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STUDY ON THE VIABILITY OF THE YOUNG IN THE SPECIES *APIS MELLIFERA* ACCORDING TO THE SECRETORY CAPACITY OF ROYAL JELLY

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Abstract

The growth of the bee brood (the stage of the decapitated larva) is directly influenced by the ability of the nurse bees to secrete royal jelly. The secretion of the main precursors of royal jelly is made in the hypopharyngeal glands, located in the bee's head. After the nursing stage (bee capable of raising new generations of larvae), the hypopharyngeal glands regress and are responsible only for the secretion of invertases that ensure the transformation of nectar into honey. The study aimed to establish the degree of variation in the viability of the brood, correlated with the feeding with royal jelly from nurse bees, fed on diets with low protein intake (low in pollen). The study conducted in an apiary with 20 bee families, Apis mellifera carpatica, in autumn, with a lack of pollen in nature. A positive correlation established between the two factors studied. The degree of development of the hypopharyngeal glands that influence the condition of bee larvae as well as their viability. Monitoring in practice the level of protein in the feeding of nurse bees and the periods with greater or lesser need may be a criterion for determining the strength of bee families.

Key words*: Apis mellifera carpatica, hypopharyngeal glands, larval brood, viability.*

INTRODUCTION

After hatching, the young bee feeds on honey and pollen, performing various activities in the bee colony (cleaning cells for laying eggs, ventilating the hive, sanitizing, etc.). Within 6-7 days, the hypopharyngeal glands reach a maximum development (Klose et al., 2017), based on protein consumption, being capable of secretion, and the bee changes its function becoming a nurse bee (Browers, 1982) (Figure 1). This function allows the differentiated feeding of bee larvae, of different ages. Nurses also feed on the larvae of beekeepers (drones and queens), the composition of the food being different from that of the bee larvae. Usually, only perfectly formed nurse bees feed these larvae (Al-Ghamdi, 2011).

The period of maximum secretion lasts until around the age of 15-18 days, the bee changing its function into a collecting bee. Their role is to bring the nectar into the hive and then to participate in its transformation into honey, the hypopharyngeal glands having only the role of secreting an enzymatic fraction, necessary for inversion. Due to intense activity and wear, the bees die around the age of 35 days.

Bees hatched in late August and September play an important role in preserving the bee colony until next season. These bees have a lifespan of about 6 months, ensuring the growth of the brood, starting with the middle of January.

The longer life is mainly due to the lack of juvenile growth activity (high degree of wear) and the lack of long-distance flying activity outside the hive.

The hypopharyngeal glands are paired glands, acinous, with external secretion. The main function is the secretion of royal jelly, having a role in the secretion of an enzyme that participates in the inversion of nectar in honey. Each gland consists of a long tube (half the length of the body, when unfolded) to which more than 1000 acini are connected by peduncles. The structure of the acini is given by the pyramidal cells, responsible for the production of royal jelly, placed with the apex facing the inside of the acini. Elimination of the secretion from the cell is done through a cellular lumen. The nuclei have a variable number of nucleoli, depending on the secretory state of the cell. During maximal secretion, they migrate to the base of the cell, pushed by the secretory cisterns. The increase in the volume of acini during the activity of maximum secretion is given by the accumulation of royal jelly in cisterns, which have different points of formation (similar to the formation of crystals in a metal) reaching to converge in a single cistern inside the cell, eliminating secretion. through the lumen of the cell and then through the lumen of the acini into the gland tube (Corby-Harris & Snyder, 2018).

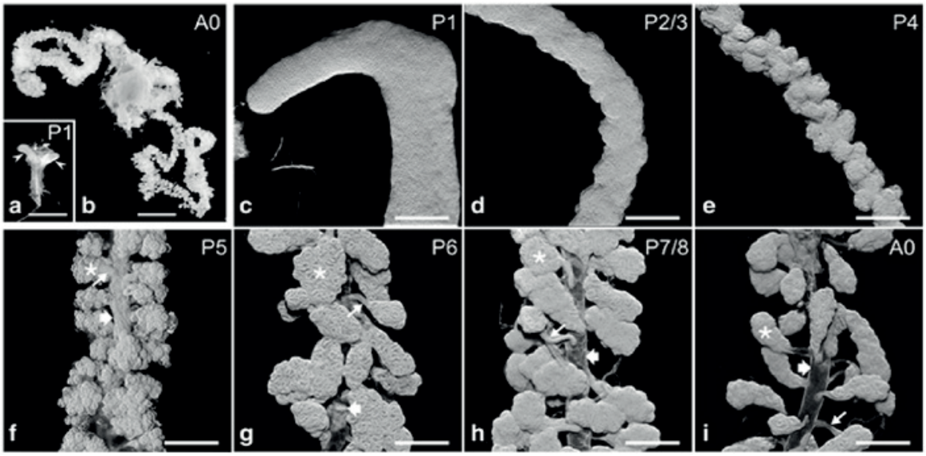

Figure 1. Hypopharyngeal gland morphogenesis (after Klose et al., 2017)

After secretion, the cell has a flabby, elongated shape, capable of secreting enzymes necessary to invert the nectar in honey.

MATERIALS AND METHODS

Carpathian bees (*Apis mellifera carpatica*) were used as a mountain ecotype harvested from bee families in full activity (active season) and from bee families entering winter (inactive season - October). Bees were also harvested during the winter from a colony located in an enclosure with controlled temperature and humidity conditions.

The summer bees were harvested from the honeycomb area with uncapped brood, and in winter, randomly from the hive.

The glands were gutted and used in observations in two ways:

- permanently prepared on the slide (hematoxylin-eosin staining);
- freshly prepared between slide and slide, in saline solution (weak Giemsa coloration).

The observations were made with an MC5 microscope, using the x20, and x40 objective lens.

In the controlled room, the conditions specific to the development period of the bee colony (spring) were simulated.

Different foods were administered. Simple pollen and honey, ad libitum, were used as feed for the Colony 1. A pollen-based recipe enriched with amino acids and vitamins, ad libitum, was used for Colony 2.

RESULTS AND DISCUSSIONS

The hypopharyngeal glands are named after Snodgrass, who in 1956 confirmed that the ducts open on the suboral plate of the hypopharynx. They act as secretions for most components of royal jelly, feeding on bee larvae (Snodgrass, 2018).

The secretion of royal jelly is conditioned by the presence of larval pheromones, by conditioned reflex. released by each larva. The study of the degree of development of these glands is closely related to the developmental capacity of the larval brood as well as the production of breeders.

The study aims to analyse the differences between the quality of the offspring obtained

during the active season and that obtained during the inactive season. Clarification of these aspects is important for the spring development period of bee families, especially in the conditions of climate change that manifests itself in recent decades (Browers, 1983; Zarić et al., 2022).
The results of this study can be capitalized on by developing new techniques for the maintenance of bee families or adapting existing techniques to the new climatic conditions. There are no recent studies showing the influence of climate change on the growth of larval broods during the spring development of bee families.

Bees harvested during the active season
The hypopharyngeal glands in 5-7 days old bees are very well developed (stage 4). But, in the developmental periods of bee families, when there is abundant feed, the hypopharyngeal glands develop even earlier (5 days) (Figure 2).

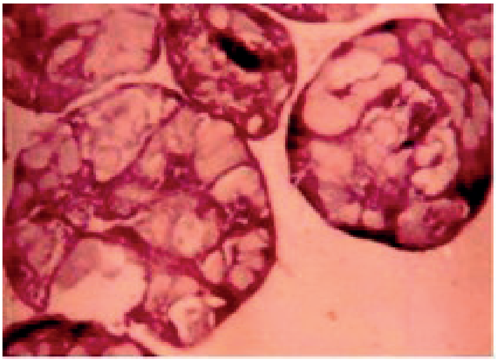

Figure 2. Glands in stage 4 (x40 objective lens)

The glands completely occupy the front surface of the bee's head, they are strongly contorted, the acini are voluminous, well developed, spherical in shape, the cells are clearly highlighted, even with a small objective lens (x20) (Figure 3). As the glands develop, the nucleus is pushed to the edge of the cell.
The bees were harvested at different times depending on the quantity and quality of pollen in the wild.
It was observed that during periods of pollen deficiency, although the hypopharyngeal glands were well developed (stage 4), the cisterns had a semi-mimicked consistency.

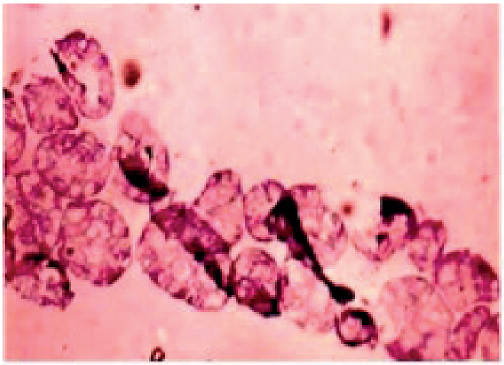

Figure 3. Glands in stage 4 (x20 objective lens)

At the age of 17-18 days, the glands underwent an involution, reaching stage 1, the secretory activity consisting only in the elimination of the enzymatic fraction necessary for the inversion of the nectar, the period corresponding to the passage of the bees in the stage of harvesting (Figure 4).

Bees harvested during the winter - in the apiary
Bees hatched in late August and the first half of September have perfectly developed hypopharyngeal glands at the age of 6 days, due to the smaller surface area of the brood.
Bees hatched in the last decades of September have incompletely developed hypopharyngeal glands (stages II-III).
The glands remain in this stage until around January 10-20, when the brood appears in the bee colony, after which it develops to its maximum capacity, there being no differences compared to the bee glands hatched during the active period.

Bees harvested during the winter - in the room with controlled conditions
Between October 15 and April 15, two bee families were maintained on the premises, simulating spring conditions (T = 15-25°C, about 80% humidity, characteristic light regime with twilight simulation).
Colony 1 was fed with honey and honeycombs at discretion, and Colony 2 was forced to consume more pollen by using an attractant.
As a result of differentiated feeding, Colony 1 started growing the brood around January 7, the brood area being smaller than in the apiary

families. Due to the growth of a smaller brood area that could not ensure generational change, it did not last until the end of the experiment (Figure 5).

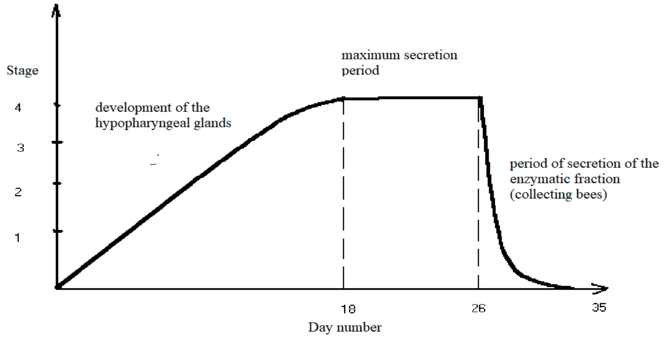

Figure 4. Development and involution of the hypopharyngeal glands in bees

Colony 2, in which an overdose of protein food was performed, started raising the young around November 20th. Although the area of the brood was comparable to that of the colony 1, a rhythmic growth of the brood was observed, the periods with brood alternating with the periods without brood (Figure 6).

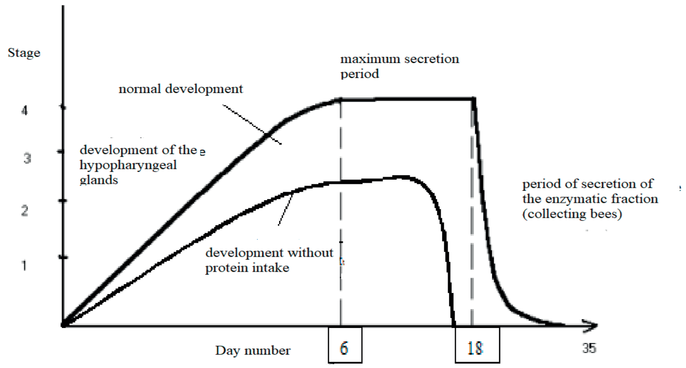

Figure 5. Development and involution of the hypopharyngeal glands in apitron with and without protein intake

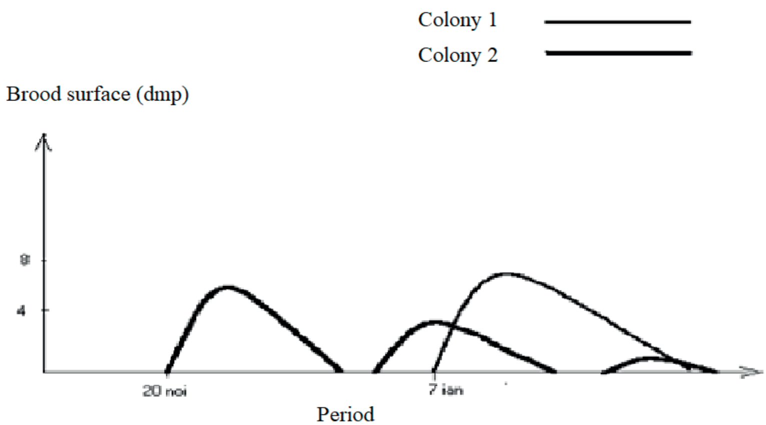

Figure 6. Raising the brood in the apitron in two differentially fed colonies (protein intake)

The colony 2 lasted until March 5, when in fact there were no more offspring.
The hypopharyngeal glands in bees of colony 1 remained incompletely developed (stages I-II) and there were no bees with glands in stage III. When the seedlings appear, the glands develop up to stage III. Acins were incompletely developed, and the cells had nuclei with fewer nucleoli. The cisterns had a semi-identical consistency.
In bees of colony 2, the hypopharyngeal glands developed up to stage 4, but the consistency of the cisterns was similar to stage 3.
The brood raised by both families had protein deficiencies, having a low survival rate, not being able to grow another generation of brood.

CONCLUSIONS

The results of the study show differences between the hypopharyngeal glands of bees hatched in the active season and those that over winter. In bees kept on the premises with controlled conditions, specific phenomena of protein deficiency were observed, although the protein feed was overdosed and the brood was present (factor that triggers the secretion of glands). The young raised by these families were crawling, showing protein deficiencies, a phenomenon materialized in the delayed development of the glands and the impossibility of raising a new generation. The change in environmental factors in apitron induced minor or insignificant changes.
The second generation of bees raised in the enclosure, although they had glands corresponding to stage 4, did not have the matte consistency of the tanks (specific to this stage) and the nuclei had a smaller number of nucleoli, indicating a low metabolic capacity.
The quality of protein food, in this case pollen, induces the proper development of the hypopharyngeal glands. This aspect can be linked to the observation that the first generations of bees in a season able to raise in turn other strong generations of bees, are those that feed on the fresh pollen from nature, the spring pollen.
Protein substitutes, widely used in beekeeping, ensure poor development of the hypopharyngeal glands.

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