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Apimix and Apipasta the New Trend in Bee Feeding and Creation of New Bee Colonies

Research Article

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ABSTRACT

The nutritional needs of worker bees are supplied by nectar carbohydrates, protein, and other nutrients in pollen. This paper aims to study the impact of Apimix and Apipasta feeding on the productive parameters of newly created bee colonies and to trace their health status concerning 6 viruses and the cause of nosematosis. Bee colonies of the local honeybee *Apis mellifera* settled in Langstroth hives system were used. The following groups were created: control group) feeding with sugar solution (sugar water 1:1) without additives; experimental group I) feeding with Apimix and experimental group II) feeding with Apipasta. It was found out that the stimulation feeding with liquid food Apimix significantly increases (P<0.01) the strength of the bee colonies in the period 13.07. – 29.09.2018. Also, significant positive correlations (r=0.55 and r=0.64, P<0.01) between the amount of sealed worker bee brood and the amount of collected pollen after feeding with Apimix and Apipasta, respectively. *Nosema ceranae* and *Nosema Apis* and 6 honey bee viruses were not detected in the bee colonies.

KEY WORDS amino acids, Apimix, Apipasta, bee colonies, bee feeding, vitamins.

INTRODUCTION

Beekeeping depends on environmental resources. Beekeepers commonly supplement honey bee colonies' nutrition with pollen or nectar substitutes to encourage colony strength and to prevent malnutrition. The nutritional needs of worker bees are supplied by nectar carbohydrates, protein, and other nutrients in pollen (Nicolson, 2011; Taha El-Kazafy *et al.* 2019). By consuming fresh or stored pollen honeybees received amino acids, lipids, vitamins, and minerals necessary for their growth and development (Smart *et al.* 2016). Pollen nutrition and different bee feeding diets affect the bee lifespan (Guler *et al.* 2018) and their resistance to pathogens (Tritschler *et al.* 2017). It has been shown that bee colonies after poor nutrition suffer from increased rates of infection (Branchiccela *et al.* 2019). For this reason, beekeepers feed pollen substitutes to colonies to increase colony strength and reduce colony susceptibility to pathogens such as *Nosema* spp. Furthermore, bee colonies with inappropriate nutrition have a risk of experiencing negative effects associated with pathogen infections (Fleming *et al.* 2015). Supplementary feeding of the bee colonies in the lack of nectar in nature could be done with sugar solution with different concentration, inverted sugar syrup, honey-sugar dough, vitamins, products which stimulate the bee colonies (Zhelyazkova and Nenchev, 1995; Zhelyazkova and Nenchev, 2001; Ivanova, 2005; Shumkova, 2016; Eşanu *et al.* 2018; Nasr *et al.* 2018). In the sugar syrup could be added vitamins, microelements, bee pollen, bee bread, extracts of coniferous trees to stimulate the growth and development of the bee colonies (Ishmuratova, 2002). Protein supplementation in the beekeeping practice

is common in cases of pollen deficiency. The protein foods have a highly beneficial effect on the immunity of the bees, brood rearing, and development of the bee colonies. In some cases, the supplements for bees are mixtures of amino acids and vitamins. Glavinic *et al.* (2017) assessed the potential of supplements with amino acids and vitamins to protect honeybees from immunosuppression induced by *Nosema ceranae* infection. The results indicated that the tested product had the potential to modify the expression of immune-related genes in infected bees. The authors Şahinler *et al.* (2015) studied the effect of vitamin E supplement on queen cell acceptance rates in bee colonies. Bee pollen is a mixture of flower pollen which is very rich in B complex vitamins. Soares de Arruda *et al.* (2013) reported results for B complex vitamins (B_1, B_2, B_6, and PP) in pollen samples. Honeybees have specific requirements for the amino acids leucine, isoleucine, and valine (Brodschneider and Crailsheim, 2010). Proline is not an essential amino acid but it is important for the bees during the flight (Micheu *et al.* 2000).

Although different products containing carbohydrates, amino acids, and vitamins have been used in beekeeping, the effect of Apimix and Apipasta on the productive parameters of bee colonies has not been studied. In this regard, the paper aims to study the impact of Apimix and Apipasta feeding on the productive parameters of newly created bee colonies and to trace their health status concerning 6 viruses and the cause of nosematosis.

MATERIALS AND METHODS

Bee colonies of the local ecotype honeybee *Apis mellifera* settled in the *Langstroth hives* system were used (Radoslavov *et al.* 2017). The experiment was conducted at the apiary of Research Center of Stockbreeding and Agriculture – Smolyan, Bulgaria. The following groups (each of 5 bee colonies) were created control group) feeding with sugar solution (sugar/water 1:1) without additives – 30 L; experimental group I) feeding with Apimix – 30 L;
Experimental group II) feeding with Apipasta – 10 kg. This experimental group received an additional 5 L Apimix after 15 September. This food is needed as food supplies for the bee colonies during the winter.

Apimix is a liquid syrup. It contains a fructose component, amino acids (alanine, isoleucine, leucine, lysine, arginine, aspartic acid, phenylalanine, methionine, cystine, proline, glutamic acid, serine, glycine, threonine, histidine, tyrosine, valine) and vitamins from B group (B_3, B_5, B_1, B_2, B_6, B_7, B_8) (https://zukanapicola.com/en/products/apimix/).
Apipasta is a pasty food made mainly of sucrose which is attractive to bees and easy to use by beekeepers. It consists of microcrystals with an average size of 15 µm. Each crystal is covered with a layer of glucose syrup, specially designed to be easily digested by the bees. It also contains amino acids (alanine, isoleucine, leucine, lysine, arginine, aspartic acid, phenylalanine, methionine, cystine, proline, glutamic acid, serine, glycine, threonine, histidine, tyrosine, valine) and vitamins from B group (B_3, B_5, B_1, B_2, B_6, B_7, B_8) (https://zukanapicola.com/en/products/apipasta/).

The bee feeding has begun since their creation. This is the time when the queen cup of the wax comb is transferred into the new bee colonies.
In 12 days a total of 8 measurements were taken. A measuring frame (size of the squares are 5×5 cm) was used. The following parameters were identified:

- amount of bees (strength of the bee colony) in kg – number of frames occupied by bees. One frame of multihull hive contains approximately 200 g bees. This is calculated after a lot of control measurements.
- quantity of honey (in kg) and bee pollen in the beehives (cm^2).
- amount of sealed worker bee brood (number of cells) – in 1 cm^2 there are 4 worker cells in the honeycomb. There are 100 worker cells in the area of 25 cm^2.
- average daily egg-laying of queen bees (number of cells) – it is determined by the amount of sealed brood at an estimated 100 worker cells with sealed brood in a square of the measuring frame.

Average daily egg-laying= (number of squares with sealed brood×100) / 12

Where:
12: number of days when the honeybee is in a phase before cocoon and cocoon stage (sealed brood).
• the day of the hatching of the bee queen.
• the day when the bee queen starts to lay eggs.
• wax construction of honeycombs – number of newly created wax honeycombs in the hives.

Dates are presented as day and month. All measurements of the bee colonies were during the period 13.07. – 29.09.2018.

Health status of the bee colonies

The presence of *Nosema* spp. and *Nosema Apis* and six honey bee viruses – deformed wing virus (DWV), acute bee paralysis virus (ABPV), chronic bee paralysis virus (CBPV), sacbrood virus (SBV), Kashmir bee virus (KBV), and black queen cell virus (BQCV) were studied by a reverse transcription-polymerase chain reaction (RT-PCR) according to Shumkova *et al.* (2018a) and Shumkova *et al.* (2018b). Bee colonies were not treated with preparations against Nosema disease 6 months before the studies. Bee samples were taken from each bee colony. All bee colonies

were examined before the experiment and they did not have clinical symptoms of bee disease.

Statistical analysis

The data were 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-tests. The correlation coefficient (r) and the coefficient of determination (R^2) were calculated with SPSS software version 20 for Windows (SPPS, 2011). Significant differences were considered at $P < 0.01$.

RESULTS AND DISCUSSION

The results for the strength of the bee colonies after stimulation with the products Apimix and Apipasta are presented in Figure 1.

At the beginning of the experiment (13.07.), the strength of the bee colonies was about 0.80 kg in the control and experimental groups bee colonies. In all subsequent measurements, the strength of the group fed with Apimix liquid syrup was higher than the control, and the group fed with Apipasta. According to Wang *et al.* (2014), bees that receive amino-rich food have a longer life span.

During the period 05.08. – 16.08. the highest strength of the bee colonies was observed in the group received Apimix. It is about 1.3 times higher than the control group. A statistically significant influence (ANOVA, $F=3.94$, $P<0.01$) for the effect of Apimix on the strength of bee colonies was observed. No significant value was found for the group fed with Apipasta.

Bee colonies fed with Apipasta received an additional 5 L of Apimix after 15.09. This is needed for the bee colonies to have enough food for the winter. It could be noted that after this period, both groups were equal regarding the parameter strength of the bee colonies. At the end of the study on 29.09. bee colonies that received Apimix had 1.2 times higher strength compared to the other two groups. This is important for the wintering bee colonies because they will have enough young bees.

In October, the strength of the bee colonies is gradually decreased. This is a normal biological process and preparation for the winter. The strength of the bee colonies which received Apipasta was similar to those of the control group. At the beginning and at the end of the experiment they were almost identical. Statistically significant differences were found between the control group and the Apimix group ($P<0.01$) in the period 13.07. – 29.09. Statistically significant differences ($P<0.05$) between the control group and the bees received Apipasta were observed on 05.08.

Taranov (1986) reported that when the bees received sugar syrup they have 18.7% less brood and collect 24.6% less honey than the bee colonies which received honey as food.

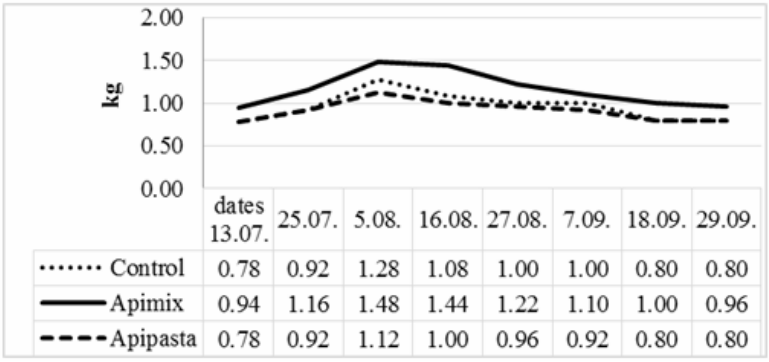
Sugar syrup as a stimulant is effective when the bees can carry fresh pollen or when protein is added to the food. The growth of young larvae can only occur with a certain amount of protein. If there is a lack of protein food in the hive or nature, bees use their protein reserves (Bilas and Benevolenscaia, 2002). In the present study, the effect on the quantity of honey after feeding with Apimix and Apipasta showed no significant values. The results for the amount of honey from the three groups were similar (Figure 2).

Figure 3 shows the results for the amount of the collected bee pollen in the bee colonies.

In the spring period, the strength of the bee colonies and the effective use of early bee pasture was directly proportional to the protein reserves in the colony. Bees can rear brood without pollen flow for only two weeks. They use reserves of their organism which leads to their exhaustion. Bee colonies provided with optimal protein food can rear 27.4% more brood and collect 40% more honey than the bee colonies with protein deficiency (Krivtsov *et al.* 1999). When the protein food is insufficient bees grow less drone brood or they do not grow at all because their larvae require five times more food than the worker's larvae. Lebedev and Billas (1994) report that in the absence of pollen, worker bees reduce or stop feeding the bee drones. According to Taranov (1986), in pollen deficiency, the honey bees are non-viable with underdeveloped hypopharyngeal and wax glands and fat bodies.

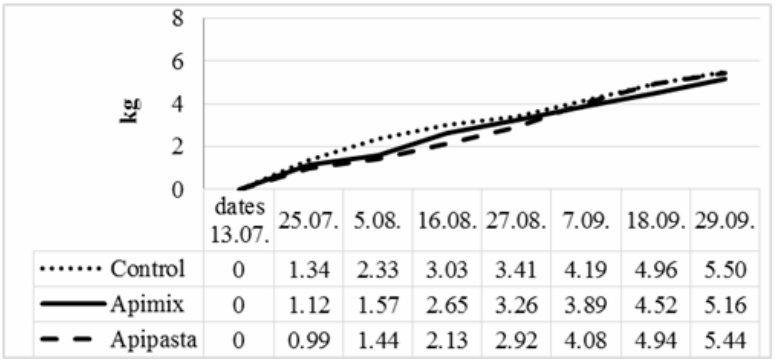
At the beginning of the experiment, the amount of pollen in both groups increased sharply and reached its highest values. The next step of a sharp rise is 05.08 (Figure 3). During this period, the bee colonies show the highest strength (Figure 1). On one hand, it can be supposed that reducing the amount of pollen in the bee colonies is connected with feeding on the bee brood. On other hand, this may depend on low pollen flow from the honey plants in the area. In this case, if the amount of pollen in the environment is reduced or absent feeding with protein or amino acids is necessary. No significant differences in the amount of collected pollen by bees were found after feeding with Apimix and Apipasta.

Indeed, the bee colonies do not store a large amount of bee pollen. They regulate the amount of pollen in the colony according to their current needs and the amount of uncapped brood (eggs and larvae) (Hoover and Ovinge, 2018).



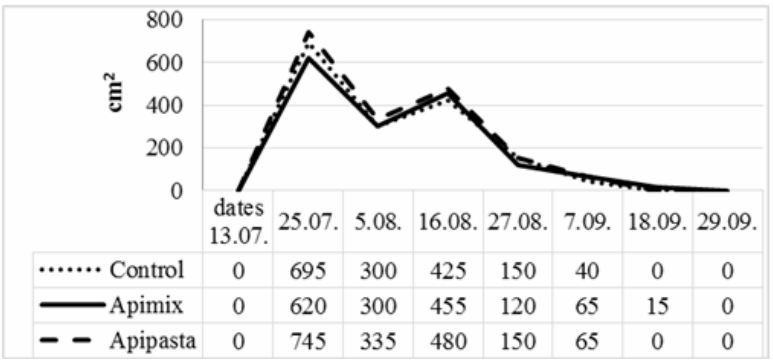
dates 13.07.	25.07.	5.08.	16.08.	27.08.	7.09.	18.09.	29.09.
Control	0.78	0.92	1.28	1.08	1.00	1.00	0.80	0.80
Apimix	0.94	1.16	1.48	1.44	1.22	1.10	1.00	0.96
Apipasta	0.78	0.92	1.12	1.00	0.96	0.92	0.80	0.80

Figure 1 Average values for the strength of the bee colonies (kg)



dates 13.07.	25.07.	5.08.	16.08.	27.08.	7.09.	18.09.	29.09.
Control	0	1.34	2.33	3.03	3.41	4.19	4.96	5.50
Apimix	0	1.12	1.57	2.65	3.26	3.89	4.52	5.16
Apipasta	0	0.99	1.44	2.13	2.92	4.08	4.94	5.44

Figure 2 Quantity of honey in the beehives (kg)



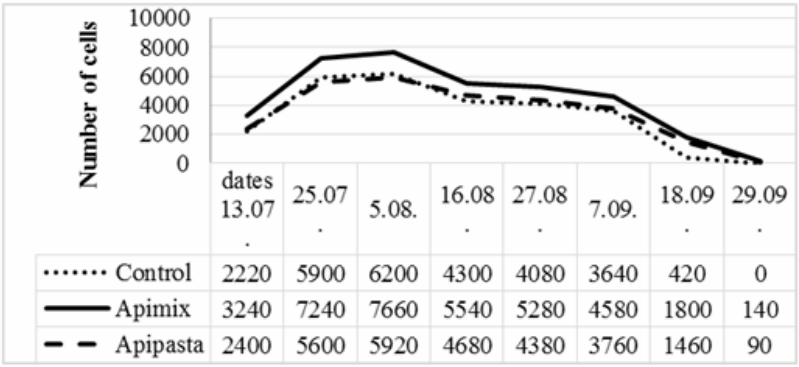
dates 13.07.	25.07.	5.08.	16.08.	27.08.	7.09.	18.09.	29.09.
Control	0	695	300	425	150	40	0	0
Apimix	0	620	300	455	120	65	15	0
Apipasta	0	745	335	480	150	65	0	0

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

According to the results obtained, a positive effect on the quantity of the sealed worker bee brood was found after feeding with Apimix during the whole period (Figure 4).

On 05.08. the group fed with Apimix has 1.2 times more sealed worker bee brood than the control group. The bee colonies receiving Apimix had significantly more amount of sealed worker bee brood as compared to the control group (ANOVA, $F=3.95$, $P<0.05$). The statistically significant influence of the product Apipasta was not found. In this regard, feeding rich in amino acid foods is beneficial to create conditions for bee brood rearing and a sufficient amount of physiologically young bees in the autumn period. These bees should not be exhausted by processing sugar syrup.

They have a well-developed fat body that will allow them to survive the winter. Significant positive correlations were established between the amount of sealed worker bee brood and the quantity of collected pollen in the experimental group received Apimix ($r=0.55$, ($P<0.01$), $R^2=0.30$), and received Apipasta ($r=0.64$, ($P<0.01$), $R^2=0.41$) (Figure 5).



	dates	25.07	5.08.	16.08	27.08	7.09.	18.09	29.09
	13.07	.		.	.		.	.
Control	2220	5900	6200	4300	4080	3640	420	0
Apimix	3240	7240	7660	5540	5280	4580	1800	140
Apipasta	2400	5600	5920	4680	4380	3760	1460	90

Figure 4 Amount of sealed worker bee brood (number of cells)

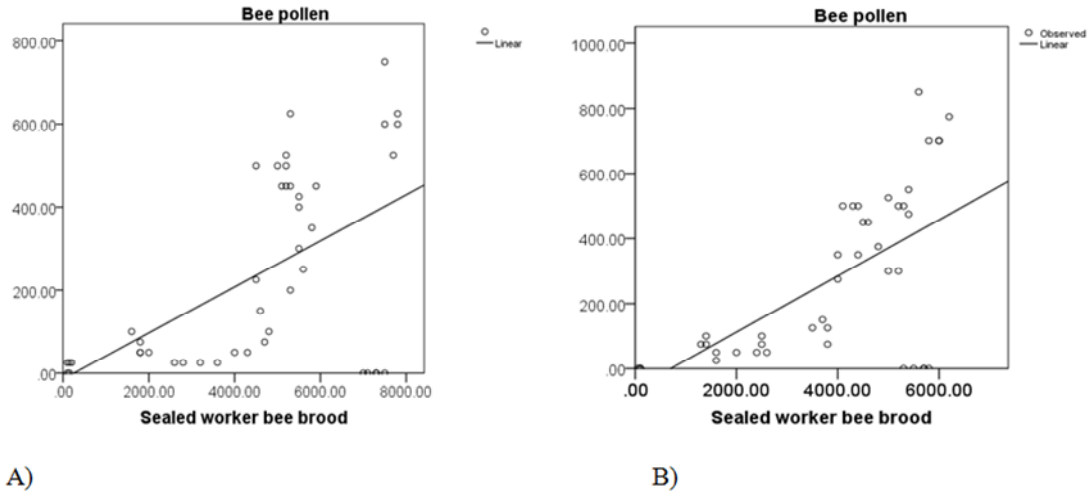


Figure 5 Linear correlation between the amount of the sealed worker bee brood and the amount of collected bee pollen A) bee colonies fed with Apimix (r=0.55, P<0.01 and B) bee colonies fed with Apipasta (r=0.64, P<0.01)

Coefficients of determination were also presented. For the Apimix group, 30% of the amount of the sealed worker bee brood depends on the quantity of collected bee pollen. The coefficient of determination is 41% for the Apipasta group.

It is expected that amino-rich and vitamin-rich foods such as Apimix and Apipasta stimulate the bee colonies to rare more bee brood. This leads to the increased strength of the bee colonies and reflects on the quantity of collected and transferred bee pollen in the hives. A positive correlation between the two parameters was also found.

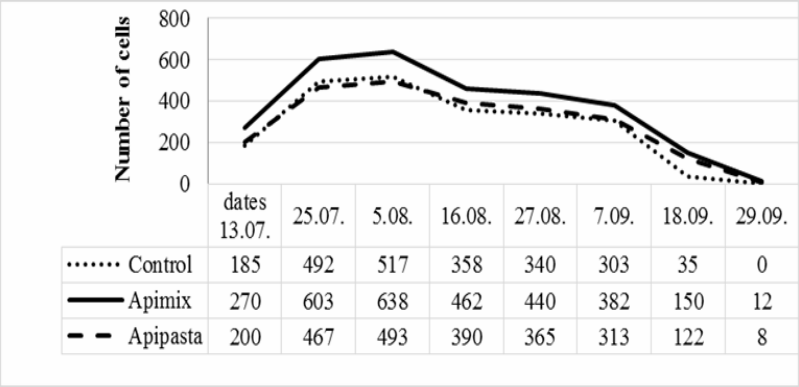
Extensive feeding of fertilized bee queens with royal jelly leads to intense laying eggs (1500-2500 eggs per day). The weight of all the eggs laid by the bee queen exceeds its live weight up to 2 times. The bee queen from colonies fed with Apimix showed the highest lying activity during the whole period (Figure 6). It can be assumed that these bee colonies have well developed hypopharyngeal glands that produce a larger quantity of royal jelly for the bee queens.

The results showed that the bee queens from the colonies that received Apimix laid eggs 24 hours earlier than the bee queens from the other two groups.

According to the results obtained bee colonies that received Apimix bees build twice more wax honeycombs than the control group. The results for Apipasta are similar to those of the control group (Figure 7). Considering that for the build of wax honeycombs the bees secreted 0.038 kg wax (Nenchev and Zhelyazkova, 2010), it can be assumed that Apimix has a stimulating effect on the wax glands development of the bees.

For the health status of bee colonies, bees have been sampled for the presence of six bee viruses, *Nosema ceranae*, and *Nosema apis* (Table 1). The results showed that these pathogens were not found in all bee colonies. The local ecotype honeybee *Apis mellifera macedonica* is resistant to a lot of bee viruses and diseases (Shumkova *et al.* 2018b).

For this reason, the control group is also negative against bee diseases.



dates	13.07.	25.07.	5.08.	16.08.	27.08.	7.09.	18.09.	29.09.
Control	185	492	517	358	340	303	35	0
Apimix	270	603	638	462	440	382	150	12
Apipasta	200	467	493	390	365	313	122	8

Figure 6 Average daily egg-laying of queen bees (number of cells)

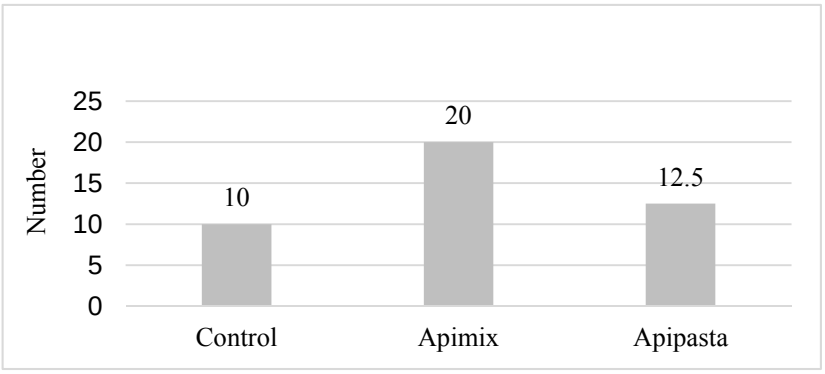


Figure 7 Number of built wax honeycombs

Table 1 Health status of the bee colonies[1]

Pathogens	Control group bee colonies, n=5	Experimental group fed with Apimix, n=5	Experimental group fed with Apipasta, n=5
Deformed wing virus (DWV)	-	-	-
Bee paralysis virus (ABPV)	-	-	-
Chronic bee paralysis virus (CBPV)	-	-	-
Sacbrood virus (SBV)	-	-	-
Kashmir bee virus (KBV)	-	-	-
Black queen cell virus (BQCV)	-	-	-
Nosema ceranae	-	-	-
Nosema apis	-	-	-

[1] Legend: - a negative result for the pathogen.

The composition of the products Apimix and Apipasta are very diverse. They contain different amino acids and vitamins which are important for a proper immune system function. Some essential amino acids are needed for the synthesis of peptides in immune pathways (Schmid-Hempel, 2005; DeGrandi-Hoffman and Chen, 2015). The present study can improve our understanding of how bee colonies fed with dietary supplementation prevent bees from pathogens.

CONCLUSION

Stimulation feeding with liquid food Apimix significantly increases ($P<0.01$) the strength of new bee colonies in the period 13.07. – 29.09. Significant positive correlations ($r=0.55$ and $r=0.64$, $P<0.01$) between the amount of sealed worker bee brood and the amount of collected pollen after feeding with Apimix and Apipasta, respectively. Bees fed with Apimix build more wax honeycombs than the bees

from the other two groups. *Nosema ceranae* and *Nosema apis* and 6 honey bee viruses such as deformed wing virus (DWV), acute bee paralysis virus (ABPV), chronic bee paralysis virus (CBPV), sacbrood virus (SBV), Kashmir bee virus (KBV), and black queen cell virus (BQCV) were not detected in the bee colonies.

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