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Application of stimulating products in autumn feeding and wintering of the bee colonies (*Apis mellifera* L.)

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Abstract

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The effect of stimulanting products on some parameters which characterize the wintering of the bees such as degree of the worker honeybees fat body, amount of dead bees and quantity of food consumption in the winter was investigated. For stimulating feeding of the bee colonies the products "Apimix", "Apipasta", "Probee-41" and "Baikal EM1" were used. The experiment was carried out in 2018 during the autumn feeding of the bee colonies at Research Center of Stockbreeding and Agriculture, town of Smolyan. Samples of worker bees were taken to determine the condition of the fat body at the end of the experiment before preparing of the bees for winter period. The degree of fat body development was determined by 5-point scale proposed by Maurizio (1954). For the characterization of the parameters of the wintering, two reviews of the bee colonies were carried out (during wintering in October 2018 and in March 2019). Strength of the bee colonies (amount of bees in the beehive) and amount of honey supplies (the capped honey in the honeycombs) were reported. It was found that the autumn feeding of the bee colonies with the addition of the microbiological product "Baikal EM1", the plant product "Probee 41" and "Baikal EM1", the honey consumption for 1 kg of bees in the winter was reduced. The highest percentage of dead bees (winter loss) in the control groups of bee colonies C_1 and C_2 was established ($P \le 0.05 - P \le 0.01$). Winter loss under 20% was found in the colonies which received "Probee 41" and "Baikal EM1" during the autumn feeding.

Keywords: honeybees; stimulating products; fat body; wintering

Introduction

Two main periods are defined in the life of the bee colonies – active and season of relative rest (autumn-winter period). The final stage of the active season is the preparation of the bee colonies for the winter. For the climate in Bulgaria, the preparation for the winter starts in the middle of August and it ends in October. October is a transitional period when the bees pass from active to passive period. The important factors for the successful wintering of the bee colonies are: the physiological condition of the bees (wintering with young bees); quantity and quality of food supplies (wintering with nectar honey and bee pollen with good quality).

The hatched worker bees at the end of September to the middle of October are equals to young honeybees and they live longer (5-6 months). In the autumn the honeybees have well-developed hypopharyngeal glands (HfG) and fat bodies, which store reserve substances such as proteins, lipids and glycogen. As a result of presented studies in the XX century (1960-2000), a number of correlations have been established between the stored food supplies in the bee body and some indicators which are important for the development of the bee colonies: higher amount of nitrogen in the bee body (fat body, respectively), the more resistant to bee diseases, less amount of consumed food in the winter, more bee brood rearing in the next spring (Zherebkin & Shagun, 1971; Zherebkin, 1974; Yakovleva, 1978; Kunert & Crailsheim, 1988; Bilash, 1990; Lebedev & Bilash, 1994). These correlations are an indication for the essential role of the fat body (its morphological changes and its degree of development) for the wintering bees. The fat body is an organ that performs a number of metabolic processes (Snodgrass, 1976; Roma et al., 2010; Hoshizaki, 2013; Aljedani, 2018). Another important function of the fat body is to store and release energy according to the energy needs of the bees (Arrese & Soulages, 2010).

Winter resistance is an important indicator for successful wintering of the bees. In Bulgaria, according to Regulation 35/2004 on the terms and conditions for carrying out the selection and reproduction in beekeeping, the winter resistance is a controlled parameter. The evaluation of the winter resistance of the bee colonies is done by the following parameters: the amount of food consumption in the winter; the amount of dead bees in the winter (Nenchev & Zhelyazkova, 2010).

During the preparation the bee colonies for wintering the quality food is important for their normal development. The addition of stimulating products in the autumn feeding is common in apiculture in the reduction or absence of bee pollen and nectar in the nature. The use of high-protein foods and vitamins has an established beneficial effect on the development of the bee colonies. In this regard, in the autumn feeding of the bee colonies pollen and the addition of stimulating products lead to storage of food reserves in the fat body of the worker bees. This reserve is necessary for the bee brood rearing which begins at the end of the winter before the first flowering plants.

The aim of the present study is to determine the effect of the stimulating products "Apimix", "Apipasta", "Probee-41" and "Baikal EM1" in the autumn feeding of the bee colonies on some parameters which characterize the wintering of the bees such as degree of the worker honeybees fat body, amount of dead bees and quantity of food consumption in the winter.

Materials and Methods

The study was conducted during preparation of the bee colonies for wintering in 2018 and in the beginning of 2019 in the Experimental apiary of Research Center for Animal Husbandry and Agriculture – town of Smolyan. The study includes 30 bee colonies, settled in Langstroth-Rut hives, 15 new created bee colonies (formed in 2018) and 15 basic bee colonies (formed before 2018) separate in two control groups and four experimental groups.

For the autumn feeding of the bee colonies "Apimix", "Apipasta", "Probee 41", "Baikal EM1" and sugar solution 1:1 (sugar and water) were used. "Apimix" and "Apipasta" have been applied to the experimental groups of new created bee colonies, and "Probee 41" and "Baikal EM1" to basic experimental bee colonies.

"Apimix" is a liquid which contains fructose component, essential amino acids and vitamins (B_1 , B_2 , nicotinic acid B_3 , pantothenic acid B_4 , B_6 , biotin B_7 and inositol B_8).

"Apipasta" is a pasty food made mainly of sucrose. It is very attractive to the bees and easy to use. It consists of microcrystals with an average size of 15 μ m. Each crystal is covered with a layer of glucose syrup, specially designed to be easily digested by the bees. It also contains essential amino acids and B vitamins (B₁, B₂, B₃, B₅, B₆, B₇, B₈).

"Probee 41" is a natural product. It is an aromatic herbal solution. It contains wheat, lavender, walnut, eucalyptus, juniper, avocado, rosemary and garlic oils.

"Baikal EM1" is a microbiological preparation consisting of a complex of living beneficial microorganisms. The application of the preparation is universal. This complex product of living beneficial bacteria is indispensable for soils and water, plants, bees, birds, animals and fish, including humans. The application of effective microorganisms (EM) in apiculture provides a significant improvement in the overall condition of bees which increases the bee products quality.

The bee feeding was from 15.07 to 15.09.2018, and for "Baikal EM1" from 05.08. to 15.09.2018. The feeding was carried out by the following scheme:

• Control groups: feeding with sugar solution (1:1) without additives – total amount of 30 l;

• Experimental group: feeding with addition of "Apimix" – total amount of 30 l;

• Experimental group: feeding with solid food "Apipasta" (10 kg) and "Apimix" (5 l);

• Experimental group: feeding with "Probee 41" at a dose of 5 ml in 1 l sugar solution (1:1) until 10 l sugar solution per bee family and spraying the honeycombs in every 15 days until the wintering with 1 part "Probee 41" and 3 parts water;

• Experimental group: feeding with "Baikal EM1" at a dose of 20 ml/10 l sugar solution (1:1).

The total amount for each bee colony is 10 l. In October, before the preparation for winter (according to the manufacturer's instructions), the honeycombs were sprayed with this product twice times per night at a dose of 2 ml/l water with 20-25 ml per bee colony. Before the preparation for winter, samples of worker bees were taken to determine the condition of the fatty body. The degree of fat body development of 406 bees was defined by the 5-degree scale presented by Maurizio (1954) - Fig. 1.

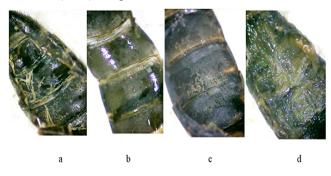


Figure 1. Different degrees of development of the fat body: *a*) 2nd degree; *b*) 3rd degree; *c*) 4th degree; *d*) 5th degree.

For the characterization of the parameters of the wintering, two reviews of the bee colonies were carried out – during wintering (in October 2018) and in March 2019. The following parameters were reported:

- Strength of the bee colonies (amount of bees in the beehive) – in frames tightly covered with bees and calculated in kg (in 1 frame from Langstroth-Rut hive has 0.200 kg bees);

- Amount of honey supplies – measuring the capped honey in the honeycomb with a measuring frame with size of the squares of 5×5 cm and calculating in kg (in 8 squares of the measuring frame the honey is 0.350 kg).

Based on the data obtained from the reviews, the following indicators were calculated (Regulation 35/2004 on the terms and conditions for carrying out the selection and reproduction in beekeeping (Nenchev & Zhelyazkova, 2010):

- The amount of food consumption from the bee colony in the winter – the difference between the amount of honey during wintering and the amount of honey in the spring review. To the amount of consumed food is added the amount of sugar dough which bees used in the winter.

- Food consumption per unit of bees with the formula:

$$M = C / 0.5 (A + B),$$

where:

M – consumed food per unit of bees (frames, number, kg) in winter;

C – the total amount of consumed food, kg;

A – strength of the bee colony in the autumn (frames, number, kg);

C – strength of the bee colony in the spring (frames, number, kg).

- Quantity of dead bees (winter loss) - difference between the strength of the bee colony in the autumn and in the spring. The relative value (%) of this indicator is more objective. The following formula is used:

P = (A - B) / A.100,

where:

P – dead bees (winter loss);

A – strength of the bee colony in the autumn;

C – strength of the bee colony in the spring.

An assessment of the bee colonies was made on the signs characterizing their winter resistance according to the criteria of Regulation 35/2004. The data was processed statistically with the program Statistics.

Results and Discussion

Degree of the development of the worker honeybees fat body

The results obtained show that the mean values for the degree of the development of the worker honeybees fat body in the studied groups ranged from 2.44 to 2.89 points (Fig. 2). Bees which received "Baikal EM1" had the highest degree of development of the fat body - 1.2 times higher than the bees from the control group. A relatively high but statistically not significant average value for the development of fat body was reported in the bees fed with "Probee 41". Not significant differences in the mean values of this parameter were observed in bee colonies received "Probee-41" and "Apipasta" compared to the control group (Fig. 2).

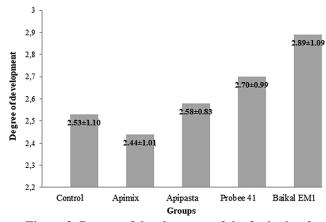


Figure 2. Degree of development of the fat body of worker bees after feeding with stimulating products.

Statistically significant differences were found between the groups: "Baikal EM1" and the control group ($P \le 0.05$); "Baikal EM1" and "Apimix" ($P \le 0.01$); "Baikal EM1" and "Apipasta" ($P \le 0.05$). The results showed that the autumn feeding of the bee colonies before wintering period with the addition of the microbiological product "Baikal EM1" and the plant product "Probee 41" positively influence the development of the worker honeybees fat body. The effect of "Apipasta" on worker honeybees fat body from the new created bee colonies (in 2018) is similar. Probably, these products stimulate the storage of food reserves in the fat body which is precondition for normal wintering and bee brood raising in the end of January and February (before the flowering of the first honey plants).

Amount of food consumption in the winter by the bee colonies

The amount of food consumption during the winter by the new created bee colonies in 2018 (nucleus colonies): The results for the amount of food consumption in the winter by the new created bee colonies in 2018 are shown in Table 1. It can be seen from the Table that the amount of honey in the bee colonies from the three groups during wintering is from 5.164 to 5.500 kg. Significant difference ($P \le 0.01$) in the amount of food supplies was established between C₁/Am and Am/Ap.

According to the criteria for the honey quantity provided in the beehive for the winter (2.0-2.5 kg of frame tightly covered with bees (Nenchev & Zhelyazkova, 2010), the honey stocks listed in Table 1 are below the optimum - for the strength of the bee colonies 0.800-0.960 kg (Table 2) the required amount of honey is 8-10 kg. In this respect, 4 kg food (sugar dough) is added to each new created bee colony in the winter (Table 1). Taking into account the amount of food available in the new created bee colonies in 2018 during the spring review (2019), it was found that the most honey had consumed by the bee colonies control group – 1.14 times more than the group received "Apimix" and 1.07 times more than the group fed with "Apipasta". The obtained differences for the total food consumption are significant between Ap/C₁ at P \leq 0.05 and between Am/C₁ at P \leq 0.001, respectively.

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Table 1 shows that the highest amount of honey for 1 kg of bees was consumed in the new created bee colonies from the control group - with 3.189 kg more than the group received "Apimix" and with 1.371 kg more than the colonies fed with "Apipasta". The differences for the mean values between the three groups are statistically highly significant ($P \le 0.001$).

The amount of food consumption during the winter by the basic bee colonies (formed before 2018): The amount of honey of the three groups basic bee colonies (created before 2018) during wintering is between 7.232 and 8.374 kg (Table. 1). The differences in the mean values of the studied parameter between the control and the other three groups are statistically highly significant ($P \le 0.001$). The amount of honey supplies for the winter period is below the optimum for colonies with strength 0.900-1.200 kg bees (Table 2). For this reason, another 2 kg sugar dough is provided for each bee colony in the winter (Table 1).

The difference between the provided food in the winter in the beehive and the available bee honey in the spring review shows that the bee colonies of the three groups use a total of 7.350 to 7.708 kg honey. The largest amount of food consumption per 1 kg of bees was found in the bee colonies from

Groups	Parameters					
	Quantity of honey in autumn review – kg	Amount of food given in the winter – kg	Quantity of honey in spring review – kg	Total amount of consumed food – kg	Consumed food per 1 kg of bees	
Bee colonies formed in 2018 (nucleus colonies) – x±SD						
$Control_1(C_1)$	5.500±0.212	4.000 ± 0.0	2.638 ± 0.385	6.812±.0177	10.185±0.599	
"Apimix" (Am)	5.164±0.086	4.000±0.0	3.202±0.196	5.962±0.126	6.996±0.779	
"Apipasta"(Ap)	5.440±0.105	4.000 ± 0.0	3.096±0.291	6.344±0.363	8.814±0.117	
Р	Am/C ₁ ** Am/Ap **		$Am/C_1 *$	$\frac{\text{Ap /C}_{1} *}{\text{Am/C}_{1} * * *}$	Am/C ₁ *** Ap /C ₁ *** Am/Ap ***	
Bee colonies formed before 2018 ("basic" colonies) – x±SD						
$Control_2(C_2)$	7.232±0.215	2.000 ± 0.0	1.876 ± 0.367	7.356±0.315	9.402±0.912	
"Probee 41" (Pb)	8.166±0.212	2.000±0.0	2.582±0.194	7.584±0.176	7.507±0.847	
"Baikal EM1" (B)	8.374±0.111	2.000 ± 0.0	$2.666 {\pm} 0.508$	7.708±0.432	7.167±0.451	
Р	Pb/C ₂ *** B/C ₂ ***		Pb/C ₂ ** B/C ₂ *		Pb/C ₂ ** B/C ₂ ***	

Table 1. Amount of consumed food from bee colonies during the winter period (kg)

 ${}^{*}P \! \le \! 0.05 \quad {}^{**}P \! \le \! 0.01 \quad {}^{***}P \! \le \! 0.001$

the control group (C₂). The differences in the mean values between the control and the experimental groups for this parameter are with an average ($P \le 0.01$) and high ($P \le 0.001$) statistically significant (Table. 1).

Based on the data from Table 1 and the criteria in Regulation 35/2004, the following estimation could be made for the food consumption in winter for 1 kg of bees: bad wintering bee colonies for the control group C₁; satisfactorily wintering bee colonies for groups fed with "Apipasta", "Probee 41" and C₂; well-wintering bee colonies for the groups receiving "Apimix" and "Baikal EM1".

Quantity of dead bees (winter loss) in the winter in the bee colonies

Quantity of dead bees (winter loss) in the winter in the new created bee colonies in 2018: In Table 2 is showed the quantity of dead bees in the winter (winter loss) of new created bee colonies in 2018. It can be seen that the strength of the bee colonies during wintering is in the range of 0.800-0.960 kg, which is equivalent to 4-4.8 frames. Based on these data, it can be assumed that bee colonies are at the lower limit for medium-sized bee colonies.

The comparative analysis of the data for the amount of bees in the beehives indicates that in the spring review the values for this parameter are lower in the three groups compared to the values in the wintering -0.260 kg for the control group, 0.200 kg for the experimental group fed with "Apimix" and 0.160 kg for "Apipasta" group, respectively (Table. 2). As could be seen from Table 2 the highest percentage of dead bees (winter loss) is in the control group which is 1.3-1.6 times more than the experimental groups which received stimulating products. The lowest percentage of dead bees was found in the bee colonies fed with "Apimix". Significant differences were found between Am/C_1 and Am/Ap at $P \le 0.01$.

Quantity of dead bees (winter loss) in the winter in the basic bee colonies created before 2018: The data on the number of bees in the beehive during wintering (0.900-1.200 kg is 4.5-6 frames) show that the bee colonies created before 2018 (basic bee colonies) have medium strength (Table 2). In the spring review, a decrease strength was found in all three groups bee colonies as follows: 0.220 kg for the control group; 0.200 kg for the bee colonies which received "Probee 41"; 0.240 kg for bee colonies fed with "Baikal EM1".

The highest percentage of dead bees (winter loss) in the control group (C_2) which is 1.2-1.4 times more than the experimental groups received "Probee 41" and "Baikal EM1" (Table 2). The lowest percentage of dead bees was found in bee colonies fed with "Probee 41". A low significant difference for this parameter (P ≤ 0.05) is found between Pb/C₂.

The estimation of the bee colonies for the amount of the dead bees (winter loss) accordance to the criteria of Regulation 35/2004 is as follows: Bad wintering bee colonies from the control group C₁; well wintering bee colonies from the groups fed with "Apimix", "Apipasta", "Probee 41", "Baikal EM1" and C₂.

In conclusion, the results of the study show that the studied stimulating products "Apimix", "Apipasta", "Probee 41" and "Baikal EM1" have different positive effects on the parameters which characterize the wintering of the bee colonies – state of the worker honeybees fat body, food consumption per unit of bees and dead bees (winter loss).

The data obtained in this study for the positive effect of the probiotic "Baikal EM1" on the development of the fat body of

Table 2. Amount of dead bees from bee colonies during the winter period (winter loss)

Groups	Parameters				
	Quantity of bees in autumn review – kg	Quantity of bees in spring review – kg	Dead bees (winter loss) – %		
	Bee colonies formed in 2013	8 (nucleus colonies) – x±SD			
$Control_1 (C_1)$	0.800±0.00	0.540±0.05	32.5±6.85		
"Apimix" (Am)	0.960±0.09	0.760±0.09	21.0±2.24		
"Apipasta"(Ap)	0.800±0.00	0.640±0.09	25.0±0.00		
Р	Am/C ₁ ** Am/Ap**	Am/C ₁ **	Am/C ₁ ** Am/Ap**		
	Bee colonies formed before 20	018 ("basic" colonies) – x±SD			
$Control_{2}(C_{2})$	0.900±0.100	0.680±0.109	24.66±5.439		
"Probee 41" (Pb)	1.120±0.109	0.920±0.109	$18.02{\pm}1.807$		
"Baikal EM1" (B)	1.200±0.122	0.960±0.089	19.88±3.151		
Р	$\frac{Pb/C_2 **}{B/C_2 **}$	$\frac{Pb/C_2}{B/C_2} **$	Pb/C ₂ *		

 $P \le 0.05 \ P \le 0.01 \ P \le 0.001$

worker honeybees and the winter resistance of the bee colonies enrich the well-known information for the application of the product in the beekeeping. From the research conducted in Russia, it has been found that the use of "Baikal EM1" increases the laying activity of the bee queen (the amount of the bee brood, respectively) and the honey production of the bee colonies (Zaitsev & Trotsenko, 2011; http://www.baikal-em1. com/ pchelarstvo/; www.em-russia.ru/opyt-vnedreniya-v-rossii-0), shows high efficacy against varroatosis and the health of bee colonies (Borodin et al., 2013; studylib.ru); reduces the percentage of the dead bees in the bee colonies (http://www. baikal-em1.com/pchelarstvo/; studylib.ru).

Conclusions

Based on the results obtained it was found that: the autumn feeding of the bee colonies with the addition of the microbiological product "Baikal EM1", the plant product "Probee 41" and the product "Apipasta" before the wintering has a positive effect on the development of the worker honeybees fat body; the amount of honey consumption in the winter for 1 kg of bees has the statistically significant highest values ($P \le 0.01 - P \le 0.001$) for the bee colonies from the control groups (C_1 and C_2); in the bee colonies which received with their food before wintering stimulating products "Apimix", "Apipasta", "Probee 41" and "Baikal EM1", the honey consumption in the winter was reduced, which is an important factor for normal wintering of the bees; the percentage of dead bees (winter loss) is the highest in the control groups of bee colonies C_1 and C_2 (P $\leq 0.05 - P \leq 0.01$); winter loss under 20% is found in the colonies which received "Probee 41" and "Baikal EM1" during the autumn feeding, low is the percentage (21%) of dead bees in the winter for the bee colonies fed with "Apimix"; according to the criteria in Regulation 35/2004 on the terms and conditions for carrying out the selection and reproduction in beekeeping, the estimation of the wintering bees colonies (food consumption in the winter for 1 kg of bees and winter loss) is as follows: bad wintering bee colonies for control group C₁; from satisfactory to wellwintering families for the group C₂ and the groups fed with "Apipasta" and "Probee 41"; well-wintering bee colonies for groups received "Apimix" and "Baikal EM1"; the data confirm and complete the available information on the positive impact of microbiological product "Baikal EM1" on the development, health status and winter resistance of the bee colonies; the reported positive effects of the other products "Apimix", "Apipasta" and "Probee 41" on the parameters characterized the winter resistance of the bee colonies give opportunity to expanding studies with them.

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