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Influence of IMMUNOSTART HERB feeding on the bee colonies development

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Abstract

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To date, various plant-based supplemental forages, which contain key molecules, for bees are being used worldwide to improve the bee health. The aim of the study was to determine the effect of the product IMMUNOSTART HERB on some physiological and biochemical parameters and the development of the bee colonies during their spring feeding. The study was conducted at the experimental apiary of the Research Center of Stockbreeding and Agriculture, Smolyan, Bulgaria. The experiment included local honey bees *Apis mellifera* L., settled in the Langstroth-Rut system. For the feeding of the bee colonies is used a natural product IMMUNOSTART HERB based on herbal extracts. It contains natural flavonoids, polyphenols, polysaccharides, mucus substances, amino acids, essential oils, vitamins, minerals and vitamin C. The feeding of the bee colonies in the spring was carried out as follows: the experimental group was fed with IMMUNOSTART HERB at a dose of 10 ml in 100 ml sugar solution (sugar/water 1:1) 4 times at intervals of 7 days; the control group received only sugar solution. Statistically significant differences were found for the strength of the bee colonies and the amount of sealed worker bee brood in the experimental group fed with IMMUNOSTART HERB compared to the control group. A linear positive correlation between the fresh bee head weight and the degree of development of the hypopharyngeal glands was found. The higher values of total protein in the haemolymph in the experimental group were found to be indicative of the positive effect of the product IMMUNOSTART HERB on the immunity of bees. According to this study, the supplementary feeding of the bee colonies with IMMUNOSTART HERB is good to start about 60 days before the first main pasture in the region.

Keywords: IMMUNOSTART HERB; bee feeding; bee colony development; hemolymph; hypopharyngeal glands

Introduction

The high bee colony losses of *Apis mellifera* has attracted the attention of researchers from all over the world. The reasons are complex and many factors can influence them such as pathogens (*Nosema spp.,* viruses), parasites (*Varroa destructor*, *Acarapis woodi*), the use of antibiotics, pesticides, nutritional stress (lack of honey plants), technological errors in the beekeeping and interactions between these factors (Genersch, 2010; Ratnieks et al., 2010; Wu et al., 2011). In the recent study, Neov et al. (2019) summarizes the impact of biotic and abiotic factors on the health and behavior of bees to limit the stressors.

The nutritional stress is connected to the agricultural intensification and the increase of agricultural land with monocultures. This deprives the honey bees from the necessary polifloral pollen, which is required for their proper feeding (Crailsheim, 1990). The pollen feeding affects the bee lifespan (Di Pasquale et al., 2013), their immunocompetence (Alaux et al., 2010) and their resistance to pathogens

(DeGrandi-Hoffman et al., 2010; Basualdo et al., 2014). The nutritional stress has a long-term negative effect on the bee colonies and sometimes they are not well developed in the spring. The nutritional stress with the infection with *Nosema spp.* together has a strong impact on the bee colonies strength with consequences in both short and long-term (Branchiccela et al., 2019).

The morphological and physiological status of the hypopharyngeal glands is age-dependent and depends on the division of the roles in the bee colony (Lee et al., 2019). The hypopharyngeal glands are exocrine glands located in the front of the head. In the nursing bees, this gland has a high secretory activity and contributes to the production of royal jelly. The hypopharyngeal glands of the worker bees decrease in size, secrete at a lower rate, and produce a different protein blend including enzymes involved in carbohydrate metabolism (Klose et al., 2017). Different factors may influence the hypopharyngeal gland development of the honey bees (Huang & Otis, 1989). The most important is the nutritional factor. Protein sources are extremely important for the physiological development of the hypopharyngeal glands, especially for the young worker bees. Pollen is the main source of protein for them (Zahra & Talal, 2008). In addition, plant extracts also have a good effect on the development of hypopharyngeal glands.

Insufficient nutritional resources can weaken the immune system of the bees and the bee colonies are more susceptible to disease. The term "precision nutrition" already exists. It studies the bee nutrition in the search for key molecules that could enhance the response of the bees to one or more stress factors. The bee diet could be precisely designed with the right choice of different combinations of key molecules. This could be achieved through adding the molecule itself or adding the necessary molecules to induce the synthesis of a particular signal. In this way, "precision nutrition" with precision beekeeping could be developed (Negri et al., 2019).

To date, various plant-based supplemental forages for bees which contain key molecules are being used worldwide to improve the bee health. However, additional studies are needed to prove the effects of these products on the bee's body.

The aim of the study was to determine the effect of the product IMMUNOSTART HERB on some physiological and biochemical parameters and the development of the bee colonies during their spring feeding.

Materials and Methods

The study was conducted at the experimental apiary of the Research Center of Stockbreeding and Agriculture, Smolyan, Bulgaria located at an altitude of 1070 m in the Smolyan valley. The experiment included local honey bees *Apis mellifera* L., settled in the Langstroth-Rut system. For the feeding of the bee colonies is used a natural product IMMUNOSTART HERB based on herbal extracts. It contains natural flavonoids, polyphenols, polysaccharides, mucus substances, amino acids, essential oils, vitamins, minerals and vitamin C. This is a Bulgarian product produced by Extractpharma Ltd.

The spring feeding of the bee colonies was conducted during the period 11.04. – 10.06.2019. The experiment involved one control and one experimental group. Each group contained 5 bee colonies. All bee colonies were equal in regards to the amount of bees (strength), brood and food supplies.

The feeding of the bee colonies in the spring was carried out as follows: the experimental group was fed with IMMUNOSTART HERB at a dose of 10 ml in 100 ml sugar solution (sugar/water 1:1) 4 times at intervals of 7 days. The solution was sprayed on the bees and honeycombs; the control group received only sugar solution (sugar/water 1:1) without additives.

All bee colonies received a total of 5 L sugar solution during the experimental period, at intervals of 2 – 3 days. A total of 6 measurements were done in every 12 days with a measuring frame and size of the squares are 5×5 cm.

The following parameters characterizing the development of the bee colonies were determined:

1) Amount of bees (strength of the bee colony) in kg – approximately in the number of frames

2) Amount of sealed worker bee brood (number of cells) – a measuring frame with the size of the squares 5×5 cm is used. In 1 cm^2 there are 4 worker cells in the honeycomb. There are 100 worker cells in the area of 25 cm^2.

3) Quantity of bee pollen in the beehives (cm^2).

At the end of the experiment, 46 bees (age 5 – 10 days) were taken from each bee colony. The fresh weight of the bee heads was measured with an analytical balance (BEL Engineering). The degree of development of the hypopharyngeal glands was determined on the same worker bees from each bee colony. The hypopharyngeal glands were found with a shallow cut in the head. They were removed and placed on a glass slide. Both lobes of the hypopharyngeal glands were evaluated with a binocular loupe LAB-20, OPTIKA – Italy. The degree of development of hypopharyngeal glands was established using the 4-point Hess scale (Maurizio, 1954).

Bee hemolymph were collected from young non-flying worker bees. The hemolymph was obtained by suction with

a pasteur pipette at the border between the 2nd and 3rd abdominal tergites. Total protein content was determined by the Biuret method. The hemolymph samples were stored at -20°C until the analysis.

The data was expressed as mean ± standard deviation and analyzed by one-way analysis of variance (ANOVA). Significant differences were considered at $P < 0.05$ by Student's t-test. The correlation coefficient (r) was calculated with SPSS software version 23 for Windows. Significant differences were considered at $P < 0.05$.

Results and Discussion

At the beginning of the study (11.04.2019), the bee colonies from the experimental and control groups are identical in strength (amount of bees in beehive) (Figure 1). There is a gradual increase in the amount of bees for all bee colonies, which is a normal process in their spring development. The data showed that until 05.05.2019 the strength of the bee colonies was almost equal. After this date, the amount of bees was increased in the experimental group fed with IMMUNOSTART HERB. Statistically significant differences were found in the strength of the bee colonies from the experimental group in the last three measurements – 17.05. ($P < 0.05$), 29.05. ($P < 0.001$), 10.06. ($P < 0.001$). At the end of the spring study (about 10 days before the main pasture from meadow vegetation), bee colonies fed with supplemental forage for bees IMMUNOSTART HERB had about 1 kg more bees than the control group. This means that experimental colonies had about 10000 more young worker bees and will be able to participate actively in the main pasture. Stimulating effect on the bee colonies after spring feeding was observed by Zhelyazkova et al. (2008; 2010). The authors studied the Bulgarian product "Apisaniran". It contains

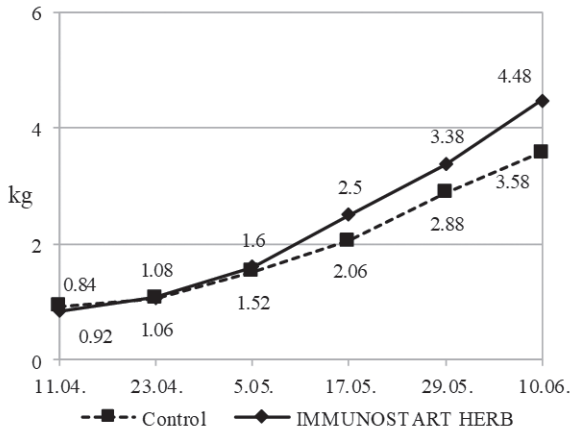


Fig. 1. Strength of the bee colony (amount of bees) in kg

essential oils of wormwood, *Artemisia absinthium* and pine needles. The combination of these extracts has stimulating effect on the bee colonies. When the bee pollen is insufficient in the spring, "Apisaniran" can replace it and stimulate the bee colonies.

During the spring testing, a clear positive effect was observed for the amount of the sealed brood in the experimental group received IMMUNOSTART HERB (Figure 2).

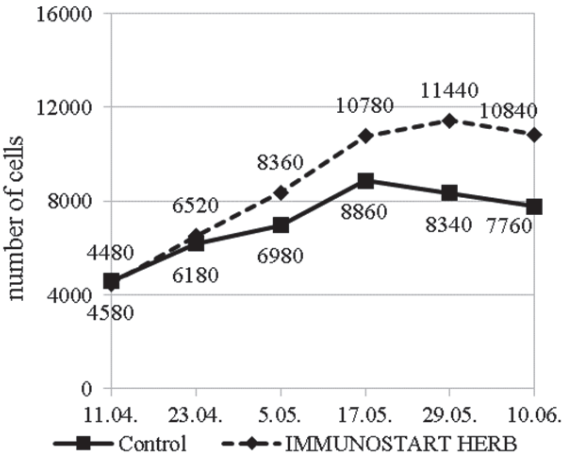


Fig. 2. Amount of sealed worker bee brood (number of cells)

Significantly highest values ($P < 0.001$) for the amount of sealed worker bee brood in the bee colonies fed with IMMUNOSTART HERB have been established for the last three measurements. Our results are in agreement with these of Andi & Ahmadi (2014). They found a significant higher value ($P < 0.05$) for the brood area when the bee colonies are fed with vitamin C. In addition, the authors observed a significant increase in the bee weight and protein content in the experimental group fed with vitamin C. Furthermore, Pătruică et al. (2011) found significantly higher values ($P < 0.01$) for the amount of sealed worker bee brood in the experimental group bee colonies which received shrub and savory extracts in the sugar syrup. In earlier studies, the results of Hristakov (2012) and Hristakov et al. (2013) showed that stimulating feeding with the addition of *Tribulus terrestris* L. at a dose of 10 mg per 1 kg of bees had a statistically positive effect on the strength of the bee colonies and the amount of sealed worker brood. Moreover, Zhelyazkova et al. (2009) studied the stimulating effects of the products "Green TM" and „Ecophil-P" on the indicators that characterize the development of bee colonies – the strength and the amount of brood. In this regard, the feeding of the bee colonies with products, which stimulate their development is appropriate. These

bees could be involved in the collection of nectar and pollen, as well as in the construction of wax combs. Regarding the use of immunomodulators in beekeeping practice Gurgulova et al. (2001) administered the product „Interstim" at doses of 0.1, 0.5 and 1% to the sugar syrup. The authors found an increased amount of brood and productivity of the bee queens.

The results for the amount of pollen collected by the bee colonies are presented in Figure 3. As can be seen the bee colonies receiving IMMUNOSTART HERB as a supplement have higher amount of bee pollen in the beehives.

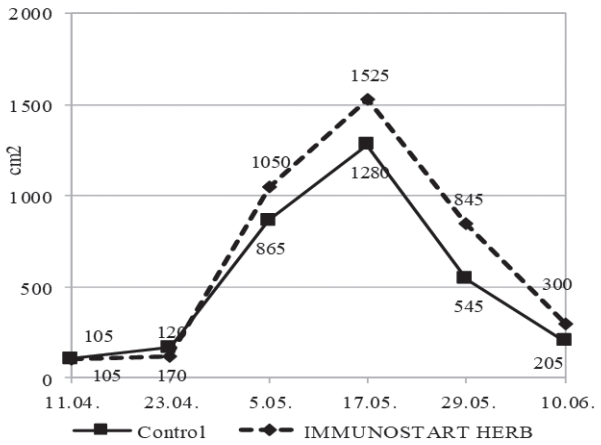


Fig. 3. Quantity of bee pollen in the bee colonies, cm²

The effect of the product IMMUNOSTART HERB on the bee colonies was also studied to the fresh head weight and the degree of development of the hypopharyngeal glands of worker bees. The results of the one-way ANOVA showed a significant effect ($P < 0.001$) of the use of IMMUNOSTART HERB on the fresh head weight (Table 1, Figure 4).

The results for the degree of development of the hypopharyngeal glands of worker bees are shown in Figure 5. The bee colonies fed with IMMUNOSTART HERB have a higher degree of development of hypopharyngeal glands than the control group fed with sugar syrup only.

Significant differences in the development of hypopharyngeal glands after feeding of the bee colonies with protein sources such as beebread, pollen and other protein substi-

Table 1. The effect of the use of IMMUNOSTART HERB on the fresh head weight of the worker bees (n = 92)

Source of Variation	df	MS	F
Between Groups	1	0.00003	15.659***
Within Groups	90	0.000002	
Total	91		

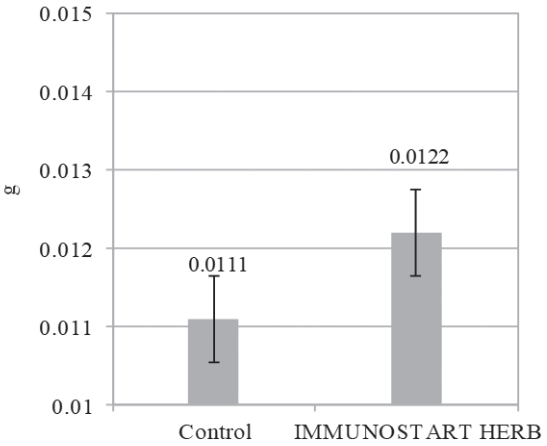
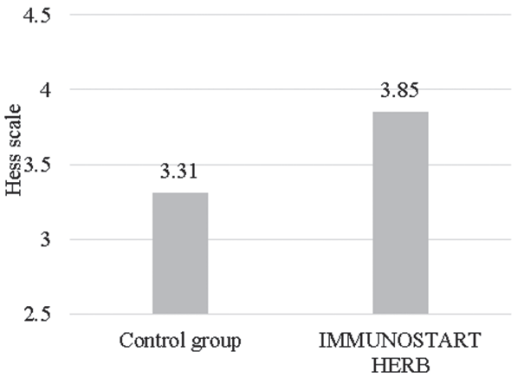
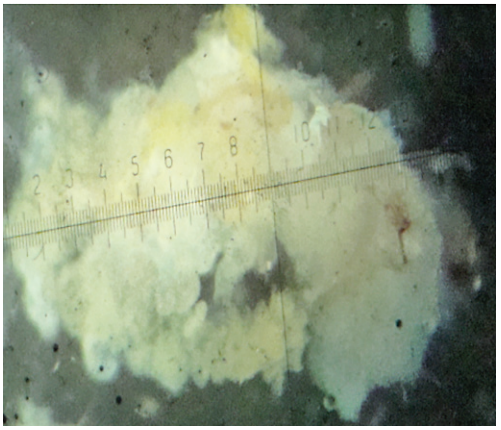


Fig. 4. Mean values and standard deviations of the bee head weight, g



A)



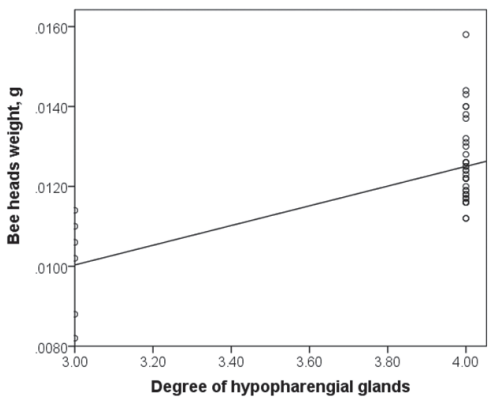
B)

Fig. 5. Degree of development of the worker bees hypopharyngeal glands (in Hess scale) A), 4-degree of worker bees hypopharyngeal glands B)

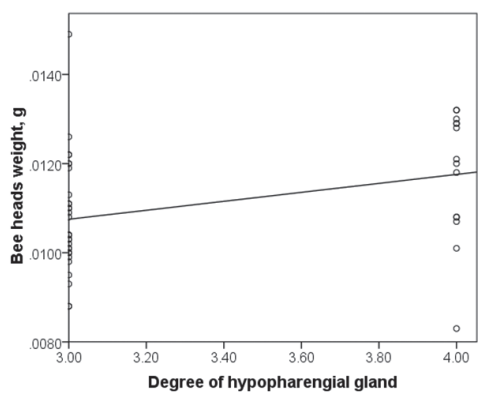
tutes have been investigated by Al-Ghamdi et al. (2011), DeGrandi-Hoffman et al. (2010). Shumkova (2016) established maximum values for the development of the hypopharyngeal glands for bees receiving plant-based stimulating product „Apidas". The results obtained are similar to those of the present study. In an earlier study, Zahra & Talal (2008) found that vitamin C at a dose of 250 mg/1.5 L sugar syrup had a positive effect on the development of hypopharyngeal glands and the raising of more bee brood.

The results obtained prove that the product IMMUNOSTART HERB has a positive effect on the development of the hypopharyngeal glands and increases the secretion of royal jelly from them.

Positive linear correlations were also found between the fresh bee heads weight and the degree of development of the hypopharyngeal glands of bee workers from the two groups (Figure 6 A and B). Škerl & Gregorc (2015) also found a positive relationship between bee head weight and actin diameter of the hypopharyngeal glands in bees (r^2=0.81, P < 0.0001). Therefore, the bee heads weights can serve as an indicator for the development of the hypopharyngeal glands in them.



A)



B)

Fig. 6. Correlation between the weight of the bee heads and the degree of development of the hypopharyngeal glands of worker bees in the control group r^2 = 0.417, P < 0.05 (A) and in the experimental group r^2 = 0.403, P < 0.001 (B)

The results for the total protein content in the hemolymph are shown in Figure 7.

The results obtained show that the total protein content of the worker bee hemolymph was 47% higher in the bee colonies receiving IMMUNOSTART HERB stimulating product compared to the control group (Figure 7). Based on the results obtained, it can be assumed that the use of the product IMMUNOSTART HERB may affect the immunity and the resistance of bee disease.

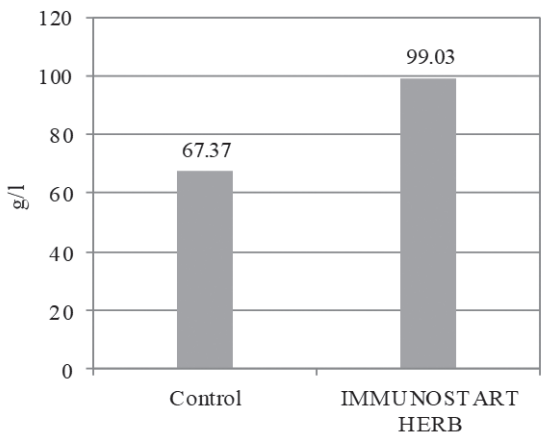


Fig. 7. Total protein content (g/L) in haemolymph of worker bees

The results obtained in present study are confirmed from different authors. In the recent study, Kunc et al. (2019) found that in the summer period the total protein concentration in worker bee haemolymph ranges from 17 to 42 mg/ml. In the winter it is from 49 to 87 mg/ml. According to these authors, total protein concentration positively correlates with the antibacterial activity of hemolymph, both in the summer and in the winter. In the summer the protein concentration positively correlates with vitellogenin concentration, a marker of lifespan in bees. Gurgulova et al. (2001) found that the immunomodulator "Interstim" stimulates protein synthesis in the worker bee haemolymph and it is a good inducer of lysozyme. According to the authors, this leads to an increase in the natural resistance of bee colonies to various diseases in the experiments in Bulgaria.

According to this data it can be supposed that an increase of the total protein in the haemolymph of worker bees receiv-

ing the product IMMUNOSTART HERB leads to increasing their disease resistance.

The spring feeding with IMMUNOSTART HERB affects the egg-laying activity of the queen bee. After one month of use, the amount of sealed worker bee brood increases, which also affects the strength of the bee colonies. The presence of a large number of flying bees increases the amount of pollen in the beehives. The higher levels of pollen in the experimental group allow worker bees to use more nutritious food and they have higher value of the degree of development of the hypopharyngeal glands. The obtained results give opportunity for further studies with IMMUNOSTART HERB for better understanding of health status of bees, morphological characteristics and prevention from various diseases. IMMUNOSTART HERB as a natural product based on herbal extracts suitable for organic beekeeping.

Conclusion

Statistically significant differences were found for the strength of the bee colonies and the amount of sealed worker bee brood in the experimental group fed with IMMUNOSTART HERB compared to the control group.

The results of the one-factor analysis showed a significant influence ($P < 0.001$) on the use of the preparation IMMUNOSTART HERB on the fresh bee head weight. In addition, a higher value of the degree of development of the hypopharyngeal glands was found in the experimental group compared to the control group. A linear positive correlation between the two indicators was also found.

The higher values of total protein in the haemolymph in the experimental group were found to be indicative of the positive effect of the product IMMUNOSTART HERB on the immunity of bees. IMMUNOSTART HERB probably contains key molecules that are effective for the immunological protection of the bees.

According to this study, the supplementary feeding of the bee colonies with IMMUNOSTART HERB is recommended to start about 60 days before the first main pasture in the region.

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