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Effects of Antioxidant food Supplement on aging in *Bombyx mori*

Amritha Sudhakaran, Sumisha Kunnathuparambil and Chempakassery Devasia Sebastian*
Dept. of Zoology, Calicut University P.O, University of Calicut, Malappuram, Kerala, INDIA

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Abstract

Aging is the accumulation of deleterious changes occurring in an organism over time. It causes as a result of free radical deposition in the body which is fought out by the antioxidants. Present study mainly focuses on the dietary supplementation of antioxidants, ascorbic acid and tyrosine in the aging of Bombyx mori larvae. Total protein, hydrogen peroxide and catalase activity of haemolymph and fat body have been estimated at time interval of 24 hrs for a period of 4 days. The results obtained were statistically analysed. It was found that ascorbic acid and tyrosine acted as a potential dietary antioxidant supplement which caused significant changes in the organism's body, by scavenging the free radicals formed in the body as a result of metabolic reactions.

Keywords: *Bombyx mori,* tyrosine, ascorbic acid, aging, protein, catalase.

Introduction

Aging is defined as the progressive loss of functioning by decreasing fertility, increasing mortality and disability. It happens as the result of accumulation of free radicals which is oxygen derived molecules produced during the oxidation of food molecules. Free radicals when initiated propagate by involving in chain reactions with other less reactive types. The resulting chain reaction compounds accumulate in the body and thus increase the potential for cellular damage. Feeding of exogenous anti-oxidants increases the lifespan of organisms by decreasing age-independent susceptibility to death. To fight against toxic free radicals and other reactive oxygen species (ROS), cells have developed antioxidant defence system consisting of the enzymes super oxide dismutase (SOD), and glutathione peroxidase, and small molecules like glutathione[1]. These enzymes destroy toxic peroxides.

The purpose of antioxidants is to prevent ROS concentrations from reaching a higher level in a cell that cause damage. Antioxidants offer resistance against oxidative stress by scavenging free radicals, inhibiting lipid peroxidation etc[2]. Studies regarding the catalase activity showed that it was widely distributed in various tissues of *Bombyx mori* larvae. They also detected a relatively high activity in the fat body[3]. Fatbody plays a physiological role like that of the liver in mammals[4]. Antioxidant enzymes and small molecular weight antioxidants perform an effective response against oxidants in insects. Due to lack of glutathione peroxidase activity in insects, catalase activity provides the sole enzymatic mechanism for removal of H_2O_2[5,6].

Experimental aging studies have been done in various groups of animals such as insects, nematodes, rodents *etc*. accepting the universality of aging process in multicellular animals. Among vitamins, vitamin C is the more effective antioxidant and has got many protective effects, but it cannot be stored in the body so that must be taken frequently[7,8]. Attempts have been made in sericulture with nutrients such as proteins, carbohydrates, amino acids, vitamins, hormones, antibiotics etc for better performance and it succeeded getting high yield and quality cocoons. Silkworm fed on mulberry leaves supplemented with nitrogen and ascorbic acid showed higher growth rate and lower mortality[9]. The effect of vitamin supplementation on growth of silkworm has been investigated by many researchers[10,11]. It was concluded that ascorbic acid is an indispensable vitamin in the diet of silkworm and other polyphagous insects[12,13].

Material and Methods

Bivoltine silkworm hybrid, *Bombyx mori* Elite –CSR 2X4 was used for the present study. The newly hatched larvae of silkworm were reared in plastic rearing trays in the laboratory with proper hygienic condition[14]. Larvae were fed with the leaves of mulberry plant, *Morus alba*. The temperature in the rearing room was maintained at 27 ± 1ºC and humidity between 70-80%. Soon after the fourth moult the larvae were segregated into two groups in different trays as control and experimental. The control group was maintained with normal leaves while experimental were fed with ascorbic acid (10mM solution) treated leaves. In both case feeding were done five times in a day. For various analyses, haemolymph and fat body samples were extracted from appropriated number of normal and treated larvae separately, after anaesthetized slowly with diethyl ether.

The haemolymph was extracted from thoracic leg by amputation and fat body was dissected out in ice cold insect ringer and homogenized in a tissue homogenizer[15]. The analyses were carried out at 24h interval during the fifth instar period, ie., 0 hr, 24 hr, 48 hr, 72 hr and 96 hr.

Estimation of protein was done as per Lowry *et al*. by using crystalline bovine serum albumin (fraction V, Sigma) as standard[16]. Estimation of hydrogen peroxide was done based on the formation of soluble coloured peroxi titanium complex in a reaction of hydrogen peroxide with potassium titanium oxalate[17]. The determination of catalase activity was based on estimating the amount of residual hydrogen peroxide in the assay mixture after incubation of a known amount of hydrogen peroxide with the enzyme extract for a fixed time interval[17]. The values obtained were statistically analyzed for their significance in variation between normal and experimental larvae.

Results and Discussion

Total protein in the haemolymph (figure 1a) and fat body (figure 1b) of the normal and treated larvae showed a sharp increase throughout the experimental period. The variation was highly significant when 48h, 72h and 96h larvae were considered.

The H_2O_2 (figure 2a and 2b) and catalase activity (figure 3a and 3b) levels in the haemolymph and fat body of the normal and treated larvae showed a gradual increase throughout the experimental period. The H_2O_2 levels in the treated larvae showed a reduced rate of increase when compared to normal. The difference was significant when the levels at 96h while highly significant at 48h and 72h. The levels in the tyrosine treated larvae showed a reduced rate of activity when compared to normal. The difference was significant when the levels at 48h and 96h while very significant at 72h.

The present study was conducted to evaluate the antioxidant effect of ascorbic acid (vitamin C) and tyrosine on total protein, hydrogen peroxide and catalase activity both in haemolymph and fat body of *Bombyx mori* larvae during its final larval instar. It is well established that ascorbic acid is a non-enzymatic antioxidant and tyrosine has proven effect as an antioxidant.

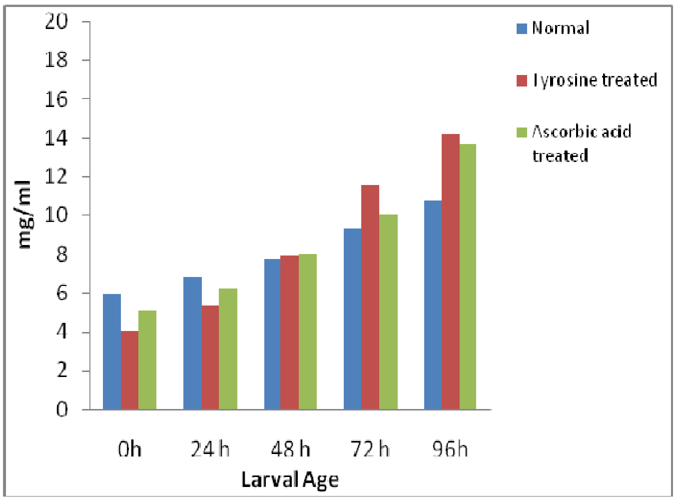

Figure-1a
Total protein in the haemolymph of *B. mori* larvae

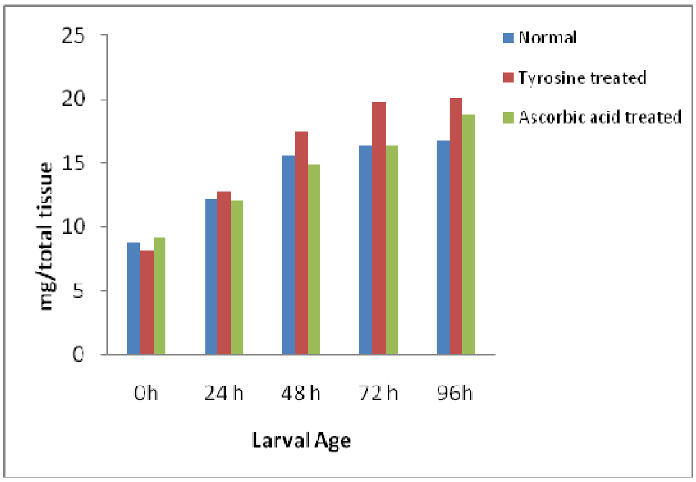

Figure-1b
Total protein in the fat body of *B. mori* larvae

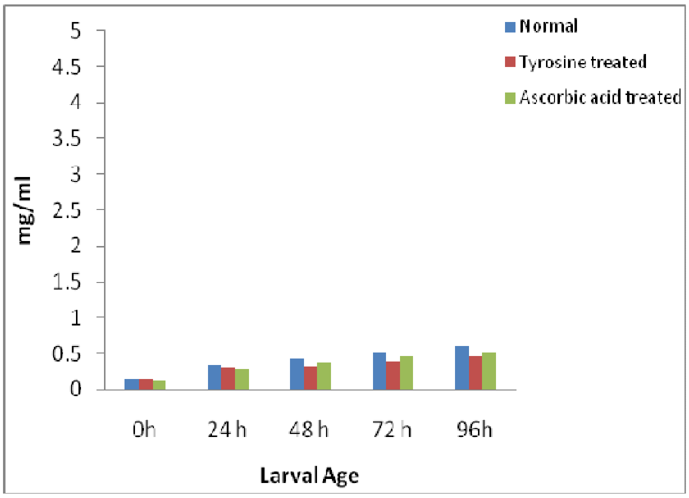

Figure-2a
Hydrogen peroxide activity in the haemolymph of *B. mori* larvae

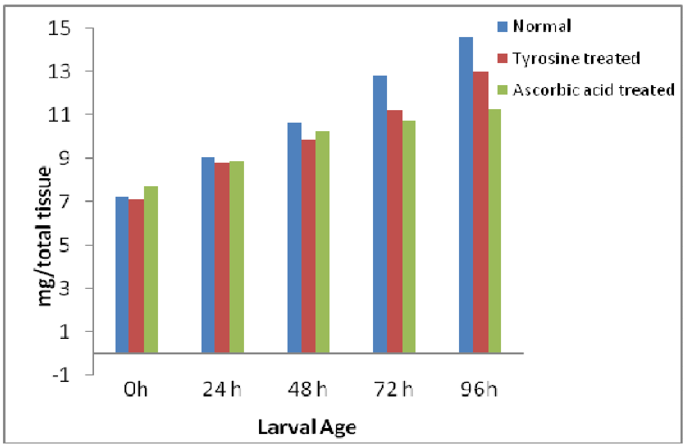

Figure-2b
Hydrogen peroxide activity in the fat body of *B. mori* larvae

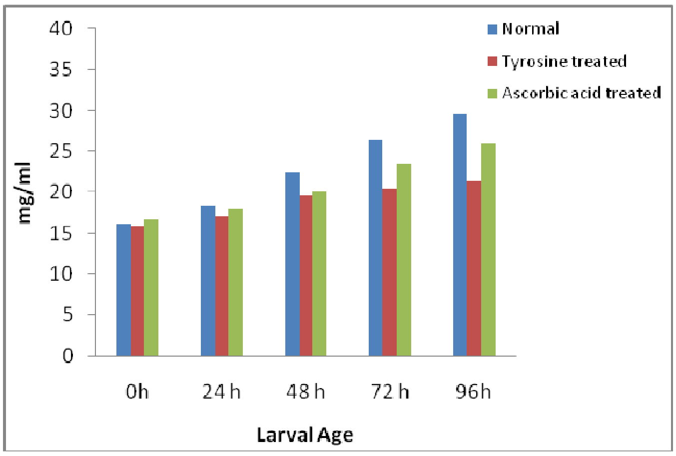

Figure-3a
Catalase activity in the haemolymph of *B. mori* larvae

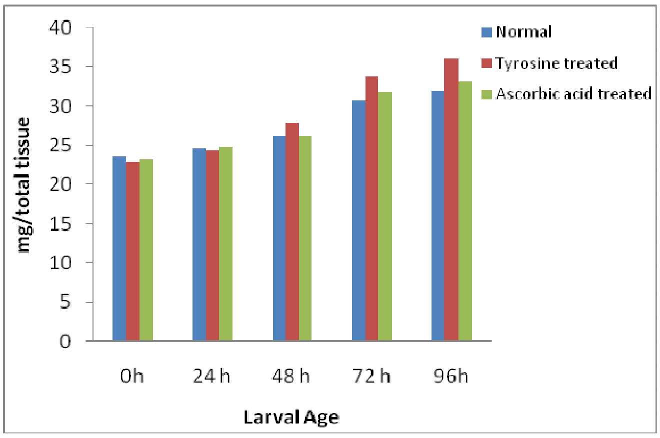

Figure-3b
Catalase activity in the fat body of *B. mori* larvae

Discussion: In silkworms, the simple protein and amino acids derived from ingested food is absorbed into and transported through the haemolymph. This protein is then carried to fat body and from where its metabolism takes place. For insects, fat body is the principal site of protein synthesis and is the major source haemolymph proteins [18]. Investigations of haemolymph proteins are of particular interest as they provide a background to analyze the synthetic activity associated with the differentiation process in developing organism.

Prior to first feeding after fourth moult, the tissue samples were taken for the 0 hr analysis. At this time protein concentration was found to be less. As feeding proceeds the proteins in the haemolymph gradually increases and it is in tune with feeding. Being the extracellular fluid, the haemolymph can function as a medium for easy exchange of metabolites with the fat body. In normal fed larvae the total protein in unit volume of haemolymph was increased from 0 hr to 72 hr this increase is in accordance with active feeding and increased growth of larvae[19]. But this much hike is not seen at the stage between 72 to 96 hrs. This is because of the decline in feeding as well as metabolism in insects at last stages prior to pupation. During the later stages of larval life the fat body ceases its synthetic activity and there happens a protein sequestration from haemolymph into fat body for reserve on the light of forthcoming pupation. The protein concentrations of plasma increases upto 200 mg/ml at the end of the actively feeding phase of third instar larval life, and then it falls slowly during the wandering period of late larval life with a tremendous decrease near pupation[20].

In the spinning insects, there is a necessary to maintain a high level of tissue protein in the final instar for and which is the main source for cocoon production. Fat body of insects play an important role in the synthesis, storage and translocation of protein. Fat body in insect larvae function both as a storage centre for fat, carbohydrate and protein and is the principle site for intermediary metabolism of proteins in the final instar [21,22]. The conformational change in structure of the protein during its temporary retention in the haemolymph is necessary before being stored in the fat body[23].

The total haemolymph protein level in larvae treated with ascorbic acid and tyrosine was increased during the development of the final instars at a rate more than that of normally fed larvae. The difference was found to be significant since 48h of treatment and became highly significant prior to pupation. This variation implies that the ascorbic acid treatment made the larva in a more physiologically active condition with an increased rate of protein metabolism. The enhanced physiology of protein reveals that the antioxidant action of ascorbic acid and tyrosine which prevents deleterious effects of aging processes. Similar to haemolymph, protein level increase takes place in the fat body as the larval development progresses. The rise in total fat body protein in larvae was significant at 96h of ascorbic acid treatment which indicates fat body tends to continue its synthetic phase even prior to pupation on the administration of antioxidant.

The accumulation of hydrogen peroxide is associated with aging process of organism. The normal larvae showed an increase in H_2O_2 level in haemolymph and fat body in a gradual rate. Generation of H_2O_2 associated with life expectancy is proved by different workers. The silkworm supplemented with ascorbic acid and tyrosine maintained high level of H_2O_2 in feeding stages and showed a sharp dip in the non-feeding stages. H_2O_2 levels in ascorbic acid and tyrosine treated larvae showed a significant reduction indicating the scavenging action on free radicals. The decline in catalase activity is associated with aging process of organism. Systolic enzyme catalase is a component of antioxidant defence system that converts H_2O_2 to water and protects the cell from oxidative damage[24].

In control larvae catalase activity increased with age, normally as that occurs in all organisms. This is because of the increase in H_2O_2 level as aging proceeds. Catalase activity increased with age and decreased during the later part of life. The increase in level of catalase activity in the haemolymph of normal and treated larvae in the active feeding stages and its decline in the non feeding stages observed in the present study can be explained in the above context.

The treated silkworms exhibited a significant reduction in haemolymph catalase activity in tune with reduced levels of H_2O_2 in tissue. This indicate that there is a corresponding increase in the antioxidant activity with an increase in pro oxidant generation in treated ones .This implies that the supplementation of ascorbic acid resulted in an increase in the antioxidant activity in insects. The high specificity of the enzyme in normal and treated larvae during initial stages of fifth instar larval period in turn provides an explanation for the total reduction of H_2O_2 titre during the same period. The fat body catalase activity was higher than that of haemolymph in normal as well as treated larvae. Its difference have been attributed to several factors like their physiological functions, the risk of exposure to oxidative damage and the balance between synthesis and degradation of proteins during normal metabolism. In precise, the administration of ascorbic acid and tyrosine as a food supplement has a positive effect in the defence of a living organism against the deleterious effects of pro oxidants. The antioxidant effect and scavenging role of this compound is promising in aging studies.

Conclusion

Accumulation of hydrogen peroxide in haemolymph and fat body is a natural phenomenon of aging. The sharp decline of H_2O_2 levels in the ascorbic acid and tyrosine supplemented larvae demonstrates that it has a property to scavenge the free radicals. The rise in catalase activity during larval development was directly related to the formation of pro oxidant in the larvae. The present results proved that the ascorbic acid and tyrosine can check the deleterious effects of aging through its antioxidant activity.

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