity through changes in pattern of chitin deposition in the integument of fifth instar larvae of silkworm, Bombyx mori (L) (PM x CSR2) *World Scientific News* 37 (2016) 179-201.

[49] Vitthalrao B. Khyade and Atharv Atul Gosavi (2016).Utilization of mulberry leaves treated with seed powder cowpea, *Vigna unguiculata* (L) for feeding the fifth instar larvae of silkworm, *Bombyx mori* (L) (Race: PM x CSR2). *World Scientific News* 40 (2016) 147-162.

[50] Khyade, V. B. (2004). Influence of juvenoids on silk worm, Bombyx mori (L). Ph.D. Thesis, Shivaji University, Kolhapur, India

[51] Khyade, V. B. and Ganga V. Mhamane (2005). Vividh Vanaspati Arkancha Tuti Reshim Kitak Sangopanasathi Upyojana. *Krishi Vdnyan.* 4: 18-22

[52] Khyade, V. B.; Poonam B. Patil; M. Jaybhay; Rasika R. Gaikwad; Ghantaloo,U. S. ;Vandana D. Shinde; Kavita H. Nimbalkar and Sarwade, J. P. (2007). Use of digoxin for improvement of economic parameters in silk worm, Bombyx mori (L).Bioinfrmatics (Zoological Society of India) www.abebooks.com/Bioinformatics-Pandey-Sadhana-Pande. Parent, A; Carpenter MB (1995). "Ch. 1". Carpenter's Human Neuroanatomy. Williams & Wilkins. ISBN 978-0-683-06752-1.

[53] Kristin L. Bigos, Ahmad R. Hariri, Daniel R. Weinberger (2015). Neuroimaging Genetics: Principles and Practices. Oxford University Press. p. 157. ISBN 0199920222. Retrieved January 2, 2016.

[54] Cosgrove, KP; Mazure CM; Staley JK (2007). "Evolving knowledge of sex differences in brain structure, function, and chemistry". *Biol Psychiat* 62 (8): 847-855. doi:10.1016/j.biopsych.2007.03.001. PMC 2711771. PMID 17544382.

[55] Azevedo, F.A.C., Carvalho, L.R.B., Grinberg, L.T., Farfel, J.M., Ferretti, R.E.L., Leite, R.E.P., Filho, W.J., Lent, R., Herculano-Houzel, S. (2009). "Equal numbers of neuronal and nonneuronal cells make the human brain an isometrically scaled-up primate brain." *Journal of Comparative Neurology* 513(5): 532-541. doi:10.1002/cne.21974.PMID 19226510.

[56] Kandel, ER; Schwartz JH; Jessel TM (2000). Principles of Neural Science. McGraw-Hill Professional. p. 324. ISBN 978-0-8385-7701-1.

[57] Jones R (2012). "Neurogenetics: What makes a human brain?". *Nature Reviews Neuroscience* 13(10): 655. doi:10.1038/nrn3355. PMID 22992645.

[58] From the National Library of Medicine's Visible Human Project. In this project, two human cadavers (from a man and a woman) were frozen and then sliced into thin sections, which were individually photographed and digitized. The slice here is taken from a small distance below the top of the brain, and shows the cerebral cortex (the convoluted cellular layer on the outside) and the underlying white matter, which consists of myelinated fiber tracts traveling to and from the cerebral cortex.

[59] Swaminathan, Nikhil (29 April 2008). "Why Does the Brain Need So Much Power?". Scientific American. Scientific American, a Division of Nature America, Inc. Retrieved 19 November 2010.

[60] Quistorff, Bjørn; Secher, Niels; Van Lieshout, Johanne (July 24, 2008). "Lactate fuels the human brain during exercise". *The FASEB Journal* 22(10): 3443-3449. doi:10.1096/fj.08-106104. Retrieved May 9, 2011.

[61] Obel, LF; Müller, MS; Walls, AB; Sickmann, HM; Bak, LK; Waagepetersen, HS; Schousboe, A (2012). "Brain glycogen-new perspectives on its metabolic function and regulation at the subcellular level.". *Frontiers in neuroenergetics* 4: 3. doi:10.3389/fnene.2012.00003. PMC 3291878. PMID 22403540.

[62] Clark, DD; Sokoloff L (1999). Siegel GJ, Agranoff BW, Albers RW, Fisher SK, Uhler MD, eds. Basic Neurochemistry: Molecular, Cellular and Medical Aspects. Philadelphia: Lippincott. pp. 637-670. ISBN 978-0-397-51820-3.

[63] Dorland's (2012). Dorland's Illustrated Medical Dictionary (32nd ed.). Elsevier. p. 784. ISBN 978-1-4160-6257-8.

[64] Easton, J. D. Albers; et al. (2009). "Definition and evaluation of transient ischemic attack: a scientific statement for healthcare professionals from the American Heart Association/American Stroke Association Stroke Council; Council on Cardiovascular Surgery and Anesthesia; Council on Cardiovascular Radiology and Intervention; Council on Cardiovascular Nursing; and the Interdisciplinary Council on Peripheral Vascular Disease. The American Academy of Neurology affirms the value of this statement as an educational tool for neurologists". *Stroke* 40(6): 2276 2293. doi:10.1161/STROKEAHA.108.192218.PMID 19423857.

(Received 03 February 2016; accepted 16 February 2016)