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Enhancing the productivity of honey bee colonies through the use of an immunomodulator

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Abstract. Providing honey bees with a diet enriched with biogenic metal compounds reduces the risk of infectious diseases, enhances resistance, improves the queen's reproductive capacity, and strengthens colonies. This study aimed to examine colony strength, productivity, brood development, and honey quality when using an immunomodulator. Colony strength was higher with the immunomodulator by 8.3% on 10 May, 7.1% on 15 May, 6.3% on 25 May, and 5.6% on 5 June. Pollen collection significantly increased by 11.9% on 10 May, 28.4% on 15 May ($P < 0.05$), 17.7% on

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25 May (P < 0.05), and 32.2% on 5 June (P < 0.05). Brood development increased by 5.3% on 10 May, 11.92% on 15 May, 19.6% on 25 May (P < 0.05), and 23.4% on 5 June (P < 0.05). Queens' live weight increased by 1.8% on 10 May, 2.5% on 15 May, 4.5% on 25 May, and 5.15% on 5 June in groups receiving the immunomodulator. When an immunomodulator was used for winter feeding, colony strength increased by 9.6%, honey production by 5.1%, pollen volume by 20.4%, brood quantity by 35.3%, and queens' live weight by 9.4% compared to the control. In October, colony strength increased by 18.7% (P < 0.05), honey production by 2%, pollen volume by 30.1%, and brood quantity by 50% in the experimental groups. The total honey weight increased by 14.4% and centrifuged honey by 15.5%. The use of the immunomodulator resulted in high quality honey, including a 20.9% increase in diastase activity and a 9.8% reduction in moisture content. A positive effect on the microscopic profile was noted, with increased adhesive and phagocytic activity of haemocytes against pathogens and an overall rise in immune cell count in bees. The practical significance of the study lies in enhancing the immune defences and productivity of honey bee colonies while ensuring high-quality and safe honey production

Keywords: immunomodulator; honey bee colony strength; honey yield; brood; queens live weight; pollen; honey quality

INTRODUCTION

Increasing the productivity of honey bee colonies is a crucial objective for beekeepers. Therefore, providing additional support to these insects during critical periods of their life is essential. Nutrient deficiencies within the bee's organism, particularly during overwintering, can lead to weakened immunity and increased susceptibility to diseases. Despite the numerous infectious diseases that can affect bees, researchers M. López-Uribe *et al.* (2020) identified a limited number of genes associated with genetically determined immune responses. This suggests that honey bees possess a unique species-specific mechanism for combating pathogens. However, the study did not specify the exact components responsible for the bees' natural immunity, nor the potential for their support and stimulation.

Scientists R. Underwood *et al.* (2023) conducted a study employing a systematic approach to assess the efficacy of three beekeeping management systems: traditional, organic, and chemicalfree. The research revealed that honey bee colony survival rates were 2.8 times higher in traditional and organic systems compared to chemical-free management. These results highlight the critical role of management system selection in colony preservation. However, the study primarily focused on parasitic diseases, leaving gaps in data concerning bacterial and fungal infections. Further research by L. Bataglia *et al.* (2022) demonstrated that the immunity of worker bees significantly declines with age and strenuous labour. This decline correlates with a reduction in juvenile hormone levels and an increase in haemolymph vitellogenin. Vitellogenin, a zinc-binding glycoprotein, plays a role in stimulating the bee's immune system. Consequently, the introduction of zinc into the bee's diet may support their resistance.

Field studies conducted by C. Rudelli *et al.* (2024) have demonstrated a correlation between pollen reserves, vitellogenin, and hexamerin levels in bees. In October, a decrease in iron and zinc levels coincided with an increase in *Varroa* mite infestation, negatively impacting bee overwintering success. Research into bee immunity and its relationship with the environment and nutrition is of paramount importance to both scientists and beekeepers. Consequently, there is ongoing research into safe immunomodulators to support bee immune defences throughout the honey flow season and during winter. The concentration of trace elements in honey bee haemolymph is linked to their prevalence in the surrounding environment (Fry *et al.,* 2023).

Studies by D. Fèvre and P. Dearden (2024) provide evidence that nutrition directly influences queen reproductive activity, colony productivity, overwintering, and overall health. The specific dietary components that are crucial remain to be determined. In a scientific review, H. Moura *et al.* (2020) identified essential biogenic metals for each animal species, which play a role in the functioning of organismal systems. It has been established that bees use magnetic iron oxide particles for their navigational system. There is a need to broaden the spectrum of biogenic metals that can be utilised to enhance honey bee colony strength and increase brood production. The application of plant-based and probiotic supplements has been shown to increase animal productivity. Calves were administered a premix of biogenic metal nicotinates (Shkromada *et al.* 2021). The results of these studies demonstrated a positive impact of biogenic metals on animal metabolism, productivity, and an increase in serum levels of zinc, iron, copper, and selenium. An immunostimulatory effect on young animals was also confirmed. This raises the need to investigate the effects of biogenic metals on honey bees (Fotina *et al.* 2024).

The enrichment of bee products (pollen) with biochemical components such as proteins, lipids, carbohydrates, carotenoids, and sporopollenins was determined by A. Kenđel and B. Zimmermann (2020) using infrared spectroscopy. The experimental results confirmed that the saturation of pollen with beneficial substances depends on bee nutrition. Research by

H. Shahid *et al.* (2023) has established the high antioxidant and anti-inflammatory properties of iron metal oxides obtained from honey. Honey bee pollen contains a rich array of trace elements, making it a viable bioindicator for environmental assessment, with demonstrated accuracy and precision (Erdoğan *et al.* 2023). Experimental results have identified key mineral components of pollen, including manganese, iron, zinc, selenium, and chromium. The mineral composition of pollen varies depending on the collection area and any supplemental feed provided to the bees.

Researchers R. Hussain *et al.* (2023) tested silver and zinc nanoparticles against fungal and bacterial infections in honey bees. Resistant honey bee pathogens, such as *Paenibacillus larvae*, *Melissococcus plutonius*, and *Ascosphaera apis*, exhibited sensitivity to Ag and Zn oxides. Bees obtain macro- and microelements from pollen, water, and nectar. A diverse pollen diet can positively influence honey bee health (Lee *et al.* 2024). However, the effects of phytochemicals and trace elements on honey bee physiology remain largely unexplored. Therefore, this study aimed to determine the impact of an immunomodulator on honey bee colony development, overwintering, productivity, and honey quality.

MATERIALS AND METHODS

Experiment design. The experiments were conducted in the "Innovative Technologies" laboratory of the Faculty of Veterinary Medicine at Sumy National Agrarian University and bee farms in the Sumy Region. In spring 2024, the following indicators characterising the development of honey bee colonies were examined: colony strength, brood development, pollen and honey production, and queen live weight. Ten honey bee colonies were selected for the study based on the principle of analogues, divided equally into experimental and control groups. The control group bees received a sugar solution (1:1). The experimental group bees received a sugar solution (1:1) supplemented with an immunomodulator based on germanium succinate, zinc, and cobalt, at a rate of 2.5 g of the preparation per honey bee colony in 0.5 L of syrup.

Honey quality analysis. Following the honey flow season, an inspection and preparation of honey bee colonies for overwintering were carried out. The honey underwent veterinary and sanitary examination according to DSTU 4497-2005 (2007). Honey quality and safety were assessed using organoleptic and laboratory methods. The organoleptic evaluation included assessments of consistency, colour, taste, aroma, and the presence of mechanical impurities. Laboratory methods were used to determine moisture content and diastase number.

Assessment of honey bee colonies' condition during winter preparation. Five spring feedings of the bees were conducted at 5-day intervals. The degree of honey bee colony development was determined using measurement frames.

Haemolymph analysis of bees following immunomodulator application. Following the application of the immunomodulator, haemolymph microscopy was performed using a scanning electron microscope to examine quantitative cellular changes and the haemocytes' immune response to bee pathogens. Haemolymph was extracted from the bee's heart. The obtained haemolymph was centrifuged at 1,500 revolutions per minute. A 2.5% glutaraldehyde solution was used for fixation. A buffer solution (NaH_2PO_4) was used to stabilise the resulting solution. Microscopy was conducted using a PEM 106 device (JSC SELMI, Sumy, Ukraine) at electronoptical magnifications ranging from 200 to 5,000 times, according to the methodology described by M. Bozhokin *et al.* (2021).

Statistical analysis. Experiments were conducted using Microsoft Excel 2010, and all obtained results were subjected to statistical analysis using the Fisher-Student method, considering statistical errors and a significance level of more than 95% ($p < 0.05$). During the study, the principles of humane treatment of experimental insects were followed, following DSTU EN ISO/IEC 17025:2019 (2021), adhering to the rules of bioethics and humane treatment of animals 2010/63/EU (Hartung, 2010), European Convention... (1986) Law of Ukraine No. 249 (2012)

RESULTS AND DISCUSSION

It was established that at the beginning of the experiment, the indicators of the control and experimental groups of honey bee colonies did not significantly differ. A gradual increase in colony strength was observed in both groups (Table 1).

Table 1*. Indicators of honey bee colony development with immunomodulator supplementation, M ± m, n = 10*

Experiment	Groups	Colony strength, kg	Pollen volume, cm^2	Brood, units	Queen bees' live weight, mg
10 May 2024	Control	1.1 ± 0.1	90.6 ± 15.5	3,410 ± 303.8	265.6 ± 7.5
	Experimental	1.2 ± 0.2	101.4 ± 21.4	3,590 ± 205.8	270.4 ± 4.7
15 May 2024	Control	1.4 ± 0.2	128.5 ± 24.9	6,070 ± 610.1	268.4 ± 7.6
	Experimental	1.5 ± 0.1	165.0 ± 24.7*	6,794 ± 665.4	275.1 ± 4.9
25 May 2024	Control	1.6 ± 0.1	200.0 ± 43.1	10,080 ± 653.6	272.4 ± 7.4
	Experimental	1.7 ± 0.2	235.5 ± 53.6*	12,060 ± 568.6*	284.6 ± 5.3
5 June 2024	Control	1.8 ± 0.2	248.5 ± 56.8	12,500 ± 836.8	273.6 ± 7.4
	Experimental	1.9 ± 0.1	328.5 ± 97.3*	15,420 ± 654.2*	287.7 ± 4.7

Note: **P < 0.05 – significant compared to the control*
Source: *compiled by the authors*

The experiment clearly demonstrates the difference in honey bee colony strength before and after immunomodulator supplementation. The strength of honey bee colonies in the experimental groups showed a non-significant increase during the following periods: 8.3% on 10 May, 7.1% on 15 May, 6.3% on 25 May, and 5.6% on 5 June. Pollen volume was higher in the experimental group: 11.9% on 10 May, 28.4% on 15 May (*P < 0.05), 17.7% on 25 May (*P < 0.05), and 32.2% on 5 June (*P < 0.05), compared to the control group without immunomodulator supplementation. A positive effect of the biogenic metal-based immunomodulator was observed on subsequent young bee generations. A trend towards increased reproductive activity of queen bees and an increase in sealed brood was observed in the experimental groups. The amount of brood was higher in the experimental groups compared to the control group without immunomodulator supplementation: 5.3% on 10 May, 11.92% on 15 May, 19.6% on 25 May (*P < 0.05), and 23.4% on 5 June (*P < 0.05). In the control group, larvae developed poorly, and some died before pupation. A nonsignificant trend of increased queen bees' live weight was observed with the use of the immunomodulator. It was found that in the immunomodulator-treated groups, the live weight of queen bees was higher: 1.8% on 10 May, 2.5% on 15 May, 4.5% on 25 May, and 5.15% on 5 June, compared to the control. The next study examined the development of honey bee colonies during preparation for overwintering (August) and during overwintering (October) (Table 2).

***Table 2**. Development of honey bee colonies during preparation for overwintering (August) and during overwintering (October) under the influence of immunomodulator, M ± m, n = 10*

Experiment	Groups	Colony strength, kg	Honey production, kg	Pollen volume, cm^2	Brood, units	Queen bees' live weight, mg
August	Control	2.1 ± 0.1	11.7 ± 0.6	131.6 ± 20.6	5,780 ± 556.8	234.8 ± 7.5
	Experimental	2.3 ± 0.1	12.3 ± 0.8	158.5 ± 24.1	7,820 ± 406.7*	256.8 ± 4.8
October	Control	1.6 ± 0.2	9,432 ± 0.57	101.1 ± 5.5	60 ± 33.1	-
	Experimental	1.9 ± 0.1*	9,620 ± 0.43	131.5 ± 4.1	90 ± 22.3	

***Note:** *P < 0.05 – significant compared to the control*
***Source:** compiled by the authors*

It was demonstrated that at the end of the honey flow season, honey bee colonies in the experimental group exhibited higher levels of colony strength, queen bees' live weight, and brood quantity compared to the control group. It was found that honey bee colony strength in the experimental groups was higher in August by 9.6% and in October by 18.7% (*P < 0.05) compared to the control. Honey production was higher in the experimental group by 5.1% in August, at the time of feeding, and by 2% in October, during overwintering. Pollen volume in August was 20.4% higher, and in October, 30.1% higher than in the control. The greater amount of brood in the experimental colonies indicates that these groups have younger bees or bees with enhanced immunity. Such bees are better equipped to survive overwintering. It was established that the brood quantity in the experimental groups was higher in August by 35.3% and in October by 50% compared to the groups without immunomodulator supplementation. A non-significant increase in queen bees' live weight was observed at the beginning of feeding in August, by 9.4% in the experimental honey bee colony groups. Studies have shown that the experimental group produced more honey than the control group (Table 3).

***Table 3**. Average honey yield per honey bee colony per season, M ± m, n = 10*

Groups	Total honey weight, kg	Centrifuged honey, kg
Control	75.30 ± 3.45	55.18 ± 3.20
Experimental	86.12 ± 2.56*	63.75 ± 2.34*

***Note:** *P < 0.05 – significant compared to the control*
***Source:** compiled by the authors*

At the end of the honey flow season in August, an increase in total honey weight of 14.4% and centrifuged honey of 15.5% was observed in the experimental groups compared to the control. The potential impact of immunomodulator application on honey quality was also assessed (Table 4).

***Table 4**. Honey quality determination results following immunomodulator application, M ± m, n = 10*

Indicator	Groups	
	Experimental	**Control**
Moisture, %	15.7 ± 0.5	17.4 ± 0.4
Diastase number, Gothe units	17.9 ± 0.1*	14.8 ± 0.2
Inverted sugar, %	73.8 ± 0.2	72.9 ± 0.1
Honeydew	Not detected	Not detected
Consistency	Liquid	Liquid
Consistency	Light amber	Light amber
Aroma	Pleasant	Pleasant
Taste	Pleasant	Pleasant
Mechanical impurities	Absent	Absent

***Note:** *P < 0.05 – significant compared to the control*
***Source:** compiled by the authors*

During the veterinary and sanitary examination of honey samples, it was found that honey from both experimental and control groups exhibited high-quality indicators. However, the experimental group's honey had a higher diastase number (20.9% increase) and a lower moisture percentage (9.8% decrease), which positively affected honey storage and quality. Haemolymph examination using scanning electron microscopy revealed that no infectious disease pathogens were found in the haemolymph of bees treated with the immunomodulator (Fig. 1). In the haemolymph of control group bees, phagocytosis of the Nosema pathogen was observed (Fig. 2). When examined under a scanning electron microscope, no disease-causing pathogens were observed in the haemolymph of bees treated with the immunomodulator, compared to the group of bees that did not receive the supplement. In the experimental haemolymph, a haemocyte, acting as an immune cell, exhibited signs of phagocytosis, specifically attracting and destroying the Nosema pathogen (Fig. 3). Additionally, there was a trend towards an increased number of haemocytes following immunomodulator application.

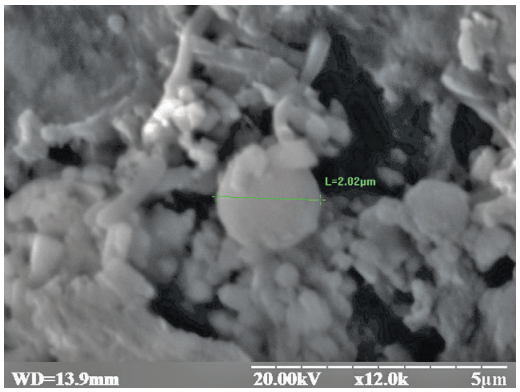

***Figure 1**. Haemocyte activity in bee haemolymph*
***Source:** authors' photo*

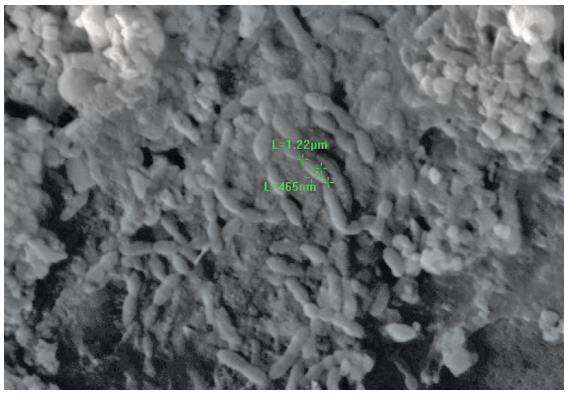

***Figure 2**. Nosema apis pathogen in the haemolymph of control bees*
***Source:** authors' photo*

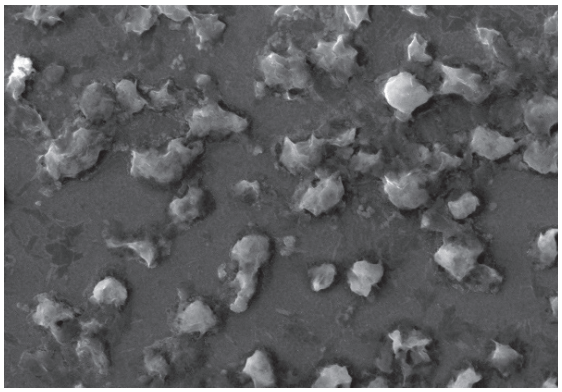

***Figure 2**. Destruction of Nosema apis after phagocytosis by haemocytes in the lymph of control bees*
***Source:** authors' photo*

Field studies demonstrated that immunomodulator application increased bee productivity, resistance, and fecundity. The immunomodulator had no negative impact on honey quality. Honey bee colony

strength significantly increased by 5.6-8.3% in the immunomodulator-treated groups from May to June. Pollen volume significantly increased by up to 32.2% in the experimental groups. Brood quantity increased by 23.4% in June in honey bee colonies treated with the microelement-based immunomodulator. Researchers G. Zhang *et al.* (2015) investigated whether bees obtain sufficient zinc from their environment. They confirmed that supplemental zinc enhances bee survival, royal jelly production, and larval health.

Studies have shown that the live weight of queen bees was higher in groups supplemented with microelements (Table 1). Similar results were obtained by researchers T. Fotina *et al.* (2022) when using mineral microelement supplements of zinc, copper, and manganese in poultry diets, which resulted in increased poultry live weight and enhanced immunity. Measurements taken in honey bee colonies during preparation for overwintering and during overwintering (Table 2) showed that honey bee colony strength was higher in the experimental groups compared to the control. When feeding honey bee colonies for overwintering, honey bee colony strength increased by 9.6%, honey production by 5.1%, pollen volume by 20.4%, brood quantity by 35.3%, and queen bees' live weight by 9.4%.

At the beginning of overwintering, the experimental groups showed higher values compared to the control group: colony strength was 18.7% higher (*P < 0.05), honey production was 2% higher, pollen volume was 30.1% higher, and brood quantity was 50% higher. Research by G. Glavan *et al.* (2024) demonstrated that zinc supplementation in bees did not cause toxic effects, even at high doses. However, as the results of this study show, therapeutic doses of a mineral-based immunomodulator positively influenced bee productivity and brood development in August. Researchers G. Ribeiro *et al.* (2023) confirmed experimentally that zinc supplementation in bee feed stimulates royal jelly production, which promotes brood survival. Additionally, the study of G. Cullen *et al.* (2023) supports that the diet composition of nurse bees influences larval development, reproductive potential, and disease resistance.

It was established that the average honey yield per honey bee colony at the end of the honey flow season was 14.4% higher in the experimental groups (Table 3). Furthermore, the amount of centrifuged honey increased by 15.5% compared to the control group. Research by M. Behjatian Esfahani *et al.* (2023) supports that adding microelements to bee diets increases their productivity and brood development. It was experimentally proven that the immunomodulator application had no negative impact on honey quality (Table 4). On the contrary, the diastase number was 20.9% higher compared to the control, and the moisture content was 9.8% lower. The physicochemical properties of honey are important as they determine the product's value in comparison to international standards (Sęk *et al.* 2023; Nikitina & Zasiekyn, 2024).

Researchers R. Pavlović *et al.* (2024) have established that micronutrient deficiencies in bee diets can lead to bacterial and fungal diseases. Therefore, the immune response of bees is affected by micronutrient shortages, which results in an increase in infectious diseases. As demonstrated in this study, infectious disease pathogens were not detected in the haemolymph of bees treated with the immunomodulator (Fig. 1). In the haemolymph of control group bees, phagocytosis of the Nosema pathogen was observed (Fig. 2). An increase in the number of haemocytes was observed in bees treated with the immunomodulator. Thus, to enhance the defensive capabilities of honey bee colonies, their productivity, and obtain high-quality and safe honey, it is advisable to use the immunomodulator at a rate of 2.5 g of the preparation per honey bee colony in 0.5 L of syrup.

CONCLUSIONS

Studies have demonstrated that the use of an immunomodulator resulted in increased honey bee colony strength during the following periods: 8.3% on 10 May, 7.1% on 15 May, 6.3% on 25 May, and 5.6% on 5 June. Pollen production significantly increased: 11.9% on 10 May, 28.4% on 15 May (*P < 0.05), 17.7% on 25 May (*P < 0.05), and 32.2% on 5 June (*P < 0.05). Queen reproductive capacity increased: 5.3% on 10 May, 11.92% on 15 May, 19.6% on 25 May (*P < 0.05), and 23.4% on 5 June (*P < 0.05). Additionally, queens live weight increased in the immunomodulator-supplemented groups: 1.8% on 10 May, 2.5% on 15 May, 4.5% on 25 May, and 5.15% on 5 June. When feeding honey bee colonies for overwintering, the immunomodulator increased honey bee colony strength by 9.6%, honey production by 5.1%, pollen volume by 20.4%, brood quantity by 35.3%, and queens bee live weight by 9.4% compared to the control. During overwintering in October, the experimental groups showed higher values: colony strength was 18.7% higher (*P < 0.05), honey production was 2% higher, pollen volume was 30.1% higher, and brood quantity was 50% higher. Furthermore, the experimental groups showed an increase in total honey weight of 14.4% and centrifuged honey of 15.5% compared to the control.

Veterinary and sanitary examination of the honey obtained using the immunomodulator demonstrated high quality, including a 20.9% increase in diastase number and a 9.8% decrease in moisture content. Scanning electron microscopy of bee haemolymph revealed the absence of infectious disease infections. The *Nosema apis* pathogen, undergoing phagocytosis, was detected in the haemolymph of the control group of bees. Future research will focus on determining the level of parasitic and bacterial infections in bees using the immunomodulator.

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CONFLICT OF INTEREST

None.

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Підвищення продуктивності бджолиних сімей за рахунок використання імуномодулятору

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Анотація. Забезпечення медоносних бджіл збагаченим раціоном на основі біогенних сполук металів зменшує ризик виникнення інфекційних захворювань, сприяє підвищенню резистентності, репродуктивної здатності матки та сили сімей. Метою дослідження було дослідити силу сімей, продуктивність, розплід, якість меду за використання імуномодулятору. Сила бджолиних сімей була більше у період 10 травня – на 8,3 %, 15 травня – на 7,1 %, 25 травня – на 6,3 %, 5 червня – на 5,6 % за використання імуномодулятору. Вірогідно збільшився видобуток пилку 10 травня на 11,9 %, 15 травня – на 28,4 % (*P < 0,05), 25 травня – на 17,7 % (*P < 0,05), 5 червня – на 32,2 % (*P < 0,05). Розплід збільшився 10 травня – на 5,3 %, 15 травня – на 11,92 %, 25 травня – на 19,6 % (*P < 0,05), 5 червня – на 23,4 % (*P < 0,05). Жива маса маток збільшилась 10 травня – на 1,8 %, 15 травня – на 2,5 %, 15 травня – на 4,5 %, 5 червня – на 5,15 % у групах з додаванням імуномодулятору. При застосуванні імуномодулятора для підгодівлі сімей на зимування сила збільшилась на 9,6 %, видобуток меду – на 5,1 %, обсяг пилку – на 20,4 %, кількість розплоду – на 35,3 %, збільшення живої ваги маток – на 9,4 %, порівняно з контролем. У жовтні місяці сила сімей збільшилась на 18,7 % (*P < 0,05), продукція меду – на 2 %, обсяг пилку – на 30,1 %, розплід – на 50 % у дослідних групах. Загальна вага меду збільшилась на 14,4 % та центрифугованого – на 15,5 %. За використання імуномодулятору отримали високу якість меду, в тому числі вище діастазне число – на 20,9 % та нижчий відсоток водності на 9,8 %. Відмічено позитивний вплив на мікрокартину, де спостерігається висока адгезивна та фагоцитарна активність гемоцитів до збудників хвороб та загальне збільшення кількості імунних клітин бджоли. Практичною цінністю роботи є підвищення захисних сил бджолиних сімей, їх продуктивності та отримання якісного та безпечного меду

Ключові слова: імуномодулятор; сила бджолиної сім'ї; видобуток меду; розплід; жива вага матки; пилок; якість меду