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Changes with Age in Amino Acids and some Mineral Constituents of Worker Honeybees

Introduction

Although honeybees (*Apis mellifera* L.) has been chemically analysed for dry matter and nitrogen content (Groot, 1953; Haydak, 1959; Dietz, 1971; Konopaka and Muszynska, 1981), mineral content (Dietz, 1971; Nation & Robinson, 1971; Matsuyama et al., 1974), little informations are known about their amino acids except for those of Fujii et al., 1962; McLellan, 1976; El-Shakaa, 1985 who studied the amino acid contents of adult worker honeybees.

The nitrogen content increase during the early part of the life of a bee (Dietz, 1971a; Ursu, 1981). Variations in the amino acid composition of insects have been attributed either to the effects of flight metabolism (Bursell, 1963) or to dietary absorption into the haemolymph (Auclair, 1959). Furthermore such products as uric acid, the principal end-product of nitrogen metabolism in insects, was partly converted to glycine by acid hydrolysis (Shannon, 1971), this convertion may be responsible for some of the variation of this amino acid. Also, highly variable results are to be expected for cystine, as it is susceptible to destruction during acid hydrolysis (McLellan, 1976).

Minerals are not essential in the diet of adult honeybees for the growth and development of their hypopharyngeal gland. However, the possibility that sufficient minerals are acquired during the larval period cannot be ruled out. In addition, impurities often found in test diets may also provide the insect with the necessary minerals (Haydak and Dietz, 1965). On the other hand, National and Robinson (1968) found that addition of a small amount of pollen ash to an artificial diet for adult honeybees, *Apis mellifera*, improved their ability to rear blood.

The mineral constituents of bees generally declined with age Dietz Z. 1977a. Mineral contents in nurse bee were higher than that obtained in forager bees during different seasons in dry weight and nitrogen content of worker bees with age, especially in and within the same seasons (El-Shakaa 1985), the thoraces (Haydak 1959; Dietz and Hadak 1965). However, the dry weight and the nitrogen content in older bees (28 days) without digestive tracts were decreased (Groot, 1953).

This study is part of obtain the necessary information for amino acid and mineral constituents of honeybee workers at different ages.

Materials and Methods

In August, a comb of emerging brood was selected at random from a normal colony in the experimental apiary of the faculty of Agriculture, Zagazig University. Comb was placed in the incubator at 33—34 °C and left over-night. The next day, newly emerged bees were marked with a white paint spot on thorax (without the use of any anaesthetic) and returned to the same colony. At 5 day intervals afterwards

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marked bees were removed from the colony killed with chloroform and removed the white paint then dired at 70 °C until reached a constant weight. Before direct analysis, samples were ground as finely as possible.

Analysis for Crude Protein:

The microkjeldahl technique for nitrogen was used to determine the crude protein (N X 6.25) content according to the procedure of (A.O.A.C., 1965).

Analysis of Amino acids:

All analyses were performed on an LKB 451 Alpha Plus Amino Acid Analyser (Andrews and Balder, 1985).

Analysis for Minerals:

Potassium, sodium, magnesium, calcium, manganese, copper, zinc and iron were determined in sample solution. Analysis were carried out by a Pye Unicam Model Sp 1900 Atomic Absorption Spectrophotometry.

Phosphorus was determined in the samples by the colorimetric method as described by Murphy and Riely (1962).

Results and Discussion

Data for the crude protein and amino acid contents (calculated as g/100 g drywt.) of worker bees during different ages are shown in Table 1. It could be concluded that there are great variations in protein content of honeybee workers. The highest protein content appeared in 1st day of the life (69.0 %), followed by 10-days old bees (56 %). Twenty days old bees had the lowest protein content (41.3 %). Concerning the protein amounts of 5th and 15th days of the life, it were approximately similar (48.5 and 48.3 %, respectively).

The nitrogen content during the early life of a bee is high and declined with age. The results obtained by Ursu (1981) may explain the data obtained in the present study. Ursu (1981) found in the bodies of young bees the protein content was 72.1—76.6 %. On the 8th—10th day of the life the protein amount decreased by 5—11 % in spring and by 1.9—8.3 % in autumn. As bees become older the protein content gradually decreases.

Most of the protein in flying insects in flight muscle, of which one third is mitachondrial protein (Bartelink and De Kort, 1973) although the protein levels varied greatly in this way, the percentage of individual amino acids was relatively constant, differing by less than 2 % between different ages, with the exception of threonine, lysine and aspartic acid (less than 3 %); glutamic acid, glycine and alanine, the differences more than 3 %. Glutamic acid was the predominant amino acid in all samples, except 5 days old bees that alanine was considered the highest one. Aspartic acid, leucine and glycine were also found in highe levels. The present investigation was coincided with the results obtained by McLellan (1976). Cystine was found the lowest amino acid with no considerable variations in the worker bees during different ages. Variations in the amino acid composition of insects have been attributed either to the effects of flight metabolism (Bursell, 1963) or to dietary absorption into the haemolymph (Auclair, 1959), and in worker honeybees the relative activity of glands producing larval food may be also implicated. It is possible that in worker bees all three factors are involved in the variations observed (Table 1). Furthermore as uric acid, the principle end-product of nitrogen metabolism in insects, is partly converted to glycine by acid hydrolysis (Shannon, 1971), this conversion may be re-

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Table 1

Percentage of crude protein and total amino acids (g/100 g dry weight) of honeybee workers during different ages.

Protein % Amino acids	Age (days)					Total	General mean
	1	5	10	15	20		
Crude protein	69.0	48.5	56.0	49.3	41.3	264.1	52.82
Aspartic acid	4.36	3.17	5.20	3.95	3.37	20.05	4,01
Threonine	2.75	1.80	2.55	1.80	1.44	10.34	2.07
Serine	3.77	2.73	3.03	2.38	1.92	13.83	2.77
Glutamic acid	7.50	4.38	6.55	5.30	4.26	27.99	5.59
Proline	4.52	3.66	3.62	4.01	3.05	18.86	3.77
Glycine	5.53	4.42	3.83	3.58	2.52	19.88	3.98
Alanine	6.69	4.70	4.50	3.66	3.17	22.72	4.54
Cystine	0.32	0.34	0.40	0.22	0.24	1.52	0.30
Valine	3.61	2.78	2.64	2.39	1.80	13.22	2.64
Methionine	1.03	0.70	1.04	0.72	0.74	4.23	0.85
Leucine	4.64	3.47	4.40	3.97	2.75	19.23	3.85
Isoleucine	3.11	1.82	2.63	2.47	1.70	11.73	2.35
Tyrosine	2.61	1.52	2.15	1.57	1.37	9.19	1.84
Phenylalanine	1.86	1.29	1.90	1.27	1.53	7.85	1.57
Lysine	4.11	2.04	3.20	2.01	1.48	12.84	2.57
Histidine	2.14	1.49	1.40	1.39	0.85	7.28	1.47
Arginine	2.41	1.44	2.65	1.76	1.44	9.70	1.94
Total recovered	129.96	90.25	107.69	91.69	74.94	494.53	494.56

sponsible some of the variation of this amino acid. Also, the low level is to be expected for cystine, as it is susceptible to destruction during acid hydrolysis.

Concerning the mineral contents, the results are given in Table 2.

Table 2

Mineral constituents of the whole bodies of worker bees during different ages.

Element (mg %)	Age (days)					Total	Mean
	1	5	10	15	20		
Phosphorus	1276.4	1446.0	1176.9	861.2	577.7	5338.2	1067.64
Potassium	1720.0	1378.4	1189.2	1003.9	790.8	6082.3	1216.46
Sodium	313.5	296.5	127.4	97.9	52.8	888.1	177.62
Magnesium	264.9	90.9	83.5	76.0	98.4	613.7	122.74
Calcium	263.6	293.9	129.4	134.2	26.7	847.8	169.56
Manganese	7.3	1.8	9.6	7.0	6.8	32.5	6.50
Copper	2.1	0.7	0.6	1.9	0.5	5.8	1.16
Zinc	14.9	10.8	14.0	9.7	10.1	59.5	11.90
Iron	72.3	36.3	47.7	15.7	35.5	207.2	41.44
Total	3935.0	3555.3	27778.0	2207.5	1599.3	14075.1	2815.02

The results of the present investigation showed that considerable variations in mineral constituents of worker bees during different ages. The mineral contents of bees generally decline with age (Dietz, 1971a). The highest total mineral contents appeared in one day old bees (3935.0 mg %) and decreased gradually until reached the lowest contents in 20 days old bees (1599,3 mg %). Also, El-Shakaa (1985)

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found that the total mineral contents in nurse bees were higher than that obtained in forager bees. However, these variations may be due to response to diet, physiological age condition, and possibly other factors (Nation and Robinson, 1971) and biochemical metabolisms of worker bee (Matsuyama et al., 1974).

As for the levels of minerals, a wide variations were observed. Potassium and phosphorus are the most abundant minerals in all samples under study. Sodium calcium and magnesium are present in moderate quantities and relatively constant. Iron are found in smaller quantity. Honeybee workers contained very small amounts of zinc and manganese. Copper was the lowest mineral contents. A similar relationship was reported by Lederle (1920), except that the phosphorus content of bees was higher than that of potassium. The present data are in very good agreement with those reported by Dietz (1971). However, his data for iron are higher. Also, the results obtained by Nation and Robinson (1971) and Matsuyama et al., 1974 were closed to the present work.

As a conclusion, the change in food from pollen with its fairly high mineral content to nector or honey with a much lower mineral content undoubtly affects the mineral content present in older bees.

Summary

The protein content, amino acid composition and minerals of worker honeybee that sampled every 5 day intervals until 20 days old.

The nitrogen and mineral contents during the early life of a bee were high and declined with age. Total protein content ranged from 41.3 % in worker bees 20 days old to 69.0 % in newly emergence. Results of amino acid analyser indicated that glutamic acid was the predominant amino acid in all samples, except 5 days old bees that alanine was considered the highest one. Cystine was considered the lowest amino acid with no appreciable differences in worker bees during different ages.

The mineral constituents of bees generally decline with age.

The highest total mineral contents appeared in newly emergence (3.93 %) and decreased gradually until reached a minimum in 20 days old bees (1.60 %). Potassium and phosphorus are the most abundant mineral constituents of worker bees. Sodium, calcium, magnesium and iron are present in considerably smaller quantities. Zinc and manganese remain relatively constant during different ages, except the latter very low in 10 days old bees. Copper was the lowest mineral content in all samples. The preliminary data indicate that the changes in the protein content and mineral constituents of honeybees are probably connected with their activities or the division of labor.

Zusammenfassung

Der Gehalt an Protein, Aminosäuren und Mineralen bei Honigbienen wurde in Abständen von 5 Tagen bis zum Alter von 20 Tagen untersucht. Der Gehalt an Stickstoff und Mineralen war hoch während des frühen Lebensalters der Bienen und sank mit zunehmendem Alter ab. Der gesamte Proteingehalt reichte von 41,3 % bei 20 Tage alten Arbeiterbienen bis 69,0 % bei neu geschlüpften. Die Ergebnisse der Aminosäuren-Analyse zeigten, daß Glutaminsäure bei allen Proben die vorherrschende Aminosäure war außer bei den 5 Tage alten Bienen, bei denen Alanin den höchsten Stand erreichte. Cystin war die niedrigste Aminosäure ohne nennenswerte Unterschiede bei den Arbeiterinnen verschiedenen Alters.

Die Mineralbestandteile der Bienen nehmen allgemein mit dem Alter ab. Der höchste Gesamtanteil von Mineralen war mit 3,93 % bei den neu geschlüpften Bienen festzustellen; er nahm allmählich ab bis zu einem Minimum von 1,60 % bei den 20 Tage alten Bienen. Kalium und Phosphor sind die häufigsten Mineralbestandteile der Arbeiterinnen. Natrium, Kalzium, Magnesium und

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Eisen sind in wesentlich geringeren Mengen vorhanden. Zink und Mangan bleiben in den verschiedenen Altersstufen relativ gleich, nur daß das letztere bei 10 Tage alten Bienen sehr niedrig liegt. Kupfer wies bei allen Proben den geringsten Anteil auf. Diese vorläufigen Daten lassen erkennen, daß der Gehalt an Protein und Mineralen bei den Bienen wahrscheinlich mit ihren Tätigkeiten oder mit der Arbeitsteilung in Zusammenhang steht.

Резюме

Установлены содержание белков и состав аминокислот и минеральных веществ у рабочих пчел с интервалами 5 дней до 20-суточного возраста. Содержание азота и минеральных веществ в ранних фазах развития пчел было высокое и снизилось с возрастом. Общее содержание белков колебалось от 41,3 % в 20-суточных рабочих пчелах до 69,0 % в вышедших молодых пчелах. Результаты анализа аминокислот показали, что глутаминовая кислота преобладала во всех образцах за исключением 5-суточных пчел, у которых содержание аланина было самое высокое. Содержание цистина было самое низкое из аминокислот в рабочих пчелах с незначительными разницами во время разных стадий развития.
В общем содержание минеральных веществ пчел снижается с их возрастом. Наивысшее общее содержание минеральных веществ установлено в вышедших молодых пчелах (3,93 %) и постепенно снизилось до минимума в 20-суточных пчелах (1,60 %). Содержание калия и фосфора наивысшее в рабочих пчелах. Количество натрия, кальция, магнезии и железа намного ниже. Содержание цинка и марганца остаются сравнительно постоянными в разных стадиях развития, за исключением последнего, содержание которого было очень низкое во всех образцах. Эти предварительные данные показывают, что, по всей вероятности, изменения содержания белка и минеральных веществ медоносных пчел связаны с выполнением их функций или с разделением труда.

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Besprechung

AUDREY Z. SMITH: **A History of the Hope Entomological Collections in the University Museum, Oxford.** — Oxford: CLARENDON Press, 1986. 172 S., 17 Taf. — (Oxford University Museum Publication; 2). — 16,5×24 cm. — Preis 15.— £.

FREDERICK WILLIAM HOPE (1797—1862) war nach Abschluß seines ersten Studiums (BA 1820, MA 1823) zunächst Reverend. Aus gesundheitlichen Gründen gab er diese Tätigkeit aber bald auf und widmete sich dem Studium der Naturwissenschaften in Oxford sowie dem Sammeln zoologischer Objekte und der einschlägigen Literatur, wobei die Entomologie den Schwerpunkt bildete. CHARLES DARWIN nannte ihn „my father in Entomology". Sein wissenschaftliches Erbe bestand neben zahlreichen entomologischen Veröffentlichungen aus den Jahren 1831—48 (besonders über Coleoptera) aus einer sehr umfangreichen und wertvollen Insektensammlung und Bibliothek, die er 1849 der Universität Oxford vermachte. Die Sammlung umfaßte 31 Schränke und enthielt Teilkollektionen von bekannten Entomologen wie DRURY, HAWORTH, BILLBERG und anderen. Sammlung, Archiv und Bibliothek wurden unter der Bezeichnung „Hope Department of Zoology (Entomology)", seit 1978 „Hope Entomological Collections", im Universitätsmuseum Oxford untergebracht, gepflegt, laufend erweitert und wegen ihres reichen Bestandes an Typen und historischen Exemplaren viel genutzt.

Die Autorin, seit 1937 Bibliothekarin der HOPE-Sammlungen, faßt im vorliegenden Band die Geschichte dieser Institution zusammen. Sie berichtet aus dem Leben und Wirken von HOPE, über die Entstehung der Sammlungen sowie Schenkungen und Erweiterungen 1849—1983, über Kuratoren, Konservatoren und HOPE-Professoren (sehr ausführlich über J. O. WESTWOOD) wie auch über die Bibliothek (heute 13000 Einzelwerke, 400 Periodika, 56000 Separate).

Von besonderem Wert sind die Anhänge mit vielen bisher nicht publizierten Informationen: Appendix A (S. 67—96) verzeichnet die vorhandenen Archivalien wie Briefe, Manuskripte, Sammlungsverzeichnisse, Erwerbungslisten, Zeichnungen, Fotos von zahlreichen Zoologen und Entomologen; Appendix B (S. 97—163) enthält eine Zusammenstellung der Sammler und der Objekte, die durch Schenkung oder Kauf von den Anfängen bis in die jüngste Zeit in die HOPE-Sammlungen gelangten.

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