EFFECT OF FEEDING POSTURE ON MID-GUT COLUMNAR EPITHELIAL CELL NUCLEUS AND MITOCHONDRIA IN MUGA SILK MOTH (*ANTHERAEA ASSAMENSIS*) LARVA: A TRANSMISSION ELECTRON MICROSCOPIC STUDY

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ABSTRACT
Feeding posture (vertical/horizontal) has been found to have remarkable impact on food consumption, food assimilation, growth, molting etc. of the larvae of the 'Muga' silk moth *Antheraea assamensis*, endemic to Northeastern states of India[1].Transmission electron microscopy revealed that due to less food consumption at conventional tray-feeding (horizontal feeding posture) under indoor rearing condition, a number of abnormalities in ultra structural features of mid-gut columnar epithelial cell nucleus and mitochondria take place. Loss of contour, development of constrictions, evagination, invagination and breakage of membrane etc. were some of the abnormalities in nucleus. In mitochondria, vacuolization, inner and outer membrane distortion, de-shaping, loss of electron density etc. were evident. Significance of these abnormalities in the larva is discussed with the help of relevant literature.

Keywords: Feeding posture; silk moth; mid-gut; TEM; Nucleus

INTRODUCTION

The feeding behavior of herbivorous insects appears to be widely different depending upon the types of leaves, feeding conditions, shape of leaves etc. Lepidopteran larvae represent one of the most important groups of leaf consumers in nature, but detailed observations on the feeding behavior are limited to a few out of thousands of species in the order[2].

One of the commercially important lepidopteran species, *Antheraea assamensis*, popularly known as "muga" silk moth is a multi - voltine, sericogenic insect, endemic to Assam and adjoining states of Northeast India. The commercial rearing of 'Muga' silk moth larvae is conducted during May–June and October–November because the climatic conditions during the period are favorable for growth and development of the larvae.

The dependence on favorable seasonal conditions led to the compulsion of choosing only two commercial rearing although the insect has six broods in a year. Due to the same reason the larvae are preferred to be reared outdoor, which however, subjects the delicate larvae to environmental constrains such as rain, hailstorms and a number of predators e.g., birds. To overcome these problems, domestication or indoor rearing of the larvae appears to be extremely important. However, several attempts in the past [3] were far from being satisfactory at the commercial level. One of the reasons for less success in indoor rearing of the "muga" silk moth larvae appears to be the adoption of the same procedure as has been followed in indoor rearing of the domesticated silk moth species, *Samia Cynthia ricini* (Eri silk moth) and *Bombyx mori* (Mulberry silk moth). A study conducted by us revealed that the head cuticle of "muga" silk moth larvae contains some gravity receptor (dorsal Campaniform sensilla) which are absent on the head cuticle of *Samia Cynthia ricini* and *Bombyx mori* [4] Besides this, a number of peculiarities have been reported in the "muga" silk moth larvae in terms of its eco-physiology, behavior, general morphology, ultra structural features etc. [3, 5-9].

The detection of gravity receptors on the head cuticle of the "muga" silk moth larvae and behavioral studies under indoor and outdoor conditions suggests that the vertical posture of the worm is its preferred feeding posture. Providing a vertical feeding posture to the worm under indoor rearing conditions could improve the food consumption, food assimilation, larval weight, cocoon weight etc. and also could reduce the larval life span, molting period and spinning time [1]. It was thus confirmed that the conventional tray feeding of the larvae at horizontal posture is to a great extent, responsible for its poor rearing performance under indoor condition. Since the low food consumption is likely to affect the metabolism significantly, it was felt that a study on the cellular and ultra structural features of mid-gut cells of the larvae will be relevant. The mid-gut of lepidopteran larvae is morphologically differentiated through out its length [10-13]. The epithelium of mid-gut is composed of four different cell types, columnar, goblet, regenerative and endocrine [14]. It is known that the major portion of the mid-gut epithelium consists of columnar cells and the mitochondria present there plays important role in the absorption and metabolism of nutrients [14].

Similarly, Nucleus is one of the most prominent cellular organelles, and, its shape and size are important in cellular function [15].

The present study was therefore undertaken to investigate ultra structural features of nucleus and mitochondria of the mid- gut columnar epithelial cell in relation to possible physiological stress in *A. assamensis* due to low food consumption at conventional horizontal feeding posture under indoor rearing condition.

MATERIALS AND METHODS

Insect Material The first larval stage samples of the muga silk moth, *Antheraea assamensis* were collected immediately after hatching from the field Station of Central Silk Board, Muga Seed Development Project (MSDP), P–31 Unit, Nongpoh, Meghalaya, India, and were reared in different feeding posture under indoor condition through out the larval period.

Vertical Feeding Posture:

In the vertical posture, the larvae were reared in twigs, along with leaves of *Machilus bombycina* (host plant), placed in water-filled bottles.

Horizontal Feeding Posture:

For providing horizontal feeding posture, the larvae were kept in bamboo trays with twigs along with leaves of *Machilus bombycina*. The twigs in both bottles and trays were changed frequently to maintain freshness of the leaves. The outdoor reared larvae were used as control. The experiment was carried out in the field station of Muga Seed Development Project (MSDP), Central Silk Board, Nongpoh, Meghalaya, India.

Transmission Electron Microscopy:

The mid-gut samples excised from the fifth stage of control (outdoor reared) as well as vertically and horizontally fed indoor reared larva were prepared for Transmission Electron Microscopy as follows.

The samples were cut into small pieces of approximately 1mm × 1mm in size and were fixed in Modified Karnovsky's fixative[16]having the composition of 250ml of 0.2M sodium cacodylate buffer, 20g of Para formaldehyde dissolved in it at 60°C, bringing the volume to 480ml by double distilled water. To this 20ml of 25% glutaraldehyde and 12.5g of anhydrous calcium chloride was added.

After four hours in the above primary fixative, the samples were washed thoroughly in 0.1M sodium

cacodylate buffer. Post fixation was carried out in 1% osmium tetroxide in the same buffer for one hour at 4°C.

Specimens were dehydrated in ascending grades of acetone (30%, 50%, 70%, 80%, 90%, 95% and dry acetone) with two changes of fifteen minutes each. The samples were then cleared off the acetone by propylene oxide for 30 minutes.

Infiltration was carried out gradually in different proportions of propylene oxide with the liquid resin [Araldite CY 212, 10ml; DDSA (Dodecenyl succinic anhydride), 10ml; DMP–30 {Tri–(di-methylaminomethyl) phenol}, 0.4m and Di butyl phthalate, 1ml]. Embedding of tissue were carried out in the Araldite embedding medium using beem-capsule The embedding blocks were kept at 50°C in an embedding oven for 24 hours. The temperature was then raised to 60°C and the embedded tissues were kept for 48 hours to complete polymerization.

Ultra-thin sections (600–800Å) were cut in an RMC Ultra-Microtome, MT–X, with a diamond knife. The sections were collected on copper grids and stained with 50% alcoholic solution of Uranyl acetate for 10 minutes at room temperature in the dark, followed by Lead nitrate for 5 minutes [17]. The stained sections were examined in a Jeol JEM 100CX II Transmission Electron Microscope at an accelerating voltage of 80Kv.

RESULTS AND DISCUSSION

Remarkable differences were observed in the ultra structural features of nucleus and mitochondria of mid-gut columnar epithelial cells of the larvae of *Antheraea assamensis* reared at horizontal feeding posture under indoor rearing conditions, as compared to control (outdoor reared larvae) and indoor reared larvae at vertical feeding posture.

Nucleus

Control: The nucleus of the mid-gut columnar epithelial cell of the fifth larval stage of the silk moth collected from their natural habitat in the field revealed normal features. The contour of the nucleus was round or oval with the nuclear membrane showing normal structures such as regular outline and the absence of any prominent membrane protrusion or invagination. Dense chromatin, characteristic of normal nucleus was also evident (Fig. 1).

Indoor Reared Horizontally Postured larvae: When the larvae were reared under indoor conditions with conventional tray rearing, it was observed that the nucleus of some of the mid-gut columnar epithelial cells had undergone remarkable changes in certain features as compared to the control. The round contour, as observed in normal outdoor reared larvae was lost. In some cases, the nuclear membrane was found to develop constriction leading to the de-shaping of nucleus. Breakage was observed in the membrane in the constricted region (Fig.2). In some of the mid-gut columnar epithelial cells on the other hand, the contour of the nucleus was found to be changed to such an extent that the nucleus assumed an amoebic shape. There were some evagination and invagination of the nuclear membrane at places (Fig.3). 35 to 40% of the mid-gut columnar epithelial cells showed the aforementioned abnormalities in the nucleus in 50 sections (from different individual larvae) examined.

Indoor Reared Vertically Postured larvae: In indoor reared vertically postured larvae, no abnormality was observed in the nucleus. The contour was either oval or round as in the normal nucleus. No abnormality in the nuclear membrane, either in the form of protrusion or invagination could be detected (Fig.4).

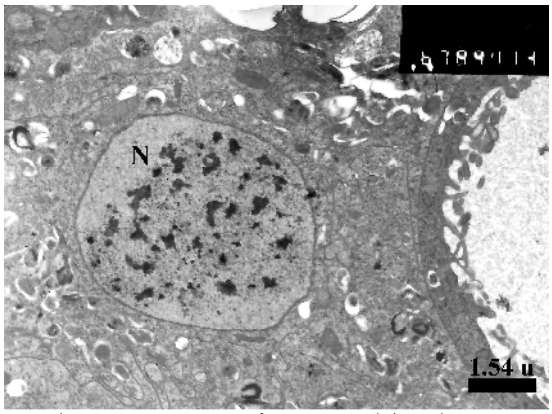

Fig.1.Normal shape of Nucleus (N) in Mid-gut Columnar Epithelial cell of Outdoor reared larva of A.assamensis

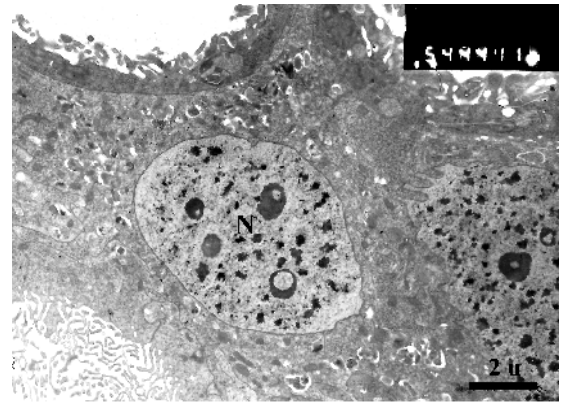

Fig. 4. Normal shape of Nucleus (N) in mid-gut columnar epithelial cell of A. assamensis at Indoor vertical feeding posture

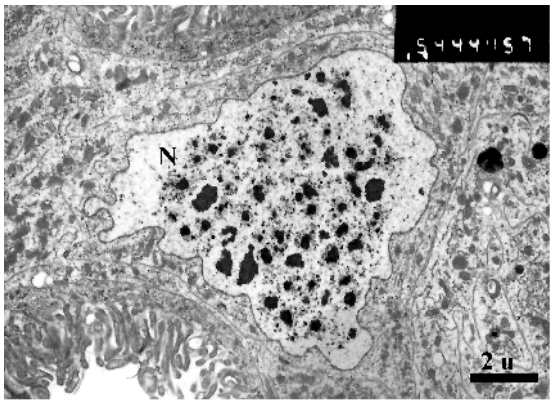

Fig 3. Amoebic shape of Nucleus (N) at Indoor horizontal feeding posture.

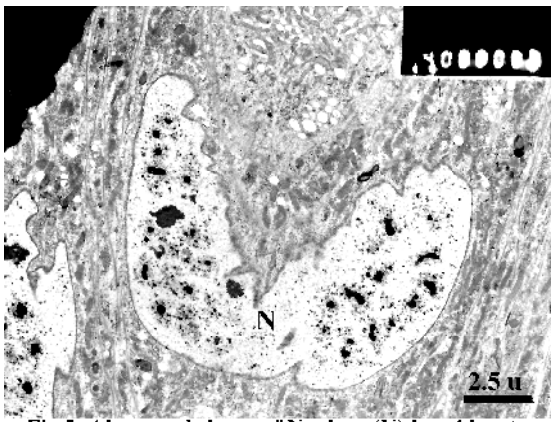

Fig 2. Abnormal shape of Nucleus (N) in mid-gut columnar epithelial cell of A. assamensis at Indoor Horizontal feeding posture

Mitochondria:

Control:

In the mid-gut columnar epithelial cells of control larvae collected from the field, normal structural features of the mitochondria were observed. The shape and size of the mitochondria was normal. The outer and inner mitochondrial membranes were intact. High electron density was exhibited by the matrix and the number of cristae was quite high. No abnormality such as membrane distortion or vacuolization was observed in the mitochondrial matrix (Fig.5).

Indoor-Reared Horizontally Postured larvae:

In the indoor-reared horizontally postured larvae, remarkable abnormalities were observed in mitochondrial population. Some of the mitochondria showed breakage of the outer membrane at places, while some showed distortion of the inner cristae. In some cells the shape of the mitochondria was found to be disturbed due to the disorganization of the outer membrane. Vacuolization was observed in the matrix of many of the mitochondria and electron density in the matrix of some was less as compared to control (Figs.6, 7). The abnormalities were observed in about 45% of the cells out of 50 sections (from different individual larvae) examined.

Vertically–Postured Indoor Reared larvae:

In the mid-gut columnar epithelial cells of indoor reared vertically postured larvae, most of the mitochondria were found to be normal in their ultra structural features. The inner and outer membranes were found to be intact and the electron density of the mitochondrial matrix was similar to that of the control and higher than that of the larva fed at horizontal feeding posture. Vacuolization was observed in less than 5% of the mitochondrial matrix (Fig.8) out 50 sections (from different individual larvae) examined.

The abnormal features of mid-gut columnar epithelial cell nucleus and mitochondria, resulting from remarkably low food intake due to wrong feeding posture (conventional tray feeding)[1] appears to have a number of adverse effects on the physiology of digestion in the larvae of the silk moth, *Antheraea assamensis*.

The abnormal features of the nucleus in terms of loss of uniform contour, development of constriction in the nuclear membrane, breakage of the membrane at places etc. give enough evidence that low food consumption of the larvae at horizontal posture (conventional tray feeding) under indoor rearing has resulted in some cellular stress in the mid-gut. Pertinent here is to mention that the role of abnormal nuclear shape in impaired mechano–transduction has been reported by some authors [18]. Further, it is worth mentioning that defective nucleo cytoskeletal integrity is reported to cause decreased mechanical stiffness in some cells [19]. Besides these, the mechanical connection between nucleoplasm, cytoskeletal filaments etc. and structural stability of nucleus is well acknowledged in existing literature [20]. The relationship between the defects in nuclear structure and body function even in higher vertebrates has been reported [21]. Further, reports on structural and mechanical properties of the nuclear lamina in certain pathological conditions are available in existing literature [22]. Life-span of some organisms has been reported to be linked with nuclear architecture [23]. Abnormal nuclear shape was reported to reflect mitotic instability [24], which is bound to affect the body growth adversely. In mid-gut epithelium of some hemipteran insects, abnormalities in nucleus have been linked to degenerating cells [25]. Thus it appears that the remarkable abnormalities in mid-gut columnar epithelial cell nucleus detected in horizontally fed larvae under indoor rearing conditions is indicative of poor functioning of the cells, causing disturbances in digestion and assimilation. This in turn is bound to cause poor body growth in horizontally fed indoor reared larvae, which was confirmed in earlier studies [1] carried out in our laboratory. The absence of any major abnormality in the mid-gut columnar epithelial cell nucleus of larvae fed at vertical posture resulting in normal digestion and body growth supports this conjecture.

The abnormalities observed in the mitochondrial shape; distortion and breakage of inner as well as outer membrane of mitochondria in the mid-gut columnar epithelial cells of horizontally fed larvae under indoor rearing conditions appear to be directly related to the abnormal functioning of mid-gut resulting in poor digestion, assimilation and growth. This is logical because of the fact that physiological process within the mitochondrion is facilitated by a continuous matrix in the inner chamber, just as these processes benefit from the increased surface area provided by the cristae and the continuation of inner membrane into the cristae [26]. In view of the significance and role of mitochondria in cellular metabolism and enzymatic activities, the observed abnormalities in mitochondria in mid-gut columnar epithelial cells of horizontally fed indoor reared

larvae suggests that the larvae experience severe cellular stress due to their defective feeding posture. Vacuolization observed in the mitochondrial matrix of the larvae fed at horizontal posture is significant because of the fact that autophagic cell death is morphologically characterized by an accumulation of vacuoles [27]. Further, the role of abnormalities in mitochondria in both necrotic and apoptotic cell death is amply highlighted in existing literature [28].

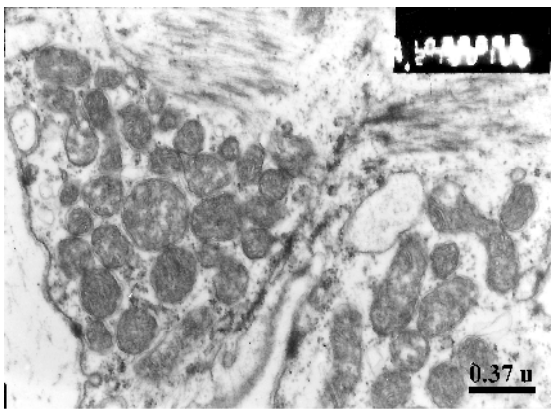

Fig. 5.Mitochondria with well defined membranes (Inner and Outer) and electron dense matrix in Mid-gut columnar epithelial cell of outdoor reared larva

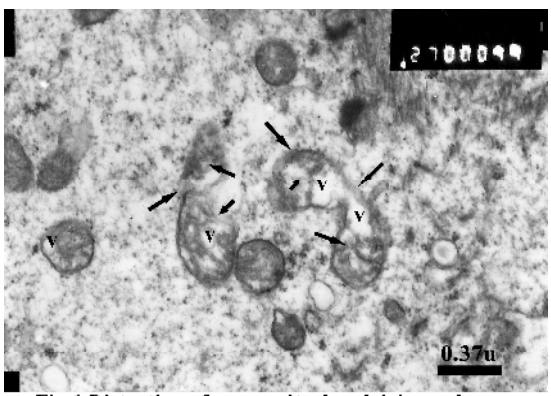

Fig 6.Distortion of outer mitochondrial membrane (long arrow), inner mitochondrial membrane (short arrow), vacuolization (V) and low electron density of mitochondrial matrix. (Indoor horizontal feeding posture).

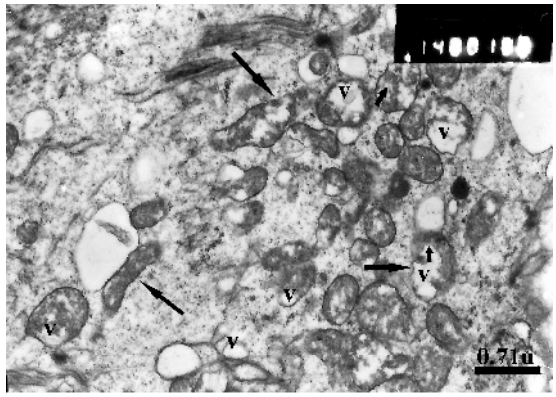

Fig 7.A portion of the mid-gut columnar epithelial cell showing larger population of mitochondria with distortion of outer (long arrow), inner (short arrow) membrane and vacuolization (V) in mitochondrial matrix at indoor horizontal feeding posture.

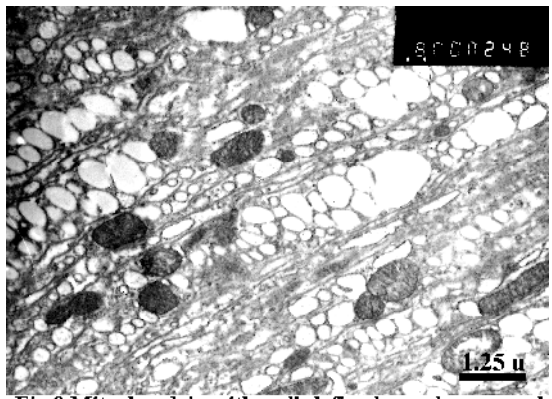

Fig 8.Mitochondria with well-defined membranes and electron dense matrix in mid-gut columnar epithelial cell of indoor reared larva at vertical feeding posture

In horizontal feeding posture, the food consumption of the silk moth larvae is extremely low and is close to starvation. In this context, the evidence for selective mitochondrial autophagy under starvation is worth mentioning [29].

It has been reported that starvation can induce mitochondrial autophagy, which is required for the synthesis of proteins essential to survival under starvation condition [29]. The high electron density of mitochondrial matrix in control and in larvae with vertical feeding posture is suggestive of maximally condensed

structural organization and high energy state orthodox conformation of mitochondria [30].In contrast, the low electron density of mitochondrial matrix in larvae with horizontal feeding posture suggests low energy state conformation of mitochondria, which indicates severe adverse affect on energy status of the mid gut epithelial cell relevant to the physiology of digestion and assimilation. In this context it is worth mentioning that another important cell organelle, i.e. Microvillus in mid-gut epithelial cell also exhibited remarkable abnormalities in indoor reared A*ntheraea assamensis* larva in its horizontal feeding posture [31].

CONCLUSION

The study reveals that feeding posture of the muga silk moth larvae under indoor rearing conditions which acts as a guiding factor for the food consumption [1], also affects the ultra structural features of the mid gut columnar epithelial cell nucleus and mitochondria.

The structural abnormalities of the nucleus in the silk moth larvae reared by conventional feeding posture (horizontal feeding posture) are reflective of cellular stress due to low food consumption. Similarly, the mitochondrial abnormalities suggest the possibilities of apoptotic or necrotic cell death, at least in some cells of the mid gut. It can thus be concluded that the conventional tray feeding is not at all advisable for indoor rearing of the silk moth larvae, and, vertical feeding posture is extremely important for the same. The study further suggests the importance of Electron Microscopy in Bio resource management through ultra structural understanding.

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