

Factors Affecting On The Food Metabolism In Some Honey Bee Races

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Abstract: The food metabolism activity in between some honey bee races were tested through determination the acid phosphatase concentration, total proteins, and the protein differentiation in the haemolymph, preitrophic membrane and whole midgut tissues of honey bee workers of Egyptian race (*Apis mellifera lamarckii*), Carniolian race (*A. m. carnica*) and Italian race (*A. m. ligustica*). The acid phosphatase concentration was detected as a hydrolytic enzyme activity and energy carrier. The total protein and the electrophoretic proteins were estimated to correlate between their concentrations and the acid phosphatase activity. The total acid phosphatase concentration recorded the highest values in the haemolymph of honey bee workers followed with midgut tissues and preitrophic membrane particularly with Egyptian bee race followed with Carniolian and Italian bee races related that with the total protein concentration in the midgut present.

Key words: Honey bees, *Apis mellifera*, Food metabolism, Digestive enzymes, Acid phosphatase, Proteins, Haemolymph, Midgut tissues, Preitrophic membrane

INTRODUCTION

The productivity of honey bee colonies depends upon many factors as race of bees, rich nutrition of pollen resources, good preparation of such colony to new season and controlling bees from diseases. Honey bee require proteins, carbohydrates, lipids, vitamins, minerals and water. These nutrients must be in the diet in a definite qualitative^[25]. The food metabolism play an important role in bee life. The physiological digestion different in bee body composition; percentage of glycogen, lipids and proteins^[15]. The source of feeding honey bees effect on induction haemolymph enzymes^[3]. Fourteen enzymes activity were determined in the midgut of *Apis indica* and 7 in the crop. These enzymes were; alpha- amylase, 4 alpha glucosidase, 3 protease, 5 aminopeptidases and lipase^[1]. The digestive enzymes of honey bee workers may have been contributed by microorganisms. The highest concentrations of these digestive enzymes present in the midgut of honey bee workers were leucine aminopeptidase, acid phosphatase and alpha-Glucosidase^[11]. The protein directly involved with carbohydrates and energetic metabolism were; alpha glucosidase, glucose oxidase and alpha amylase, whose are members of same family of enzymes, catalysing the hydrolysis of the glucosidic linkage of starch, alcohol dehydrogenase and aldehyde dehydrogenase^[24]. The acid phosphatase activity was present apparently as a result

of cellular autolysis^[9]. Mucopolysaccharides were found in the basal membrane of the midgut epithelium cells and preitrophic membrane of *Apis mellifera carpathica*. The main function of the preitrophic membrane was to distribute the digestive enzymes evenly through the midgut^[28].

The aim of this investigate is to study some factors affecting on the food metabolism in some honey bee races.

MATERIALS AND METHODS

This work was carried out in the Dept. of Apiculture, Plant Protection Research Institute, Dokki, Cairo, Egypt, during summer season 2006. Fifteen honey bee colonies were conducted for this study. The tested bee colonies were classified into three groups as follows; Egyptian race (*Apis m. lamarckii*), Carniolian race (*A. m. carnica*) and Italian race (*A. m. ligustica*) (five colonies per each). The worker bees used in this experiment were captured from nurse combs of the tested bee colonies. The following procedures were carried out.

1- The Haemolymph Collection: The pure haemolymph samples were collected according to the following technique based on the method of Gilliam and Shimanuki,^[10] as follows; 1- Anaesthetized bee samples in cold condition at + 4° C (one hundred of

nurse bees/ colony). 2- Separated out the head antennae. 3- Slightly pressure on the thorax and collecting the extracted haemolymph through micropipette and kept it after adding traces of an anticoagulation powder at -4° C till used.

2- The Whole Midgut Tissue: Fifty individuals of the whole midgut tissues from nurse bees of the tested honey bee colonies were dissected out in presence of saline solution (0.9% Na Cl) and stored at -20° C. The mid gut extract was obtained by the homogenized and centrifuged at 10 000 rpm for 5 min. period. The supernatant was dissolved in 100 µl of distilled water and kept at -20° C till used. The technique for collecting the whole midgut tissue based on the method of Cavalcante and Landim^[5].

3-The Preitrophic Membrane: The preitrophic membrane with its food contents were separated and collected from the midgut of the nurse bees according to method of Cavalcante and Landim^[5].

The Tested Chemical Analysis: The following procedures were conducted on the haemolymph samples, supernatant of the hole midguts and the preitrophic membrane.

- 1- Separated the proteins was routinely performed on a gradient Polyacrylamide Gel Electrophoresis (SDS-PAGE) according to method of Weber & Osborn^[26] and Kubicz & Galuska^[19].
- 2- Estimated the total protein concentration according to the method of Bradford^[4].
- 3- Determined the acid phosphatase enzyme activity according to method of Hillmann^[14].

RESULTS AND DISCUSSIONS

Data presented in Table(1) and Fig. (1) indicated clearly differences in the electrophoretic protein patterns between the haemolymph, peritrophic membrane and hole midgut tissue in honey bee workers of different tested Egyptian, Carniolian and Italian races.

The haemolymph of honey bee workers of Egyptian race recorded 23 protein bands with molecular weights (MW) ranged between 33.517-195.768 (kDa.), while Carniolian workers recorded 22 protein bands with (MW) ranged between 33.306 & 181.003 (kDa.), whereas the lowest value was recorded with Italian race which recorded 21 protein bands with (MW) ranged between 33.737 and 195.348 (kDa.). Fifteen protein bands were presented in the midgut tissue of nurse honey bee workers of *A.m. lamarckii* and *A. m. carnica* with (MW) ranged between 33.381&132.847 (kDa.), and 32.776 &130.62 (kDa.) respectively, while honey bee workers of *A. m. ligustica* recorded the lowest 13 protein bands with (MW) ranged

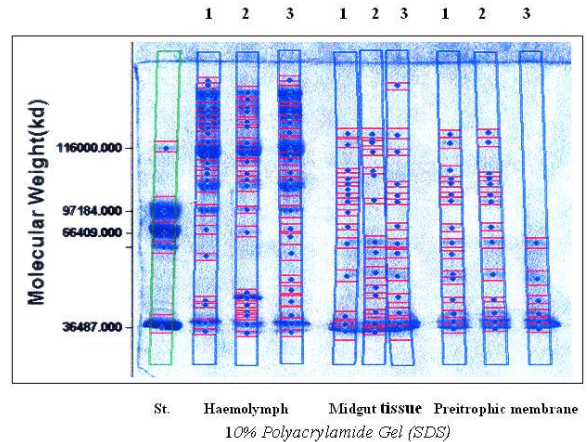


Fig. (1): The Electrophoretic protein patterns of the haemolymph, midgut tissue and preitrophic membrane of worker honey bees.

St: Protein standard.

1- Egyptian race.

2- Carniolian race.

3- Italian race.

between 33.621&131.148 (kDa.).The preitrophic membrane of worker honey bees of *A. m. lamarckii* recorded highest 16 protein bands with (MW) ranged between 33.919 – 131.808 (kDa.), followed with *A. m. carnica* which recorded 14 protein bands with (MW) ranged between 33.675&133.722 (kDa.), while the lowest value was detected with honey bees workers of *A.m. ligustica* which recorded 6 protein bands with (MW) less than 62 kDa., and ranged between 34.068 -61.967 (kDa.).

From obtained results showed in Table (2), it could be concluded that the total protein concentration was higher in the haemolymph and midgut tissues of honey bee workers of *A. m. lamarckii* followed by those recorded with Carniolian and Italian bee races. Italian race recorded equal protein concentration in the mid gut tissues and preitrophic membrane. Clear significant differences in the total protein concentration present in the haemolymph and preitrophic membrane were recorded between different tested bee races.

Data illustrated in Table (3) revealed highest equal levels of the total acid phosphatase concentration present in the haemolymph of nurse honey bee workers of different bee races in comparison with those recorded in the preitrophic membrane and mid gut tissues, while higher enzyme concentration was recorded in both midgut tissues and preitrophic membrane of Egyptian race followed with Carniolian and Italian races, respectively. Equal secretion of the acid phosphatase enzyme was recorded in the midgut tissues and the preitrophic membrane of Italian bee workers, that may be related to the equal protein determined in these organs. Significant differences in the total acid phosphatase

Table 1: The molecular weights (kDa.) of the Electrophoretic proteins of different organs of honey bee workers and different bee races.

Band No.	Protein St.	Haemolymph			Midgut tissues			Preitrophic membrane		
		<i>A.m. lamarckii</i>	<i>A.m. carnica</i>	<i>A.m. ligustica</i>	<i>A.m. lamarckii</i>	<i>A.m. carnica</i>	<i>A.m. ligustica</i>	<i>A.m. lamarckii</i>	<i>A.m. carnica</i>	<i>A.m. ligustica</i>
1		195.768		195.348						
2		190.170								
3		177.619	181.003	176.210						
4			172.574							
5		160.604	162.589							
6		158.155	158.218	156.283						
7		152.848		151.088						
8		143.984	147.046	145.082						
9		140.344	142.850							
10		135.172		135.597	132.847	130.624	131.148	131.808	133.722	
11						126.063				
12		126.900	126.797		122.908	123.529		123.608	125.529	
13			121.927	120.874						
14	116.00	115.726	119.460	116.856		155.444	116.689			
15		109.762	111.404	112.021						
16		105.177			102.743	101.168		102.634		
17		101.647	102.137	102.077		97.801			100.009	
18		97.069	97.713	97.523	96.513			96.411	96.980	
19					93.305					
20		92.856	93.960	92.858	89.898		92.847	93.522	93.083	
21					86.470		86.063	86.234	86.194	
22					82.472	82.435	82.471	82.526	82.448	
23	97.184	78.285	79.382	78.816	76.560					
24		68.644	67.870	69.315	68.941		69.274	68.639	68.788	
25	66.409			65.554						
26	45.000				61.455	61.513	61.532	61.705	61.768	61.967
27		56.894				57.292	57.309	57.089		
28								49.448		
29				48.522	49.163	49.616	49.043	47.885	49.210	48.139
30			44.188	45.044		44.750				
31		41.630	42.635	41.604		42.319	42.183	41.406	41.128	41.469
32		40.302	40.512							

Table 1: Continued.

33		38.560	37.847						
34		37.651		37.070	37.783	37.851	37.730	37.631	37.715
35	35.641	35.473	35.673	35.000	34.835	35.194	35.563	35.386	35.784
36	36.487	33.517	33.306	33.737	33.381	32.776	33.621	33.919	33.675
Total protein bands	23	22	21	15	15	13	16	14	6

A.M.: *Apis mellifera*.
St.: Standard.
kDa.: Kilo Dalton.

Table 2: Total protein concentration of the haemolymph, preitrophic membrane and hole midgut tissues of nurse honey bee workers at different bee races.

Race of bees	Determination of Total protein concentration		
	Haemolymph (mg/ml)	Midgut tissues (mg/gm)	Preitrophic membrane (mg/gm)
<i>Apis mellifera</i>			
<i>lamarckii</i>	37.40 a	30.5 a	27.3 b
<i>A.m. carnica</i>	30.25 b	30.3 a	24.5c
<i>A.m. ligustica</i>	30.25 b	30.0 a	30.0 a
LSD _{0.05}	0.411	F=0.367	0.199

Table 3: Total acid phosphatase concentration of the haemolymph, preitrophic membrane and midgut tissues of nurse honey worker bees at different honey bee races.

Race of bees	Determination of Total acid phosphatase concentration (Diagnosticum Kit)		
	Haemolymph (U/L)	Midgut tissues (U/gm)	Preitrophic membrane (U/gm)
<i>Apis mellifera</i>			
<i>lamarckii</i>	0.125	0.022a	0.017a
<i>A.m.carnica</i>	0.125	0.017b	0.015a
<i>A.m.ligustica</i>	0.125	0.013c	0.013a
LSD _{0.05}	F=0	0.00399	F=1.5

U/L: Unit/ Liter= (/min.x750).

The changes of absorbance/minute (A/min.)

concentration in the midgut tissues were recorded between tested bee races. It could be conducted that presence relation ship between acid phosphatase enzyme secreted in the midgut tissues and total protein concentration found.

From obtained results it could be concluded that honey bee workers of Egyptian race distinguished by higher protein bands present in the haemolymph, midgut tissues and preitrophic membrane followed with Carniolian and Italian bee races, respectively. The higher levels of the total protein concentration in the haemolymph, midgut tissues and preitrophic membrane were recorded with Egyptian bee workers followed with Carniolian and Italian bees, respectively. The total acid phosphatase concentration recorded the highest values in the midgut tissues followed with preitrophic membrane particularly with

Egyptian race followed with Carniolian and Italian bee races and its secretion depends on protein values in the midgut present as the haemolymph protein concentration.

It could be summarized that the food metabolism levels were more activity in the honey bee workers of Egyptian race followed with Carniolian and Italian races, respectively depends on protein concentration present in the midgut and those found in the haemolymph. Thus may be reflects on the digestion levels markedly with acid phosphatase enzyme. It could be estimated that the higher concentration of the acid phosphatase enzyme of honey bees workers related with the amount of the protein diet consumed. The different metabolism activity between bee races may be attributed to one or more than factor as different cellular secretion rate and protein concentration in the midgut present. Its noticeable to not that utilization value of the food consumed play the important role in the digestive process thought different between bee races. The protein consumption would initiate both digestive enzymes synthesis and bee glands secretion.

The obtained results are agreement with the finding of. Miao *et al.*^[21] they found significant differences in the digestive enzymes between *Apis mellifera ligustica* and *Apis cerana cerana*. The total protein patterns and acid phosphatase activity in the haemolymph of queens, drones and workers honey bees were differences between sexes and castes^[22]. Romoser and Stoffolano^[23] reported that succinate, malate, fumarate, lactate, pyruvate, ∞- ketoglutarate and several organic phosphate compounds are the major acids found in insect blood. The first seven of them are formed during the kreb cycle. They had been suggested that these acids are important in balancing the cations in the blood. Jimenez and Gilliam^[17,18] related the highest concentrations of these enzymes and the trypsin activity of honey bee of *Apis mellifera* with the amount of protein diet consumed, bees aged and season of year. Barabanova^[2] related the differences in the enzyme

activity, protein and carbohydrate composition of bee blood to seasonal and the developmental differences. Landim *et al.*^[20] determined the nuclear acid phosphatase activity in the somatic and germ cells of honey bee worker ovaries and in the midgut cells of metamorphosing bees and related the differences of the nuclear functional state to the cell functions in these tissues. Gilliam *et al.*^[11] and Jimenez & Gilliam^[16] decided that the enzymes present in highest concentrations of honey bees midgut were leucine aminopeptidase, acid phosphatase and alpha-glucosidase. Alkaline phosphatase activity was localized on the elongate microvilli of the striated border and within large electron- lucent microbodies of the ventriculus of the honey bees. Standifer^[25] recorded that the digestion process of honey bee workers depends on the activity of enzymes present in secretions of the salivary, postcerebral and hypopharyngeal glands and in the secretions of the midgut epithelial cells. Weirich *et al.*^[27] found that some enzymes activity was higher in the ventriculus than those found in the haemolymph of honey bees workers. Delage and Darchen^[8] compared 19 digestive enzymes of the salivary glands and the midgut of the *Melipona beecheii* with the results for *Apis mellifera* and for an African stingless bees, *Apotrigona nebulata* and concluded that although these 3 species have comparaple glands and fundamentally identical types of diets, the differences in enzymes complements suggest that these bee have different metabolisms probably as the result of adaptations to foraging on different floral resources. Gregorc and Bowen^[12] found free acid phosphatase and alkaline phosphatase in the basal area of the midgut epithelial cells and the former also occurred in the haemocoel. Duca *et al.*^[9] assumed the activity of the acid phosphatase enzyme after infected the midgut of honey bee workers by *Nosema* spores to the cellular autolysis. Gregorc and Bowen^[13] reported that bacteria which passed through the midgut epithelium cells of honey bee workers produced signs of lytic function in the haemocoel. Chorbinski^[6] found not significant differences between the activities of non- specific esterase, alkaline, phosphatase and acid phosphatase in the midgut of worker bees fed on Apitol and exposure to Apistan strips. Chorbinski and Tomaszewska^[7] found decreased in the acid phosphatase and alkaline phosphatase activities in the epithelial cells of honey bee midgut after application of Warrosect.

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