EFFECT OF THE ACCESSORY PROTEIN FEEDING ON HONEY BEE WORKER TISSUES AND HYPOPHARYNGEAL GLANDS

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Abstract

The accessory protein diets major applied with most bee-keepers; dried medical yeast, baby milk powder and pollen grains were investigated in order to test their physiological reflections on worker honey bee tissues and glandular secretion of the royal jelly the final secretion of the hypopharyngeal glands which indicated privileged chemical properties with all tested foods particularly with the pollen grains caused increase the secretary globule densities, synchronism that with presence physiological active exceeding in the proliferating of the small digestive cells of the mid gut tissues and the fat bodies in the integument of the body cavity. The milk as supported protein let to higher synthesis of the connective tissues of the hypopharyngeal glands tissue followed with the medical dried yeast. The cytoplasmic albuminoidal granules of the fat cells and the Oenocytoids showed more densities with all tested protein diets particularly with pollen grain treatment.

INTRODUCTION

Feeding honey bee colonies with propped protein nutrition consider definite matter particularly at pollen and nectar dearth periods and to exaltation the utilization of the food present and help bee colonies to surmount on the malnutrition particularly at unsuitable conditions. The pollen substitute used appeared to be a valuable pertinacious food cause some changes in the cellular system of the honey bees (Szymas and Jedruszuk,2003). Faithful reproduction of pollen natural feed of all bees is not easy because it contains a wealth of substances of these protein is the basic components. It contain along with endogenous amino acids and all exogenous amino acids essential for bees (Rogala and Szymas,2004[a]) . Pollen grains differ widely in their size, shape, ratio of the digestible contents and chemical composition (Velthuis,1992). The normal protein source for honey bee workers as bee bread and date palm pollen were the best sources for hypopharyngeal gland development (Abdilla,2005). The development and physiological activity of the food glands vary with work of bees which being fully functional when the work is serving in the hive as nurse bees feeding larvae and queens. The hypopharyngeal gland consider the best parameter reflects nutrition qualitative in honey bee food with best assimilation in gut.

It often used to describe the physiological status of honey bee workers and consider arbitrary scale classification have been estimated by different methods as acinus's size (Hrassning and Crailsheim, 1998). Several mal factors can be affected on worker bee gland secretions caused sever damage to hypopharyngeal, mandibular and head salivary glands affected on their products. This study is interested to investigate the physiological reflects of the accessory protein nutrition on honey bee worker tissues and glandular secretions and to how far it could utilized from them.

MATERIALS AND METHODS

I-Tested bee colonies

The present work was carried out in the apiary of the Plant Protection Research, Institute Apiculture Research Center during late summer season, 2009.

Twelve honey bee colonies of Carniolian hybrids were conducted for this study. The bee colonies were divided into four groups (Three colonies / each treatment). Three groups were fed on sugar solution (½ liter / colony in rat of (2 w sugar: 1 V water) contained one gram of the following protein diets; dried pollen grains, dried medical yeast and baby milk powdered without fats. These diets were added to the 1st , 2nd and 3rd treatments, respectively. The other 4th group was fed only with sugar solution (as control). These nutritious were replaced every three days during the experimental period which persist two months. Doolittle's scientific queen rearing method (Doolittle,1864) was carried out in the end of the experimental period /each tested groups which fed daily on the previous tested diets/ group.

II-Morphological studies

Fifty individuals of nurse bees of unknown age were collected directly from workers fed the queen larvae in unsealed queen cells from the queen builder colonies. The hypopharyngeal glands were separated in presence of saline solution (0.09% Na Cl) as the method described by Wang and Moeller, (1971). Length and width of the acinus's gland were measured as well as the mean surface area according to the formula of Maurizio, (1954); The surface area = II x ab/2 (II=3.14, a= maximum length, b= maximum width).

III-Histological studies

Serial sections of tested nurse bees were made using the method described by Gad,(1951). The histological changes were evaluated in both of the ventriculus, hypopharyngeal glands and fat bodies.

VI- Biochemical analysis

The royal jelly was collected from queen cells 3 days old and analyzed according to the method of Lammelli, (1970) using the Polyacrylamide Gel Electrophoresis technique (PAGE) .

RESULTS AND DISCUSSION

I- The ventriculus

The tested ventriculus of worker bees fed on different protein diets showed higher physiological specific differences. The generated cells which analyzed and generated others found in end of the Epithelial cells of the ventriculus of worker bees showed higher numbers with the nutrition of the pollen grains (Fig.1d) than hose recorded with other treatments (Fig.1 a,b,c). A large number of the proliferated of the small digestive cells with worker bees fed on the pollen grains than those fed on other different protein diets or control. These liberated cells are most probably charged with digestive enzymes. The Peritrophic membrane was conglutination with the walls of mid gut tissues in treated bee workers with the pollen grains indicated the active state. The Epithelial cells contains Calcium particles were present in the mid gut tissues of worker honey bees fed on the milk protein. The Calcium appears in the form of minute crystals massed in the inner ends of the ventricular cells (Fig.1c). Calcium is commonly thus excreted by insects, its excretion is probably merely a process of getting rid of the Calcium unavoidably taken in with the food , the insect having little need of Calcium in its tissues (Snodgrass,1984). Sort of the food present to honey bee food can be reflects on the digestive physiological activities.

II-The hypopharyngeal glands (HG)

Separated the hypopharyngeal glands (HG) from tested worker bees showed higher measurements with the feeding on various protein diets than those fed only on the sugar solution with significant differences (Table1). Their maximum measurements were detected with the dried medical yeast treatment . The cytoplasmic tissues of the (HG) were more compacted in the cytoplasm formulation particularly with the baby milk powered treatment with nucleus had not clear appearance followed with the medical yeast treatment (Fig.2c&d). The secret globules of the (HG) showed more densities and large size with pollen grains nutrition (Fig.2d) than those fed on other protein diets (Fig.1a&,b). It could be concluded that all tested proteins recorded the higher measurements, while pollen grains positively affected on the secret globules development of the (HG) and qualified of the secret (HG) globules.

TABLE (I) : The hypopharyngeal glands measurements of the worker honey bee fed on different protein diets.

Treatment	Acinus's of the Hypopharyngeal Glands (μ)		
	Length	Width	Area (μ2)
Sugar syrup (control)	108.174c	81.8516d	13901.1d
Dried medical yeast	121.451a	90.638b	17282.8a
Pollen grains	105.929d	94.529a	15721.0b
Baby milk powder	115.287b	84.29c	15256.5c
$LSD_{0.05}$	1.882	1.838	1.338

III- Fat bodies

Fat cells the main source of the fountain energy provide the insect body its requirements, while Oenocytoids seem to have something to do with production of wax in the abdominal wax glands of worker bees showed clearly differences with all tested protein diets. The cytoplasmic tissue of the fat cells showed clear borderlines between their reservoirs with the protein milk than other proteins and control one. While the cytoplasmic albuminoidal granules of the fat cells and the Oenocytoids showed the more densities with all tested protein diets particularly with pollen grains treatment (Fig.3).

VI- Royal Jelly Analysis

The royal jelly as end secretion collected of nurse bees fed on different protein diets were analyzed for detected how long these treatments can be affected on its components. Data presented in Table (2) and Fig.(4) showed that there are clear differences between different protein diets and sugar syrup treatment. Also between pollen grains and other tested protein diets from the other side. Medical yeast and baby milk powder revealed presence 7 protein bands in the royal jelly while pollen grains treatment excellence by presence 8 protein bands more by one protein which had 12 (kDa.), molecular weight. That may play an essential role for enzymes secretion.

TABLE (II) : Royal jelly separated proteins of worker bees fed on different protein diets.

Band Protein No.	Royal jelly proteins (kDa.)				
	Protein standard	Sugar syrup (control)	Medical yeast	Pollen grains	Baby milk powder
1	66	66	66	66	66
2		64	64	64	64
3		62	62	62	62
4	45	50	50	50	50
5	14			12	
6			10	10	10
7			8	8	8
8		5	5	5	5
Total		5	7	8	7

kDa., Kilo Dalton.

From general obtained results, it could be concluded that milk proteins consider the more important supplemental nutrition followed with the medical dried yeast. It force supported the connective tissues can be utilized particularly at the critical periods of the bee life. The Calcium particles present in the epithelial cells of the mid gut exceeded excretion getting ride of them. In the other side the pollen grains as accessory natural proteins have important private nutrient for the glandular secretion to creation new proteins known royal jelly. Bastos *et al.*,(2004) suggested that pollen grains as a nature product contains specific fats not found in the other tested nutrition foods that may be play an important role in the physiological activities reverberate on the digestion process and functional structure of the tissues and bee gland secretion. It consider as a good source of unsaturated fatty acids. The worker body tissues showed the higher physiological responses especially with the pollen grains and milk protein additions. It could be established that use the milk proteins as supplemental diets help to quickly building the connective tissues, while pollen grains can be encourage hypopharyngeal gland royal jelly secretion.

Brouwers,(1982) decided that neither size of the acini's of the (HG) nor the protein content can be consider a reliable parameter for (HG) development, while Rogala and Szymas, (2004)[b] reported that faithful reproduction of pollen, natural feed of all bees, is not easy because it contains a wealth of its substances. Of these, protein is the basic component. It contains, along with endogenous amino acids and all exogenous amino acids essential for bees. In addition the poorest developed pharyngeal glands and fat bodies were recorded in bees which were fed a non-supplemented pollen substitute. The haemocyte numbers in the haemolymph as the nutritional value of the pollen substitute protein was increased. Hrassning and Crailsheim (1998),

reported that the development of (HG) is considered as parameter reflects nutritive quality in honey bee food with best assimilation in gut. Zakaria, (2005) found sharply decreased in the enzymes activity of (HG), honey sac contents and the haemolymph of infected honey bee workers with the nosema disease that may be affected on the physiological statues of honey bee members. Cost and da Cruz-Landim , (2005) recorded that the hypopharyngeal gland is rich in enzymes. Fifteen different enzymes were found in the extracts, with only a few quantitative differences between the bee species. Some of the enzymes present in the extracts may have intracellular functions, while others seem to be digestive enzymes. Honey bees use protein of pollen mainly to provide structural elements of muscles, glands such as; hypopharyngeal and venom glands and other tissues. The changes occurred in integument structure of honey bees provide insights that permit an improved physiological characterization for staging pupal and pharate adult development (Neto *et al.*,2009).

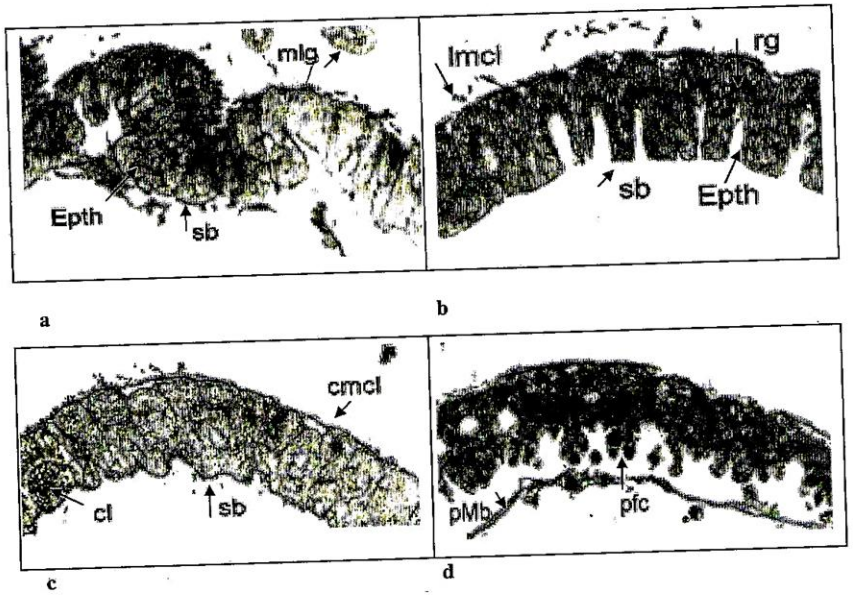


Fig.(1): Histological details of the ventriculus of worker bees fed on different protein diets. **A**: The sugar solution t. **B**: The dried medical yeast. **C**: Baby powder milk. **D**: Pollen grains. **rg.**: Regenerative cells. **Epth**. Epithelium Cells. **Pfc**: proliferating of small digestive cells. **pMb**: Peritrophic membrane. **sb**: started bordered. **cl**: Calcium particles. **lmcl**: longitudinal muscles. **cmcl**: Circular muscles. **mlg:** Melpighian tubules (**x**- 400).

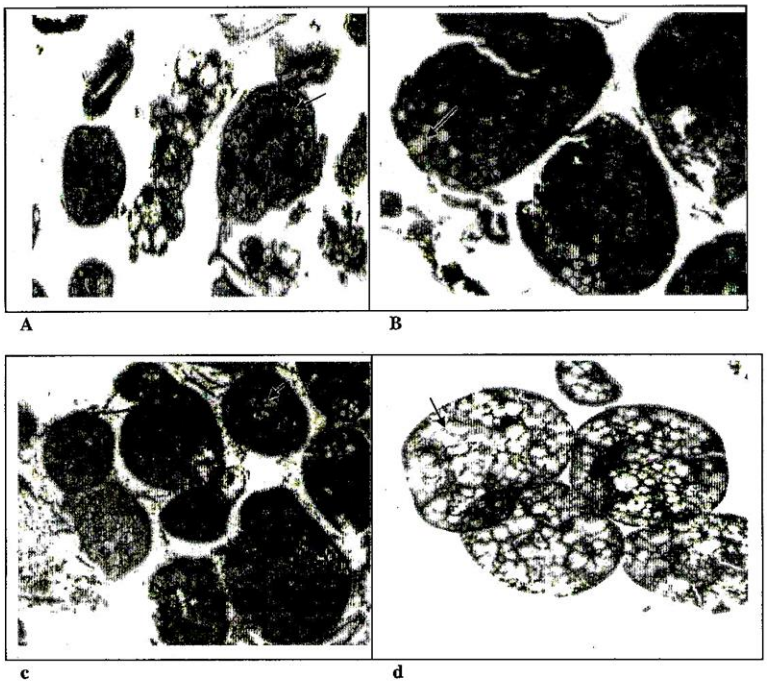


Fig.(2): The hypopharyngeal gland lobules of worker honey bees fed on different protein diets; **A**: Only sugar solution as control. **B:** Dried medical yeast. **C**: Baby milk powder. **D**: Pollen grains. Secrete globule cells (black arrow). (**x**-400).

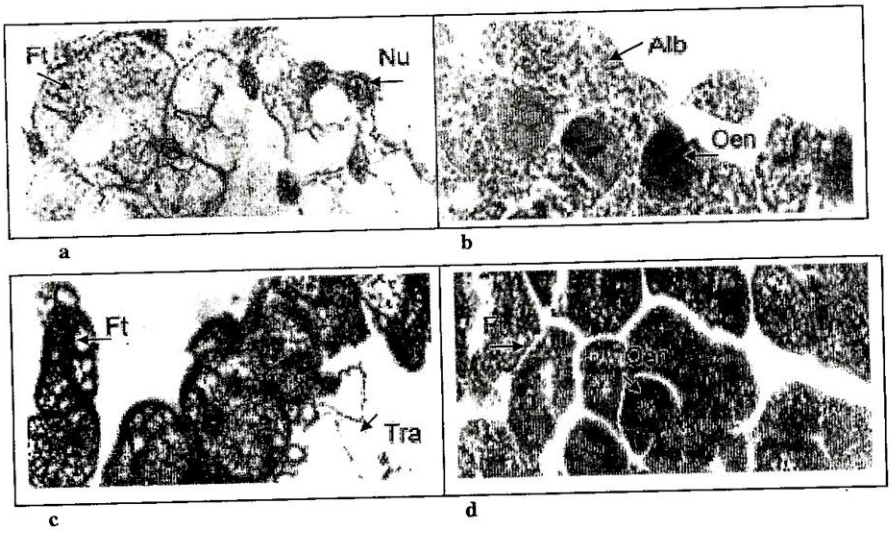
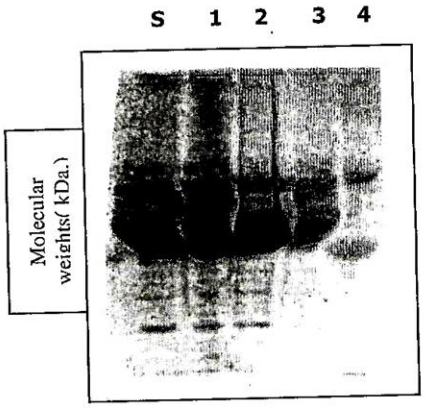


Fig.(3) : The fat bodies of worker bees fed on different protein diets; **a:** Sugar solution. **b:** Dried medical yeast. **c:** Baby milk powder. **d:** Pollen grains. **Alb:** Albuminoidal granules. **Ft:** Fat cells. **Tra:** Trachea. **Oen** : Oenocytoids. **Nu:** Nucleus (x-160).



10% Polyacrylamide Gel Electrophoresis

Fig.(4): Polyacrylamide Gel Electrophoresis of the royal jelly proteins fed on different protein diets.

1- Sugar solution (control). 2- pollen grains.

3- Medical yeast. 4- Baby milk powder.

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تأثير التغذية البروتينية الإضافية على أنسجة شغالات نحل العسل وغدد الغذاء الملكى

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التغذية البروتينية الإضافية الأكثر استعمالا لدى النحالين مثل الخميرة الجافة، اللبن المجفف وحبوب اللقاح تم بحثها لدراسة التأثير الفسيولوجى لها على أنسجة شغالات نحل العسل والغدد المفرزة للغذاء الملكى. ولقد حققت تلك التغذية تأثيرات متميزة فى خصائصها مع كل المعاملات خاصة مع حبوب اللقاح والتى أدت الى زيادة نشاط الفصوص الإفرازية لهذه الغدد وإفراز الغذاء الملكى ، تزامن هذا مع وجود نشاط للخلايا الحرة الإفرازية داخل القناة الهضمية وكذلك الأجسام الدهنية. اللبن المجفف المضاف كغذاء بروتينى يليه التغذية بالخميرة الجافة أديا الى سرعة تخليق وبناء الأنسجة الضامة فى نسيج غدد الغذاء الملكى. حبيبات الألبيومين فى الأجسام الدهنية وكذلك ال (Oenocytoids) أظهرت كثافة عالية مع كل المعاملات البروتينية خاصة مع حبوب اللقاح.