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Honeybee Nutrition

Review of research and practices

A report for the Rural Industries Research and Development Corporation

by John Black

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Foreword

This review identifies the current status of knowledge on the nutrition of honeybees and how this knowledge could be applied to improve the focus of research and the practice of honey production in Australia.

Suboptimal nutrition is frequently associated with the use of eucalyptus species with high nectar flows, but with small quantities of pollen and/or low quality pollen. The major production issues related to the nutrition of honeybees in Australia are outlined in the Report. The sources of nutrients, their intake and digestibility by honeybees and differences in nutritional quality between pollens are discussed. The nutrient requirements of honeybees are determined as quantitatively as possible and used to suggest specifications for artificial nectar and pollen substitutes.

The contribution of previous and current RIRDC funded research to knowledge of honeybee nutrition is outlined and preliminary recommendations made on directions for future research and application of knowledge to practice within the honeybee industry. Following a two-day meeting with apiarists and scientists involved in honeybee research, a revised integrated program for honeybee nutrition research is recommended for RIRDC to consider for funding.

This report, an addition to RIRDC's diverse range of over 1500 research publications, forms part of our Honeybee R&D program, which aims to improve the productivity and profitability of the Australian beekeeping industry.

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Peter O'Brien
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Contents

Executive Summary

Background

- This review was commissioned by the Rural Industries Research and Development Corporation with the following objectives:

  - Develop a review of all nutrition research, which has been undertaken for bees and if possible relate this to practical use by beekeepers in Australia.

  - Assess how the current and past research RIRDC has funded fits into an overall understanding of honeybee nutrition.

  - Develop an integrated framework for bee nutrition research in Australia and identify priority areas.

Major review findings

- The productivity of honeybees within Australia can be affected adversely by three main issues relating to nutrition.
  (i) The 'skinny bee' syndrome, which occurs particularly when honeybees work eucalyptus species with high nectar flows and small quantities or poor quality pollen. The number of bees within the colony and their vitality fall markedly with this syndrome.
  (ii) The inability to exploit new honey flows due to inadequate colony size prior to flowering caused by a previous 'skinny bee' syndrome, prolonged periods without flowering plants or following winter.
  (iii) The severity of bee diseases, particularly nosema, American and European foulbrood and the varroa mite is increased during periods of poor food supply and high hive activity.

- The nutritional status of a colony has a marked effect on the growth and development of individual bees, their lifespan, foraging capacity, brood rearing, sex differentiation and resistance to diseases. The protein content of individual bees has been recommended, particularly in Australia, as a means for assessing the nutritional status of individual colonies.

- There are many reports on the weight and protein contents of honeybees at different stages of development. The variation in published values for protein content is particularly large ranging from 21-76% when expressed on a dry matter basis and from 11-23% when expressed on a fresh bee basis. Factors contributing to the variation include water content of bees, pollen intake, maturity of the food glands, when the bee defecated, whether the alimentary tract was removed prior to analysis and the method of analysing for protein. The effects of nutritional status on the protein content of individual bees appear to be minor and the extremely wide range reported in the literature cannot be explained by differences in nutrition. Alternatively, there is strong evidence that the weight of bees, particularly at emergence, is influenced significantly by the nutritional status of the colony. However, weight of bees at emergence is also affected by the number of nurse bees per larva, which changes with season. Measurement of dry weight of emerging bees would appear to be a more reliable procedure than protein content for assessing the nutritional status of a colon, but some allowance may need to be made for the ratio of nurse bees to larvae, which increases towards the end of the brood rearing period.

- Longevity of honeybees, as measured by the time taken for 50% of caged bees to die, has been shown to vary from –4 to +41 days when different pollen sources were compared with diets of sugar alone. Longevity can be influenced readily by altering the nutrition of young bees, but altering the nutrition of foraging bees appears not to affect their longevity. Worker bees have

been observed to have lifespans from less than 20 days to greater than 200 days. Longevity of hive bees is determined primarily by the period the bees spend in the hive before commencing foraging. It has been hypothesised that a worker bee will die once it has flown a distance of 800 km, without a decline in the efficiency of flight performance prior to death.

- The brood rearing capacity of a colony is influenced substantially by nutrient availability. Development of ovaries and the number of ovarioles per ovary in larvae are reduced under conditions of poor nutrition. Similarly, the number of eggs laid by queen bees is reduced and the sex ratio of eggs laid swings significantly in favour of females in underfed colonies. Drones from colonies with low pollen stores take longer to reach sexual maturity and there is evidence from bumblebees that the production of spermatozoa declines in undernourished colonies. Overall larval survival is reduced in poorly fed colonies with increased cannibalism, particularly of eggs and three-day old male larvae. Furthermore, larvae are capped earlier and the emerging bees are of lighter weight.

- Nectar, honeydew and pollen are the major sources of nutrients for honeybees. Variations in the quantities, chemical composition and digestibility of components of these nutrient sources are described. A large number of studies, including several in Australia, have been conducted to determine the composition of pollens. The protein content of pollens ranges from 2.9 to 53.5% and there is at least a four-fold range in the proportion of individual essential amino acids in pollen proteins. The concentration of lipids varies from almost zero to over 20% and fatty acids with chain lengths from C_8 to C_{22} have been observed. The predominant fatty acids in most pollen sources are linoleic, palmitic and linolenic. Pollens from eucalyptus species are particularly low in lipids with most having less than 2% total lipid. A variety of sterols are also found in pollens including cholesterol and 24 methylene cholesterol.

- The carbohydrates in pollen are predominantly fibrous materials with cellulose content ranging from 1.2 to 15% and sporopollenin from 1.8 to 23%. Approximately 2% of pollen carbohydrates are soluble hemicellulose material. The starch content of pollen ranges from 2 to 3% and sugars are around 0.5%. The mineral content of pollen ranges from 1 to 6.5% and covers the range of macro and micro-elements found in plant tissue. Potassium was the most abundant mineral representing over 50% of total ash in some pollen samples. The range in content of individual minerals found in Australian pollen samples was from 3 to 20-fold. Pollens are rich in water soluble vitamins, but contain only low concentrations of fat soluble vitamins. There is a wide range in the vitamin content of pollens and several vitamins including niacin, folic acid, ascorbic acid and pyridoxine are not stable and deteriorate over time. Freezing of pollen slowed, but did not stop the loss of vitamin activity over time.

- The composition of pollen is not constant for any plant species, but varies from site to site and year to year with many factors affecting growing conditions including soil moisture, fertility and ambient temperature.

- Adult bees commence consuming pollen within 6-10 hours from emergence. Pollen consumption reaches a peak around day 9 and falls to extremely low amounts in foraging bees. The pattern of pollen consumption follows the need for protein and other nutrients during growth and for hypopharyngeal gland activity. The weight of pollen in the midgut of worker bees increases from around 1 mg/bee at day 1 to 4 mg/bee 8-10 days after emergence. Pollen grains are digested by removal of the pollenkitt and the protoplasm, through the germination pores and by disruption of the solid wall with osmotic shock. Release of the inner protoplasm of pollen grains depends on the extent of wall disruption and results in dry matter digestibility ranging from 30 to 90%. The few measurements of pollen protein digestibility indicate that, within any pollen sample, it is less than for dry matter.

No lipases are found in newly emerged honeybees, but the concentrations increase to reach a maximum in 8-day old bees. Substantial amounts of fatty acids, sterols and waxes are found in the faeces of bees with concentrations of oleic, palmitic, linoleic and linolenic acids being particularly high in bee excreta. The most common sugars in pollen, fructose, glucose and sucrose are highly digested, but many other sugars are either not digested or are toxic to bees. The fibrous components of pollen are not digested and are excreted. Foraging bees, but not younger bees, possess starch digesting enzymes. Thus, starches can be utilised successfully only by foraging bees.

- The nutritional value of pollens for honeybees varies widely with floral source and between years, particularly due to variation in the amount consumed. However, some pollen sources may lack sufficient protein, individual amino acids, minerals, vitamins or lipids for maximum productivity. Other pollens may be toxic because of excess minerals, amino acids or other compounds. Visual, aromatic, tactile and metabolic cues influence the attractiveness of pollens to honeybees. Pollens in the yellow colour range are most preferred. Bees are also attracted to some portions of the acetone soluble lipid fraction of pollen and several chemical attractants have been identified. However, bees are not attracted to the volatile compounds released from pollen. Pollen aggregates that are too large are not attractive. Honeybees also demonstrate a 'nutritional wisdom' and avoid pollens that will result is a marked imbalance of metabolites in the haemolymph.

- An attempt was made within the review to quantify the nutrient requirements of honeybees. Energy requirements, expressed as requirements for glucose, were estimated for maintenance, hive activity, flying, thermoregulation and comb building. Based on measurements of oxygen consumption by individual bees or small groups of bees, the glucose requirement for a 40,000 bee breeding colony was estimated to be 2 kg/day and for a non-breeding colony 0.5 kg/day. These values equate to a total glucose requirement of approximately 240 kg/year for maintenance of the colony. This estimate is considerably higher than those in the published literature of 70-80 kg of honey/year. The estimate made in this review may have been high because of poor assumptions of colony dynamics, measurements of oxygen consumption being made on small groups of bees or literature values not considering the nectar metabolised by foraging bees during flight.

- Protein requirements for each stage of growth were estimated. The requirement for growth and maintenance of young nurse bees was estimated to be approximately 0.5 mg ideal protein/day and this increased to greater than 1 mg/day when food gland secretions and the efficiency of digestion and metabolism were included. Approximately 4 mg/day of good quality pollen would be required to satisfy the requirement of nurse bees. Literature estimates of colony pollen consumption vary widely from 6 to 55 kg/year. Honeybees require the same essential amino acids as mammals, but have a lower requirement of sulphur containing amino acids because of the small amount of keratin synthesised. The amino acid pattern of protein required by honeybees suggested by de Groot (1953) appears to be satisfactory, but could not be independently verified. Nevertheless, the concentration of essential amino acids suggested by de Groot (1953) added to only 28% of total protein requirements. Comparison of the amino acid pattern in pollen compared with the de Groot estimates is not satisfactory for assessing the adequacy of a specific pollen type. Estimates of the protein content of the pollen, its intake by bees and amino acid digestibility are required to determine whether daily amino acid requirements are being met.

- Honeybees have specific requirements for linoleic acid, linolenic acid and sterols. Although no estimates of requirements for the essential fatty acids could be found in the literature, the requirement for growth of bees was estimated to be 1.6 mg/g pollen for linoleic acid and 5.5 mg/g pollen for linolenic acid. However, the values need to be increased when accounting for the large amounts of these fatty acids excreted by adult bees. Sterol requirements can be met by 0.1% of either cholesterol or 24-methylene cholesterol in the diet.

- Honeybees have requirements for a large number of macro and micro minerals. However their requirements for calcium and sodium are substantially less and their requirement for potassium substantially greater than for mammals and birds. The lower requirement for calcium results from the lack of a skeleton and the lower requirement for sodium from the lack of a sodium pump used for maintenance of body temperature in mammals. Estimates are made in the review for the individual mineral requirements of honeybees.

- The following vitamins have been shown to be essential for honeybees: biotin, choline, folic acid, inositol, niacin, pantothenic acid, pyridoxine, riboflavin, thiamine, vitamin B_{12}, vitamin A and vitamin K. Although ascorbic acid can be synthesised by bees, a response in bee performance has been observed when this vitamin is added to supplements. Quantitative estimates of the requirements of bees for vitamins have not been established. However, except for the fat soluble vitamins A and K, others are easily excreted when fed in excess. Suggested vitamin concentrations required in a pollen substitute are presented in the review.

RIRDC honeybee projects

- Early research on honeybees in Australia defined the major problems associated with nutrition of bees under Australian conditions and particularly the importance of low pollen production and quality from many eucalypt and related species.

- A significant portion of RIRDC funded research (DAN-134A, DAW-91A, DAW-100A) has described the nutrient composition of pollen from Australian plants. An extremely comprehensive database on protein, amino acid, fatty acid, mineral and vitamin content of these pollens has been developed. Attempts to identify the nutritional value of these pollens by comparing their composition with published estimates of the nutrient requirements for honeybees have been of limited success. Literature estimates of nutrient requirements are generally poor and the adequacy of a nutrient depends on the intake of the pollen and the digestibility of the nutrient as well as its concentration in pollen. There are very limited measurements of intake of pollens and digestibility of nutrients by honeybees.

- Project ANU-57A proposes to develop NIR prediction calibrations to determine first the nutrient composition of Australian pollens and secondly the productive capacity (growth, hypopharyngeal gland development, intake and brood rearing capacity) of these pollens. The approach is technically feasible and, if successful, could be used to assess rapidly the nutritional value of individual pollen samples. However, at least 80 and preferably more samples are needed for the development of robust NIR calibrations. Nevertheless, the approach is worth pursuing because of its ease of application in practice.

- Project DAW-105A is also examining the effects of pollens on productivity as assessed by bee longevity. The pollens are being selected for a range in lipid content and the experiments appear to be first of their kind. Interpretation of the results may be difficult because other components of the pollens will vary also. However, when these experiments are taken in conjunction with fatty acid supplement experiments, a greater understanding of the importance of fatty acids in bee nutrition will result. The effects of linolenic acid contents of artificial diets are being examined as well as the effects of oleic and linoleic acids. The supplement experiments provide an opportunity for better defining the fatty acid requirements and toxicities for honeybees.

- Project DAN-214A is examining the practicality of feeding supplements to bee colonies. Results to date have been limited and point to the need for a greater understanding of when to offer supplements and of the specifications for pollen replacements. Project DAN-186A is to provide an extension publication on bee nutrition and is important for the industry. Information from this review may be useful for the project.

Initially suggested research priorities

- Two integrated research programs were suggested from the initial review:

    1. An accurate and practical method for predicting when pollen supply to a colony working high nectar flows is about to become limiting and will affect colony vitality precipitating the 'skinny bee' syndrome, with the aim of determining when to start feeding the colony.

    2. Specifications for a pollen substitute, based on readily available ingredients, that is highly attractive and meets the nutrient requirements of honeybee colonies

- A workshop of 6-10 people is recommended to revise the two proposals and develop an integrated research program within current funding capacity to deliver the described outcome for the industry.

Recommended procedures for adoption

- There is a great deal of knowledge from both the literature and held by beekeepers that could be applied more effectively to improve the efficiency and profitability of the Australian honeybee industry. Quality control and continuous improvement systems are now being applied to many areas of agriculture including the pig and beef industries.

- It is recommended that a small group of 8-10 producers, scientists and RIRDC personnel hold a 2-day Workshop to examine the practicality of introducing a HACCP approach to the management of honeybees in Australia.

Final recommendations for an integrated honeybee nutrition research program

- A 2-day Workshop was held with industry representatives and scientists in October 2004 to identify the factors that have the greatest impact on productivity and profitability of the Australian honeybee industry and to refine the areas most needing research.

    The most critical factors affecting profitability of a honeybee enterprise were identified as:
    - Advanced knowledge of where and when floral resources will be available. This knowledge is essential for strategic planning within a business and allows identification of the most profitable and order of honey flows to target.
    - Correct number of healthy bees in a hive at the right time relative to targeted honey flows. The process involves changing throughout the year the number and condition of bees in a colony to ensure there are sufficient bees in high condition prior to allocation to targeted honey flows, while bee numbers are reduced during long periods of floral dearth.
    - Lower costs of production. Many of the tasks undertaken in beekeeping are labour intensive and involve large travelling distances. Processes that reduce the need for travelling and reduce overall costs would be valuable to the industry.

- The research priorities identified at the Workshop were determined following extensive discussion. Priority was established by the 10 people present using a scoring system. The area with highest priority was allocated 1 and the area with lowest priority allocated 4 by each person. The research priorities with mean score and range in scores were as follows:

    - Develop a practical and economically viable pollen substitute (mean 2.0; range 1-3)
    - Refine recommendations for sugar feeding including timing, feeding systems, sugar concentrations, etc. (mean 2.1; range 1-4)

- Measurement of potential onset of ‘skinny bee’ syndrome (mean 2.6; range 1-4)
- Advanced prediction of timing and location of future floral resources (mean 3.3; range 1-4)

- Further discussion with apiarists and scientists following the workshop and at the 2005 RIRDC Honeybee R&D meeting about the needs of industry and the research areas which are likely to have the greatest impact on profitability resulted in the following recommended integrated nutrition research program. The priority order is influenced by strong evidence that because of the flat response (payoff) curves in most agricultural systems, the greatest gains in profitability come from quantum changes resulting from the development of new technologies rather than from Research & Development that improves decision making about technologies currently used within an industry (Pannell, 2004).

  a) Develop an effective, economically viable pollen substitute and methods for feeding to colonies

  b) Refine recommendations for effective feeding of sugar supplements, including methods of feeding, amounts needed to maintain or change bee numbers in a colony over a specified period, concentrations of sugar supplements and methods for preserving sugar solutions.

  c) Investigate the feasibility of using existing technologies including historical weather records and satellite imagery for forecasting the site and timing of future floral resources.

  d) Continue development of NIR calibrations for assessing the productive capacity of pollen samples.

  e) Develop effective methods for identifying early onset of the ‘skinny bee’ syndrome both in the field and under laboratory conditions.

- It is recommended that research into areas a), b) and c) commences as soon as funds are available within RIRDC, whereas research into area d) is delayed until project ANU-57A is closer to completion and research into area e) is delayed until there has been substantial progress with developing an effective pollen substitute and reliable sugar feeding techniques.

Introduction

This review was commissioned by the Rural Industries Research and Development Corporation (RIRDC) to identify the current status of knowledge on the nutrition of honeybees and how this knowledge could be applied to improve the focus of research and the practice of honey production in Australia. Suboptimal nutrition is frequently associated with the use of eucalyptus species with high nectar flows, but with small quantities of pollen and/or low quality pollen. The major production issues related to the nutrition of honeybees in Australia are outlined in the Report. The sources of nutrients, their intake and digestibility by honeybees and differences in nutritional quality between pollens are discussed. The nutrient requirements of honeybees are determined as quantitatively as possible and used to suggest specifications for artificial nectar and pollen substitutes. The contribution of RIRDC funded research to current knowledge is outlined and recommendations made on directions for future research and application of knowledge to practice within the honeybee industry. The estimates for requirements per bees within honeybee colonies for individual essential nutrients were refined following completion of the initial review. In addition, a 2-day workshop was held between apiarists and honeybee scientists to refine the recommendations for research. The final recommendations for an integrated research program to be considered for funding by RIRDC was determined after the 2005 honeybee R&D meeting in May 2005.

Honeybee production issues in Australia related to nutrition

The 'skinny bee' syndrome

Many Australian eucalypt species including iron bark, yellow box, grey gum and others produce large quantities of high quality honey, but often at the expense of the vitality and size of the bee colony. Manning (2002) states, "The problem of beehive populations collapsing during *E. wandoo* forest nectar flows has been recognised over many years". Kleinschmidt and Kondos (1978) describe a strong colony of 60,000 bees producing 138 kg of honey from an iron bark nectar flow that resulted in a reduction in colony size to 45,000 bees over 12 weeks. In some situations, the vitality of a colony working eucalyptus stands can become so poor that the bees virtually cease working the flow while nectar remains. The egg laying capacity of the queen bees decline and in some cases ceases (Kleinschmidt and Kondos, 1978), the size of emerging bees declines substantially (Somerville, 2000) and longevity of the bees can be halved from 46-50 days to 20-26 days (Kleinschmidt and Kondos, 1978). A substantial amount of protein can be lost from the colony with 1,500 g being lost from the colony reported by Kleinschmidt and Kondos (1978). The loss in protein content of the colony was due partly to a decline in bee numbers, but the protein content of individual bees can decline also by up to 20% (Kleinschmidt and Kondos, 1978).

Colonies that have worked these eucalyptus flows can take a considerable time to rebuild before they are able to be used to collect nectar from other flows. Kleinschmidt (1986) reported colonies with bees containing only 20% crude protein in their dry weight taking 12 weeks to rebuild to a condition sufficient for working another nectar flow. Other colonies with 40% crude protein content took only four weeks to rebuild. The honeybees used in Australian apiculture are of European origin. The quantity and nutritional quality of pollen from eucalyptus species is often far less than that from the plants where the bees evolved. In addition, nectar flows are strictly seasonal in most regions of Europe (McLellan, 1978), whereas they can occur at any time of the year in many parts of Australia.

Low bee numbers in colonies prior to a targeted honey flow

Honeybee colonies with a small number of bees fail to take full advantage of new nectar flows. Honey production increases substantially as bee numbers within a colony increase from around 35,000 to 60,000 when there is a good nectar flow (Kleinschmidt, 1986). Colony bee numbers within Australian beehives frequently decline to levels where the ability of the hive to collect nectar is depressed. Colony numbers of less than 25,000 can occur following periods of working floral species with large nectar flows, but inadequate pollen, during long periods of a dearth in floral sources and following winter (Kleinschmidt, 1986). These colonies must be 'rebuilt' and numbers restored to near 50,000 per colony before they are sufficiently robust to fully utilise new nectar flows (Kleinschmidt, 1986; Somerville, 2000). Methods of providing nutritional supplements for periods of up to four weeks before anticipated new nectar flows would significantly improve the productivity of many colonies (Somerville, 2000). The most efficient management of the hive is to ensure minimum energy use during periods of nectar dearth, but to have the ability to build the colony to 50,000 or more bees prior to introduction to targeted flows.

Susceptibility to and effects of diseases

Bee colonies are susceptible to several parasites and diseases, which can seriously affect their growth and survival of the colony. The most important diseases for bees are the protozoan *Nosema apis*, the bacterial diseases European foulbrood (*Streptococcus pluton*) and American foulbrood (*Paenibacillus larvae*), the fungus chalkbrood (*Ascophaera apis*), the mite (*Varroa destructor*) and viral diseases

such as sacbrood. Many bee diseases such as nosema can have a marked effect on productivity of the colony (Kleinschmidt, 1986) and others including severe infestation with the varroa mite can result in death of the colony (Janmaat *et al*., 2000). There is evidence that diseases such as nosema and varroa mite cause a decline in brood rearing, weight of emerging bees, longevity and size of the hypopharyngeal glands (Wang and Moeller, 1970; Schneider and Drescher, 1988; Janmaat *et al*., 2000).

The severity of many diseases affecting honeybee colonies can fluctuate throughout the year. For example, European foulbrood was found to have serious effects on colonies during early spring in southern New Jersey USA, but the effect disappeared with the onset of major nectar flows in early summer (Herbert and Shimanuki, 1984). Similarly, Kleinschmidt (1986) observed a substantial increase in nosema spores from 0-100,000/bee when the colonies were first moved to a winter ironbark flow in western Queensland to approximately 5.4 million spores/bee after two months of working the heavy honey flow. However, frequently diseases in bees appear to remain benign unless the colony is subjected to nutritional stress or infected with another disease agent (Ewald, 1983).

Several experiments have demonstrated a strong interaction between the nutritional status of a colony and the severity of diseases affecting the honeybee and other bee species. Rinderer and Elliott (1977) provided individual honeybees with a 5.0 μl droplet of a 33% sucrose solution either alone or containing $4.3\text{-}6x10^5$ spores of the *Nosema apis* protozoan. The nosema treated and untreated bees were fed in cages either a 50% sucrose solution or the sucrose solution with a paste of mixed pollen in experiment 1 or a paste of yeast product, sucrose, cellulose and an alcohol extract of pollen in experiment 2. The longevity of the bees was observed and expressed as the time taken for half the bees in each treatment to die. The number of nosema spores in each bee at death was recorded. The results presented in Table 1 show that the presence of nosema substantially reduced the longevity of the bees. The proportional effect of the disease on longevity was greater for the bees provided with pollen or protein rich food compared with sucrose alone. However, bees receiving the pollen or protein food had a lifespan almost double those receiving sucrose alone. Bees receiving the supplements also contained significantly more spores at death than those receiving only the sucrose solution. An examination of the number of nosema spores in bees from the pollen supplemented and non-supplemented groups that died at equivalent ages from 14-19 days showed significantly more spores in the pollen fed ($1.3x10^7$) than the non-supplemented groups ($3.4x10^6$). These results show that although the provision of pollen increased the multiplication of nosema spores, the bees receiving pollen were more resistant to the effects of the disease and their lifespan was increased by approximately 50% from 14 to 21 days.

Table 1. Longevity and spore counts of groups of honeybees fed or not fed *Nosema apis* spores and supplied or not supplied with a high protein food. After Rinderer and Elliott (1977)

Treatment	**Longevity[a] (days)**	**Nosema spores/bee at death ($x10^5$)**
	Experiment 1	
Spores + pollen	21.0	180
No spores + pollen	46.8	-
Spores + no pollen	14.0	29
No spores + no pollen	21.3	-
	Experiment 2	
Spores + protein food	22.8	140
No spores + protein food	33.3	-
Spores + no protein food	14.0	95
No spores + no protein food	22.3	-

[a]Longevity was measured as the time taken for half the bees in the treatment replicate to die.

Janmaat *et al*. (2000) investigated the effect on brood production and foraging strategies of providing an additional pollen frame to honeybee colonies either lightly or moderately infested with the mite *Varroa jacobsoni*. The moderately infested colonies reared significantly less brood than the lightly infested colonies. Initially, the moderately infested colonies had more bees collecting pollen, but each bee carried a smaller load than for the lightly infested colonies. However, as the need for pollen by the colonies increased, a significantly smaller number of bees were recruited for pollen foraging in the moderately infested than in the lightly infested colonies. The efficiency with which pollen was converted into brood was also less in the moderately infested colonies. The provision of additional pollen stores did not change total brood production but increased the efficiency of pollen conversion into brood in both the moderately and lightly infested colonies. However, the magnitude of the effect of extra pollen on the efficiency of brood production was greater in the moderately infested than lightly infested colonies. The results from this experiment show that infestation of honeybee colonies with varroa mite substantially reduced brood production and altered the pollen foraging strategies, but the efficiency of brood production in the moderately infected colonies was increased by the provision of additional nutrients in pollen stores.

Vandenberg (1994) examined the effect of various sugar, pollen, pollen supplement and natural hive provisions on the infectivity of the chalkbrood fungus, *Ascosphaera aggregata*, for larvae of the alfalfa leafcutting bee (*Megachile rotundata*). The results, expressed as the number of spores required for the death of 50% of the larvae (lethal challenge, LC50), are presented in Table 2. The experiment showed clearly that the susceptibility of larvae to the fungus was influenced significantly by the nutrients provided to the larvae.

Encapsulation of parasites with several layers of haemocytes (blood cells) and modification of humoural responses are the main defence that insects mount against disease challenges (Hoffmann *et al*., 1994). The effectiveness of the encapsulation system in insects including some bee species appears to be positively related to their nutritional status (Lochmiller, 1996) and is depressed with an increase in other essential activities such as foraging (Konig and Schmid-Hempel, 1995). Similarly, high rates of parasite encapsulation have been found to reduce the reproductive capacity of insects (Carton and David, 1983). In a more recent study, Schmid-Hempel and Schmid-Hempel (1998) found that moderate restriction of a nutritionally adequate diet without a change in other activities did not alter immunocompetence in the bumble bee. These authors suggest that the main impact of reduced nutrition on immunocompetence is through the increase in other activities such as foraging or temperature regulation. However, the effects of pollen deficiencies on immunocompetence were not studied in the experiments.

Table 2. Effect of diet used to rear larvae from the alfalfa leafcutting bee on the number of spores from chalkbrood resulting in the death of 50% of the population. After Vandenberg (1994)

Diet	20% Pollen + sugars	33% Pollen + pollen substitute	33% pollen + sugars	Natural hive provisions
LC50[a] (spores/larvae)	79	138	1549	219

[a]LC50 is the Lethal Challenge expressed as the number of spores/larvae required for the death of 50% of the larvae.

Effects of nutrition on productive functions of bees

Growth and body composition

The growth and development of the honeybee has been extensively described, particularly in the early literature. A summary of the changes in weight and protein content during the life cycle of the honeybee summarised by de Groot (1953) is shown in Table 3. The weight and protein content of the imago bee increases during the first 5-8 days following emergence by from 30-60% due partially to growth, to development of the hypopharyngeal, mandibular and other glands and to changes in contents of the alimentary tract (de Groot, 1953; Crailsheim and Stolberg, 1989). Weight then falls by approximately 10% in foraging bees (de Groot, 1953) with regression of the glands and reduction in the consumption of pollen.

Table 3. A summary of changes in weight and protein content during successive developmental stages of the honeybee. Modified from de Groot (1953)

Developmental Stage	Weight			Protein		
	Fresh (mg/bee)	Dry (mg/bee)	Dry matter (%)	(mg/bee)	% dry matter	% fresh weight
Egg	0.05					
Larva	0.3-150	0.07-33	22-23	0.56-13.8	80-42	20-9
Pupa	150-117	30-18	20-15	13.8-11.2	46-62	9-10
Newly emerged	80	16	20	11.9	74	15
Nurse bee	72	22	30	16.3	74	23
Foraging bee	66	21	32	15.0	71	23

There is an extremely large variation in the many publications reporting measurements of the protein content of honeybees, which has been recommended widely within Australia as a means for assessing the nutritional status of a colony (Kleinschmidt and Kondos, 1976). A selected number of these published values are presented in Table 4 to illustrate the extent of the variation. The protein content of bees ranged from 21 to 76% when expressed on a dry matter basis and from 11 to 23% when expressed on a fresh basis. de Groot (1953) provides evidence that the dry matter content of bees increases from around 20% at emergence to 30% for the nurse bee and 32% for the foraging bee. This change in dry matter content of the honeybee as it matures would help explain some of the variation in the nitrogen content when expressed on a fresh basis as is seen in Table 3. However, there appears to be little information on the changes in dry matter content of bees under normal conditions although de Groot (1953) states that the variance in fresh weight of bees is greater than the variance in dry weight. The dry matter content may change in relation to activity, weather conditions, content of the crop and alimentary tract and other factors. Muszynaska and Bornus (1983) found that the water content of bees from colonies with good over-wintering success was significantly lower than for those with poor over-wintering success.

Table 4. Published range in the protein content of honeybees

Bee type	Bee weight (mg)		Bee protein			Tract removed	Author
	Fresh	Dry	(mg)	% fresh	% dry		
Emerged		17.9	11.4		64	Y	Haydak (1936)

Drone		50	37.5		75	Y	De Groot (1953)
Worker 14d		22.4	16.4		73		
Worker 28d		20.7	15.8		76		
Emerged		14.7	3.8		26	N	Hepburn *et al*. (1979)
Emerged		17.1-21.1	11.3-14.3		66-68	N	Kunert & Crailsheim (1988)
Variable					21-67		Kleinschmidt & Kondos (1978)
Emerged	80		11.9	15		Y	De Groot (1953)
Nurse	72		16.3	23			
Forager	66		15.0	23			
Emerged				11-13			Manning (2002)
Nurse+forager				16-23			

Other sources of variation in the published values for the protein content of bees relate to whether the alimentary tract was removed and the method used for analysing for nitrogen or protein. Significant amounts of pollen and other material are stored in the midgut and rectum of bees. Hrassnigg and Crailsheim (1998) showed that the dry weight of the midgut ranged from approximately 2 to 6 mg depending on the amount of pollen consumed, whereas the dry weight of the rectum and its contents ranged from approximately 1 to 3 mg depending primarily on age. Weight of the rectum is influenced mainly by the age at which bees start foraging flights as they do not usually defecate unless flying. Hrassnigg and Crailsheim (1998) also found that there was a strong negative correlation between the weight of the midgut and the percentage of protein it contained.

Thus, part of the difference in protein content of whole bees when expressed as a percentage could be explained by pollen consumption in relation to age of the bee and the size of the brood as it affects the size of hypopharyngeal glands. Part of the observed difference in body protein content could be explained also by whether the bee had commenced foraging flights, whether it was collected before leaving or after returning to the hive and whether the alimentary tract was removed before analysis. Kunert and Crailsheim (1988) discuss some of the issues associated with the methods used for protein analysis, which are also likely to contribute significantly to the variation in reported protein content of bees. Nevertheless, the extremely wide range in the published values for protein content of bees expressed on a dry matter basis is difficult to explain.

De Groot (1953) analysed 600 samples of 5-10 newly emerged bees from several colonies over a period of four years and found that dry weight ranged from 13.2 to 18.5 mg/bee while protein ranged from 9.3 to 14.6 mg/bee. Even if the lowest and highest values for weight and nitrogen content were assumed to apply to the same group of bees, protein content of the bees would range only from 70.5 to 78.7%. In addition, de Groot (1953) fed newly emerged bees a sugar candy diet alone for 10 days and then provided them with a 20% pollen supplement for the following 15 days. The mean protein content of the bees increased from 12 mg after 10 days of receiving the sugar only diet to 16.2 mg after 13 days of pollen supplement. The corresponding change in the dry weight of the bees was from 17 to 23 mg. Thus, the protein content of the bees changed only from 71 to 74% despite a severe nutritional restriction caused by the sugar only diet. De Groot (1953) removed the alimentary tract from all bees prior to analysis for protein.

Kunert and Crailshiem (1988) also collected and analysed newly emerged bees over two years from May to September in the northern hemisphere. Both the weight and protein content of the bees fluctuated with food supply, but protein ranged only from 62 to 68% of dry matter when the alimentary tract was not removed. Protein percentage closely mirrored the weight of the bees with two exceptions. The main exception was when the weight of the bee increased prior to winter with an increase in the percentage of fat and triglycerides and a decrease in the percentage of protein. Bees

that survive the winter were shown to be heavier and had higher concentrations of triglycerides, fats, glucose and glycogen in their bodies than those that died during winter.

It is difficult to explain the large variation reported by Kleinschmidt and Kondos (1978) in the protein content of bees foraging in Australia without detailed information on the actual values for bee weight and body composition. However, the results from the other experiments discussed above suggest that more information about the nutritional status of a colony could be obtained from measurements of bee weight than from measurements of protein percentage. Again, the effect of the alimentary tract contents would need to be considered unless the measurements are taken on newly emerged bees before pollen is consumed. Until the causes of variation in water content of bees are better defined, the most accurate means of assessing the nutritional status of a bee colony would appear to come from measurements of dry weight at emergence.

There is clear evidence that the nutritional status of a colony and pattern of nutrient supply can affect the weight of honeybees at emergence (Levin and Haydak, 1951). Schmid-Hempel and Schmid-Hempel (1998) provided bumble bees with a constant excess supply of food from 1000h to 1600 h every day, a variable supply when excess food was either made available or not available in random two hour blocks from 1000h to 1600 h, or a poor food supply when food was available daily from 1000h to 1300 h only. The average weight of worker bees receiving the good, variable and poor food supply was significantly different at 122, 129 and 103 mg, respectively. The experiment demonstrates that restricted food supply reduced the size of worker bees. However, it showed also that bees respond to a randomly variable food supply by increasing pollen collection and producing heavier bees. The increase in pollen collection was through the commitment of more bees to foraging rather than increasing the amount of pollen collected by each bee. A similar experiment by Sutcliffe and Plowright (1988) where foraging time for bumble bee colonies was restricted to 8, 14 or 24 hours each day showed a linear effect of nutrient supply on the size of the emerging bee as measured by the length of radial cells on the wings.

Schmickl and Crailsheim (2001) demonstrated also an effect of pollen restriction on honeybee colonies by either producing artificial rain at the entrance to the hive thereby preventing the bees form foraging for 5 days or by removing pollen stores for 5 days. The rate of larval survival declined from 83% to 53%, cannibalism of 3-day old larvae increased substantially, the time to capping larvae declined by approximately 1 day and the protein content of the capped larvae was reduced. Similarly, Blaschon *et al*. (1999) showed that honeybee colonies subjected to alternative 5-6 day periods of artificial rain and no rain produced significantly smaller larvae during periods of rain. Furthermore, Szabo (1980) demonstrated a highly significant correlation between foraging activity of honeybees and weight gain of the colony.

Although the experiments described above demonstrate that nutrient supply affects the size of emerging bees, results from Levin and Haydak (1951), de Groot (1953), Kleinschmidt and Kondos (1978), Eischen *et al*. (1982) and Kunert and Crailsheim (1988) indicate that the weight and nitrogen content of bees is not determined only by the amount of pollen being brought into the colony. The relative proportion of the number of larvae to be fed in relation to the number of nurse bees is also a major determinant as illustrated by an increase in the size of emerging bees when the production of brood is declining with the onset of winter.

In summary, the information reviewed suggests that measurement of the protein content of honey bees, either immediately after emergence or particularly in foraging bees, is not a reliable method for establishing the nutritional status of a colony. Measurement of dry weight of emerging bees would appear to be a more reliable procedure for assessing the nutritional status of a colony, but other factors such as the ratio of nurse bees to larvae can influence the size of emerging bees. Season, as well as nutrition, affects reproductive status of a colony and the ratio of nurse bees to larvae. De Groot (1953) observed also that the weight of newly emerged honeybees within the same colony varied significantly when sampled on consecutive days.

Lifespan and foraging capacity

Many studies have shown that longevity of bees is influenced by nutrition (de Groot, 1953, Standifer, 1967; Knox *et al*, 1971; Schmidt *et al*., 1987; Schmidt *et al*., 1995). The majority of these experiments have been conducted with caged bees, where longevity has been used as one measure of the nutritional adequacy of pollen from different plants and of other nutrient sources. For example, Schmidt *et al*. (1987) found that longevity, measured as the time for 50% of the bees to die, ranged from –4 to 41 days relative to a sugar only diet for 33 different pollen sources. Similarly, the longevity of bees has been observed to fall from 41 to 24 days when a fresh pollen source was heated for 48 hours (de Groot, 1953). de Groot (1953) used longevity to show that excess dietary protein was detrimental to the honeybee with the time taken for 50% of the bees to die decreasing from 48 to 27 days as the protein content of a sucrose-casein diet was increased from 1.25 to 10%.

The nutritional status of an individual bee during its growth and development is not the only factor affecting longevity of honeybees under natural conditions. Worker bees that survive the northern-hemisphere winter have been reported to live more than 200 days, whereas the lifespan during summer is normally around 30-40 days (Amdam and Omholt (2003). There is strong evidence that longevity is determined primarily by the period a bee spends in the hive before commencing foraging (Neukirch, 1982), which is influenced by brood size and the relative supply of nectar and pollen for the colony (Free and Spencer-Booth, 1959; Schulz *et al.*, 1998). Significant foraging activity has been reported in bees only 7 days old in starved colonies (Schulz *et al*., 1998), whereas nurse bees as old as 138 days have been reported by Haydak (1963) in over-wintering colonies. Development of foraging capacity in response to brood size and nutrient supply is influenced primarily by the concentrations of juvenile hormone and its interaction with the lipoprotein vitellogenin (Amdam and Omholt, 2003). Topical application or injection of juvenile hormone or juvenile hormone analogues to newly emerged honeybees causes them to become precocious forages (Jaycox, 1976; Robinson, 1985), whereas removal of the corpora allata, which is the site of juvenile hormone production, delays foraging (Sullivan *et al*., 1996).

Neukirch (1982) concludes that longevity of a bee after foraging commences varies depending upon the activity of the bee and the average distance flown each day until a predetermined flight distance has been achieved. Neukirch (1982) suggests that the predetermined distance for a worker honeybee is approximately 800 km and when this distance is reached the bee dies quickly without an apparent drop in flight performance. Neukrich (1982) presents evidence showing that longevity from the onset of foraging for bees caged within a 2x3x2 m flight room was approximately 25 days compared with only 10 days for free-ranging colony bees.

The distance travelled by a bee in one flight and the speed of flight is influenced by the concentration of the nectar consumed. Gmeinbauer and Crailsheim (1993) provided tethered worker bees, drones and queens with solutions of glucose ranging from 1.0 to 4.0 M and measured the distance flown to exhaustion and the speed of the flight. There was a significant positive correlation between the distance flown and the concentration of glucose solution provided. Worker bees offered 1.0 M glucose solution flew approximately 1000 m before exhaustion, whereas those offered 4.0 M glucose flew 4322 m. The flight distance to exhaustion for drones was approximately 60% of the distance for workers and the distance for the queen bees was intermediate. However, drones flew at significantly higher speeds than either the worker or queen bees, presumably because they require speed to catch queen bees on mating flights. Worker bees were the least efficient at using sugar during flight and required 105.6 μg/h/g compared with 101.1 μg/h/g for drones and only 66.7 μg/h/g for the queen bees. The bees needed to be fed within seconds of completion of the exhaustion flights to prevent them dying from lack of metabolisable substrates in their bodies.

Reproductive capacity of a colony and sex differentiation

There are reports of brood rearing decreasing markedly or ceasing when the availability or quality of pollen in bee colonies is low (Haydak, 1961a; Doull, 1973; Kleinschmidt and Kondos, 1978) and of brood rearing being stimulated by the presence of nectar (Somerville, 2000). Herbert *et al*. (1980a) showed that experimentally prepared honeybee colonies produced almost 3-fold more brood area when offered fresh, bee-collected pollen with a 50% sucrose solution compared with a starch-whey-yeast based artificial pollen and the sucrose solution. Brood area was similar to the naturally collected pollen when 2-4% lipid extract from pollen was added to the artificial pollen, because of an increase in consumption. Similarly, the provision of variable or poor food supplies to experimentally derived colonies by Schmid-Hempel and Schmid-Hempel (1998) resulted in significantly more and less bees, respectively, being produced for a variable than for a poor food supply. However, there are other reports where brood production has not increased with the provision of additional pollen to bee colonies (Janmaat and Winston, 2000).

Development of the ovary and number of ovarioles per ovary is affected by the composition of the diet offered to larval honeybees during development (Wang and Shuel, 1965). Similarly, a reduction in the feeding frequency of queen bees by their attendant workers has been shown to be directly related to a reduction in the rate of egg laying (Allen, 1960). There is also strong evidence that pollen shortage over 5-day periods to a colony reduces substantially the survival of larvae. A decrease in protein supply to nurse bees caused by artificial rain reduces secretions from the hypopharyngeal gland and stimulates the cannibalism of eggs and particularly 3-day old larvae to maintain food supplies for the older larvae in the brood (Schmickl and Crailsheim, 2001). There is evidence from experiments with bumble bees that nutrition can have a substantial effect on the development of ovaries of young queens following hibernation (Vogt *et al*., 1998). The presence of both pollen and honey in the diet of the queens increased ovary mass by 300% over 8 days, compared with only 50% for bees fed pollen or honey alone. The provision of no supplements resulted in only a 25% increase in ovary mass and therefore reduced egg laying capacity.

The provision of nutrients to a colony also affects the sex ratio of eggs laid by the queen. Sasaki and Obara (2001) manipulated the amount of a commercial protein diet available to honeybee colonies and observed a significantly lower (7%) proportion of male eggs being laid during the reproductive season when the protein supply was restricted than when protein supply was adequate (44%). However, nutrition did not affect the proportion of male eggs laid during the seasonal period of declining reproduction when the number of male eggs was already low. Similar observations showing an effect of the supply of pollen and hive reserves on the proportion of male eggs have been made for the stingless bee, *Melipona beechii* (Moo-Valle *et al*., 2001). Male honeybees are also particularly prone to cannibalism during periods of low pollen supplies (Moritz, 1999). Nutritional status has been shown to affect the number of spermatozoa produced and delivered to the queen during mating in the bumble bee *B. atratus* (Garofalo *et al*., 1986). However, Winston (1987) states that the production of spermatozoa in honeybees has not been shown to depend on the amount of protein fed to young drones. Nevertheless, drones from colonies with low pollen stores take longer to reach sexual maturity than drones from colonies rich in pollen (Free and Williams, 1975)

Sources of nutrients for bees

Nectar

Nectar is the main energy source for bees and consists predominantly of sugars and water. The most common sugars in nectar are sucrose, glucose and fructose, but nectars also include galactose, mannose, maltose and raffinose (Waddington, 1987). The concentration of sugars in nectar from different plant species can vary widely from approximately 4-70% (Waddington, 1987; Herbert, 1997). Similarly, the volume of nectar between flower species varies from extremely small amounts up to 5.5 μL (Waddington, 1987). In addition to sugars, nectar from various flowers can contain small amounts of amino acids, protein, lipids and free fatty acids, other fragrances, organic acids and minerals. The concentration of amino acids in nectar varies with flower type and tends to be higher in flowers visited by long-tongued bees rather than short-tongued bees like the honeybee because the former consume little pollen (Baker and Baker, 1975).

Nectar is carried in the crop of the bees and the proventriculus regulates the flow of material from the crop to the midgut. Sugars in nectar are used for flight and other foraging activities and nectar remaining in the crop following return of the foraging bee to the colony is used for the production of honey. The rate of empting of the fluid component of the crop of the foraging bee depends on the energy demands, the molarity of the food (Crailsheim, 1988a) and the concentration of metabolisable compounds in the haemolymph (Blatt and Roces, 2002). The midgut of honeybees contains peritrophic membranes that are secreted by cells along the gut and generate compartments. These compartments are organised differently depending on the age of the bee and the availability of nectar and pollen and influence the rate of digestion and absorption of nutrients (Crailsheim, 1988a). The membranes are particularly important for regulating the absorption of energy providing metabolites to coincide closely with the rate of energy use.

Sugars are absorbed from the midgut by a passive process, which is rapid and results in approximately half of a 1 M solution being absorbed in 5 minutes (Crailsheim, 1988b). Sugars from most flower nectars appear to be almost completely digested with virtually none appearing in faeces (Crailsheim, 1988b). There are differences in the rate of metabolism by the honeybee of sugars found in nectar with glucose and fructose being metabolised about 3 times faster than mannitol (Crailsheim, 1988b).

The concentration of sugars in nectar, the amount of nectar per flower, the variation in nectar volume between flowers and the morphology of the flowers have a significant effect on the foraging behaviour of bees (Waddington, 1987). There is strong evidence to show that the honeybee will make foraging decisions that result in the greatest net gain in energy for the colony. Honeybees tend to prefer flowers with the greatest energy content determined by sugar concentration and nectar volume, but select against flower types with a wide variation between individual flowers in nectar energy content (Real *et al*., 1983). Honeybees have a strong preference for flowers with high sugar concentration in nectar (Waddington, 1987). Other factors that influence foraging behaviour and selection of flowers are distance from the hive through its effect on energy required to fly to the nectar source plus the difficulty and energy cost in collecting the nectar. These “handling” energy costs can be significant if the nectar is difficult to collect (Heinrich, 1979). Ambient temperature also affects the energy expenditure during the nectar collecting process. Metabolic rate of the resting honeybee increases approximately 10-fold from 5 to 50 ml O_2 consumed/mg/h as ambient temperature decreases from 36°C to 5°C (Cahill and Lustick, 1976).

Honeydew

Honeydew is a rich source of carbohydrates excreted by aphids that feed on the sap of plants. Honeybees are known to collect honeydew as a carbohydrate source (Moritz, 1999). Some insects, particularly species of ants, remain close to aphids and ‘milk’ the honeydew droplets from their

bodies. However, honeybees do not actively seek out aphids, but collect droplets of honeydew from leaves and other surfaces. The composition of honeydew differs markedly from nectar. The mineral content is generally considerably higher and the honeydew contains a greater proportion of oligosaccharides such as melizitose and fructomaltose (Wolf and Ewart, 1955).

Pollen

Pollen provides bees with the main nutrients required for growth and development. Pollens contain a range of nutrients including proteins and amino acids, fats, fatty acids and sterols, carbohydrates such as sugars and starches, minerals and vitamins. Pollens also contain a range of cell wall constituents including cellulose, pectins and the sporopollenin matrix. Pollen grains range in diameter from 15 to 60 μm, with the larger grains usually containing more available nutrients (Waddington, 1987). The pollen grain consists of several structural components (Stanley and Linskens, 1974; Klungness and Peng, 1984a) as follows:

(i) An internal protoplasm consisting of proteins, lipids, sterols and small amounts of starch and sugars
(ii) The intine which is a true cell wall composed predominantly of cellulose and pectic acids
(iii) The exine, which is secreted onto the outside of the intine by the anther and consists primarily of cellulose with a sporopollenin matrix that provides rigidity and unique morphology to the pollen grain, and
(iv) The pollenkitt which is a soluble layer of lipids, proteins and sugars attached to the outer surface of the exine.

Composition

A large number of studies has been conducted to determine the chemical composition of pollen from many parts of the world including USA (Auclair and Jamieson, 1948; Loper *et al*., 1980; Buchmann, 1986; Schmidt *et al*., 1987), USSR (Grigoryan *et al*., 1969), New Zealand (Day *et al*., 1990), Spain (Serra Bonvehi and Jorda, 1997) and Australia (Rayner and Langridge, 1985; Kleinschmidt, 1993; Somerville, 2001, Manning and Harvey, 2002). The studies in Australia have been extensive and include pollen from 75 plant species by Kleinschmidt (1993) and 194 pollen samples from 55 plant species by Somerville (2001). Many of the analyses have been for protein and amino acid content, but others have investigated fat and fatty acid concentrations, sugars and starches, fibrous components, vitamins and minerals.

Protein and amino acids

There is an extraordinarily large range in the protein and amino acid composition of pollen analysed. For example, the reported range in protein content of pollen is from 2.9% for pistillate kiwifruit (*Actinidia deliciosa*) pollen (Day *et al*., 1990) to 53.4% for the Solonaceae plant *Solanum douglassii* (Buchmann, 1986). The protein content of pollen from flowers without nectar such as found in members of the *Solanum* and *Lycopersicon* genera, where bees obtain pollen through apical pores in the anther by vibrating the anther using indirect flight muscles, appear to have higher protein content than flowers with nectar (Buchmann, 1986). The range in protein content observed by Somerville (2001) for pollen collected in Australia was 9.2% for flatweed (*Hypochoeris radicata*) to 37.4% for one sample of pollen from Paterson's curse (*Echium plantagineum*). The range in protein content of pollen from eucalypt species obtained by Kleinschmidt (1993) was from 24% to 37%.

Eighteen amino acids have been measured in bee-collected pollen and again there is a wide variation between pollens in the relative proportion of individual amino acids. For many amino acids the range is 2-4-fold when expressed in relation to total nitrogen or protein (McCaughey *et al*., 1980; Serra Bonvehi and Jorda, 1997; Somerville, 2001). The biggest ranges in essential amino acids observed in Australian collected plants were seen in the sulphur containing amino acids, lysine, histidine and arginine (Somerville, 2001).

Lipids, fatty acids and sterols

The fat content of pollen also shows wide variation from 0% for red stringybark (*Eucalyptus macroorhyncha*) to 11.4% for flatweed in the studies of Somerville (2001). The range in fat content recorded by Serra Bonvehi and Jorda (1997) for pollen collected in Spain was 4.80 to 7.18%, while the range reported by Day *et al*. (1990) from New Zealand was 0.17% for pistillate kiwifruit to 13.4% for hawkweed (*Hieracium pilosela*). Singh *et al*. (1999) has recorded lipid concentrations as high as 20.3% in oven-dried pollen samples collected in India.

Fatty acids with carbon chain lengths from C_8 to C_{22} have been measured in pollen. Serra Bonvehi and Jorda (1997) found the highest mean concentration for C18:2 (linoleic acid, 31.3%) followed by C16:0 (palmitic acid, 22.7%) and C18:3 (linolenic acid, 19.1%). Manning and Harvey (2002) examined the lipid content of pollen from six eucalyptus species in Western Australia and found the lipid content was low relative to the pollen of many European plants with a range from 0.59 to 1.9%. The dominant fatty acid in these eucalyptus species was also linoleic acid (36-48% of lipid), with palmitic and oleic (C18:1) acids being the next most dominant.

Cholesterol and 24-methyl cholesterol are the major sterols in pollens, but were not found in all those examined by Standifer *et al*. (1968). The most dominant sterols in 15 plant pollens investigated by Standifer *et al*. (1968) were 24-methyl cholesterol, B-sitosterol, stigmasterol and cholesterol, but campesterol and sitosterol have also been found (Herbert,1997).

Pollen grains are coated with an oily and frequently yellow coloured material called the ‘pollenkitt’. The pollenkitt is rich in lipids and fatty acids, which are often attractive to bees. Lipids are also found within the centre of the pollen grains. Dobson (1988) found that the lipids of the pollenkitt were predominantly neutral including triglycerides, free fatty acids, sterols and sterol esters, whereas those found in the internal protoplasm component of the pollen were exclusively polar lipids including phospholipids, glycolipids and flavonoids.

Carbohydrates

Pollens contain a considerable amount of carbohydrates mainly in the form of fibrous material. Sera Bonvehi and Jorda (1997) found the average fibre content of honey bee collected pollens in Spain was 13.7 % with a range from 10.6% to 15.9%. The majority of this fibre was composed of insoluble components (11.3 %) such as cellulose, while 2.3 % was composed of soluble components, presumably mainly hemicellulose in the form of arabinoxylans, glucans and pectins (Stanley and Linskens,1974). Kwiatkowski and Lubliner-Mianowska (1957) reported that the cellulose content of pollen from 28 species of plants ranged from 1.2 to 14.9 % of dry weight and the sporopollenin content ranged from 1.8 to 23%.

Pollens also contain small amounts of starch and sugars. The pollen collected by bees in Spain contained an average of 2.13 % starch, with a range from 1.79 % to 2.63 % and an average of 0.4 % sugars (Sera Bonvehi and Jorda, 1997). The sugars were primarily fructose, glucose and sucrose with trace amounts of trehalose, isomaltose, maltose, raffinose, erlose and melezitose.

Minerals

Pollen is the dominant source of minerals for honeybees with only minor amounts normally coming from nectar (Somerville and Nicol, 2002). Pollens contain from around 1 to 6.5% ash with the mineral elements covering the range found in plant tissues (Herbert, 1997). The predominant elements found in pollens are K, Na, P, S, Ca and Mg (Herbert and Miller-Ihli, 1987; Sera Bonvehi and Jorda, 1997; Somerville and Nicol, 2002). However, 22 trace elements including Si, Al, Mn, Mo, Cu, Fe, Ni, Ti,

Cr, Zr, Be, B, Zn, Pb, Ag, As, Sn, Ga, Sr, Ba and U have been measured in pollen collected by honeybees in Russia (Grigoryan *et al*. 1969).

Potassium represented an average of 59% of the minerals in bee collected pollens in Spain (Sera Bonvehi and Jorda, 1997) with a range between pollens from 2.5 to 6.4 g/kg (mean 4.2 g/kg). Somerville and Nicol (2002) and Manning (2002) similarly found potassium to be the most dominant mineral in Australian pollen. However, the range in potassium content of pollen collected in Eastern Australia by Somerville and Nicol (2002) was 2.2 to 38 g/kg with the very high value being found in onion weed (*Asphodelus fistulosus*). The variation between pollen sources in the content of individual minerals can be particularly large. For example, Somerville and Nicol (2002) found the iron content of pollens to vary from 14 to 520 mg/kg. Other elements analysed varied across the pollen samples by 3.4 fold for sulphur, 5.7 fold for phosphorus, 8.6 fold for calcium, 12.3 fold for magnesium, 30 fold for sodium, 21 fold for zinc, 22 fold for manganese and 14 fold for copper.

Vitamins

Pollens have been reported to be rich in the water soluble vitamins thiamine, riboflavin, pyridoxine, pantothenic acid, niacin, folic acid, biotin, inositol and ascorbic acid (Nielsen, 1955; Barbier, 1971; Herbert, 1997). However, pollens appear to contain only small quantities of fat soluble vitamins. Vivino and Palmer (1944) measured low concentrations of vitamins D and E, but no presence of vitamins A and K in a number of pollen samples. Manning (2001a) measured the concentrations of thiamine, ascorbic acid, riboflavin, niacin, pyridoxine and folic acid in two eucalyptus species in Western Australia.

Vitamin concentrations in pollen, similar to other components, show a large variation between pollen sources. For example, Herbert *et al*. (1985) found that the ascorbic acid content of bee collected pollen in north-eastern USA varied from 136 to 1943 mg/kg throughout the year. Similarly, Herbert *et al*. (1987) found that the thiamine content of pollen varied widely depending on time of year and floral source. Stanley and Linskens (1974) reported the vitamin E content of pollen from *Echinops* species to vary from 21 to 170 mg/kg. The range in values reported by Manning (2001a) for pollen samples from *Eucalyptus accedens* and *Corymbia calophylla* were 21-207 mg/kg for ascorbic acid, 10-34 mg/kg for thiamine, 6.1-6.5 mg/kg for riboflavin, 1-35 mg/kg for niacin, 2-4 mg/kg for pyridoxine and 10-22 mg/kg for folic acid.

There is evidence that several vitamins in pollens are not stable and deteriorate over time. Hagedorn and Burger (1968) studied the effects of pollen aging on the content of several water soluble vitamins and found that the concentrations of niacin, folic acid, ascorbic acid and pyridoxine declined over 1-4 years, whereas the concentrations of riboflavin, pantothenic acid and thiamine remained relatively constant (Table 5). The major decline in the content of niacin and folic acid occurred after 2 years of storage. Freezing the pollen for one year slowed the rate of deterioration of folic acid, but after 2 years of storage there was little difference in the vitamin concentrations in frozen and unfrozen samples. The concentration of pantothenic acid was reported to be higher in the dried than the frozen pollen stored for 1 or 2 years.

Table 5. Effect of storage on the vitamin content of pollen (units/g dry weight)

Vitamin	Fresh	1 year frozen	1 year dried	2 year frozen	2 year dried	4 year dried
Niacin	116.05	105.55	97.33	104.31	106.95	83.65
Riboflavin	13.32	14.21	13.13	13.31	14.07	12.41
Folic acid	14.89	16.00	14.45	15.81	15.73	7.80
Pantothenic acid	17.31	16.07	20.42	18.26	20.00	17.79
Thiamine	15.62	14.18	11.35	14.44	11.08	13.27

Ascorbic acid	492	475	377	255	259	254
Pyridoxine	8.78	8.79	6.76	8.31	7.34	4.58

Effect of environment on the composition of pollen

The composition of pollen is not constant for any plant species, but varies with many factors affecting the growing conditions including soil moisture, fertility and ambient temperature (Herbert, 1997). Somerville (2001) collected 61 samples of pollen from Paterson's curse (*Echium plantagineum*) over three seasons and from a range of geographical areas in Australia and found that the protein content of the pollen ranged from 28.1 to 37.4% with an average of 33%. There were significant differences in protein content and of the contents of methionine, isoleucine, lysine and cystine between years. There were also significant differences between years when samples were collected from the same site.

A similar wide variation within floral species has been observed for some minerals and vitamins. For example, Manning (2001a) observed the mean iron content of pollen collected from *Eucalyptus wandoo* to be 181 mg/kg in winter, 120 mg/kg in spring and 145 mg/kg in summer. The pattern in concentrations was not constant across mineral elements with corresponding values for manganese being 25.6 mg/kg in winter, 25.0 mg/kg in spring and 35.5 mg/kg in summer. Other mineral elements such as boron, copper, zinc, phosphorus and potassium showed smaller differences between seasons. The pattern of variation observed with iron was similar to that seen for ascorbic acid where winter, spring and summer values were 206, 44 and 102 mg/kg respectively. The range in values for the same plant species appears to be less for lipids. Manning and Harvey (2002) found only a small range in the lipid and fatty acid content of *Eucalyptus wandoo* collected from three sites and three times of the year in Western Australia. The largest variation from winter, spring and summer collected pollen was for myristic (0.3, 1.20, 0.00 mg/kg), linoleic (29.9, 17.8, 13.4 mg/kg) and linolenic acids (1.30, 0.8, 0.7 mg/kg).

Intake and Digestibility

Pollen

Pollen consumption by the honeybee commences shortly after emergence with continuous consumption in around 80% of the bees occurring by 6 hours under laboratory condition and 10 hours for bees in colonies (Dietz, 1969). Pollen intake increases from relatively low amounts on days 1 and 2 after emergence to reach a peak around day 9 and then fall to low levels in foraging bees (Crailsheim *et al*. 1992). During high brood rearing activity, the weight of pollen in the midgut of honeybees increased from less than 1 mg/bee at day 1 after emergence to approximately 4 mg/bee at days 8-10 and then fell to negligible amounts from day 15 until death (Crailsheim *et al*., 1992). Pollen intake has been related closely to the growth of the bee and the development and activity of the hypopharyngeal gland and declines once the worker bees commence foraging activities (Crailsheim and Stolberg, 1989). Pollen consumption peaks at around four days of age in drones where it is used primarily for body growth, is reduced to low amounts by day 8 and is negligible once flights begin (Szolderits and Crailsheim, 1993).

Pollen grains consumed by bees enter the crop and pass by contraction of the proventriculus to the midgut where they are subjected to the action of digestive enzymes. A pollen bolus takes from 1-3 hours to pass through the midgut to the hindgut and rectum (Klungness and Peng, 1984b), with the rate of passage being influenced by the molarity of the food and amount consumed (Crailsheim, 1988a). The pollenkitt layer of the pollen is removed as the pollen passes through the proventriculus and little remains attached to the pollen in the anterior section of the midgut (Klungness and Peng, 1984a). The germination pores of the pollen then become swollen in the anterior midgut region and, with dandelion (*Taraxacum officinale*) pollen, loss of protoplasm through the pores and its digestion occurs as the pollen moves through the midgut over the next 2 hours (Peng *et al*., 1985).

Pollen grains from some plant species are completely disrupted through osmotic shock, which releases the contents of the protoplasm for digestion (Kroon *et al.*, 1974; Klungness and Peng, 1984b). Other pollen grains undergo softening and disruption of pectic acids and hemicellulose of the intine, become weakened and rupture releasing the protoplasm (Peng *et al*., 1985). However, some pollen grains with thicker cell walls appear to lose little protoplasm through the germination pores and remain largely undigested by the time they reach the rectum (Klungness and Peng, 1984b; Peng *et al*., 1985). The material in the rectum consists of undigested pollen cell walls and large quantities of slurry consisting predominantly of undigested lipids and waxes from the pollenkitt and protoplasm and complex proteins (Klungness and Peng, 1984a; Peng *et al*. 1985).

The digestibility of pollen grains has been reported to range from 30 to 70% (Martinho, 1975, cited by Klungness and Peng, 1984a) and depends largely on the degree of disruption of the intine and exposure of the pollen protoplasm to digestive enzymes. The digestibility of proteins, starch and sugars within the protoplasm appears to depend largely on the extent of rupture of the pollen walls and exposure to digestive enzymes (Klungness and Peng, 1984a). The relatively low nutritional value of dandelion pollen has been attributed in part to the low removal of protoplasm from the intine and therefore low digestibility of its protein and lipid contents (Peng *et al*., 1985).

Nitrogen and protein

Only one experiment investigating the digestibility of protein from pollen by the honeybee was found in the literature search. Pollen predominantly from three floral species collected by honeybees in Tuscon, Arizona was used in an experiment, which showed an apparent nitrogen digestibility of 83% using a faecal collection technique (Schmidt and Buchmann, 1985). The digestibility of pollen dry matter in the same experiment was 89%. These results indicate that the digestibility of pollen protein may be slightly less than for pollen dry matter, but more experiments would be needed to confirm this assumption because pollen grains vary widely in dry matter digestibility. Schmidt and Buchmann (1985) estimated the digestibility of dry matter and protein using both faecal collection and a Cr_2O_3 marker technique and found substantially higher values with faecal collection than with the marker technique. Respective values for apparent nitrogen digestion were 83% for faecal collection and 72% for the marker technique. The conduct of digestibility studies in honeybees is not easy, but these results suggest that the marker technique used by Schmidt and Buchmann (1985) is not as satisfactory as total faecal collection.

Measurement of the total protein and amino acid content of pollen may not be an accurate method for assessing the relative nutritional value of pollen from different sources for honeybees because protein digestibility is closely linked to the extent of protoplasm release within the midgut. The evidence presented by Klungness and Peng (1984a) and Schmidt and Buchmann (1985) suggests that dry matter digestibility in honeybees of pollen from different sources can range from 30 to 90%.

Trypsin and chymotrypsin like activity has been measured in the intestine of the honeybee (Moritz and Crailsheim, 1987). Proteolytic activity in the intestine increases soon after emergence irrespective of the protein content of the food consumed, but the activity is enhanced with protein containing diets (Crailsheim and Stolberg, 1989). Proteolytic activity in honeybees within colonies reaches a peak around 8 days of age and is related closely to the intake of pollen and development of the body and hypopharyngeal glands (Grogan and Hunt, 1980; Crailsheim and Stolberg, 1989). However, the changes in proteolytic activity are not dependent on the presence of either brood or a queen bee (Crailsheim and Stolberg, 1989). Although proteolytic activity in the intestine of honeybees falls once they commence foraging and pollen intake becomes negligible, considerable activity remains for the digestion of proteins transferred in food gland secretions from nurse bees through trophallactic contact. Grogan and Hunt (1980) reported that the decline with age of the honeybee in trypsin concentration in the midgut was greater than for chymotrypsin and that there was a strong correlation between chymotrypsin concentration in the gut of bees and chymotrypsin concentrations in the pollen consumed.

Honeybee larvae do not possess protease enzymes and proteolytic activity appears only in the last stage pupal stage (Moritz and Crailsheim, 1987). Larvae receive the majority of protein from the gland secretions of nurse bees rather than from pollen. Moritz (1999) speculates that this dependence of larvae on gland secretions from nurse bees rather than on pollen for their protein supply is due to the closed rectum in larvae, which is an adaptation to reduce parasitism and disease within the hive.

The proteolytic activity of enzymes in honeybees, like other animals, is affected by endopeptidase inhibitors such as bovine trypsin inhibitor, Kunitz soybean trypsin inhibitor (Burgess *et al*., 1996) and potato protease inhibitors (Malone *et al*., 1998). Trypsin inhibitors added to a 60% sucrose solution offered *ad libitum* to caged bees significantly reduced the activity of the midgut endopeptidases trypsin, chymotrypsin and elastase, but increased the activity of the exopeptidase leucine aminopeptidase (Burgess *et al*., 1996). Longevity of the bees decreased from approximately 60 days for 100% dead to less than 20 days when the concentration of trypsin inhibitors in the sucrose solution was increased from 0 to 1.0 %. A similar decrease in the activity of trypsin, chymotrypsin and elastase, but not leucine aminopeptidase, has been observed when potato serine proteinase inhibitors, POT-1 and POT-2, were offered to caged bees in either artificial pollen or a sucrose solution (Malone *et al*., 1998). Longevity was significantly reduced when the bees were offered the potato protease inhibitors at a concentration of 1% in pollen or 0.2% in the sucrose solution. Trypsin and potato protease inhibitors have been incorporated into a range of economically important plants using gene transfer techniques and have been shown to be effective against numerous insect pests. If these plants are released commercially and significant amounts of the inhibitors accumulated in pollen, they could have detrimental effects on the longevity of honeybees working the transgenic crops. Trypsin inhibitors are found also in numerous pulse seeds including soybeans. Products made from these pulse seeds need to be heated to destroy the inhibitors before inclusion in pollen supplements (Erickson and Herbert, 1980).

Fats

Loidl and Crailsheim (2001) provided evidence of lipase activity in the intestines of honeybees by measuring free fatty acid concentration before and after feeding a glucose-triolein mixture. The triolein provided 0.91 μg/bee of triacylglycerols, which is near the highest amount recorded as being supplied from pollen. There appears to be no lipase activity in newly emerged bees, but it increases steeply in bees up to 3-days old and reaches a maximum in 8-day old colony bees. Lipolytic activity falls in foraging bees to approximately half the levels found in 8-day old nurse bees. In the experiment of Loidl and Crailsheim (2001), approximately 50% of the triolein was measured as free fatty acids in the midgut of honeybees. However, the value cannot be used as an accurate estimate of the extent of fat digestion by honeybees because of absorption from the midgut and differences between various fat types.

The extent of lipid digestion in the honeybee is likely to be influenced by the fatty acid chain length and degree of saturation, and the extent of disruption of the pollen exine. Although quantitative values could not be found in the literature, results from microscopic and histological examination indicates that substantial quantities of lipids, sterols and waxes remain undigested in the rectum of honeybees consuming pollen (Phillips, 1924; Klugness and Peng, 1984a, 1984b). These lipid materials are believed to be of both protoplasmic and pollenkitt origin (Peng *et al*., 1985). Robinson and Nation (1970) found that over 30% of the fresh weight of adult bee excreta consisted of lipid with a predominance of oleic, palmitic, linoleic and linolenic fatty acids.

Some of the volatile lipids and yellow coloured carotenoids associated with the pollenkitt may be excreted unaltered and act as phagostimulants or attractants for directing foraging bees to specific pollen sources (Dobson and Peng, 1997). The presence of lipid material in the faeces of honeybees is in strict contrast to pollen-specialist bees such as the solitary bee, *Chemostoma florisomne*, which has been found to rapidly and completely digest all lipid material in pollen (Dobson and Peng, 1997).

Carbohydrates

There have been few quantitative studies on the digestibility of carbohydrates by honeybees. However, evidence from microscopic, histochemical and longevity studies indicate that carbohydrates are digested to varying degrees by bees and that some carbohydrates are toxic. The most common sugars in pollen, fructose, glucose and sucrose are completely digested by honeybees and readily utilised in metabolic processes (Crailsheim 1988b). Mannitol is transported at identical rates to glucose across the gut wall by passive diffusion, but is utilised at about a third the rate as glucose (Crailsheim, 1988b). There is evidence from longevity studies that honeybees can also utilise maltose, trehalose and melezitose, but cannot utilise the pentoses, arabinose and xylose, or raffinose, galactose, glucuronic acid, galacturonic acid lactose and stachyose (Phillips, 1924; Barker, 1977). Many of the more complex carbohydrates including dextrins, inulin, glycogen and pectins are not utilised by the honeybee (Phillips, 1924; Barker, 1977).

Cellulose, sporopollenin and other pollen cell wall constituents appear also to pass intact to the rectum and are not digested (Klungness and Peng, 1984a). Although bacteria and fungi have been isolated from the digestive tract of foraging honeybees (Gilliam and Valentine, 1976), there is no evidence of cellulase activity in honeybees from either bee or microbial origin and the cellulose component of pollen remains undigested (Klungness and Peng, 1984b). However, there is evidence that pectic acids within the pollen walls are removed during passage along the digestive tract (Klungness and Peng, 1984b), but the pectic acids are not utilised by honeybees (Barker, 1977).

Some sugars and more complex carbohydrates are toxic to bees. Barker (1977) fed to honeybees syrups of various carbohydrates at concentrations from 2-10% in a 50% sucrose solution and determined toxicity from the number of deaths recorded in caged bees at 8 and at 16 days after being offered the syrups. The carbohydrates were ranked in order of increasing toxicity as follows: raffinose < galactose < glucuronic acid < galacturonic acid < lactose < stachyose < pectin. Herbert and Shimanuki (1978a) confirmed that lactose was toxic to honeybees when included in pollen substitutes.

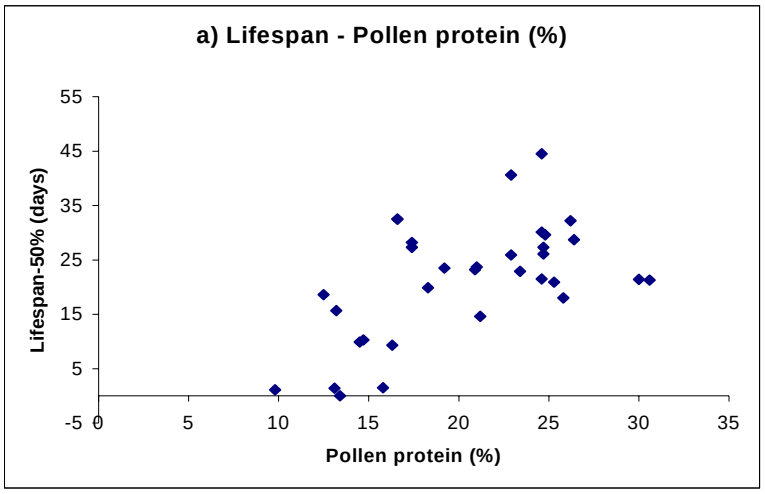
There are contradictory reports in the literature about the ability of honeybees to utilise starch. Klungness and Peng (1984a) concluded from histochemical studies that starch in pollen is efficiently digested and absorbed. However, early studies by Phillip (1924) based on measurements of longevity and iodine staining suggested that honeybees cannot utilise starch. Other studies report that potato starch can be used successfully for brood rearing when added to pollen substitutes (Harp, 1978). Similarly, starch encapsulated, pollen lipid extracts have been well utilised when incorporated into pollen substitutes (Herbert *et al*. 1980a). The ability of worker honeybees to digest starch may depend on their age and specific hive activities. Ohashi *et al*. (1999) provides evidence from mRNA studies that at least three carbohydrate digesting enzymes, α-amylase, α-glucosidase and glucose oxidase are produced in the hypopharyngeal gland of foraging bees but not of nurse bees. Amylase converts starch into glucose and glucose oxidase converts glucose into gluconic acid and peroxide. The gluconic acid is used to maintain the acidity of honey and, together with hydrogen peroxide, have an important anti-microbial action for the preservation of honey. These three carbohydrate digesting enzymes have been shown to represent the major proteins present in the hypopharyngeal gland of foraging honey bees, which is in marked contrast to the proteins present in the hypopharyngeal gland of nurse bees (Kubo *et al*., 1996). Amylase is secreted also by the salivary glands and other digestive organs of the honeybee (Ohashi *et al*., 1999; XiaoQing *et al*., 2000), but there is little information on their quantitative significance in the digestion of starch. In summary, these results indicate that starches can be utilised successfully by foraging bees, but would be of less nutritional value for younger bees.

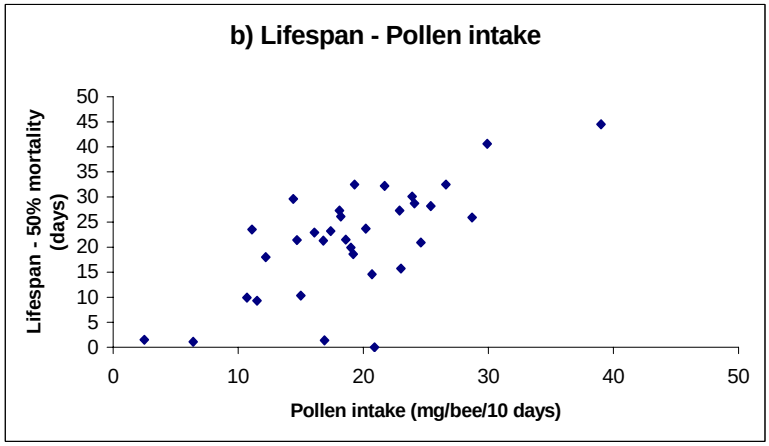
Nutritional value of pollen

Variation in productivity from pollens

The nutritional value for honeybees of pollen from different floral sources and for the same floral source over different years has been shown to vary widely. These assessments have been based on measurements of longevity (Standifer, 1967; Knox *et al*. 1971; Schmidt *et al*., 1987), hypopharyngeal gland development (Standifer, 1967; Standifer *et al*, 1970; McCaughey *et al*., 1980) and brood rearing capacity (Herbert *et al*., 1970; Campana and Moeller, 1977; Loper and Berdel, 1980).

Schmidt *et al*. (1987) offered caged bees pure samples of bee-collected pollens from 25 plant species and 8 pollen mixtures and found that longevity as measured by days to 50% mortality relative to bees offered sucrose alone ranged from -4 to +40 days. A similar wide variation in hypopharyngeal gland development from 1.82 to 3.5 on a scale from 0 to 4 was observed when pollen from 11 different plants was offered to caged honeybees by Standifer (1967). Some pollen sources, such as dandelion (*Taraxacum officinale*) were found to be incapable of producing brood whereas pollen from other plants like saguaro (*Cereus giganteus*) produced 240 brood cells sealed/bee/day (Loper and Berdel, 1980). A low correlation has been observed between longevity and hypopharyngeal gland development when both were measured concurrently in the same experiment, suggesting that the nutrient requirements and suitability of pollen for young nurse bees differ from those of older foraging bees (Standifer *et al*., 1960).





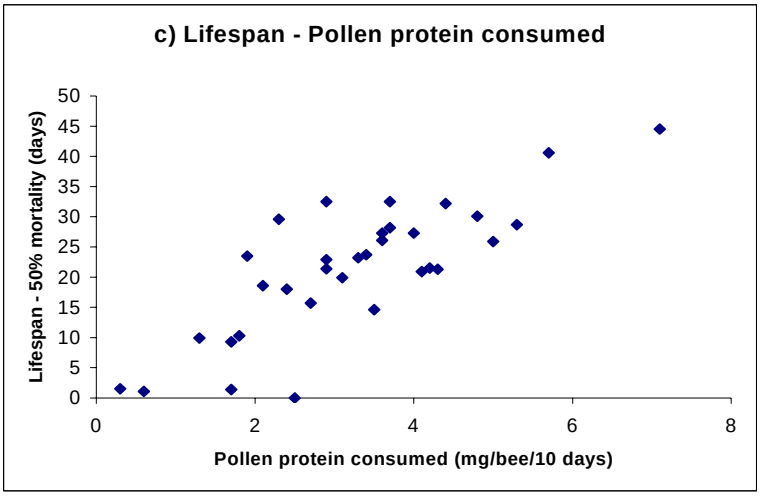


Figure 1. Relationships between lifespan (days to 50% mortality when shortest value =0) of caged bees offered 33 different floral pollen species and mixtures and a) pollen protein content (R^2 = 0.15), b) pollen intake (R^2 = 0.34) and c) pollen protein consumed (R^2 = 0.52). Calculated from Schmidt *et al.* (1987).

Schmidt *et al*. (1987) found that increased lifespan was not correlated with physical characteristics of pollen grains such as size, spininess or whether the dispersal vector for the plant pollen was wind or insects. Lifespan showed little correlation with the season the pollen was collected. It also showed only a small positive relationship with pollen protein content (R^2 = 0.15). However, longevity showed a significant relationship with pollen consumption (R^2 = 0.34), and was even more closely related to pollen protein consumption (R^2 = 0.52) as shown in (Figure 1). Similarly, McCaughey *et al*. (1980) found a strong relationship between development of the hypopharyngeal gland and pollen protein consumption (R^2 = 0.77, Figure 2). Loper and Berdel (1980) also observed a stronger relationship between the number of brood cells sealed and pollen protein intake (R^2 = 0.46) than with pollen protein content (R^2 = 0.32, Figure 3). Although Campana and Moeller (1977) did not measure the protein content of pollen from five floral sources, they showed that the area of brood sealed was closely related to total daily pollen consumption by the colony. However, the efficiency of brood rearing ranged from 7.9 to 13.1 brood reared/g pollen consumed suggesting that there were variations between the pollen sources in their brood rearing capacity.

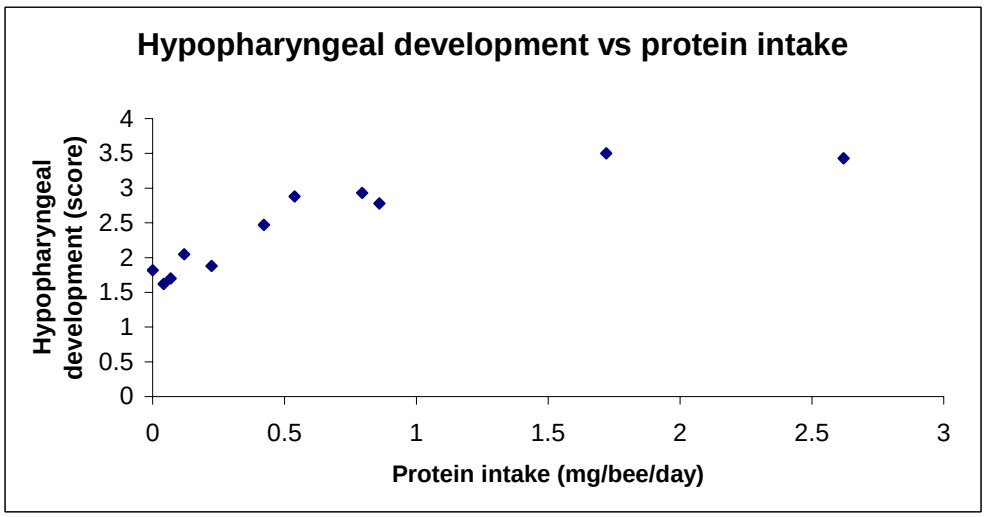


Figure 2. Relationship between hypopharyngeal gland development and protein intake by bees from a range of pollen samples. Calculated from McCaughey *et al.* (1980).

The analyses outlined above suggest that the attractiveness of pollen to honeybees and the amount eaten are more important for determining nutritional value than the protein content. However, because the correlation between the performance characteristics measured and pollen protein intake was not unity, other factors such as the amino acid, mineral, vitamin, essential-fatty acid or sterol contents of the pollen are likely to influence honeybee productivity measures. There are examples where the nutritional value of individual pollens has been improved by overcoming imbalances of amino acids, minerals, vitamins and sterols in pollens.

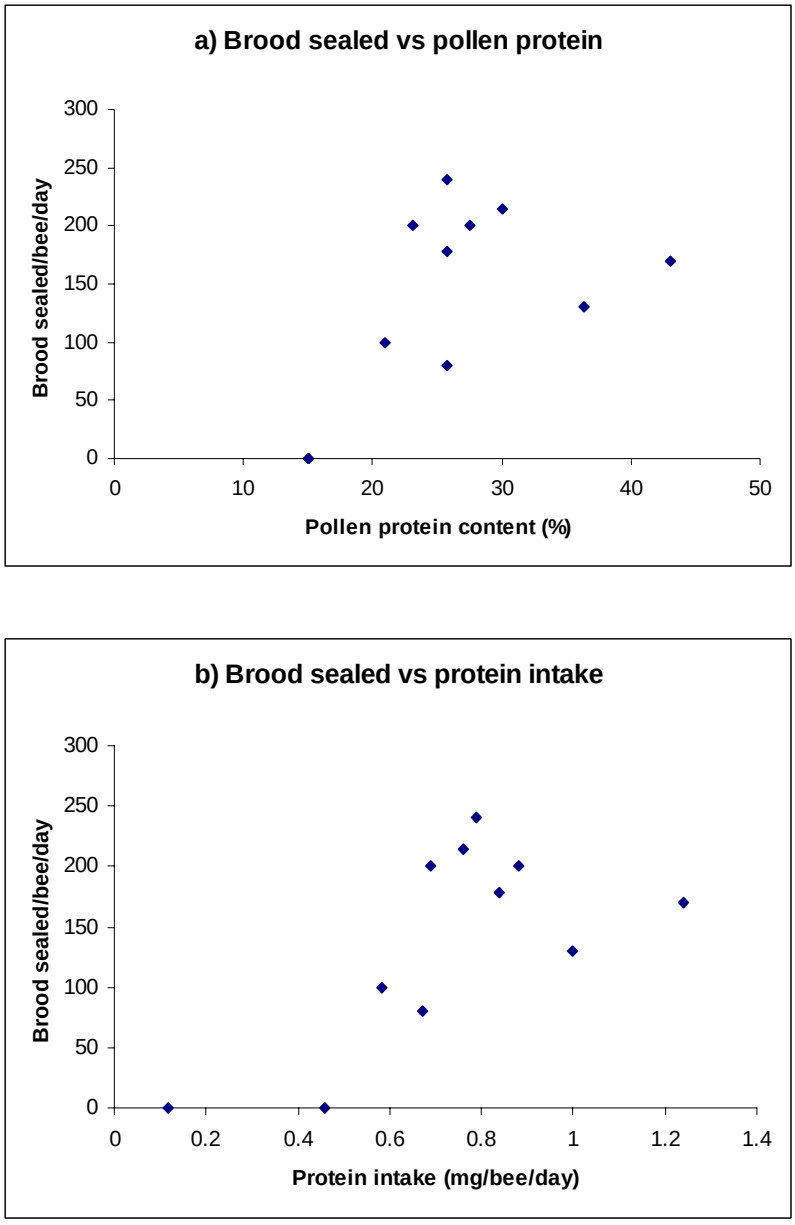


Figure 3. Relationship between brood rearing capacity and a) pollen protein content (R^2 = 0.32) and b) pollen protein intake (R^2 = 0.46) for a range of pollen samples. Calculated from Loper and Berdel (1980).

Herbert *et al*. (1970) found that supplementation of dandelion pollen with L-arginine, but not tryptophan or phenylalanine returned brood rearing capacity of this pollen to near that observed for colony housed bees. However, when Loper and Berdel (1980) conducted a similar experiment, no improvement in brood rearing capacity of dandelion pollen was observed with the addition of arginine.

There is strong evidence that the mineral concentrations in some pollen sources are so high that there is a negative effect on brood rearing and productivity. Herbert and Shimanuki (1978b) found that the brood rearing capacity of honeybees offered artificial diets declined once the concentration of ash from bee collected pollen exceeded 2% dry matter and almost ceased with high levels of mortality when the diets contained 8% pollen ash. The ash content of pollen has been found to range from 1–6.5% (Herbert, 1997). Anderson (1997, 2002) has suggested that 'disappearing disorder' seen in honeybee hives during some years in southern Queensland is most likely due to high concentrations of trace elements, particularly iron, in nectar and pollen. Alternatively, Manning (2002) has suggested that pollens from eucalyptus species in Western Australia may lack sufficient manganses for full productivity of honeybees.

Marshall and Haydak (1958) showed that there were marked differences between pollen sources in the longevity and growth rate of larvae and in development to adults during experiments with the solitary bee, *Osmia lignaria*. Pollen from gumweed (*Grindelia quarrosa*) did not allow development of the larvae past the third instar stage. The addition of vitamin-free casein to the gumweed pollen increased longevity of the larvae, but did not increase growth rate or maturation to adulthood, suggesting that factors other than protein were limiting the nutritional value of gumweed pollen for the solitary bee. However, when yeast, egg yolk, cholesterol or combinations of these ingredients were added to the casein-supplemented pollen, larval growth and maturity to the adult stage was improved to near that of the best pollens examined. These experiments suggest that some pollen sources lack essential vitamins found in yeast and sterols, such as cholesterol.

In summary, the nutritional value of pollens for honeybees varies widely with floral source, particularly because of variation in the amount consumed. However, some pollen sources may lack sufficient protein, individual amino acids, minerals, vitamins or lipids for maximum productivity. Other pollens may be toxic because of excess minerals, amino acids or other compounds (de Groot, 1953; Schmidt *et al*., 1987).

Pollen attractiveness

Honeybees have a clear ability to identify plant pollens from other materials, but there are reports of bees collecting fungal spores (Schmidt *et al.*, 1987; Shaw, 1990), starch encapsulated insecticide pellets (Herbert *et al*., 1980a) and other small particles, particularly at times of pollen dearth. Pollen sources can vary widely in their attractiveness to honeybees (Levin and Bohart, 1955; Doull, 1966; Boch, 1982). For example, Doull (1966) showed that the pollen from some eucalyptus species, particularly long leaf box (*Eucalyptus goniocalyx*), has low attractiveness for honeybees and is rarely taken when offered with other pollen sources. Combs of pollen from this eucalyptus species have been observed to remain untouched with in a hive for a period of 12 months (Doull, 1966).

Visual, aromatic, tactile and metabolic cues have been identified as influencing the attractiveness of pollen to honeybees. Boch (1982) found a strong correlation between colour of pollen and its preference by foraging bees and suggested that the yellow colour range was a common visual signal for attracting bees. These observations were supported by earlier research from Daumer (1958, cited by Boch, 1982). There is evidence also from Schmidt *et al*. (1987) that the attractiveness of pollen from *Populus* declines as it changes in colour from light tan to very dark brown on storage at room temperature. Lepage and Boch (1968) showed that honeybees were attracted strongly to dishes with cellulose powder to which a range of yellow coloured substances had been adsorbed including a pigment extracted from mixed pollen, vegetable colour added to margarine, ginger and turmeric extracts, mineral yellow or riboflavin. However, the same strong attractiveness to pollen within the yellow colour range was not observed for hive bees, prompting Boch (1982) to speculate that foraging bees which consume only small amounts of pollen may have different pollen attraction cues than nurse bees which have a high pollen requirement.

A large number of studies have been conducted in an attempt to isolate the chemical compounds that attract honeybees to pollen. There is strong evidence that chemicals attractive to honeybees reside in the lipid fraction of the pollenkitt (Dobson, 1988) and can be extracted with lipid solvents. Doull

(1966) demonstrated that lipid extracts from mixed pollen when placed on glass dishes attracted bees to these dishes. When patches of a comb containing long leaf box (*Eucalyptus goniocalyx*) pollen were treated with the lipid extract, bees completely consumed the pollen that had previously been rejected for 12 months, but only in the areas where the extract had been applied. Robinson and Nation (1968) demonstrated that the consumption of artificial diets by caged bees increased when either whole pollen lipid extracts or fractions soluble in cold acetone were added to the diets. However, the addition of either cold acetone insoluble phospholipids or an extract of volatile substances from the pollen did not increase attractiveness of the diets. This experiment indicates that the chemicals which attract honeybees reside in the neutral lipid or sterol component of the pollen.

Several compounds have been suggested to act as the attractants in pollen for honeybees including 24-methylene cholesterol (Herbert *et al*., 1980), an acylated polar carotenoid (Lepage and Boch, 1968) and octadeca-*trans*-2,*cis*-9,*cis*-12-trienoic acid (Hopkins *et al*., 1969). However, other components such as linoleic and linolenic acid may play some role in attracting honeybees (Singh *et al*., 1999). Despite the indication that these specific compounds are attractive to honeybees, these compounds are rarely used in pollen substitutes and bee collected pollen is commonly added to artificial diets to enhance attractiveness and consumption.

The attractiveness of pollens depends also upon the ease with which they can be gathered. Bees have difficulty collecting particles greater than 0.5 mm in diameter (Somerville, 2000). Doull (1966) found that some pollens such as from *Eucalyptus camaldulensis* absorbed water quickly and can clump together to form particles too large for the bees to collect. However, other pollens such as *Echium plantagineum* do not absorb water as quickly and remain easier for bees to collect under a wider range of climatic conditions. Doull (1966) observed that temperature and humidity affected the clumping of pollens and altered the percentage of different pollen species collected by honeybees. Doull (1974) showed that the addition of a solution of sucrose, fructose and glucose increased the attractiveness of pollen compared with the addition of water only. However, pollen to which water had been added was more attractive than dry pollen. Bees offered dry foods have been shown to use secretions from their labial glands to moisten the food, which slows the rate of intake (Simpson, 1964). Pollen mixed with sucrose, or fructose and glucose in equal proportions was as attractive to the honeybee as pollen mixed with honey (Doull, 1974). The latter is a common industry practice to increase the attractiveness of artificial pollen substitutes.

There is strong evidence that honeybees alter their intake and diet preferences depending on their metabolic status and this will affect the attractiveness of food sources including pollens. Thus, the attractiveness of any pollen or pollen substitute may change depending on the current metabolic status of the honeybee. Inouye and Waller (1984) found that honeybees showed a strong positive preference for 30% sucrose solutions containing only phenylalanine when 24 amino acid, phenolic acid or flavonoid solutions were compared with sucrose alone. Contrary to the results of Inouye and Waller (1984), Kim and Smith (2000) found that glycine stimulated feeding preferences. These authors argued that honeybees like other insects alter feeding preferences depending on the relative concentrations of carbohydrates and proteins or amino acids in their haemolymph. The feeding preference will be modified to maintain a desired balance between nutrients ingested and nutrients used in metabolic processes. Thus, when diets are deficient in any essential nutrient there will be a preference for foods containing that nutrient. Herbert *et al*. (1980a) reported that the consumption of pollen substitutes was increased substantially when they were supplemented with 4% of the lipid fraction from bee collected pollen. However, if the imbalance cannot be rectified through a choice of an alternative feed, intake of all diets will decline. For example, de Groot (1953) showed that honeybees reduced their consumption of diets lacking in tryptophan and arginine. Similarly, Hagler (1990) reported that onion cultivars with high concentrations of potassium in nectar were unattractive to bees.

Nutrient requirements of bees

Main processes requiring nutrients

Nutrients are required by the honeybee colony to provide energy for the survival of individual bees, for maintenance of hive temperature and particularly brood temperature close to 36°C, for flight and for communication activities of foraging bees. Energy is used also through fanning actions of wings to remove excess carbon dioxide from the hive. Nutrients, particularly amino acids and fatty acids, are required to provide the components for reproduction and the growth of bees within the colony and to cover inevitable metabolic losses. Energy is lost through the biochemical processes converting absorbed nutrients to body tissues. Minerals and vitamins are needed as co-factors to many essential biochemical reactions. Nutrients are required also for the provision of substrates for construction of the combs for brood cells and food storage. Excess nectar and pollen are stored as honey and bee bread within the combs as nutrient reserves for the colony during periods of low temperatures, wet weather and when there is a dearth of flowering plants. Water is also an essential nutrient for maintaining hydration of individual bees, for regulation of humidity within the colony and for evaporative cooling in hot weather.

Energy

Carbohydrates, particularly sugars, are the main source of metabolic energy for the honeybee as determined from measurements of respiratory quotients (Southwick, 1983; Storey, 1985). However, excess dietary amino acids can also be utilised for energy metabolism (Crailsheim, 1988c). Energy requirements of honeybees are commonly measured in terms of oxygen consumption, but these values can be converted readily to glucose consumption by assuming that 747 ml oxygen results from the oxidation of 1 g of glucose (Gmeinbauer and Crailsheim, 1993).

The energy requirements of individual honeybees depend largely on ambient temperature and the extent and type of activity (Cahill and Lustick, 1976; Southwick, 1991; Stabentheiner *et al*., 2003), but are influenced also by the number of bees in a cluster (Southwick, 1985) and by ambient barometric air pressure (Withers, 1981).

Maintenance requirements

Measurements of the minimum amount of energy required for survival of honeybees, or maintenance requirements, have been made using young bees immediately following emergence when 1-7 hours old and by using older bees at rest. Newly emerged honeybees have poorly developed flight muscles and are incapable of endothermic heat generation through contraction of the flight muscles as is common in older bees. Thus, measurements of oxygen consumption by resting bees within a few hours from emergence have been assumed to represent closely the minimum maintenance energy requirement of individual honeybees (Stabentheiner *et al*., 2003). Oxygen consumption by these young bees was shown to increase from 0.212 µl O_2/minute/bee at 10°C to 1.12 µl O_2/minute/bee at 25°C, 2.09 µl O_2/minute/bee at 35°C and 3.03 µl O_2/minute/bee at 40°C (Stabentheiner *et al*., 2003). Young bees exposed to temperatures of 10°C succumbed to chill coma because of their inability to heat their bodies through endothermic processes associated with flight muscle contraction.

Maintenance energy requirements of older bees with mature flight muscles appear to be higher than for newly emerged bees. Oxygen consumption measured by Stabentheiner *et al*. (2003) for adult mid-aged (7-13 day-old) and foraging bees with minimal activity at 35°C was 17.45 and 5.55 µl O_2/minute/bee, respectively. A similar value of 16.4 µl O_2/minute/bee was obtained under normal atmospheric pressures for resting adult bees by Withers (1981) when a standard bee fresh weight of 80 mg is assumed. There appeared to be little consistent effect of ambient temperature from 10°C to

35°C on the oxygen consumption of the mature, inactive bees (Stabentheiner *et al.*, 2003). The mean oxygen consumption recorded for both 7-13 day-old and foraging bees with minimal activity at 15, 25, 30 and 35°C was 12.7 µl O_2/minute/bee. However, Cahill and Lustick (1976) measured a 10-fold increase in oxygen consumption of resting bees as the temperature was decreased from 36°C to 5°C.

Energy expenditure during activity

Activity level was shown by Stabentheiner *et al*. (2003) to have a significant effect on oxygen consumption in adult bees. These authors classified activity into 4 levels from small or no movements to low activity of grooming or walking a few steps, medium activity when walking at slow or medium speeds and high activity when walking at fast speeds and taking short flights. Oxygen consumption for foraging bees at 35°C increased from 5.55 µl O_2/minute/bee with small or no activity to 21.07 µl O_2/minute/bee with low activity, 30.30 µl O_2/minute/bee with medium activity to 41.15 µl O_2/minute/bee with high activity. Slightly higher values were measured for nurse, mid-aged bees than for foraging bees where energy expenditure in the highly active bees was 52.27 and 41.15 µl O_2/minute/bee, respectively. As activity increased there was a marked effect of ambient temperature on oxygen consumption. For foraging bees with high activity, oxygen consumption increased from 41.15 µl O_2/minute/bee at 35°C to 133.12 µl O_2/minute/bee at 15°C.

Numerous measurements have been made of the oxygen consumption of hovering and flying honeybees. There appears to be little difference in energy expenditure between hovering and free flight. Values summarised by Gmeinbauer and Crailsheim (1993) and Withers (1981) for hovering and flight range from approximately 105 µl O_2/minute/bee to 160 µl O_2/minute/bee when standard bee fresh weight is assumed to be 80 mg. The average value for oxygen consumption bees from these reports for hovering and flying was about 130 µl O_2/minute/bee.

Thermoregulation and air quality

Honeybees expend a considerable amount of energy maintaining the temperature within the hive to around 35-36°C. During cold weather, worker bees increase heat production by continuous contraction of the flight muscles without wing movement and, during hot weather, wing muscle contraction is associated with fanning movement of the wings (Heinrich, 1993). When the hive is too hot or the humidity too low, water-collecting bees regurgitate water droplets to cover their combs with a thin layer of water, which evaporates to increases the cooling capacity of the colony (Schmaranzer, 2000). The optimum humidity for brood rearing is between 90 and 95% (Doull, 1976). Wing fanning is also stimulated by high concentrations of carbon dioxide to increase circulation within the hive when air movement is low during periods of colder weather (Seeley, 1974).

Particular effort is made by worker bees to maintain the temperature of the brood and capped brood cells. Temperature of brood either above or below 36°C for any appreciable time can result in developmental abnormalities or death (Winston, 1987). Bujok *et al*. (2002) observed that, in cooler weather, worker bees undergo alternate periods of lying motionless, except for fast respiration movements of the abdomen, with their thorax pressed tightly to the cap of brood cells and periods of moving about without thorax contact to the brood cells. The motionless periods extended up to 9 minutes and during this time average thorax temperature was observed to range from 38.1°C to 42.2°C compared with a mean of 36.3°C during periods of movement. The temperature of the brood caps below the motionless bees was up to 3.2°C higher than for caps without bee thorax contact. This behaviour ensures that the temperature of the capped brood remains within strict limits when conditions are cold.

Starks and Gilley (1999) observed an alternate behaviour for worker bees under conditions of high radiation on the colony. Worker bees shield the brood comb from high radiation by positioning themselves on the hot interior region of the hive wall. The temperature of individual bees can rise as

high as 50°C without affecting survival (Coelho, 1991). Starks and Gilley (1999) showed that although workers shielded both honey and brood combs from the radiant heat source, a significantly greater number of bees protected the brood cells.

O'Donnell and Foster (2001) observed that worker bumble bees responded to changes in ambient temperature within a hive by altering the performance of fanning or brood incubation tasks. Changes in the proportion of bees undertaking each task were critical in temperature control for the colony. Although individual worker bees could undertake either task, there appeared to be differences between bees in the temperature at which they commenced a particular task. The authors suggest that a difference between individual bees in the threshold temperature for commencing a task resulted in a continuing increase in the intensity of the task within the colony as ambient temperature conditions, either cold or hot, became more severe.

Comb building

Combs for the storage of honey, bee bread and brood are constructed from wax secreted by wax glands found in worker bees. Wax glands are most developed in bees between 12 and 18 days of age. The wax is secreted as small scales from either side of the four abdominal segments on the underside of the bee. The wax is produced primarily during warm weather when nectar flows are high. Whitcomb (1946) estimates that approximately 8.4 grams of honey are required to produce 1 gram of beeswax. There is evidence that dietary protein is required also for the production of beeswax. Worker bees fed sugar solution only lost 20% of their body protein over 15 days of intensive wax production (Taranov, 1959; cited by Gray, 1997).

Whitcomb (1946) obtained information from four colonies and found that an average of approximately 0.7 kg of wax was produced each day during the summer period from mid July to mid September in Louisiana USA. An average of 5.9 kg of honey was used daily to produce the wax. During the same period, approximately 2.1 kg of honey was extracted daily from the colonies. Thus, a total of 8 kg of honey was used each day by the colonies for the production of wax and storage.

Colony energy requirements

The information presented above can be used to estimate the total glucose needs of a colony if various assumptions are made about the size of the colony, the number of brood, proportion of the total time each bee is active and the number and distance of flights. Such calculations provide a test of the accuracy of the estimated energy requirements.

The assumed oxygen requirements for each activity are given in Table 6. The colony was assumed to have 40,000 adult bees and 20,000 brood during the breeding season. However, at other times the colony was assumed to have only 20,000 adults and no brood. The estimated number of flights was determined from observations by Szabo (1980) at 70,000/day for an active colony with 40,000 adult bees during the breeding period. The number of flights was assumed to be only 2,000/day during the non-breeding period. The duration of each flight was assumed to be 3 minutes. Adult bees were assumed to be either highly active or inactive and the proportion of time in active mode was assumed to be 25% during the breeding period and 5% at other times. These estimates for the proportion of bees undertaking activity, and considered to be 'working' at any time, were based on statements by Starks and Gilley (1999).

Estimated daily glucose requirements for survival of the colony during the breeding and non-breeding periods are shown in Table 7. The estimated glucose requirement for maintenance of the 40,000 honeybee breeding colony was approximately 2 kg/day and for the 20,000 honeybee non-breeding colony 0.5 kg/day. Assuming that the colony is in its breeding phase for 3 months of the year, these calculations suggest that the total glucose requirement for survival of the colony would be approximately 240 kg/year. In addition to the sugar needed for survival of the colony, amounts as

much as 6 kg/day or 550 kg/year are required for comb construction and 80 to 140 kg extracted as honey from Australian hives.

Table 6. Estimated oxygen requirements for the main activities of a honeybee colony

Bee class or activity	Oxygen consumption ($\mu l\ O_2$/minute/bee)	Comments
Larvae	2.1	Value for newly emerged bee (Stabentheiner *et al*., 2003). Assume same maintenance energy requirements for larvae and pupae with average near that of the newly emerged bee.
Inactive adult bee	12.5	Approximate average values for mid-aged and foraging bees (Stabentheiner *et al*., 2003). A value of 5.5 was assumed during non-breeding periods.
Active adult bee	41.0	Approximate average values for mid-aged and foraging bees (Stabentheiner *et al*., 2003).
Flying/hovering foraging bee	130.0	Approximate average values for mid-aged and foraging bees (Stabentheiner *et al*., 2003).

Table 7. Estimated daily oxygen consumption and glucose requirement for a breeding and a non-breeding colony of honeybees

Bee type/activity	Number	O_2 Consumption (l/day)	Glucose requirement (g/d)
		40,000 honeybee breeding colony	
Inactive adult bees	32,000	576	771
Active adult bees	8,000	472	632
Total brood	20,000	60	81
No flights/day	70,000	27	36
Total colony requirement		1,136	1,521
		20,000 honeybee non-breeding colony	
Inactive adult bees	19,000	150	201
Active adult bees	1,000	59	79
Total brood	0	0	0
No flights/day	5,000	1	1
Total colony requirement		210	281

Winston (1987) estimated that a honeybee colony requires about 80 kg/year of honey for survival. Herbert (1997) estimated that honeybee colonies in northern USA require approximately 43 kg honey over the summer period and 70 kg over a whole year for survival. These estimates are well below the value of 215 kg/year predicted above. However, if the requirements for the nine month non-breeding period alone are considered, the value predicted from first principles of 77 kg is similar to previous estimates. The disparity between the calculations made for the whole year in this report and those suggested by Winston (1987) and Herbert (1997) could be due to several reasons. The estimated oxygen requirements for different hive activities may be too high, particularly as many of the measurements were made with either single bees or small groups of bees. The assumptions about level of activity within the hive may be incorrect and/or Winston (1987) and Herbert (1997) may not have taken into account the contribution of nectar collected by foraging bees to their energy needs.

Protein and amino acids

Protein or more specifically amino acids are essential for the growth of honeybee larvae through food provided by nurse bees and for growth of the imago from emergence until it reaches maximum size and development between 5 to 8 days of age. Amino acids are required also to replace those lost endogenously from the gut as undigested enzymes and sloughed cells and those utilised through inevitable catabolism associated with protein turnover (Crailsheim, 1986). As described above, inadequate pollen or protein supplied to a colony will reduce reproductive performance, brood rearing and the size of individual bees at emergence. Protein supply to young bees can also affect longevity, but appears to have little effect on the longevity of foraging bees (de Groot, 1953).

Amino acid requirements

There have been few estimates of the protein or amino acid requirements of honeybees. De Groot (1953) has conducted the most extensive study of the amino acids required by the honeybee. He measured the growth and nitrogen retention of bees from approximately 2 days after emergence up to 14 days of age to demonstrate that the amino acids essential for the honeybee were identical to those required by mammals. Amino acids that must be provided in the diet of honeybees are arginine, histidine, lysine, tryptophan, phenylalanine, methionine, threonine, leucine, isoleucine and valine. Similar to mammals, cystine can replace some of the requirement for methionine and tyrosine can replace some of the requirement for phenylalanine. In addition, carnosine was shown to be capable of replacing histidine. De Groot (1953) demonstrated also that honeybees could utilise the d-isomers of only histidine, methionine and phenylalanine. All other amino acids must be provided in the l-isomeric form.

De Groot (1953) did not attempt to define the minimum protein content of a diet needed for maximum growth of the young bee because the growth rates achieved under the laboratory conditions were well below those observed for bees reared under natural conditions. However, de Groot (1953) did estimate the minimum requirements for each essential amino acid relative to the total amino acid supply by first removing each essential amino acid in turn and replacing it in increasing concentrations. The minimum requirement was determined by assessing the amino acid concentration coincident with the commencement of the plateau in nitrogen retention as the amino acid content of the diet was increased. The estimated minimum concentrations were rounded to the nearest 0.5% units and are given in Table 8.

The amino acid content of royal jelly and the relative proportions of amino acids from the de Groot estimate and royal jelly are also shown in Table 8. The comparison indicates that the concentration of essential amino acids in royal jelly is on average 1.57 times greater than the concentrations suggested by de Groot for young growing honeybees. The largest discrepancy in amino acid pattern was for lysine, which on a relative basis was 43% higher in the royal jelly than in the de Groot estimate for growth of young bees. The relative proportion of methionine was 19% lower, but the de Groot estimates for the other amino acids were of similar proportionality to royal jelly.

A comparison between the de Groot (1953) estimates of essential amino acid requirements for young growing bees and the concentrations of these amino acids in royal jelly indicates that a higher concentration of essential amino acids relative to total protein may be required for growth of larvae. However, the relative proportions of essential amino acids required for growth of larvae through brood food and growth of the young imago are likely to be similar except perhaps for lysine. The method used by de Groot (1953) to estimate amino acid requirements for young adult honeybees should provide a sum of all components of amino acid requirement including those needed for tissue growth, food and other gland secretions, endogenous faecal losses and inevitable catabolism. Thus, an exact correspondence between the amino acid pattern of royal jelly and minimum needs for a young bee would not be expected.

Table 8. Estimates of the minimal concentrations of essential amino acids required for growth of the honeybee as determined by de Groot (1953) compared with the proportions in royal jelly (de Groot, 1953). The concentrations of each amino acid in royal jelly relative to the de Groot estimates and the relativity of amino acid concentrations between the de Groot estimates and royal jelly are shown.

Essential amino acid	De Groot estimate of minimum concentration needed for growth of honeybees (% total amino acids)	Amino acid content of royal jelly (% total protein)	Concentration in royal jelly/ De Groot estimate (A)	A values/ mean value for A
Arginine	3.0	5.1	1.70	1.09
Histidine	1.5	2.2	1.47	0.94
Lysine	3.0	6.7	2.23	1.43
Tryptophan	1.0	1.3	1.30	0.83
Phenylalanine	2.5	4.1	1.64	1.05
Methionine	1.5	1.9	1.27	0.81
Threonine	3.0	4.0	1.33	0.85
Leucine	4.5	7.7	1.71	1.09
Iso-leucine	4.0	5.3	1.33	0.85
Valine	4.0	6.7	1.68	1.07
Mean			**1.57**	**1.00**

De Groot (1953) compared his estimates of the relative proportions of essential amino acid required for the young growing honeybee with those established for rats, human infants and chickens. His estimates for both lysine and methionine tended to be lower than for the other species. The low methionine requirement could be explained readily through the lack of keratin production in the bee compared with the need for growth of hair and feathers in mammals and birds.

Amino acid requirements for foraging bees are likely to differ from those estimated for young growing bees or nurse bees, because foraging bees have no need for new tissue growth or food gland secretions. The primary need for amino acids in foraging bees is to replace those lost from body secretions and gut sloughing through the faeces and through inevitable catabolism associated with body protein turnover. No estimates appear to have been made of the relative proportions of amino acids needed by the honeybee to replace those lost through these endogenous processes. However, de Groot (1953) measured the nitrogen lost from nurse bees and foraging bees when they were provided with diets consisting only of sugar candy. These results can be used to calculate the total endogenous nitrogen losses for nurse and foraging bees. The endogenous nitrogen loss for nurse bees was estimated to be 6.4 μg N/day/bee or 40 μg protein/day/bee when a standard bee fresh weight of 80 mg is assumed. Corresponding values for foraging bees are 3.44 μg N/day/bee or 21.5 μg protein/day/bee.

Protein requirements

Few direct estimates of the protein requirements of honeybees have been made. De Groot (1953) found that maximum average longevity of caged bees could be achieved with sugar diets containing 1.0% casein, but not with diets containing 0.63% casein. Similarly, maximum longevity could be achieved with sugar diets containing 2% mixed pollen or 2% white Dutch clover pollen. Herbert *et al.* (1977) offered caged honeybees pollen substitutes containing 5, 10, 23, 30 or 50% protein in patties mixed with sucrose and cellulose powder and found the optimum protein concentration for rearing sealed brood was 23%. However, contrary to the experiments of De Groot (1953), a 50% sucrose

solution was provided *ad libitum* to the bees during the experiment by Herbert *et al*. (1977). Consumption of the sucrose would have diluted considerably the protein consumed from the patties.

De Groot (1953) provided strong evidence to show that sugar diets containing 5% or more casein were detrimental to longevity, with average lifespan being approximately half that observed when bees were offered a diet containing 1.25% casein. Similarly, Herbert *et al*. (1977) showed that pollen substitutes containing 50% protein depressed brood rearing. The amount of diets consumed by the bees was not measured by De Groot (1953), but results from Schmidt *et al.* (1987) presented in Figure 1 suggest that longevity of honeybees continued to increase with intake of pollen protein up to at least 0.7 mg/bee/day. The results presented in Figure 2 from McCaughey *et al*. (1980) suggest that maximum development of hypopharyngeal glands occur with an intake of approximately 1.2 mg/bee/day of protein from different pollen sources. Similarly, results from Loper and Berdel (1980) presented in Figure 3 suggest that at least 0.8 mg/bee/day of mixed pollen protein is needed for maximum brood rearing capacity of caged bees.

Information on the changes in protein content of individual bees during their life and on the endogenous protein losses at each life stage can be used to estimate the total requirements for an 'ideal' protein that has the perfect amino acid pattern and is completely absorbed and available for metabolism. The calculations for protein gain in Table 9 are based on the information from de Groot (1953) presented in Table 3. Brood cells are assumed to be capped when the larvae are 6 days old and the pupae emerge at day 21. The loss of weight and protein during the pupal stage is ignored in the calculation as it is accounted for in the larvae at the time of larval capping. However, no account was made for any protein lost in material shed during the four larval moults. The nurse bee stage was assumed to end 10 days after emergence and the adult bees to die when 40 days old. The loss of weight and protein during the foraging stage is not taken into account directly as it is assumed to decrease the endogenous protein losses of the foraging bee. The endogenous protein losses for nurse and foraging bees are given above at 40 and 21.5 μg protein/day/bee, respectively. Endogenous losses for the larvae are assumed to be negligible because of the lack of a rectal opening.

The calculations suggest that the honeybee gains a total of 18.3 mg protein over its lifetime and when endogenous losses are taken into account this figure rises to 19.35 mg or an average of 0.485 mg/adult bee/day over a lifespan of 40 days. The calculations suggest that the total requirement for an ideal protein for nurse bees is approximately 0.5 (0.444+0.040) mg/bee/day. This value compares with around 1.2 mg/bee/day suggested from Figure 2 for pollen protein requirements for maximum hypopharyngeal gland development. The values are relatively similar if it is assumed the digestibility of pollen protein and its 'Biological Value' (amino acid pattern match) are each 70%. (0.5/0.7/0.7 = 1.02). Assuming that pollen contains 25% protein, the pollen intake for an adult nurse bee would be approximately 4 mg/bee/day. This value compares with measurements of pollen intake by colony bees of 3.4 and 4.3 mg/adult bee/day made by Crailsheim *et al.* (1992).

The figures presented in Table 9 can be used to calculate the total protein requirements of the colony if the number of bees of different ages is known. Crailsheim *et al*. (1992) estimated that the total yearly protein requirement of a colony was 2.8 kg for one colony and 3.7 kg for another colony. Crailsheim *et al*. (1992) translated these estimates into yearly requirements of 13.4 kg and 17.8 kg pollen, respectively, per colony per year. The estimates by Crailsheim *et al*. (1992) of total pollen requirements for bee colonies are considerably lower than the estimate of 55 kg pollen/colony/year by Kleinschmidt and Kondos (1976). Cralisheim *et al*. (1992) present estimates from other authors ranging from 6 to 55 kg pollen/colony/year.

The information in Table 9 could be used also to estimate the requirements for individual amino acids for each stage of the growth cycle if the amino acid composition of larvae immediately prior to capping, nurse bees at maximum hypopharyngeal activity and foraging bees was known. However, there are few measurements of the amino acid composition of honeybees at different stages of development. Spataru (1969) has determined the content of 12 amino acids in the body of adult bees

and these values could be used to calculate amino acid requirements. Due to the lack of information on the amino acid composition of honeybees and the fact that essential amino acids in estimates made by de Groot (1953) add only to 28% of total protein, the amino acid composition of royal jelly is assumed when calculating the amino acid composition required for a protein substitute later in this report.

Table 9. An estimation of the daily requirement for ideal protein by the honeybee

Stage	Weight (g/DM)	Protein content		Protein gain		Endogenous Loss (mg/day)
		(%)	(mg/bee)	(mg)	(mg/day)	
Egg	0.05	-				
Capped Larvae	33	42	13.86	13.86	1.54[a]	
Emerged bee	16	74	11.84	-	-	
Nurse bee	22	74	16.28	4.44	0.444[b]	0.040
Foraging bee	21	71	14.91	-	-	0.0215
Total				18.3		

[a] Larvae capped at day 9 and pupae are not fed
[b] Nurse bees cease brood feeding when they are 10 days of age

Protein and amino acid content of pollen compared with estimated requirements

Several authors have measured the protein and amino acid composition of pollens from specific floral sources to assess their suitability for honeybees, including extensive studies of Australian plants by Somerville (2001) and Manning (2001a). The protein content of pollens examined by Somerville (2001) ranged from 11 to 35%. The pollens were classified as having poor quality when the protein content was less than 20% based on the conclusions by Kleinschmidt and Kondos (1976). Pollens with protein contents from 20 to 25% were classified as average quality. The results presented in Figures 1 and 3 suggest that there is little direct relationship between the protein content of pollen sources and either longevity or brood rearing capacity of bees. These functions were more dependent on total protein intake than on the content of protein in pollen, primarily because of differences in consumption of pollens. However, maximum brood rearing did not occur with pollens containing less than 25% protein (Figure 3).

Somerville (2001) found that the isoleucine content of 74% (42 of 57 samples) of the eucalypt and related species examined was below the levels recommended by De Groot (1953), whereas the proportions of all other essential amino acids exceeded the recommendations. Manning (2001a) found that the proportion of histidine as well as isoleucine in pollen from Western Australian Jarrah (*Eucalyptus marginata*) was below the recommendations made by De Groot (1953). Somerville (2001) suggests that the lack of isoleucine could pose a problem when honeybees are relying on pollens from eucalypt species.

The research by De Groot (1953) established the proportions of each essential amino acid required for maximum growth by the young adult honeybee relative to total amino acids (protein) in diet. In other words, he established only the 'ideal' pattern of amino acids in protein for young bees. Pollen with a ratio of an essential amino acid relative to total protein that is below the value recommended by DeGroot (1953) does not mean necessarily that the supply of the amino acid from this pollen will limit performance when consumed by honeybees. Provided the imbalance is not too excessive, it simply means that more of the protein must be consumed to meet the total amino acid requirement of the bee. Thus, the protein content of the pollen and amount consumed are also important for determining whether an amino acid will be deficient at any specific time.

The following must be established in order to determine whether a particular sample of pollen will satisfy the amino acid requirements of honeybees in different stages of life.

- The amount of each essential amino acid and total protein needed for metabolism at the tissues for the particular physiological state. The individual life stages can be averaged to determine the requirements over the lifetime of one bee or for a colony.
- The amount of each essential amino acid and total protein in the pollen.
- The digestibility and absorption for metabolism of each amino acid and total protein in the pollen.
- The total intake of the pollen.

There is extensive information on the amino acid contents of pollens from Australian plants. However, there appears to be little information on the digestibility of protein from pollens and none on the digestibility of individual amino acids by honeybees. It can be assumed that the digestibility of protein in pollens varies widely because there is a wide range in the digestibility of dry matter in pollen as discussed above (Klungness and Peng, 1984a). There appears also to be little information except for the work of Doull (1966) on the attractiveness and daily intake of Australian pollens.

Fats, fatty acids and sterols

Dietary lipids have a range of functions in honeybees. Lipid substances are oxidised in honeybees as a source of energy, used for the synthesis of cell membrane phospholipids and for fat storage depots particularly preceding non-feeding periods associated with pupation, over-wintering and in maturing queens for egg laying (Dadd, 1973). Robinson and Nation (1970) found that lipids accounted for 6.6% of the fresh weight of 6 day old worker larvae and 2.6% of the fresh weight of emerging worker bees. The most prominent fatty acids present in the lipid component of both larvae and adult bees were found to be oleic (40% and 62%, respectively for larvae and adults), palmitic (42% and 18%) and stearic (10% and 14%).

There is evidence that some lipid compounds are an essential component of the diet of honeybees. Herbert *et al*. (1980a) found that honeybees fed a substitute diet containing at least 2% lipid extracted from pollen produced significantly more brood than those without the lipid extract. However, some of this observed response may have been associated with an increased intake of pollen containing lipid. Weaver (1964) showed that brood rearing was not depressed in one colony when bees were fed whole pollen from which the lipid had been extracted, whereas brood rearing was depressed in other colonies fed the lipid extracted material.

Although saturated and mono-unsaturated fatty acids can be synthesised by insects from simple precursors derived from food, they cannot synthesise several polyunsaturated fatty acids essential for phospholipid components of cell membranes (Dadd, 1973). Both linoleic and linolenic acids have been shown to be essential dietary ingredients for insects including the honeybee (Dadd, 1973). Furthermore, honeybees have an obligatory requirement for dietary sterols for the production of the moulting hormone ecdysone. Herbert *et al*. (1980b) found that the sterol requirements of the honeybee could be satisfied from either cholesterol or the plant sterol 24-methylene cholesterol. However, bees consuming diets with other plant sterols, stigmasterol, sitosterol and campesterol, produced significantly less brood than those with either cholesterol or 24-methylene cholesterol. Svoboda *et al*. (1981) found no evidence of these other plant sterols being converted to 24-methylene cholesterol within the bee. A concentration of 0.1% sterols used in the synthetic diets by Herbert *et al*. (1980b) appears to be sufficient to meet the requirements of honeybees for brood rearing.

No estimates of the requirements of honeybees for linoleic or linolenic acid could be found. Less than 2% of the fatty acids in larvae, pupae and adult bees have been found to be linoleic acid (Robinson and Nation, 1970). However, the concentration of linolenic acid was shown to exceed 7% of the lipid in adult worker bees or approximately 1% of dry weight. Assuming that the dry weight of the nurse bee

at maximum size is 22 mg (Table 3), the total amount of linolenic acid accumulated in the bee is about 0.22 mg. Assuming growth of the nurse bee from emergence to maximum size covers 10 days, the minimum requirement for linolenic acid would be around 0.022 mg/bee/day. Pollen intake of nurse bees has been measured at approximately 4 mg/bee/day (Crailsheim *et al*., 1992). Thus, pollen would need to contain approximately 5.5 mg of linolenic acid/g to provide the amount required. A similar calculation indicates that the requirement for linoleic acid is 1.6 mg/g pollen. However, Robinson and Nation (1970) found that 19% of the fatty acids in adult bee excreta were in the form of linoleic acid and 13% as linolenic acid. Thus, the amount of these fatty acids to be provided in the diet would exceed the estimated requirements of the tissues because of a relatively low digestibility of these fatty acids. An estimate of the requirements for linolenic and lenoleic acid relative to protein and in a pollen substitute was revised after completion of the initial review and is given in Table 13.

Manning (2001a) measured an average of only 0.48 mg/g linolenic acid in the pollen from eucalyptus and related species in Western Australia, which is approximately 10-fold less than the calculated tissue requirements. However, the value measured for linoleic acid in the pollen from Western Australia was 4.5 mg/g pollen and well in excess of the calculated requirement. Such calculations do not prove that pollen from these eucalyptus species is limiting growth and productivity of honeybees because of a lack of linolenic acid. It is possible also that the fatty acids may have been stored in the body in excess of requirement in the bees analysed by Robinson and Nation (1970). However, evaluation of the concept could be undertaken by adding linolenic acid to pollen from these Western Australian plants.

Several of the fatty acids found in pollen and honeybee excreta have been shown to have substantial antimicrobial activity. Feldlaufer *et al*. (1993) showed that capric, lauric, myristic, linoleic and linolenic acids had antimocrobial properties against American foulbrood (*Paenibacillus larvae*). Other fatty acids including palmitic, stearic and oleic did not show antimicrobial properties. Hornitzky (2003) examined the effectiveness of an extremely wide range of fatty acids on their antimicrobial properties for both American foulbrood and European foulbrood (*Melissococcus pluton*). Fifteen fatty acids were inhibitory to the growth of the American foulbrood organism and eight inhibitory for European foulbrood. High concentrations of both linoleic and linolenic acids in pollens collected by honeybees and in their excreta appear to provide strong protection for the colony against bacterial (Manning, 2001b) and fungal (Pandey *et al*., 1983) infections.

Minerals

Although bees like other insects and animals have requirements for minerals which are essential for numerous biochemical reactions within the body, the actual dietary requirements for minerals by bees have not been well established (Herbert, 1997). Nation and Robinson (1968) showed that the brood rearing capacity of bees was improved when pollen ash was added to an artificial diet. Following further studies of the mineral contents of pollen, royal jelly and worker bees, Nation and Robinson (1971) suggested that artificial diets should contain 3% ash on a dry matter basis with 5000 mg/kg of potassium, 200 mg/kg sodium, and 1000 mg/kg each of magnesium and calcium. However, subsequent studies by Herbert and Shimanuki (1978b) in which pollen ash was included in an artificial diet at concentrations from 0.5 to 8% suggested that the optimum ash concentration for brood rearing by bees was only 1% of dry matter. The recommended mineral concentrations suggested by Herbert and Shimanuki (1978b) were substantially lower than those made by Nation and Robinson (1971) being 1000 mg/kg for potassium, 500 mg/kg for calcium, 300 mg/kg for magnesium and less than 50 mg/kg for sodium, zinc, manganese, iron and copper.

Mineral requirements of bees cannot be established satisfactorily from an analysis of the mineral content of bee collected pollen because of the large variability between pollen samples as described above. The recommended mineral requirements made by earlier workers could have been influenced by the particular pollen samples used to produce ash for the experiments. There is strong evidence that the mineral concentrations in some pollen sources are so high that they have a negative effect on

brood rearing and productivity as shown by Herbert and Shimanuki (1978b) in artificial diets and by Hagler (1990) for onion cultivars with high concentrations of potassium.

Attempts to use commercial salt mixtures designed for vertebrates, such as Wesson's salt mixture, have proved to be unsatisfactory for honeybees and have resulted in depressed brood rearing (Nation and Robinson, 1968; Herbert and Shimanuki, 1977). Salt mixtures designed for vertebrates have been found to contain insufficient potassium and markedly excess calcium and sodium for insects including honeybees (Herbert and Shimanuki, 1978b). The lack in insects of either a bony skeleton or need for an active sodium pump to maintain body temperature provides good reasons why the calcium and sodium requirements of bees should be substantially less than for mammals and birds.

Traditionally, nutrient requirements of animals have been determined by feeding increasing amounts of a nutrient in otherwise sufficient diets and measuring the response in a selected variable such as growth rate, reproductive performance or longevity. Such studies are arduous and particularly difficult for determining the requirements for trace minerals because of the ease of contamination from the environment.

An alternative method for estimating the requirements of minerals available for metabolism by the honeybee is to determine the amount of each mineral deposited in the body during growth and to add inevitable endogenous losses. The method has been used to estimate the mineral requirements of domesticated mammals and birds and has been shown to be accurate for those minerals not stored in body tissues when absorbed in amounts in excess of metabolic requirements such as copper in the liver of sheep. The method involves estimating the change in weight of a bee over its lifespan and the mineral composition of the bee at different stages of growth. There are two important assumptions. First, that the bees used to estimate mineral content are not being fed mineral deficient diets and do not store minerals in excess of requirements. Secondly, the secretions in the form of royal or worker jelly needed to feed the larvae are not specified explicitly but are assumed to contribute to the growth of individual bees when requirements are expressed on a per bee basis for the whole colony.

The mean weights of honeybees at different stages in the lifecycle were given in Table 3. Mean values for the mineral composition of honeybees at emergence and as nurse/worker bees, derived from Nation and Robinson (1971) and Manning (2002), are given in Table 10.

Table 10. Mean mineral composition (mg/kg dry matter) of newly emerged bees and nurse/worker bees

Mineral	Newly emerged[1]	Nurse/worker[2]	Worker[3]
Phosphorus	9000	8333	-
Potassium	15000	9333	6675
Sodium	1000	1000	987
Calcium	500	666	390
Magnesium	1000	1000	1010
Sulphur	5500	6000	-
Boron	5	3.8	-
Copper	21	25	22
Iron	69	191	114
Manganese	2.65	88	186
Zinc	75	119	108

[1] Calculated from Manning (2002) assuming that newly-emerged bees have a dry matter content of 0.2 (Table 3).
[2] Calculated from Manning (2002) assuming that nurse/worker bees have a dry matter content of 0.3 (Table 3).
[3] From Nation and Robinson (1971) the mean of spring and autumn measurements.

The concentrations of phosphorus, sodium, magnesium, copper and zinc across bee types and references are similar, whereas the concentration of potassium was substantially greater in the observations by Manning (2002) than by Nation and Robinson (1971). Calcium concentrations measured by Manning (2002) were also about 30% higher than those measured by Nation and Robinson (1971). The concentrations of iron and magnesium appeared to increase substantially as bees increased in age. Iron is known to accumulate in granules in the trophocytes of fat bodies in maturing bees. The concentration of iron increases from emergence of the honeybee imago until the bee is approximately 9 days of age under normal dietary conditions (Kuterbach and Walcott, 1986). Although the rate of increase in iron content of the fat bodies is affected by the amount of iron in the diet, maximum iron content appears not to be affected and is reached at the time the worker commences foraging behaviour. The iron rich granules are thought to play a role in the orientation of the bee in relation to the earth's magnetic field (Kuterbach and Walcott, 1986). Manning (2002) has suggested the low manganese concentration, particularly in the emerging bee could reflect a nutritional deficiency at times for honeybees in Western Australia.

The requirement of each mineral for growth to maturity can be calculated by assuming a minimum essential concentration of the mineral in the body of the mature bee and the mature bee weighs 22 mg dry matter. An initial calculation of requirements for available minerals is presented in Table 11. The estimates of the dietary requirements are likely to differ from the values presented because of small endogenous losses of minerals and because most mineral sources will not be fully available for absorption due to complexes formed with phytates and other components of pollen. The estimates for mineral requirements were revised following the Workshop and other considerations and presented in Table 13 later in the report. Table 11 provides an estimate of the requirements of a smaller number of trace elements than are known to have essential physiological roles and be required in the diets of mammals. The additional elements which are essential for animals, but for which no estimates of their contents in honeybees could be found include chlorine, iodine, bromide, fluorine, selenium, chromium, cobalt, arsenic, molybdenum, nickel, silicon, tin and vanadium (Underwood, 1997). Determination of the concentration of these elements in the body of honeybees would provide the information needed to make an estimate of their requirement for bees.

Table 11. Estimated minimum available mineral requirements for the growth of honeybees

Mineral	Assumed mineral content of mature bee (mg/kg)	Available mineral requirement for growth			*E. wandoo* Pollen (mg/kg)[b]	*C. calophylla* Pollen (mg/kg)[b]
		(μg/bee)	(mg/g available protein)	(mg/kg pollen)[a]		
Phosphorus	8500	187	11.5	2872	3200	4200
Potassium	8000	176	10.8	2703	4200	5400
Sodium	1000	22	1.35	338	300	100
Calcium	500	11	0.68	169	900	600
Magnesium	1000	22	1.35	338	500	900
Sulphur	6000	132	8.1	2027	2300	3000
Boron	5	0.11	0.007	2	10	19
Copper	23	0.51	0.031	8	15	22
Iron	150	3.3	0.203	51	181	124
Manganese	150	3.3	0.203	51	26	35
Zinc	100	2.2	0.135	34	52	79

[a] Protein content of pollen assumed to be 25%
[b] Mineral content of pollen from Manning 2001a

The mineral contents of wandoo (*Eucalyptus wandoo*, winter sample) and redgum (*Corymbia calophylla*) as determined by Manning (2001a) are included in Table 11 for comparison with estimated requirements. The comparison suggests that the sodium content of redgum may be below requirement and manganese may be insufficient in both pollens. However, sodium is known to accumulate in the tissues of mammals and this may have also been the case for the bees used for the calculation of requirements.

Vitamins

There is clear evidence that honeybees have a requirement for a range of vitamins. Nation and Robinson (1968) and Haydak (1970) showed that honeybees offered artificial diets without vitamins failed to rear brood. The addition of inositol or gibberellic acid (Nation and Robinson, 1968), niacin and riboflavin (Haydak, 1949), pyridoxine (Anderson and Dietz, 1976; Haydak and Dietz, 1972), vitamin A and vitamin K (Herbert and Shimanuki, 1978c) to the diets of honeybees have been shown to increase brood rearing success. Although ascorbic acid appears to be synthesised by bees, brood rearing capacity has been increased by the addition of more than 400 mg/kg to an artificial diet (Herbert *et al*., 1985). Herbert and Shimanuki, 1978c) did not demonstrate a requirement for either vitamin D or E for brood rearing by honeybees fed an artificial diet.

Vitamins are also essential for development of the hypopharyngeal glands of bees with requirements being established for pantothenic acid, thiamine and riboflavin (Pain, 1956 cited by Herbert, 1997; Herbert and Shimanuki, 1978d). The pantothenic acid, niacin and pyridoxine content of larval food produced by nurse bees falls markedly as nurse bees increase in age from 11-18 to 34-54 days of age (Haydak, 1961b; Standifer and Mills, 1977). However, there was no change in the concentration of ascorbic acid in larval food as the nurse bees aged. There is no evidence that longevity of bees is affected by the vitamin content of diets consumed (Herbert, 1997).

Although it has been demonstrated that honeybees have requirements for some vitamins there have been few attempts to quantify these requirements. Anderson and Dietz (1976) estimated that bees require 5.4 μg of pyridoxine to rear one larva to the sealed stage. Herbert *et al*. (1985) showed that

the greatest brood rearing occurred when artificial diets were supplemented with 2000 mg/kg ascorbic acid. However, the authors suggest that the effects on brood rearing may have been due, at least partially, to the high antioxidant capacity of ascorbic acid.

Table 12 provides the vitamin mixtures used in artificial diets fed to bees by Haydak (1957), Weaver (1974) and by Herbert and Shimanuki (1977) with the latter updated using the results from the ascorbic acid (Herbert *et al*. (1985) and fat soluble vitamins (Herbert and Shimanuki, 1978c). Results from Weaver (1974) suggest that the growth of larvae improved when vitamin content of the diet was increased three-fold. Similarly, soybean flour supplemented with the Haydak (1957) vitamin mix improved brood rearing but not development of the hypopharyngeal glands of caged bees (Hagedorn and Moeller, 1968). There is little consistency between the two sources in recommended inclusion rates for vitamins in artificial diets for honeybees except for riboflavin. Evidence from mammals and birds suggests that water-soluble vitamins are seldom toxic when given in excess of requirements because they are either metabolised or excreted from the body. It is possible that some of the vitamins are included in the mixtures at concentrations in excess of requirements because there are few experiments where the response of bees to graded inclusions of vitamins have been studied.

The vitamin concentrations in honeybee collected pollen from wandoo and redgum in Western Australia (Manning, 2001a, Table 12) show that the values for all vitamins measured except folic acid and thiamine were well below the amounts included in the artificial diets by Haydak (1957), Weaver (1974) and Herbert and Shimanuki (1977). The vitamin contents of fresh and 4-year old pollen analysed by Hagedorn and Burger (1968) is also shown in Table 12. Hagedorn and Moeller (1968) observed that brood rearing success and development of the hypopharyngeal glands were reduced in honeybees offered soybean flour based diets containing older compared with fresh pollen. Hagedorn and Burger (1968) suggest that differences observed may have resulted form the decline in ascorbic acid content of the pollen with storage. Haydak (1949) showed that niacin and riboflavin also limited brood rearing of honeybees offered soybean flour based diets. The best brood rearing performance observed by Haydak (1949) was obtained with the addition of brewer's yeast, which is known to have high concentrations of many water-soluble vitamins. However, without more detailed information on the requirements of individual vitamins determined by measuring the responses to increasing concentrations of vitamins in diets, it is not possible to state categorically whether the vitamin concentrations in some pollen source limit productivity of honeybees.

Table 12. Composition of the vitamin mixtures (mg/kg) used in artificial diets fed to honeybees by Herbert and Shimanuki (1977), updated for ascorbic acid (Herbert *et al*., 1985) and fat soluble vitamins A and K (Herbert and Shimanuli, 1978c), Haydak (1957) and Weaver (1974). The vitamin mixtures are compared with values measured in two eucalypts from Western Australia (Manning, 2001a) and fresh and 4-year old pollen by Hagedorn and Burger (1968)

	Herbert and Shimanuki (1977)	Haydak (1957)	Weaver (1974)		Pollen values		
			As used	3-fold increase	Manning (2001a)	Hagedorn & Burger (1968)	
						Fresh	4-year old
Biotin	2.8	-	4	12	-	-	
Choline chloride	3103.3	-	150	450	-	-	
Folic acid	26	5.2	4	12	20-22	15	7.8
Inositol	1101.1	-	150	450	-	-	
Nicotinic acid	190.2	124	150	450	1-35	116	84
Ca Pantothenate	135.1	32.2	150	450	-	17.3	17.8
Pyridoxine hydrochloride	26.5	11.4	15	45	2-4	8.8	4.6
Riboflavin	42	10.2	15	45	6.1-6.5	13.3	12.4
Thiamine hydrochloride	20	22.5	15	45	10-34	15.6	13.3
Vitamin B_{12}	0.05	-	1	3			
Ascorbic acid	2000	146	150	450	21-207	492	254
Vitamin A	0.4	-	-	-	-	-	-
Vitamin K	0.4	-	-	-	-	-	-
Glutathione[1]			10	30	-	-	
Carnitine[1]			2	6	-	-	
$_pNH_2$ Benzoic acid[1]			2	6	-	-	

[1] Not vitamins, but added to the mixture by Weaver (1974).

Water

Water is an essential component of the diet for honeybees. Caged bees offered water have increased longevity compared with bees deprived of water (Gray, 1997). Special foraging bees exist within colonies for the collection of water (Schmaranzer, 2000). Water is used by nurse bees in the dilution of honey during its processing as larval food, but the amount of water used by the colony for this process depends greatly on the supply of fresh nectar. Water is used also for incorporation into the growing larvae and young bee and also for regulation of hive temperature and humidity as described above.

Although the quantity of water required by an adult bee has not been determined, Gray (1997) estimates that the water requirement of an average colony during spring brood rearing in North America is approximately 150 ml/day, while Herbert (1997) suggests 200 ml/day. However, for strong colonies, especially in hot environments, the water requirement was estimated to be around 1 kg/colony/day (Gray, 1997). In colder climates, honeybees utilise water that condenses on the inside of the hive. Nelson (1983) estimated that up to 5 litres per month may be collected by colonies through this method.

Approximately 800 foraging bees making an average of 50 flights/day could collect 1 kg water daily. The provision of sufficient water is particularly important during the transport of colonies and during the initial period following a new location of a colony as the foraging bees can take some time to locate a water source.

A colony can store water for a few days when foraging flights are prevented. The water is stored within the honey sacs of the water foraging bees, which remain inactive and occupy areas surrounding the brood area. Water from these bees is transferred to other bees for use within the brood area when needed.

Artificial feeding of bees

Nectar substitutes

The energy requirements of honey depleted colonies can be replaced by sealed honey combs, diluted liquid honey or sugar solutions. Herbert (1997) suggests that liquid honey should be diluted to produce a solution containing 50-60% honey. However, the most commonly used and suitable carbohydrate to feed honeybees as an energy source is a 30-50% sucrose solution. Sucrose is highly attractive to honeybees (Doull, 1974), completely digested and metabolised (Crailsheim 1988b) and is relatively cheap to supply. Dry sugar is less attractive than solutions of sugar, because the honeybee must first moisten the dry compounds prior to uptake into the honey crop (Simpson, 1963). Honeybees can also utilise readily solutions containing glucose and fructose (Crailsheim 1988b). High fructose corn syrup containing predominantly fructose and glucose is used commonly in the USA as a carbohydrate supplement for bees (Herbert, 1977).

Pollen substitutes

A large number of substitutes for pollen have been investigated and the effects of a wide range of protein sources and other additives on bee productivity have been tested (see Haydak, 1967; Herbert, 1997). However, despite many decades of research, pollen substitutes remain less effective than most sources of fresh pollen particularly for brood rearing. Low attractiveness of artificial pollen substitutes and consequently low intake is thought to be the major cause for the reduced productivity of bees offered pollen substitutes in place of mixed bee collected pollens. Consequently, pollen substitutes commonly contain significant amounts of fresh pollen and either honey or sucrose to increase attractiveness for bees (Doull, 1974; Somerville, 2000). However, it is clear also from the information presented above on the nutrient requirements of honeybees and their digestive capabilities, that specific amino acids, fatty acids, sterols, vitamins and minerals must be included in a supplement and that some materials are either toxic at specific concentrations or cannot be digested. Physical and chemical properties that influence the attractiveness of materials for bees have also been identified.

Based on this review, the following specifications for a pollen substitute can be proposed. All requirements are specified on a dry matter basis

Protein and amino acids

An adult nurse bee needs to consume at least 1 mg/day of a protein with a suitable amino acid pattern. A protein concentration of approximately 25% in a pollen substitute would satisfy this requirement because the consumption of pollen by nurse bees is approximately 4 mg/day. The same essential amino acids required by mammals must be included in the substitute for bees, but the proportion of sulphur containing amino acids in the protein can be less because of the low keratin synthesis. The amino acid pattern provided by de Groot (1953) for royal jelly is assumed to be suitable for proteins included in pollen substitutes. Free amino acids added to a substitute should be of the l-isomer except for histidine, methionine and phenylalanine, which can be provided also as the d-isomer. Significant amino acid imbalances will reduce intake of the substitute.

Protein sources included in a substitute should have a digestibility of at least 70% as assessed in domestic animals because the digestive effectiveness of proteins in bees appears to be similar to mammals. Care should be taken to ensure that trypsin inhibitors are deactivated and that amino acid availability is not reduced by over heating.

Carbohydrates

The most suitable carbohydrates to be added to a pollen substitute are sucrose, glucose and fructose. Lactose, galactose, stachyose and some other plant sugars are toxic to honeybees when included as significant proportions of the diet. Starches can be utilised by foraging bees, but not younger bees and should not be included at high concentrations in pollen substitutes available to younger bees. Although fibrous compounds such as cellulose, arabinoxylans and glucans are not digested by the honeybee, they are processed successfully by the digestive tract and excreted. However, high concentrations of fibrous compounds in a substitute may reduce the rate of passage of material through the gut and reduce intake. Pectins should be avoided because of possible toxicity.

Lipids and sterols

The honeybee has an obligatory requirement for linoleic and linolenic acids and for either cholesterol or 24-methylene cholesterol. The minimum requirements for growth of linoleic and linolenic acids were estimated to be 1.6 and 5.5 mg/g of pollen substitute. However, because significant quantities of these fatty acids are found in excreta and act as antimicrobial agents, the concentrations included in a pollen substitute should be increased by 50% to 2.4 and 8.25 mg/kg, respectivley. A concentration of 0.1% cholesterol or 24-methylene cholesterol should be sufficient to meet the honeybee needs for production of essential hormones. Concentrations of these sterols above 1% of the diet have been shown to depress brood rearing. The total concentration of lipid within a pollen substitute should be between 5 and 8%.

Minerals

Mineral mixtures developed for domestic mammals and birds are unsatisfactory for honeybees because of high concentrations of calcium and sodium and low concentrations of potassium. There is strong evidence that excess minerals can be toxic to honeybees. A suggested mineral composition for honeybees was shown in column 5 of Table 11, but following the completion of the initial review the estimation of mineral requirements was modified as shown in Table 13.

Vitamins

The following vitamins have been shown to be essential for honeybees: biotin, choline, folic acid, inositol, niacin, pantothenic acid, pyridoxine, riboflavin, thiamine, vitamin B_{12}, vitamin A and vitamin K. Although ascorbic acid can be synthesised by bees, a response in bee performance has been observed when added to supplements. Quantitative estimates of the requirements of bees for vitamins have not been established. However, except for the fat soluble vitamins A and K, others are easily excreted when fed in excess. The suggested vitamin concentrations for a pollen substitute are given in column 5 of Table 12 for the water soluble vitamins and in column 2 for vitamins A and K.

Attractiveness

An extremely essential criterion for a pollen substitute is that it is attractive to bees and consumed in sufficient quantities to meet daily nutrient requirements. Factors that will improve attractiveness include a limit to maximum size of ingredient particles to 0.5 mm diameter, colour in the yellow range, addition of specific lipid fractions that may include linoleic and linolenic acids, octadeca-*trans*-2,*cis*-9,*cis*-12-trienoic acid and 24-methylene cholesterol. Pollen supplements containing unsaturated fatty acids should be either mixed frequently or stored at cold temperatures to prevent the fatty acids from being oxidised and becoming rancid. There should be no significant nutrient imbalances and the substitute should be moistened, preferably with either honey or sucrose in the ratio of 70:30 sugar to water.

Ingredients for pollen substitutes

Several animal and plant protein sources have been used as substitutes for pollen protein (Haydak, 1967) with soybean flour, dried brewers yeast and dried skim milk being the moderately effective. However, the soybean product must have been heated for a period to remove the adverse effects of trypsin inhibitors on protein digestion (Erickson and Herbert, 1980). Somerville (2000) has outlined potential ingredients available within Australia for pollen substitutes. However, there are many reports in the literature described above where pollen substitutes have been found to be less effective than the best pollen samples because of deficiencies in essential fatty acids, sterols, vitamins and minerals and low attractiveness. The requirements for pollen supplements outlined above and revised in Table 13 should assist the selection of ingredients that better meet the needs of honeybees.

RIRDC honeybee nutrition projects

The major issues facing honeybee producers in Australia are the 'skinny bee' syndrome, poor colony vitality prior to a new honey flow and exacerbation of hive diseases during periods of poor nutrition. A great deal of the earlier research in Australia, particularly by Kleinschmidt and other State Department research workers, defined these problems. The importance for these production issues of low production and poor nutritional value of pollens from some eucalyptus and related species was identified from these earlier studies.

A significant part of RIRDC funded research related to these production issues has been directed towards describing the nutrient composition of pollen from floral species found in Australia. Other projects such as CSE-85A and DAV-157A have investigated the effects of nutritional supplements on the reproductive capacity of queen bees and severity of hive diseases. The likely contribution of excess iron in nectar and pollen as a cause for 'disappearing disorder' in colonies in south-central Queensland has also been determined. Project DAN-193A has investigated the antimicrobial effect of a range of isolated fatty acids on hive diseases of bacterial origin.

An extremely comprehensive database has been established on the protein, amino acid, fatty acid, mineral and vitamin content of a wide range of pollen samples from Australian plants used for honey or pollen production, particularly in projects DAN-134A, DAW-91A and DAW-100A. This research is as comprehensive as for any other part of the world and is possibly more extensive than other countries for commercially important plants. Information on the fatty acid composition of pollens in Australia is particularly extensive compared with literature reports from other countries.

The composition of pollens from Australian plants has been compared with published estimates of requirements to assess their nutritional value for honeybees. Such an approach depends greatly upon the accuracy of the estimates for requirements. Unfortunately, the estimates for nutrient requirements of honeybees within current literature are not extensive and are often based on experiments that have not been designed specifically for the purpose.

A considerable effort has been made within the RIRDC projects to estimate the adequacy of amino acids within Australian pollens. Although the pattern of amino acids required by young growing honeybees has been established by De Groot (1953), a comparison with the amino acid patterns found in pollens does not indicate satisfactorily whether a particular pollen sample has sufficient amino acids to meet the needs of growing bees. As explained in the review, an amino acid deficiency for a growing bee depends on the pattern of essential amino acids as well as the protein content, daily intake of pollen and digestibility of the amino acid. If bees consume sufficient of an essential amino acid to meet the daily requirement, productivity will not be reduced despite the amino acid having a relative concentration less than determined as the 'ideal' pattern by de Groot. There have been few measurements of pollen intake or the digestibility of amino acids by bees offered pollens from Australian plants. The current research has provided an excellent base for selecting pollen samples to test whether they have an amino acid deficiency by undertaking studies on intake and amino acid digestibility.

Similarly, the importance in terms of bee productivity of fatty acids concentrations measured in Australian pollens (DAW-91A, DAW-100A) is difficult to ascertain because of a lack of estimates for requirements of essential fatty acids for growth and antimicrobial properties. The same argument applies to measurements of the mineral and vitamin contents of pollens. However, the concentrations of minerals causing toxicity are also important to determine. Knowledge of the intake of pollens by bees is of major importance for determining the nutritional adequacy of Australian pollens.

Project ANU-57A has taken an alternative approach for determining the nutritional value of pollens. It is proposed that NIR calibrations are developed to predict first the nutrient composition of pollens and secondly the productive capacity (growth, hypopharyngeal gland weight and intake) of pollens. It

is technically feasible to develop NIR calibrations for predicting the nutrient composition of pollens, although several hundred samples may need to be included in the calibration for it to be robust for a range of important nutrients. Similarly, it is technically feasible to develop NIR calibrations for predicting the productivity of individual pollen samples. However, at least 80 samples would need to be examined experimentally with measurement of productivity criteria. A considerable research effort would be required to achieve this outcome, which is unlikely to occur by the proposed finish of the project in December 2004.

As explained above, predicting the nutrient composition of a pollen sample is not necessarily of great value unless the intake and digestibility of the pollen can also be predicted. However, the need to know composition, intake and digestibility is negated if NIR scans of pollen samples are related to a direct measure of bee productivity. Thus, there is value in the approach proposed for project ANU-57A if it is successful.

Project DAW-105A is also examining a productivity measure (longevity) in pollens varying widely in lipid content. Longevity is not always associated closely with other measures of productivity including growth rate, brood rearing or hypopharyngeal gland development and results will need to be interpreted carefully. However, this experiment appears to be the first to investigate the effects of pollens selected for a variation in lipid content on bee productivity. Proving that lipid content of pollen affects longevity may be difficult because other nutrients within the pollen samples will vary. However, this project is examining also the effect of free fatty acids added to artificial diets on longevity. Currently the effects of oleic and linoleic acids are being investigated. Although oleic acid is the dominant fatty acid in the body of bees, there is good evidence that insects can synthesis monounsaturated fatty acids from common metabolites. An investigation of the responses to linolenic acid may be of greater value. The supplement experiments provide an opportunity for better defining the fatty acid requirements for honeybees.

Project DAN-214A is examining the practicality and effect on productivity of providing sugar and artificial pollen supplements for building colony numbers prior to working new nectar flows. Early experiments have been of limited success, partly because of disease occurrences. Clear definition of the characteristics of colonies that may respond to supplementary feeding, the composition of pollen supplements and the amounts of pollen supplement and sucrose to feed remain issues to be resolved.

Early identification of colonies in the first stages of decline is of great importance for alleviating the ‘skinny bee’ syndrome. Kleinschmidt recommended that the protein content of bees expressed as a percentage of weight could be used as an indicator of the nutritional status of a colony. However, it is clear from the review that there is little relationship between nutritional status and protein percentage of honeybees. Dry weight of newly emerged bees was suggested as the best current measure for assessing the nutritional status of a colony. Manning (2002) in a projected funded by the Western Australian Department of Agriculture showed that the protein and lipid contents of bees expressed as a percentage of fresh weight declined in colonies fed pollen supplements but tended to increase in unfed colonies. These results are difficult to interpret, but may suggest that nutrient and ingredient specification for pollen supplements and knowledge of when to feed supplements is limited.

Project DAN-186A is to provide an extension publication on bee nutrition. It is to include findings from RIRDC projects. This is an important publication and may be able to draw on some of the information presented in the review. However, most of the review is at a scientific rather than practical level.

Initially suggested research priorities

Two major research outcomes were recommended initially to address the main nutritional issues for the Australian honeybee industry. However it was recognised that further discussion with apiarists and scientists should be undertaken to refine the recommended integrated research program.

1. ***To determine when to start feeding a colony by developing an accurate and practical method for predicting when pollen supply to a colony working high nectar flows is about to become limiting and will affect colony vitality precipitating the 'skinny bee' syndrome.***

   The proposed research strategy would require measurements on a significant number of colonies that move from a status of high vitality to a status of low vitality consistent with severe 'skinny bee' syndrome.

   Changes in the vitality of the colony could be assessed by measuring characteristics such as:
   - Brood area
   - Dry weight of newly emerged bees
   - Hypopharyngeal gland weight or size
   - Colony bee numbers
   - Others to be determined

   The primary aim of the research program would be to identify characteristics of individual bees, of the colony or of the pollen supply that change prior to a marked reduction in colony vitality. Such characteristics may include:
   - Colony pollen reserves
   - Pollen load of returning foraging bees
   - Number or proportion of foraging bees collecting pollen
   - Begging behaviour of nurse bees towards returning foraging bees
   - The nutritional quality of collected pollen (as estimated from NIR or other procedures in relation to its productive ability)
   - The intake potential of collected pollen (as estimated from NIR or other procedures)
   - The dry weight or body composition of foraging bees, either before or after foraging flights

   Careful consideration is required of the number and most appropriate measurements that should be made. The research program would be directed towards identifying the most suitable correlates with an objective measure of colony vitality. The principal aim would then be to develop a rapid technique for measuring these characteristics in the field. The most common rapid measurement techniques being used or being developed for use in agriculture are NIR, ELISA, e-noze and image analysis technologies. Many of these technologies are now being developed as portable instruments for field use. A decision would be required as to which of these techniques would be appropriate for the bee industry.

   All pollen samples collected by the bees in the experiment, individual foraging bees, nurse bees, etc. that are measured would also be scanned by the selected instruments to allow subsequent development or application of predictive calibrations.

   Concurrent with these observations, other projects would be undertaken to develop rapid predictive calibrations for characteristics such as the following:

   - Relative intake of Australian pollens by honeybees
   - The productive value of Australian pollens (growth promotion, hypopharyngeal gland development, etc)

- Chemical composition and nutrient contents of Australian pollens
- Digestibility of Australian pollens by honeybees
- Dry weight and composition of foraging honeybees

The above provides an outline of the type of research that could be undertaken to meet the desired outcome. However, detailed consideration needs to be given to the most appropriate measurements and rapid analysis technologies to be developed and applied.

Several of the current RIRDC projects including ANU-57A, DAW-100A, DAW-105A and DAN-214A cover aspects of the approach outlined. Three of these projects are due to be completed by the end of 2004 and should continue, but the proposed research should be reassessed in relation to achieving the longer-term goal.

2. ***Specifications for a pollen substitute based on readily available ingredients, that is highly attractive and meets the nutrient requirements of honeybee colonies***

   Current pollen substitutes are not as effective as desired. The following strategy is proposed to improve the effectiveness of pollen supplements for use in Australia.

   - Review and modify the recommended nutrient requirements of honeybees as outlined in this report and develop nutrient and physical specifications for an effective pollen substitute.

   - Identify economically available ingredients for inclusion in pollen substitutes to meet the specifications and that are attractive to honeybees.

   - Test the pollen substitutes for attractiveness and intake by honeybees.

   - Evaluate the practicality and economics of application of selected pollen substitutes in colonies predicted to be at risk of 'skinny bee' syndrome and for maintaining the vitality and productivity of these colonies.

3. ***Recommendation:*** A workshop of 6-10 people to be held to revise the two proposals above and develop an integrated research program within current funding capacity to deliver the described outcome for the industry.

Recommended procedures for adoption

There is a great deal of knowledge from both the literature and held by beekeepers that could be applied more effectively to improve the efficiency and profitability of the Australian honeybee industry. Quality control and continuous improvement systems are now being applied to many areas of agriculture including the pig and beef industries. The principles are taken from the Hazard Analysis Critical Control Point (HACCP) system used widely in manufacturing to ensure that products always meet specifications and in the health industry to minimise the risk of errors.

The principles behind the HACCP system are first to identify those processes that are essential to the success of the business. The method used for determining whether a process actually is essential, is to identify the hazards (things that will go wrong and the consequences of them going wrong) if the process is not carried out correctly. Only those processes with significant hazards should be developed further

The next step is to identify the Critical Control Point for each process. The Critical Control Point is the last point in a process where the hazard can be averted and product quality, level of productivity or sustainability, including financial, of the system maintained. However, to know whether a Critical Control Point has been reached, specific components within a system must be measured. These measurements must be taken at predetermined times and the measured value must fall within predetermined critical limits. These measurement limits are set either from recommended best practice or from the best scientific knowledge currently available. If the measured values fall outside these critical limits, there is a significant risk of failure to the system and the identified hazards will occur.

The principles behind the HACCP system are to control the risk of not optimising productivity by implementing previously identified corrective actions that must be carried out immediately a measurement limit is breached. By using this quality control system, the management of risk is made simple because the corrective actions have been identified before the adverse event occurs and can be readily carried out with the knowledge that the hazard has been controlled.

Several important aids and processes are required to ensure the HACCP system can be applied readily to an enterprise. The first are tools. These tools have three functions:

- A device or process that is necessary to make a specific measurement.
- A device, process or software used to interpret the measured value and determine whether it is outside the critical limits.
- A device, process or software used to identify which of the corrective actions is the most appropriate in the circumstances and will maintain productivity and maximise profitability.

The second essential process needed for the HACCP system to be properly applied and risks controlled, is a method to ensure that the identified measurements have actually been made and recorded. Typically, within manufacturing systems, the measurements are made by one person, and a second person will check that the measurements were recorded and were within the critical limits. If the measured values were not within the critical limits, the second person checks that the right corrective action was carried out. Failure to make measurements at the predetermined times and failure of checking by another person, are the most common reason for breakdown in quality control systems that lead to hazards actually occurring. A great deal of continuing discipline is required by all people working in an enterprise to ensure that the measurements, records and checks are carried out.

There is a large potential benefit to the honeybee industry from rigorous application of existing knowledge. The HACCP system provides a framework to apply such information.

- A device or process that is necessary to make a specific measurement.

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There is a large potential benefit to the honeybee industry from rigorous application of existing knowledge. The HACCP system provides a framework to apply such information.

Recommendation: A small group of 8-10 producers, scientists and RIRDC personnel hold a 2-day Workshop to examine the practicality of introducing a HACCP approach to the management of honeybees.

Final recommended integrated honeybee nutrition research program

Workshop with apiarists and scientists

A 2-day Workshop was held with industry representatives and scientists in October 2004 to identify the factors that have the greatest impact on productivity and profitability of the Australian honeybee industry and to refine the areas most needing research. The meeting agreed that the primary goals of beekeepers were to maximise profit in terms of $/year and maintain an acceptable lifestyle by reducing the time away from home while allowing sufficient time to enjoy the Australian bush.

The most critical factors affecting profitability were identified as follows:

- Advanced knowledge of where and when floral resources will be available.

  This knowledge is essential for strategic planning within a business and allows identification of the order of honey flows to target in relation to the quantity, quality and price of honey and distance travelled to the floral source.

- Correct number of healthy bees in a hive at the right time relative to targeted honey flows.

  The process involves changing throughout the year the number and condition of bees in a colony to ensure there are sufficient bees in high condition prior to allocation to targeted honey flows, while bee numbers are reduced during long periods of floral dearth. The number of bees in a colony can be regulated through nutrition, reproductive capacity of the queen and minimising diseases. A major problem affecting productivity of bees on eucalyptus honey flows can be a deterioration of reproductive capacity of the colony due to lack of pollen and onset of the 'skinny bee' syndrome. A system that identified the timing, amount and type of nutrients to be supplied to manipulate the healthy bees in a colony to a predetermined number before exploitation of a floral resource would be extremely valuable for the industry.

- Lower costs of production

  Many of the tasks undertaken in beekeeping are labour intensive and involve large travelling distances. Processes that reduce the need for travelling would be valuable to the industry.

- Optimum number of hives

  Profitability is influenced significantly by the number of hives of highly productive bees operated by an enterprise. The number of hives is often dictated by the size of the available trucks.

Priorities for research were identified at the Workshop following extensive discussion about the research areas that would have the greatest impact on profitability. Priority was established by the 10 people present using a scoring system. The area with highest priority was allocated 1 and the area with lowest priority allocated 4 by each person. The research priorities, with mean score and range in scores, were as follows:

a) Develop a practical and economically viable pollen substitute (mean 2.0; range 1-3)

b) Refine recommendations for sugar feeding including timing, feeding systems, sugar concentrations, etc. (mean 2.1; range 1-4)

c) Measurement of potential onset of 'skinny bee' syndrome (mean 2.6; range 1-4)

d) Advanced prediction of timing and location of future floral resources (mean 3.3; range 1-4)

Although the process resulted in a priority for the suggested research areas, it was clear that there were wide divergences in opinion about the most important area for research with each area except the development of a pollen substitute receiving the highest and lowest priority from individuals at the Workshop. Consequently, further consultation was held following the Workshop with scientists and apiarists to refine the recommended integrated research program for RIRDC funding.

Recommended integrated research program

The priority order for the final recommended research program, particularly relating to identifying future floral resources, was influenced by the strong evidence that because of the flat response (payoff) curves in most agricultural systems, the greatest gains in profitability come from quantum changes resulting from the development of new technologies rather than from Research & Development that improves decision making about technologies currently used within an industry (Pannell, 2004). The following final recommendations are made in priority order for a research program on honeybee nutrition for funding by RIRDC.

a) Develop an effective, economically viable pollen substitute and methods for feeding to colonies

b) Refine recommendations for effective feeding of sugar supplements

c) Investigate the feasibility of using existing technologies for forecasting the site and timing of future floral resources

d) Continue development of NIR calibrations for assessing the productive capacity of pollen samples

e) Develop effective methods for identifying early onset of the 'skinny bee' syndrome both in the field and under laboratory conditions with the aim of determining the most appropriate timing for feeding sugar and pollen supplements

The research strategies, likely resource needs and timing are given for each research area.

Development of a pollen substitute

A pollen substitute would be of substantial value to the Australian honeybee industry because of the small amounts and poor quality of pollen produced by many eucalyptus species. Development of an effective pollen substitute would reduce substantially the need to move bees to restore colony vitality when working certain high nectar flows. Although pollen supplements are available to the honeybee industry, there is no completely effective product and all contain significant amounts of bee collected pollen. In addition, the use of mineral mixtures designed for domestic livestock is likely to have been detrimental for honeybees.

An effective pollen substitute must be attractive to foraging bees, contain sufficient essential nutrients to meet the daily mean nutrient requirements of bees in a colony in 3-4 grams consumed by nurse bees per day and must not contain substances toxic to bees. Factors affecting attractiveness of pollens have

been reviewed, but further research is needed to identify the most effective and economically viable method for making a supplement attractive to foraging bees.

Specifications for a pollen substitute

Nutrient requirements

Traditionally, nutrient requirements of animals have been determined by feeding increasing amounts of a nutrient in otherwise sufficient diets and measuring the response in a selected variable such as growth rate, reproductive performance or longevity. Such studies have not been conducted for honeybees except for the work by de Groot (1953) to establish the relative requirements for essential amino acids.

An alternative method for estimating the requirements of nutrients available for metabolism by the honeybee is to determine the amount of each nutrient deposited in the body during growth to maturity and to add inevitable endogenous losses from the body for individual nutrients. The method has been used to estimate the protein, amino acid, fatty acid and mineral requirements for domestic mammals and birds and has been shown to be accurate for those nutrients not stored in body tissues when absorbed in amounts in excess of metabolic requirements such as copper in the liver of sheep.

The method involves estimating the change in weight and nutrient composition of a bee over its lifespan, the addition of nutrient losses during moulting and also from daily endogenous losses mainly from the gut.

There are several important assumptions with the method:

- The bees used to estimate nutrient content have not been fed diets deficient in the nutrients of concern
- Honeybees do not store nutrients in excess of their requirements
- The secretions in the form of royal or worker jelly needed to feed the larvae are not specified explicitly but are assumed to contribute to the growth of individual bees when requirements are expressed on a per bee basis for the whole colony.

Evidence is provided in Table 3 for the increase in weight of the honeybee and for the composition of adult nurse bees. Although honeybees loose weight during their foraging phase of life, this loss is ignored in the calculation of requirements because it is assumed to reduce the endogenous nutrient losses. The mature dry weight of a nurse bee is assumed to be 22 mg. Endogenous losses have been estimated only for nitrogen by de Groot (1953). A daily endogenous loss of 10% of minerals deposited has been assumed, whereas a loss of 50% of requirements has been assumed for the essential fatty acids, linoleic and linolenic acid. No allowance has been made for the nutrients lost during moulting in the calculations described. Although these losses are likely to be small, they should be added to the estimates of requirements if values can be obtained. An analysis of the nutrient content of all material shed from the bee during its growth to emergence could be undertaken to improve the estimates of requirements.

The method described above is inappropriate for vitamins because vitamins are not deposited within the body but are needed continuously for metabolic processes within the bee. Hence, estimates of vitamin requirements of a honeybee diet based on the values of Weaver (1974) for the three-fold increase given in Table 12 have been used in the recommended nutrient requirements of honeybees given below.

The nutrient requirements per bee within a colony are expressed first in relation to the estimated protein requirements and then converted to mg/kg of supplement assuming that the ideal pollen substitute contains 25% protein and a nurse bee can consume 4 mg pollen or pollen substitute per day. Although, de Groot (1953) estimated the essential amino acid proportions in total protein needed by honeybees, the total essential amino acid requirement from his estimates added to only 28% of total protein requirements. These values would appear to be too low based on the requirements of other animals, so the amino acid composition of royal jelly has been used to determine the proportion of total protein requirements that need to be supplied from each essential amino acid.

Table 13. Nutrient requirements as a proportion of protein requirement and as mg/kg of a pollen substitute

Nutrient	Content in adult bee (mg/kg dm)	Nutrient requirement				
		Body growth[1] (µg/bee)	Endogenous losses (µg/bee)	Total (µg/bee)	Total (mg/g protein)	Total (mg/kg pollen substitute)
Protein	740000	18300	1045[2]	19345	1000	250000
Arginine					51	12750
Histidine					22	5500
Lysine					67	16750
Tryptophan					13	3250
Phenylalanine					41	10250
Methionine					19	4750
Threonine					40	10000
Leucine					77	19250
Iso-leucine					53	13250
Valine					67	16750
Arginine					51	12750
Histidine					22	5500
Linolenic acid	10000	220	110[3]	330	17.059	4264
Linoleic acid	2857	63	31[3]	94	4.874	1218
Sterol					4	1000[4]
Phosphorus	8500	187	18.7[5]	205.7	10.633	2658.3
Potassium	8000	176	17.6	193.6	10.008	2501.9
Sodium	1000	22	2.2	24.2	1.251	312.7
Calcium	500	11	1.1	12.1	0.625	156.4
Magnesium	1000	22	2.2	24.2	1.251	312.7
Sulphur	6000	132	13.2	145.2	7.506	1876.5
Boron	5	0.11	0.011	0.121	0.006	1.6
Copper	23	0.506	0.0506	0.5566	0.029	7.2
Iron	150	3.3	0.33	3.63	0.188	46.9
Manganese	150	3.3	0.33	3.63	0.188	46.9
Zinc	100	2.2	0.22	2.42	0.125	31.3
Biotin					3	12[6]
Choline chloride					112.5	450
Folic acid					3	12
Inositol					112.5	450
Nicotinic acid					112.5	450
Ca Pantothenate					112.5	450
Pyridoxine hydrochloride					11.25	45
Riboflavin					11.25	45
Thiamine hydrochloride					11.25	45
Vitamin B12					0.75	3
Ascorbic acid					112.5	450
Vitamin A					0.1	0.4
Vitamin K					0.1	0.4

[1] Weight of adult nurse bee assumed to be 22 mg dry matter (de Groot 1953); losses during moulting not included.

[2] Endogenous loss assumed to be 40 and 21.5 µg protein/day/bee, respectively, for nurse and foraging bees (de Groot 1953) and nurse bee stage assumed to be 10 days from emergence, while foraging bees are assumed to die at 40 days from emergence.

[3] Endogenous losses of essential fatty acids assumed to be 50% of requirement for body growth because of high rates of excretion observed (Robinson and Nation, 1970)

[4] Sterol (cholesterol or 4-methylene cholesterol) requirements of honeybees estimated to be 0.1% of a pollen substitute (Herbert *et al*. 1980b).

[5] Endogenous mineral losses assumed to be 10% of requirements for body growth – assumption has little supporting evidence.

[6] Estimates provided per kg pollen supplement by Weaver 1974.

Specifications for a pollen substitute

The following criteria need to be met when developing a pollen substitute:

- Must be attractive to honeybees:
    - yellow colour (can be a food colouring agent),
    - < 0.5 mm particle size,
    - contain lipids that are attractive to bees. Some attractants have been identified such as linoleic and linolenic acids, octadeca-*trans*-2,*cis*-9,*cis*-12-trienoic acid and 24-methylene cholesterol. However, the attractiveness of essential oil extracts including almond oil need to be investigated as a simple means of making the pollen substitute attractive.
    - sweet flavour from sugar or honey
    - moistened (no more than 90% dry matter)
- Must avoid substances toxic to honeybees including lactose, galactose, stachyose, pectins, protease inhibitors such as pepsin inhibitors and tannins, no more than 2% starch
- 25-30% protein with right essential amino acid balance and at least 70% digestible in mammals
- approximately 5% lipids with sufficient linoleic, linoleinic and cholesterol to meet requirements
- Sufficient minerals and vitamins to meet requirements as set out in Table 13
- 10-20% fibre can be handled by the honeybee digestive system
- An antioxidant may need to be added to preserve the essential fatty acids if the substitute is to be stored for long periods.
- High sugar or honey concentrations should act as antimicrobial agents and prevent fungal and bacteria growth. However, it is possible that antimicrobial agents will need to be added.

A pollen substitute may have the following specifications:

- 25-30% protein meeting essential amino acid requirements as specified in Table 13
- 5 % + lipid meeting essential fatty acid and sterol requirements as specified in Table 13
- 1-1.5% minerals and vitamins meeting requirements as specified in Table 13
- < 2% starch
- 10-20% fibre
- 40-60% sugar and/or honey

Components of a research program

- Determine the nutrients that are lost in material shed in moults during the development of a honeybee to emergence and recalculate nutrient requirements. Although this activity would result in a more accurate estimate of nutrient requirements of honeybees and improve the specifications for a pollen substitute, it is considered to be of less importance to the development of a pollen substitute than the subsequent activities listed. The activity could be removed if funds available are limited.

- Undertake experiments to establish the attractiveness to foraging honeybees of lipids, fatty acids, essential oils and other substances as well as the importance of yellow colours to identify methods for ensuring that pollen substitutes will be attractive and collected by foraging bees.

- Identify ingredients available in Australia that could be incorporated into pollen substitutes to meet nutrient requirements and to be attractive to honeybees.

- Formulate a range of pollen substitutes that meet the specifications outlined above and evaluate their effectiveness by measuring intake and performance of small numbers of caged bees.

Performance indicators could include growth rate, pharyngeal gland development or head weight, longevity and brood rearing capacity.

- Select the most promising pollen substitutes from the cage trials and evaluate their effectiveness in the field as free standing compounds placed some distance from colonies. Measure the intake and performance of colonies with and without pollen substitutes and determine the cost effectiveness of the substitutes.

- Select those substitutes that have the greatest impact on productivity for commercial development.

Time and resources needed

A 3-year project is proposed initially, employing a scientist and one support staff, particularly a PhD student.

Year 1:

i) Quantify the nutrients lost from a colony in material moulted from larvae-pupae and re-estimate nutrient requirements.
ii) Undertake experiments with inert material such as cellulose powder to identify substances that are attractive to honeybees and then establish whether the substances remain attractive when added to ingredients likely to be used in pollen substitutes. An important decision point for the project is identification of additives to a pollen substitute that are highly attractive to honeybees.
iii) Formulate pollen substitutes that meet the specifications set out above and commence testing their productive capacity with caged bees.

Year 2:

i) Continue evaluation of alternative pollen substitutes with caged bees and select the most successful formulations for evaluation under field conditions.
ii) Commence evaluation of several selected formulations under field conditions.
iii) Refine formulations where necessary and retest under cage conditions.

Year 3:

i) Test preferred formulations under a wider range of field conditions and particularly under circumstances that are likely to result in the 'skinny bee' syndrome.
ii) Quantify the benefits and costs of the preferred formulations.
iii) Promote the use of the best formulations by the industry and encourage the development of a commercial product.

Refine recommendations for sugar feeding

Although the feeding of sugar supplements is practiced widely overseas, further information is required under Australian conditions to refine recommended practices for beekeepers. It is particularly important to know the amount of sugar needed by colonies of different sizes to maintain bee numbers during short periods of nectar dearth or to stimulate breeding and expansion of bee numbers following periods of prolonged nectar dearth such as after winter. Sugar needs by honeybees will be influenced by environmental temperatures, while dynamics of the change in colony population size will be affected by the initial size of the colony, the egg laying capacity of the queen and the disease status of the colony. The over-feeding of sugar should be avoided to prevent sucrose being capped as a reserve and decreasing the quality of honey produced.

Research activities

- Calculate the energy requirements for actively reproducing colonies of different size and at different temperatures, including the energy cost of retrieving the sugar supplement. The following estimates for oxygen consumption (ml/bee/min) were made following a revision of the values produced in Table 6 for colonies at 15˚C or 30˚C (Table 14). Further refinement of these estimates should be made within the new project.

Table 14. Revised estimates of the oxygen requirements of honeybees when at environmental temperatures of 15˚C or 30˚C

Bee class & activity	Oxygen consumption (ml/bee/min)	
	15˚C	**30˚C**
Larvae/pupae	2.1	2.1
Inactive adult	12.5	6
Active adult	130	41
Flying/foraging	200	130
Wax production (ml/g)	6275	6275

- Estimate the amount of sugar needed daily by a colony to increase numbers to that desired for a 3 or 6 week nectar dearth and after over-wintering. A preliminary spreadsheet model has been developed to estimate the daily requirements of sugar needed by a colony under different temperature regimes and for different periods of nectar dearth. The effects of disease status and particularly through its effect on egg laying capacity and longevity could be estimated using the model. The assumptions used to calculate sugar requirements each day to increase a colony from 20,000 bees to a maximum (equilibrium) number are given in Table 15 and Table 16.

Table 15. Assumptions made about colony dynamics for prediction of total energy and sugar requirements for expanding the size of a colony from 20,000 bees at the commencement of feeding

Longevity of bees from emergence - normal	42 days
Longevity of bees over winter	95 days
Lag from commencement of feeding to first egg lay by queen	3 days
Age larvae pupate	6 days
Age from egg lay to bee emergence	21days
Number of cells/brood comb side	3300
Number of brood comb sides/colony	14
Maximum proportion of brood cells filled	0.85
Age nurse bee commences to feed larvae	3 days
Age nurse bee ceases to feed larvae	14 days
Number of nurse bees/larva	3
Glucose to oxygen conversion (g/l O_2)	1.3387

Table 16. Assumptions made about colony activity for prediction of total energy and sugar requirements for expanding the size of a colony from 20,000 bees at the commencement of feeding when at 15˚C or 30˚C

Variable	15˚C	30˚C
Proportion of nurse bees active/day	0.50	0.25
Proportion of foraging bees active/day[a]	0.10	0.20
Number of flights/active forager/day	15	40
Flight time (min)	1	1

[a] Assume 50% of foraging bees are actively foraging for 10 hrs/day at 30˚C and 5 hrs/day at 15˚C.

When the assumptions given in Tables 14 to 16 are used in the model, the predicted changes in a colony starting with 20,000 bees are given in the following Figures (Figure 4 – bee class numbers; Figure 5 – number of eggs laid/day with limits being set by brood cell space; Figure 6 – number of larvae and pupae; Figure 7 – daily sugar requirements for colonies at 15˚C or 30˚C when there is no other energy source). The model predicted that it would take approximately 3 months to increase the number of bees in the colony from 12,500 bees at the minimum numbers to 50,000 bees. The daily sugar requirements increased from approximately 0.55 kg to 2.0 kg over the same period when bees were held at 30˚C. However, corresponding sugar requirements for bees at 15˚C were predicted to increase from 1.0 to 3.8 kg/day.

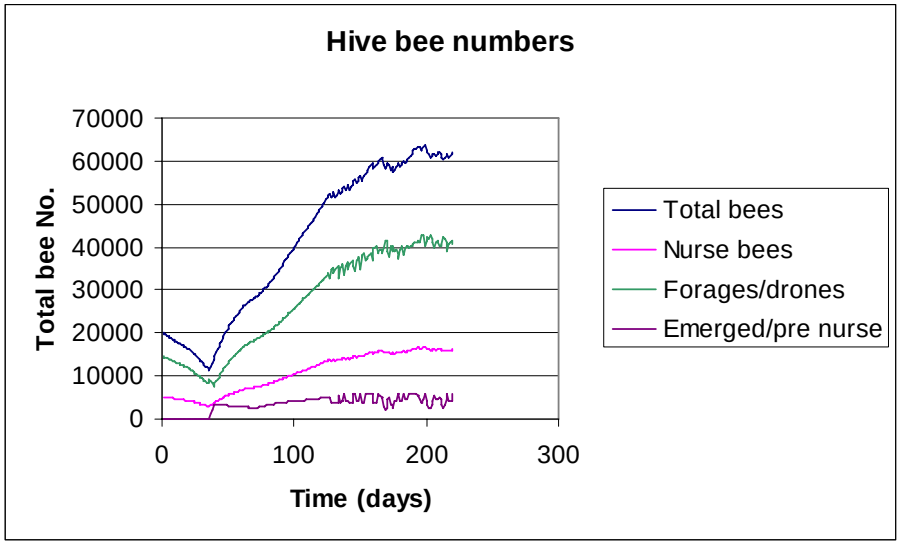

Figure 4. Predicted number of bees of different types within a colony using the assumptions in Tables 14-16.

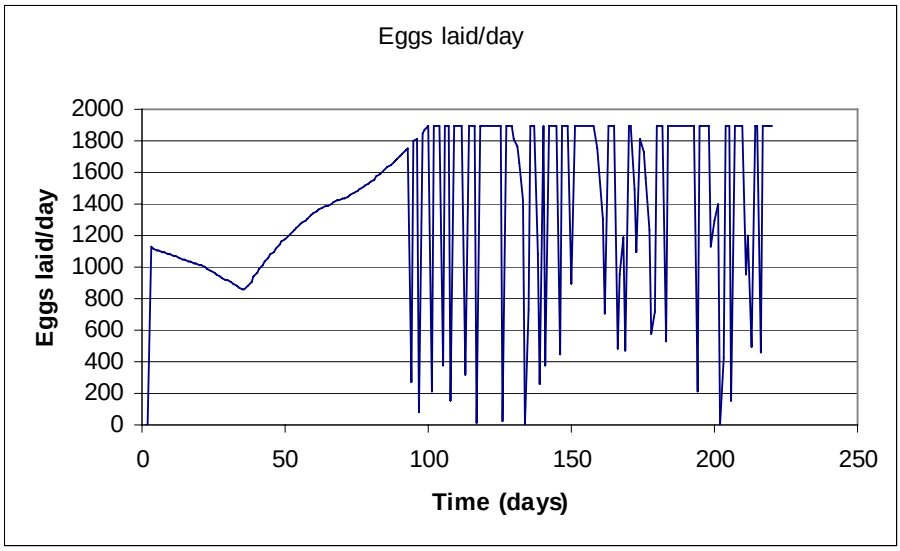

Figure 5. Predicted numbers of eggs laid daily within a colony using the assumptions in Tables 14-16. The initial limitation to egg laying capacity was number of nurse bees and then available brood cells which produced an irregularity in daily lay pattern.

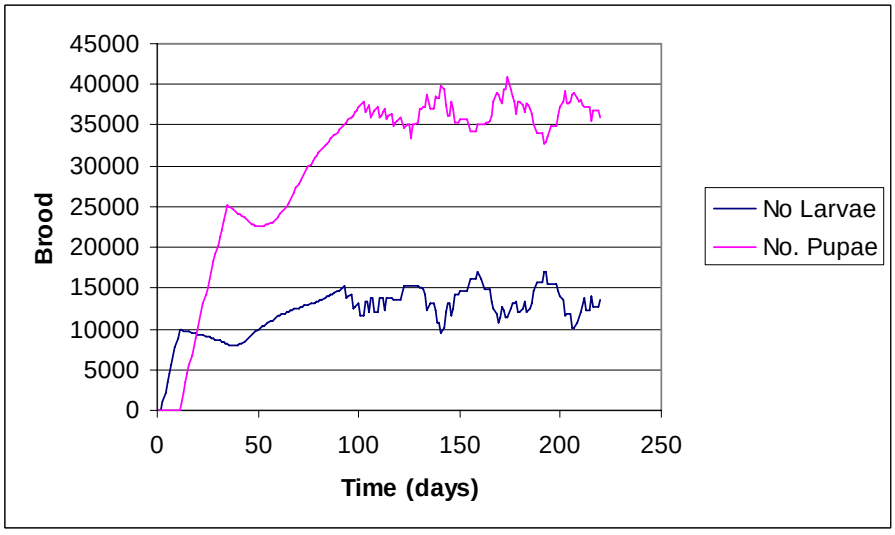

Figure 6. Predicted number of larvae and pupae within a colony using the assumptions in Tables 14-16.

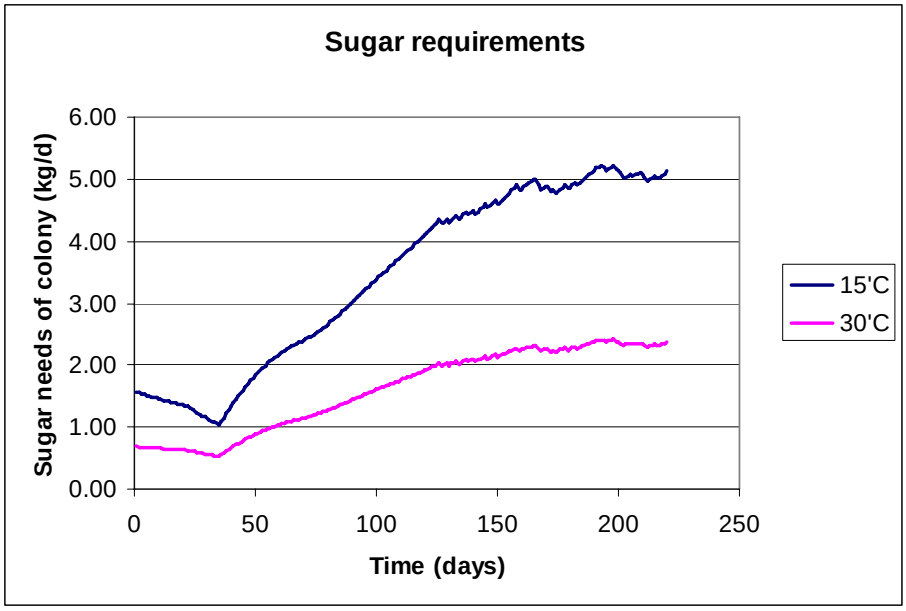

Figure 7. Predicted daily sugar requirements of a colony at either 15˚C or 30˚C using the assumptions in Tables 14-16.

Further refinement of this model is needed within the proposed project to more accurately predict the daily requirements of sugar needed by a colony of any size to change the number of bees by a specified amount for a nectar dearth of different times and for different environmental temperatures.

- Undertake research to develop practical recommendations for when to start feeding, where to place the supplements, the most appropriate concentration of sugar and sterilisation-preservation procedures by measuring sugar uptake and responses in colony numbers and bee health with alternative feeding procedures. It is particularly important to examine the effect on productivity of:
    - Feeding sugar syrup as slow release or from a bulk source
    - Feeding sugar inside or outside the hive
    - Different concentrations of sugar syrup
    - Preserving the syrup with H_2O_2, metabisulphite or other food preservatives

- Develop and assess recommendations for feeding sugar supplements under a wide range of field situations across Australia.

- Publish final recommendations

Time and resources needed

A 2-year project is proposed initially employing a scientist and assistant with collaborating apiarists-scientists from different States and regions to test the recommendations over a wide range of environmental conditions.

Year 1:

i) Refine the model for predicting the sugar requirements and colony population dynamics. Use the refined model to predict the amount of sugar needed to change the number of bees within a colony over a specified range with varying periods of nectar dearth and different environmental temperatures. There may be value to the industry in enhancing the input-outputs from the model to make it available to apiarists for

predicting sugar requirements and population changes under a wide variety of conditions. This would take approximately 5 days work.

ii) Undertake experiments under field conditions to define the best methods for feeding and preserving sugar. These experiments would need to be conducted at a range of sites and at different times of the year to evaluate sugar feeding strategies for various times of nectar dearth and various environmental temperatures.

Year 2:

i) Continue to evaluate sugar feeding strategies across different regions of Australia and at different times of the year. Collaborate with apiarists and scientists from different States and different regions.

ii) Refine recommendations for sugar feeding and draft a publication for distribution across the industry.

Feasibility of using existing technologies for forecasting floral resources

This project has been raised in priority from the list developed at the apiarist-scientist workshop because of the belief that the most significant improvements in profitability in an industry come from the adoption of new technologies that will deliver quantum changes in productivity. The Workshop identified forecasting of the timing and location of future floral resources as the most important process affecting profitability of the industry. A number of technologies that could be applied to the bee industry are used in other industries and the feasibility of modifying them for the bee industry may bring about such a quantum change to productivity.

The following activities may be included in a feasibility study to identify potential new technologies for forecasting flowering times.

- Evaluate the potential of using existing Bureau of Meteorology weather records from up to 4000 sites across Australia for forecasting species flowering times and locations based on historical information. An important component of this proposal is identification of the extent and reliability of past records for determining the species in flower and the extent and period of flowering. These records may be kept by apiarists, herbaria, forestry organisations and/or university botany schools. If the records are available a thorough statistical analysis would be required to identify patterns in climatic conditions over several years that stimulate extended flowering of specific species with high yields of nectar. The records could be used also to identify the post flowering conditions that affect the sustainability of nectar flows for different floral species.

- Evaluation of the potential for using satellite images for forecasting species flowering times and locations based on historical information. Extensive historical records of flowering are also required for development of methods for forecasting the site and timing of future floral resources using satellite imaging. The satellite imaging technology has been used to predict vegetation types and stages of growth for other situations around the world and may be applicable to the honeybee industry.

- Evaluation of other existing technologies as appropriate. The potential use of other existing technologies for forecasting future floral resources should be investigated using lateral thinking and investigative techniques.

- Write a report on the feasibility of using existing technologies for forecasting floral resources and recommend further research that would be needed to make the technology applicable to the Australian honeybee industry.

- The recommendations should include the outline of a strategy for making potential technologies available to the honeybee industry and for monitoring their capacity to improve profitability.

Time and resources needed

The project should initially be a 1 year feasibility study that recommends promising technologies to be developed in a follow-up project for the Australian industry.

Refine NIR calibration for assessing productivity of pollen

It is logical to build on the research already undertaken in project ANU-57A to complete the measurement on a sufficient number of additional pollen samples to allow the development of robust NIR calibrations for predicting the productive capacity of Australian pollens. However, the number of samples needed to ensure that the NIR calibration is robust and applicable to a wide range of pollen samples from Australia can be best assessed after completion of the current research within ANU-57A project. The research activities would include:

- Measurement, using the current ANU-57A project protocol, on up to 40 additional pollen samples and the refinement of NIR calibrations for predicting growth and head weight (as an indicator of hypopharyngeal gland development), longevity and brood rearing.

- Adding a number of mixed bee collected pollen samples to the experiments and testing the accuracy of the calibrations for these samples.

- Testing the calibrations on pollens collected over several years because NIR predictions tend to vary between years for other agricultural applications.

- Transfer the calibrations to other laboratories or make other arrangements for them to be widely available to the honeybee industry.

The initial phase of the extended project involving measurements on an additional 40 pollen samples plus several samples of mixed bee collected pollen and development of the NIR calibrations should take approximately one year. Further testing of the calibrations on pollens from new seasons and transfer of the calibrations should continue for a further 2 years.

It is recommended that the research is reconsidered towards the completion of the current project ANU-57A.

Identifying early onset of the ‘skinny bee’ syndrome

An ability to assess accurately the potential onset of the ‘skinny bee’ syndrome would assist greatly the knowledge of when to commence sugar syrup supplementation and/or the provision of pollen substitutes. Although, experienced apiarists can detect changes in the vitality of a colony, a rapid and reliable method for predicting the onset of the syndrome would be valuable for both industry practice and for assessing changes in bee vitality during research projects. Research activities would be:

- Assessment of alternative variables that reflect accurately the nutritional status of a colony including bee ‘fatness’ score, brood area, number of bees in a colony or change in bee numbers, bee weight at emergence, bee protein content, dry comb area, new nectar storage area, number of bees foraging, pollen load carried by foraging bees etc. Experiments would be conducted with colonies under a range of field and controlled situations.

- Work with image analysis specialists to develop calibrations that could be used to quantify visual assessments.

- Select the most appropriate measurements for assessing colony numbers and vitality for testing on a large number of field sites.

- Develop practical protocols for application of the selected methods and promote their use across the industry.

The project is likely to take 2-3 years. However, commencement of the project should be delayed until and effective pollen substitute has been developed and sugar feeding recommendations refined.

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