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THE IMPACT OF THE NUTRITIVE VALUE OF POLLEN ON THE DEVELOPMENT, REPRODUCTION AND PRODUCTIVITY OF HONEY BEE (*APIS MELLIFERA* L.)

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Abstract

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The objective of the study was to investigate the impact of the nutritive value of pollen on the development, reproduction and productivity of the honey bee (*Apis mellifera* L.). The pollen collected in different periods of the year has a different protein value for honeybees. The protein content in the examined samples through the season ranged from 13.88% to 25.02%, and the average value was 19.45%. In spring providing pollen with an average protein content of 24.35%, bee colonies can maintain a high level of reproduction and development, whereas during autumn, when the average is 15.57%, reproduction and development of colonies is reduced. During the period, when flowering plants provide pollen with protein content of 23.60%, honey and pollen productivity are higher, than period provide pollen with 16.24% protein content, although in which time the bee colonies had built wax foundation combs and reared the greatest amount of brood. There is relationship between the nutritional value of pollen and the development, reproduction and productivity of bee colonies.

Key words: Apis mellifera, nutritive value of pollen, development, reproduction, productivity**,** Belozem

Introduction

Honey is the energy source of honey bees and the pollen – the source of protein, vitamins, enzymes, lipids, minerals and others. Protein is important for the development, reproduction and productivity of bee colonies. Pollen is a natural source of protein for bees. Without the presence of flowering plants and supply of pollen in the local natural environment, bee families slow growing brood. Haydak (1961) established that a lack of pollen or a lowering of its nutritional value over long-term feeding negatively affects the physiological development of bees. If pollen is absent, or is kept for a long period at 18-26^{o}C, the amount of brood reared in colonies will be reduced (Stroykov, 1963). In order for a bee colony to undertake its vital processes, it must be sufficiently supplied with quality protein food. The nutritional value of pollen varies between different plant species, and this affects the growth and productivity of a bee colony. Honey bee longevity, brood rearing and honey production are all reduced when protein availability is insufficient (Crailsheim, 1990; Herbert, 2000). The level of protein in bees' food significantly affects the amount of brood reared, the protein content of bee hemolymph, the metabolism, and the quality of food for the larvae according to Levchenko (Stashenko, 1988). Low protein content in pollen also reduces the resistance of honeybees to diseases (Matilla and Ottis, 2006).

The purpose of this study is to correlate the crude protein content of pollen to development, reproduction and productivity of honeybee (*Apis mellifera*).

Materials and Methods

Bee colonies

The study was carried out using 5 bee colonies, made equal on March 26th housed in 10-frame Langstroth-Ruth hives. At the end of each month the colonies were inspected, with the following taken into account:

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A. Taking into account the number of frames covered by bees, there are 1580±100 bees on a frame, we multiply by 1580 according to Nikolia (2013).

B. Quantity of capped and uncapped workers brood - using measuring frame with squares of size 5x5 cm (25 cm^2 area). Taking into account that in 1cm^2 of comb there are 4 worker cells, then in each of the squares there are 100 worker cells, then multiply the number of squares by 100 according to Lavrehin and Pankova (1983).

C. Honey productivity (kg) - was found as the difference between the weight of the full combs and the empty ones after centrifugation of the honey. The total productivity of colony was determined by the total honey collected from all the extractions.

D. Pollen productivity (kg) - was given as the amount of pollen collected with pollen traps.

Collection of pollen

Pollen traps were placed in 5 bee hives and the pollen pellets were harvested every 1–2 days. The collection of pollen started on April 24 and continued until September 30. Each sample collected was marked with a predetermined color for each hive and date. The collected pollen was placed in plastic bags and stored in a freezer at -20°C until the time of analysis. The multifloral bee–collected pollen samples were harvested from an apiary located in Belozem (Bulgaria) in a central area of the Upper Thracian Lowland, 20 km east of Plovdiv.

Preparing the pollen samples for analysis

Every single month was with two reporting periods (except April - only one): I period from 1st to 15th day of the month and II period from 16th to 30/31st day of the month. The collected pollen from the bee colonies was mixed together according to the accurate period. The random sample using for analysis for each period was taken using the quarter method of sampling. In this method was spread the pollen on paper and divided it into four equal quarters, then removed two diagonally opposite quarters. The remaining material was mixed and quartered until the sample was reduced to the desired size.

Determination of protein content

Eleven vials with mixed pollen collected from all the colonies were analysed. For nitrogen content determination, the pollen was analysed using the Kjeldahl method, which is separated into three steps. During the first step, digestion, a quantity of 1g of pollen was placed into the tubes of a digestion unit (K-435 Buchi) with 20 ml of strong sulphuric acid (H_2SO_4, 95-98%) in the presence of a catalyst – a Kjeldahl tablet (Copper sulphate, Potassium sulphate, Sodium sulphate, Titanium dioxide, Wuelfel), which assists the conversion of nitrogen in proteins to ammonium ions. When the digestion was complete, the samples were removed and left to cool at room temperature. After cooling, the ammonia was distilled in the presence of NaOH (KjelFlex, 360 Buchi) and collected in a solution of boric acid (H_3BO_3, 99.5-100.5%, Merck), which was then titrated against 1 M HCl (Mettler Toledo, T – 50). The crude protein content was estimated using the factor 5.60 (Rabie et al., 1983).

Determination of water content

The method of drying at 103°C under reduced pressure was used for the determination of moisture content in bee–pollen samples. A quantity of 1g of pollen was accurately weighed in a porcelain-evaporating dish and was placed at 103°C in a vacuum oven for 2.5 hours. The water content was estimated as the difference in weight of the dried samples before and after introduction into the drying oven.

Water % = $M\text{-}M_1/M\text{-}M_2 \times 100$

M= dish and sample weight (g)

M_1= mass weight with sample after drying (g)

M_2 = weight before adding the sample analysed (g)

The determination of moisture content in the pollen is used to reveal the remaining dry matter content and to express the protein value as a percentage of dry matter.

Results and Discussion

The study results show that the protein content of mixed pollen varied for the two reporting periods of each month and for each single month. The percentage of protein content in the examined mixed pollen samples ranged from 13.88% to 25.02% as shown on Figure 1. During spring (April to May)

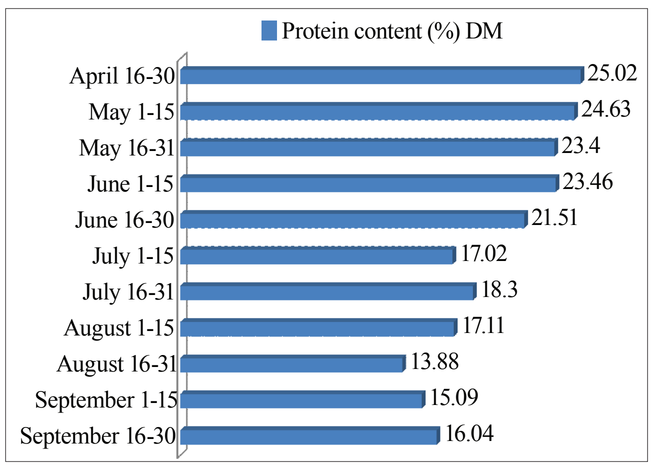

Fig. 1. Protein content (%) of mix bee – collected pollen in different harvesting periods

the protein content is average 24.35%, during summer (June-August) is 18.55% and during autumn (September) is 15.57% as shown on Figure 2. There is a tendency to reduce the crude protein content of pollen in Summer-Autumn compared to spring. Statistical analysis of the data of Figure 2 at ($p \leq 0.05$) accuracy of confidential limits for means proves difference of the spring season as compared to summer and autumn, while Summer and Autumn are coincide. It can therefore identifiable two periods with different nutritional value of pollen: Spring and Summer-Autumn. The overall average protein content in the analysed samples was 19.45%. The results of this study agree with Stanley and Liskens (1974), Herbert and Shimanuki (1978) and Szczesna (2006) who found that average protein content approaches 20%, also the result is close to Liolios et al. (2013), who found an average protein content of 20.79%.

During the period from March 26th to the end of June, the bees have increase amount of brood as shown on Figure 3, when the crude protein content of pollen is highest, average is 23.60%. That result coincides to Herbert (2000), who mentions that a protein content of 20-23% in pollen is ideal for dietary requirements of honeybees. The most essential difference in reproduction was recorded in the period from March 26th to the end of April, when the protein content was 25.02%, which coincide to Standifer (Roulston and Cane, 2000) and Sommervile (2001), who found average of 25%. According to Kleinschmidt and Kondos (1976), a high level of productivity and viability can be maintained in the presence of a sufficient amount of pollen containing not less than 25% protein. The statistical analysis of the data in Figure 3 showed that there is positive, high and significant correlation ($r=0.84$, $p \leq 0.05$) between the protein concentration of pollen and the rearing amount of brood in the bee colonies. The most amount brood is reported up to 38 400 brood cells at the end of May, while at the end of September down to 7600. The differences of laying eggs of the queens could be genetic, although they are sisters. Despite all, the inspecting bee colonies increased and reduced rearing of brood together, depending on nutrition value of the pollen. During the period of July-September, the protein content of pollen significantly reduced to an overall average of 16.24%, which corresponds to results derived from the research of Bonvehi and Jorda (1997), who found an average protein content of pollen samples from Spain of close to 16%. If the nutritional value of pollen is reduced, a sharp drop in reproduction of colonies is noted.

The data provided in the Figure 4 demonstrates that bee colonies showed the largest development in June, when the number of bees were up to 47 400. This is owing to large amount of brood reared in April-June due to the high nutritional value of pollen during this period. In contrast, during July-September when the protein average is 16.24%, development of the bee colonies is reduced twice to 23 700 bees. The statistical analysis of the data in Figure 4 showed that there is a positive but no significant correlation ($r=0.30$, $p \leq 0.05$)

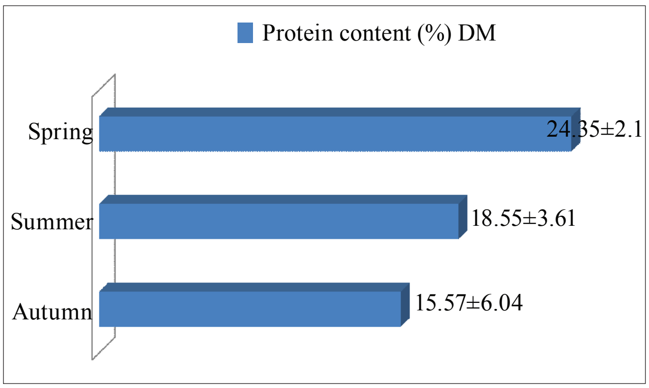

Fig. 2. Protein content (%) of mix bee – collected pollen in different seasons

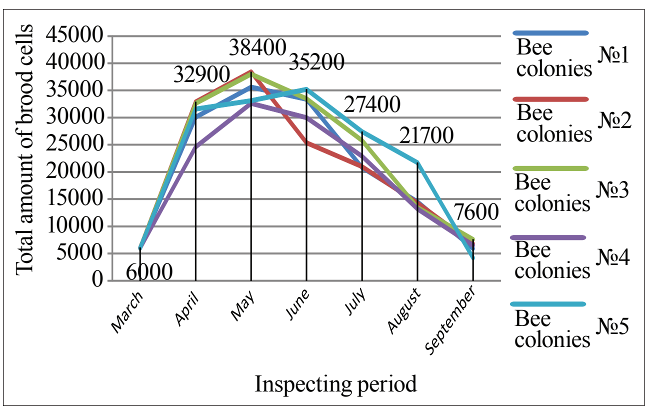

Fig. 3. Dynamics of the reproduction in the bee-colonies for each month

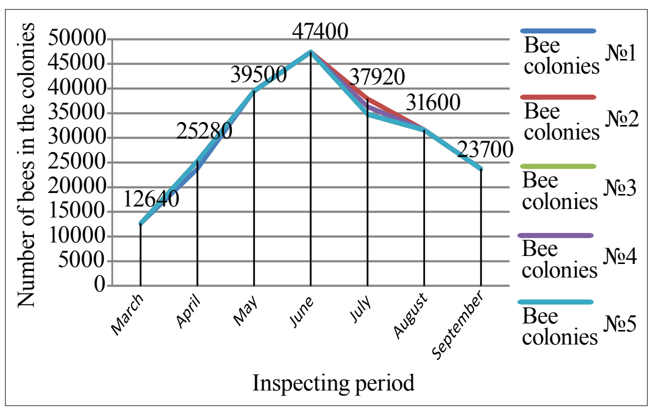

Fig. 4. Dynamic of the development (population) of the bee colonies (number of bees)

Table 1
Honey and pollen production, kg

Bee colony	Honey/Pollen production April-June, kg	Honey/Pollen production July-September, kg	Total honey/pollen production, kg
№1	19.9/1.2	16.2/0.8	36.1/2.0
№2	21.01/1.0	18.05/0.6	39.6/1.7
№3	24.5/3.2	17.4/1.9	41.9/5.2
№4	21.3/1.6	17.4/1.0	38.7/2.6
№5	23.3/2.2	13.9/1.4	37.2/3.6

between the protein concentration of pollen and the development of the bee colonies. The reason is the biggest amount of brood rearing in May but hatched in June.

The presented results in Table 1 show that the bees collected the most honey and pollen during the period when the protein content is at highest. Plenty of references indicate that, during the spring season when there is strong growth of the bee brood and population, the amount of pollen collected by bees increases (Camazine, 1993; Dreller et al., 1999; Dreller and Tarpy, 2000). During the period April-June, when flowering plants provide pollen with high protein content, honey and pollen productivity were higher than period July-September. Although in which time (April-June) the bee colonies had built wax foundation combs and reared the greatest amount of brood. The range observed in the nutrient-protein value of pollen is valuable information for beekeepers. Knowing the flora of the beekeeping area where the hives are located and assuming the protein value of polliniferous plants occurring in this area, beekeepers can, using the appropriate methods, assist the development of colonies. This study could also be used to assist the reproduction and productivity of colonies; for example, the transfer of colonies to areas where there is an abundance of plants with pollen high in protein content would help their development.

Conclusions

Pollen collected by honeybees has a different protein value at different periods of the year. In 11 mixed pollen samples the protein content ranged from 13.88% to 25.02%, and the average value was 19.45%. In spring providing pollen with an average protein content of 24.35%, colonies can maintain a high level of reproduction and development. In contrast, during the autumn when the protein average is 15.57%, reproduction and development of the bee colonies is reduced.

During the period, when flowering plants provide pollen with protein content of 23.60%, honey and pollen productivity are higher, than period provide pollen with 16.24% protein content, although in which time the bee colonies had built wax foundation combs and reared the greatest amount of brood. There is a relationship between the nutritional value of pollen and the development, reproduction and productivity of bee colonies.

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