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**Original article**

# Nitrogen and mineral constituents of honey bee worker brood during pollen shortage

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**Abstract** – Bee colonies were prevented from collecting pollen, and the effect on brood rearing and on the N, P, K, Ca, Na and Mg contents of pupae was studied. Under these conditions brood rearing was reduced and fully stopped, which lead to a decrease in population size, whereas control colonies with access to pollen developed normally. Only a few significant differences were found in chemical analyses in pupae and worker bees of colonies with and without access to pollen. We conclude that the major feature of honey bee response to pollen shortage is a termination of brood rearing, and that those pupae still reared contain similar quantities of nitrogen and of most minerals as pupae reared during good foraging conditions. © Inra/DIB/AGIB/Elsevier, Paris

***Apis mellifera* / brood rearing / pollen / nitrogen / minerals**

## 1. INTRODUCTION

Honey bees (*Apis mellifera* L.) feed mainly on nectar and pollen. Pollen is the main source of protein for the whole colony. During weather conditions that prevent forager bees from harvesting pollen a protein shortage may occur. Adult bees from brood reared under such conditions had less dry substance, less protein content and shorter lifespans [4, 13]. However, when larval food was analysed [12] no correlation was found between its protein content and the lifespan of the bees which emerged later. It is known that bees feed on pollen within the first day after emergence, and that during their first 3 days the workers' nitrogen content increases from 2 to 3 mg [18]. Shorter lifespans, lower nitrogen contents or lower dry weights may therefore result from having no, or little, access to pollen after emergence of worker bees during periods of poor or no pollen

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harvest. If worker bees were experimentally excluded from feeding on pollen shorter lifespans were observed [14]. We measured for the first time numbers and qualities including dry weight and content of nitrogen and minerals subsequent to a pollen shortage on pupae; thereby excluding effects of feeding on pollen after emergence of the bees.

## 2. MATERIAL AND METHODS

### 2.1. Bees

Two (colonies 5 and 8) of four honey bee colonies similar in population size and stored honey and pollen were allowed to collect pollen freely between 23 April and 11 May 1987 (controls). Two other colonies (numbers 144 and 200) were confined separately under a tent of wire mesh (8 × 3 × 2.5 m) where no pollen was available (test). All colonies had ample honey stored for the duration of the test and were chosen for their similar amounts in stored pollen. The amount of pollen collected by the control colonies was determined using pollen traps [10]. Samples of worker bees for chemical analysis were obtained under conditions when in test colonies the last larvae, no larvae, or fresh larvae were reared (*figure 1*). The samples of pupae were likewise divided into subsets of: larvae fed before or at the onset of the test; the last pupae; and new brood reared after the end of the pollen shortage (*figure 1*). By estimating the number of worker bees and the surface of open and sealed brood the populations of the colonies were monitored [11] on the dates shown in *figure 2*. Additional data such as the growth of colonies per period were calculated as described by Bühlmann [2].

### 2.2. Sampling for chemical analysis

Samples of 200 worker bees of mixed age from combs, and samples of 50 pupae (dark eyes = Pd, Rembold et al. [15]) were collected per colony (*figure 1*). The bees were killed with ethyl ether, their alimentary tracts removed, and the bees and alimentary tracts were analysed separately.

### 2.3. Preparation of samples

All samples of pollen, pupae, bees and alimentary tracts were dried for 48 h at 108 °C, and homogenized. Nitrogen analysis of the samples was performed according to the Kjeldahl block digestion method after measurement of the dry weight [9]. For analysis of the sample P, Na, K, Ca and Mg content, 0.2–0.4 g of dry material were dried for 2 h at 150 °C, cooled to room temperature, then slowly heated for 3 h to 510 °C and maintained at that temperature for 4 h. The ash was dissolved in 2.5 mL 1 mol/L HCl diluted with $H_2O$ to attain a final volume of 25 mL. The element content of this solution was determined by atomic adsorption photometry (flame adsorption). The wave lengths used were 589.0 nm for Na, 766.5 nm for K, 422.7 nm for Ca and 285.2 nm for Mg. For the analysis of P the solution was mixed with $MoNa_2O_4$ and ascorbic acid to build Molybdenum Blue and measured photometrically at 820 nm.

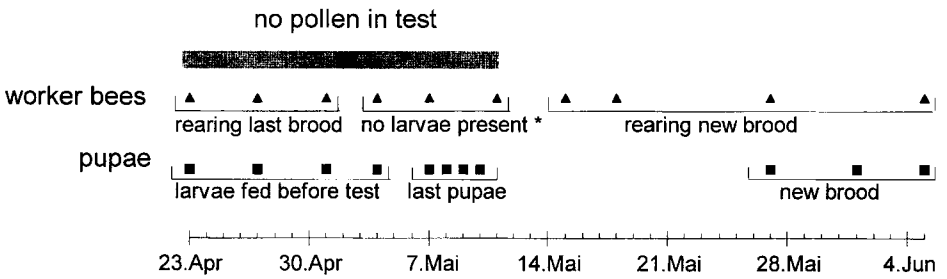


**Figure 1.** Sampling dates in control and test colonies during experiment: sampling of worker bees (triangles, $n = 200$) and of pupae (Pd, squares, $n = 50$) for chemical analyses. * On 1 May 1- and 2-day-old larvae were observed, but these were removed on 4 May.

## 2.4. Statistics

The development of the populations in control and test colonies of worker bees and of brood cells was obviously different and therefore not statistically analysed (*figure 2*). An ANOVA test with a 'repeated measures design' was used to detect significant differences between series of values obtained by chemical analyses from control and test colonies. This method takes into account the fact that the values were not independent but obtained from successive samples of the same population, i.e. the four colonies. Thus, the succession of values over time for each colony rather than individual values were analysed, and the two test colonies were compared to the two control colonies.

# 3. RESULTS

## 3.1. Population development

Populations of worker bees and brood cells during the total of the experiment are shown in *figure 2*. The most obvious effect of the pollen shortage was seen in the decrease in brood cells from 1 May to 11 May in test colonies. Thereafter the workers resumed collecting pollen and subsequently rearing brood. With a delay of 2 weeks, the population of worker bees also declined in test colonies, which was the result of the low numbers of brood reared.

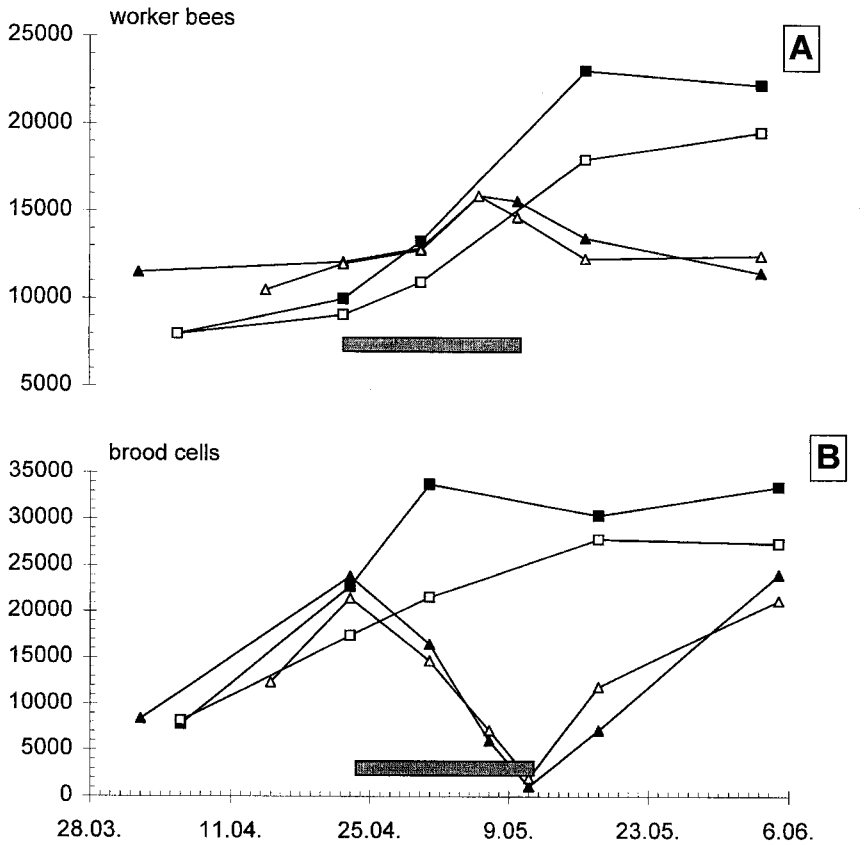


**Figure 2.** Populations of worker bees (A) and brood cells (B). Grey bars represent artificial pollen shortage in test colonies from 23 April to 11 May. Solid squares: control colony 5, open squares: control colony 8, solid triangles: test colony 144, open triangle: test colony 200.

### 3.2. Pollen provision in control colonies

The amount of pollen collected by the control colonies and its nitrogen and mineral constituents are given in *table I*. In our study a pupa with dark eyes (Pd) contained 1.94 mg nitrogen (mean of all values measured in control colonies, *table II*). Between 23 April and 18 May the two control colonies harvested 5 147 and 3 489 mg pollen. During the same period they reared 51 900 and 35 700 larvae, respectively. Thus, per larva a total of 100 or 98 mg pollen (3.72 mg or 3.42 mg nitrogen) was available in the colonies.

### 3.3. Chemical analysis

To give an idea of the values found in control colonies, we present the means of all samples taken in the control colonies (*table II*). *Table III* indicates bee stages and compounds with significant differences between test and control colonies (ANOVA repeated measures design, $P > 0.05$). Significant differences found are presented graphically in *figures 3–5*. The values found in pupae for Na ($P < 0.01$, *figure 3A*) and Ca ($P < 0.05$, *figure 3B*) followed significantly different courses in test colonies during the whole sampling period compared to those in control colonies. There was no significant difference in the nitrogen content of pupae between test and control groups as shown in *figure 3C*. Worker bees from test colonies (with their alimentary tracts removed) were heavier in dry weight when rearing no larvae than those from control colonies ($P < 0.05$, *figure 4A*) and they contained less Na over the whole sampling period ($P < 0.01$, *figure 4B*). As regards the other parameters studied we found no significant differences between test and control colonies. The alimentary tracts (AT) of the worker bees were significantly lighter in dry weight when not rearing brood in test colonies than in controls ($P < 0.05$, *figure 5A*), and contained significantly less nitrogen ($P < 0.05$, *figure 5B*) and Mg ($P < 0.05$; *figure 5C*) than those of control colonies due to the empty AT in bees from test colonies.

**Table I.** Pollen collected by control colonies and its content of nitrogen per colony and sampling period (A) and total mineral constituents between 6 April and 5 June 1987 (B).

| Date | Number of days | Colony 5 pollen (g) | Nitrogen (mg) | Colony 8 pollen (g) | Nitrogen (mg) |
|---|---|---|---|---|---|
| (A) | | | | | |
| 6.04.87 | – | – | – | – | – |
| 23.04.87 | 17 | 614 | 22 600 | 239 | 11 100 |
| 1.05.87 | 8 | 4 018 | 185 900 | 2 238 | 93 500 |
| 18.05.87 | 17 | 1 130 | 56 000 | 1 251 | 60 000 |
| 5.06.87 | 18 | 3 152 | 162 300 | 3 431 | 172 000 |

| | Colony 5 | Colony 8 |
|---|---|---|
| (B) | | |
| Pollen (g) | 8 921 | 7 159 |
| Content (mg) of | | |
| N | 426 700 | 336 600 |
| P | 55 300 | 46 800 |
| Na | 254 | 197 |
| Ca | 20 750 | 16 860 |
| K | 72 600 | 61 000 |
| Mg | 12 300 | 10 200 |

**Table 2.** Means and standard deviations of all values found in chemical analyses in control colonies (amounts per bee or pupa).

| | Adult bees | | Aliment. tracts | | Pupae | |
|---|---|---|---|---|---|---|
| | mean | ± s.d. | mean | ± s.d. | mean | ± s.d. |
| Dry weight (mg) | 27.26 | 5.48 | 9.27 | 0.81 | 23.06 | 1.94 |
| N (mg) | 2.36 | 0.16 | 0.55 | 0.04 | 1.85 | 0.10 |
| P (μg) | 191.55 | 11.72 | 104.64 | 13.06 | 190.27 | 8.59 |
| Na (μg) | 13.47 | 1.86 | 4.31 | 2.49 | 10.71 | 0.97 |
| Mg (μg) | 23.33 | 1.71 | 22.46 | 3.73 | 26.33 | 0.95 |
| K (μg) | 226.58 | 25.80 | 202.13 | 43.44 | 319.03 | 13.32 |
| Ca (μg) | 10.83 | 1.65 | 38.32 | 6.68 | 12.15 | 0.46 |

**Table 3.** Significant differences (ANOVA test, repeated measures design, $P < 0.05$) found between test and control either in the whole series of sampling or in one of the subseries.

| | Adult bees | Aliment. tracts | Pupae |
|---|---|---|---|
| Dry weight | sign | sign | – |
| N | – | sign | – |
| P | – | – | – |
| Na | sign | – | sign |
| Mg | – | sign | – |
| K | – | – | – |
| Ca | – | – | sign |

## 4. DISCUSSION

The populations of worker bees in control colonies developed normally. In the same year (1987), the populations of 16 colonies were estimated in the same region using the same method. In the same period these colonies reached mean populations of 21 000 worker bees [5]. The decline in the populations observed in the test colonies and starting at 7 May, however, is highly unusual for the season. In test colonies 54 550 and 57 160 bees emerged between 23 April and 5 June, while in control colonies 87 000 and 71 050 bees were calculated from population estimations. The difference was due to the suspension of rearing brood during the pollen shortage. In the two control colonies 34 000 and 28 000 brood cells were observed at the beginning of June, which corresponds to similar numbers of brood cells in 16 other colonies in the same year (30 000 brood cells, Fluri and Imdorf [5]). The number of brood cells in the test colonies declined rapidly as soon as pollen was no longer available to the colonies. It increased again as soon the bees had access to pollen. The presence of eggs was observed, but no larvae were found in the last week of pollen shortage.

In our study a pupa with dark eyes (Pd) contains 1.94 mg nitrogen (mean of all values measured in control colonies, *table II*). Assuming an efficacy of nitrogen utilization of

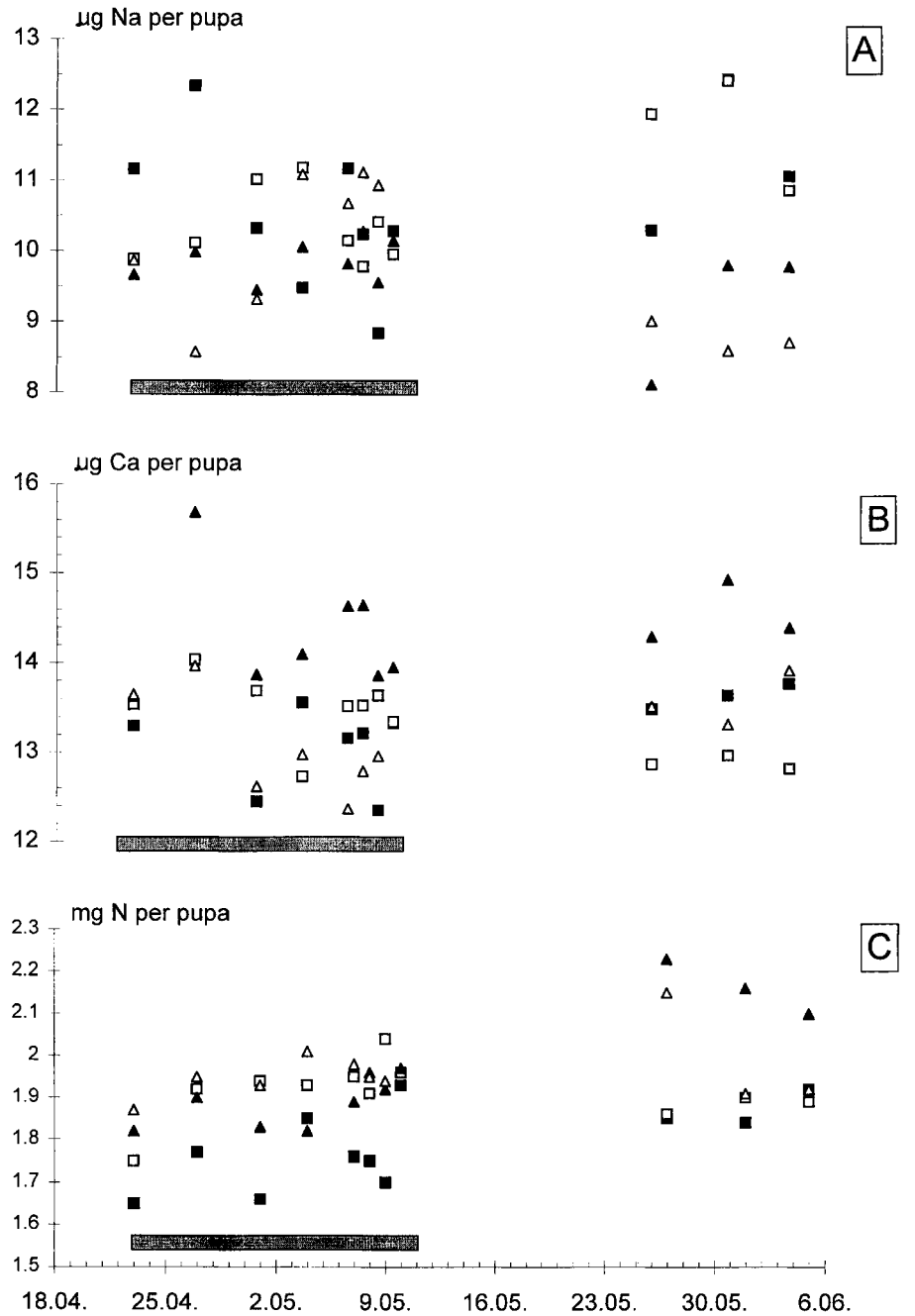


**Figure 3**. Content of Na (A), of Ca (B) and of nitrogen (C) of pupae. Significant differences between test and control were found for Na in the whole series as well as in the subseries 'larvae fed before test' and 'new brood' ($P < 0.05$), for Ca in the whole series ($P < 0.05$), but no difference was found for nitrogen. Symbols as in *figure 2*.

80 % as reported by Schmidt and Buchmann [16] and confirmed by our own unpublished results, a pupa requires 2.4 mg of nitrogen. Haydak [6] calculated a minimum need of 3.21 mg N to rear one worker bee. Between 23 April and 18 May the two control colonies harvested 5 147 mg and 3 489 mg pollen, containing 193 and 122 g nitrogen, respectively. During the same period they reared 51 900 and 35 700 larvae, respectively. Thus per larva 100 or 98 mg pollen (3.72 mg or 3.42 mg nitrogen) were available. Our control colonies collected sufficient amounts of pollen and we may assume that, although adult bees also feed on pollen, the pupae were fed sufficiently. If not, the number of brood cells reared in these control colonies would not have grown as observed (*figure 2*). Thus, the lack of significant differences in the chemical contents of pupae in test and control colonies is not due to insufficient nutrition in the control colonies. The major difference between test and control colonies lies in the number of immatures reared during times of pollen shortage.

These results allow us to conclude that those individuals reared in the test colonies

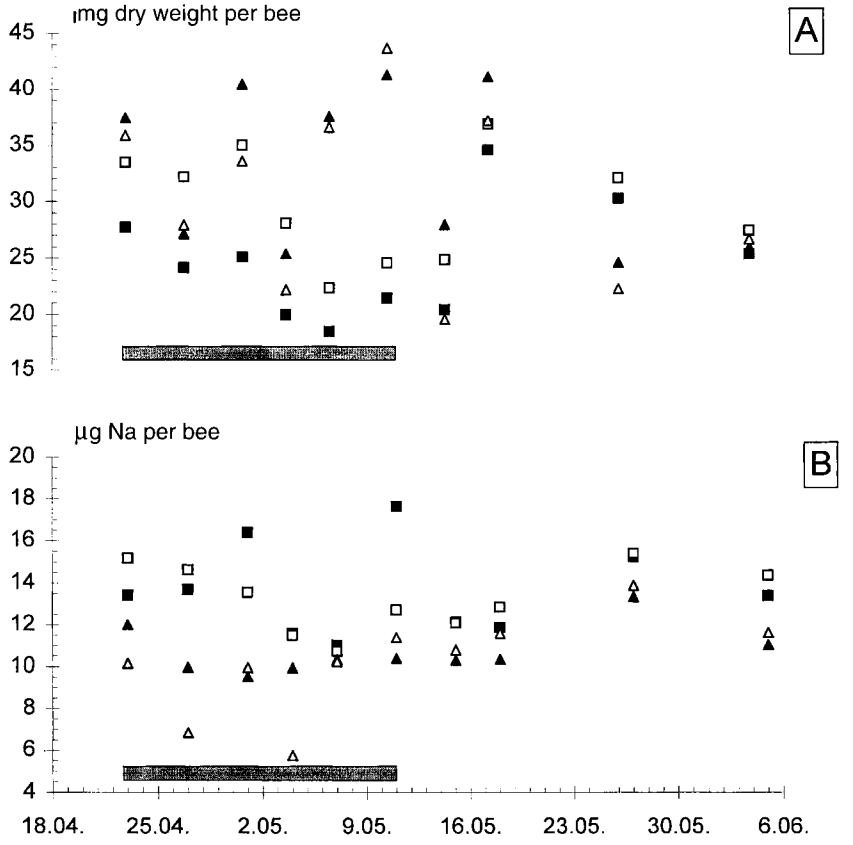


**Figure 4.** Dry weight (A) and content of Na (B) of worker bees with their alimentary tracts removed. Significant differences between test and control were found in dry weight in the subseries of 'no brood present' ($P < 0.05$) and in Na for the whole series and the subseries of 'rearing last brood' ($P < 0.05$). Symbols as in *figure 2*.

during the period of pollen shortage developed similarly to the control colonies. That is, in cases of pollen shortage, the nurse bees feed larvae as long as possible. Bee colonies fed on a pure carbohydrate diet manage to rear brood for 1 week [6], during which time worker bees mobilize proteinaceous material from their body. When a lack of nutrients would result in undernourished offspring, they stop rearing brood and even remove the larvae (e.g. [6, 17]). In the hypopharyngal glands of worker bees larval food jelly is synthesized and the glands' activity depends, among other factors, on the pre-

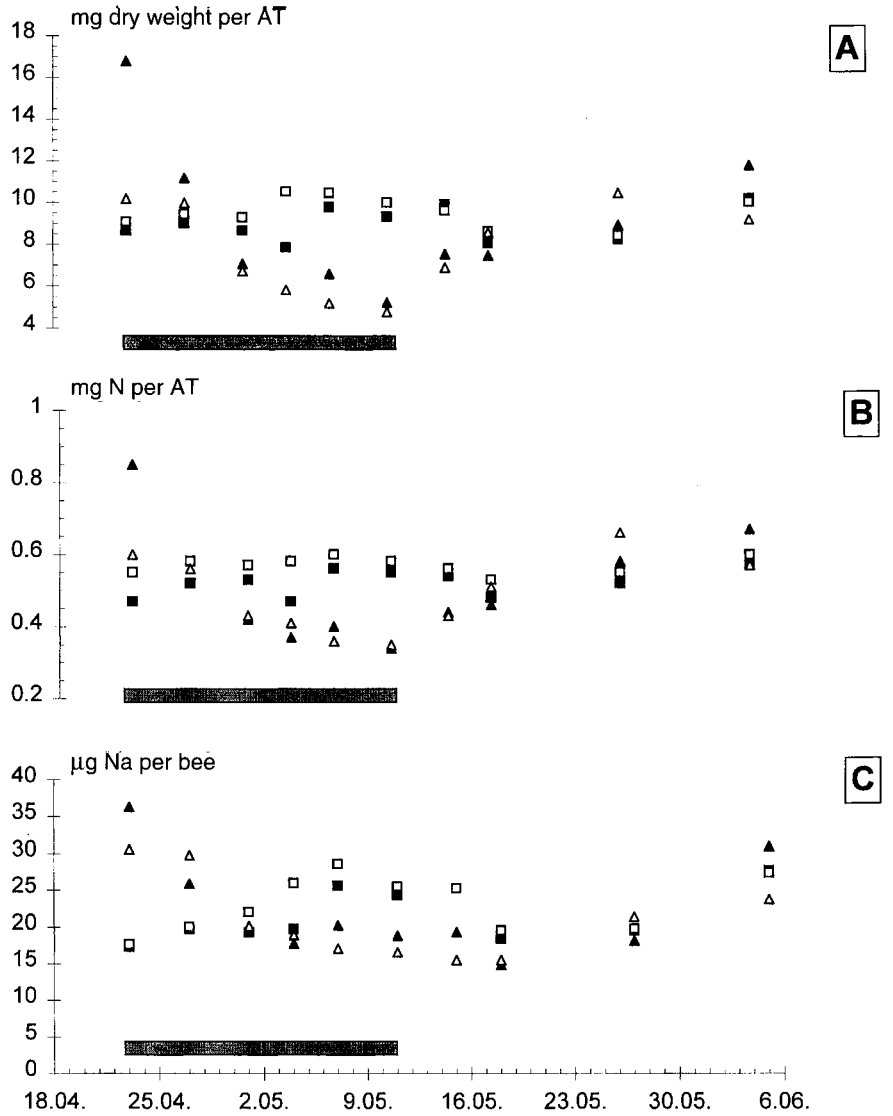


**Figure 5.** Dry weights (A), content of nitrogen (B) and Mg (C) of alimentary tract AT of bees. Significant differences between test and control were found for dry weight, content of nitrogen and Mg each in the subseries of 'no brood reared' ($P < 0.05$). Symbols as in *figure 2*.

sence of pollen [1]. If no pollen was present, the hypopharyngal gland activity of nurse bees was reduced [1] and, thus, the total of the colony's capability to rear brood is reduced.

Inspection of test colony 144 on 1 May showed 5 400 open brood cells with eggs and up to 2-day-old larvae but none older. In test colony 200, 5 000 open brood cells with eggs or 1-day-old larvae were observed on 1 May. On 4 May, eggs only but no larvae were observed. On 7 May 3 800 and 5 800 eggs, respectively, and on 11 May 1 200 and 2 000 eggs were found in test colonies, but again no larvae. Thus, both queens continued to lay eggs, while worker bees removed hatching larvae. After a delay of some days, the queens reduced their egg laying. It seems that the worker bees regulate the presence of larvae under limited food conditions. Removal of brood by worker bees is well documented and, especially when larvae are cannibalized, may be used as last reserves of nutritients in a colony during starvation or malnutrition [17].

Some studies cover mineral requirements for bees rearing brood (e.g. [7, 8]. For mineral contents of adult worker bees our results give similar ranges of P, K, Ca, Mg and Na contents to those reported by Dietz [3]. However, very little information is available on mineral contents of pupae. We found two minerals, Na and Ca, in significantly different amounts in pupae from control and test colonies. For both substances, the statistical differences were found over the whole course of the experiment (*figure 3*). Nevertheless, we can not interprete these differences as obvious effects of pollen shortage. If pollen shortage had an effect on the contents of nitrogen and mineral constituents of the brood, one would expect in this experimental design that any differences observed during the pollen shortage would diminish when pollen is again available.

We did not find lower nitrogen contents in those pupae reared during the pollen-free period. Kunert and Crailsheim [13] found that newly emerged summer bees, reared under good foraging conditions, had significantly higher protein contents than those reared under poor ones. Their results confirmed Haydak [6] who reported that newly emerged worker bees reared in colonies on a pollen-free diet had lower nitrogen contents than bees reared in colonies having access to pollen. A possible explanation for these differences might be the different stages of worker bees which were examined under different experimental conditions. Or, poor foraging conditions can be caused by bad weather, i.e. in addition to low income of pollen cold temperatures could also influence brood rearing. Furthermore, when analysing larval food jelly, Kunert and Crailsheim ([13] and refs therein) found high variances in protein content, but no correlations between the protein content of larval food and weight, protein content or lifespan of the later emerging bees.

Only a few significant differences were found in the chemical analyses between samples of control and test colonies. Significant differences in adult worker bees fed with or without pollen reported in the abovementioned reports are not directly comparable to our findings. Our bee samples were of unidentified age, whereas in the above studies the emerging workers were examined. In addition, the contents of nitrogen found in our analyses were within the ranges reported by Kunert and Crailsheim [13] and by Haydak [6]. The conclusion that the lower protein content of emerging bees is due to the larvae being fed poorer jelly or smaller amounts of jelly by the nurses contradicts our results.

The nitrogen and mineral constituents of pupae found in this study provide no conclusive evidence for an effect of pollen shortage on brood quality. However, it seems that instead of providing brood with insufficient food jelly bees reduce rearing brood and remove larvae during pollen shortage. By rearing healthy brood only the colony may minimize losses of nutritients and energy during periods of bad foraging conditions.

## ACKNOWLEDGEMENTS

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**Résumé – Teneur en azote et en éléments minéraux du couvain d'ouvrières (*Apis mellifera* L.) en cas de pénurie de pollen.** Pour étudier la réaction des colonies d'abeilles à une pénurie de pollen on a placé deux colonies sous une cage (test) dépourvue de toute source de pollen et deux autres à l'extérieur (témoins), qui pouvaient récolter du pollen. Le nombre d'ouvrières et de cellules de couvain a été estimé à plusieurs reprises (*figure 2*). On a prélevé des échantillons d'ouvrières et de nymphes aux yeux foncés (= Pd selon Rembold et al. [15]) et analysé leur teneur en N (méthode Kjedahl) et en P, Ca, Na et Mg (photométrie de flamme). Les colonies expérimentales sont suspendu l'élevage de nouveau couvain quelques jours après le début de la pénurie de pollen (*figure 1*). En conséquence la quantité de couvain et, avec quelque retard, le nombre d'ouvrières ont régressé par rapport aux colonies témoins. En revanche, les ouvrières et les nymphes de toutes les colonies avaient la même teneur en azote. La teneur des nymphes en éléments minéraux n'était que faiblement significativement différente entre les colonies expérimentales et les témoins. Les *figures 3* à *5* donnent les résultats qui présentent une différence significative entre les colonies expérimentales et les témoins. Les teneurs en azote et en éléments minéraux des nymphes ne prouvent pas de façon décisive une quelconque action de la pénurie de pollen sur la qualité de couvain. Au contraire, les ouvrières semblent suspendre l'élevage de couvain et éloigner le couvain restant plutôt que de fournir une nourriture insuffisante aux larves. En élevant exclusivement du couvain sain, les colonies d'abeilles évitent des pertes d'énergie et de nutriments en cas de miellée insuffisante © Inra/DIB/AGIB/Elsevier, Paris

***Apis mellifera* / élevage couvain / pollen / azote / élément minéral**

**Zusammenfassung – Stick- und Mineralstoffgehalt von Bienenbrut bei Pollenmangel.** Es wurde ermittelt, wie Bienenvölker auf einen Pollenmangel reagieren. Dazu wurden 2 Völker in ein Zelt gebracht, in dem sie keine Pollenquellen fanden (Test), während zwei andere Bienenvölker frei fliegen und Pollen sammeln konnten (Kontrolle). Die Anzahl Arbeiterinnen und Brutzellen der Völker wurde in Intervallen geschätzt. Zudem wurden Stichproben von Arbeiterinnen und von Puppen mit dunklen Augen (Pd, nach Rembold et al. [15]) auf ihre Gehalte an N (Kjedahl), P, K, Ca, Na und Mg (Flammen-Adsorption) analysiert. Die Versuchsvölker stellten innerhalb von wenigen Tagen nach Einsetzen des Pollenmangels die Aufzucht von neuer Brut ein (*Abb. 1*). Entsprechend ging ihre Brutmenge und mit Verzögerung ihre Anzahl Arbeiterinnen im Vergleich zu den Kontrollvölkern zurück. Hingegen wiesen Arbeiterinnen und Puppen aller Völker ähnliche Gehalte an N auf. Puppen wiesen im Gehalt an Mineralstoffen nur wenig signifikante Unterschiede zwischen Versuchs- und Kontrollvölkern auf. Ergebnisse mit signifikanten Unterschieden zwischen Test- und Kontrollvölkern sind in den *Abb. 3* bis *5* dargestellt. Die Gehalte an Stickstoff und Mineralstoffen in Puppen ergeben keinen schlüssigen Beweis für Auswirkungen von Pollenmangel auf die entsprechende Brutqualität. Vielmehr scheinen Arbeiterinnen eher die Brutaufzucht einzustellen und die verbleibende Brut zu entfernen, als daß sie Larven mit ungenügender Nahrung versorgen würden. Durch die ausschließliche Aufzucht gesunder Brut kann das Bienenvolk bei ungenügender Tracht Verluste von Energie und Nährstoffen vermeiden. © Inra/DIB/AGIB/-Elsevier, Paris

***Apis mellifera* / Brutaufzucht / Pollen / Stickstoff / Mineralstoff**

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