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Spring Growth Rates of Bee Families and the Level of Certain Amino Acids in Bees-Feeders with Stimulating Feeding

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Abstract: It was found that the spring development of bee colonies most actively occurs against the background of the compositional stimulating feeding of honey mead with the addition of the milk mixture "Nanny 2 with a prebiotic" in a complex with cobalt sulfate (group 4), which, in comparison with the background level, increased the average daily egg-laying capacity of queen bees by the second count by 2.48 times and at the height of the season - 3.03 times (in control 2.02 and 2.28 times) with an absolute value of 1814.0 and 2209.0 eggs/day (in control 1459.0 and 1650.0 eggs/day). At the same time, the growth index of the family strength, which is a vector biological component indicating economically useful traits, was maximum during all observation periods in the 3rd and especially in the 4th group, the peak values of which show that from March 18 to May 21 they can be used as families-educators and families of the control (1st group) and 2nd groups - from April 9 to May 21. It was revealed that the abundant secretion by the pharyngeal glands of milk sufficient for feeding 3-4 larvae in worker bees of 9 days of age in spring generation is carried out against the background of stimulating feeding with honey mead containing milk mixture "Nanny 2 with a prebiotic" or with sulphate cobalt with a degree of vitality of their glands in 3.98 points and 3.89 points, respectively (in control 3.74 points). The degree of development of the pharyngeal glands in overwintered workers of the autumn generation recorded in the range from 2.5 to 2.64 points shows that, as nursing bees, they can feed only one larva. To ensure the secretory function of the pharyngeal glands in the hemolymph of 9-day-old worker bees who are nursing bees, the level of lysine should be higher, in comparison with overwintered workers, by 2.37-2.79 times, histidine by 2.47-2.85 times, glycine - 2.32-2.46 times.

Keywords: Worker Bees, Queen, Egg-Laying Capacity, Amino Acids

Introduction

The decline in bee colonies all over the world, caused by the autumn gathering of families, lead to a decrease in the production of honey and biologically active beekeeping products used in general therapy and prevention of various diseases of an infectious and non-infectious nature. This indicates the need for the development and implementation of new technologies in the management of the vital activity, growth, and development of bee colonies in the spring and summer periods. Stimulating feeding with protein fillers, in frame beekeeping, can become a biological and technological tool not only in increasing the productivity of bee colonies but also in raising full-fledged offspring in subsequent generations of bees (Lebedev *et al*., 2000; Melnik *et al*., 2006; Pshenichnaya, 2011; Ursu and Leonov, 1976; Ursu and Eremia, 1984).

This is especially relevant concerning the reproduction of the spring generation of honey bees, which should participate in the composition of families-educators in the feeding of larvae data for queen rearing and in user families to grow a full generation of summer bees, providing the productive performance of bee colonies on the main honey flow (Khrapova *et al*., 2019; Chupakhin and Kustrya, 2003a).

As a result of the above noted for the spring stimulation of egg production in queen bees, in addition to protein fillers and mineral complexes, probiotics began to be used as part of stimulating feeding. However, in our opinion, it is better to add prebiotics to the composition of feeding, which is found in the composition of milk formulas used for baby food. This will make it possible to receive a full-fledged generation of spring and summer bees, which will be successfully used in the breeding of over-early queen bees in nursing families.

The research aims to optimize the rates of spring growth of bee colonies and the level of some amino acids in feeding bees based on the use of stimulating supplements with protein fillers, in combination with cobalt sulphate and the mixture of "Nanny 2 with prebiotics".

Materials and Research Methods

The object of the study was the bee families of the Carpathian breed, which were kept in 16 frame beehives with beds on frames measuring 435* 300 mm at the educational and experimental apiary of the RSAU-MTAA named after K.A. Timiryazev and the Association of beekeepers of the Samarkand region. Bee colonies for experiments were organized according to the recommendations of the FSBI "FNC of Beekeeping" and the Department of Aquaculture and Beekeeping of the RGAU-MSHA named after K.A. Timiryazev. In the selected families of pairs of analogues of 15 pcs., in each, in the spring of 2019, 2020, and 2021. spring stimulating fertilizing was carried out, which was subsequently used to form families of caregivers involved in the reproduction of bee colonies. The 1st group of bee colonies was a control group, they were fed 1:1 sugar syrup of 300 mL every day from February 24 to April 2. Bee families of the 2nd - 4th groups received stimulating fertilizing with the addition of ingredients affecting egg production, brood cultivation, and the functional state of the families. So, for bee families of the 2nd group, $CoSO_4$ was added to sugar syrup, at the rate of 2 mg per 1 liter of syrup, in the 3rd group - the same amount of CoSO4 was added to honey cooked in a ratio of 1:1, the 4th group - 2 mg of $CoSO_4$ was added to honey cooked in a ratio of 1:1 and 5 g of milk mixture "Nanny 2 with prebiotics".

The average daily egg production of queen bees was calculated using the formula using the data of the content of printed brood: Msr. = n*100/12. The degree of development of the pharyngeal glands in overwintered and young bees for 9 days. The treatment was carried out on histopreparations, taking into account the development of the alveoli and excretory ducts.

The detection of the content of asparagine amino acid, glycine, lysine, and histidine in the hemolymph of working individuals of the control and experimental groups was carried out according to generally accepted methods (Melnik *et al*., 2006; Pshenichnaya, 2011; Ursu and Leonov, 1976; Chupakhin and Kustrya, 2003b; Sadek, 2006; Noctor and Feuer, 1998) on an automatic amino acid analyzer of the Elite Lachrom VWR Hitachi brand in an accredited research laboratory of the Department of Aquaculture and Beekeeping of the K.A. Timiryazev Russian State Agricultural Academy.

Research Results

The used stimulating feeding to varying degrees influenced the studied biological parameters of bee families and workers in the spring period (Table 1). It was found that the compositional feeding of honey mead with the Nanny 2 with prebiotics (group 4) or with cobalt sulphate (group 3) has the greatest impact on the rates of spring growth and development of bee colonies. So, the background level of the average daily egg-laying capacity of queen bees at the beginning of the experiments fluctuated in the 1st - 4th groups in the range from 723 to 732 eggs/day. By the second count on March 9, the described parameter increases sharply in all groups. However, its highest level was recorded in the 3rd and especially, in the 4th groups.

Compared with the background level, the average daily egg-laying capacity increased in the 1st group by 2.02 times, in the 2nd group - by 2.1 times, in the 3rd group - by 2.35 times, in the 4th group - by 2.48 times. At the same time, the queen bees of the 4th group for the given observation period exceeded the average daily egg-laying capacity of the sisters of the 1st control group by 1.24 times, the 2nd group – by 1.17 times, and the 3rd group – by 1.07 times. The queen bees of the 3rd group were 1.17 times ahead of their mates from the 1st group and the 2nd group – by 1.1 times.

By March 21, the described parameter continued to increase. The maximum level of average daily egg-laying capacity of queen bees in all groups was recorded by the fourth observation period. However, their levels in the context of the 1st control and 2nd - 4th experimental groups differed markedly.

So, by April 2, the average daily egg-laying capacity in the 4th group of queen bees was 2209.0 eggs/day, in the 3rd group - 1940.0 eggs/day, in the 2nd group - 1940.0 eggs/day, in the 1st control group - 1650.0 eggs/day. At the same time, the peak value of the egg production recorded in the 4th group exceeded the similar parameter of the 1st group by 1.34 times, the 2nd group - by 1.18 times,

and the 3rd group - by 1.14 times. In the subsequent observation period, a slight decline was recorded in this trait of queen bees, but their numerical indicators remained at the level of previous values, showing the stabilization of the reproductive index of queens in the context of the studied groups.

The effect of stimulating feeding in the context of groups can be expressed by the index of egg production of queen bees both concerning the initial value and between groups at the end of the experiment. In the 1st group, the average daily egg production index relative to the background level was 2.27, in the 2nd group - 2.48, in the 3rd group - 2.69, and in the 4th group - 3.02. Consequently, the genetic potential of the reproductive capabilities of queen bees is well realized in the 4th group.

The increase in the reproductive properties of the queen bees contributed to the growth of the strength of the bee colonies. So, according to the results of experiments carried out for three years, in the period from February 25 to May 21, there was an active increase in the strength of bee colonies. The most active growth in the mass of families, expressed through the streets occupied by worker bees, was recorded in the 3rd and especially in the 4th groups by April 30. At the given observation period, the strength of families increased, compared to the background level, in the 1st group by 1.88 times, in the 2nd group - by 2.05 times, in the 3rd group - by 2.42 times, in the 4th group - by 3.05 times.

The maximum level of this parameter recorded in the 4th group exceeded the similar values of the control group (1st group) by 1.57 times, 2nd group - by 1.48 times, 3rd group - by 1.26 times. The described parameter reached the highest level by May 21, amounting to 16.0 bee spaces in group 1, 18.0 bee spaces in group 2, 21.0 bee spaces in group 3, and 24.0 spaces in group 4 streets. At the same time, the growth index of the strength of families in the 1st group compared to the background value was 2.0, in the 2nd group - 2.31, in the 3rd group - 2.72, in the 4th group - 3.1 ... This indicates that the bee colonies of the 3rd and 4th groups from March 18 to May 21 can be used as caretaker families and the 1st and 2nd groups - from April 9 to May 21.

Results of the study of the degree of development of the pharyngeal glands in 9-day-old working bees are presented in Table 2. Analysis of the data presented in Table 3 shows that the smallest parameters of the development degree of the pharyngeal glands had overwintered workers. Here their level of development ranged from 2.5 to 2.64 points. This describes that workers with this level of state of the pharyngeal glands can feed only one larva.

With the appearance of the first generation of the spring generation of worker bees, the degree of development of the pharyngeal glands changes the direction of increase. However, the degree of development of the pharyngeal glands was the highest in the 4th and 3rd groups, amounting to 3.5 and 3.4 points, respectively. In working bees of the 3rd and 4th generations, the described parameter, indicating the state of the pharyngeal glands in the context of the studied groups, reaches almost peak levels. So, in working individuals of the 4th generation on May 9, the degree of development of the pharyngeal glands was 3.74 points in the 1st group, 3.8 points in the 2nd group, 3.89 points in the 3rd group, and in the 4-th group-3.98 points. Consequently, the full secretion of milk by the pharyngeal glands for feeding larvae in 9-day-old worker bees occurs against the background of stimulating feeding with honey mead containing milk mixture Nanny 2 with a prebiotic or with sulphate cobalt.

The results of the study of the content of irreplaceable and nonessential amino acids in the hemolymph of worker bees participating in brood feeding against the background of applied feeding with various fillers are presented in Table 3.

Analysis of the content of the studied essential and nonessential amino acids in 9-day-old workers in bee colonies against the background of stimulating feeding showed that their lowest level is recorded in overwintered worker bees. So after wintering on February 27 in the hemolymph of worker bees, the content of lysine ranged from 1250.40 to 1273.25 μmol/L, histidine from 81.50 to 85.65 μmol/L, glycine from 110.40 to 115.20 μmol/L., aspartic amino acid from 350.60 to 360.40 μmol/L.

With the appearance of the first generation of young bees, the level of amino acids in the hemolymph increases. The maximum frequency increase in the level was recorded for the amino acid lysine, which doubled in comparison with overwintered bees. So, by March 7, in 9-day-old worker bees, the lysine level, in comparison with the previous observation period, increased in the 1st group by 1.95 times, in the 2nd group - by 1.93 times, in the 3rd group - by 2.06 times, in the 4th group - 2.17 times.

A similar trend was recorded for other amino acids, but the level of their increase was less pronounced. So, by March 7, the level of histidine in group 1 increased 1.25 times, glycine - by 1.42 times, aspartic amino acid - by 1.18 times. In group 2, the level of the described amino acids increased by 1.21, 1.45, and 1.2 times, respectively, in group 3 - 1.27, 1.62, 1.18 times, 4 group - 1.34 times, 1.72 times, 1.27 times.

By March 28, the studied essential and nonessential amino acids in the hemolymph reached the peak level in the second generation of 9-day-old bees in the 3rd and 4th groups. On April 18, in the 1st and 2nd groups, the peak values in the content of amino acids were recorded in worker bees of the third generation. At the same time, the numerical values differed in the context of the control and experimental groups. So in the 3rd group, the lysine level reached 3330.80 by the indicated date (March 28), and in the 4th group - 3530.70 μmol/L, exceeding the control value by 1.1 and 1.17 times, respectively. In the 1st and 2nd groups, the described indicators reached 3170.30 and

3190.70 μmol/L, inferior to the same indicator of the 4th group by 1.1 and 1.09 times.

The content of histidine by March 28 in the studied worker bees of the 3rd and 4th groups was 208.8 and 232.1 μmol/L, glycine - 265.80 and 283.7 μmol/L and aspartic amino acid - 652.60 and 690.2 μmol/L.

In subsequent periods, the level of the studied amino acids is subject to insignificant changes, however, their content in the hemolymph of bees in the context of groups is stabilized. At the same time, their maximum levels are preserved in the 3rd and especially the 4th groups. So in 9-day-old bees of the fifth generation in the 3rd group, the hemolymph content of lysine was 3268.6, histidine - 207.3, glycine - 263.0, aspartic amino acid - 630.0 μmol/L. In the 4th group - 3473.1, 228.2, 271.3, 634.1 μmol/L, respectively. Consequently, to ensure the secretory function of the pharyngeal glands in the hemolymph of 9-day-old worker bees who are feeding bees, the level of lysine should be higher, compared with overwintered workers, by 2.37-2.79 times, histidine by 2.47 -2.85 times, glycine - 2.32-2.46 times, aspartic amino acid - 1.81-1.94 times.

Table 1: Indicators of the average daily egg production of queen bees in bee colonies against the background of stimulating feeding

	Accounting dates, eggs/pcs.				
Groups of families	Background 25. II	9.03.	21.03	2.04	14.04
1st, control	723.0±5.10	1459.0±3.20	1617.0±4.17	1650.0±2.53	1640.0±2.49
2nd	738.0±1.80	1547.0±2.10**	1850.0±3.42**	1879.0±1.75***	1830.0±2.17***
3rd	725.0±3.50	1703.0±2.90***	1889.0±4.05***	1940.0±2.65***	1950.0±3.41***
4th	730.0±2.20	1814.0±3.35***	1995.0±3.17***	2209.0±3.200***	2205.0±1.40***

Note. Here and below in the tables: * -P ≥0.95; ** - P ≥0.99; *** -P≥0.999 compared to the control group

Table 2: The state of the pharyngeal glands in 9-day-old workers in bee families against the background of stimulating feeding, points

		Generations of 9-day-old worker bees and census dates				
Family groups	Overwintered bees, 27. II	1 7. III	2 28. III	3 18. IV	4 9. V	5 30. V
1st, control	2.60±0.02	3.00±0.01	3.50±0.01	3.70±0.02	3.74±0.03	3.78±0.02
2nd	2.50±0.01	3.30±0.02	3.60±0.04	3.75±0.03	3.80±0.02	3.81±0.03
3rd	2.64±0.02	3.40±0.03*	3.70±0.02*	3.80±0.02*	3.89±0.03*	3.91±0.02*
4th	2.57 ± 0.03	3.50 ± 0.02 **	3.90 ± 0.03 **	3.90 ± 0.01 **	3.98 ± 0.02 **	3.99 ± 0.01 ***

Note. Here and below in the tables: * -P ≥0.95; ** -P ≥0.99; *** -P≥0.999 compared to the control group

Table 3: The content of some essential and nonessential amino acids in 9-day-old workers in bee colonies against the background of stimulating feeding, μmoL/l

		Generations of 9-day old worker bees and census dates				
Family groups and types of feeding	Overwintered worker bees 27.II	1 7.III	2 28. III	3 18.IV	4 9.V	5 30.V
Lysine content						
1st, CC – control	1260.15	2451.60	3015.0	3170.30	3050.43	3043.10
2nd, CC + $CoSO_4$	1273.25	2460.20	3020.6	3190.70	3087.20	3090.40
3rd, MS + $CoSO_4$	1250.40	2578.90	3330,80	3313.30	3273.15	3268.60
4th, MS + NANNY with prebiotic + $CoSO_4$	1265,00	2743.80	3530.70	3481.50	3485.90	3473.10
Histidine content						
1st, CC – control	82.86	103.40	137,60	138.16	137.18	133.50
2nd, CC + $CoSO_4$	85.65	106.35	139.50	141.45	139.70	135.40
3rd, MS + $CoSO_4$	84.70	107.63	208.80	203.30	205.60	207.30
4th, MS + NANNY with prebiotic + $CoSO_4$	81.50	109.5	232.10	227.75	225.90	228.20
Glycine content						
1st, CC – control	110.40	157.00	210.30	214.17	212.50	217.40
2nd, CC + $CoSO_4$	112.70	163.20	203.50	215.70	231.2	229.30
3rd, MS + $CoSO_4$	114.50	185.40	265.80	259.10	260.58	263,00
4th, MS + NANNY with prebiotic + $CoSO_4$	115.20	198.30	283.70	276.5	270,80	271.30
Aspartic amino acid content						
1st, CC – control	352.30	415.70	520.50	544.60	538.30	532.70
2nd, CC + $CoSO_4$	350,60	419.30	530.40	555.30	540.10	539.80
3rd, MS + $CoSO_4$	360.40	425.60	652.60	630.20	625.50	630,00
4th, MS + NANNY with prebiotic + $CoSO_4$	355.10	452.30	690.20	634.50	630.70	634.10

Conclusion

Abundant secretion by the pharyngeal glands of milk sufficient for feeding 3-4 larvae in worker bees of 9 days of age in spring generation is carried out against the background of stimulating feeding with honey mead containing milk mixture "Nanny 2 with a prebiotic" or with sulphate cobalt with a degree of development of their glands of 3.98 points and 3.89 points, respectively (in control 3.74 points). The degree of development of the pharyngeal glands in overwintered workers of the autumn generation recorded in the range from 2.5 to 2.64 points shows that they, as nurse bees, can feed only one larva.

The most intense increase in the level of essential and non-essential amino acids occurs in the hemolymph of workers of the spring generation, compared with overwintered bees of the autumn generation, against the background of stimulating feedings with honey mead containing milk mixture "Nanny 2 with a prebiotic" or with cobalt sulphate. It has been established that to ensure the secretory function of the pharyngeal glands in the hemolymph of 9-day-old worker bees who are feeding bees, the level of lysine should be higher than that of overwintered workers by 2.37-2.79 times, histidine by 2.47-2.85 times, glycine - 2.32-2.46 times.

Author's Contributions

All authors equally contributed in this work.

Ethics

This article is original and contains unpublished material. The corresponding author confirms that all of the other authors have read and approved the manuscript and no ethical issues involved.

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