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Review article

The protein balance of the honey bee worker

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Summary — For honey bee workers, protein requirements and consumption, digestion, intestinal absorption and utilisation all depend on the worker's age and functional status in the system of division of labor within the colony. Workers at the stage of nurses play a pivotal role not only in protein digestion but also in its distribution. They are well equipped for digestion of pollen, are rich in protein and produce large amounts of jelly, which they distribute to almost all members of the colony.

protein metabolism / nutrition / digestion / polyethism / Apis mellifera

INTRODUCTION

The honey bee is one of the few insects whose commercial importance lies in being cultivated, not exterminated. This led to intensive investigations of its nutritional needs. Many papers dealt with the types of food foraged by the colonies, the influence of artificial food or the fate of food-stuffs taken up by bees. Nevertheless, many questions concerning protein metabolism remain unanswered, due to the complexity and plasticity of the social system of the bees.

Temporal polyethism of the worker caste depends primarily on the age of the insect; the division of labor is also influenced by the availability of food inside and outside the hive, on the actual needs of the colony (Rösch, 1925, 1930; Lindauer, 1952; Ribbands, 1952; Free, 1965; Michener, 1969; Seeley, 1982; Winston and Neilson Punnet, 1982; Kolmes, 1985) and on season (Merz et al, 1979). Hormone levels influence the beginning of different age-dependent tasks (Robinson, 1987; Robinson et al, 1989). In the last few years more and more genetically determined controlling mechanisms have been investigated (Calderone and Page, 1988; Frumhoff and Baker, 1988; Robinson and Page, 1988; Kolmes et al, 1989; Robinson et al, 1989; Rothenbuler and Page, 1989).

This review describes the protein metabolism of workers with special attention paid to their age and function.

PROTEINACEOUS NUTRIENTS

When worker larvae hatch, they are provided with jelly from the brood food glands of the nurses (Hanser and Rembold, 1964; Rembold, 1974) until they reach an age of 3.5 d. Afterwards, they receive a mixture of jelly, honey and pollen; this latter food mixture has less protein but more carbohydrates (von Rhein, 1956; Kunert and Crailsheim, 1987). Approximately 1/3
of the dry weight of the jelly fed at first consists of high molecular weight proteins (Habowsky and Shuel, 1959; Patel et al., 1960). The percentage of protein in the food for the young larvae depends not only on the age of the larvae but is also subject to seasonal fluctuations: during 1 summer a decline from more than 50% (protein of dry weight) to less than 30% was demonstrated in 2 colonies investigated in parallel (Kunert and Crailsheim, 1987).

Soon after emergence, young bees start to consume honey and beebread from an area near their brood nest (Lotmar, 1938; Lindauer, 1952; Dietz, 1969; Haydak, 1970; Hagedorn and Moeller, 1967). Beebread is the pollen that has been collected by the foragers and prepared for storage in the combs. It is mixed with secretion from workers (Pain and Maugenet, 1966; Maurizio, 1969; Herbert and Shimanuki, 1978) and contains various microorganisms (Pain and Maugenet, 1966; Gilliam et al., 1989). The nutritive value of pollen stored in this manner is higher than that of laboratory-stored pollen (Beutler et al., 1949; Van Der Vorst and Jacobs, 1980). In addition, the newly emerged workers receive food from older bees in trophallactic interactions; Perepe-lova (1928) observed that bees of 1–3 d of age were fed by others whose age ranged from 7–14 d. Free (1957, 1959) found that the young bees also received food from workers of other ages; however, he also confirmed that nurses of 7–14 d were predominant in the group of food providers.

From about the third day of life, the number of receiving and giving interactions are similar in number, but generally there is rather more transfer from old to young bees than in the opposite direction (Free, 1957). As the age class from 7–14 d provides the protein for the larvae, it could be assumed from the above findings that newly emerged bees receive not only honey but also protein-rich products of the nurse's brood glands.

These trophallactic transfers also occur when bees are caged (Free, 1957; Korst and Velthuis, 1982; Moritz and Hallmen, 1986). Such laboratory experiments were used to prove the transfer of proteinaceous food between nurses and 1 d-old bees (Crailsheim, 1990a). This transfer was also shown in small free-flying colonies (Crailsheim, 1990b).

Pollen is the main natural source of protein for bees; nectar collected by bees contains rather low concentrations of amino acids (Baker and Baker, 1973). The pollen consumption of the workers increases until they reach the age when they become nurses (Lotmar, 1938; Lindauer, 1952; Pain, 1961), after which pollen consumption tends to decrease.

During the winter when there is no food available outside the hive, bees consume the stored food, ie honey and beebread. Beebread is important for surviving the winter as well as for the start of colony re-population in the spring (Wille et al., 1987).

One way of obtaining protein is cannibalism. In certain situations, such as lack of proteinaceous food, workers may eat eggs and brood (Haydak, 1935, Myser, 1952, Newton and Michl, 1974). This food can lengthen the lifespan of the cannibal bees themselves (Weiss, 1984), or it can be used for brood rearing or feeding the queen (Weiss, 1984; Webster and Peng, 1987; Webster et al., 1987).

QUALITY OF PROTEIN

That pollen is necessary for a colony has been known for a long time (Eckert, 1942). Two authors laid down the essential foundations for all subsequent work on protein requirements and metabolism in the early
fifties. Maurizio (1954) described the importance of pollen for the colonies and Groot (1953) described the nutritional value of various sources of protein for the colonies as well as the requirements of individual bees. He classified the following 10 amino acids as being essential for the growth of the honey bee: arginine, histidine, lysine, tryptophan, phenylalanine, methionine, threonine, leucine, isoleucine and valine; the largest requirements were for leucine, isoleucine and valine.

Thus, the nutritional value of the pollen for bees is primarily defined by its absolute and relative content of essential amino acids. Accordingly, pollen from different plants has a different nutritional value for bees. This could be demonstrated by measuring such diverse parameters as the growth and development of the fat body (Maurizio, 1954), the development of the hypopharyngeal glands (Maurizio, 1954; Standifer, 1967), lifespan (Maurizio, 1954; Wahl, 1963; Schmidt et al, 1987) or brood rearing (Wahl, 1963; Herbert et al, 1970). Caged bees show the ability to distinguish between different kinds of pollen. They prefer protein-rich pollen (Schmidt and Johnson, 1984) and react to the presence of phagostimulants in pollen (Schmidt, 1985).

Less important for bees in nature than for artificial feeding of bees is the fact that the age of pollen (Dietz and Haydak, 1965; Hagedorn, 1968) and the way it was collected – ie whether the pollen was collected by humans or by bees (Maurizio, 1954) – influences its value for bees.

**INTESTINAL TRANSPORT, DIGESTION AND ABSORPTION**

Pollen is ingested either fresh or from bee-bread. There is no digestion of protein in the crop, as salivary and hypopharyngeal glands do not produce proteolytic enzymes (Arnold and Delage-Darchen, 1978). Amino acids from nectar do not penetrate the crop wall (Crailsheim, 1988a). Pollen grains are quickly transported through the crop to the proventriculus which regulates the passage of all nutrients into the midgut. In agreement with their intensive pollen consumption, the worker caste has the largest proventriculus as compared to the drones and to the queen (Pabst and Crailsheim, 1990). Transport of liquids is regulated according to requirements (Crailsheim, 1988b, c); solid food, ie pollen, is compressed into bolusses by the proventriculus which are then transported through the stomodeal valve, a flexible pipe (Trappman, 1923) into the midgut (Whitcomb and Wilson, 1929; Bailey, 1952; Schreiner, 1952; Barker and Lehner, 1972; Klungness and Peng, 1984a; Peng and Marston, 1986). There, the pollen bolusses are surrounded by peritrophic membranes. They do not leave the tube of peritrophic membranes (endoperitrophic space) but pass through the anterior intestine into the rectum (Schreiner, 1952). The time required for passage through the midgut depends on the amount of foodstuffs in this section, on the age of the bee and on the way the bees are kept; the length of time from ingestion until pollen, found in the rectum ranged from a few h up to more than 1 d. The long duration is necessary because pollen is difficult to digest, due to its tough wall (Barker and Lehner, 1972; Klungness and Peng, 1984b; Peng et al, 1986). Some types of pollen leave the midgut without being utilized completely (Peng et al, 1985). For a pollen mixture, offered in a formulated diet, an apparent digestibility of 77% was shown by Schmidt and Buchmann (1985).

The peritrophic membranes form compartments for digestion (Moritz and Crailsheim, 1987) as also described for other
insects (Terra et al., 1979; Terra and Ferreira, 1981; Terra, 1988, 1990) and contain immobilized aminopeptidases (Peters and Kalnins, 1985). The chemical composition of the membranes depends on the age of the bees (Pabst et al., 1988).

Upon entering the midgut, pollen is thought to be broken down by osmotic shock (Kroon et al., 1974) and/or by the effects of proteolytic enzymes. In another bee (Melipona quadrifasciata), osmotic shock is less pronounced and only causes an opening of the pores on the pollen grains (Martinho, 1975). Gut proteolytic enzymes are only present to a limited extent in the last pupal stage and in emerging bees, but they increase rapidly in the first hours of imaginal life (Moritz and Crailsheim, 1987). Pavlovsky and Zarin (1922) were the first to investigate extensively the proteolytic enzymes in the gut of the honey bee; they found several types. Giebel et al. (1971) described 4 endopeptidases with different properties. Dahlman et al. (1978) only found 3 endopeptidases, 1 trypsin-like and 2 chymotrypsin-like. Grogan and Hunt (1980, 1984) first proved differences in gut proteolytic activity between house bees (those who did not yet forage) and foragers; Moritz and Crailsheim (1987) showed age-dependence and a close correlation with soluble protein content in the midgut. Trypsin-like and chymotrypsin-like enzymes showed their maximum activity at pH 8.5 and around pH 8 respectively. The highest levels of proteolytic activity were found in the nurse bees; this activity declined when bees started to forage. Low levels were found in bees in winter; the same population of bees showed a slight increase when bees again began to forage and to perform brood-care tasks in spring. Proteolytic activity in the gut of workers is also influenced by the exigency of the bees' social system. Neither a queen nor open brood was necessary for normal development of intestinal proteolytic activity when bees lived in colonies, but proteolytic levels were much lower if bees were caged, even in groups and with sufficient proteinaceous food. The composition of the diet also influenced proteolytic levels (Crailsheim and Stolberg, 1989).

Only little proteolytic activity was found in the midgut tissue, somewhat more in the ectoperitrophic space and most in the endoperitrophic space where pollen is transported (Moritz and Crailsheim, 1987; Jimenez and Gilliam, 1989). The latter authors postulate a countercurrent flow in the endo- and ectoperitrophic spaces as described for other insects (Terra and Ferreira, 1981; Terra et al., 1985; Terra, 1988, 1990). Such a flow enables the enzymes to be utilized optimally and explains why only small amounts of liquid pass from midgut into the rectum of bees (Crailsheim, 1988b).

There is less information about the absorption of substances through the midgut wall of the honey bee than for other insects (Turunen, 1985). The transport of sugars was shown to be passive by simple diffusion (Crailsheim, 1988d). Subsequent in vivo experiments demonstrated the concentration dependence of leucine transport as well as inhibition of this transport by the structurally similar amino acid isoleucine, but not by glycine, arginine or glutamic acid (Crailsheim, 1988a). This finding of a rather specific carrier mechanism could be confirmed by in vitro experiments which furthermore proved that the transport of leucine is not energy-dependent (Haszonits and Crailsheim, 1990). In vivo as well as in vitro experiments had previously shown different transport kinetics in bees in summer and in winter; transport being faster in summer. These results are in good agreement with data about protein metabolism. Morphological studies offer an explanation for the lower transport capacity.
for amino acid in winter. Freeze-etch studies showed a lower number of particles in the plasma membrane of midgut epithelial cells in winter bees (Pabst and Crailsheim, 1987); such particles are described in mammals as being carriers or membrane-bound enzymes.

PROTEIN UTILISATION AND TURNOVER

Large amounts of protein are required during larval development; 1 larva needs about 50 mg jelly to reach a weight of 35 mg (von Rhein, 1956). During about 3.5 d, the high protein content of a 3 mg-larva is reduced from nearly 19% (of fresh weight), to about 9% (Kunert and Crailsheim, 1987).

If the nursing bees themselves lack proteinaceous food, they can continue to nurse larvae, but only for about 1 week. All larvae fed after this period were found to be unable to reach maturity and were removed by the bees. Protein-deprived nurse bees had lower nitrogen levels in all 3 body sections, as compared to nurses before the onset of protein deprivation. The largest reduction (~11%) was seen in the abdomen. Haydak (1935) concludes from these results that the nurses use their own tissue protein for rearing the larvae. Larvae that were nursed deficiently but reached maturity, developed into dwarfed adults (Jay, 1964).

After emergence, bees require protein for imaginal tissue differentiation. They consume a great deal of pollen and receive proteinaceous food from other workers. Upon emerging, they have a nitrogen content of 1.74 mg; this increases to 2.65 mg in 21–38 d-old bees (Haydak, 1934). A comparison of protein- or nitrogen-content values of emerging workers taken from different authors with different methods is given by Kunert and Crailsheim (1988). The protein content at emergence depends on the availability of food outside the hive during the nursing season; the difference between bees nursed during bad pollen harvesting conditions and between those nursed during good conditions amounts to more than 13%.

At the moment of emergence, bees which were destined to survive the winter had a higher protein content than summer bees (Kunert and Crailsheim, 1988). They are reared during late summer and autumn, a period with less pollen available outside the hive but with considerable amounts of beebread inside. Furthermore, much fewer larvae are reared compared to the period from May to July.

During the hive-bound period, the most remarkable protein increase was found in the heads; nurses had about twice the amount found in newly emerged bees (Haydak, 1934). This is due to the system of work allotment in the honey bee community. Workers at the age of nurses have well-developed hypopharyngeal glands (Krathky, 1931; Moskovljevic-Filipovic, 1952; Groot, 1953; Maurizio, 1954; Beams et al, 1959; Fluri et al, 1982; Mortiz and Crailsheim, 1987; Fergusson and Winston, 1988; Suzuki, 1988; Crailsheim and Stolberg, 1989; Knecht and Kaatz, 1990) which are superbly equipped for synthesis of protein-rich secretions (Brouwers, 1982; Takenaka and Kaatz, 1987; Webster et al, 1987; Webster and Peng, 1987; Knecht and Kaatz, 1990; Crailsheim, 1990a). The secretion products of these glands are then distributed throughout the colony. The development of these glands is stimulated by a signal from the brood (Huang and Otis, 1989; Huang et al, 1989) and suppressed by juvenile hormone (Jaycox et al, 1974; Fluri et al, 1982; Liu, 1989) at least at high dosages (Sasagawa et al, 1989). Workers with the highest rates of protein
synthesis also exhibit the highest protein levels (4.3%) in the haemolymph; this is reduced to 1.7% in foragers (Sinizki and Lewtschenko, 1971). In winter the protein levels are again higher than in summer; this is due to a lower titer of juvenile hormone (Fluri et al, 1982). In summer, when workers stop producing jelly upon reaching the age of foragers, the shrunken hypopharyngeal glands produce other proteins, mainly enzymes (Simpson et al, 1968; Halberstadt, 1980; Sasagawa et al, 1989). The correct development of hypopharyngeal glands depends on proper nutrition (Soudek, 1927; Free, 1961; Maurizio, 1962; Standifer, 1967; Herbert et al, 1977) and on the existence of a social system (Crailsheim and Stolberg, 1989). When Schmidt and Buchmann (1985) offered such a proper pollen-containing diet to a small colony in a flight cage, they determined an average need of 3.07 mg N for 1 bee of the colony per 28 d. This corresponds to 19.2 mg protein.

The fat body is the general storage organ in the bee larva and contains large amounts of protein. Its composition changes with age (Krajewska and Hryniewieckaszyfter, 1988). Subsequently, it provides material for the newly developing imaginal tissues in pupae (Schnelle, 1923). After emergence, the protein content of the fat body increases until the worker reaches the nurse stage in summer. Protein content is then lower in foragers and highest in bees that survive the winter (Shehata et al, 1981; Fluri and Bogdanov, 1987; Southwick, 1989). Winter bees have higher protein levels in the haemolymph and more protein in their hypopharyngeal glands than foragers in summer (Fluri et al, 1982). Thus, both compartments function, together with the fat body, as protein storing organs. Many proteins circulating in the haemolymph are produced by the fat body (Lensky and Rakover, 1983).

Injections of radioactively labelled amino acids or feeding of labelled protein enable us to determine the site and rate of protein synthesis as well as catabolism. The high activity of hypopharyngeal glands was demonstrated in vivo with tracer methods (Webster and Peng, 1987; Webster et al, 1987; Knecht and Kaatz, 1990; Crailsheim, 1990a). Ninety min after an injection of 14C-phenylalanine, nurses had more than 25% of the radioactivity in the protein fraction of their caput, less than 10% in the thorax and more than 25% in the abdomen. Foragers showed similar results in the thorax and abdomen, but contained somewhat less than 10% in the caput. More than half of the tracer found in the protein fraction of the head of nurses was in the hypopharyngeal glands after 6 h (Crailsheim, 1990a).

The half-life of total body protein was shortest in foragers (11–13 d), was about 50% longer in nurses and was about 4 times longer in bees in winter that were taken from the outside of a wintercluster (Crailsheim, 1986). The reduction of metabolism in winter bees (Omholt and Lonvik, 1986) is a useful adaptation to the lessening of the duties of these bees. Many of them remain almost motionless during long periods on the outside of the cluster and therefore should not need much protein turnover. Nurses in summer that have to do the work of digestion for a large part of the colony need much protein and have a faster turnover. Their enzymatic equipment is adapted to this level of protein metabolism.

Foragers are highly active flyers and cover on average 21.5 km per d (Neukrich, 1982) which requires the metabolic equivalent of more than 6 mg sugar solely for flight (Heran and Crailsheim, 1988); such an amount of metabolic work is certainly bound to increase protein turnover. Accordingly, they are able to absorb more
leucine and probably more other amino acids than winter bees (see previous section).

Honey bees are known to use carbohydrates for their energy requirements during flight (Nachtigall et al, 1989). Nevertheless, a considerable fraction of $^{14}$C from $^{14}$C-amino acids was shown to be exhaled as $^{14}$CO$_2$ (Webster et al, 1987; Crailsheim, 1988a, 1990a) in bees that were resting or walking, showing that amino acids are ultimately broken down and utilized for energy metabolism. The latter function in other situations than flight requires further investigation.

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A l'âge de 8 j, dans une colonie moyenne et dans des conditions normales de miellée, elles occupent la fonction de nourrices, pour laquelle elles consomment d'énormes quantités de pollen. Elles sont alors abondamment pourvues de protéases intestinales et le transport des acides aminés de l'intestin dans l'hémolymphe est réalisé par des mécanismes spécifiques. Ce transport – qui a été étudié pour la leucine – se passe de la même façon chez les nourrices et les butineuses, mais il est plus lent chez les abeilles de la grappe hivernale. Les glandes nourricières des nourrices sont bien développées et produisent une nourriture larvaire riche en protéines, distribuée au couvain et à la reine, mais aussi aux ouvrières de tous âges. Quand les abeilles passent au stade de butineuses, leurs glandes hypopharyngiennes diminuent et changent de fonction.

La circulation des protéines corporelles des nourrices est semblable à celle des butineuses pendant la période de miellée, mais elle est beaucoup plus rapide que chez les abeilles d'hiver.

**Résumé — Le métabolisme des protéines chez l'ouvrière d'abeille.** Il existe, au sein de la caste de ouvrières, des différences dans le métabolisme des protéines dues à des facteurs tels que l'alimentation, l'âge des abeilles, leur fonction au sein de la colonie.

Les abeilles récemment écloses écosent densités croissantes et reçoivent également de la nourriture larvaire de la part des congénères de la ruche plus âgées. Leur teneur en protéines s'élève, pendant cette période, puis reste à un niveau élevé jusqu'à la fin de leur vie. La teneur en protéases intestinales et la taille des glandes hypopharyngiennes augmentent continuellement.

Im Alter von etwa 8 Tagen haben sie in einem durchschnittlichen Volk bei norma-
len Trachtverhältnissen die Funktion von Ammen, in der sie sehr große Mengen an
Pollen fressen. Sie sind hierzu reichlich mit intestinalen Proteasen ausgestattet,
und der Transport von Aminosäuren aus dem Darm in die Hämolymphpe wird durch
spezielle Trägermechanismen bewerkstel-
gt. Dieser Transport – untersucht für die
Aminosäure Leucin – geschieht bei Am-
menbienen und bei Sammlerinnen in
ähnlicher Weise, bei Bienen die in der
Wintertraube leben jedoch langsamer. Die
Futtersaftdrüsen der Ammen sind groß
und produzieren eiweißreichen Futtersaft,
der an die Brut, die Königin, aber auch an
Arbeiterinnen aller Altersstufen verfüttert
wird. Beim Übergang zur Ausflugtätigkeit
werden die Hypopharynxdrüsen kleiner
und ändern ihre Funktion.

Der Umsatz des Körperproteins von
Ammenbienen ist während der Trachtzeit
dem von Flugbienen ähnlich, er geschieht
aber wesentlich rascher als bei Arbeiterin-
nen, die im Winter leben.

Proteinstoffwechsel / Ernährung / Ver-
dauung / Polyethismus / *Apis mellifera*

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