CONCLUSION:

Like most honeybees, *A. c. indica* exhibit a complete development or complete metamorphosis. Development from egg to new worker normally takes two to three weeks (Tales from the Hive, 2000; Bishop, 2005). The brood of *A. c. indica* colony is made up of egg, larva and pupa (Mishra, 1995; Rahman, 2014). The larva eat and grow, completely depending and being fed with royal jelly; pollen and honey mixture by workers and also shed their skin a few times as they grow. The pupa prepares for life and moult as an adult and goes through all the changes it needs to emerge as an adult. The life of young worker bees is mainly spent inside or within the hive with pollen as their main source of food especially during the nursing period. After 21 days the worker bees start their life as foragers and mainly depend on nectar and honey which is high in carbohydrates as their source of food which is especially needed as energy for flight (Mishra, 1995; Rahman, 2014).

During development, the digestive tract of worker bee, showed external differences between adults and larvae. The larva is characterized by a simple undifferentiated gut having a midgut and a hindgut, whereas, the foregut starts to appear and differentiate in the pupal stage and become completely differentiated in the adult.

The foregut first appeared as a small outgrowth consisting of the crop and proventriculus in the white eyed pupa; the four proventricular lips were observed in the pink eye pupa. It becomes completely differentiated in the brown eye pupa where it is highly musculated. The adult foregut epithelium is folded and lined with a cuticle that becomes highly fused and in the area of the lips of the proventriculus, the cuticle is thicker and provided with spines.
The larval midgut begins to differentiate and degenerate in the late larval instar. The pupa midgut is vacuolated and the larval midgut epithelium is thus superseded by the pupal epithelium. The early pupal midgut alterations are characterized by ruptured digestive cells; loss of microvilli and contents released off into the lumen. The greatest alterations of the midgut cells occur during the pupal stage in the present study. The presence of the regenerative cells at the basement membrane in the midgut was also noted and are considered to be the generative cells that will give rise to a new midgut epithelium during development (Neves et al., 2002, Cruz-Landim and Calvacante, 2003; Barsagade and Kelwadkar, 2008). In the present study it was also observed that the larval peritrophic membrane was eliminated in the pupal stage but formed again in the late brown eye pupa. The midgut epithelium changes in the larval and pupal stage. The digestive cell microvilli and the amount of organelles like mitochondria, endoplasmic reticulum, granules and lysosomes increased together with vesicles of different electron densities. The midgut increased in length during the mid-pupa attaining a maximum length in the late brown eye pupa and prominent external annelations being observed as in adult.

The simple larval hindgut consists of an ileum and a rectum which increase in length and completely differentiates in the brown eye pupa. The region between the midgut and the anterior hindgut remains closed during larval development until the pre-pupal stage (Snodgrass, 1956; Cruz-Landim and Mello, 1981). Very little changes were detected in the ileum whereas; the rectal pads start to appear in the white eyed pupa. The hindgut becomes completely differentiated in the late brown eyed pupa. Various infoldings were also detected in the hindgut cell at the ultrastructural level and these
infoldings are associated with many mitochondria which thus relate to water and ion absorption (Garayoa et al., 1999 a, b; Serrão and Santos, 2006).

Most of the degeneration and differentiation occurs mainly in the midgut than either the foregut or hindgut. Both the foregut and hindgut epithelium are covered with a cuticle whereas the midgut has a peritrophic membrane that envelops it and it is the main site of digestion and absorption of food (Snodgrass, 1965; Cruz-Landim and Mello, 1970).

Thus the development of the digestive tract in the worker bee, A. c. indica supports similar observations on A. mellifera (Dabrovsky, Cruz Landim and Cavacante, 2004; Santos et al., 2009; Zakaria, 2010) and the stingless bee Melipona quadrifasciata anthidioides (Cruz-Landim and Mello, 1970; Neves et al., 2002).

There were differences in the nutritional components like proteins, sugars, lipids and vitamins between larva, pupa and adult during the development. These nutritional differences, which affects the development of the larva, pupa and adult workers of A. c. indica, which according to many literatures point to different nutritional requirements for workers and among workers themselves according to their activity and task differences (Beetsma, 1979; Brouwers, 1984; Boot et al., 2006; Rahman and Hajong, 2013).

In the present study, high protein content in larval stage newly emerged and nurse bee indicated that workers required a high-protein diet for satisfactory development when they are young and during brood rearing. Larva required higher protein content for their development while a diet restricted to nectar or honey is inadequate for larval growth (Louveaux, 1963; Haydak, 1970; Zerbo and Silva de
Moraes, 1996). The high level of proteins in nurse also correlates with the HPG development that is required to synthesize proteins for brood feeding (Brouwers, 1982; Fluri et al., 1982; Moritz and Crailsheim, 1987; Deseyn and Billen, 2005; Rahman et al., 2013). Dietary protein during development in honeybees also directly influence some aspects of immune function (Crailsheim and Stolberg 1989; Alaux et al., 2010; Brodschneider and Crailsheim 2010; DeGrandi-Hoffman et al., 2008; 2010) as well as learning and memory ability (Wright et al. 2007, 2009; Wright 2011).

In terms of sugar, forager, carrying out external activities has significantly higher sugar content than the other age groups. The same was also supported by histochemical analysis showing that the gut of forager bees stained strongly to PAS. This high sugar content is due to a switch in the diet i.e. the consumed only nectar and honey which are rich in fructose and glucose (Crailsheim et al., 1992, Zerbo and Silva de Moraes, 1996). Nectar is a stored carbohydrate source, honey that is used in fuelling colony metabolism (Winston, 1987; Anderson et al., 2011).

From the present study, the lipid content was found to be higher in the larva which may indicate lipids reserved for the starvation pupal period while in the adult workers; nurse bees had higher lipid contents. The high lipid content in nurse bee supports studies of Rahman and Hajong (2013) in A. c. indica where it was reported that lipids are mostly utilized by the young worker bees. This is also in consistent with the findings of Toth et al. (2009) and Crailsheim et al. (1992) where the nurse worker had higher lipid quantity.

In the presence study, vitamin C (ascorbic acid) and vitamin B₂ (riboflavin) were detected from the honeybee worker during development. Honeybees prefer a range of vitamins (Black, 2006), like ascorbic acid, pantothenic acid, niacin, thiamine and
riboflavin which are needed and are important for the development of the hypopharyngeal glands, protein synthesis and it also affects brood rearing and growth of the larvae (Haydak, 1949; Haydak and Dietz, 1972; Weaver, 1974; Anderson and Dietz, 1976; Herbert et al., 1985; Herbert and Shimanuki, 1978d; Herbert, 1997; Zahra and Talal, 2008; Amiri-Andi and Ahmadi, 2014).

The nutritional requirements of individual honeybees within the hive vary with their life-stage, with larvae primarily requiring protein (Ward et al., 2008) and adult honeybees requiring greater carbohydrate and less protein (Mayack and Naug, 2010). The enzymatic study shows that there are differences during development due to differences in the diet of the brood and adult worker. During the development, it was observed that the gut activities of most enzymes started to increase in the oldest pupae, but it was relatively low in the pupal stage of the worker bee, A. c. indica. The high levels of protease activity in larva and nurse workers, suggests that these individuals required protein in their diet, similar to reports in case of A. mellifera workers (Maurizio, 1954; Standifer, 1967; Szolderits and Crailsheim, 1993) and in stingless bee Scaptotrigona postica (Zerbo and Silva de Moraes, 2001). The carbohydrate enzymes activities (amylase, invertase and trehalase) in the gut of A. c. indica showed that the activity is higher in the adult stage than that of the larval and pupal stage. This high level of activity found in the adult gut which is maximum in the forager worker correlates to its higher capacity to digest high sugar diet and is the main carbohydrate-degrading enzymes (Zerbo and Silva de Moraes, 1996; Zoltowska et al., 2011). The enzyme lipase and phosphatase are active in the larva mainly due to high protein concentration in the gut and haemolymph and helps in tissue development (Zakaria, 2007; Barsagade et al., 2009; El-Ebiarie, 2011).
The colony forming unit value for yeast and fungi was more in larva than in case of adults, whereas, the gram bacteria and lactobacillus count was more in the adult in the present study. In honeybees, beneficial microflora occurred naturally providing metabolites such as intestinal enzymes, vitamins, antimicrobial substances, organic acids and lipids that contribute to the conversion of pollen and the stabilization of bee bread; aids in immune-related function that obstruct colonization by pathogens (Gilliam, 1979b; Yatsunami and Echigo, 1984; Gilliam et al., 1988b; Gilliam et al., 1989; Burgett, 1990; Berg, 1996; Gilliam, 1997). From the pure culture of fungi and yeast, strains of the genus Penicillium, Aspergillus and Mucor for fungi and for yeast only one strain was identified that belonged to the genus Saccharomyces. According to Gilliam et al., 1988b; beneficial fungi in the hive (e.g. Penicillium and Aspergillus sp.) have the ability to inhibit the growth of pathogenic hive fungi; produce antibiotics that contribute to the bee bread storage/conversion process. The pollen that is collected is mixed with saliva containing enzymes and microorganisms from the honey bee gut and nectar, and thereby the mixture becomes “beebread” which is a nutrient storage medium that resistant to pathogenic microbes (Human and Nicolson, 2006). The growth and development of the colony relies heavily on this process because the stored beebread is later consumed by nurse bees and converted to royal jelly, a pre-digested and nutrient rich food distributed throughout the colony to the queen, workers and developing larva (Winston, 1987).

From the present study, it was observed that the life cycle of A. c. indica is very similar to that of A. mellifera. They go through a number of growth stages and reached adulthood by completing their larval and pupal stages. Their gut cells also shows great alterations during the metamorphic period that partly also change due to change in
nutrition. Changes in organic compounds like proteins, lipids, sugars and vitamins within the individual worker play a very important role in the regulation of honeybee development, growth, function and social behaviour. Thus, the young and the adult diet typically differ, and in another way benefit and prevent the older bees to competing with the brood for resources (Tales from the Hive, 2000) and there will be less competition for nourishment among individuals of the colony (Wang and Byarlay, 2015).

Honeybees forage on flowering plants and can accrue all of their nutritional requirements from the pollen and nectar (Herbert and Shimanuki 1978c; Morgano et al. 2012). The nutritional composition is also largely dependent on the local and seasonal availability of pollens from different plant species. However, not all flowering plants offer the same amounts or blends of nutrients. Thus, the availability and diversity of forage available to honeybees will vary not only with the local landscape composition, but also on the nutritional content of the pollen and nectar that these plants provide (Keller et al., 2005 a, b). According to Donkersley et al. (2014); spatial variation in the nutritional composition of beebread also correlates with several landscape types. This may affects the biochemical compounds and there will be differences in the level of these biochemical compounds from one region to another in the area.