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CORRECTION OF HYGIENIC ACTIVITY OF BEES

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The hygienic cleaning of honeybees is a genetic defensive reaction which inhibits the spread of infections on an apiary. The level of its activity determines the protective abilities of bee colonies and can be adjusted by the application of the adequate in composition stimulants used in the bee colonies in a special way. It has been found out that the application of PDE (Placenta Denatured Emulsified) by the «modified method» (by feeding and aerosol spraying) activates the development of bee colonies and their sanitary and hygienic activity to a greater extent than by simple feeding. The first experimental group was given PDE by feeding; the second group was given PDE by feeding and aerosol spraying in a dose of 1 ml per 100 ml of 50 % syrup in the amount of 100 ml per beeway; the first control group was given sugar syrup without PDE by the «modified method»; the second control group was kept without any stimulation. The changes of hygienic cleaning of honey bees in Varroatosis and infections honeybee brood were evaluated after stimulation.

The level of hygienic cleaning in bee colonies was evaluated by the «perforation test» 24 hours after the application of PDE by different methods and without any stimulation. The degrees of hygienic cleaning in the bee colonies were the following: 95.3 % in the group where PDE was applied by the «modified method»; 94.7 % in the group where PDE was applied by the feeding; 90.3 % in the group which was given syrup without PDE, and 79.7 % in the group without any stimulation. The increase of the level of hygienic cleaning in bee colonies after the application of PDE by the «modified method» improved their ability to resist infectious diseases of the honeybee brood.

Keywords: BEEKEEPING, HONEYBEES, BEEWAY, PDE (PLACENTA DENATURED EMULSIFIED), STIMULATION, HYGIENIC CLEANING OF BEES, «PERFORATION TEST», DISEASES OF BROOD, PREVENTION

КОРЕКЦІЯ ГІГІЄНІЧОЇ АКТИВНОСТІ БДЖІЛ

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Гігієнічна поведінка медоносних бджіл є генетичною захисною реакцією, яка стримує розповсюдження інфекцій на пасіці. Рівень її активності визначає захисні можливості бджолосімей та може бути скорегований шляхом застосування повноцінних за складом стимуляторів та особливим способом їх задавання бджолосім'ям. Встановлено, що застосування плацентарного препарату ПДЕ (плацента денатурована емульгована) «модифікованим методом» (згодовуванням та аерозольно) активізує розвиток бджолосімей та їх санітарно-гігієнічну активність в більшій мірі ніж шляхом згодовування. Першій дослідній групі ПДЕ задавали згодовуванням; другій — «модифікованим методом» (згодовуванням і аерозольно) в дозі 1 мл на 100 мл 50 % цукрового сиропу по 100 мл на вуличку бджіл; першій контрольній групі — задавали цукровий сироп без ПДЕ «модифікованим методом»; друга контрольна група була без всілякої стимуляції. Зміни гігієнічної очистки бджіл при Вароозі та інфекціях розплоду медоносних бджіл оцінювали після проведення стимуляції.

Рівень гігієнічних очистки в бджолосімей оцінювалася тестом «перфорація» через 24 годин після задавання ПДЕ різними методами та без проведення стимуляції. Ступінь гігієнічної очистки в бджолиних сім'ях були відповідно: 95,3 % — у групі, що отримувала ПДЕ «модифікованим методом»; 94,7 % у групі, що отримувала ПДЕ згодовуванням; 90,3 % — у групі, що отримувала сироп без ПДЕ та 79,7 % — у групі без проведення стимуляції. Підвищення гігієнічної очистки

бджолосімей після використання ПДЕ «модифікованим способом» підвищила їх здатність протистояти інфекційним захворюванням розплоду медоносних бджіл.

Ключові слова: БДЖІЛЬНИЦТВО, МЕДОНОСНІ БДЖОЛИ, ВУЛОЧКИ БДЖІЛ, ПДЕ (ПЛАЦЕНТА ДЕНАТУРОВАНА ЕМУЛЬГОВАНА), СТИМУЛЯЦІЯ, ГІГІЄНІЧНА ОЧИСТКА БДЖІЛ, «ТЕСТ-ПЕРФОРАЦІЯ», ХВОРОБИ РОЗПЛОДУ, ПРОФІЛАКТИКА

КОРРЕКЦИЯ ГИГИЕНИЧЕСКОЙ АКТИВНОСТИ ПЧЕЛ

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Гигиеническое поведение медоносных пчел является генетической защитной реакцией, которая сдерживает распространение инфекций на пасеке. Уровень ее активности определяет защитные функции пчелосемей и может быть скорректированный путем применения полноценных по составу стимуляторов и особым способом их задавания пчелосемьям. Установлено, что применение плацентарного препарата ПДЭ «модифицированным методом» (скармливанием и аэрозольно) активизирует развитие пчелосемей и их санитарно-гигиеническую активность в большей степени, чем путем скармливания. Первой опытной группе задавали ПДЭ скармливанием; второй — «модифицированным способом» (скармливанием и аэрозольно) в дозе 1 мл на 100 мл 50 % сахарного сиропа по 100 мл на улочку пчел; первой контрольной группе — задавали сахарный сироп без ПДЭ «модифицированным методом»; вторая контрольная группа была без стимуляции. Изменения гигиенической очистки пчел при Вароозе и инфекциях расплода медоносных пчёл оценивали после проведения стимуляции.

Уровень гигиенической очистки в пчелосемьях оценивали тестом «перфорация» через 24 часа после задавания ПДЕ разными методами и без проведения стимуляции. Степень гигиенической очистки в пчелиных семьях были соответственно: 95,3 % — в группе, которая получала ПДЭ «модифицированным методом»; 94,7 % в группе, которая получала ПДЭ скармливанием; 90,3 % — в группе, что получала сироп без ПДЭ и 79,7 % — в группе без проведения стимуляции. Повышение гигиенической очистки пчелосемей после применения ПДЭ «модифицированным методом» повысила их способность противостоять инфекционным болезням расплода медоносных пчёл.

Ключевые слова: ПЧЕЛОВОДСТВО, МЕДОНОСНЫЕ ПЧЕЛЫ, УЛОЧКИ ПЧЁЛ, ПДЭ (ПЛАЦЕНТА ДЕНАТУРИРОВАНАЯ ЭМУЛЬГИРОВАННАЯ), СТИМУЛЯЦИЯ, ГИГИЕНИЧЕСКАЯ ОЧИСТКА ПЧЁЛ, «ТЕСТ-ПЕРФОРАЦИЯ», БОЛЕЗНИ РАСПЛОДА, ПРОФИЛАКТИКА

Ukraine has unique climatic conditions for the successful development of strong and viable bee colonies on the conditions that risks and infectious diseases of bee broods are minimized. The situation is complicated by the presence of associated infections on an apiaries, which cause some difficulties in diagnosis and therapy and require the use of different drugs. Therefore, to prevent the diseases on an apiary, it is advisable to take less aggressive preventive measures for bee colonies. First of all, this is disinfection, which provides the containment of spread of the infection, but does not play any decisive role in the protection of bees [1–2].

Due to the special vital activity of bees, which are in a constant contact with various pathogens that exist in the environment, it's impossible to fully exclude the epizootic chain in bee colonies. Thus, to resist diseases it's quite reasonable to take less aggressive preventive measures for the bee colonies on the apiaries. The term «immunity» is seen as the protective properties of bee colonies and their representatives. To create the protective mechanism, it is necessary to have the strong bee colonies with a high level of sanitary

cleaning; owing to this the removal of dead brood as a source of infection will be fulfilled in time. Therefore the search for ways of improvement of the hygienic cleaning of bees is relevant today [3–5].

The hygienic cleaning of bees or sanitary cleaning is the basis of adaptation and protection of natural bee colonies. Some scientists determine the term «hygienic cleaning» as a form of resistance to such infections as the bee pest, foul brood, mycosis and *Varroozis*. Such disease resistance is provided by early removal of the pathogens before they affect the other honeybees or the brood. The assertion that by activation of the sanitary ability of honeybees the sickness rate can be reduced and the use of drugs can be avoided is in the focus of attention of the researchers all over the world [6–8].

There is a classification to determine the hygienic level of bee colonies. The colony is considered hygienic if it is able to remove 70 % of the affected brood within 24 hours (other authors claim 90 % barrier) and unhygienic if it removes less than 40 % (70 % according to the other authors), which is a critical indicator, especially for winter [9].

Such matters as the connection of the hygienic cleaning of bees with genetics, breeds, habitats, and the ability of honeybees to recognize the diseased brood have been the subjects of debates nowadays. Bee colonies with a high degree of cleaning are recommended for breeding. It is also known that hygienic behavior depends on the strength of a bee colony, forms its viability, which in its turn depends on the climatic conditions, the quality of food supply and other factors. The use of immune drugs may be considered one of the ways of strengthening the hygienic cleaning. In our previous studies, the positive influence of PDE on the economic indicators and on the changes in the organism and hemolymph structure was proved. Its toxicity has been studied, its dose has been defined; the «modified method» of its application for the honeybees has been developed and patented [10–13].

The purpose of our work was to study possible ways of correction of sanitary and hygienic cleaning of honeybees through the application of PDE using the «modified method» (by feeding and aerosol spraying) in spring and assess the clinical signs of infestation of bee colonies with brood infectious diseases against a background of intensification of the hygienic cleaning.

Materials and Methods

The test was conducted on a private apiary of Chernihiv region in April 2013, after the exhibition of bee colonies had taken place. On this apiary, the honeybees were kept in multiblock beehives where the treatment against ticks was provided in autumn; the queens were replaced in time, the research and disinfection took place according to the plan, the honeycombs were discarded; honey flow took place in the environmentally friendly — forest, meadow and field - areas.

The object of study was 104 bee colonies of different strength, the native Ukrainian gray breed, bee queens aged 1.5–2.5 years, PDE (Placenta Denatured Emulsified) produced by «Biotekhindustriya» Ltd., changes in the sanitary and hygienic cleaning after the application of PDE in different ways, the analysis of the degree of *Varroatosis* infestation and the presence of «diversity» of symptoms in the brood of bees.

At the beginning, the four groups of bee colonies were formed for experiments and control. In each group, the types of families were isolated according to their strength: 10 strong (8–9 beeways), 10 average (6–7 beeways) and 6 weak (5 and fewer beeways). The first experimental group (E 1) was given PDE by feeding and the second experimental group (E 2) — by the application of the «modified method» (by feeding and aerosol spraying) in a dose of 1 ml per 100 ml of 50 % sugar syrup in the amount of 100 ml per beeway within two weeks. The first control group (C 1) was given sugar syrup without PDE of the «modified method» (by feeding and aerosol spraying) and the second control group (C 2) was kept without any stimulation (tabl. 1). The hygienic cleaning of bee colonies

and brood infestation of honey bees were evaluated after applying the additives.

The hygienic cleaning was studied by the «perforation test» of brood cells on the frame taken from the middle of the hive. The needle was inserted into the depth of 0.5 cm in each of 100 cells of the sealed brood on the frame segment sized 5x5 cm. This zone was marked and the number of the treated cells was counted 12 and 24 hours later.

The analysis of the hygienic ability of honeybees in bee colonies was evaluated by the "perforation test" according to the following indicators: above hygienic or high degree of cleaning — more than 95 %; hygienic enough — 90–95 %, medium hygienic — 80–90 %; unhygienic — below 80–70 % of treated cells with the pathologic brood of bees 24 hours later.

The presence of clinical signs of the infected brood was determined visually by the presence of «irregular brood» and mummified larvae during the research period. The extensiveness of *Varroatosis* infestation (number of mites per 100 bees) was taken into account. The changes in the bee colony strength before and after the application of PDE were evaluated. These experiments were treated statistically with the help of the Student's factor [14].

Results and Discussion

The experiments have confirmed the dependence of the intensity of development of bee colonies in spring upon their well-timed PDE stimulation. On the basis of the results of previous studies, the following methods of PDE application for bee colonies have been proposed (tabl. 1).

Table 1

The methods of application PDE with sugar syrup and without PDE

Period of experiment - spring	Groups of honey bees and their strength verified by experiment and control	PDE application (dose, frequency)		Methods of PDE application
		Basic PDE application	Additional PDE application	
	experimental			
	The 1st experimental group (**E 1***): Weak, n = 6 Average, n = 10 Strong, n = 10	1 ml of the PDE per 100 ml of 50 % sugar syrup per frame covered with bees by seven day as eaten, depending on the strength and needs of the colony, weather conditions and bloom intensity of honey plants	was not applied additionally	The feeding method of syrup with PDE
	The 2nd experimental group (**E 2****): Weak, n = 6 Average, n = 10 Strong, n = 10	1 ml of the PDE per 100 ml of 50 % sugar syrup per frame covered with bees by seven day as eaten, depending on the strength and needs of the colony, weather conditions and bloom intensity of honey plants.	in addition, by during the inspection or examination, the empty open frames for brood and honey are sprinkled with syrup with PDE and the PDE by aerosol spraying for better eating three times within two weeks	The «modified method» (feeding and aerosol spraying) of syrup with PDE
	control			
	The 1st control group (**C 1*****): Weak, n = 6 Average, n = 10 Strong, n = 10	100 ml of 50 % sugar syrup per frame covered with bees; 7 times a day as eaten, depending on the strength and needs of the colony, weather conditions and bloom intensity of honey plants. Drug was not applied	In addition, during the inspection or examination, the frames are sprinkled with syrup by aerosol spraying for better eating 3 times within 2 weeks. Drug was not applied	The «modified method» (feeding and aerosol spraying) of syrup without PDE
	The 2nd control group (**C 2 ******): Weak, n = 6 Average, n = 10 Strong, n = 10	Bee colonies only exist in natural conditions (nectar and honey). The PDE and syrup were not applied		Without any stimulation

Note: E 1*, E 2** — experimental groups and C 1***, C 2**** — control groups of bee colonies

The effectiveness of various stimulation methods are listed below (tabl. 2). Thus, on the average, after the application of PDE by the «modified method» in experimental group E 1 (feeding and aerosol spraying) the number of beeways increased by 1 beeway compared with experimental group E 1 (feeding method); increased by 1.3 beeways compared with control group C1 (feeding of syrup by the «modified method») and increased by 1.8 beeways compared with control group C 2 (without any stimulation).

Table 2

The hygienic cleaning and strength of bee colonies by different methods of PDE application (M ± m, n=6/10)

Period of experiment	Groups of honey bees and their strength verified by experiment and control		The strength of the colonies in beeways, number		Sanitary-hygienic cleaning. «Perforation test» control of cell cleaning 12 and 24 hours later, %	
			before the use of the PDE	after one month of the use PDE	**12 hour**	**24 hour**
Spring (April)	**E1***	Weak, n=6	4.5±0.31	7.4±0.24	71±0.78	89±1.69
		Average, n=10	5.5±0.2	8.0±0.20	81±1.29	96±0.67
		Strong, n=10	8.5±0.2	9.0±0.10	93±0.58	99±0.30
	Average data for group E1		**6,2**	**8.1**	**81.7 %**	**94.7 %**
	E2**	Weak, n=6	5.5±0.24	8.5±0.13	78±2.68	91±1.24
		Average, n=10	6.4±0.12	9.2±0.15	87±2.18	96±1.10
		Strong, n=10	8.5±0.18	9.5±0.00	96±1.21	99±0.30
	Average data for group E2		**6,8**	**9.1**	**87.0 %**	**95.3 %**
	C1***	Weak, n=6	5.5±0.20	6.5±0.22	70±1.75	81±0.97
		Average, n=10	6.5±0.21	7.5±0.13	82±2.21	91±1.69
		Strong, n=10	8.5±0.20	9.5±0.00	91±0.83	99±0.33
	Average data for group C1		**6,8**	**7.8**	**81.0 %**	**90.3 %**
	C2**	Weak, n=6	5.5±0.20	6.0±0.00	67±1.44	71±1.37
		Average, n=10	7.0±0.21	7.5±0.20	70±0.93	82±1.79
		Strong, n=10	8.0±0.20	8.5±0.17	78±2.21	86±1.87
	Average data for group C2		**6.8**	**7.3**	**71.7 %**	**79.7 %**

Note: E 1*, E 2** — experimental groups and C 1***, C 2**** — control groups of bee colonies

The application of PDE by the «modified method» showed the better results, especially for weak colonies (fig.1). Thus, on the average, for the weak colonies, after the application of PDE by the «modified method» in experimental group E 1 the number of beeways increased by 1.1 beeways compared with experimental group E 1.1 (feeding method); increased by 2.0 beeways in comparison with control group C1.1; and increased by 2.5 beeways compared with control group C 2.1 (without any stimulation).

Thus, on the average, for the strong colonies (fig. 1), after the application of PDE by the «modified method» in experimental group E1.1 the number of beeways increased by 0.5 beeway compared with experimental group E 1.2 (feeding method); increased by 0.0 beeway in comparison with control group C 1.1; and increased by 1.0 beeway compared with control group C 2.1 (without any stimulation).

That resulted from the fact that the bees are probably capable to adjust the intensity of development in accordance with the conditions in which they reside. Thus, the lack of nectar can be an instinctive deterrent for a queen to lay eggs and for swarming of bee colonies.

The results of the «perforation test» showed that the strong bee colonies, stimulated with PDE by feeding and the «modified methods», performed their sanitary functions by 94.7–95.3 %, which is considered the sufficient and high levels of cleaning; the control group of bee colonies receiving sugar syrup by the «modified method» — by 90.3 % — the average level of cleaning; the colonies without any stimulation — by 79.7 % — the low level of cell cleaning.

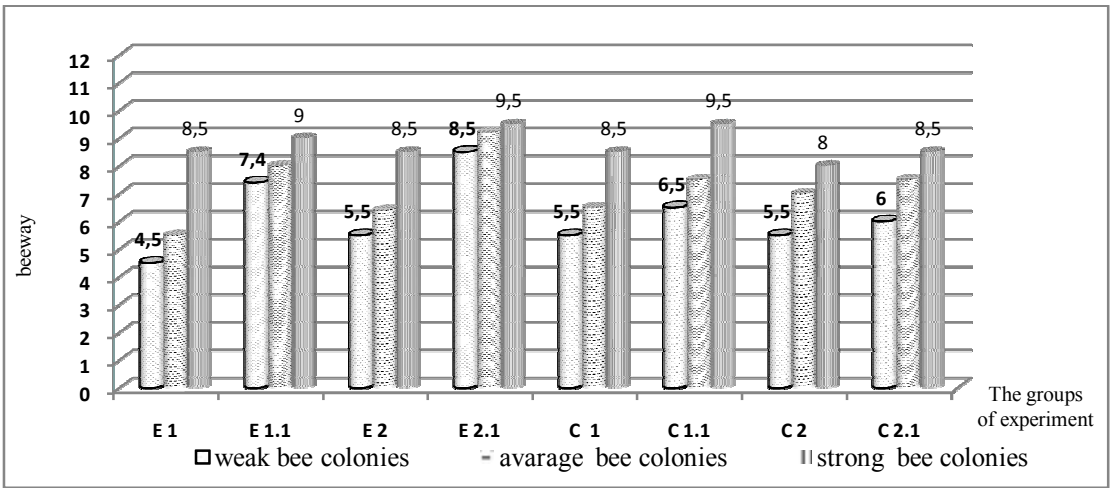

Fig. 1. The correlation of strength of bee colonies in different methods of the PDE application:
E 1 — the experimental group before PDE application by the feeding method of syrup with PDE;
E 2 — the experimental group before PDE application by the «modified method» of syrup with PDE;
C 1 — the control group before syrup application by the «modified method» without PDE;
C 2 — the control group before any stimulation, under natural conditions;
E 1.1, E 2.1, C 1.1, C 2.1 — after the application of different stimulation

The comparison of the results of the test has shown that 12 hours later, the total degree of hygienic cleaning was 84.4 %; 24 hours later — 95.0 % in experimental groups E 1 and E 2 and 76.4 % and 85.0 % in control groups C 1 and C 2 (fig. 2).

The interesting thing is that the bees of the experimental and control groups cleared 80.4 % of the perforated cells in the first 12 hours of the test. This fact proves the development of sanitary and hygienic properties in all bee colonies and their dependence on the external factors that affect the viability of bee colonies. Active ability of honeybees to clean the comb cells from the dead brood does not lead to a complete recovery of colonies but it is an effective deterrent to the spread of infectious brood diseases.

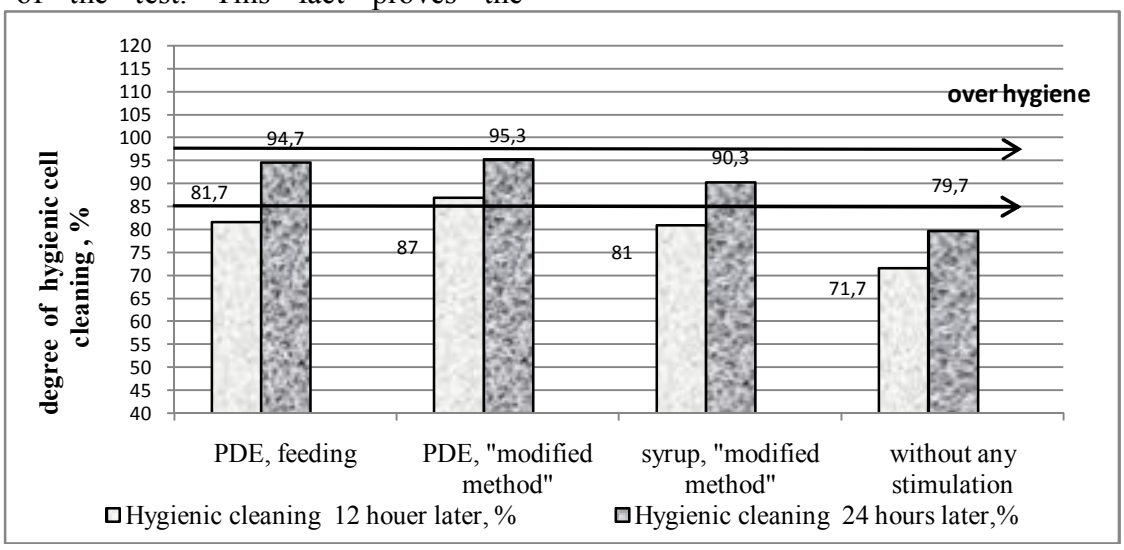

Fig. 2. Evaluation of hygienic cleaning of bee colonies by the «perforation test» in different methods of PDE application

The presence of the mummified larvae and immature bees in large numbers at the bottom of the hive, the «irregular brood» in the brood cells in many bee colonies are the signs of clinical development of *Foulbrood*, fungal infections, *Varroatozis* or other diseases. This indicates that bee colonies are no longer able to suppress the infectious process themselves and require complex veterinary-sanitary measures. The observation of the above mentioned symptoms showed that the signs of *Varroatozis* in the weak bee colonies were 2–3 %, in the strong bee colonies — 1–2 % before the experiment. The «irregular brood» and the presence of *Chalkbrood* or individual cells with the signs of larvae decay (1–5 cells on the combs) were observed in April in 10 % of weak bee colonies, in 5 % of average bee colonies and in 1–2 % of strong colonies.

The clinical signs of brood infestation were reduced after the correction of the hygienic cleaning of bees with the application of PDE by the "modified method". They were absent in strong colonies. In average bee colonies, the figures were 1–2 %. In weak colonies, the clinical signs of diseases reduced to 3 %.

The clinical signs of brood infestation reduced less considerably in the bee colonies without any stimulating. So, in weak colonies, these figures dropped from 10 % to 8 %. This proves the fact that after the stimulation by natural factors alone, the weak colonies have less ability to resist diseases. The degree of *Varroatozis* infestation in weak colonies increased from 3 to 5 % and in other weak and average colonies that received PDE remained within the limits of 1–2 %.

Conclusions

1. The «modified method» of PDE application (by feeding and aerosol spraying) with syrup is an additional motivating factor for bee colony development. So, compared with the «modified method», during April, the number of beeways in all groups increased on the average by one beeway due to PDE feeding; the number of beeways increased by 1.3 beeways due to syrup feeding by the «modified method»; the number of beeways increased by 1.8 beeways — in bee colonies without any stimulation. In weak bee colonies the number of beeways increased by 1.1; 2; 2.5 beeways respectively, indicating the need for the required support of weak families.

2. The «modified method» can improve the hygienic cleaning of bee colonies. When conducting the «perforation test», the daily hygienic cleaning of the bee colonies in the group where PDE was applied by the «modified method» was 95.3 % and by simple feeding with PDE — by 94.7 % which correspond to the high level of cleaning; the application of syrup without PDE by the «modified method» showed 90.3 % of hygienic cleaning — the average level; bee colonies without any stimylation — 79.7 % which correspond to the low level of hygienic cleaning.

3. The bee colonies with high and average levels of hygienic cleaning (above 90 %) have a strong protective reaction, which resists the development of brood infections. The bee colonies with low level of cleaning (less than 90 %) can be regarded at risk of disease and therefore need support. The clinical signs of infectious brood diseases were absent after the stimulation in strong experimental colonies; in average colonies they were at the level of 1–2 %; in weak colonies that received syrup — 2 %; in bee colonies without any stimulation there were 8 % of affected bee colonies.

Prospects for Further research. The «modified method» of the stimulant application with PDE as an example can be recommended for the correction of the hygienic cleaning of bees in practical beekeeping. It is planned to assess the effectiveness of other stimulants on the hygienic cleaning of honeybees in the application of the «modified method».

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