DISCUSSION

Research on the biological activity and chemistry of antifeedants is heavily emphasized because of their biodegradable nature and relative safety for useful organisms in the environment. Unlike insecticides that kill outright both pests and predators, the antifeedants obtained from plants are relatively safe for natural enemies which subsist on pests rather than on plants. Feeding and reproduction in insects are very closely related to nutritional factors. The consumption of food is necessary for all the aspects of insects performance, i.e., growth and development, reproduction, defense, movement and survival (Slansky and Rodriguez, 1987).

The repellent and antifeedant effects of neem derivatives on sucking and chewing insect pests of rice have been reviewed in depth (Saxena, 1989). Pradhan et al. (1962) showed that absolute antifeedant property of Locusta migratoria was exhibited at 0.05% suspension of NSKE while 0.001% was needed to inhibit the feeding of Schistocerca gregaria. The present investigation have shown that application of AZA on the host leaves disrupt the normal process of feeding. This behaviour significantly reduces the duration of feeding and quantity of food ingested. This deterrent effect resulting in reduced feeding on treated leaves was clearly evident in the poor growth and development of A. crenulata. The insects were apparently repelled by AZA as they did not settle to feed on treated leaves as on control leaves.

It is well known that terpenoids are one of the largest groups of plant secondary compounds known to have insecticidal and repellent properties. AZA
is well-known for its insect antifeedant and growth regulation actions (Saxena, 1989). The antifeedant effects of AZA were assessed directly using a leaf-disc choice and no choice test. In the present study, AZA was largely responsible for both antifeedant (behavioural) and growth-regulatory (physiological) activities in *A. crenulata*. In the case of chronic growth bioassay, AZA may be exerting an overall effect through both behavioural and physiological means (Isman *et al.*, 1990). Although toxic effects of AZA against number of insects have been reported (Schmutterer, 1990; Mordue and Blackwell, 1993; Koul *et al.*, 1994), the present results clearly indicate that AZA severely affects nymphal growth as well as adults of *A. crenulata* when reared on AZA treated leaves.

In the present investigation, the antifeedant effects of AZA were induced at lower concentration. For instance, oral cannulation bioassay - a forced feeding method-used recently on several insect species along with other bioassays (topical and injection) also revealed that AZA activity varies with the mode of application (Simmonds *et al.*, 1990), a phenomenon well demonstrated for secondary plant compounds (Cottee *et al.*, 1988). However, it is evident from this and other status that the antifeedant effects of AZA in many phytophagous insects can be induced at very low concentrations (Barnby and Klocke, 1987). Several studies have established that AZA inhibits moulting by interfering with the production of ecdysteroid prior to apolysis. Mortality in *A. crenulata* fed on AZA treated leaves is often closely linked with feeding and the concomitant lack of growth. AZA resulted in a lack of growth and an apparent blockage in ecdysteroid release (Mordue *et al.*, 1986). Death in this instance therefore occurred before the moult.

Results presented indicate that the impact of AZA on the food utilization efficiency was dosage-dependent, and that the consumption and conversion effi-
ciency were highly correlatable with the gut enzymes activity of *A. crenulata*. Instars fed with AZA coated leaves consumed less food, resulting in loss of body weight and were less efficient in converting the ingested and digested food into biomass (ECI and ECD) compared to the controls. These results are similar to those reported for larvae of European corn borer, *Ostrinia nubilalis* (Arnason *et al.*, 1985) and for the tobacco budworm, *Heliothis virescens* (Barnby and Klocke, 1987).

Reduced relative growth rate (RGR) of the treated insects indicate that some physiological parameter of utilization had been affected by the AZA treatment. CI and RGR decreases with increasing AZA concentration as would be expected from the involvement of chemoreceptors (Barnby and Klocke, 1987; Koul and Isman, 1991). The reduced ECI and ECD result from a reduction in the efficiency to convert food stuffs into growth, perhaps by a diversion of energy from production of biomass into detoxification (Koul and Isman, 1991; Timmins and Reynolds, 1992). The prolongation of food in the gut may increase AD due to increased exposure to digestive enzymes (Barnby and Klocke, 1987). Results obtained for AD in AZA - treated insects corroborates the earlier findings of Fagoonee (1984), Arnason *et al.* (1985) and Barnby and Klocke (1987) in other lepidopteran insects. Increase in the level of AD is directly proportional to the concentration of AZA. This may be in response to a decreased rate of passage of food through gut as it was shown to occur in *L. migratoria* (Mordue *et al.*, 1985).

The feeding experiment clearly demonstrates that feeding deterrence was the principal mode of action responsible for reduced growth. This conclusion is corroborated by the results of direct no-choice and choice bioassays for feeding deterrence where substantial feeding inhibition on the treated leaf discs was ob-
served. Significant decrease in dietary utilisation and concomitant decrease in growth following AZA treatment showed the post-ingestive toxic effects. AZA have both behavioural and physiological action and serve as reasonable growth inhibitor to nymphs which may reflect an antifeedant action. A decrease in growth rate following treatment appears mostly due to decrease in net dietary utilisation (Koul and Isman, 1991; Isman, 1993).

The present findings that with increasing concentration of AZA, the food consumption rate, conversion rate and body weight of the nymphs decreased, suggests that besides antifeedant properties which reduce the food consumption rate, AZA possesses digestive enzyme inhibiting components to reduce the conversion rate (Barnby and Klocke, 1987; Timmins and Reynolds, 1992). Higher activity of enzymes in midgut of control insects was due to consumption as well as utilization of large quantities of food. Imbalance in the enzyme-substrate complex and inhibition of peristaltic movement of the gut (Hori, 1969) might have inhibited the enzyme quantity in midgut of AZA treated insects.

Excretion of increasing number of faecal pellets may be due to the increased feeding and increased rate of passage of food through the gut (Abisgold and Simpson, 1988). Hence, in this study, slow rate of feeding and decreased rate of passage of food through the gut due to the treatment with AZA suggest the higher level of accumulation of allelochemicals in the gut which might obstruct the digestive physiology. AZA's antifeedant effect was reflected in the reduced feeding activity and lower production of excreta on the treated leaves.

AZA treated leaves fed last-instar nymphs of *A. crenulata* either prolonged the intermoult stage or abolished adult moult completely, depending on the dose. Induction of permanent nymphs has been observed before in hemimeta-
bolous insects (Sieber and Rembold, 1983; Garcia and Rembold, 1984). In the present study, AZA treatment prolonged the duration of surviving nymphs associated with reduced growth. Delays in moulting, resulting in prolonged developmental period in insects treated with AZA is due to antiprothoracicotropic effect (Fagoonee and Lauge, 1981; Warthen and Uebel, 1982; Barnby et al., 1989). AZA in the diet of *A. crenulata* severely retarded the growth and development of surviving insects.

The present data suggest that AZA probably disrupts the moulting, with incomplete sclerotization and pigmentation of new cuticle of *A. crenulata*. The developmental effect of AZA are attributed to a disruption of endocrine events. Complete moult inhibition has been shown to be due either to a total blockage of haemolymph ecdysteroids or to a delay in the appearance of the last ecdysteroid peak, with or without a reduction in peak height, and a slow abnormal decline in the peak (Redfern et al., 1982; Sieber and Rembold, 1983; Mordue (Luntz) et al., 1986).

In the present study, the fifth instar male nymphs fed with AZA treated castor leaves failed to moult or moulted to severely deformed adults. At lower concentration most nymphs completed the moult as severely deformed adults with crumpled and curled wings and in some cases these individuals were very slow in completing the moult, and probably began to sclerotize new cuticle before ecdysis was complete. At higher concentration, the nymphs died without completing the moult and in some cases such nymphs lived for over 40 days. These results paralleled those obtained with *Oncopeltus* (Dorn et al., 1986) and *Melanoplus* (Champagne et al., 1989).
The present observation clearly demonstrates that AZA treated over-aged nymphs which have a greatly extended instar may survive for several weeks. Such insects have not metamorphosed and thus have not achieved the imaginal competence for adult physiology and development (Pener and Shalom, 1987; Van der Horst et al., 1989). The cuticular melanization, resulting in characteristic black spots, is often seen in AZA treated *A. crenulata*. Such blackening, may be due to absence of juvenile hormone and low levels of ecdysteroid titre (Hori et al., 1984; Koul et al., 1987).

Insect growth regulation is the most important physiological effect of neem. V instar male *A. crenulata* fed on castor leaves treated with AZA produced malformed adults and when this did not occur, the nymph either died during development or the adults from the surviving nymphs were abnormal. High mortality and considerable failure of ecdysis are also the characteristic features as a result of AZA treatment in this study. Some adults that moulted from treated nymphs appeared morphologically normal but exhibited higher daily mortality. Certain juvenilising actions (other than delay of moults) such as absence of moults and morphological modifications recall the action of AZA (Gujar and Mehrotra, 1983). Overall mortality may nevertheless be accounted for by the combined effects of partial or total starvation, toxicity and either absence of moults or abnormal moults.

In the present study, higher concentrations of AZA reduced the longevity markedly. However, lower concentration caused male impotency. Mating was apparently blocked by a disturbance of the haemolymph pressure which hindered the proper erection of the aedeagus. The involvement of neurohormones in the regulation of haemolymph pressure and a possible influence of AZA on such process is established (Dorn et al., 1986).
The fecundity of normal females was strongly reduced when mated with AZA treated males. It could be shown that this was due to mating failure. Since the secretion of the erection fluid is apparently under endocrine control (Dorn et al., 1986) an AZA induced hormonal imbalance could account for the male impotence. The results show clearly that in respect to reproductive capacity, AZA has a stronger effect in males. The mating interruption caused by AZA in males is the primary factor in the inhibition of reproduction in A. crenulata.

Transport enzyme like ATPases are very essential for transport of glucose, amino acids etc. and any impairment in their activity will surely affect the gut activity as well as its motility. ATPases are membrane bound enzymes. The role of membrane lipids and their micro environmental changes at the physical and chemical level may be responsible for the differential response observed at the level of ATPase activity under AZA treatment. By the action of ATPase, the membrane especially in the intestinal epithelium helps for the transport and reabsorption of metabolites and nutrients and also for the secondary transport of ions and non-electrolytes (Lechleitner and Philips, 1988; Fogg et al., 1991). In the present study, the ATPase activity in the gut was significantly reduced by AZA treatment. The ATPase inhibition may affect active ion transport leading to alteration in electrolyte regulation. Under AZA treatment decrease in enzyme activity denotes reduced metabolism in the insect and may be due to the toxic effects of the AZA on membrane permeability especially in the gut epithelium (Mordue (Luntz) et al., 1985 ; Babu et al., 1995b). ALP is mainly seen in the intestinal epithelium of animals and its primary function is to provide phosphate ions from mononucleotide and ribonucleo proteins for a variety of metabolic processes (Mc Comb et al., 1979 ; Sakarov et al.,
1989). ALP is involved in the transphosphorylation reaction. In the present study, the decrease in ALP activity in the midgut of AZA fed *A. crenulata* suggests that AZA had decreased the absorption of ions and also might have inhibited transphosphorylation reaction. ACP catalyse the hydrolysis of phosphate esters of all acid medium. Decreased level of this enzyme at higher concentration of AZA suggests a reduced phosphorus liberation for energy metabolism, decreased rate of metabolism as well as decreased rate of transportation of metabolites and may be due to the direct effect of AZA on enzyme regulation (Babu *et al.*, 1995b).

In the midgut lumen, phosphatidylcholine is hydrolyzed by the enzymes, released from free fatty acids (lipase, phospholipase) (Turunen, 1988). From the data obtained in the present study, the activity of digestive enzymes were significantly reduced by AZA and it inhibited the hydrolyzation of phosphatidylcholine. Enzymes secreted from the midgut mucosa enter the endoperitrophic space, and it is possible that they are involved in polar lipid synthesis. AZA severely inhibit the digestive enzyme secretion leading to suppression of lipid synthesis. Reversibility is well established in the function of some vertebrate digestive enzymes (Thomson and Dietschy, 1981).

The midgut is that part of the alimentary canal in which the cells secrete digestive enzymes and absorb nutrients as well as play an important role in ion transport (Martoja and Ballan-Dufrancis, 1984). Light and Electron microscopic studies were conducted to examine the effects of AZA treatment on the midgut of *A. crenulata*. The onset of the histological changes to the gut varied with dose and there was a gradual trend and a characteristic pattern. The well defined lining of brush border or 'microvilli' in controls suggested physiologically active cells,
involved in both secretion of enzymes and absorption of food (Dalton et al., 1951; Ferreira et al., 1981). These features are lost in treated insects. In treated A. crenulata, the epithelial cells showed the characteristic symptoms of the cytoplasm acquiring a less granular appearance, the brush border being irregular, the numbers of mitochondria decreased, the endoplasmic reticulum reduced and disposed into tight whorls and lipid bodies no longer being present.

In the present study midgut of AZA treated A. crenulata showed changes in the shape of epithelial cells from tall columnar to low cuboidal, the gradual loss of apical brush border, swollen mitochondria with swollen cristae, less dense and dilated endoplasmic reticulum bearing similarities to the cytoplasmic responses of columnar cells of the midgut of B. mori and A. aegypti to B. thuringiensis crystal toxin (Endo and Nishiitsu - Uwo, 1980; Singh et al., 1986). Accumulation of autophagic vacuoles in large numbers were observed in gut cells of AZA treated insects. These vacuoles, comprising a membrane surrounding materials such as mitochondria and endoplasmic reticulum are often associated with stress, senescence or cell death (De Priester, 1971; Dunn, 1990).

Morphological studies on dying cells reveal two patterns of cell death, 'apoptosis' and 'necrosis', the latter being due to toxic agents in the cell (Wyllie et al., 1984). In the present study, AZA has been shown to cause some of the initial effects of necrosis particularly associated with the swelling of the cell and organelles, vesication of membranes and dilation of rough endoplasmic reticulum, although extreme and rapid effects on cell and organelles were not seen. AZA causes slow necrosis of the epithelial cells, associated perhaps with a blockage of mitosis, as in epidermal cells and imaginal discs, resulting in a reduction in the number of regenerative cells of the nidi (Nasiruddin and Mordue (Luntz),
Such necrotic and perhaps senescent epithelial cells together with swollen and disrupted gut musculatures would produce a fragile gut unable to function normally in terms of digestive efficiency and capacity.

The most important function in the insects is the manufacture of the spermatozoa, in the male reproductive system. In the present study, due to AZA treatment the various reproductive biochemical parameters such as protein, carbohydrate, lipid, fructose, cholesterol, amino acids and other enzymatic profiles were markedly decreased. The significant decrease in the rate of reproductive maturation caused by AZA treatment appear to be a direct consequence of poor nutrition in the treated insects and also associated feeding physiology that influence the spermatogenesis.

There was alteration of free amino acids by AZA treatment in haemolymph, ARG and fat body of *A. crenulata*. Amino acids such as proline, glutamic acid, alanine, aspartic acid and serine were absent in ARG of AZA (0.5 ppm) treated leaves fed insect. Frank and Happ (1976) demonstrated that proline, glutamic acid, aspartic acid and alanine are quantitatively important amino acids in the ARG and spermatophore of *Tenebrio molitor*. Since Orthopteran insects produce spermatophores (Leopold, 1976) it is probable that proline, glutamic acid, aspartic acid and alanine detected in control *A. crenulata* contribute to the formation of spermatophore during mating. Happ (1987), similarly demonstrated that proline-rich secretory proteins from the ARG of *T. molitar* is indistinguishable from the structural protein of the spermatophore.

Chen and Oechslin (1976) have demonstrated the accumulation of glutamic acid in the paragonial gland of *D. nigromelanica* and suggested a neurophysiological function for the amino acid. In the present study, absence of the glutam-
mic acid and other amino acids in AZA treated insects suggest that AZA inhibited the neurophysiological function leading to suppression of protein metabolism in *A. crenulata*.

In the present study AZA caused significant reductions in qualitative and quantitative proteins in whole body, reproductive organs and haemolymph of *A. crenulata*. The reduction was perhaps due to the interference of AZA on the hormones regulating protein synthesis (Sieber and Rembold, 1983; Dorn et al., 1986). The study with AZA against *A. crenulata* also confirms that deprivation of protein qualitatively and quantitatively during the critical stages of growth probably resulted in hampering the regular metamorphosis resulting in abnormal insects. The lower level of proteins may be attributed to less feeding, as feeding alone provides the basic substrate for any biosynthetic process.

Several studies have shown that the proteins from the male accessory reproductive glands are transported during copulation (Leopold et al., 1971; Chen, 1984). It is known that the male ARG's produce the proteins which are used in the assembly of spermatophore, a structure which serves as the vehicle for the transfer of sperm from male to female (Wigglesworth, 1936). In the present investigation, after the treatment with AZA there is reduction in the proteins produced and hence transfer of sperm from male to female is hindered. In the treated insects the male accessory secretion transferred during copulation was affected and this secretion seems to have an important role in the regulation of the physiology of the female and may contain several stimulatory substance (Pickford et al., 1969).

The qualitative protein profiles of control and treated insects in the haemolymph and ARG of male showed electrophoretically different protein pat-
Gillot and Friedel (1976) proposed that the secretory proteins are synthesized in the fat body, released into the haemolymph and taken up by the accessory gland cells. In the present study, AZA severely affected the ARG proteins, which may lead to reduced fecundity. Generally, the secretory proteins of the accessory gland in *A. crenulata* have two reproductively important functions; they act both as stimulant for oviposition and as energy source for the developing oocytes. This accessory gland secretions may be involved in the formation of the spermatophore which may contribute to the seminal fluids and modify female oviposition behaviour (Pickford *et al.*, 1969; Friedel and Gillot, 1976).

Qadri and Narsaiah (1978) have found the inhibition of RNA and DNA profiles in AZA treated cockroaches. Similar results were obtained in this study also. RNA and DNA concentration were drastically reduced in nymphs and adult of *A. crenulata* by AZA. Decreased level of DNA and RNA concentration in whole body of male nymphs and adult *A. crenulata* fed on AZA treated leaves suggest that AZA strongly inhibits the DNA and RNA synthesis. Fritzysche and Cleffmann (1987) showed cell proliferation to be inhibited by AZA, with RNA synthesis being strongly affected. In the present study, the decreasing level of nucleic acids and protein by AZA treatment hindered the biochemical and physiological process of *A. crenulata*. The decrease in the nucleic acids in AZA treated insects probably suggest that AZA inhibit the metabolic process like protein synthesis. According to Chen (1971) protein synthesis is directly related to RNA which in turn is DNA dependent. In the present study the RNA/DNA concentration was decreased, which corresponds with decrease in protein synthesis in the AZA treated insects.
In insects, fat body appears to be the major organ where various phenomena like storage, synthesis and degradation occur systematically and it serves as the main storage organ for the nutrient reserve, as far as carbohydrate, lipid and protein are concerned (Hill and Goldsworthy, 1968). Lipids play some significant role in insect metabolism, as they form the major secretory products. Lipids serve as structural components of membranes, and they are essential components in the function of the cuticle (Blomquist and Dillwith, 1985). But after AZA treatment, the rate of lipid biosynthesis and the synthesis of specific lipid classes also would be expected to decline.

In the present study AZA treatment decreased total lipid content and altered major classes of lipids. It suggest the probable impairment of membrane permeability of the testis, seminal vesicle and ARG’s. Various phospholipid and neutral lipid classes were also adversely affected, suggesting the depletion of reserved fats in *A. crenulata* under the influence of AZA treatment. In the present study, the gradual decline in total lipid can be attributed to decreased rate of synthesis and greater demand for energy during metamorphic transformation from nymph to adult in *A. crenulata*.

Lipids form the major secretory products of accessory sex organs (Mann, 1964). Observation of lipid release from insect fat body and its complexing with haemolymph protein were reported (Tietz, 1962). The increased level of total carbohydrate and lipid in nympha1 stages may act as potential reserve energy sources for nympha1 - adult development.

The quantitative and qualitative profiles of lipids in the ARG, fat body and haemolymph were adversely affected by AZA, probably suggesting the depletion of the reserved fats in *A. crenulata* under the influence of the AZA. Chino
and Gilbert (1965) proposed that a haemolymph - protein diglyceride complex provided the mechanism for lipid transport in insects. Present study indicates the hinderance of lipid transport as a result of AZA treatment. The decreased concentration of total lipids and major lipid classes noted during present investigation suggest the toxicity of AZA elicited on the fat body, since fat body is the main source of plasma lipoprotein production and metabolism (Chino, 1985). Recently, Moreira et al. (1994) reported that, transfer of phospholipid from lipophorin was inhibited by AZA.

Food deprivation exerts an appreciable impact on fat body carbohydrate as suggested by Goldsworthy (1969) in Locusta. The decreased carbohydrate level in the reproductive organs, fatbody and haemolymph, may be due to reduced consumption by AZA treatment. Decline of sugar component in fat body might be due to its transport to storage site via haemolymph to meet energy requirement for detoxification and metabolism of the candidate compound (Barnby and Klocke, 1987). It is accepted that the fructose serves as source of energy for various activities of insect (Steele, 1982). The present study has revealed that the fructose content of the male reproductive organs depleted significantly after AZA treatment.

JH is demonstrated to have a general stimulatory effect on protein synthesis in the fat body of several insect species such as R. prolixus, L. migratoria, Blaberus discojalis (Coles, 1964 ; Dhadialla and Wyatt, 1983). In the present study, the protein concentration in the fat body, haemolymph and ARG was significantly reduced by AZA as well as electrophoretic observations revealed there was alteration in the number of bands in the haemolymph and ARG of AZA treated insects. This disturbances in the protein metabolism suggest that AZA inhibit
the protein synthesis through the disturbance of neuroendocrine system. In the present studies it was found that an additional protein fraction was evident in the haemolymph of 10th day old adult normal male insect. The extra protein fraction may be male specific protein. This specific protein fraction was absent in AZA treated male insect haemolymph. This resulted in the mating disruption in AZA treated insects leading to reduced fecundity and also mating was not achieved in higher concentration of AZA treatment. In the present study, the fecundity was significantly decreased in the normal females, which were mated with higher concentration of AZA treated males. This may be due to that AZA inhibits the secretory proteins leading to suppression of oviposition stimulant.

Fatbody and haemolymph are known to contribute the precursor materials that are used up in the elaboration of secretion by the male ARG in some insects (Friedel and Gillott, 1976; Ranganathan and Padmanabhan, 1994). Sterols are important for growth, development and reproduction in insects and are essential for the integrity of the cell membrane and synthesis of ecdysteroids from cholesterol. The cholesterol level was decreased in the reproductive organs of *A. crenulata* after AZA treatment. This may be due to interference of AZA in cholesterol synthesis.

In the present investigation, AZA reduced the ACP and ALP activities in reproductive organs indicating some relation of these enzymes to the metabolism of these compounds. The decrease in the activities of acid and alkaline phosphatases in the testis and seminal vesicle may be due to the inhibitory role of AZA on spermatogenesis and sperm survival (Schluter and Schulz, 1983).

In general, lactate dehydrogenase has been shown to play an important role in the mitochondrial lactate - pyruvate shuttle system for transferring the
reducing equivalent from cytosol to mitochondria for generation of energy necessary for motility and survival of germ cells. The decreased level of LDH in male reproductive organs may thus suggest that the reproductive function was severely inhibited by AZA. AZA caused spermatocytes to degenerate and disrupt the germarium and severely affect the growth and maturation of sperms. It suggests AZA's direct effect on the testicular membrane, rendering the tissue incapable of developing spermatocysts. Similar results were obtained by Shimizu (1988) in the in vitro study of spermiogenesis in diapausing Mamestra brassicae males where 3 ppm AZA caused spermatocytes to degenerate, even in the presence of 20-hydroxyecdysone.

From the present investigation it may be concluded that the antifeedant activity of AZA may be primarily through chemoreception and also reduced food intake due to toxic effects. With increasing concentrations of AZA, the food consumption, conversion efficiencies and body weight of the nymphs decreased suggesting that besides antifeedant properties it also possesses digestive enzyme inhibiting components. There was a marked prolongation in nymphal period, therefore AZA is an ecdysis and growth-inhibitor. Electron microscopic studies revealed the extent of damage of AZA on midgut cell organelles. The swelling of mitochondria, appearance of autophagic vacuoles, a loss of microvilli in certain regions and fewer mitochondria were evident in AZA treated midgut epithelial cells.

The decrease in various biochemical profiles such as protein, carbohydrate and lipid concentrations after AZA treatment suggests that AZA may inhibit their metabolisms and interfere with the neuroendocrine control of development. AZA affects the activities of nucleic acids and results in concomittant reduction in
the protein levels. Reduction in quantitative and qualitative amino acid profiles suggest that AZA blocks the synthesis of secretory protein. The reduced activities of acid and alkaline phosphatases and lactate dehydrogenase in reproductive organs of AZA treated insects is believed to inhibit the transport of metabolites. Light microscopic studies also reveals the structural changes in testis and seminal vesicle after AZA treatment which may be due to the direct effect on gonads and also through neuroendocrine system.