Nutrition and health in honey bees

Robert Brodschneider, Karl Crailsheim

Apidologie 41 (2010) 278–294
© INRA/DIB-AGIB/EDP Sciences, 2010
DOI: 10.1051/apido/2010012

Available online at:
www.apidologie.org

Review article

Nutrition and health in honey bees*

Robert BRODSCHNEIDER, Karl CRAILSHEIM

Department of Zoology, Karl-Franzens-University Graz, Universitätsplatz 2, 8010 Graz, Austria

Received 15 September 2009 – Revised 4 February 2010 – Accepted 11 February 2010

Abstract – Adequate nutrition supports the development of healthy honey bee colonies. We give an overview of the nutritional demands of honey bee workers at three levels: (1) colony nutrition with the possibility of supplementation of carbohydrates and proteins; (2) adult nutrition and (3) larval nutrition. Larvae are especially dependant on protein and brood production is strongly affected by shortages of this nutrient. The number of larvae reared may be reduced to maintain the quality of remaining offspring. The quality of developing workers also suffers under conditions of larval starvation, leading to slightly affected workers. Larval starvation, alone or in combination with other stressors, can weaken colonies. The potential of different diets to meet nutritional requirements or to improve survival or brood production is outlined. We discuss nutrition-related risks to honey bee colonies such as starvation, monocultures, genetically modified crops and pesticides in pollen and nectar.

malnutrition / pollen / protein / carbohydrates / supplemental feeding

1. INTRODUCTION

Adequate nutrition is a honey bee colony's basis for growth and development. De Groot (1953) reported the amino acid requirements for honey bee growth and development, and Haydak (1970) extensively reviewed the general dietary requirements of honey bees. This knowledge led to the formulation of special diets that support colony development (Tab. I). Because landscapes have become increasingly characterized by agriculturally intensive monocultures, and since honey bee pollination services often occur within a human-defined ecosystem, bees nutritional needs may not be provided for properly (Naug, 2009). Thus the question arises if and how bees should be provided with supplemental food when nutritional deficits occur.

Honey bees are social insects, often regarded as super-organisms (Seeley, 1989). Thus, nutrition can be investigated on three levels – colony nutrition, adult nutrition and larval nutrition – with increasing complexity, because disorders in prior stages affect subsequent stages, and vice versa. Poor colony pollen stores may hinder adults from feeding larvae properly or from rearing all larvae to adulthood. Hence the quality or the number of adults in the next generation may be poor, which could affect colony nutritional state and thus influence subsequent brood rearing (Fig. 1). In a colony, the nutritional levels are closely connected through numerous adult-brood interactions and trophallactic contacts. Trophallaxis describes the social transfer of food from one adult individual to another, partly in a directed manner, and partly generating a common stomach that enables all bees to obtain knowledge of the nutritional status of the colony (Crailsheim, 1991, 1998). Both larvae and adults are highly dependent on colony food stores, and adult honey bees may adapt their foraging or brood-care strategies according to the respective need and supply of carbohydrates and proteins (Schmickl and Crailsheim, 2004).

Corresponding author: R. Brodschneider,
Robert.Brodschneider@Uni-Graz.at
* Manuscript editor: Yves Le Conte

Table I. Additional (a) or exclusive (e) amount of protein consumed in different diets provided through supplemental feeding of honey bee colonies of different sizes. Data are inferred from published articles. Where possible, main ingredients, protein content of each diet, and colony characteristics are given.

Diet consumption per unit (g/d)	Diet	Protein content	Colony size	Reference
0.7–3.6 (e)	Various synthetic diet formulations	-	Nuclei (46 × 19 × 27 cm)	Herbert and Shimanuki (1977)
9.1	Pollen	-	500 g bees	
3.9 (e)	Wheast	5%	Nuclei (46 × 19 × 27 cm) 500 g bees	Herbert et al. (1977)
6.6 (e)		10%		
11.1 (e)		23%		
9.9 (e)		30%		
8.0 (e)		50%		
8.5–15.2 (e)	Various pollen	-	12-frame hives	Campana and Moeller (1977)
2.5–4.5 (e)	Synthetic diet with various amounts of pollen ash	-	Nuclei (23 × 19 × 27 cm) 400 g bees	Herbert and Shimanuki (1978b)
0.9–2.6 (e)	Synthetic diet with various fat soluble vitamins	-	Nuclei (23 × 19 × 27 cm) 400 g bees	Herbert and Shimanuki (1978c)
7.5–8.7 (e)	Wheast	23%	Nuclei (23 × 19 × 27 cm) 4000 bees	Herbert (1980)
2.8 (e)	Yeaco	23%		
4.2–4.8 (e)	Soybean	23%		
6.3–9.6 (e)	Whey-yeast with various amounts of pollen lipids	23%	Nuclei (23 × 19 × 27 cm) 4000 bees	Herbert et al. (1980a)
10.8 (e)	Pollen	-		
0.8–1.6 (e)	Various pollen	12–19%	Nuclei (26 × 13 × 20 cm), 300 bees	Loper and Berdel (1980a) Loper and Berdel (1980b)
0.4 (e)	Yeaco-20	-		
1.3–2.0 (e)	Various pollen	-		
3.2–8.4 (e)	Lactalbumin/yeast	23% 200–600 g bees	Nuclei (23 × 19 × 27 cm)	Herbert and Shimanuki (1982)
6.9–8.9 (e)	Synthetic diet with various amounts of vitamin C	-	Nuclei (23 × 19 × 25 cm) 4000 bees	Herbert et al. (1985)
18.0–29.5 (e)	Pollen, sucrose, invert sugar	12.4%[1]	5-frame hive, 4320 bees	Schmidt and Buchmann (1985)
6.5–10.4 (e)	Pollen	-	Nuclei (23 × 19 × 25 cm) 4000 bees	Herbert et al. (1988)
4.1–5.6 (e)	Synthetic diet	-		
0.4–6.1 (e)	Pollen substitute	-		
1.6 (e)	Bee bread	-	120 caged workers, average age 1–6 d	Cremonez et al. (1998)
1.4 (e)	Soybean/yeast	-		
1.8 (e)	Pollen	-		
1.5 (e)	Corn meal	-		
0.5–0.6 (e)	Various fresh pollen	14.9–30.1%	150 caged workers, average age 1–14 d	Pernal and Currie (2000)
0.4–0.5 (e)	Various 1-y-old pollen	15.0–29.9%		
0.1 (e)	Fresh *Pinus* pollen	14.0%		
0.1 (e)	1-y-old *Pinus* pollen	14.0%		
0.2 (e)	Bee Pro®	29.9%		
13.9 (a)	Soy flour, yeast, milk protein, linseed oil	-	Colonies	van der Steen (2007)

Table I. Continued.

Diet consumption per unit (g/d)	Diet	Protein content	Colony size	Reference
21.4 (a)	Pollen (Winter)	-		
37.6 (a)	Pollen (Summer)	-		
10.3–20.7 (a)	Three substitutes (Winter)	8.3–26%	Colonies	DeGrandi-Hoffman et al. (2008)
33.5–34.1 (a)	Two substitutes (Summer)	8.3-16.5%		
12.6–23.9 (a)	Brood builder	-	12 000 bees	Pankiw et al. (2008)
26.5–28.6 (a)	Brood builder (+ Brood Pheromone)			
35.3–58.6 (a)	Roasted soy flour, pollen, sugar, honey	-	Full colonies	Avni et al. (2009)
16.0 (a)	Pollen	-	3-frame hives, 6000 bees	Brodschneider et al. (2009a)
22.7 (a)	Pollen (large surface)	-		
7.5 (a)	Feedbee®	15%		
14.8 (a)	Feedbee® (large surface)	15%		

[1] Calculated from nitrogen using 6.25 as conversion factor (Roulston and Cane, 2000).

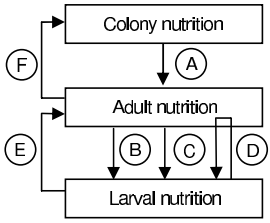

Figure 1. A schematic representation of the three levels of honey bee nutrition, dependencies, and possible effects of protein malnutrition. A: dependency of adults on colony food stores; B: investment in larval quality; C: regulation of larval number; D: cannibalism; E: impact of larval nutrition on next adult generation; F: impact of adults on colony nutrition.

Physiological and nutritional needs of workers, queens and drones differ somewhat (Hrassnigg and Crailsheim, 2005). In this review we focus on the nutritional demands of honey bee colonies and the demand of adult workers and worker larvae, because their survival and quality are responsible for the colonies' productivity and health. The health of honey bee colonies is not only defined by the absence of diseases, but also by the presence of many well-nourished individuals capable of producing progeny and resisting stressors such as parasites, infections, insecticides and periods of dearth.

2. CARBOHYDRATES

2.1. Colony nutrition

The natural carbohydrate source of honey bees is nectar or honeydew, collected by foragers from plants, transported to the hive and finally stored in sealed cells as honey. The transformation from nectar to honey is gradual and begins during the returning flight (Nicolson and Human, 2008). In the colony the water content is further reduced to 16–20%, and enzymes (invertase, diastase and glucose oxidase) are added, which account for the sugar composition of honey: on average 38% fructose, 31% glucose and other di- and trisaccharids (Doner, 1977). Annual honey yields vary widely, depending on climate, beekeeping operation and forage availability, and are reported to be 19.2 kg (McLellan, 1977), 24.3–31.3 kg (Avni et al., 2009) or more than 50 kg for carbohydrate-supplemented colonies (Severson and Erickson, 1984). Annual yields may reach 96–124 kg per colony in Canada (Mattila and Otis, 2006a). This long-term provisioning and food preservation enables the

colonies to survive long periods without food flow from the environment. The energetic cost of overwintering is high. Seeley and Visscher (1985) assessed the weight loss of small colonies – mostly due to honey consumption – to be at least 20 kg between July and April in a temperate climate. Weight loss, and therefore energetic cost, is higher during times of winter brood rearing (0.84 kg/week) compared to the cost of thermoregulation necessary for the colonies' survival without brood (0.42 kg/week).

2.2. Adult nutrition

Carbohydrates meet the energetic expenses of honey bees. Adult workers are strongly dependent on colony food stores and do not survive long periods without feeding as they do not have considerable carbohydrate, protein or lipid reserves in their bodies (Kunert and Crailsheim, 1988; Hrassnigg and Crailsheim, 2005). Adult bees, in contrast to larvae, have low glycogen stores (0.05–0.47 mg per worker, Hrassnigg and Crailsheim, 2005). When in need of energy (e.g., before foraging flights), workers provision themselves with sugars in their honey crop, which they obtain from honey stores or via trophallactic contacts.

An adult honey bee worker needs about 4 mg of utilizable sugars per day for survival (Barker and Lehner, 1974). Caged bees fed carbohydrates exclusively survive best on sucrose (LT_{50} = 56.3 days) compared to honey (31.3 d) or high-fructose corn syrup (HFCS, 37.7 d, Barker and Lehner, 1978). Workers at foraging age have the enzymes necessary to use polysaccharides (starch) for flight metabolism (Hrassnigg et al., 2005). Some sugars, like mannose (Staudenmayer, 1939), galactose, arabinose, xylose, melibiose, raffinose, stachyose and lactose (Barker and Lehner, 1974; Barker, 1977), are toxic to bees. These sugars can be found in considerable amounts in pollen (see references in Barker, 1977), nectars of Tiliaceae or Theaceae (Barker, 1990) or exudates of tulips (Barker and Lehner, 1976). About 40% of the sugars found in soybeans, which are used as pollen substitutes, are toxic to bees (Barker, 1977). Toxicity is reduced when honey bees have plenty of nectar or when these carbohydrates are experimentally diluted to under 4% with 50% sucrose solution (Barker, 1977).

Another substance toxic to bees is hydroxymethylfurfural (HMF), formed from the acid-catalized dehydration of hexose sugars, especially fructose, and formed in honey as a result of heat treatment or storage. High HMF levels must also be considered as a risk in the feeding of invert sugars or HFCS. A good estimate of the toxicity of HMF was published by Jachimowicz and El Sherbiny (1975): Sugar solutions containing 150 ppm HMF result in 58.7% mortality within 20 days in caged bees, whereas solutions containing 30 ppm cause a mortality of only 15.0%, which was not significantly different from the controls (12.5%). HMF levels of 30 ppm can therefore be regarded as safe for bees. Recently, the HMF content of commercially available HFCS was determined to be between 3.1 and 28.7 ppm by LeBlanc et al. (2009). They also demonstrated that HFCS stored at 40 °C for 69 days may reach 250 ppm, a concentration that significantly reduced longevity compared to HFCS with lower concentrations of HMF (57–200 ppm). The authors rightly opposed a comparison of bee mortality between HFCS and sucrose, but bees in their experiment survived better on sucrose.

2.3. Larval nutrition

A larva is regularly inspected by nurse bees and fed if necessary, so that it is always sufficiently provided with food. The sugar content (fructose and sucrose) of brood food is 18% in the first three days of larval development, then 45% in the last two days. Rortais et al. (2005) calculated that 59.4 mg of carbohydrates are fed to one worker larva during its development. Carbohydrate need would be higher when taking into account the costs of adult bees to provide the stable environment necessary for brood rearing (like comb building or thermoregulation). A lack of carbohydrates limits the number of larvae reared in spring, when nectar sources are poor and

winter stores already depleted, or after harvesting the honey without adequate replacement of carbohydrates. Little is known about lethal or sublethal effects of HMF or toxic sugars on larval development.

2.4. Feeding carbohydrates

Carbohydrates are fed to colonies routinely after harvesting honey or during periods of dearth. This is accomplished by feeding sucrose solution, invert sugars, HFCS (see also Sect. 2.2) or various fruit syrups (Neupane and Thapa, 2005) inside the hive. Grape syrup causes dysentery and reduces longevity, and its use is therefore not recommended (Barker and Lehner, 1978). After autumn feeding, colonies in a temperate climate consume about 20–25 kg of syrup between November and April (Severson and Erickson, 1984). Syrup is consumed by hive bees, and the applied carbohydrates are integrated into the colony's food flow and transported to food storage combs (DeGrandi-Hoffmann and Hagler, 2000). Free (1965) found bees feeding sugar from a feeder to be younger than foragers, and Brodschneider et al. (2007) demonstrated that bees consuming sugar solutions inside the hive during summer are mostly of the same age as nectar receivers.

Severson and Erickson (1984) found no deleterious effect of HFCS feeding to colonies in a temperate climate. They investigated early season weight gains, season honey production and brood quality (head and thorax dry weight). Deleterious effects are most likely to be observed when a colony obtains carbohydrates almost exclusively from HFCS, as is the case when colonies are fed before winter. Colonies supplemented with HFCS in autumn had less sealed brood in spring compared to colonies supplemented with sucrose, but this setback in spring build-up did not affect honey production later in the season. These findings do not prove that HFCS is harmless, as the authors did not report the HMF content of the supplementary feed and only one winter was considered. Future research should include other climatic regions. However, in contrast to experiments with caged honey bees, free-flying colonies can dilute high HMF levels when other carbohydrate sources are present. Especially in hot regions with only few nectar sources available, feeding of HFCS that has been stored for a long time should be avoided (LeBlanc et al., 2009). Surveys conducted among Austrian beekeepers (Brodschneider et al., 2010) also suggest that the type of carbohydrates fed to colonies (sucrose, invert sugar or starch syrup) did not affect overwintering mortality of colonies when supplementation was done between July and October.

3. PROTEIN

3.1. Colony nutrition

The only natural protein source for honey bees is pollen. Colonies collect 10–26 kg of pollen per year (Wille et al., 1985) and Crailsheim et al. (1992) estimated the pollen requirement of two 10-frame colonies to be 13.4 and 17.8 kg per year, respectively. In contrast to honey, only a small amount of pollen is stored in the colony at any given time, and stores quickly diminish during non-foraging periods (Schmickl and Crailsheim, 2001, 2002).

In the colony, honey bees mix pollen with regurgitated nectar, honey and glandular secretions to produce bee bread, which differs from freshly collected pollen, in having a lower pH and less starch (Herbert and Shimanuki, 1978a; Ellis and Hayes, 2009). The nutritive value of bee bread to honey bees is higher than that of fresh bee collected, laboratory stored, or frozen pollen with few exceptions (Hagedorn and Moeller, 1968; Herbert and Shimanuki, 1978a; Dietz and Stevenson, 1980; Cremonez et al., 1998; Pernal and Currie, 2000). The shift in the quality of pollen stored in the colony (bee bread) is attributed to microorganisms associated with the honey bee (Gilliam, 1997). Vásquez and Olofsson (2009) suggested that lactic acid bacteria from the honey bee stomach belonging to the genera *Lactobacillus* and *Bifidobacterium* are involved in the fermentation process of bee bread and may be responsible for improving the nutritive value by producing vitamins.

The protein content of pollen from different species and regions varies widely (2.5–61%, Roulston et al., 2000). Roulston and Cane (2000) discuss the problems of determining the nutritive value of pollen through measurements solely of crude protein content in the laboratory. Honey bees need to ingest ten amino acids described as essential to their diet (De Groot, 1953). Amino acid requirements are highest for l-leucine, l-isoleucine and l-valine, and limitations of one of the essential amino acids in the food protein limit colony development. Pollens from different plants have different nutritive values for bees. To determine the value to honey bees, it is necessary to conduct bioassays of different pollen or protein diets and their effects on brood production (Campana and Moeller, 1977; Loper and Berdel, 1980a, b; Dietz and Stevenson, 1980), life span (Schmidt et al., 1987) or other physiological parameters (see Sects. 3.2.1 and 3.4).

3.1.1. Malnourished colonies

Colonies without pollen supply maintain brood rearing only for a short time, first by using up the stored bee bread and later by depleting their body reserves (Haydak, 1935). Honey bees have developed a mechanism to react to changes in the ratio of pollen supply and protein demand of brood: they cannibalize brood and thereby gain protein which they use to feed other larvae. Young larvae, in which little investment has been made up to that point, are cannibalized, and older larvae are maintained (Schmickl and Crailsheim, 2001, 2002). If the pollen dearth continues, no more brood can be produced.

3.2. Adult nutrition

Proteins make up 66–74% of the dry matter of adult workers (Hrassnigg and Crailsheim, 2005). Protein content increases during the first few days (De Groot, 1953) due to protein anabolism (Crailsheim, 1986), and slightly decreases in older bees. A worker bee consumes on average 3.4–4.3 mg pollen per day, with a peak at the age of nurses (Crailsheim et al., 1992). The amount of protein in the haemolymph also increases over the first few days after emergence and is determined to be 11.4–27.6 μg/μL (Cremonez et al., 1998) or 6.0–9.4 μg/μL (De Jong et al. 2009) for 6-day-old bees fed various pollens or protein diets.

The protein content of bees also varies depending on the season. In late summer, the last generation of bees produced shows distinct physiological characteristics (De Groot, 1953; Crailsheim, 1986; Kunert and Crailsheim, 1988) and a markedly increased lifespan compared to short-lived summer bees (Maurizio, 1954). This difference is accompanied by the bees' capability of building up high levels of haemolymph proteins, which allow them to survive several months on carbohydrates only. Vitellogenin, the main storage protein in the haemolymph and precursor for many other proteins (Amdam et al., 2003), reaches concentrations of up to 80 μg/μL in 'simulated' winter bees (produced in summer colonies without brood) older than 20 days. The importance of this lipoprotein for the onset of foraging by workers, longevity and overwintering has been demonstrated (Amdam and Omholt, 2002, 2003; Amdam et al., 2004).

3.2.1. Malnourished adults

The importance of protein provisions to adult bees has been demonstrated by many authors (see Crailsheim, 1990, for additional references). Cage studies established that adult honey bees can survive for a very long time on carbohydrates (see Sect. 2.2), which they need for energy metabolism, but bees allowed to also feed on pollen show greater longevity (Maurizio, 1954; Schmidt et al., 1987; Schmidt et al., 1995; Alqarni, 2006; Manning et al., 2007). The age-specific protein demand of honey bees is remarkable: young adults undergo physiological changes such as the maturation of flight muscles (Hersch et al., 1978), and suboptimal pollen diets delay the time that workers reach their maximum thorax masses (Hagedorn and Moeller, 1968). Pollen ingestion is also necessary to develop hypopharyngeal glands (Maurizio, 1954; Alqarni, 2006) and ovaries (Hoover

et al., 2006). The development of both is positively correlated with protein consumption (Pernal and Currie, 2000). A protein-free diet reduces the metabolic activity of haemocytes and increases the number of haemocytes (especially granular haemocytes), which may be a mechanism to compensate for the lack of protein in the haemolymph or the lower metabolic activity of haemocytes (Szymas and Jedruszuk, 2003). Alaux et al. (2010) confirmed this increase in haemocyte concentration and found glucose oxidase activity (an indicator of social immunity) and mass of fat body (an indirect indicator of immunocompetence of individual bees) to depend on protein nutrition.

3.3. Larval nutrition

To rear one larva, 25–37.5 mg protein (or 125–187.5 mg pollen) is needed (Hrassnigg and Crailsheim, 2005). Pollen is only fed directly to larvae in a small amount; the majority of protein a larva obtains is processed brood food from adult honey bees. Babendreier et al. (2004) calculated the portion of protein directly derived from pollen to be about 5%. Nurse bees have developed hypopharyngeal glands and the enzymatic equipment to process protein derived from pollen into a high-quality larval food (Moritz and Crailsheim, 1987) that allows honey bee larvae to grow rapidly. The number of feedings and the food applied by nurses is adjusted to the age of the larva. Young larvae are visited and fed less often than older lavae (Haydak, 1970; Schmickl and Crailsheim, 2002).

3.3.1. Malnourished larvae

Colonies terminate brood rearing rather than produce malnourished pupae (see Sect. 3.1.1), and according to Imdorf et al. (1998) this maintains the quality of pupae that are produced. In contrast, other authors found that malnourished larvae are reared into adults with impaired quality. This has been demonstrated drastically under experimental conditions, where starved larvae developed malformations (Jay, 1964). Impairments have also been demonstrated for life span and dry weight (Eischen et al., 1982), protein content (Kunert and Crailsheim, 1988), morphometrics (Herbert et al., 1988) and wing and body size (Daly et al., 1995; Brodschneider et al., 2009b, c).

To determine precisely the effect of larval nutrition on adult honey bees one can use feeding protocols to rear larvae in vitro (Rembold and Lackner, 1981; Aupinel et al., 2005). Honey bees reared according to these protocols have reduced body size and weight, especially dry weight of the thorax, compared to sister bees reared in a colony. Their flight capacity (measured in tethered flight in a roundabout) was similar to the controls, with minor drawbacks in high performance (Brodschneider et al., 2009b). Other effects of larval malnutrition on lifetime flight performance or onset of foraging can not be excluded.

Ovaries of workers reared under low-pollen conditions are less developed compared with those of workers reared by colonies given access to a high-pollen diet. Hoover et al. (2006) investigated the effect of larval nutrition on the ovaries of queenright workers and also demonstrated a carry-over of nutrients from the larval to the adult stage. When both groups were fed the same mixture of royal jelly and honey as adults, the ovaries of the bees reared under poor conditions did not develop as well as those of the others.

Larval nutrition may also affect behaviour and physiology of workers. Mattila and Otis (2006b) obtained longer-lived workers in one year and shorter-lived workers another year, when they supplemented colonies with protein in spring. This contrast between years suggests that other factors in addition to protein nutrition are responsible for their results. Bees reared under supplemented conditions also performed brood-related behaviours more often than did those reared under control conditions.

3.4. Feeding proteins

Protein-rich diets may be fed to honey bee colonies to enhance colony growth in

spring (Mattila and Otis, 2006a), in times of low or single source pollen income (e.g. Schmidt et al., 1995), or in areas or periods in which only poor-quality pollens are available (Somerville and Nicol, 2006). Supplementation in fall extends brood rearing before winter, but it does not facilitate wintering of fall-reared bees. Consequently, fall supplemented colonies did not perform better the next spring (Mattila and Otis, 2006a). Standifer et al. (1977) differentiated between pollen supplements (artificial high-protein diets containing pollen) and pollen substitutes (artificial high-protein diets containing no pollen but rather protein from soybean, brewer's yeast, milk, or algae, among others). Pollen substitutes may be a more cost-effective alternative to feeding pollen. Feeding pollen from other colonies incurs the risk of spreading pathogens, which can be mitigated by irradiating pollen.

Many studies have investigated worker bee preference for protein diets by measuring consumption. The reported consumption of diets varies widely (Tab. I) most likely due to biological reasons (type of diet, colony size, brood rearing, time of year, presence of other food sources inside or outside the hive). Furthermore, comparability between different experimental designs is low, because behaviour and nutritional physiology differ greatly among full colonies, nuclei and caged honey bees. Standardized definitions of these units and of the experimental procedures are needed. As discussed before, protein consumption is highest in young bees, which results in higher average consumption of caged honeybees during short investigation periods (Cremonez et al., 1998) compared to longer experiments (Pernal and Currie, 2000). Daily diet consumption is not more than 10 g for nuclei and up to 58.6 g for colonies (Tab. I). When we assume a total protein need of 40 mg per larva reared (Hrassnigg and Crailsheim, 2005) and a protein content of 20% of a given diet, we can roughly estimate that, per gram diet consumed, a colony gains enough protein to rear five larvae from egg to sealed stage. Of course, this is only true if all the protein is consumed by nurse bees and used to produce brood food without loss of protein. Thus it is an over-estimation as other adults obtain protein for their basic turnover as well (Crailsheim, 1986). Honey bees utilize pollen proteins very efficiently. We assume the digestibility of protein diets to be lower or similar to the 80% reported for pollen by Schmidt and Buchmann (1985). Thus, we reduce our estimation of how many larvae a colony could rear to four larvae per gram diet consumed. This calculation demonstrates that protein supplementation only results in high numbers of progeny when the applied diet is consumed in a substantial amount.

Consumption is a good first indication of the acceptance of supplemental diets. Pollen or lipids from pollen-containing diets (Herbert et al., 1980a) are consumed more readily than other substitutes due to phagostimulants in pollen (Tab. I, Hopkins et al., 1969; Schmidt and Hanna, 2006), making pollen without a doubt the most suitable additive for honey bee colonies. Fermented diets are consumed more readily than unfermented diets (Ellis and Hayes, 2009). Consumption also depends on the method of application, and beekeepers can easily adopt this to force the consumption of diets: patties enriched with soy flour (Avni et al., 2009), a non-soy-based pollen substitute or pollen-only patties are consumed more readily when the surface area of the patties is enlarged (Tab. I; Brodschneider et al., 2009a). This is because a larger surface gives more bees access to the patty, and the number of feeding bees positively correlates with consumption.

Loper and Berdel (1980a, b) demonstrated variations in protein consumption and sealed brood production in response to various pollen or protein diets fed to dwarf colonies of about 300 bees. In one experiment, they calculated a consumption of 0.31–0.99 mg protein per bee per day, and in another 0.12–1.24 mg, respectively. Schmidt and Buchmann (1985) reported an average consumption of 3.07 mg nitrogen during their 28-day experiment using a colony confined to a flight cage, which accounts for 0.69 mg daily protein consumption. This amount also corresponds to the value calculated by Brodschneider et al. (2009a) using a three-frame hive containing 6000 bees and brood and the ability to forage normally: 0.49 mg for the pollen supplement (assuming

the protein content of pollen to be 15%) and 0.38 mg for a commercial pollen substitute (Tab. I). Because both diets were present simultaneously, the total supplemental protein intake was 0.87 mg per bee per day in this experiment.

Supplemental diets were critically evaluated measuring physiological parameters of adult bees. Protein diets increase haemolymph protein levels in caged honey bees to values considered to be similar to natural nutrition (De Jong et al., 2009). Caged bees fed (ad libitum) a pollen substitute had fewer haemocytes compared to bees fed pollen or controls from a colony, and haemocytes exhibited lower metabolic activity compared to controls from the colony (Szymas and Jedruszuk, 2003). This test may provide a good diagnostic tool, but the authors did not report whether their substitute was consumed equivalently to pollen. Alqarni (2006) found longevity and hypopharyngeal gland development of caged honey bees fed protein diets (especially the pollen-enriched diet) to be similar, but not superior, to that of bees fed bee bread.

A colony can rear brood only when all essential nutrients are present in the bees' diet. Therefore both the consumption of protein diets and the number and quality of individuals reared by colonies fed with pollen substitutes are important. Honey bees can rear brood from protein diets other than pollen, and Herbert et al. (1977) demonstrated that the optimum protein level of diets required by caged honey bee colonies to rear brood is 23–30%; a protein level of 50% should be avoided. Nevertheless authors are inconsistent about whether supplemental feeding significantly increases brood production in free-flying colonies. Doull (1980a, b) found no increase in the number of reared bees in his year-long study. Mattila and Otis (2006a) reported an earlier start and increased brood rearing of colonies supplemented with pollen or pollen substitute in spring. DeGrandi-Hoffmann et al. (2008) found at least one pollen substitute in one of two trials to increase brood rearing compared to a pollen supplement. Avni et al. (2009) found no significant increase "vis-à-vis" a control group only fed carbohydrates with one exception. Pankiw et al. (2008) added brood pheromone to colonies fed a protein supplement during two winters in Texas and found increased brood areas, adult populations and diet consumption compared with colonies with protein supplement only. Doull (1980a, b), and Mattila and Otis in one of three years (2006a) found higher honey yields in supplemented colonies, which could be due partly to the carbohydrates introduced. Doull attributed higher honey yields to increased longevity of bees from supplemented colonies. This result was supported by van der Steen (2007), who found higher longevity of bees reared by colonies supplementally fed with a pollen substitute.

4. OTHER NUTRIENTS

We have focused on carbohydrates and proteins, as these macronutrients are well investigated, but it is also essential that honey bees ingest lipids, vitamins and minerals (Haydak, 1970). Supplemental feeding can support colonies with additional fats, vitamins and minerals, but the actual requirements and optimal levels needed by honey bees remain relatively unexplored. A significant challenge in studying these requirements is the formulation of clear-cut chemically defined diets for colonies (Herbert and Shimanuki, 1977, 1978b) and larvae reared under laboratory conditions. The latter has not yet been accomplished because diets remain semi-defined (Rembold and Lackner, 1981; Aupinel et al., 2005).

Honey bees obtain lipids exclusively from pollen, and the lipid content of pollen from various species ranges between 0.8% and 18.9% (Roulston and Cane, 2000). The importance of lipids as attractants in pollen was previously reviewed (Sect. 3.4), and oils are often added to artificial protein diets. Bees reared more brood when 2–4% lipid extracts from pollen were added to their diet (Herbert et al., 1980a). Lipids are mainly metabolized during the brood stage of honey bees and are regarded as an important energy source, and as precursors for further biosynthesis (Cantrill et al., 1981). Sterols in pollen are essential to honey bees, and workers convert ingested phytosterols to 24-methylenecholesterol (the

honey bees' major sterol), sitosterol and isofucosterol (Svoboda et al., 1982). They can not convert phytosterols to cholesterol. It has been demonstrated that diets containing 0.1% cholesterol or 24-methylenecholesterol supported bee survival and brood production, although honey bees also reared small amounts of brood without any dietary sterols (Herbert et al., 1980b). Regardless of the sterols present in the bees' diets, workers have the unique ability to transfer sterols selectively to larvae through brood food and hence maintain fairly consistent levels of 24-methylenecholesterol (Svoboda et al., 1986). This might involve the depletion of the workers' endogenous sterol pools. Toth et al. (2005) verified a link between nutritional state and behaviour; nurses have high abdominal lipid stores, which are depleted prior to the onset of foraging. These researchers could experimentally induce precocious foraging by treatment with a fatty acid synthesis inhibitor. When regular foragers were experimentally reverted to nurses, they did not regain high lipid stores, suggesting a reduced performance of these nurses (Toth and Robinson, 2005). Manning et al. (2007) tested the effect of enhancing low lipid pollen (from redgum trees) with fatty acids on the longevity of caged honey bees. They found a decreased life span of bees fed on concentrations of oleic acid higher than 2% but a greater tolerance of bees towards linoleic acid.

Water soluble vitamins, in contrast to fat soluble, are common in pollen (Roulston and Cane, 2000), but the content of vitamin C, for example, varies throughout the season according to different floral sources. No relationship has been found between the vitamin C level of pollen collected by free-flying honey bees and brood production (Herbert et al., 1985). Furthermore, prepupae reared by bees constrained to a vitamin C-free diet contained similar vitamin C levels than did those reared by bees fed vitamin C-enhanced diets. Therefore, honey bees (or their symbiotic microorganisms) are assumed to be able to synthesize vitamin C. Conversely, the presence of pyridoxine in a colony's diet is essential for the development of larvae. According to Anderson and Dietz (1976), nuclei were only able to rear brood when fed a diet containing 4 mg pyridoxine per 500 g, and 5.4 μg of pyridoxine was necessary to rear one larva to the sealed stage. A mixture of the fat soluble vitamins A, D, E and K in the diet substantially improved the amount of brood produced, although these vitamins are not regarded essential (Herbert and Shimanuki, 1978c). The same result was obtained when only vitamins A or K were seperately added to the diet.

Honey bees obtain inorganic elements mainly from pollen, and according to Imdorf et al. (1998) bees reared during pollen shortages contain similar quantities of most minerals compared to bees reared during favorable foraging conditions. This suggests other important sources of minerals like nectar and water or the existence of endogenous mineral pools in adults. Brood rearing significantly increased when Herbert and Shimanuki (1978b) added 1% of pollen ash to a synthetic diet, but levels exceeding 2% appeared to be disadvantageous. The authors recommended a diet containing 1000 ppm potassium, 500 ppm calcium, 300 ppm magnesium and 50 ppm each of sodium, zinc, manganese, iron and copper for further investigation of the mineral requirements of honey bees.

Water is indispensable for bee survival, and honey bees may collect water at high costs in some situations. However we do not discuss water homeostasis, because it has been reviewed recently (Nicolson, 2009).

5. NUTRITION-RELATED RISKS FOR THE HONEY BEE

5.1. Starvation

Exceptionally long non-foraging periods and insufficient or untimely feeding of carbohydrates after harvesting honey make starvation one of the most common reasons for honey bee colony winter mortality (Brodschneider et al., 2010; vanEngelsdorp et al., 2010). However, this is a common and well-known fact in colony management and it is the responsibility of beekeepers to avoid. The effects of protein malnutrition on colonies, adults and larvae were discussed in Sections 3.1.1, 3.2.1 and 3.3.1, respectively.

Starvation influences the behavioural development of honey bees: food shortage induces early onset of foraging in young workers, which affects the life span of the foraging population and colony demography (Schulz et al., 1998). Parasites, which also exert nutritional stress on honey bees, can intensify the effect of starvation. Alternately, in starving colonies nutritional stress caused by parasites is amplified. Workers infected with *Nosema ceranae* exhibit elevated hunger levels and changes in trophallactic behaviour (Naug and Gibbs, 2009). The lower energetic state caused by the parasite diminishes survival of bees in starving colonies and might play a role in the disappearing of foragers (Mayack and Naug, 2009). Another parasite, *Varroa destructor*, has been shown to reduce the vitellogenin storing capacity of infested workers and has a severe impact on their overwintering ability (Amdam et al., 2004).

5.2. Monocultures

Honey bees characteristically gather mixtures of pollens from many different species (Wille et al., 1985; Dimou and Thrasyvoulou, 2009), which support a balanced and diverse diet. Colonies used for pollination in agricultural areas face a less diversified diet of pollens, and this particular diet may not provide all essential nutrients. Only few uniform pollen diets – sweet clover (Campana and Moeller, 1977) or mustard (Singh and Singh, 1996) – are considered better than a diet of mixed (even poor) pollens, and generally single-pollen diets are inferior to mixed diets (Schmidt, 1984; Schmidt et al., 1987; Alaux et al., 2010). Schmidt et al. (1995) concluded from their experiments that bees used for pollination, having access only to sunflower or sesame pollen, are likely to be stressed and should be provided additional pollen or protein sources. Diverse pollen diets may compensate for deficiencies in essential nutrients in the pollen of one species (the poverty of arginine in dandelion pollen, for example, Herbert et al., 1970). If only pollen lacking this essential nutrient is available, honey bees can not compensate by consuming more pollen of such poorer quality. This nutritional stress, among other factors, may be responsible for high colony mortalities (Naug, 2009).

5.3. Transgenic products

The non-target impact of genetically modified crops on honey bees is poorly understood. Many authors (reviewed in Malone and Pham-Delègue, 2001) studied the effect of dietary transgene products on honey bees, because of the possible threat emanating from plants containing *Bacillus thuringiensis* (Bt) toxins or protease inhibitors. Malone et al. (2004) demonstrated that the feeding of insecticidal transgenic plant proteins to caged bees did not affect survival and hypopharyngeal gland development. Very high concentrations of Cry1Ab proteins may affect workers' learning or feeding behaviour (Ramirez-Romero et al., 2008), but longevity is not reduced. From the calculations of Babendreier et al. (2004) we can assume that the amount of Bt-toxin from maize pollen that a larva ingests during its development is far below toxicity.

5.4. Pesticide residues in nutrients

When foraging in the proximity of landscapes used extensively for agriculture, honey bees unavoidably bring chemicals applied for plant protection into their colonies by collecting contaminated nectar and pollen. Several studies (e.g. Rortais et al., 2005; Johnson et al., 2010) estimated the potential risk of insecticides ingested by honey bees through contaminated pollen and nectars. Assuming maximum exposures, it has been demonstrated that toxins may reach levels considered lethal or sublethal to bees. Recently, leaf guttation drops of corn plants germinated from neonicotinoid-coated seeds have been demonstrated to contain insecticides at concentrations that are lethal to honey bees (Girolami et al., 2009). However, the importance of this must be investigated once the likelihood of bees collecting water from these leaf guttation drops has been clarified.

5.5. Poisoning by plants

Some plants, including those that are recommended honey crops produce substances that are poisonous to honey bees. For example, poisonings from protoanemonin present in flowers in the Ranunculaceae have been suspected in relation to the condition of May disease. A toxic saponin is found in the nectar and pollen of *Aesculus* species (Hippocastanaceae). A thorough discussion of this topic can be found in Barker (1990). The problem regarding sugars that are toxic to honey bees has been indicated in Section 2.2.

6. DISCUSSION

Carbohydrates, proteins, lipids, vitamins and minerals available to honey bees are factors responsible for the amount of progeny produced, longevity and health of adults and for the survival and productivity of a colony. Colonies facing a limitation of an essential nutrient, such as pollen in general, or an essential amino acid or vitamin in particular, will cease brood production and may not survive if not supplied with the missing nutrient.

Recent honey bee colony losses reintroduced nutrition as a focus of research as it was speculated that poor nutrition may be a crucial or subtle (co-)factor in the occurrence of such losses (Oldroyd, 2007; Naug, 2009). Last year, vanEngelsdorp et al. (2009) demonstrated that workers from colonies affected by CCD showed no alteration in protein nutritional state. Still, honey bee nutrition bears many potential threats including starvation (Brodschneider et al., 2010), reduced diversity of diets due to monocultures, and pesticides brought to the colony with food.

Our understanding of how essential lipids, vitamins or minerals are for brood rearing and development of adults is still limited. First, we know that the addition of some substances at particular concentrations supports brood rearing, but not how progeny from colonies lacking this substance perform in the long-run. Second, we know that colonies are inexplicably able to rear brood even when substances regarded as essential are missing (Herbert et al., 1980b). Also, researchers often stress that workers are able to mobilize endogenous reserves. However, we still know little about these mechanisms. Can depleted stores be replenished, or do workers satisfactorily forage after exhausting their endogenous pools? In this context, little is known about the role of the mutualistic flora of the honey bee intestinal tract (Gilliam, 1997; Forsgren et al., 2010) and how antibiotics fed to colonies may affect the benefits of the microflora for honey bees.

Honey bees have evolved many strategies to cope with parasites and pathogens, but if they are nutritionally stressed, they face a major battle. Therefore, future research must take into account the interaction of possible nutrition-related effects with other factors, such as the influence of nutrition on susceptibility or tolerance of honey bees to parasites, pathogens and pesticides, the energetic stress of bees caused by parasites (Amdam et al., 2004; Mayack and Naug, 2009) and the role of nutrition in building up the honey bee's immune system (Randolt et al., 2008). The studies of Szymas and Jedruszuk (2003) and Alaux et al. (2010) raise the important question of how the honey bee's ability to defend pathogens is hindered when they are malnourished and how this ability may be improved by adequate nutrition.

The honey bee colony as a super-organism comprises three levels of nutrition (Fig. 1). The fact that malnutrition can happen on any level reveals an important consideration about nutritional stress: larvae reared during the shortage of an essential nutrient may develop into short-lived adults or adults with slightly impaired brood rearing and foraging abilities (Maurizio, 1954; Dustmann and von der Ohe, 1988; Mattila and Otis, 2006b; Brodschneider et al., 2009b). This impairment might range from being very subtle to substantial. Little is known about sublethal larval malnutrition in combination with sublethal adult malnutrition. Moreover, the abilities of honey bees can be impaired by stressors other than nutrition, like low temperature during pupal development (Tautz et al., 2003), parasitism (Kralj et al., 2007) or sublethal pesticide effects. We assume that the effects of these different factors may combine to pose a substantial threat.

To achieve well-fed and healthy colonies we recommend balanced nutrition for colonies, especially when they are placed in a difficult environment or used for pollination (Schmidt et al., 1995; Pernal and Currie, 2000; Somerville and Nicol, 2006). Balanced nutrition is best supported by growing a diversity of plants, even near agricultural areas, as a natural mixture of different pollens is the optimal source of proteins and vitamins for honey bees (Decourtye et al., 2010). Where this is not possible, feeding adequate supplemental diets is recommended, even if they are of poorer quality than natural pollen, because the diets can provide many essential nutrients. To our knowledge there are no reports that diets prepared meeting the criteria presented in this review harm the bees, although the inferiority of bees or colonies fed exclusively on artificial diets compared to those fed natural pollen has been demonstrated.

Nutrition et santé des abeilles.

malnutrition / pollen / protéines / carbohydrates / nourrissement

Zusammenfassung – Ernährung und Gesundheit bei Honigbienen. Eine ausgewogene Ernährung mit ausreichend Proteinen, Kohlenhydraten, Fetten, Vitaminen und Mineralstoffen ist notwendig für das Überleben eines Bienenvolkes, die Entwicklung der Arbeiterinnen und die Aufzucht von Brut. Im Superorganismus Honigbiene sind diese drei Ebenen der Ernährung eng miteinander verknüpft (Abb. 1), und Defizite in einer dieser Ebenen wirken sich negativ auf die anderen aus.

Für das Überleben des Volkes sind vor allem Kohlenhydrate notwendig. Eine Arbeiterin benötigt pro Tag etwa 4 mg verwertbaren Zucker. Allerdings sind nicht alle Zucker verwertbar, einige sind für Bienen giftig. Ebenfalls giftig ist Hydroxymethylfurfural (HMF) das sich bei thermischer Zersetzung und langer Lagerung aus Zuckern bildet. Der HMF Gehalt erhältlicher Maissirupe liegt zwischen 3,1 und 28,7 ppm, kann aber durch Lagerung bei zu hohen Temperaturen drastisch ansteigen und die Mortalität von Bienen erhöhen.

Pollen ist die natürliche Proteinquelle von Bienen. Daraus bilden Ammenbienen ein proteinreiches Futter für die Brut. Ist nicht genügend Pollen vorhanden, reduziert das Bienenvolk die Zahl der produzierten Larven durch Kannibalismus. Ein Mangel von Protein in der Larval- oder Adultnahrung führt zur reduzierten Entwicklung der Brutfutterdrüsen und Ovarien sowie einer kürzeren Lebensdauer. Proteinmangel während der Larvalernährung führt darüber hinaus zu beeinträchtigter Thoraxentwicklung, Flugleistung und Verhaltensänderungen. Bei Pollenmangel können dem Bienenvolk andere Proteinquellen angeboten werden, Tabelle I zeigt die pro Tag konsumierten Mengen unterschiedlicher Diäten, deren Bestandteile, Proteingehalt und die Größe der untersuchten Einheit. Ein Proteingehalt zwischen 23 und 30 % hat sich als zur Brutaufzucht geeignet erwiesen. Unseren Berechnungen zufolge erhält ein Volk mit jedem konsumierten Gramm etwa die Menge Protein die 4 Larven bis zur Verdeckelung benötigen.

Pollen liefert ebenfalls Fette, die vor allem in der Larvalentwicklung benötigt werden. Honigbienen können Sterole nicht selbst herstellen, und verfüttern überwiegend 24-Methylen-Cholesterin an die Brut. Das tun sie, unter Verwendung von Körperreserven auch dann, wenn kein Cholesterin in der Nahrung vorhanden ist.

Arbeiterinnen (oder symbiontische Mikroorganismen) sind in der Lage Vitamin C zu synthetisieren. Pyridoxin, ein Vitamin aus dem B-Komplex, ist hingegen notwendig für erfolgreiche Brutaufzucht. Obwohl fettlösliche Vitamine nicht essentiell für die Honigbiene sind, steigert ihre Anwesenheit in der Diät die Menge an produzierter Brut.

Neben dem Verhungern oder der erwähnten Mangelernährung stellen einseitige Ernährung durch Monokulturen, genetisch modifizierte Pflanzen oder vom Menschen oder der Pflanze produzierte Giftstoffe die mit der Nahrung eingetragen werden Gefahren für die Honigbiene dar.

Mangelernährung / Pollen / Protein / Kohlenhydrate / Zufütterung

REFERENCES

Alaux C., Ducloz F., Crauser D., Le Conte Y. (2010) Diet effects on honeybee immunocompetence, Biol. Lett., DOI:10.1098/rsbl.2009.0986.

Alqarni A.S. (2006) Influence of some protein diets on the longevity and some physiological conditions of honeybee *Apis mellifera* L. workers, J. Biol. Sci. 6, 734–737.

Amdam G.V., Omholt S.W. (2002) The regulatory anatomy of honeybee lifespan, J. Theor. Biol. 216, 209–228.

Amdam G.V., Omholt S.W. (2003) The hive bee to forager transition in honeybee colonies: the double repressor hypothesis, J. Theor. Biol. 223, 451–464.

Amdam G.V., Hartfelder K., Norberg K., Hagen A., Omholt S.W. (2004) Altered physiology in worker honey bees (Hymenoptera: Apidae) infested with the mite Varroa destructor (Acari: Varroidae): a

factor in colony loss during overwintering? J. Econ. Entomol. 97, 741–747.

Amdam G.V., Norberg K., Hagen A., Omholt S.W. (2003) Social exploitation of vitellogenin, Proc. Natl. Acad. Sci. 100, 1799–1802.

Anderson L.M., Dietz A. (1976) Pyridoxine requirement of the honey bee (*Apis mellifera*) for brood rearing, Apidologie 7, 67–84.

Aupinel P., Fortini D., Dufour H., Tasei J.N., Michaud B., Odoux J.F., Pham-Delègue M.H. (2005) Improvement of artificial feeding in a standard in vitro method for rearing *Apis mellifera* larvae, Bull. Insect 58, 107–111.

Avni D., Dag A., Shafir S. (2009) The effect of surface area of pollen patties fed to honey bee (*Apis mellifera*) colonies on their consumption, brood production and honey yields, J. Apic. Res. 48, 23–28.

Babendreier D., Kalberer N., Romeis J., Fluri P., Bigler F. (2004) Pollen consumption in honey bee larvae: a step forward in the risk assessment of transgenic plants, Apidologie 35, 293–300.

Barker R.J. (1977) Some carbohydrates found in pollen and pollen substitutes are toxic to honey bees, J. Nutr. 107, 1859–1862.

Barker R.J. (1990) Poisoning by plants, in: Morse R.A., Nowogrodzki R. (Eds.), Honey bee pests, predators, and diseases, Cornell University Press, Ithaca, N.Y. and London, pp. 306–328.

Barker R.J., Lehner Y. (1974) Acceptance and sustenance value of naturally occurring sugars fed to newly emerged adult workers of honey bees (*Apis mellifera* L.), J. Exp. Zool. 187, 277–285.

Barker R.J., Lehner Y. (1976) Galactose a sugar toxic to honey bees found in exudate of tulip flowers, Apidologie 7, 109–112.

Barker R.J., Lehner Y. (1978) Laboratory comparison of high fructose corn syrup, grape syrup, honey, and sucrose syrup as maintenance food for caged honey bees, Apidologie 9, 111–116.

Brodschneider R., Moosbeckhofer R., Crailsheim K. (2010) Surveys as a tool to record winter losses of honey bee colonies – a 2-year case study in Austria and South Tyrol, Tyrol, J. Apic. Res. 49, 23–30.

Brodschneider R., Hrassnigg N., Vollmann J., Petz M., Riessberger-Gallé U., Crailsheim K. (2007) Liquid nutrition within a honeybee colony – who feeds? Apidologie 38, 492.

Brodschneider R., Haidmayer C., Riessberger-Gallé U., Crailsheim K. (2009a) Protein uptake in honeybee colonies supplemented with two protein diets simultaneously, Apidologie 40, 662.

Brodschneider R., Riessberger-Gallé U., Crailsheim K. (2009b) Flight performance of artificially reared honeybees (*Apis mellifera*), Apidologie 40, 441–449.

Brodschneider R., Steiner D., Moder A., Vollmann J., Riessberger-Gallé U., Crailsheim K. (2009c) Synthetic larval diet produces lighter and smaller honeybees (*Apis mellifera*), Apidologie 40, 663–664.

Campana B.J., Moeller F.E. (1977) Honey bees: preference for and nutritive value of pollen from five plant sources, J. Econ. Entomol. 70, 39–41.

Cantrill R.C., Hepburn H.R., Warner S.J. (1981) Changes in lipid composition during sealed brood development of African worker honeybees, Comp. Biochem. Physiol. B 68, 351–353.

Crailsheim K. (1986) Dependence of protein metabolism on age and season in the honeybee (*Apis mellifica carnica* Pollm), J. Insect Physiol. 32, 629–634.

Crailsheim K. (1990) The protein balance of the honey bee worker, Apidologie 21, 417–429.

Crailsheim K. (1991) Interadult feeding of jelly in honeybee (*Apis mellifera* L.) colonies, J. Comp. Physiol. B 161, 55–60.

Crailsheim K. (1998) Trophallactic interactions in the adult honeybee (*Apis mellifera* L.), Apidologie 29, 97–112.

Crailsheim K., Schneider L.H.W., Hrassnigg N., Bühlmann G., Brosch U., Gmeinbauer R., Schöffmann B. (1992) Pollen consumption and utilization in worker honeybees (*Apis mellifera carnica*): dependence on individual age and function, J. Insect Physiol. 38, 409–419.

Cremonez T.M., de Jong D., Bitondi M.M.G. (1998) Quantification of hemolymph proteins as a fast method for testing protein diets for honey bees (Hymenoptera: Apidae), J. Econ. Entomol. 91, 1284–1289.

Daly H.V., Danka R.G., Hoelmer K., Rinderer T.E., Buco S.M. (1995) Honey bee morphometrics: linearity of variables with respect to body size and classification tested with European worker bees reared by varying ratios of nurse bees, J. Apic. Res. 34, 129–145.

DeGrandi-Hoffman G., Hagler J. (2000) The flow of incoming nectar through a honey bee (*Apis mellifera* L.) colony as revealed by a protein marker, Insectes Soc. 47, 302–306.

DeGrandi-Hoffman G., Wardell G., Ahumada-Secura F., Rinderer T.E., Danka R., Pettis J. (2008) Comparisons of pollen substitute diets for honeybees: consumption rates by colonies and effects on brood and adult populations, J. Apic. Res. 47, 265–270.

De Groot A.P. (1953) Protein and amino acid requirements of the honeybee (*Apis mellifica* L.), Physiol. Comp. Oecol. 3, 197–285.

De Jong D., da Silva E.J., Kevan P.G., Atkinson J.L. (2009) Pollen substitutes increase honey bee haemolymph protein levels as much as or more than does pollen, J. Apic. Res. 48, 34–37.

Decourtye A., Mader E., Desneux N. (2010) Landscape scale enhancement of floral resources for honey bees in agro-ecosystems, Apidologie 41, 264–277.

Dietz A., Stevenson H.R. (1980) Influence of long term storage on the nutritional value of frozen pollen for brood rearing of honey bees, Apidologie 11, 143–151.

Dimou M., Thrasyvoulou A. (2009) Pollen analysis of honeybee rectum as a method to record the bee pollen flora of an area, Apidologie 40, 124–133.

Doner L.W. (1977) The sugars of honey - a review, J. Sci. Food Agric. 28, 443–456.

Doull K.M. (1980a) Relationships between consumption of a pollen supplement, honey production, and broodrearing in colonies of honeybees *Apis mellifera* L. I, Apidologie 11, 361–365.

Doull K.M. (1980b) Relationships between consumption of a pollen supplement, honey production and broodrearing in colonies of honeybees *Apis mellifera* L. II, Apidologie 11, 367–374.

Dustmann J.H., von der Ohe W. (1988) Einfluβ von Kälteeinbrüchen auf die Frühjahrsentwinklung von Bienenvölkern (*Apis mellifera* L), Apidologie 19, 245–254.

Eischen F.A., Rothenbuhler W.C., Kulincevic J.M. (1982) Length of life and dry weight of worker honeybees reared in colonies with different worker-larva ratios, J. Apic. Res. 21, 19–25.

Ellis A.M., Hayes G.W. Jr (2009) An evaluation of fresh versus fermented diets for honey bees (*Apis mellifera*), J. Apic. Res. 48, 215–216.

Free J.B. (1965) The behaviour of honeybee foragers when their colonies are fed sugar syrup, J. Apic. Res. 4, 85–88.

Forsgren E., Vásquez A., Olofsson T.C., Fries I. (2010) Novel lactic acid bacteria inhibiting *Paenibacillus larvae* in honey bee larvae, Apidologie 41, 99–108.

Gilliam M. (1997) Identification and roles of non-pathogenic microflora associated with honey bees, FEMS Microbiol. Lett. 155, 1–10.

Girolami V., Mazzon L., Squartini A., Mori N., Marzaro M., Di Bernardo A., Greatti M., Giorio C., Tapparo A. (2009) Translocation of neonicotinoid insecticides from coated seeds to seedling guttation drops: a novel way of intoxication for bees, J. Econ. Entomol. 102, 1808–1815.

Hagedorn H.H., Moeller F.E. (1968) Effect of the age of pollen used in pollen supplements on their nutritive value for the honeybee. I. Effect on thoracic weight, development of hypopharyngeal glands, and brood rearing, J. Apic. Res. 7, 89–95.

Haydak M.H. (1935) Brood rearing by honeybees confined to a pure carbohydrate diet, J. Econ. Entomol. 28, 657–660.

Haydak M.H. (1970) Honey bee nutrition, Ann. Rev. Entomol. 15, 143–156.

Herbert E.W. (1980) Effect of diet on the rate of brood rearing by naturally and instrumentally inseminated queens, Apidologie 11, 57–62.

Herbert E.W., Shimanuki H. (1977) Brood-rearing capability of caged honeybees fed synthetic diets, J. Apic. Res. 15, 150–153.

Herbert E.W., Shimanuki H. (1978a) Chemical composition and nutritive value of bee-collected and bee-stored pollen, Apidologie 9, 33–40.

Herbert E.W., Shimanuki H. (1978b) Mineral requirements for brood-rearing by honey bees fed a synthetic diet, J. Apic. Res. 17, 118–122.

Herbert E.W., Shimanuki H. (1978c) Effect of fat soluble vitamins on the brood rearing capabilities of honey bees fed a synthetic diet, Ann. Entomol. Soc. Am. 71, 689–691.

Herbert E.W., Shimanuki H. (1982) Effect of population density and available diet on the rate of brood rearing by honey bees offered a pollen substitute, Apidologie 13, 21–28.

Herbert E.W., Bickley W.E., Shimanuki H. (1970) The brood-rearing capability of caged honey bees fed dandelion and mixed pollen diets, J. Econ. Entomol. 63, 215–218.

Herbert E.W., Shimanuki H. Caron D. (1977) Optimum protein levels required by honey bees (Hymenoptera, Apidae) to initiate and maintain brood rearing, Apidologie 8, 141–146.

Herbert E.W., Shimanuki H., Shasha B.S. (1980a) Brood rearing and food consumption by honeybee colonies fed pollen substitutes supplemented with starch encapsulated pollen extracts, J. Apic. Res. 19, 115–118.

Herbert E.W. Jr., Svoboda J.A., Thompson M.J., Shimanuki H. (1980b) Sterol utilization in honey bees fed a synthetic diet: Effects on brood rearing, J. Insect Physiol. 26, 287–289.

Herbert E.W., Sylvester H.A., Vandenberg J.D., Shimanuki H. (1988) Influence of nutritional stress and the age of adults on the morphometrics of honey bees (*Apis mellifera* L.), Apidologie 19, 221–230. 26, 287–289.

Herbert E.W., Vanderslice J.T., Higgs D.J. (1985) Effect of dietary vitamin C levels on the rate of brood production of freeflying and confined colonies of honey bees, Apidologie 16, 385–394.

Hersch M.I., Crewe R.M., Hepburn H.R., Thompson P.R., Savage N. (1978) Sequential development of glycolytic competence in muscles of worker honeybees, Comp. Biochem. Physiol. B 61, 427–431.

Hoover S.E., Higo H.A., Winston M.L. (2006) Worker honey bee ovary development: seasonal variation and the influence of larval and adult nutrition, J. Comp. Physiol. B 176, 55–63.

Hopkins C.Y., Jevans A.W., Boch R. (1969) Occurence of octadeca-trans-2, cis-9, cis-12- trienoic acid in pollen attractive to the honey bee, Can. J. Biochem. Cell Biol. 47, 433–436.

Hrassnigg N., Crailsheim K. (2005) Differences in drone and worker physiology in honeybees (*Apis mellifera* L.), Apidologie 36, 255–277.

Hrassnigg N., Brodschneider R., Fleischmann P.H., Crailsheim K. (2005) Unlike nectar foragers, honeybee drones (*Apis mellifera*) are not able to utilize starch as fuel for flight, Apidologie 36, 547–557.

Imdorf A., Rickli M., Kilchenmann V., Bogdanov S., Wille H. (1998) Nitrogen and mineral constituents of honey bee worker brood during pollen shortage, Apidologie 29, 315–325.

Jachimowicz T., El Sherbiny G. (1975) Zur Problematik der Verwendung von Invertzucker für die Bienenfütterung (Problems of invert sugar as food for honeybees), Apidologie 6, 121–143.

Jay S.C. (1964) Starvation studies of larval honey bees, Can. J. Zool. 42, 455–462.

Johnson R.M., Ellis M.D., Mullin C.A., Frazier M. (2010) Pesticides and honey bee toxicity – U.S.A., Apidologie, 41, 312–331.

Kralj J., Brockmann A., Fuchs S., Tautz, J. (2007) The parasitic mite *Varroa destructor* affects non-associative learning in honey bee foragers, *Apis mellifera* L., J. Comp. Physiol. A 193, 363–370.

Kunert K., Crailsheim K. (1988) Seasonal changes in carbohydrate, lipid and protein content in emerging worker honeybees and their mortality, J. Apic. Res. 27, 13–21.

LeBlanc B.W., Eggleston G., Sammataro D., Cornett C., Dufault R., Deeby T., Cyr E.ST. (2009) Formation of hydroxymethylfurfural in domestic high-fructose corn syrup and its toxicity to the honey bee (*Apis mellifera*), J. Agric. Food Chem. 57, 7369–7376.

Loper G.M., Berdel R.L. (1980a) A nutritional bioassay of honeybee brood-rearing potential, Apidologie 11, 181–189.

Loper G.M., Berdel R.L. (1980b) The effects of nine pollen diets on broodrearing of honeybees, Apidologie 11, 351–359.

Malone L.A., Pham-Delègue M.-H. (2001) Effects of transgene products on honey bees (*Apis mellifera*) and bumblebees (*Bombus* sp.), Apidologie 32, 287–304.

Malone L.A., Todd J.H., Burgess E.P.J, Christeller J.T. (2004) Development of hypopharyngeal glands in adult honey bees fed with a Bt toxin, a biotin-binding protein and a protease inhibitor, Apidologie 35, 655–664.

Manning R., Rutkay A., Eaton L., Dell B. (2007) Lipid-enhanced pollen and lipid-reduced flour diets and their effect on the longevity of honey bees (*Apis mellifera* L.), Aust. J. Entomol. 46, 251–257.

Mattila H.R., Otis G.W. (2006a) Influence of pollen diet in spring on development of honey bee (Hymenoptera: Apidae) colonies, J. Econ. Entomol. 99, 604–613.

Mattila H.R., Otis G.W. (2006b) The effects of pollen availability during larval development on the behaviour and physiology of spring-reared honey bee, Apidologie 37, 533–546.

Maurizio A. (1954) Pollenernährung und Lebensvorgänge bei der Honigbiene (*Apis mellifica* L.), Landwirtsch. Jahrb. Schweiz 62, 115–182.

Mayack C., Naug D. (2009) Energetic stress in the honeybee *Apis mellifera* from *Nosema ceranae* infection, J. Invertebr. Pathol. 100, 185–188.

McLellan A.R. (1977) Honeybee colony weight as an index of honey production and nectar flow: A critical evaluation, J. Appl. Ecol. 14, 401–408.

Moritz B., Crailsheim K. (1987) Physiology of protein digestion in the midgut of the honeybee (*Apis mellifera* L.), J. Insect Physiol. 33, 923–931.

Naug D. (2009) Nutritional stress due to habitat loss may explain recent honeybee colony collapses, Biol. Conserv. 142, 2369–2372.

Naug D., Gibbs A. (2009) Behavioral changes mediated by hunger in honeybees infected with *Nosema ceranae*, Apidologie 40, 595–599.

Neupane K.R., Thapa R.B. (2005) Alternative to off-season sugar supplement feeding of honeybees, J. Inst. Agric. Anim. Sci. 26, 77–81.

Nicolson S.W. (2009) Water homeostasis in bees, with the emphasis on sociality, J. Exp. Biol. 212, 429–434.

Nicolson S.W., Human H. (2008) Bees get a head start on honey production, Biol. Lett. 4, 299–301.

Oldroyd B.P. (2007) What's killing American honey bees? PLoS Biol. 5, e168.

Pankiw T., Sagili R.R., Metz B.N. (2008) Brood pheromone effects on colony protein supplement consumption and growth in the honey bee (Hymenoptera: Apidae) in a subtropical winter climate, J. Econ. Entomol. 101, 1749–1755.

Pernal S.F., Currie R.W. (2000) Pollen quality of fresh and 1-year-old single pollen diets for worker honey bees (*Apis mellifera* L.), Apidologie 31, 387–409.

Ramirez-Romero R., Desneux N., Decourtye A., Chaffiol A., Pham-Delègue M.H. (2008) Does Cry1Ab protein affect learning performances of the honey bee *Apis mellifera* L. (Hymenoptera, Apidae)? Ecotoxicol. Environ. Saf. 70, 327–333.

Randolt K., Gimple O., Geissendörfer J., Reinders J., Prusko C., Mueller M.J., Albert S., Tautz J., Beier H. (2008) Immune-related proteins induced in the hemolymph after aseptic and septic injury differ in honey bee worker larvae and adults, Arch. Insect Biochem. Physiol. 69, 155–167.

Rembold H., Lackner B. (1981) Rearing of honeybee larvae in vitro: Effect of yeast extract on queen differentiation, J. Apic. Res. 20, 165–171.

Rortais A., Arnold G., Halm M.-P., Touffet-Briens F. (2005) Modes of honeybees exposure to systemic insecticides: estimated amounts of contaminated pollen and nectar consumed by different categories of bees, Apidologie 36, 71–83.

Roulston T.H., Cane J.H. (2000) Pollen nutritional content and digestibility for animals, Plant Syst. Evol. 222, 187–209.

Roulston T.H., Cane J.H., Buchmann S.L. (2000) What governs protein content of pollen: pollinator preferences, pollen–pistil interactions, or phylogeny? Ecol. Monogr. 70, 617–643.

Schmickl T., Crailsheim K. (2001) Cannibalism and early capping: strategies of honeybee colonies in times of experimental pollen shortages, J. Comp. Physiol. A 187, 541–547.

Schmickl T., Crailsheim K. (2002) How honeybees (*Apis mellifera* L.) change their broodcare behavior in response to non-foraging conditions and poor pollen conditions, Behav. Ecol. Sociobiol. 51, 415–425.

Schmickl T., Crailsheim K. (2004) Inner nest homeostasis in a changing environment with special emphasis on honey bee brood nursing and pollen supply, Apidologie 35, 249–263.

Schmidt J.O. (1984) Feeding preference of *Apis mellifera* L. (Hymenoptera: Apidae): individual versus mixed pollen species, J. Kans. Entomol. Soc. 57, 323–327.

Schmidt J.O., Buchmann S.L. (1985) Pollen digestion and nitrogen-utilization by *Apis mellifera* L. (Hymenoptera, Apidae), Comp. Biochem. Physiol. A 82, 499–503.

Schmidt J.O., Hanna A. (2006) Chemical nature of phagostimulants in pollen attractive to honeybees, J. Insect Physiol. 19, 521–532.

Schmidt J.O., Thoenes S.C., Levin M.D. (1987) Survival of honey bees, *Apis mellifera* (Hymenoptera: Apidae), fed various pollen sources, J. Econ. Entomol. 80, 176–183.

Schmidt L.S., Schmidt J.O., Rao H., Wang W., Xu L. (1995) Feeding preference of young worker honey bees (Hymenoptera: Apidae) fed rape, sesame, and sunflower pollen, J. Econ. Entomol. 88 1591–1595.

Schulz D.J., Huang Z.-Y., Robinson G.E. (1998) Effect of colony food shortage on the behavioral development of the honey bee, *Apis mellifera*, Behav. Ecol. Sociobiol. 42, 295–303.

Seeley T.D. (1989) The honey bee colony as a superorganism, Am. Sci. 77, 546–553.

Seeley T.D., Visscher P.K. (1985) Survival of honeybees in cold climates: the critical timing of colony growth and reproduction, Ecol. Entomol. 10, 81–88.

Severson D.W., Erickson E.H. (1984) Honey bee (Hymenoptera: Apidae) colony performance in relation to supplemental carbohydrates, J. Econ. Entomol. 77, 1473–1478.

Singh R.P., Singh P.N. (1996) Amino acid and lipid spectra of larvae of honey bee (*Apis cerana* Fabr) feeding on mustard pollen, Apidologie, 27, 21–28.

Somerville D.C., Nicol H.I. (2006) Crude protein and amino acid composition of honey bee-collected pollen pellets from south-east Australia and a note on laboratory disparity, Aust. J. Exp. Agr. 46, 141–149.

Standifer L.N., Moeller F.E., Kauffeld N.M., Herbert E.W., Shimanuki, H. (1977) Supplemental feeding of honey bee colonies, USDA Agr. Inform. Bull. No. 413, 8 p.

Staudenmayer T. (1939) Die Giftigkeit der Mannose für Bienen und andere Insekten, J. Comp. Physiol. A 26, 644–668.

Svoboda J.A., Herbert, E.W., Thompson M.J. Feldlaufer M.F. (1986) Selective sterol transfer in the honey bee: Its significance and relationship to other hymenoptera, Lipids 21, 97–101.

Svoboda J.A., Thompson M.J., Herbert E.W., Shortino T.J., Szczepanik-Vanleeuwen P.A. (1982) Utilization and metabolism of dietary sterols in the honey bee and the yellow fever mosquito, Lipids 17, 220–225.

Szymas B., Jedruszuk A. (2003) The influence of different diets on haemocytes of adult worker honey bees, *Apis mellifera*, Apidologie 34, 97–102.

Tautz J., Maier S., Groh C., Rössler W., Brockmann A. (2003) Behavioral performance in adult honey bees is influenced by the temperature experienced during their pupal development, Proc. Natl. Acad. Sci. 100, 7343–7347.

Toth A.L., Robinson G.E. (2005) Worker nutrition and division of labour in honeybees, Anim. Behav. 69, 427–435.

Toth A.L., Kantarovich S., Meisel A.F., Robinson G.E. (2005) Nutritional status influences socially regulated foraging ontogeny in honey bees, J. Exp. Biol. 208, 4641–4649.

van der Steen J. (2007) Effect of a home-made pollen substitute on honey bee colony development, J. Apic. Res. 46, 114–119.

vanEngelsdorp D., Evans J.D., Saegermann C., Mullin C., Haubrugge E., Nguyen B.K., Frazier M., Frazier J., Cox-Foster D., Chen Y., Underwood R., Tarpy D.R., Pettis J.S. (2009) Colony Collapse Disorder: a descriptive study, PLoS ONE 4, e6481.

vanEngelsdorp D., Hayes J., Underwood R.M., Pettis J.S. (2010) A survey of honey bee colony losses in the United States, fall 2008 to spring 2009, J. Apic. Res. 49, 7–14.

Vásquez A., Olofsson T.C. (2009) The lactic acid bacteria involved in the production of bee pollen and bee bread, J. Apic. Res. 48, 189–195.

Wille H., Wille M., Kilchenmann V., Imdorf A., Bühlmann G. (1985) Pollenernte und Massenwechsel von drei *Apis mellifera*-Völkern auf demselben Bienenstand in zwei aufeinanderfolgenden Jahren, Rev. Suisse Zool. 92, 897–914.