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Influence of Baikal EM1 preparation on the productive parameters of bee colonies (*Apis mellifera* L.) during spring and autumn feeding

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Abstract. *The aim of the present study is to investigate the effect of Baikal EM1 on the productive parameters of the bee colonies (Apis mellifera L.) during spring and autumn feeding and the chemical composition of the worker bee bodies. Two groups of bee colonies were formed (1 experimental group and 1 control group). During the spring feeding the experimental group was fed with Baikal EM1 at a dose of 5 ml/0.500 L added in the sugar syrup (sugar:water 1:1) for 4 consecutive days at the start of the experiment. Each bee colony received 5 L sugar syrup. During the autumn feeding the experimental group received Baikal EM1 at a dose of 20 ml/10 L sugar syrup. Each bee colony received 10 L sugar syrup. The control group received only sugar syrup. The spring and autumn feeding of the group fed with Baikal EM1 significantly increases the strength of the bee colonies and the amount of the sealed worker brood compared to the control group. According to the results obtained for the strength of the bee colonies and the bee brood supplementary feeding with Baikal EM1 is very effective in the autumn feeding. For these two parameters there are significant differences between the experimental and control on 29.08. ($p<0.01$), 10.09. ($p<0.05$) and 22.09.2018 ($p<0.01$). Statistically significant differences were reported for the strength of the bee colonies ($p<0.01$) and the amount of sealed worker brood ($p<0.001$) in the experimental group receiving Baikal EM1 before wintering compared to the control group. It can be expected to reveal a tendency for better spring development in the next year. Feeding with Baikal EM1 does not affect the chemical composition of worker bee bodies.*

Keywords: bee brood, chemical composition of worker bee bodies, strength of bee colonies, sealed brood, sealed worker brood

Introduction

Honey bees (*Apis mellifera* L.) play the most important role in the pollination of a number of crops and vegetables (Neov et al., 2019). In this regard, one of the main tasks in beekeeping is to increase the number of bee colonies and their productivity. Stimulation feeding of the bee colonies is especially important in the spring and in the autumn period. During the spring period, honeybees begin to grow large amounts of bee brood which will participate actively in the main pasture (Shumkova, 2016). The growth of the bee colonies depends on the nectar and pollen during the intensive flowering of the plants in the spring. Nectar from the plants is the main source of carbohydrates for bees. Bees also need proteins, lipids (fatty acids, sterols), vitamins, mineral elements and water (Nicolson, 2011). In early spring and in the late autumn, when nectar and pollen deficiency may occur in nature, additional nutrition for the bee colonies is needed (Dodologlu and Emsen, 2007). Feeding with sugar syrup is often used in beekeeping for various purposes. Usually, sugar syrup is used to stimulate the egg activity of the queen bee, to grow bee brood, to build wax combs in the spring. Ceksteryte and Racys (2006) investigated invert sugars syrups to feed wintering bees.

The favorable conditions in the beehive (increasing the number of young bees) and in the environment conditions (temperature, inflow of nectar and pollen) influence the increase the honeycomb numbers occupied by bees. However, additional stimulating feeding for the young bees is needed. Taranov (1986) proved that feeding with sugar syrup only has a smaller effect on the amount of sealed bee brood. Therefore, it is advisable to add stimulant products to the sugar syrup in the presence of pollen. According to Simidchiev (1991), food supplies are an important factor for the normal development of the bee colonies in spring. In the absence of food bees restrict the feeding of the bee queen and the larvae. This may interfere with the normal development of the bee colony. In spring honey consumption for raising bee brood is from 5 to 10 times more compared to honey consumption in winter (Nenchev and Zhelyazkova, 2010).

The nutrition of the bee colonies can influence the production of bee products (honey, pollen, royal jelly, wax, bee venom, propolis)

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with high biological and nutritional value. In recent years, different preparations have been tested as food for bees. They are based on biologically active substances. A part of them are plant extracts from various plants and essential oils. They are used to stimulate bee colonies' development and to prevent them from various diseases (Gurgulova et al., 2001; Pohorecka, 2004; Nenchev et al., 2006; Hristakov, 2012; Shumkova and Zhelyazkova, 2013; Shumkova, 2014, 2016). Electrochemically activated aqueous solutions are also used for supplementary feeding, prophylaxis of diseases, as well as for disinfection of beekeeping equipment (Gurgulova et al., 2006, 2011; Shumkova, 2014). Other products that are used in beekeeping are microbiological preparations containing a complex of useful microorganisms (http://www.baikal-em1.com/pchelarstvo/). At the same time, they are harmless to humans, animals and the environment.

The application of probiotics in beekeeping as an additive can increase the survival of bees and can improve their health status (Patruica et al., 2013). Baikal EM1 is a multipurpose microbiological preparation created by Russian microbiologists. It consists of a complex of living beneficial microorganisms. The product Baikal EM1 is a microbiological preparation and is increasingly used in animal husbandry (Lorets et al., 2015) and crop production (Atik, 2013; Allahverdiyev et al., 2018). In recent studies, Shumkova and Balkanska (2019) examined the effect of Baikal EM1 on bee hypopharyngeal and thoracic glands. They found statistically significant differences ($p<0.001$) in the height, diameter and volume of the thoracic glands in worker bees and bee drones in the experimental group.

Eremia et al. (2015) applied the probiotic product Bilaxan in different concentrations from 50 mg/l to 200 mg/l added to sugar syrup in spring feeding of the bee colonies. They found an increase in the egg-laying capacity of the queen bee, the strength of bee colonies and the amount of sealed worker bee brood of the experimental group. In another study, Eremia et al. (2016) use the probiotic products Primix-Bionorm-K and Primix-Bionorm-P to feed the bee colonies. Both products increased honey bee yields in the fed bee colonies compared to the control group. The authors Alberoni et al. (2018) used to feed bees with sugar syrup containing bifidobacteria and lactobacilli isolated from the bee gut.

However, the influence of microbiological preparations on various indicators characterizing the development of the bee colonies has been poorly studied. In the available scientific literature, no studies have been found by other authors on the application of Baikal EM1 for the nutrition of bee colonies. In this regard, the aim of the study is to investigate the effect of Baikal EM1 on the productive parameters of the bee colonies (*Apis mellifera* L.) during spring and autumn feeding and the chemical composition of the worker bee bodies.

Material and methods

Experimental design

The present study was conducted in the Spring (23 April – 10 June 2018) and in the Autumn (05 August – 10 October 2018) at the experimental apiary of the Research Center of Stockbreeding and Agriculture – Smolyan, Bulgaria. Two groups of bee colonies were formed – 1 experimental group and 1 control group. Each group consists of 5 bee colonies from the local bee *Apis mellifera* L. settled in Langstroth-Route system. All bee colonies were equal in quantity of bees (strength), brood and food supplies (honey and bee pollen).

Feeding of the bee colonies in the Spring: The supplemented feeding of bee colonies was carried out according to the following scheme:

• Control group – fed with sugar syrup (water:sugar 1:1) without additives;

• Experiment group – fed with Baikal EM1 at a dose of 5 ml/0.500 L added in the sugar syrup (sugar:water 1:1) for 4 consecutive days at the start of the experiment. Each bee colony received 5 L sugar syrup.

Feeding of the bee colonies in the Autumn: The supplemented feeding of bee colonies was carried out according to the following scheme:

• Control group – fed with sugar syrup (water:sugar 1:1) without additives;

• Experimental group – fed with Baikal EM1 at a dose of 20 ml/10 L sugar syrup (sugar:water 1:1). Each bee colony received 10 L sugar syrup.

The differences in the concentration and the quantity of Baikal EM1 are due to the different needs of the bees in the spring and autumn feeding. In October, before wintering, honeybees from the experimental group were sprayed with 20-25 ml per colony with Baikal EM1 in the evening, two consecutive nights. The preparation was applied at a dose of 2 ml/L water. Spraying with Baikal EM1 is recommended by the producer of the product for the better wintering of the bee colonies.

Monitored parameters

The following parameters are identified characterizing the development of the bee colonies:

• amount of bees (strength of the bee colony), kg – approximately in number of frames occupied by bees and calculating the mass in a statement of one frame in the the multihull hive contains approximately 200 g of bees after control measurements;

• amount of sealed worker bee brood (number of cells) – measuring frame is used. In 1 cm^2 of honey comb there are 4 work cells, and in each of the squares of the measuring frame area of 25 cm^2 the cells are 100;

• quantity of honey, kg.

At the end of the autumn experiment, from each bee colony (experimental group n=5 and control group n=5) bee samples were taken for determination of proteins (Kjeldahl method), lipids (Soxhlet method) and mineral content (muffle furnace).

Statistical analysis

The data were expressed as mean ± standard deviation. Significant differences were considered at $p<0.05$ by Student's

t-tests. The correlation coefficient (r) was calculated with SPSS software (version 23 for Windows). Significant differences were considered at p<0.01.

Results and discussion

The results for the strength of the bee colonies during the spring feeding period are presented on Figure 1. In the Spring, the egg laying of the queen bee increases with the higher daily temperature (20-22ºC), flowering of honey plants – dandelion (*Taraxacum officinale*), (*Lamium purpureum*), fruit species such as apple (*Malus domestica*), cherry (*Prunus avium*) and inflow of pollen in the beehive. Furthermore, the strength of the bee colonies gradually increases. Significant differences were found in the last two measurements of the bee colonies strength (29.05.2018, p<0.05 and 10.06.2018, p<0.01) after feeding with the product Baikal EM1. At the last measurement of the experimental group of bee colonies (10.06.2018), they reached 3.76 kg bees on average. This is 1.15 times more than the control group. Actually, the experimental group has 0.48 kg (4800 bees) more young bees which is about 2 frames with bees for the multihull hive.

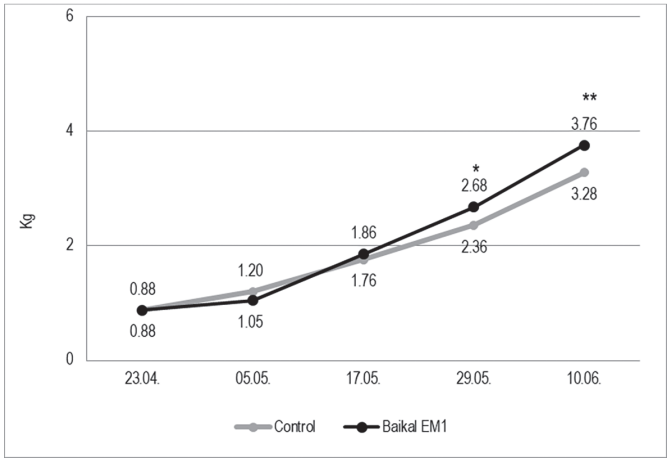


Figure 1. Strength of the bee colonies during the spring feeding (2018)
(*p<0.05; **p<0.01)

At this time in each bee colony from the experimental group (fed with Baikal EM1) there were about 40000 young bees (Figure 1). This moment of the bee colony development is of great importance. At the same time, the reserve of young bees provides an opportunity for the bee colony to use effectively the bee pasture, to collect and accumulate in the hive sufficient amount of food supplies for the period of relative rest in the winter. The strength of the bee colonies is very important for raising quality honey bees. The strong bee colonies always have better conditions for raising well-developed and fully-fledged bees (Bizhev and Nenchev, 1990). According to our previous study (Shumkova and Balkanska, 2019), bee colonies fed with Baikal EM1 have higher degree of development of the hypopharyngeal glands than the control group. Furthermore, worker bees and bee drones had significantly higher height, diameter and volume of the thoracic glands than those from the control group of bee colonies (p<0.001).

As can be seen on Figure 2, the amount of sealed worker brood increases between the period 23.04. – 29.05.2018. The amount of sealed worker brood on 17.05.2018 was significantly higher (p<0.05) than that of the control group. This confirms the results of the producer of Baikal EM1 in Russia to increase the bee brood in the hive and to stimulate the egg laying activity of the queen bee. Similar results are obtained for the administration of sugar syrup containing bifidobacteria and lactobacilli on the amount of worker brood and bee pollen (Alberoni et al., 2018).

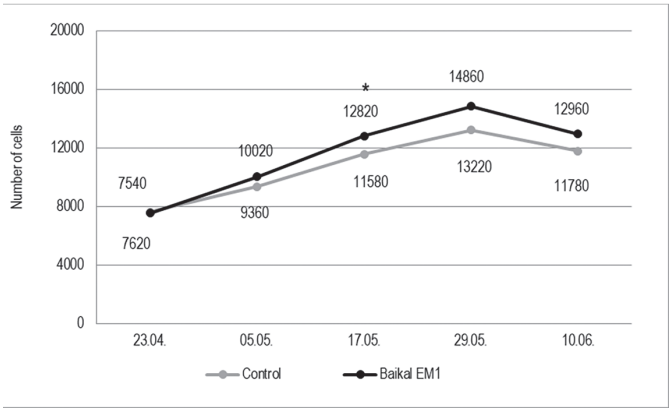


Figure 2. Amount of sealed worker brood during the spring period of feeding (2018)
(*p<0.05)

The amount of sealed bee brood decreased in all bee colonies at the last measurements (10.06.2018). The reason is changes of the environmental conditions in the study area (region of Smolyan) such as low temperature, prolonged rainfall, reduction of the nectar and pollen in the environment during the period 29.05. – 10.06.2018. However, the positive effect in Baikal EM1-fed bee colonies remains. Generally, during the spring period, bee colonies raise a larger number of young bees which will provide the necessary food supplies for the bee colony.

Negative correlation was found between the strength of bee colonies and the quantity of honey in the control group (r= -0.878, p<0.01 and in the experimental group fed with Baikal EM1 (r= -0.874, p<0.01). These results were obtained before the main pasture and the honey collection. The bee colonies were in the process of development and they consumed food supplies (honey). Gąbka (2014) found significant positive correlation between the strength of the bee colony and honey production (r= 0.456). The variations between different reports are due to the different studied period of these parameters.

The last main pasture for the experimental apiary of the Research Center of Stockbreeding and Agriculture – Smolyan is at the end of July. The altitude of the apiary is 1070 m. In 2018, due to unfavorable conditions in nature, additional feeding of the bee colonies was needed to replenish their food supplies for successful wintering. The study of the effect of Baikal EM1 on the development of bee colonies continued into the autumn period.

The comparative analysis of the results at the beginning of the study (05.08.2018) and in preparation for the winter on

22.09.2018 showed that the strength of the control group is reduced to a greater extent from 3.12 kg to 1.20 kg (Figure 3). Higher values of this indicator were observed in the experimental group fed with Baikal EM1 throughout the whole study period. Statistically significant differences in the strength of bee colonies were observed at the measurements on 29.08. ($p<0.01$), 10.09. ($p<0.05$) and 22.09.2018 ($p<0.01$). In general, the autumn stimulation feeding is intended to stimulate the hatching of young bees with good physiological conditions which will be wintering (Shumkova, 2016).

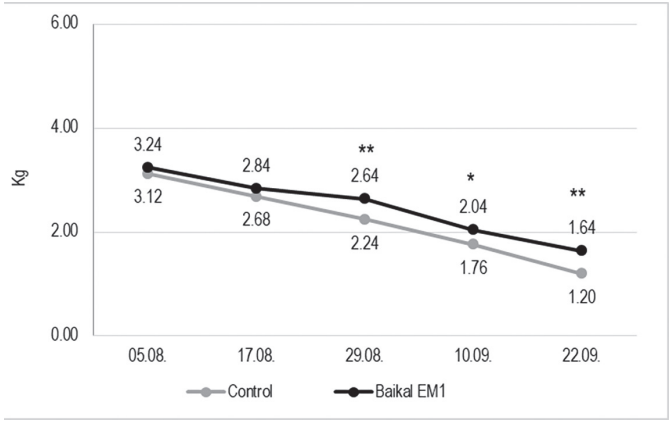


Figure 3. Strength of the bee colonies during the autumn feeding (2018)
(*p<0.05; **p<0.01)

As has been said before, Baikal EM1 stimulates hypopharyngeal and thoracic glands (Shumkova and Balkanska, 2019). The autumn feeding of the bee colonies with the addition of the microbiological product Baikal EM1 has a positive effect on the development of the worker honeybee fat body (Shumkova et al., 2019).

Negative linear correlation was found between the bee colonies strength and quantity of honey in the control group ($r= -0.969$, $p<0.01$ and in the experimental group ($r= -0.964$, $p<0.01$) (Figure 4). At the end of the season, the strength of bee colonies decreases. This is a normal biological process which is associated with an increase in the amount of food reserves (honey). It can be said that the relationship between the strength of bee colonies and the amount of brood depends on many factors that are difficult to control. Bobrzecki et al. (1994) found that bee colony strength does not have a significant influence on honey production.

Figure 5 shows a gradual decrease in the amount of sealed worker brood in the experimental and the control groups of bee colonies. This is a normal process for the autumn honey bee development due to a lower egg activity of the queen bee. It can be seen that on the second measurement (17.08.2018), which is 12 days after the start of the experiment, in both groups the amount of sealed worker brood increases (Figure 5). In the same way as in the strength of bee colonies, statistically significant differences were observed for the sealed worker brood on 29.08. ($p<0.01$), 10.09. ($p<0.05$) and 22.09.2018 ($p<0.01$) compared to the control group.

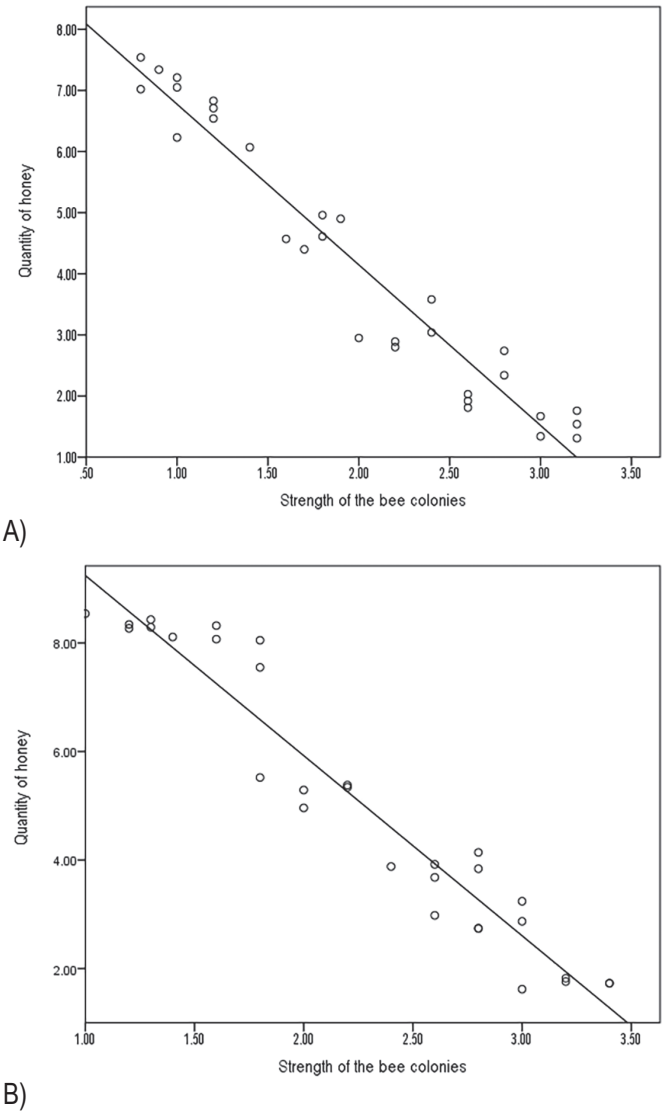


Figure 4. Linear correlation between strength of the bee colonies and quantity of honey A) in the control group ($r= -0.969$, $p<0.01$ and B) in the experimental group ($r= -0.964$, $p<0.01$)

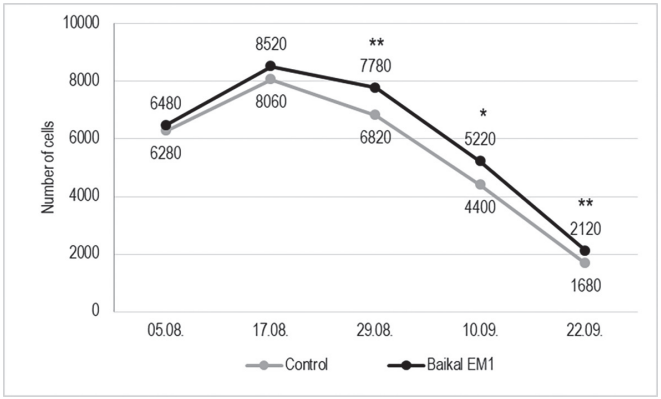
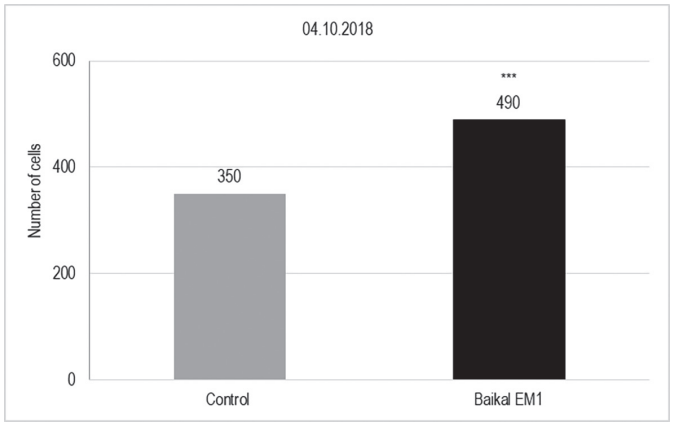


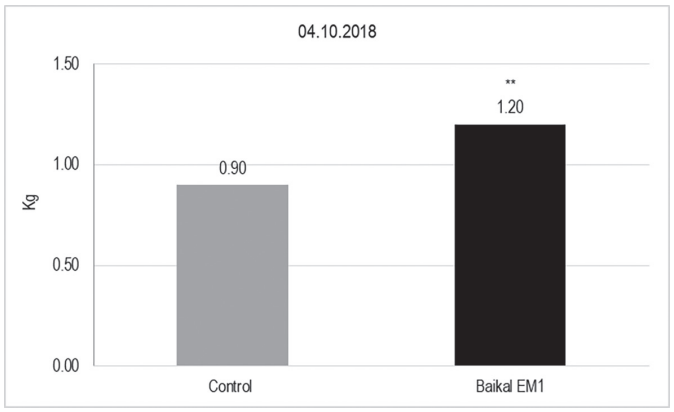
Figure 5. Amount of sealed worker brood during the autumn period of feeding (2018)
(*p<0.05; **p<0.01)

Baikal EM1 has a lower stimulating effect to bee strength and bee brood compared to other results at the same apiary with different products such as Apidas, Vita Feed Gold, Oligofosi and Anolyte-7 in the spring feeding (Shumkova, 2012).

The results of the last survey for the strength of the bee colonies and the amount of sealed bee brood before wintering are presented on Figure 6A and B. Statistically significant differences were reported for the strength of bee colonies ($p<0.01$) and the amount of sealed worker brood ($p<0.001$) in the experimental group receiving Baikal EM1 before wintering compared to the control group. According to the results obtained for the strength of the bee colonies and the bee brood, additional feeding with Baikal EM1 is very effective in the autumn feeding. For these two parameters there are significant differences between the experimental and control groups in all last four measurements (including this before wintering of bees).



A)



B)

Figure 6. Strength of the bee colonies (A) and amount of the sealed worker brood before wintering (B), (**p<0.01; ***p<0.001)

The results for the chemical composition of the worker bee bodies are presented in Table 1. Not statistically significant differences were found in the chemical composition of the body of worker bees. In our previous study, statistical differences ($p<0.05$) were observed for the protein and lipid content in the bee bodies from bee colonies fed with supplemented foods (Shumkova, 2016). Additional supplemented feeding can influence the chemical composition of the worker bee bodies but in the present study it was not confirmed.

Table 1. Chemical composition of the worker bee bodies (mean±SD)

Parameter	Control group (n=5)	Baikal EM1 group (n=5)	Significance
Water content, %	63.55±6.42	67.35±5.36	NS
Protein content, %	43.89±8.27	49.62±8.10	NS
Lipid content, %	8.24±3.31	10.10±1.59	NS
Mineral content, %	3.80±1.03	4.82±0.82	NS

*NS – not significant

Conclusion

The spring and autumn feeding of the group fed with Baikal EM1 significantly increases the strength of the bee colonies and the amount of the sealed worker brood compared to the control group of bee colonies. According to the results obtained for the strength of bee colonies and bee brood, supplementary feeding with Baikal EM1 is very effective in the autumn feeding. For these two parameters there are significant differences between the experimental and control bee colonies on 29.08. ($p<0.01$), 10.09. ($p<0.05$) and 22.09.2018 ($p<0.01$). Statistically significant differences were reported for the strength of the bee colonies ($p<0.01$) and the amount of sealed worker brood ($p<0.001$) in the experimental group receiving Baikal EM1 before wintering compared to the control group. It can be expected to reveal a tendency for better spring development in the next year. Feeding with Baikal EM1 does not affect the chemical composition (water, protein, lipids and mineral content) of worker bee bodies.

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