FATTY ACID COMPOSITION OF POLLEN AND
THE EFFECT OF TWO DOMINANT FATTY ACIDS (LINOLEIC AND OLEIC)
IN POLLEN AND FLOUR DIETS ON LONGEVITY
AND NUTRITIONAL COMPOSITION OF HONEY BEES (Apis mellifera)

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This thesis is presented for the degree of
Doctor of Philosophy
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Murdoch University

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My thesis is dedicated to my dear father

Keith Manning
(28 March 1927 – 26 December 2006)
Who fought a valiant fight
against the inevitable outcome
of a terminal disease
to live as long as he could.

His last note
DECLARATION

I declare that this thesis is my own account of my research and contains as its main content work which has not previously been submitted for a degree at any tertiary education institution.

Robert Manning

DECLARATION

This thesis is the culmination of research conducted for projects: DAW-91A; DAW-100A; DAW-105A funded by the Rural Industries Research and Development Corporation.

Project supervisor:
Associate Professor Bernard Dell

The experimental work was carried out at the Western Australian Department of Agriculture and Food, South Perth.
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**GENERAL DISCUSSION**

**References**
A dearth of nectar and pollen (carbohydrate and protein) reduces the population of a feral honey bee colony [photo: L Manning, Bibra Lake, 2006]
ABSTRACT

The size of the apiculture industry in Western Australia (W.A.) is one of the smallest in the nation but the production of pollen and honey per colony is the highest in Australia. The overwhelming value of the bee industry to the community is through pollination. The pollination service benefit provided by honey bees (*Apis mellifera*) in Australia has an estimated value of $AUS1.7 billion (1999 - 2000). The economic yields from crops, such as almonds and cucurbits, depend entirely on the activity of honey bees.

Access to flora is essential to maintain productive colonies for pollination services. Pollen and nectar from flowers provide the nutritional components for colonies of honey bees to breed, but pollen is more important as it provides the colony with its source of protein. Protein content is changed by pollen lipid content which can vary from 0.8 to 18.9 %. Lipids are composed of fatty acids and a number are highly antimicrobial and play an important role in colony hygiene, whilst others are nutritionally crucial for honey bee development.

Australian honey bee colonies utilise areas of native flora where a diversity of pollen species exist or hives are placed with agricultural crops that are based on European plants grown in monocultures, e.g. canola. Anecdotal evidence suggests that, in terms of breeding bees, some pollen species are much better than others and that bee health and longevity can be compromised if pollen is derived from single plant species. Protein analysis of pollen has been conducted on a wide range of species over the last two decades. However, lipid content and its analysis for fatty acids, which was reviewed for this thesis, have only been conducted on a few species. An initial investigation into the fatty acid composition of the pollen of W.A. eucalypts revealed the genus was characteristically high in linoleic acid concentration and ranged from 35.7 – 48 % (2.77 – 5.81 mg/g). Of the six species that are important to W.A. beekeepers, *Eucalyptus wandoo* (whitegum) and *E. accedens* (Powderbark Wandoo), a taxonomically similar species, showed similar levels of arachidic acid, whilst all three *E. wandoo* flowering varieties (summer, winter and spring) were the lowest in linolenic acid. *Corymbia calophylla* (redgum) was significantly higher in myristic and linolenic acids and *E. patens* (blackbutt), *E. marginata* (jarrah) and *E. diversicolor* (karri) had similar fatty acid profiles. European honey bees have evolved with plant species that have pollen that contain much higher levels of lipids, which are dominated by linolenic acid, than eucalypts. By contrast, the pollen of eucalypts, the most targeted plants by W.A. beekeepers, and other Australian plants are typically higher in linoleic than linolenic acid.
Given the influence of lipids on protein content and that fatty acid concentration varied amongst some of the important eucalypt species, a much wider study of pollen from plant species that are important to beekeepers was conducted. The first aim of the project, a national pollen survey, was undertaken in which 577 samples of pollen were collected. A total of 73 different fatty acids were identified. Of these, only five: palmitic, stearic, oleic, linoleic and linolenic were common to all 577 samples of pollen.

The second aim of the thesis was to investigate the effect of two commonly found fatty acids in pollen and their concentration at which longevity and life-span of honey bees, and development of the hypopharyngeal gland were deleteriously affected. An associated objective was to determine whether a range of soya bean flours, the main ingredient of an artificial bee diet that can replace pollen but differs in lipid concentration, had a similar effect. The aim encompassed two projects. In the first, two fatty acids (oleic and linoleic acid) were added at concentrations from 0 to 16 % to the low-fat, bee-collected pollen from *C. calophylla*. Eight different lipid-enhanced diets were created and each fed to bees confined in cages (mini-colonies containing 1400 bees). Oleic and linoleic acids were chosen because they are two of the five commonly found fatty acids in pollen. Oleic acid is the dominant fatty acid in honey bees and is a monounsaturated fatty acid. Linoleic acid is a dominant fatty acid in eucalypt pollen and is a polyunsaturated fatty acid that is one of two essential fatty acids that has antimicrobial activity.

The second project revolved around the problem of maintaining bee populations when apiaries are in environments that lack floral abundance due to drought or other environmental catastrophes. In these situations, beekeepers maintain their colonies by supplying artificial feedstuffs to colonies of bees. The high-protein diet ingredient of choice is imported soya bean flour and three flours containing 0.6 % (protein concentrate), 1.8 % (defatted) and 18.9 % (full-fat) lipid, were used. Locally milled lupin flour, containing 6.9 % lipid, was tested as a possible replacement for imported soya bean flour. As for soya bean flour, lupin flour was used in pure form or mixed with pollen in diets fed to bees. Flour and pollen combinations created another ten different diets fed to bees the same way as the fatty acid-enhanced pollen diets.

Along with the 8 lipid-enhanced and 10 flour and flour-pollen diets, there were two sugar-only diets, one mixed from dry cane sugar and the other, a liquid invert sugar. Two redgum pollen-only diets concluded the suite of 22 diets tested. One of these pollen diets was crushed and irradiated and was several years old whilst the other was collected fresh at the beginning of experimentation and kept frozen. Crushed and
irradiated pollen is in common use by Australian commercial beekeepers as feedback when conditions for floral abundance are adverse.

An early experimental result was an observation of distinctive bee behaviour after bees were confined in cages for six weeks where small but persistent numbers of bees were found hairless in samples. The behaviour was apparently the same as when single cohorts of emerged bees rearrange their caste repertoire, which has been reported elsewhere, but where no connection to head weight and caste type had been documented. Low head weight and hairlessness were strongly associated with each other. Low head weights are usually associated with foraging honey bees because the hypopharyngeal gland is no longer developed functionally.

Experimentally, bees were assessed for longevity to 22 different diets in 7 experiments. Laboratory analysis was conducted on the weekly samples of bees removed from cages where bees were measured for head weight (hypopharyngeal gland development) and nutritional status by analysing de-gutted bees for protein, lipid, mineral and fatty acid content. Of the 22 diets tested, pure redgum pollen diets gave the greatest life-span and those bees fed diets of pure sugar had the shortest life. Honey bees fed a low-fat protein concentrate from soya bean flour had the longest life of the flours tested. Adding pollen to soya bean flour diets improved longevity whereas the addition of pollen to lupin flour caused increased mortality. Defatted and full-fat soya bean flours gave similar longevities and, despite large differences in fat content, the response to diet of head weight was negligible to the diets and no response was elicited by the queen bee to lay eggs which also indicated failed gland development of the worker bees.

The addition of fatty acid (oleic and linoleic) to pollen at different concentrations caused significant differences in longevity. Overall, the addition of both fatty acids to pollen did not improve longevity. The addition of oleic acid to pollen greater than 2 % caused the longevity of bees to decrease, a poor head weight response and a failure of the queen to lay eggs. The addition of linoleic acid greater than 6 % to pollen diets had a similar response. As the percentage of oil was increased for both fatty acid additions, total consumption of the diet decreased.

Honey bees fed soya bean, lupin flour and sugar-only diets failed to accumulate linoleic acid in their body which was in contrast to honey bees fed pollen diets. For the sugar diet, the failure of linoleic acid accumulation in bees occurred despite bees being able to accumulate total lipid. Manganese was poorly accumulated by honey bees fed both soya bean and lupin flour diets and a sugar-only diet. The implication is that linoleic acid
and manganese need to be added separately to dietary formulations in a form as yet to be determined that will enable honey bees to accumulate these elements in the same way as bees do from consuming pollen.

Soya bean flour-based diets, which have been used by beekeepers for decades, or lupin flour require additional amounts of linoleic acid and manganese. Similarly, this might apply to sugar. Sugar can be fed to bees in great quantities to enable bees to successfully over-winter in cold climates or it can allow breeding to commence which subsequently stimulates the collection of pollen. Sugar-feeding is widely promoted for orchard pollination, especially for kiwifruit. These changes could make these dietary ingredients more effective in enabling bees to breed between nectar flows and be more productive or nutritionally healthier, but any changes would require further cage experimentation. Bee-collected pollen naturally high in concentrations of oleic acid should also be tested in longevity trials, in conjunction with pollen that is low in oleic acid. The three untested common fatty acids (stearic, palmitic and linolenic acid) should also be evaluated for honey bee longevity and nutritional status.
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PAPERS ARISING FROM THE THESIS

Published


In preparation


   a. Linoleic and oleic acid enhanced redgum (*Corymbia calophylla*) pollen diets.
   b. Soya bean and lupin flour diets and mixes containing redgum pollen.

   a. Response to diets of linoleic and oleic acid enhanced pollen from redgum (*Corymbia calophylla*).
   b. Response to pure diets of soya bean flour (high, medium and low fat) and lupin flour and flour diets mixed with redgum pollen.
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2003

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2004

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2005

(Topic - Fatty acids of pollens/longevity of bees):
1. Rural Industry Research and Development Corporation's (RIRDC) annual research conference. Australian National University, Canberra 5 - 6 May 2005.
3. Western Australian Farmer’s Beekeepers Section, annual general conference, Swan Valley Oasis, 9 - 10 June 2005.

2006

(Topic - Longevity of bees/nutrition of honey bees):

1. Rob Manning, April Rutkay, Linda Eaton, and Bernard Dell (2006) Longevity of honey bees (Apis mellifera) fed linoleic and oleic acid enhanced redgum (Corymbia calophylla) pollen diets; high and low fat soya bean flour diets and a lupin flour diet and flour diets mixed with redgum pollen. 8th Asian Apicultural Association conference, Perth, Western Australia. March 20 - 24, 2006, University of Western Australia.

2. Rural Industry Research and Development Corporation’s (RIRDC) annual research conference. Narrabundah, Canberra 1 - 2 May 2006 (Final project review for DAW - 100A and DAW - 105A).

3. Western Australian Farmer’s Beekeepers Section, annual general conference, Swan Valley Oasis, 8 - 9 June 2006.

ABBREVIATIONS

There is a mix of common names and botanic names in the following text primarily as a result of the named pollen samples beekeepers sent to me. In different States, beekeepers can call the same plant species different common names, or a common name that could be the same for two different species.

An example is the common name ‘redgum’ which can be applied to the Western Australian species Corymbia calophylla or the name beekeepers call Eucalyptus camaldulensis in eastern Australia. In this thesis, the name redgum in the text always refers to the species, C. calophylla.