NUTRITIONAL VALUE OF CERTAIN BEE BREAD TYPES AND THEIR EFFECTS ON HONEY BEE WORKERS

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\textbf{Abstract:} The nutritional value of certain bee bread types from monofloral sources was evaluated on honey bee workers under laboratory conditions, by determining the bee bread consumption and development degree of hypopharyngeal glands. The greatest rate of consumed food was recorded during the first six days (c. 79.7\%), specially the first three days (c. 44.7\%) from total consumption. The cumulative bee bread consumptions by bees during feeding period were 60.7, 58.7, 58.0, 57.1, 56.6, 51.6 and 50.6 mg/bee/21 days for caper, broad bean fennel, maize, Egyptian clover, canola and coriander bee bread, respectively. The highest degree of glands development was recorded in bees fed on canola (3.6) or broad bean bee bread (3.56), whereas the lowest score was obtained in bees fed on fennel bee bread. According to results the bee bread types could be classified into three categories depending on their nutritional values in relation to hypopharyngeal glands development as follows: the first group (most effective), the gland degree was more than 3.5, included canola and broad bean bee bread. The second category (considerably effective) ranged from 3 to 3.5, included Egyptian clover. Meanwhile, the third one (slightly effective) included maize, caper, coriander, and fennel bee bread. Since no single pollen source provides bees with all nutritional requirements. It can be recommended that honey bees used for pollinating monoculture crops e.g. maize, caper, coriander or fennel, needs to provide nutritional supplement sources to enrich their diets, to establish health colonies and good production.

\textbf{Key words:} honey bee, bee bread, consumption, hypopharyngeal gland, nutritional value.

\textbf{Introduction}

Honey bee workers collect pollen directly from the flowers and store it in the camb cells inside the hive. During collection and storage, pollen composition changes through the addition of mainly nectar and also glandular secretions. Then it undergoes a fermentative process to transfer bee bread, thereby acquiring a
specific smell and colour according to the plant species (Winston, 1987 and Roulston, 2005). This bee bread is the main source of several important nutrients and provides the bees with most of the nutritional requirements e.g., proteins (amino acids), lipids (fatty acids and sterols), vitamins and minerals. Such contents specially protein in collected pollen from different plants are variable and range from 8 to 40% (Herbert, 1992). The protein derived from bee bread is largely used to feed developing larvae and young bees up to the age of 15-18 day, to provide bees with the structural elements of muscles, glands and other tissues and is essential for the development of hypopharyngeal glands which produce royal jelly (Hydak, 1970 and Crailsheim et al., 1992). Colonies that consume more bee bread are able to rear more bees (Campana and Moeller, 1977). Colony growth can be limited by either a lack of bee bread or, by the available bee bread lacking the necessary nutrients (Loper and Berdel, 1980).

Honey bee workers start to consume bee bread within the first 42 to 52 hours after emergence (Hagedorn and Moeller, 1968). The greatest quantity of bee bread is consumed by 3-6 day-old (Zherebkin, 1965). Development of hypopharyngeal glands is influenced by the quantity and quality of protein ingested by workers (McCaughey et al., 1980 and Hrassnigg and Crailsheim, 1998). In the present study, bee bread was used since bees do not consume fresh pollen and bee bread has greater food value than fresh pollen (Stroikov, 1963).

Materials and Methods

The present study evaluates the nutritional value of seven single bee bread diets for honey bee workers, by determining the bee bread consumption and measuring the development of hypopharyngeal glands. It means to know which bee bread types actually have a high or low nutritive value for the bees. The hypopharyngeal glands parameter was used in the present study because the glands development is influenced and more sensitive to the quantity and quality of protein ingested by workers (McCaughey et al., 1980 and Hrassnigg and Crailsheim, 1998). In the present study, bee bread was used since bees do not consume fresh pollen and bee bread has greater food value than fresh pollen (Stroikov, 1963). The study was carried out at the Faculty of Agriculture, Assiut University under laboratory conditions during 2007. The first hybrid of Carniolan bees, Apis mellifera L. workers were used.
Bee bread extraction:
During the different times of 2006 season, bee bread was extracted from honey bee colonies, according to the protocol: Numbers of bee colonies were placed in seven farms in Assiut area containing monofloral species: Egyptian clover (Trifolium alexandrinum) from Musha, broad bean (Vicia faba), maize (Zea mays), caper (Brassica kaber) from Alwan, canola (Brassica napus), fennel (Faeniculum vulgare) from Abnoub and coriander (Coriandrum sativum) from Dyroot. All these sources were the common cultivated plants in Assiut Governorate, and the main pollen sources for honey bee colonies (Hussein, 1982). At the end of flowering period, the bee bread was extracted from the different bee colonies for each of monofloral farm. Bee bread from each source was stored under freezing conditions until using it. For sure, identification of bee-stored pollen types was done microscopically in comparison with standard pollen grains collected from the anther flowers (Hussein, 1983).

Determination of bee bread consumption:
To investigate the bee bread consumption by honey bees, the tested bee bread was offered to each cage. Equal amounts (10 g) of each tested bee bread was weighed into clean plastic feeders and placed into the cage. Three days later, the net weight of the remaining bee bread in the feeders was recorded to calculate the consumed bee bread and fresh bee bread was provided to replace unfed diets. This procedure was repeated 7 times at a three days interval, until bee bread consumption stopped. The cumulative bee bread consumption during the experimental period was determined are recorded.

Determination of hypopharyngeal glands development:
Measuring of hypopharyngeal glands development as an effective physiological parameter was used to evaluate the quality of bee bread consumed. Because the development of hypopharyngeal glands is strongly correlated with the amount of protein consumed by workers from bee bread diets (Haydak, 1961). The development of glands proved to be more sensitive measure of protein utilization and bee bread quality (Pernal and Currie, 2000).

To study the effect of the different types of bee bread on hypopharyngeal glands development, every three days ten bee workers were removed from each cage. This procedure was repeated seven times at a three day interval. The head of each worker was dissected under stereo-microscope (40 times magnification force) and their glands were removed to determine the degree of hypopharyngeal glands development according to the criteria suggested by Maurizio (1954). An arbitrary scale (I to IV) was used to determine the degree of development; grade I, represented undeveloped
gland and grade IV represented complete development.

**Statistical analysis:**
Mean numbers were compared according to Duncan's multiple range test and LSD at 0.05 probability (SAS Institute, 1990).

**Results and Discussions**

**Bee bread consumption:**
In all tested bee bread, the consumption patterns were nearly similar. Generally, the bees consumed the bee bread at the greatest rate during the first six days (79.5%), especially the first three days (44.7%). Thereafter, the consumption decreased sharply and stopped by the 15th day, when bee bread ingestion was no longer measurable. Similar results were obtained by Jaycox (1981) who reported that the consumption peak recorded at three to five days of age and then decreased to a low level by time. Pollen is consumed by bees until they reach aged 15-18 days (Zherebkin, 1965), that result comes in agreement with the result of the present work. Pollen consumption varies with age and function of honey bee workers. Typically, nurse bees are the main pollen consumers and have a higher proteolytic activity than other workers (Crailsheim et al., 1992 and Loidl and Crailsheim, 2001). The amounts of bee bread consumed per bee during the first three days were 27.5, 26.6, 25.7, 25.1, 25.0, 23.4 and 21.9 mg/bee for bees fed on maize, caper, Egyptian clover, coriander, broad bean, canola and fennel bee bread respectively (Table 1).

**Table(1):** Bee bread consumption by honey bee workers fed on seven types from monoflora sources, at a period of 21 days.

<table>
<thead>
<tr>
<th>Following days</th>
<th>Caper</th>
<th>Broad bean</th>
<th>Fennel</th>
<th>Maize</th>
<th>Egyptian clover</th>
<th>Canola</th>
<th>Coriander</th>
<th>Grand Mean ±SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>1-3</td>
<td>26.6</td>
<td>25.0</td>
<td>21.9</td>
<td>27.6</td>
<td>25.7</td>
<td>23.4</td>
<td>25.1</td>
<td>25.04A±1.91</td>
</tr>
<tr>
<td>4-6</td>
<td>24.5</td>
<td>14.8</td>
<td>20.1</td>
<td>23.6</td>
<td>20.3</td>
<td>20.1</td>
<td>13.7</td>
<td>19.59B±4.06</td>
</tr>
<tr>
<td>7-9</td>
<td>6.1</td>
<td>7.8</td>
<td>6.2</td>
<td>4.8</td>
<td>6.4</td>
<td>6.5</td>
<td>5.6</td>
<td>6.63C±0.85</td>
</tr>
<tr>
<td>10-12</td>
<td>2.5</td>
<td>5.6</td>
<td>8.7</td>
<td>1.0</td>
<td>1.5</td>
<td>1.4</td>
<td>5.6</td>
<td>3.76CD±2.92</td>
</tr>
<tr>
<td>13-15</td>
<td>0.5</td>
<td>5.1</td>
<td>0.6</td>
<td>0.1</td>
<td>1.4</td>
<td>0.2</td>
<td>0.5</td>
<td>1.20D±1.77</td>
</tr>
<tr>
<td>16-18</td>
<td>0.2</td>
<td>0.4</td>
<td>0.5</td>
<td>0.0</td>
<td>1.3</td>
<td>0.0</td>
<td>0.1</td>
<td>0.35E±0.44</td>
</tr>
<tr>
<td>19-21</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.00D</td>
</tr>
<tr>
<td>Total consumption mg/bee/21 days</td>
<td>60.7</td>
<td>58.7</td>
<td>58.0</td>
<td>57.1</td>
<td>56.6</td>
<td>51.6</td>
<td>50.6</td>
<td>56.19</td>
</tr>
<tr>
<td>Grand Mean</td>
<td>8.63</td>
<td>8.39</td>
<td>8.29</td>
<td>8.16</td>
<td>8.09</td>
<td>7.37</td>
<td>7.23</td>
<td></td>
</tr>
</tbody>
</table>

LSD at 0.05 probability = 1.60

Means have the same letter(s) do not significantly different at 0.05 level of probability.
By the end of feeding period, the cumulative bee bread consumptions by bees during full feeding period were 60.7, 58.7, 58.0, 57.1, 56.6, 51.6 and 50.6 mg/bee/21 days for caper, broad bean, fennel, maize, Egyptian clover, canola and coriander bee bread, respectively (Fig. 1).

**Fig.(1):** Cumulative consumption of bee bread for days bee workers which fed on seven types during the experimental period.

There were no significant differences among all treatments under the experimental conditions, although the seven types of bee bread were different in the nutritional value, specially protein content. So, the bee bread consumption did not increase by the decrease in bee bread protein. Maize bee bread which had low protein; 14-15% (Stace, 1996) was consumed nearly in the same proportions as higher-protein bee bread e.g. broad bean which has 24% protein (Somerville, 1995). The present results are confirmed by the lack of any relationship between the crude protein content of the tested diets and their relative consumption by bees, which suggested that worker bees may have no inherent mechanism through which they can discriminate the protein content of
the consumed diet (Pernal and Currie, 2000). If bees could discriminate this aspect of pollen quality, it would be expected that larger quantities of some pollen species, e.g. *Helianthus* and *Pinus*, would have been consumed to compensate for their low protein content (Waldbauer and Friedman, 1991). Schmidt and Johnson (1984) found weak correlation between bee feeding preference and the protein level of pollen diets. These results agree with the present results in showing that bees do not increase pollen consumption to compensate for reduction in dietary protein. Also, suggests that consumption may be influenced by physical or chemical factors that are unrelated to pollen quality. In the same time, the present results disagree with the results of Kleinschmidt and Kondos (1978), that showed that the large increases in pollen consumption occur when the level of pollen protein decreases by 10%, in an apparent attempt to meet protein requirements.

**Effect of bee bread types on hypopharyngeal glands development:**

The development of hypopharyngeal glands of worker bees fed on different types of bee bread are shown in Table (2). The results clearly showed significant differences in the development of hypopharyngeal glands between the workers fed on only sugar solution (control) and that fed on each of the seven bee bread types under experiment conditions.

**Table(2):** Development degree of hypopharyngeal glands in bee workers fed on bee bread seven types from monoflora sources at a period of 21 days.

<table>
<thead>
<tr>
<th>Following days</th>
<th>Hypopharyngeal glands development</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Canola</td>
</tr>
<tr>
<td>3</td>
<td>3.05</td>
</tr>
<tr>
<td>6</td>
<td>3.53</td>
</tr>
<tr>
<td>9</td>
<td>3.73</td>
</tr>
<tr>
<td>12</td>
<td>3.88</td>
</tr>
<tr>
<td>15</td>
<td>3.58</td>
</tr>
<tr>
<td>18</td>
<td>3.60</td>
</tr>
<tr>
<td>21</td>
<td>3.85</td>
</tr>
<tr>
<td>Grand Mean ±SE</td>
<td>3.6A</td>
</tr>
</tbody>
</table>

Means have the same letter(s) do not significantly different at 0.05 level of probability.
The present results indicated that the general mean of hypopharyngeal glands development was 3.6, 3.56, 3.26, 2.79, 2.71, 2.7 and 2.47 for bees fed on canola, broad bean, Egyptian clover, maize, caper, coriander and fennel bee bread, respectively, whereas, the recorded degree of the development was the poorest score (1.13) in control bees (no bee bread) (Fig. 2).

Fig. (2): Development degree of hypopharyngeal glands in bee workers fed on seven types of bee bread during the experimental period.

The highest development of hypopharyngeal glands was recorded in bees receiving canola and/or broad bean bee bread. On the other hand, the lowest score was obtained in bees fed on fennel bee bread. The present results showed two peaks for the activity of hypopharyngeal glands; the first one was at 9th day for bees fed on each of broad bean, maize, coriander or caper, and at 12th day for canola, clover or fennel. Whereas, the second peak was recorded on 21st day for all bee bread types except for caper that was at 18th day. Similar results was showed by Rembold (1969).

According to the present results, bee bread types could suggested to classify to three categories, depending on their nutritional values in relation to
hypopharyngeal glands development as follows: the first category (more effective), the degree of hypopharyngeal development was more than 3.5. This category included canola and broad bean bee bread. In the second category (considerably effective), the degree was ranged from 3 to 3.5, included Egyptian clover bee bread. While, the third category (slightly effective), the degree was less than 3, included maize, caper, coriander and fennel bee bread.

According to Brauwers (1982) and Huang (1990), the hypopharyngeal glands, organs secreting enzymes and royal jelly, quickly respond to changes in the nutritional value of feed protein. This means that the development of hypopharyngeal glands was strongly correlated with amount of protein workers consumed from pollen diets, and crude protein content of diets (Pernal and Currie, 2000).

Pollen-feeding induces the growth of these glands, generally the size of the hypopharyngeal glands showed a similar age dependance as the intensity of pollen consumption i.e. it reached a maximum in c. 10-day-old bees (Crailshieim et al., 1992). It could be stated that the young adult worker bees do not consume needed proteins, their hypopharyngeal glands so, they not develop completely and their royal jelly will not support normal growth and development of worker larvae or egg production in the adult queen. The requirement for protein decreases if worker bees discontinue nursing; between 10th to 14th day of adult life (Standifer, 1967). Crude protein is a essential dietary component for the development and well being of bee colony. If newly emerged bees are kept on a pure carbohydrate diet their hypopharyngeal glands remain undeveloped (Maurizio, 1954).

Generally collected pollen by bees have different nutritional value and do not have the same physiological effects according to floral sources (Lauveaux, 1963). Protein levels vary from 6-65% (Roubik, 1989). The minimum protein level required for honey bees is 20%, also the balance of amino acids is also important. Kleinschmidt and Kondos (1976) found that pollen with less than 20% crude protein can not satisfy colony requirements for optimum production. The present results, workers were found to feed on canola or broad bean bee bread (more affect group) gave a high degree of hypopharyngeal glands development. These pollen types have very high crude protein levels, if compared to other pollen sources. The crude protein level was 24-27% and 24% for canola and broad bean pollen (Somerville, 1995 and Stace, 1996). Workers fed on Egyptian clover bee bread gave a considerable affect, because it contains about 22.7% crude protein level (Stace, 1996). Whereas bees fed on maize, caper,
coriander or fennel bee bread gave slightly development. These may be due to decrease in crude protein level, e.g. maize bee bread contains about 14-15%, this level is less than 20% crude protein can not satisfy colony requirements for optimum production (Kleinschmidt and Kondos, 1976). Hypopharyngeal glands development in nurse bees is positively correlated with pollen consumption (Hrassnigg and Crailsheim, 1998).

Since no single pollen source provides bee with all nutritional requirements. It could be stated that honey bees used for pollinating monoculture crops e.g. maize, caper, coriander or fennel, must have a number of pollen sources available to them, or will need to provide nutritional supplement sources to enrich their diets, to remain colony health and to produce the royal jelly required to feed the queen and rear brood.

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القيمة الغذائية لبعض أنواع خبز النحل وتأثيرها على شغالات النحل

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أجريت هذه الدراسة العملية بكلية الزراعة بجامعة أسيوط في موسم 2007، بغرض دراسة تأثير سبعة أنواع خبز نحل من المصادر النباتية: النحلة الشامية والفول البلدي، وعربع (من علوان)، الشمر والكائنولا (من أنسوب)، البرسيم (من موشأ) والكزبرة (من درويت) على معدل استهلاك النعاس ونمو وتطور غدد الغذاء الملكي كدليل على القيمة الغذائية لخبز النحل. أوضحت النتائج أنه لا توجد اختلافات معينة في معدل استهلاك خبز النحل بين الأنواع المختلفة. وقد سجلت أعلى نسبة لاستهلاك الغذاء الملكي خلال الأيام الستة الأولى وكانت نسبة 79.7% وخصوصا خلال الأيام الثلاثة الأولى (44.7%) من إجمالي الاستهلاك، ثم الاستهلاك بصورة حادة بعد ذلك. وأوضحت الدراسة أن إجمالي الاستهلاك الغذائي لخبز النحل كان 70.2، 78.7، 81.7، 87.7، 56، 70.5 مقلجرد/نحلة/12 يوم للشغالات التي تغذى على خبز النحل لكل من الكابور والفول البلدي والشمر والذرة الشامية والبرسيم المصري والكائنولا على التوالي. وقد أوضحت النتائج أن جميع أنواع خبز النحل المستخدمة أعطت تطوراً لأغذية الملكي بدرجات مختلفة بصورة معنية مقارنة بالكترول (بدون خبز النحل الذي لم يعطى تطوراً لغذاء الملكي). وقد سجلت أعلى درجة لتطور الغذاء في النحل الذي تغذيه على خبز الكائنولا (3.7، لالي) خبز نحل الفول البلدي. بينما سجلت أدنى قيمة لتطور الغذاء في النحل الذي تغذيه على خبز نحل الشمر. ومن خلال النتائج أمكن تقسيم أنواع خبز النحل إلى ثلاث مجموعات ببناءً على درجة تأثيرها على غدد الغذاء الملكي: وهي: مجموعة الأولى (أكثر تأثير) وفيها تكون درجة تطور الغذاء أعلى من 3.5 وتشمل خبز نحل كل من الكائنولا والفول البلدي، والгруппة الثانية (تأثير متوسط) وفيها تكون درجة تطور الغذاء ما بين 3، و 3.5 وتضم هذه المجموعة خبز نحل البرسيم المصري، بينما المجموعة الثالثة (تأثير طفيف) وفيها تكون درجة التطور أقل من 3، وتضمن خبز نحل كل من الذرة الشامية والكابور والكزبرة والشمر. ومن هذه الدراسة يوصى الباحث بإضافة خبز نحل عالي القيمة وأقل عدد غذائية صناعية لرفع نسبتهم الغذائية في حالة استخدام النحل في المناطق الزراعية إضافة إلى تحويل النحلة الشامية والكابور والكزبرة والشمر. ونفثه تظل طافحة خبز نحل العسل قوية وبقدرية على الإنتاج. وذلك لأنه لا يوجد خبز نحل مصدر نباتي واحد كاف لإمداد طائفة النحل بكامل احتياجاتها الغذائية.