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THE IMPACT OF DIFFERENT PROTEIN CONTENT OF POLLEN ON HONEY BEE (*APIS MELLIFERA* L.) REPRODUCTION

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Abstract: The purpose of this study is to identify the impact of different protein content of pollen on honey bee (*Apis mellifera* L.) reproduction. The study was carried out using five bee colonies, housed in 10-frame Langstroth hives. At the end of each month the colonies were inspected for quantity workers brood. Pollen traps were placed into the bee hives and the pollen pellets were harvested every 2 days from April until September. Pollen from plants blooming in spring had higher protein content- average 24.2% than those from summer- average 19.3%, and autumn- avarage 20.5%. During the spring the pollen with protein content over 21%, and especially over 27% allows the colonies to maintain a high level of reproduction. The reproduction of the bee colonies is higher, when honey bees collect pollen with higher protein content. When the protein content is going down, the reproduction of the bee colonies is also restricted. If there is autumn providing pollen with high protein content, the bee colonies grow faster in the early spring of the following year as well. There is a relationship between the protein content of pollen and the reproduction of bee colonies.

Keywords: Bulgaria, honey bee, impact, pollen, protein content, reproduction.

INTRODUCTION

Pollen provides lipids, carbohydrates, vitamins, and minerals to the bee colonies and it serves as the honey bees only protein source (Roulston and Cane, 2000), and necessary for vital processes of the honey bees (*Apis mellifera*) (Dietz, 1975). Pollen from different botanical species has different nutritional value for honey bees since it can vary significantly in protein, lipid, and vitamin content (Stanley and Linskens, 1974; Sommerville, 2001; Andrada and

Tellerı´a, 2005; Oliveira et al., 2009; Estevinho et al., 2012; Yang et al., 2013). The protein food is essential for reproduction of honey bee colonies. The colony terminates the rearing of brood in the absence of flowering pollen-giving plants in the nature and of protein reserves inside the hive (Avetisyan, 1983). On the basis of certain observations carried on honey bees during this period it becomes clear that pollen, which is a product required for reproduction, is needed for the development and the reproduction of the bee colony (Mitropolskiy, 1935; Krasnopeev, 1939; Abrikosov, 1946; Taranov, 1961). It has been found that when there is a shortage of pollen, the bees rear less brood (Lavrehin and Pankova, 1983).

The longevity of honey bees, as well as the brood rearing and honey production are reduced when protein availability is insufficient (Crailsheim, 1990; Herbert, 1992). The protein content of pollen which amounts is more than 20% seems to meet the nutritional requirements of the bees (Tyurner et al., 1972; Shaw, 1999), owes to the fact that the bees collect pollen exclusively from plants with a high percentage of protein (Moezel et al., 1987; Roulston and Cane, 2000). Similarly, Herbert (1992) mentions that a protein content of 20–23% in pollen substitutes is ideal for the dietary requirements of honey bees. The protein content of bee-collected pollen varies widely depending on the plant species and different environmental conditions, such as climatic and soil factors (Herbert, 1992; Stanley and Linskens, 1974) and can range from 9.2 to 37.4% (Sommerville, 2001). The bees rear significantly more brood in the presence of pollen from different plants rather than in the presence of one type (Taranov, 1972). According to Standifer et al. (1973a), the amount of protein in the fat part of the bee's body changes in accordance with the amount of pollen consumption and in inverse analogy to the rearing brood. In a nutritive value tests based on brood produced per unit of diet consumed, differences were found even among closely related pollen species (Loper and Berdel, 1980).

When there is a sufficient amount of pollen in nature, the free cells in the combs and the lack of stored protein food in the hive practically did not affect the brood rearing, but they only activate the honey bees (Stashenko, 1988). If pollen is absent, or is kept for a long period at 18-26ºC, the amount of reared brood in colonies will be reduced (Stroykov, 1963). In series of experiments carried out by Haydak (1935) with the complete exclusion of protein food, the bees reared brood only over two-week period. Pernal and Currie (2002) concluded through a highly controlled indoor environment testing, that the bees are incapable of discriminating differences in protein content. Contrary to those findings, Levin and Bohart (1955) suggested that honey bee collecting preference and increasing protein content were directly correlated. Studies have shown that if the pollen contains copious amounts of proteins, which is more than the necessary for the rearing and reproduction of bees, it is stored as proteins in the body. This physiological phenomenon directly affects the reproduction and the lifespan of the bees (Kleinschmidt and Kondos, 1976). Herbert (2000), said that a protein content of 20-23% in pollen was ideal for the dietary requirements of honey bees. According to Radev et al. (2014) there is relationship between the nutritional value of pollen and the development, reproduction and productivity of bee colonies.

The purpose of this study is to identify the impact of different protein content of pollen on honey bee (*Apis mellifera* L.) reproduction.

MATERIALS AND METHODS

Bee colonies

The study was carried out using five bee colonies during 2012-2014, housed in 10-frame Langstroth-Ruth hives. At the end of each month the colonies were inspected, with the following taken into account: The number of frames covered by bees- there are 1580±100 bees on a frame, we multiply by 1580 according to Argena (2013). Quantity 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).

Collection of pollen

Pollen traps were placed into the bee hives and the pollen pellets were harvested every 2 days from an apiary located in Belozem 42.2°,25.033333° (Bulgaria). The collection of pollen started in April and continued until September. Each sample collected was marked with a predetermined colour for each hive and date. Every single month was with two reporting periods (except April 2012- only one): I period from 1st to 15th day of the month and II period from 16th to 30/31st day of the month.

Preparing the pollen samples for analysis

The collected pollen from the bee colonies was mixed 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 some 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

Thirty-five 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 tube with 20 ml of strong sulphuric acid (H_2SO_4, 95-98%) and heated for three and half hours in the presence of a catalyst, 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 and collected in a solution of boric acid (H_3BO_3, 99.5-100.5%), which was then titrated against 0.1 M HCl. The crude protein content was estimated using the factor 5.60 (Rabie et al., 1983) by the volume of HCL, which was taken by an instrument and added to the pollen. In this case the following formula applied:

Protein (%) = 0.791*V/m.

Where: V = the volume (ml) of HCl taken during the titration.

M = the amount of pollen (g).

Statistical analysis

The statistical analysis was conducted with Microsoft Excel 2007 (v12.0) software for Windows to correlate the protein concentration of pollen and the rearing amount of brood in the bee colonies. Differences were considered as significant at $p \leq 0.05$.

RESULTS AND DISCUSSION

Certain changes in the reproduction of bee colonies were reported after considering different spectrum of the protein content of pollen. During the three experimental years it was established that in spring period the bees increased the amount of brood until the end of May, but there were certain differences over the years. In the spring of 2012, when the crude protein content of the pollen was average 24.4% the brood cells were up to 35540 at the end of spring in 2013 with 23.1% crude protein content of pollen up to 30540 at the end of May and 2014 spring with 25.3% protein content (Table 1) up to 34500 brood cells (Figure 1).

Table 1. Protein content (%) of mixed bee collected-pollen in different seasons for three years

	2012	**2013**	**2014**
Sring	24.4 a	23.1 a	25.3 a
Summer	18.6 b	18.6 b	20.6 b
Autumn	15.6 b	23.9 a	22 a
$\bar{X}$ **for year**	**19.5**	**21.9**	**22.6**

The most essential difference in the reproduction was recorded in the spring of 2014, when the protein content was 25.3% and the bees reared brood efficiently and constantly. At the end of March the brood cells were up to 29660, in April they were up to 33180 and in May they were up to 34500 (Figure 1). The main difference was found in the high protein value of the pollen recorded in the autumn of the previous year -2013, when the crude protein content of the pollen was 23.9% (Table 1).

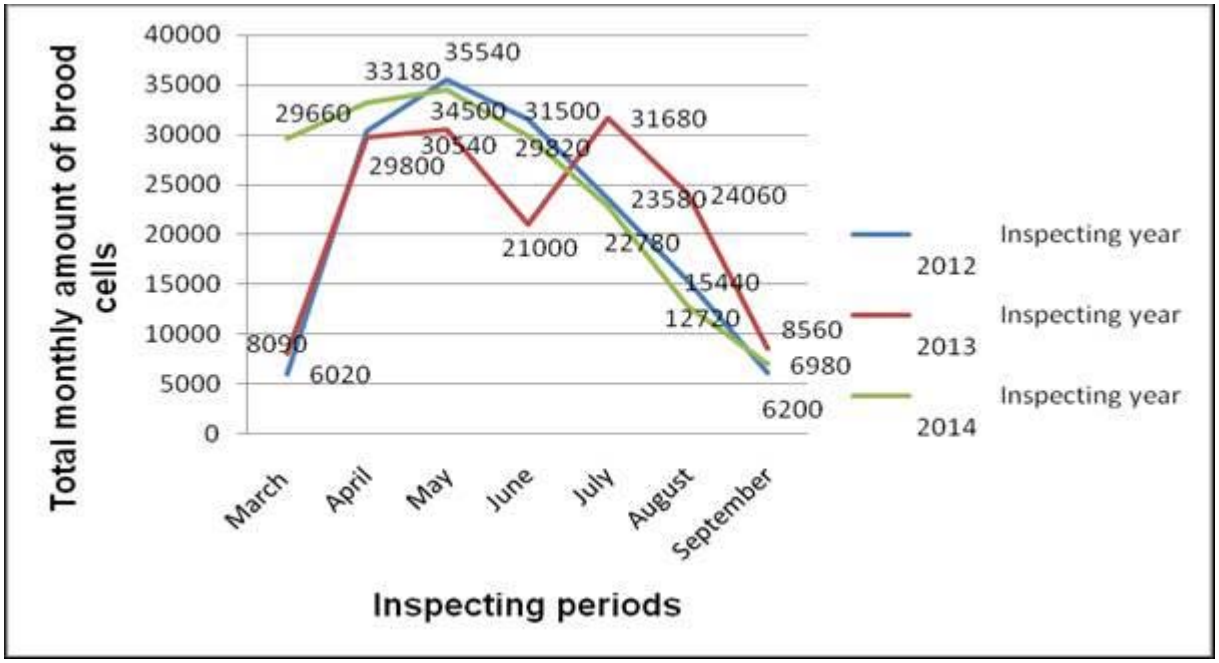

Figure 1. Dynamics of the reproduction in the bee-colonies for each month for 2012, 2013 and 2014

By comparing these data with the protein content of the pollen in the autumn of 2012, when it was recorded 15.6% (Table 1) and 8090 brood cells at the end of March 2013 (Figure 1), the important role of the protein value of pollen was established. The more pollen rich of protein was collected, the more brood was reared by the bees. Considering the fact that the new beekeeping season started at the end of August and at the beginning of September of the previous year, the amount of protein in the pollen was of great importance for rearing a large amount of brood in the early spring, when blooming of a large number of plants had not yet begun and the bees used mainly the pollen supplies from the previous year.

In the summer the reduction of protein content (Table 1) caused a reduction in the amount of brood (Figure 1). Despite the difference in the average value of the protein- 18.6% in 2012 and 20.6% in 2014 (Table 1) a gradual protein reduction from spring to summer was registered in 2012 and 2014, as shown in Table 2, and the amount of reared brood was regularly reduced (Figure 1).

The essential dynamics of the protein content was recorded 15.6% in June 2013 (Table 2) and a sharp reduction in the amount of reared brood- down to 21000 (Figure 1).

Table 2. Protein content (%) of mixed bee collected-pollen in different harvesting periods for three years

	2012	**2013**	**2014**
April 1-15		21.1	27.8
April 16-30	25	23	25.5
April (average value)	**25 a**	**22 a**	**26.7 a**
May 1-15	24.6	23.9	24.8
May 16-31	23.4	24.4	23
May (average value)	**24 a**	**24.2 a**	**23.9 a**
June 1-15	23.5	15.6	22.5
June 16-30	21.5	15.7	21.6
June (average value)	**22.5 ab**	**15.6 ab**	**22.1 ab**
July 1-15	17	16	19.2
July 16-31	18.3	19.9	21
July (average value)	**17.7 b**	**17.9 b**	**20.1 b**
August 1-15	17.1	22.8	20.7
August 16-31	13.9	21.9	18.4
August (average value)	**15.5 ab**	**22.4 ab**	**19.6 ab**
September 1-15	15.1	22.6	19.9
September 16-30	16	25.1	24.2
September (average value)	**15.6 ab**	**23.9 ab**	**22 ab**

In June 2012/ 2014 the protein content in pollen was 22.5%/ 22.1% and the reared brood 31500/ 29820. In the second period of July (16-31 of July) the increase of the protein content amounted to 19.9% from 16%, and the bees increased the amount of reared brood- up to 31680 which was in higher than that in July 2012 and July 2014. The reason is that in July 2012 and 2014 there is no such a difference of the pollen protein content among the first and second half of the month. In August the protein continued to grow (Table 2), but it was probably the wintering preparation of the colonies, which is part of their biology, that did not allow the bees to rear more brood in July. However, by comparing August 2012, and August 2014 (Figure 1) it was recorded that the bees reared more brood in the presence of higher protein content of pollen.

In the autumn of 2013 the protein amount reached higher values, compared not only to the same period of 2012 and of 2014 but also to the spring of 2013 (Table 1). A greater amount of reared brood was recorded in September 2013 in comparison to the reared brood in September 2012 and September 2014 (Figure 1). The honey bees reacted proportionally in terms of the amount of protein and the amount of reared brood.

During 2012 and 2014 the statistical analysis (Excel) of the data is showed that there was a positive, high significant correlation ($r= 0.84$, $p\leq 0.05$)/ ($r= 0.63$, $p\leq 0.05$) between the protein concentration of pollen and the rearing amount of brood in the bee colonies. The statistical analysis of the data for 2013 is showed that there was negative, but not significant correlation ($r= -0.16$, $p\leq 0.05$) between the protein concentration of the pollen and the rearing amount of brood in the bee colonies. The reason for the negative correlation was due to the fact that despite a significant increase in the amount of protein in August and in the autumn (Tables

2 and 1), when the protein was high even in the spring, the bees did not have the booming spring development and the amount of brood began to progressively reduce. In this case an inverse relationship was established in terms of the amount of protein and the amount of reared brood. This was probably due to the fact that it started preparing the bee colonies for the winter period, hatching and rearing of bees which would spend the winter, and would rear the new generations of the first spring bees. But if looking at the period May-July, where the essential dynamic of the crude protein content (Table 2) and the rearing of the brood (Figure 1) came forth, the correlation was high and significant ($r= 0.63$, $p\leq 0.05$).

The different ways in which the queens laid eggs could be genetic although they were sisters. Nevertheless, the inspecting bee colonies increased and reduced the rearing of the brood together, depending on the protein content of the pollen.

Pollen is essential for the normal growth and development of individual bees as well as for the reproduction of the colonies (Maurizio, 1950; Haydak, 1970; Herbert, 1992). Pollen from various botanical species has different protein content and for this reason the spectrum of the protein content of the pollen in the area can vary to such a large degree (Radev, 2018). Studies on the impact of protein content in pollen on the honey bee viability have already been published (Crailsheim, 1990; Herbert 2000; Radev et al., 2014). However, a long period study on impact of protein content provide wider point of view. According to Kleinschmidt and Kondos (1977), a high level of productivity and viability could be maintained in the presence of a sufficient amount of pollen containing not less than 25% protein. The result is concided with the research done by Standifer (cited in Roulston and Cane, 2000) and Sommervile (2001), who found the average value of 25%. Radev et al. (2014) found, in spring providing pollen with an average protein content of 24.35%, colonies can maintain a high level of reproduction. In contrast, during the autumn when the protein average is 15.57%, reproduction of the bee colonies is reduced, which coincided with the research work done by Crailsheim (1990) and Herbert (2000) who established that the brood rearing was reduced when protein availability was insufficient. There is a relationship between the protein content of pollen and the development of bee colonies (Radev, 2018).

The pollen is the source of protein during the feeding of the honey bees, and the proteins are crucial for the honey bees. The research indicates, that the seasonal average of protein content affects the reproduction of a bee colony. The research work conducted by Andreev (1926), Louveaux (1958), Perelson (1962) shows that the pollen of various plant species contains different amounts of protein and it makes them different in view of their nutritious value. Radev et al. (2014) found that the protein content of mixed pollen varied for the both halfs of each month and for each single month. Liolios et al. (2016) found crude protein of selected pollen sources ranged from 13.9 to 25.5%. Pollen from plants blooming in spring had higher protein content (20–24.7%) than those from summer (15.1–19.9%) and autumn (19.3-23.1%). The great amount of pollen that honey bees collected in spring and its richness in proteins could explain the strong growth of brood and population during this period.

According to Tyurner et al. (1972) before the massive nectar collection, the protein is needed for the bees to increase the strength of the colonies, and during the nectar collection for rearing new worker bees. Proteins are the bearers of life, basic substances in all organs and tissues. They also have a specific role for metabolism. The bee colonies, which intensively rear brood, can consume a full comb with pollen every 3-4 days (Doull, 1972). Campana and Moeller (1977) reported that the number of the reared honey bees depended more on the amount of food consumed than on the nutritive value. According to Levchenko (cited in Stashenko, 1988), the level of protein in the bees food significantly affects: a) the amount of brood reared, b) the protein content of bee hemolymph, c) the metabolism, and d) the quality of food for the larvae. Low protein content in pollen also reduces the resistance of honey bees to diseases (Matilla and Ottis, 2006). Deficiency of protein in the diet of the family can be one of the

reasons for the emergence of invasion and infectious diseases (Bilash, 1990). One of the best ways to fight against dangerous diseases such as nosematosis, akarapidoza, foulbrood is by feeding the bees with pollen (Lavrehin and Pankova, 1983).

In this research, is showed the impact of different protein content of pollen on honey bee reproduction according to his dynamics. The bee colonies are treated in three years. The three beekeeping seasons are taking into consideration individually and together (all in all), because the bees used pollen supplies from the previous year in the begining of the new year.

CONCLUSION

The protein content in the examined samples ranged from 13.9% to 27.8%. Pollen from plants blooming in spring had higher protein content (21.1–27.8%) than those from summer (13.9–23.5%) and autumn (15.1–25.1%). The reproduction of the bee colonies is higher, when honey bees collect pollen with higher protein content. The higher it is, the higher reproduction is. During the spring the pollen with protein content over 21%, and especially over 27% allows the colonies to maintain a high level of reproduction. When the protein content is going down, the reproduction of the bee colonies is also restricted.

Considering the fact that the new beekeeping season started at the end of August and at the beginning of September of the previous year, the amount of protein in the pollen was of great importance for rearing a large amount of brood in the early spring, when the blooming of a large number of plants had not yet begun and the bees used mainly the pollen supplies from the previous year. If there is autumn providing pollen with high protein content, the bee colonies grow faster in the early spring of the following year as well.

There is a relationship between the protein content of pollen and the reproduction of bee colonies.

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