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Improving Queen Bee Production

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by Dr Denis Anderson

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Foreword

The beekeeping industry, like other livestock industries, is embracing the use of dietary supplements to maximise production. Commercial queen bee producers, who produce large numbers of queen bees for commercial honey producing hives, are turning to dietary supplements to improve the quality of new queens. However, it has yet to be demonstrated that the use of dietary supplements during queen production delivers tangible benefits.

The objective of this project was to determine whether dietary supplements improve the quality of reared queens and drones.

This project was funded from industry revenue which is matched by funds provided by the Australian Government.

This report, a new addition to RIRDC's diverse range of over 1000 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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Contents

Executive Summary

This project addresses the use of dietary supplements in the production of queen and drone honey bees.

Commercial queen producers are increasingly feeding dietary supplements to queen and drone nurse colonies when producing large numbers of queen bees in the belief that the supplements contribute to better quality queens. These supplements, which usually consist of a protein base with or without added sugar, vitamins and minerals, increase production costs. However, it has yet to be demonstrated that the use of dietary supplements during queen and drone production delivers tangible benefits.

In this project studies were targeted at the use of dietary supplements in queen nurse colonies (also called *cell-building colonies*), queen-bank colonies (also called *queen banks*), and drone nurse colonies (also called *drone-rearing colonies*). The experimental approach used was that recommended at a honey bee nutrition workshop held in Sydney in 1998. A commercial queen producer was enlisted to assist with the project and all field experiments were conducted in the queen producer's apiaries. The colonies and materials used in the experiments were removed from every-day queen production use and the collaborating queen producer assisted directly with the setting-up of experiments, the feeding of diets and the eventual collection of queens and drones for laboratory analyses.

In the first year of the project the effect on queen quality of feeding dietary supplements to cell-building colonies was tested. Six (6) different supplements were each fed to a group of 5 standardised cell-building colonies, while another single group of 5 cell-building colonies received no supplement (controls). The supplements used were: pollen, pollen with added vitamins, a soya-flour based artificial diet, a soya-flour based artificial diet with added vitamins, vitamins in sugar syrup and commercially available pollen patties.

Four days after applying the supplements, groups of 10 genetically-related 1-day-old worker larvae were grafted into each of the cell-building colonies. The resulting queen cells were kept in their respective cell-building colony until 2 days before young adult queens were due to emerge from them, at which time they were each introduced into a 3-frame full-depth mating nucleus. All the mating nuclei were located in the same apiary. The newly emerged queens were open-mated and 21 days later caged and transported to the laboratory where the quality of some of their physical characteristics was determined. This involved weighing the queens and testing for (a) the numbers of ovarioles in their ovaries, (b) the numbers of spermatozoa in their spermatheca, (c) their levels of Nosema infection and, (d) their thoracic crude protein levels.

There was wide variation in the values of each measured characteristic. However, the physical characteristics of queens reared in cell-building colonies fed dietary supplements were no different from the physical characteristics of queens reared in cell-building colonies not fed dietary supplements. The average weight of the 144 queens examined was 0.224g (SD 0.011g), and the average number of ovarioles in their ovaries was 173 (SD 10.5). A relatively high proportion of the queens had low levels of spermatozoa in their spermatheca: 28.8% contained less than 1 million spermatozoa, 56.2% 1-3 million and 22.9% more than 3 million. The average number of spermatozoa per queen was 2.1 million with a large standard deviation of 1.28 million.

In the second year of the project the effect on queen quality of feeding dietary supplements to queen banks was tested. A total of 200 sister queens were reared by standard queen-rearing techniques, open mated and randomly assigned to one of 10 experimental groups. Queens in each group were then introduced into 1 of 10 standardised queen banks. Four (4) different dietary supplements where then each fed to two banks on a continuous basis for 2 months. These were, (a) soya-flour based artificial diet, (b) commercially available pollen patties, (c) vitamins in sugar syrup, and (d) sugar syrup. A further 2 banks were not fed dietary supplements (controls). At the end of the 2-month banking period

the quality of the physical characteristics of up to 15 banked queens from each group was determined as described above. An additional queen characteristic, the diameter of the spermatheca, was also measured.

No significant differences were observed in the physical characteristics of the queens banked in colonies fed the different dietary supplements with those of queens banked in colonies not fed dietary supplements. However, the queens rearing in this experiment (autumn reared queens) contained much higher numbers of spermatozoa in their spermatheca than queens rearing in the previous experiment (which were spring-reared queens).

In the final year of the project the effect of feeding dietary supplements on drone quality was tested. Four (4) different dietary supplements were each fed to three drone-rearing colonies on a continuos basis from 14 days prior to the commencement of drone-rearing until the time that drone larvae were capped. The supplements fed were, (a) soya-flour based artificial diet, (b) commercial pollen patties, (c) vitamins in sugar syrup, and (d) sugar syrup. A further 3 drone-rearing colonies were not fed dietary supplements (controls). New drones were reared in each of the drone-rearing colonies by standard drone-rearing procedures. When the adult drones were 20 days old, 10 were removed from each colony and examined for sperm production and a further 5 were removed and weighed. However, more than 95% of these drones were not producing semen. Therefore, the remaining drones were left to mature in the drone-rearing colonies for a further 7 days after which time 20 were captured from each colony. Of these, 10 were weighed and 10 were used to determine the numbers of spermatozoa in their semen using a haemocytometer.

The weights and numbers of spermatozoa of drones reared in colonies fed the different dietary supplements were not significantly different from drones reared in colonies not fed dietary supplements.

In conclusion, the results from the dietary supplement experiments reported here showed that the physical quality of queens and drones reared by dietary-supplemented nurse colonies were no different from that of queens and drones reared by non-supplemented nurse colonies. However, these results were no doubt influenced by experimental variables that became apparent in the experimental approach used as each experiment progressed. An alternative experimental approach is suggested for future studies.

1. Introduction

This project was developed following recommendations from a workshop on honey bee nutrition held in Sydney in May 1998. The general aim was to assess the effects of feeding dietary supplements during commercial queen production.

Queen bees are at the heart of beekeeping, but very little research has been directed at their health, nutrition and performance. The first serious research directed at queen health in Australia was in the early 1990's when a HBRDC/CSIRO sponsored project examined the aging process in adult queens and also the diseases and disorders of different life stages of queens. Several microbial pathogens were identified, but by far the commonest ailment of adult queens was 'premature aging', its underlying cause thought to be nutritional (Anderson, 1993).

Other studies on queen bees followed. A one-year pilot study in 1997-98 compared the success of introducing young autumn and spring-reared queens into normal queen-less bee colonies. Higher losses were found among the spring-reared queens (RIRDC Project: DAN-164A). A follow-up 3-year study identified factors that may affect the introduction and early performance of queens. A correlation was found between the age of young queens and their introduction success, early survival and performance in bee colonies, with increasingly older queens more likely to survive introduction and perform well. Low numbers of sperm in the queen's spermatheca was common for all ages of queens examined in the study (Rhodes and Somerville, 2003).

As stated, the current project on queens and drones was developed around guidelines set down at the 1998 nutrition workshop. Commercial queen bee producers, who rear large numbers of queens that beekeepers use in commercial honey producing hives, are increasingly feeding dietary supplements to nurse colonies during queen production in the belief that they improve the quality of new queens. However, there is much debate as to whether these practices are beneficial or cost-effective. Participants at the 1998 workshop considered that it would be worthwhile to conduct a series of trials in collaboration with a commercial queen producer to attempt to resolve this issue. The targets of the trials were to be queen nurse colonies (hereafter called *cell-building colonies*), queen-bank colonies (hereafter called *queen banks*), and drone nurse colonies (hereafter called *drone-rearing colonies*).

These 3 types of colonies are extremely important in commercial queen production. Cell-building colonies are used to rear young queen larvae. The common practice is to carefully transfer 1-2 day-old worker bee larvae (females) from horizontal worker brood cells in a nearby breeder colony into vertically placed queen cell cups attached to the underside of wooden frames inside cell-building colonies (a process called 'grafting'). Because of their vertical position in the cell cups, nurse bees in the cell-building colonies will instinctively feed the grafted worker larvae large quantities of glandular food which changes their development from worker larvae into queen larvae. As the queen larvae grow, nurse bees also elongate the walls of their cell cups with wax to accommodate their increasing size. When the larvae are ready to pupate, nurse bees place a wax capping over the cells to enclose the developing queens during their pupation stage. In the commercial queen rearing system these queen cells are generally removed from the cell-building colonies into incubators or mating nuclei (very small bee colonies) to allow the queens to complete their pupal development. However, sometimes they are left in the cell-building colonies until pupal development is well advanced at which time they may be sold to beekeepers or moved to mating nuclei.

After the queen cells are introduced to mating nuclei young adult virgin queens begin to emerge from them. These soon fly out of the nuclei to mate with up to 20 different drones (Winston 1987). Commercial queen producers attempt to control the mating of virgin queens by 'swamping' the local mating areas with drones of desired genotypes. The drones, in turn, are reared in special drone-rearing colonies. The quality of a newly mated queen depends to a large extent on the number of quality drones available for mating (a quality drone will normally produce approximately 10 million spermatozoa). A new queen must mate with many quality drones if she is to fill here spermatheca (a

sperm storage organ) with 5-7 million spermatozoa. If this is not achieved the queen will not survive long after being introduced to a normal queen-less bee colony (that is, she will be rapidly superceded).

After queens have been mated in the mating nuclei, they are captured, placed in small cages and shipped to beekeepers who introduce them into honey producing hives. However, sometimes it may be necessary for the producers to hold or store the newly mated queens. In these instances the queens are caged without candy or attendants and kept inside special colonies called 'queen banks'. There is room for 50-70 caged queens in a frame inside a single bank. Sometimes more than 200 queens are stored in a bank.

Given the perceived views about the undesirable effects that undernourished queen and drone nurse colonies may produce, it is not surprising that many queen producers have resorted to the use of dietary supplements. Proof that these supplements are beneficial would encourage their wider use.

2. Improving queen bee production – feeding dietary supplements to queen and drone-rearing colonies

2.1 Introduction

To test the effects of feeding dietary supplements to queen and drone-rearing colonies 3 experiments were carried out in collaboration with a commercial queen producer in NSW over a 3-year period. Several dietary supplements, similar to those used by commercial queen producers, where fed to groups of queen cell-building colonies (carried out in year 1), queen banks (year 2) and drone-rearing colonies (year 3). Included in each experiment were a number of untreated colonies ('controls'). At specific times after feeding the supplements, groups of queens that had been reared and held in the cell-building and queen bank colonies respectively, and drones that had been reared in the drone-rearing colonies, were captured and moved to the laboratory where the 'quality' of some of their physical characteristics was determined.

Quality is a qualitative term that, when used in relation to queens and drones, infers that they conform to certain quantitative physical and/or behavioural states. For example, quality queens are good performing queens and head large 'strong' bee colonies. Generally, this means that the queens have a high daily egg-laying rate and will survive in bee colonies for several years. Queens with these behavioural traits tend to show the following physical characteristics. They are:

- heavy;
- have high numbers of ovarioles (egg tubes) in their ovaries;
- have large numbers of spermatozoa in their spermatheca and;
- are free of the protozoan parasite *Nosema apis*.

These physical attributes are nowadays used to measure queen quality in the absence of behavioural information.

Quality has not be quantified to the same extend for drones. Nevertheless, a quality drone is considered to weigh more than a poor quality drone and to produce about 10 million spermatozoa as a mature adult.

These widely used methods for measuring queen and drone quality were used in the current study.

2.2 Effects of feeding dietary supplements to cell-building colonies

2.2.1 Methods

(a) Field Work

The field component of this experiment was carried out between 5 January and 13 February 2000. During this time the local forage condition for bees (availability of pollen and nectar) was good.

The dietary supplements fed to the cell-building colonies (described below) were those recommended by queen producers and participants at the bee nutrition workshop held in 1998. In all, 6 different supplements were each fed to groups of 5 cell-building colonies, each hived in a double-storey 10-frame Langstroth hive. As well, another single group of 5 similar cell-building colonies received no supplements (controls). Prior to the commencement of the experiment the bee and brood populations

and numbers of honey and pollen combs in the cell-builders were manipulated to a standard size. Each hive was managed by the '*Cloak Method*'.

Details of each of the 6 dietary supplements, and the ways in which they were fed to the cell-building colonies, are given in Appendix 1. In brief, the supplements were as follows:

1) pollen only (PO);
2) pollen only with added vitamins (PV);
3) soya-flour based artificial diet (SF);
4) soya-flour based artificial diet with added vitamins (SFV);
5) vitamins in sugar syrup (VO);
6) commercially available pollen patties (PP).

Studies have indicated that it takes approximately 4 days for nurse bees to consume pollen and for that pollen to be digested and changed into brood food, which is secreted by the head glands and fed to bee brood (Winston, 1987). Also, preliminary feeding trials carried out as part of the current study had shown that it took cell-building colonies about 5-7 days to consume their solid supplements. Hence the solid supplements were fed to cell-building colonies 7 days before 1-day-old worker larvae were grafted into them, while the liquid vitamin supplements were fed to cell-building colonies 4 days before larvae were grafted into them. Even using these feeding methods, not all cell-building colonies had consumed their solid supplements by the time larvae were grafted into them (see Appendix 1).

Ten one-day-old worker larvae were grafted into each of the 35 cell-building colonies with the aim of eventually obtaining 5 queens from each to test for queen quality. The grafted larvae used to produce the queens were genetically related as they were obtained from a queen that had been artificially inseminated with semen from a single drone.

The developing queens in queen cells were left in their respective cell-building colony until 2 days prior to their emergence as adults. At that time the queen cells were moved into standardised 3-frame full-depth mating nuclei located in the same apiary. The virgin queens were then open-mated and 21 days later caged and transported to the laboratory in Canberra where they were weighed and then stored at -20^{o}C to await further testing.

(b) Laboratory Work

The quality of up to 5 individual queens from each of the 7 groups of 5 cell-building colonies was tested by:

- determining their weight (this was done prior to them being stored at –20^{o}C);
- determining their ovariole numbers;
- determining the numbers of spermatozoa in their spermatheca;
- determining whether they were infected with *Nosema apis*;
- estimating their thoracic crude protein levels.

Preliminary studies had indicated that the numbers of ovarioles in one ovary of a queen did not differ significantly from those in the other ovary. Therefore, only the ovarioles in the left ovary of each queen were counted. This was done by removing the queens from storage at –20^{o}C, thawing them and dissecting their ovaries in distilled water with the aid of a dissecting microscope. The left ovary was deemed to be that on the left-hand side of a queen as she was observed and dissected from the dorsal surface with her anterior end (head) pointing away from the body of the laboratory technician carrying out the dissection. Preliminary work had indicated that it was easier to distinguish and count the ovarioles if the ovary was first placed for 30 seconds in electrophoresis gel stain (0.1% Coomassie brilliant blue R250 in 45% methanol plus 10% acetic acid), then placed in 35% ethanol and examined. This procedure stained the linings of each ovariole blue, but left the eggs inside white. To count the ovarioles, a transverse section was cut from the middle of the ovary and placed in a small watch-glass

containing 35% ethanol. The numbers of ovarioles were then carefully counted by teasing the ovarioles apart and moving each ovariole to the side of the watch glass as it was counted.

Preliminary studies had also indicated that freezing the queens did not damage the spermatozoa in their spermatheca. To determine the numbers of spermatozoa of each queen the spermatheca was removed at the same time that her ovaries were removed. The spermatheca was then placed in 100 µl of phosphate buffered saline in a small Eppendorf tube, gently teased apart, shaken well using a vortex, diluted 1:80 or 1:160 in distilled water, shaken again, and the spermatozoa counted using a haemocytometer at x160 magnification with the aid a light microscope.

To determine whether a queen was infected with *Nosema apis*, her mid and hind gut was removed at the same time that her ovaries and spermatheca were removed. The gut was then placed in 0.5 ml distilled water, mashed and examined for *N. apis* spores at x400 magnification with the aid of a light microscope.

Because the guts, ovaries and spermatheca were used for determining their physical quality, the only substantial body part left from which to obtain an estimate of crude protein in each queen was the thorax, which is mostly muscle tissue. To obtain the estimate of crude protein, the thorax of each queen was thoroughly dried by placing it in an incubator at 37^{o}C for 4 days. The dried mass, mostly dried protein, was then weighed. It should be noted that the thoraces of bees show less variability in protein content compared to abdomens (Haydak, 1935, 1937).

2.2.2 Results

The results are summarised in Table 1. None of the queens were found to be infected with *Nosema apis*.

Table 1. Summary of data collected from queen bees reared as larvae in cell-building colonies that had been fed or not fed dietary supplements. Each diet was fed to a group of 5 cell-building colonies. Shown are the numbers of queens tested from each colony, their average weights (with standard deviations in brackets), the average numbers of ovarioles in their left ovaries, the average number of spermatozoa in their spermatheca and their average thoracic dry weights. The dietary supplements were: (P) pollen, (PV) pollen with vitamins, (SF) soya-flour based artificial diet, (SFV) soya-flour based artificial diet with vitamins, (VO) vitamins in sugar syrup and, (PP) commercial pollen patties. A further group of 5 cell-building colonies were not fed diet (controls) (C). See Appendix 1 for details on diets.

Dietary Supplement – Colony No.	No of Queens Tested	Average Weight Of Queens (mg)	Average No. of Ovarioles	Average No. of Spermatozoa ($x10^6$)	Average Dry weight (Thorax) (mg)
P - 1	5	2221 (141.9)	165.4(10.6)	1.03(0.50)	21.2(0.99)
P - 2	5	2208 (119.6)	169.8(15.1)	1.20(0.61)	21.0(0.79)
P - 3	5	2184 (71.0)	169.0(17.8)	2.22(0.99)	21.1(0.79)
P - 4	5	2271 (9.7)	172.4(6.6)	1.76(0.42)	21.5(1.27)
P - 5	4	2294 (56.4))	177.5(13.0)	2.23(1.70)	20.3(0.77)
GROUP TOTALS:		**2233 (94.6)**	**170.5(12.6)**	**1.67 (0.97)**	**21.0 (0.95)**
PV - 1	5	2263 (81.9)	176.0(7.8)	1.57(0.55)	21.2(1.32)
PV - 2	5	2277 (73.0)	179.2(5.9)	1.58(0.91)	20.8(0.89)
PV - 3	5	2321 (46.3)	171.6(8.0)	1.47(0.41)	21.5(0.58)
PV - 4	2	2358 (11.31)	159.0(7.0)	1.50(0.74)	19.3(0.85)
PV - 5	4	2221 (176.5)	173.2(17.5)	0.89(0.36)	21.1(1.05)
GROUP TOTALS:		**2281 (96.5)**	**173.6(10.6)**	**1.41 (0.61)**	**21.0 (1.1)**
SF - 1	2	2108 (52.3)	171.5(0.7)	3.67(2.35)	20.2(0.64)
SF - 2	5	2211 (199.3)	182.0(12.8)	2.86(1.82)	21.4(0.47)
SF - 3	4	2421 (113.5)	178.5(5.3)	2.77(1.28)	21.1(1.08)
SF- 4	4	2415 (165.1)	172.7(8.7)	2.46(0.50)	21.3(1.48)
SF- 5	5	2273 (25.4)	170.6(9.6)	2.05(0.79)	21.3(1.22)
GROUP TOTALS:		**2299(164.4)**	**175.5(9.6)**	**2.64 (1.28)**	**21.2 (1.0)**
SFV - 1	5	2191 (53.5)	168.8(11.9)	2.54(0.99)	21.6(0.28)
SFV - 2	5	2229 (48.4)	178.2(7.4)	2.54(0.90)	20.4(0.72)
SFV - 3	4	2217 (28.9)	168.7(17.4)	1.17(0.98)	20.8(0.87)
SFV - 4	4	2285 (41.5)	172.0(9.6)	1.16(0.85)	20.9(1.11)
SFV - 5	5	2200 (116.3)	171.8(15.2)	1.36(0.61)	20.3(1.18)
GROUP TOTALS:		**2222 (69.4)**	**172.0(12.0)**	**1.80 (1.03)**	**20.8 (0.9)**
VO - 1	4	2267 (62.9)	175.0(2.7)	2.33(1.37)	21.7(0.49)
VO - 2	5	2313 (70.9)	182.0(7.3)	3.36(0.95)	21.2(0.66)
VO - 3	3	2250 (7.9)	174.3(10.0)	2.68(0.71)	21.4(0.70)
VO - 4	5	2199 (96.5)	173.0(5.6)	1.52(1.26)	21.3(0.71)
VO - 5	4	2110 (83.6)	174.2(8.0)	1.06(0.84)	20.3(1.01)
GROUP TOTALS:		**2229 (98.4)**	**175.9(7.1)**	**2.19 (1.30)**	**21.2 (0.8)**
PP - 1	0	-	-	-	-
PP - 2	3	2193 (77.0)	176.3(12.0)	2.87(0.97)	21.3(0.70)
PP - 3	1	1941	158.0	0.94	19.5
PP - 4	5	2249 (192.8)	185.2(12.7)	1.83(1.01)	21.1(2.00)
PP - 5	5	2160 (109.4)	176.4(10.7)	3.00(2.80)	21.8(0.99)
GROUP TOTALS:		**2183 (149.8)**	**178.2(12.7)**	**2.41 (1.83)**	**21.3 (1.4)**
C - 1	5	2273 (40.5)	172.6(9.9)	1.54(O.59)	22.5 (0.96)
C - 2	5	2286 (51.0)	174.0(7.5)	2.46(2.22)	21.2(1.01)
C - 3	3	2199 (115.3)	171.3(10.6)	2.36(0.79)	20.2(0.90)
C - 4	3	2375 (91.6)	172.6(7.7)	3.40(0.78)	22.4(1.28)
C - 5	5	2255 (95.3)	171.8(3.5)	3.65(1.23)	21.4(2.30)
GROUP TOTALS:		**2276 (85.6)**	**172.6(7.2)**	**2.64 (1.47)**	**21.6 (1.5)**
OVERALL AVERAGES:		**2248 (112.8)**	**173.7(10.5)**	**2.1 (1.28)**	**21.2 (1.1)**

There was wide variation in the values of each measured characteristic. The body weights, numbers of ovarioles, numbers of spermatozoa in spermatheca and dry thoracic weights of queens reared in cell-

building colonies fed dietary supplements did not differ significantly from the same physical characteristics of queens reared in cell-building colonies not fed dietary supplements. Hence, in these experiments, the feeding of dietary supplements to cell-building colonies produced no measurable effect on the quality of queens subsequently reared in them. These results are discussed below in Section 3.

2.3 Effects of feeding dietary supplements to queen banks

2.3.1 Methods

(a) Field Work

The field component of this experiment was carried out in the autumn of 2001 (March-June) when local forage conditions for bees were poor.

Two hundred (200) adult queens were produced for this experiment and they were reared using the '*Cloak Method*' in standardised, double storey 10-frame cell-building colonies. Larvae used to rear the queens were one-day-old and were grafted from a breeder colony that was headed by a queen that had been inseminated with semen obtained from a single drone. The developing queens were left in the cell-building colonies until 2 days prior to their emergence as adults. They were then moved into standardised 3-frame full-depth mating nuclei located in the same apiary. The resulting virgin queens were open-mated and 21 days later caged and randomly assigned to one of 10 different experimental groups. Each group consisted of 20 queens. The queens in each group were then introduced into the top super boxes of one of 10 double-storey banks, each of a standard size and strength.

Two of the 10 banks were fed one of 4 different dietary supplements on a continuous basis for 2 months (that is, each of 2 banks were fed the same supplement). The supplements were those recommended by queen producers and participants at the 1998 workshop on bee nutrition. Details of each are given in Appendix 1. In brief, the supplements were:

1) soya-flour based artificial diet (SF);
2) commercially available pollen pattie (PP);
3) vitamins in sugar syrup (VO);
4) sugar syrup (60% sucrose solution in tap water) (SO).

A further 2 banks were not fed dietary supplements (controls).

At the completion of the 2-month feeding period, the banked queens were re-caged with accompanying escort bees and transported to the laboratory in Canberra. Some of the banked queens died during the banking period and these were recorded as having died and eliminated from the experiment.

(b) Laboratory Examination

At the laboratory the banked queens were immediately weighed, transferred to marked Eppendorf tubes and frozen prior to further examination. At various times thereafter up to 15 randomly selected queens from each experimental group were tested for queen quality as described in Section 2.2 1 above. An additional queen characteristic, the diameter of the spermatheca, was also measured here.

2.3.2 Results

The results are summarized in Table 2. Again, none of the queens were found to be infected with *Nosema apis*.

Table 2. Summary of data collected from queen bees banked in queen-bank colonies fed or not fed dietary supplements. Each diet was fed to 2 queen-banks. Shown are the numbers of queens tested from each colony, their average weights (with standard deviations in brackets), the average number of ovarioles in their left ovaries, the average diameter of their spermatheca and the average number of spermatozoa in their spermatheca. The dietary supplements fed to the banks were: (SF) soya-based artificial diet, (PP) commercial pollen patties, (VO) vitamins in sugar syrup and, (SO) sugar syrup. A further 2 queen-bank colonies were not fed dietary supplements (controls) (C). See Appendix 1 for details on diets.

Dietary Supp.-Colony	No. of queen tested	Average Weight (g)	Average No. of Ovarioles	Average Diam. of Spermatheca (mm)	Average No. of sperm ($X10^6$)
SF - 1	15	0.1837 (0.0197)	165.85 (11.92)	1.30 (0.067)	3.80 (1.62)
SF - 2	15	0.1976 (0.0168)	156.78 (13.19)	1.26 (0.075)	3.50 (1.57)
PP - 1	15	0.2103 (0.0178)	168.67 (10.78)	1.28 (0.028)	4.02 (1.75)
PP - 2	15	0.2104 (0.0173)	174.00 (10.35)	1.28 (0.064)	3.58 (1.59)
VO - 1	13	0.1863 (0.0139)	162.43 (7.50)	1.28 (0.068)	2.22 (1.73)
VO - 2	8	0.1835 (0.0156)	153.33 (8.87)	1.26 (0.102)	3.28 (2.45)
SO - 1	15	0.2160 (0.0136)	172.92 (12.95)	1.30 (0.060)	3.09 (1.73)
SO - 2	10	0.1833 (0.0135)	158.43 (14.46)	1.24 (0.056)	3.50 (2.50)
C - 1	14	0.2169 (0.0200)	170.90 (15.29)	1.28 (0.074)	4.11 (1.69)
C - 2	10	0.1754 (0.0177)	157.71 (12.88)	1.24 (0.068)	3.98 (1.11)
Total Averages:		0.1963 (0.0166)	164.10 (11.82)	1.27 (0.066)	3.51(1.77)

Like in the previous experiment, there was wide variation in the results obtained, even from replica colonies. No significant differences were found in the physical characteristics of queens banked in colonies fed the different dietary supplements with those of queens banked in colonies fed no dietary supplements. However, there tended to less queen death in the banks that had received added protein as a dietary supplement. These results are discussed in Section 3 below.

2.4 Effects of feeding dietary supplements to drone-rearing colonies

2.4.1 Methods

(a) Field Work

The field component of this experiment was carried out in a single apiary from October to December 2003. Just prior to the commencement of the experiment there had been a drought in the local area, but during the experiment the availability of local bee forage improved.

Four (4) different dietary supplements were each fed to three drone-rearing colonies (that is, 3 different drone-rearing colonies were fed the same supplement). The supplements were those recommended by queen producers and participants at the 1998 honey bee nutrition workshop. Each supplement was fed on a continuos basis to the colonies from14 days prior to the commencement of artificial drone-rearing until the time that the experimental drone larvae had been capped. Details of each supplement are given in Appendix 1. In brief, the supplements were:

1) soya-flour based artificial diet (SF);
2) commercial pollen patties (PP);
3) vitamins in sugar syrup (VO);
4) sugar syrup (SO).

A further 3 drone-rearing colonies were not fed dietary supplements (controls).

The fifteen drone-rearing colonies were each housed in 10-frame double storey Langstroth hives. The bottom brood box and top super box of each hive was separated by a queen excluder. Each colony was headed by a related queen that had been reared in the same apiary. Before the commencement of the experiment the bee populations of each colony was standardised.

New drones were reared in each drone-rearing colony by first moving the queen from the brood box to the excluded super box which contained an empty drone comb. The queen was left to lay eggs in cells of the comb for 4 days after which time she was moved back below the queen excluder to the brood box. Twenty to twenty-one days later, adult drones began to emerge from the drone comb in the super box. When these adults were 20 days old, 15 were removed from each super box. Ten were examined for spermatozoa production and weights were measured on each of the other 5. More than 95% of the drones examined were not producing semen. Therefore, the remaining drones were left to mature in the drone-rearing colonies for a further 7 days. Then, when the drones were 27 days old, 20 were captured from each colony. Ten were frozen and then later weighed, while semen was collected immediately from each of the remaining 10.

Semen was collected from the drones by gently squeezing their abdomens until their reproductive organs everted. Their endophalli were then removed and placed into 0.25ml of insect ringers inside individual Eppendorf tubes (one endophallus per tube). The tubes were then transported to the laboratory and stored at -20^{o}C until examined.

(b) Laboratory Work

The numbers of spermatozoa in the semen of each drone was determined after removing a tube containing an endophallus from cold storage and thawing it at room temperature. The endophallus was then gently teased apart, shaken well using a vortex, diluted 1:80 or 1:160 in distilled water, shaken again, and the spermatozoa counted using a haemocytometer at x160 magnification with the aid of a light microscope.

2.4.2 Results

The results are summarized in Table 3.

Table 3. Summary of data collected from drone bees reared in drone-rearing colonies fed or not fed dietary supplements. Each diet was fed to 3 drone-rearing colonies. Shown are the numbers of drones examined and their weights when 20 and 27 days old and their spermatozoa numbers when 27 days old. The dietary supplements fed were: (SF) soya-based artificial diet, (PP) commercial pollen patties, (VO) vitamins in sugar syrup and, (SO) sugar syrup. A further 2 drone-rearing colonies were not fed dietary supplements (controls) (C). See Appendix 1 for details on diets.

Diet - Colony No.	No. of drones tested when 20 or 27 days old	Average weight of drones on day 20	Average weight of drones on day 27	Average No. Spermatozoa in drones on day 27 ($x10^7$)
SF - 1	5/6	0.24 (0.01)	0.21 (0.03)	1.53 (0.76)
SF - 2	5/10	0.23 (0.01)	0.22 (0.02)	2.03 (1.55)
SF - 3	5/8	0.24 (0.01)	0.23 (0.02)	3.66 (1.73)
	Group Averages:	0.24 (0.01)	0.22 (0.02)	2.45 (1.67)
PP - 1	5/10	0.24 (0.02)	0.21 (0.03)	2.04 (1.64)
PP - 2	5/10	0.23 (0.01)	0.22 (0.02)	1.76 (1.33)
PP - 3	5/10	0.25 (0.01)	0.23 (0.02)	2.15 (1.40)
	Group Averages:	0.24 (0.01)	0.22 (0.02)	1.98 (1.42)
VO - 1	5/6	0.21 (0.09)	0.22 (0.02)	3.13 (1.53)
VO - 2	5/10	0.24 (0.01)	0.21 (0.03)	2.86 (2.17)
VO - 3	5/9	0.22 (0.02)	0.20 (0.01)	1.49 (1.21)
	Group Averages:	0.21 (0.06)	0.21 (0.02)	2.43 (1.81)
SO - 1	5/10	0.21 (0.02)	0.21 (0.01)	2.04 (1.57)
SO – 2	5/10	0.24 (0.01)	0.21 (0.02)	1.84 (1.16)
SO – 3	5/10	0.24 (0.03)	0.23 (0.01)	2.18 (1.20)
	Group Averages:	0.23 (0.02)	0.22 (0.02)	2.02 (1.29)
C – 1	5/10	0.21 (0.01)	0.20 (0.02)	1.96 (2.11)
C – 2	5/9	0.21 (0.02)	0.21 (0.01)	3.00 (2.88)
C - 3	5/9	0.21 (0.01)	0.19 (0.02)	4.76 (0.94)
	Group Averages:	0.20 (0.02)	0.20 (0.02)	3.19 (2.37)

There was wide variation in the results obtained. No significant differences were observed in the wights and numbers of spermatozoa of drones reared in colonies fed the different dietary supplements with those of drones reared in colonies fed no dietary supplement. These results are discussed below in Section 3.

3. General discussion

The results from this project showed that the physical quality of queens and drones reared by diet supplemented nurse colonies was no different from that of queens and drones reared by non-supplemented nurse colonies. Nonetheless, the experimental approach taken here, of feeding supplements to entire bee colonies and then measuring impacts on a small number of colony members (a relatively few queens and drones), meant that there were numerous experimental variables that could not be controlled. These variables (discussed below) may have influenced the end-results and thus caution is needed when drawing conclusions and implications from the work.

As previously mentioned, the experimental approach used here was that recommended by delegates attending a workshop on bee nutrition in Sydney in May 1998. It involved using materials, methods and diets commonly used during commercial queen production to see whether there were any benefits of feeding dietary supplements during commercial queen production. Hence, a commercial queen producer was enlisted to assist with the project. The colonies used in the experiments were removed from every-day queen production use and the collaborating queen producer assisted directly with the setting-up of experiments, the feeding of diets and the eventual collection of queens and drones for laboratory analyses.

3.1 Feeding dietary supplements to cell-building colonies

In the first year of the project dietary supplements were fed to cell-building colonies during spring when there was abundant bee forage (pollen and nectar) in the local environment. Generally, these are not the conditions under which commercial queen producers would use dietary supplements. Hence all colonies in the experiment, regardless of their treatment, had access to abundant pollen and nectar. Furthermore, even though attempts were made prior to the commencement of the experiment to standardise colony genetics and population sizes, some colonies became 'stronger' than others as the experiment progressed. As well, some colonies consumed their dietary supplements faster than others. These and other variables, together with the high background level of available bee forage throughout the experiment, may have negated any benefits gained from feeding the dietary supplements. Further follow-up studies are needed to show this however (discussed below).

As the physical characteristics of queens reared in dietary-supplemented colonies were the same as queens reared in non-supplemented colonies, all queens produced for this experiment can therefore be regarded as a 'normal' batch of 144 reared queens. The average weight of these queens was 0.224g (SD 0.011g), which compares well with queen weights observed in another Australian study (0.197g-0.226g, Rhodes and Somerville, 2003) and also in overseas queens (0.214g, Van Eaton, 1996). The average number of ovarioles in the queen's ovaries (173, SD 10.5) also compares well with those observed in Australian queens by Rhodes and Somerville (2003) and in overseas queens (175 by Casagrande-Ialoretto *et al*, 1984, 154 by Woyke, 1971 and 148 by Van Eaton, 1996).

However, the numbers of spermatozoa in the spermatheca of the queens were significantly less than reported in overseas studies, with 28.8% containing less than 1 million spermatozoa, 56.2% 1-3 million and 22.9% more than 3 million. The average number of spermatozoa per queen was 2.1 million with a large standard deviation of 1.28 million. In a 6-year overseas study during the 1980's 11% of queens were found with less than 3 million spermatozoa in their spermatheca and 45-64% contained more than 5 million (Jay and Dixon, 1984). In other less extensive overseas studies Woyke (1971) reported approximately 5 million spermatozoa/queen and Van Eaton (1996) reported the average New Zealand queen contained 4.7 million spermatozoa. Interestingly, in the recent study by Rhodes and Somerville (2003) relatively high numbers of spring-reared queens (the same type examined here) were found with low numbers of spermatozoa in their spermatheca over three consecutive years. Those queens were reared in a different apiary to those reared in this study.

Hence, further follow-up studies aimed at determining the cause of poor spring matings seems warranted.

3.2 Feeding dietary supplements to queen banks

In the second year of this project, dietary supplements were fed to queen banks. This experiment was conducted in autumn when there was almost no bee forage in the local environment. As with the experiment on cell-building colonies, the colonies used as banks consumed their diets at different rates, and some became stronger than others as the experiment proceeded, despite all colonies being standardised prior to the commencement of the experiment.

When examining the physical characteristics of the banked queens no differences would have been expected in their ovariole numbers or in their numbers of spermatozoa in their spermatheca, as these characteristics would have been fully developed prior to banking. And indeed, these characteristics were much the same in queens of the different test groups. The average number of ovarioles observed in the surviving queens (164, SD 11.8) is comparable with that found in queens examined in the previous years' experiment (Section 2.2, Table 1). However, there was a marked improvement in the numbers of spermatozoa in the spermatheca of the banked queens compared with that observed in the previous years queens. The main difference between these two batches of queens was that the banked queens were autumn-reared, whereas those examined in the previous year had been spring-reared. Of the 98 surviving autumn-reared banked queens, 11.2% contained less than 1 million spermatozoa (20.8% in the previous years spring-reared queens), 23.5% contained 1-3 million spermatozoa (56.2%) and 64.8% contained more than 3 million spermatozoa (22.9%). The average number of spermatozoa per queen was 3.5 million, SD 1.77 million (2.1 million, SD 1.28 million in the spring-reared queens). These mating levels are comparable with overseas observations and suggest that, if there are problems with queen mating in Australia, it may be spring related.

The body weight of the banked queens was expected to be the main physical characteristic likely to be affected by feeding dietary supplements, particular given the poor foraging conditions available to each colony. However, the weights of queens in the diet-supplemented banks were no different from those of queens in the non-supplemented banks.

Two other interesting observations from the work presented here were that fewer queens died in banks that received protein-based diets and there was a tendency for heavier groups of queens to also show higher number of ovarioles and higher numbers of spermatozoa in their spermatheca (Table 2). This latter observation has also been observed in overseas studies (Van Eaton, 1986).

3.3 Feeding dietary supplements to drone-rearing colonies

In the final year of this project dietary supplements were fed to drone-rearing colonies. This experiment was conducted at the break of a drought when local bee forage was poor, but improving. The genetics of the drones was not as strictly controlled as for queens in the previous years' experiments, but nonetheless, the drone mothers were related and had been raised and mated in the same apiary. The same experimental variables encountered during years 1 and 2 were also encountered in this experiment.

Ruttner (1983) stated that drones reach full maturity 16 days after hatching as adults. When 20-day-old adult drones were removed from each of the drone-rearing colonies in this experiment and tested for spermatozoa numbers, more than 95% were not producing semen. Therefore, the remaining drones were kept locked in the drone-rearing colonies for a further 7 days before being tested again. After that time, approximately 65% were producing semen, indicating there is wide variation in the time taken for drones to reach maturity. Of the 27 day-old adult drones examined, the weights and numbers of spermatozoa in those reared in dietary-supplemented colonies were no different from that of drones reared in non-supplemented colonies. Perhaps all the drone rearing colonies were obtaining sufficient

forage from the local environment to more than adequately raise drones, but the uncontrolled experimental variables that become obvious during the experiment may have also contributed to the end results.

It is generally recognised that a mature individual drone produces about 10 million spermatozoa (Kaftanoglu and Peng, 1980), twice as many than is needed for a queen to become well mated. The average numbers of spermatozoa found in drones of the different test groups in this study compares well with this estimate, however the large standard deviations in the counts indicates that many drones examined were producing low numbers of spermatozoa. The issue of drone maturation times, spermatozoa numbers and its relevance to poor spring matings warrants further investigation.

3.4 Concluding remarks

The studies reported here are not the first to report no benefits from feeding dietary supplements to entire bee colonies. Another recent study in Victoria showed no gain in honey production or drop-off in the incident of European foulbrood disease by feeding dietary supplements to bee colonies (McKee, 2002). Nevertheless, it has been well demonstrated using caged honey bees that the growth and function of individual worker bees improves with the intake of external food. Hence the results obtained in the current study and in that of McKee (2002) may reflect on the composition of the dietary supplements used or the scientific methods employed to measure benefits.

The compositions of the diets used here were recommended by commercial queen producers and by an industry workshop on bee nutrition. However, not all the diets tested (especially the vitamin-based diets) are used by all queen producers. Indeed, many producers only use sugar syrup and pollen as dietary supplements and remain sceptical about the benefits from using other dietary supplements. This is probably due to the fact that very little is known about the actual nutritional requirements of queens and drones. Clearly, queen producers would benefit from more fundamental studies in this area. Such studies would be best done on caged queens and drones where experimental variables can be tightly controlled.

The general approach used here to measure benefits of feeding dietary supplements was also that recommended by commercial queen producers and an industry workshop on bee nutrition. Clearly, the approach is flawed because (a) it generates experimental variables which cannot be controlled and may influence end results and, (b) it relies on the measurement of physical attributes of queens and drones to gauge their ‘quality’ when there is no scientific evidence to show that the state of those attributes can be influenced by the intake of food. The approach is simply based on observations that have been made on adult queens in productive honey producing hives where, in general, those queens, which are regarded as ‘good quality queens’, tend to be heavy in weight, have higher numbers of ovarioles in their ovaries and higher numbers of spermatozoa in their spermatheca than queens heading poor performing colonies (Van Eaton, 1986). However, no scientific studies have shown that these physical traits of ‘good quality queens’ are a direct consequence of the way they were fed during their development. Indeed, one of the physical characteristics, the number of ovarioles in ovaries, has been shown to be highly influenced by age at which worker bee larvae development is diverted to queen larvae development (Woyke, 1971). To be able to accurately measure benefits in queens and drones from feeding dietary supplements some prior fundamental information is needed about which physical characteristic of adult queens and drones are influenced by food intake. Before more field-based research is directed at the dietary needs of queens and drones, laboratory-based research is needed to the following questions.

(a) Is the size, colour or shape of the physical characteristics of adult queens and drones influenced by their protein or carbohydrate intake during development?
(b) Are there limits to how the physical characteristics of queens and drones can be influenced by food intake during development?

(c) What effect does starvation have on the physical characteristics of queens and drones during their pre-adult and adult stages?

In conclusion, much fundamental research needs to be undertaken before the impacts of dietary supplements on artificial queen production can begin to be understood.

4. References

ANDERSON, D.L. (1993). Pathogens and queen bees. *Australasian Beekeeper*, **94**, 292-296.

CASAGRANDE-IALORETTO, D.C. CORREA BUENO, O. & SHORT, A.C. (1984). The number of ovarioles in queen bees *Apis mellifera*. *Naturalia, Sao Paulo*, **9**, 73-79.

HAYDAK, M.H. (1935). Brood rearing by honeybees confined to a pure carbohydrate diet. *Journal of Economic Entomology*, **28**, 657-660.

HAYDAK, M.H. (1937). The influence of a pure carbohydrate diet on newly emerged honeybees. *Annals Entomological Society of America*, **30** 258-262.

JAY, C. & DIXON, D. (1984). Infertile and Nosema infected honey bees shipped to western Canada. *Journal of Apicultural Research*, **23**, 40-44.

KAFTANOGLU, O. & PENG, Y. (1980). A washing technique for collection of honeybee semen. *Journal of Apicultural Research*, **19**, 205-211.

McKEE, B. (2002). Preliminary results presented at a honey bee research workshop held in Canberra, 2002.

RHODES, J. & SOMERVILLE, D. (2003). Introduction and early performance of queen bees – some factors affecting success. Rural Industries Research and Development Final Report. 37pp.

RUTTNER, F. (1983). Rearing and care of drones. In '*Queen Rearing*' (Edited by F. Ruttner). Apimondia Publishing House, Bukarest. 295-304.

VAN EATON, C. (1986). Determinants of queen quality in New Zealand commercial queens. *The New Zealand Beekeeper*, 28-30.

WINSTON, M.L. (1987). The biology of the honey bee. Harvard University Press. 281pp.

WOYKE, J. (1971). Correlations between the age at which honey bee brood was grafted, characteristics of the resultant queens, and results of insemination. *Journal of Apicultural Research*, **10**, 45-55.

5. Appendix 1

Details of the dietary supplements that were fed to 6 groups of 5 cell-building colonies[1]

Code	Dietary Supplement	Amount of supplement fed to each of 5 cell-building colonies	Amount of supplement consumed by each of the 5 cell-building colonies[2]
PO	Irradiated Western Australia pollen	300g	300g, 300g, 300g, 300g, 300g
PV	Irradiated Western Australia pollen plus 2.5% vitamins	300g	300g, 300g, 280g, 300g 295g
SF	Full fat Soya-flour (1 part), irradiated Western Australia pollen (1 part), terula yeast (1 part)[3,5]	300g	195g, 95g, 215g, 225g, 170g
SFV	Full fat Soya-flour-flour (1 part), irradiated Western Australia pollen (1 part), terula yeast (1 part) Plus 2.5% vitamins.[4]	300g	235g, 140g, 110g, 105g, 210g
VO	Vitamins in a 60% sucrose solution using tap water[6]	200ml	200ml, 200ml, 200ml, 200ml, 200ml
PP	Commercially available pollen pattie (kindly donated for the trail by Mr Rod Palmer, Queensland).	300g	300g, 300g, 300g, 300g, 260g

[1] : The PO, PV, SF, SFV and PP diets were each fed to a group of 5 cell-building colonies 7 days before queen cells were grafted into them, whereas the VO diets were each fed to one group of 5 cell-building colonies 4 days before queen cells were grafted into them.
[2] : Some colonies had not consumed all their diets by time queen cells were grafted into them.
[3] : The irradiated pollen was ground to a fine powder in a blender before being made into soft patties by adding irradiated honey. The irradiated pollen was supplied by Mr Rod Palmer, Queensland, while the irradiated honey was supplied by a commercial queen producer.
[4] : Same details as 3. The vitamins were Solominavit®
[5] : The Soya-flour used was Defiance®, full-fat flour, enzyme active, manufactured by Defiance Milling Co. P/L, Toowoomba, QLD. It was heated for 5 minutes at low temperature (to deactivate the enzyme) before being added to the rest of the diet.
[6] : The vitamins added were Solominavit®

16