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ORIGINAL ARTICLE

Nutritional balance of essential amino acids and carbohydrates of the adult worker honeybee depends on age

Pier P. Paoli · Dion Donley · Daniel Stabler · Anumodh Saseendranath · Susan W. Nicolson · Stephen J. Simpson · Geraldine A. Wright

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Abstract Dietary sources of essential amino acids (EAAs) are used for growth, somatic maintenance and reproduction. Eusocial insect workers such as honeybees are sterile, and unlike other animals, their nutritional needs should be largely dictated by somatic demands that arise from their role within the colony. Here, we investigated the extent to which the dietary requirements of adult worker honeybees for EAAs and carbohydrates are affected by behavioural caste using the Geometric Framework for nutrition. The nutritional optimum, or intake target (IT), was determined by confining cohorts of 20 young bees or foragers to liquid diets composed of specific proportions of EAAs and sucrose. The IT of young, queenless bees shifted from a proportion of EAAs-to-carbohydrates (EAA:C) of 1:50 towards 1:75 over a 2-week period, accompanied by a reduced lifespan on diets high in EAAs. Foragers required a diet high in carbohydrates (1:250) and also had low survival on diets high in EAA. Workers exposed to queen mandibular pheromone lived longer on diets high in EAA, even when those diets contained 5× their dietary requirements. Our data show that worker honeybees prioritize their intake of carbohydrates over dietary EAAs, even when overeating EAAs to obtain sufficient carbohydrates results in a shorter lifespan. Thus, our data demonstrate that even when young bees are not nursing brood and foragers are not flying, their nutritional needs shift towards a diet largely composed of carbohydrates when they make the transition from within-hive duties to foraging.

Keywords Honeybee · Amino acid · Nutrition · Protein-to-carbohydrate ratio · *Apis mellifera* · Diet

Introduction

All animals require a dietary source of essential amino acids (EAAs) which are used for growth, somatic maintenance, and reproduction. EAAs are obtained by consuming the protein found in other animals or plants and are in greatest demand during periods of growth early in life (Behmer 2009; Tigreros 2013). In contrast to juvenile animals, adults mainly require amino acids for basic somatic functions (e.g. production of enzymes, peptide or amine signalling, tissue repair, immune function) or reproduction, and their needs for EAA decline with age (Millward et al. 1997; van de Rest et al. 2013). Reproduction, in the form of allocation of resources to eggs or offspring by females (O'Brien et al. 2002) or the donation of nuptial gifts and the production of sperm by males (Voigt et al. 2008), also places demands on the acquisition of amino acids in adult diets, which is often manifested as a trade-off between lifespan and protein/amino acid

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Here, we tested how caste determined the adult worker honeybee's nutritional requirements for EAAs using the principles in the GF (Simpson and Raubenheimer 1993; Simpson et al. 2004). By confining cohorts of workers to single diets composed of specific proportions of sucrose and the ten EAAs required by honeybees (i.e. protein was identified), the ITs of young bees (days 0–4 from emergence within the colony) and foragers (collected from outside the colony). Diet was limited to carbohydrates (sucrose) and amino acids because these are the main components of honey (Anklam 1998; Hermosin et al. 2003), one of the foods eaten by young workers within the colony and the only food consumed by foragers (with the exception of glandular secretions received during trophallaxis; Crailsheim 1988). To identify costs associated with over consumption of specific nutrients, we also measured how dietary intake of EAAs influenced survival. To confirm that the costs associated with overeating EAAs was a result of physiological changes associated with age and behavioural caste, we exposed newly eclosed workers to synthetic QMP and measured food consumption and survival on diets high in EAA. This study is the first to show that the nutritional requirements of honeybees change as a function of age and behavioural role, and has implications for dietary intervention strategies designed to improve honeybee health.

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Here, we tested how caste determined the adult worker honeybee's nutritional requirements for EAAs using the principles in the GF (Simpson and Raubenheimer 1993; Simpson et al. 2004). By confining cohorts of workers to single diets composed of specific proportions of sucrose and the 10 EAAs required by honeybees (i.e. protein was identified the ITs of young bees (days 0–4 from emergence within the colony) and foragers (collected from outside the colony). Diet was limited to carbohydrates (sucrose) and amino acids because these are the main components of honey (Anklam 1998; Hermosin et al. 2003), one of the foods eaten by young workers within the colony and the only food consumed by foragers (with the exception of glandular secretions received during trophallaxis; Crailsheim 1988). To identify costs associated with over consumption of specific nutrients, we also measured how dietary intake of EAAs influenced survival. To confirm that the costs associated with overeating EAAs was a result of physiological changes associated with age and behavioural caste, we exposed newly eclosed workers to synthetic QMP and measured food consumption and survival on diets high in EAA. This study is the first to show that the nutritional requirements of honeybees change as a function of age and behavioural role, and has implications for dietary intervention strategies designed to improve honeybee health.

Materials and methods

Animals

Frames of newly emerged workers were removed from two colonies of *Apis mellifera* "Buckfast" hybrid honeybees kept in outdoor colonies at Newcastle University. Each frame was placed in a box in a controlled temperature room at 34 C and 60 % relative humidity. Newly emerged bees were brushed off the frame each day; foraging workers were collected daily at the hive entrance by capturing individuals in plastic sample tubes. For each cohort, 20

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lates with age within the colony. After eclosion, adult worker bees perform within-colony activities such as cleaning, food storage, rearing larvae, and attending the queen (Winston 1987). These bees continue to perform within-hive behaviours as a function of their exposure to the queen's mandibular pheromone (QMP): bees near the queen remain more nurse like whereas bees exposed to less of the queen's pheromones become foragers (Pankiw et al. 1998). Honeybees are unique among social insects, because nurses provision larvae, other workers, and the queen with glandular secretions as food—royal jelly—which is the main source of protein that larvae receive for the first three instars after hatching (Winston 1987). Because most adult bees are sterile, their nutritional requirements for amino acids should be considerably less than those of queens or drones. However, the production of royal jelly is likely to place metabolic demands on young bees (e.g. nurses) for dietary amino acids (Crailsheim 1990): how much that demand exceeds their own requirements for somatic maintenance is unknown. As young bees mature and pass through their behavioural ontogeny, they stop eating pollen and lose the ability to digest solid proteins (Crailsheim 1986, 1990; Lass and Crailsheim 1996; Moritz and Crailsheim 1987); they also stop producing glandular secretions and feeding larvae and start building wax comb, packing food into the cells, guarding the colony entrance and eventually become foragers (Winston 1987). Furthermore, during this period, their physiology changes substantially: their mandibular glands and ovaries atrophy (Winston 1987) and their fat body reduces (Seehuus et al. 2007; Ament et al. 2008). How much their nutritional optimum depends on their behavioural caste and age has not yet been tested.

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Here, we tested how caste determined the adult worker honeybee's nutritional requirements for EAAs using the principles in the GF (Simpson and Raubenheimer 1993; Simpson et al. 2004). By confining cohorts of workers to single diets composed of specific proportions of sucrose and the ten EAAs required by honeybees (i.e. protein was identified), the ITs of young bees (days 0–4 from emergence within the colony) and foragers (collected from outside the colony) were identified. Diet was limited to carbohydrates (sucrose) and amino acids because these are the main components of honey (Anklam 1998; Hermosin et al. 2003), one of the foods eaten by young workers within the colony and the only food consumed by foragers (with the exception of glandular secretions received during trophallaxis; Crailsheim 1988). To identify costs associated with over consumption of specific nutrients, we also measured how dietary intake of EAAs influenced survival. To confirm that the costs associated with overeating EAAs was a result of physiological changes associated with age and behavioural caste, we exposed newly eclosed workers to synthetic QMP and measured food consumption and survival on diets high in EAA. This study is the first to show that the nutritional requirements of honeybees change as a function of age and behavioural role, and has implications for dietary intervention strategies designed to improve honeybee health.

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