The Effect of Prebiotic and Probiotic Feed Supplementation on the Wax Glands of Worker Bees (*Apis Mellifera*)

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Abstract

This paper presents the effects of acidifying substances (lactic acid or acetic acid), Enterobiotics products (*Lactobacillus acidophilus* LA-14 and *Bifidobacterium lactis* BI-04) and Enterolactis Plus (*Lactobacillus casei*) on the wax glands of worker bees. The research was conducted in Timis County, Romania, between March 25 and April 20, 2011, on 110 colonies of bees (*Apis mellifera carpatica*), allocated to 11 experimental treatment groups. Colonies in the experimental groups were given three weekly feeds of sugar syrup supplemented with acidifying substances (lactic acid or cider vinegar) and/or probiotic products (Enterobiotics or Enterolactis Plus). Three weeks after the administration of the experimental diets, 10 worker bees from each treatment group were sampled for histological examination of their wax glands. Gland development was shown to be influenced by administration of prebiotic and/or probiotic supplements. Wax gland cell sizes ranged from 25.1 microns for the control group to between 27.8 and 31.8 microns in the group fed with acidifying substances and between 26.9 and 29.2 microns in bees fed with probiotic products. Bees supplemented with both lactic acid and probiotic product (group LE₉ and LE₁₀) showed mean wax cell sizes of 31.8 microns.

Keywords: histological study, prebiotic and probiotic products, worker bee.

1. Introduction

Annual colony wax production is an important selection criterion in apiculture [1]. Prebiotic and probiotic supplementation of colony spring sugar syrup feeds has been shown to result in better colony development as evidenced by an increase in populated brood comb area [2-5]. It also leads to an improvement in bee health through reducing the innoculum of potentially pathogenic intestinal bacteria and repopulation of the intestinal tract with the benign species present in the probiotic supplement [6].

This study was aimed at investigating the effect of use of prebiotics (lactic and acetic acids) and probiotics (Enterobiotics and Enterolactis Plus) as supplements in bee colony spring stimulation feeds on honeybee wax gland development.

2. Materials and methods

The biological material consisted of colonies of bees (*Apis mellifera carpatica*), allocated to 11 experimental treatment groups each of 10 colonies, each of approximately equal vigour and with queens of the same age. The experiments were conducted in Timis County, Romania,
between March 25 and April 20, 2011, the bee colonies being housed in multistage hives. For three weeks, the colonies were fed sugar syrup supplemented with acidifying substances (lactic acid or acetic acid) and/or probiotic products (Enterobiotics or Enterolactis Plus) according to the scheme shown in Table 1.

### Table 1. Experimental treatment scheme

<table>
<thead>
<tr>
<th>No</th>
<th>Treatment group</th>
<th>Feed composition</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Sugar syrup (ml)</td>
</tr>
<tr>
<td>1.</td>
<td>Control group (C)</td>
<td>1000</td>
</tr>
<tr>
<td>2.</td>
<td>Experimental group 1 (EG₁)</td>
<td>1000</td>
</tr>
<tr>
<td>3.</td>
<td>Experimental group 2 (EG₂)</td>
<td>1000</td>
</tr>
<tr>
<td>4.</td>
<td>Experimental group 3 (EG₃)</td>
<td>1000</td>
</tr>
<tr>
<td>5.</td>
<td>Experimental group 4 (EG₄)</td>
<td>1000</td>
</tr>
<tr>
<td>6.</td>
<td>Experimental group 5 (EG₅)</td>
<td>1000</td>
</tr>
<tr>
<td>7.</td>
<td>Experimental group 6 (EG₆)</td>
<td>1000</td>
</tr>
<tr>
<td>8.</td>
<td>Experimental group 7 (EG₇)</td>
<td>1000</td>
</tr>
<tr>
<td>9.</td>
<td>Experimental group 8 (EG₈)</td>
<td>1000</td>
</tr>
<tr>
<td>10.</td>
<td>Experimental group 9 (EG₉)</td>
<td>1000</td>
</tr>
<tr>
<td>11.</td>
<td>Experimental group 10 (EG₁₀)</td>
<td>1000</td>
</tr>
</tbody>
</table>

The syrup sugar was administered in the same amount to all experimental variants three times: at the beginning of the experiment, at 7 days and at 14 days. The amount of sugar syrup used was 1.4 l / colony / week.

Three weeks after the administration of the sugar syrup a sample of 10 workers was collected from each treatment group for histological evaluation. In the laboratory each bee abdomen was incised anteriorly and posteriorly before being fixed in 4% formalin for 5 days. The samples were transferred to 80% ethanol before double staining with eosin-haematoxilin [7]. 4 micron sections were cut using a Leyca Microtome and viewed under an IOR binocular microscope.

Statistical processing of results was performed using the Minitab 14 software package.

### 3. Results and discussion

Results showed a favourable effect of prebiotic and probiotic feed supplementation on the development of cerides (wax flakes). Bees in the control group showed wax cells arranged in cords with vacuolar cytoplasm and large densely staining nuclei. This vacuolar appearance reflects the presence of cerides, cell secretion products which, because of their hydrophobic character, appear as distinct cytoplasmic droplets. Many anucleate hypertrophied wax cells (fig 1) of mean diameter 25.1 microns (Table 2) were in evidence.

![Figure 1. Control group. Wax cells at different stages of the secretory cycle (400x)](image1)

![Figure 2. Treatment group 1. Wax cells (400x)](image2)
Bees in groups EG1, EG2, EG3 and EG4 showed the presence of wax cells in much higher numbers than the control group. These cells had a more uniform appearance, with a spherical central core rich in chromatin. The cytoplasm was laden with small, lipid-like droplets (Figure 2). Mean diameters for wax cells of bees fed with acidifying syrup fell between 27.8 and 31.8 microns, all groups showing a statistically significant difference from the control group except for treatment group 4 (Table 2).

### Table 2. Mean and standard deviation values for wax cell diameters observed in bees fed with prebiotic and probiotic products (N=110)

<table>
<thead>
<tr>
<th>Prebiotic products</th>
<th>Probiotic products</th>
<th>Prebiotic and probiotic products</th>
<th>Neither prebiotic nor probiotic products</th>
</tr>
</thead>
<tbody>
<tr>
<td>EG1</td>
<td>29.7±0.9*</td>
<td>EG6</td>
<td>EG9, 32.2±2.01**</td>
</tr>
<tr>
<td>EG2</td>
<td>31.8±1.08**</td>
<td>EG6</td>
<td>EG10, 31.4±1.99**</td>
</tr>
</tbody>
</table>
| EG3                | 28.2±1.95*         | EG7                              | C= control group; EG1= lactic acid (2 ml) treatment group; EG2= lactic acid (2.5 ml) treatment group; EG3= acetic acid (20 ml) treatment group; EG4= acetic acid (30 ml) treatment group; EG5= Enterobiotics (1.25 g) treatment group; EG6= Enterobiotics (2.5 g) treatment group; EG7= Enterolactis Plus (1.2 g) treatment group; EG8= Enterolactis Plus (2.4 g) treatment group; EG9= lactic acid (2.5) and Enterobiotics (2.5 g) treatment group; EG10= lactic acid (2.5) and Enterolactis Plus (2.4 g) treatment group.

* p<0.05
** p<0.01

The wax cells of the bees in the treatment groups fed with probiotic products (EG5, EG6, EG7 and EG8) were found in different stages of their secretory cycle. Their morphology ranged from spherical and polygonal with spherical nuclei to large, irregular, densely staining cells lacking nuclei but having lipid vacuole rich cytoplasm (Figure 3). Their mean size was 26.9-29.2 microns. Bees fed on Enterobiotics supplemented syrup showed wax cell diameters larger than those found in the control group, with the differences being statistically significant (EG5 and EG6, Table 2).

Bees fed syrup supplemented with both lactic acid and a probiotic product (EG9 and EG10) showed ceride diameters significantly larger (p<0.01) than those of bees fed pure sugar syrup.

### 4. Conclusions

1. Feeding bee colonies on sugar syrup incorporating different dosages of acidifying substances (lactic and acetic acids) led to the development of wax cells between 10.75% and 26.69% greater in diameter than those of bees in the control group.
2. Bees given probiotic supplement in their feed (treatment groups EG5, EG6, EG7 and EG8) developed wax cells between 7.17% and 16.33% greater in diameter than those in the control group.
3. Supplementation of feed with both lactic acid and a probiotic product led to a better development of wax cells, these being between 25.09% and 28.28% greater in diameter than those found in bees fed pure sugar syrup.
4. This greater development of wax cells as a result of feeding bees with prebiotic and probiotic products are correlated with increased glandular activity resulting in increased colony wax production. Wax production is an important criterion for the selection of breeding families.
Acknowledgments

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References