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Investigations

Microbiocenosis of the Intestinal Tract of Honey Bees and its Correction

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Abstract: We have studied microbiota of the intestinal tract of honey bees and the effect of various preparations and feed additives, in particular hydrohemol. It was established that feeding of hydrohemol in a mixture with candy caused a decrease in the pathogenic microbiota of the digestive tract of bees. This creates the most favorable conditions for the further development of the bee colonies and, as a result, an increase in their honey production.

Keywords: Honey Bee, Microorganisms, Microbiota, *Apis Mellifera*, Lactic Acid Bacteria, Microbiocenosis, Pathogenic Bacteria, Pathogenic Fungi, Intestinal Microbiota

Introduction

Health status of the bee family members is one of the fundamental factors affecting its strength and productivity. Bees, as well as other biological objects, are subject to various infectious and non-contagious diseases and therefore are constantly at risk of developing diseases. Bees are under the threat of not only the disease, but also complete extinction as a species, as the last decade has been marked by the spread of the phenomenon of mass death of bees in Europe and the USA (Onischuk *et al*., 2016; Tantillo *et al*., 2015). This phenomenon has been called the collapse of bee colonies. It is assumed that these are various biogenic and abiogenic factors, including microorganisms, that make up the microbiota of the bee organism that causes mass death of bees (Evteeva, 2009; Corby-Harris *et al*., 2014; Sobol *et al*., 2017).

Microbiota of bees is largely determined by the habitat of insects and therefore its members are not only saprophytic, but also potentially pathogenic microorganisms that cause the development of the diseases such as escherichiosis, hafniosis, cytrobacteriosis, salmonellosis, etc (Moran, 2015; Radchenko *et al*., 2016; Koshchaev *et al*., 2017).

The presence of such a biological feature in honey bees as the absence of emptying of the large intestine during the winter period causes the risk of developing of some infectious diseases during or towards the end of wintering.

Meanwhile, it should be assumed that microbiocenosis of the intestinal tract of bees is formed during the whole active period of the family's life. Thus, the family's health will depend on the composition of the microbiota in adult and young bees preparing for wintering. Consequently, their reproductive and productive activity in the next season will also depend on it (Stankus, 2014; Tozkar *et al*., 2015).

In order to mitigate negative effects of wintering, various feeding methods are widely used in beekeeping. In addition to sugar or honey, they include antibiotic-like substances, minerals, stimulants and vitamins affecting both the bees themselves and microbiota of their digestive tract (Pashayan, 2008; Rousseau and Giovenazzo, 2016; Chasovshchikova *et al*., 2017). Therefore, when developing feeding, one should consider how their use will affect normal intestinal microbiota. Due to the foregoing, the study of intestinal microbiocenosis of the honey bee and

the development of ecologically safe methods of its regulation are topical issues for modern beekeeping.

The aim of the trials was to study the state of intestinal microbiocenosis in honey bees in the conditions of the South of the Russian Federation and to develop an effective method for its correction.

Materials and Methods

The work was carried out at the Faculty of Veterinary Medicine of the Kuban State University of Agriculture and the Apiary "IP Ovsyannikov A.A." (Mostovskoy area of the Krasnodar Region) (Serdyuchenko, 2013).

Object of the study was the honey bee of Carpathian breed - *Apis mellifera carpatica*. In the studies and experiments, 196 bee colonies and 1,000 bees were used. During the work, 1,576 bacteriological studies were conducted and quality and quantity contents of the intestinal microflora of the bees were assessed.

For this purpose, 10 live bees from each bee family were euthanized with subsequent preparation of their intestines. Then the intestines were placed in a sterile weighing cup, weighed and thoroughly homogenized in a sterile porcelain mortar in 1% peptone water in the ratio of 1:10. Then the series of consecutive 10-fold dilutions were prepared on 1% peptone water with 0.1% agar-agar. From the dilutions obtained, a graduated pipette was applied to the surface of well-dried selective culture media and bacterial inoculations in the volume of 0.025 mL were made in form of 3 isolated droplets. After 24-72 h of incubation at 37°C, the grown colonies were counted and the number of microorganisms in 1 g of intestinal contents of bees was calculated according to the formula (Bochkov *et al*., 1989).

The study of the microbial landscape of the surfaces of various objects of the hive and drinking bowls was carried out on the area of 1 cm^2 in 3 points of each object with sterile cotton swabs. After that, swabs being in the test tubes with 2.5 mL of 1% peptone water were rinsed by vigorous shaking for 10-15 sec. Then the swabs were squeezed against the walls of the tube and removed with addition to the tube of 2.5 mL of peptone water with 0.2% agar-agar. Then the series of consecutive 10-fold dilutions in the peptone water with 0.1% agar-agar were prepared followed by bacterial inoculation on selective media, as was mentioned above. Registration of the increased colonies was performed 24-72 h after incubation at 37°C. Calculation of the number of microorganisms was carried out according to the formula (Starostina *et al*., 1997a; 1997b).

The following selective culture media were used: Endo agar for isolation of enterobacteria, Kvasnikov agar with 6% ethanol for isolation of lactobacilli, yolk-salt agar for isolation of staphylococci, nutritive agar with potassium tellurite for isolation of enterococci, CPC agar for isolation of Pseudomonads and other non-fermenting bacteria, author's media (patent of the Russian Federation No. 2407783) for isolation of microscopic fungi, Wilson-Blair media for isolation of clostridia, Blaurok media with neomycin for isolation of bifidobacteria. In addition, we used blood agar to isolate streptococci and meat-peptone agar to isolate bacilli.

The effect of enrofloxacin on the microbiota in the digestive tract of bees was studied through bacteriological studies of bee intestines' suspension. The qualitative and quantitative indicators of microbiota in the digestive tract of the bees was assessed using the method of drip count of microbial cells with planting on differentially diagnostic media: Endo, Saburo, Kvasnikov, cetylpyridinium chloride agar, yolk-salt agar, meat-and peptone agar.

Identification of isolated bacteria was carried out according to cultural, morphological, tinctorial and biochemical properties. Biochemical test-systems of the company Pliva-Lachema Diagnostika were used: Enterotest, NEFERMtest, EN-Kokkustest, ANAEROTEST, STAFITEST. Taxonomical identification of bacteria and fungi was performed by using special catalogs, determinants and guidelines.

The state of the intestinal tract of bees was assessed according to the point method (Shagun, 1983):

- I point - intestinal wall is very thin, it tears up easily, filled with watery flaky contents, intestine tears up easily on extraction from the abdomen of the bee
- II points - intestinal wall is loose, filled with liquid, homogeneous, easily spreading excrements, it seems possible to extract only a part of the intestine from the bee's abdomen
- III points – intestine is removed completely, its walls do not tear up, it is filled with weakly flowing homogeneous excrements
- IV points - intestine completely retains its structure, it is extracted easily in a full range, its wall is elastic, it does not tear up, it keeps excrements well, on the outer side there is musculature with tracheas in the form of white threads. Faeces are formed and dense

Reproductive capacity and honey production of bee colonies were determined by the methods recommended by the Research Institute of Apiculture (Methods of conducting scientific research in beekeeping, 2006).

For the estimation of the sealed brood, a grid with a size of 5×5 cm was used, taking into account the number of complete squares occupied by the brood on each side of the cell frame. In each square there are approximately 100 cells with brood.

Honey productivity was determined by weighing of the frames on the postal weights before and after evacuation of

honey. The difference in weight of the frames was calculated by the amount of honey pumped out.

The results of the studies were subjected to mathematical and biometric processing by using the computer software *Microsoft Excel* 2010. With the help of this program, we have calculated mean statistical values, mean arithmetic error, significance of the degree of reliability and the degree of correlation and also converted the obtained data into graphs and diagrams.

Results and Discussion

Microbiocenosis of the Intestinal Tract of Adult Bees

Results of the studies showed (Table 1) that enterobacteria, lactic acid bacteria, staphylococci, enterococci, pseudomonas, yeast and mold fungi live in the intestinal tract of an adult honey bee. In different months of the year these microorganisms have an unequal quantitative presence and enterococci and mold fungi are even absent in some of them. So enterococci were not detected from September to November and mold fungi - from August to February.

The most numerous group of microorganisms were staphylococci and enterobacteria, the number of which averaged 5.4-5.5 log CFU/g. The least part was presented by lactobacilli (3.2+0.6 log CFU/g) and mold fungi (2.4+0.3 log CFU/g). Bifidobacteria, streptococci, bacilli, clostridia were not identified.

Typical feature of the state of the intestinal microbiocenosis of adult bees was a sharp decrease in the number of all representatives by the beginning of wintering (September-November) and, on the contrary, the maximum increase in their number towards the end of wintering (February-March).

Microbiota isolated from the intestinal tract of adult bees was represented by 6 species of bacteria and 3 species of fungi. It was established that enterobacteria isolated from adult bees consisted of *Enterobacter aerogenes* and *Escherichia coli*, lactobacteria – of *Lactobacillus plantarum*, staphylococci-of *Staphylococcus warneri*, enterococci - of *Enterococcus faecalis*, pseudomonas - of *Pseudomonas fluorescens*, yeast - of *Candida glabrata*, mold fungi – of *Aspergillus niger* and *Aspergillus ustus*. The prevailing position in the microbiocenosis of bees was occupied by: *Enterobacter aerogenes*, *Staphylococcus warneri*, *Pseudomonas fluorescens* and *Candida glabrata*. Their number was the highest in the community of other microorganisms.

Microbiocenosis of the Intestinal Tract of Young Bees

Microbial colonization of any biological object begins from the moment of its interaction with the environment. Bees are not an exception in this respect. It should be assumed that finally the intestinal microbiota of the honey bee is formed with the beginning of its flight from the hive and independent feeding. Microbiota of young bees (i.e., bees from the moment they leave the pupa and before the first flight from the hive) is formed exclusively at the expense of those microbiota that live in nectar, pollen, on the hive and adult specimens (Egorova, 1971; Skrynnik, 1971; Chekryga, 2008).

Results of our studies have shown (Table 2) that in the intestinal tract of young bees there are microorganisms of the same groups as in adult specimens. However, not all of them were constantly identified throughout the year.

Typical feature of the intestinal microbiocenosis of young bees consisted of two signs revealed by us. Firstly, microbiota isolated from young bees was represented in a much smaller quantitative expression than in adult species and secondly, in the intestinal tract of young bees there were no lactobacilli in the period from September to February.

Enterococci were the most numerous group of microorganisms in the intestinal tract of young bees, the mean value of which was 5.1+0.4 log CFU/g. The number of enterobacteria, staphylococci, pseudomonads and yeast fungi was smaller and was equal to 4.3-4.8 log CFU/g. The smallest group consisted of lactobacilli (1.8+0.4 log CFU/g) and mold fungi (0.9+0.3 log CFU/g).

The minimal quantitative presence of microorganisms in the intestine of the young bees was observed in September-October and the maximum in February-April.

Intestinal microbiota of young bees, in the species composition, is similar to microbiota of adult bees. At the same time, *Enterobacter aerogenes*, *Staphylococcus warneri*, *Pseudomonas fluorescens* and *Candida glabrata* were constantly isolated from the digestive tract of young bees. It allowed us to attribute them to resident microbiota. *Esherichia coli*, *Lactobacillus plantarum*, *Enterococcus faecalis* and mold fungi (*Aspergillus ustus* and *Penicillium glaucum)* were not identified in certain periods of the year, which suggested the transient nature of these microorganisms.

Microbiological State of the Components of the Internal Contents of Hive and Drinkers

Studies performed by several authors indicated that microbiocenosis of adult bees was mainly formed by microbiota of the honey plants being in contact with insects on a daily basis. At the same time, microbiocenosis of young bees was formed at the expense of food they receive from nurse bees and direct contact with adult working bees (Smirnov, 1982; Gilliam, 1997; Goerzen, 1991).

Meanwhile, we believe that the formation of the intestinal microbiocenosis of young bees can also take place due to the microbiota that lives on various components of the internal contents of the hive and due to the microbiota of the water that adult bees deliver to the hive for drinking and cooling (Cherepov and Cherepov, 1997). To prove our assumption, we conducted bacteriological studies of washings from aperture, frame, bottom and walls of the hive and also drinkers for bees.

Table 1: The quantitative composition of microorganisms in the contents of the intestines of adult bees throughout the year

	Quantity of microorganisms, lg CFU/g						
Month	Enterobacteria	Lactobacilli	Staphylococci	Enterococcus	Pseudomonas	Yeast	Moldy fungi
September	4.7±0.4	2.3±0.4	4.0±0.8	-	3.7±0.4	3.7±0.4	-
October	5.3±0.4	2.0±0.2	5.0±0.8	-	4.7±0.4	4.7±0.4	-
November	5.7±1.2	1.3±0.9	5.7±0.4	-	5.3±0.4	5.3±0.4	-
December	5.7±1.2	2.0±1.4	5.7±0.4	2.7±0.4	6.0±0.2	5.3±0.4	-
January	6.0±0.8	3.0±0.8	6.3±0.4	4.3±0.9	6.0±0.8	5.7±0.9	-
February	6.3±0.9	3.7±0.9	6.3±0.4	6.0±1.4	6.3±0.9	5.7±0.9	-
March	5.3±0.4	5.0±0.8	6.3±0.4	6.3±0.9	5.7±0.4	6.0±0.8	2.7+0.4
April	5.3±0.4	5.0±0.4	6.0±0.3	6.3±0.9	5.3±0.4	6.0±0.8	2.0+0.1
May	5.3±0.4	4.0±0.8	6.0±0.3	6.3±0.4	5.3±0.4	5.7±0.4	2.0+0.2
June	5.3±0.4	3.7±0.4	5.7±0.4	6.3±0.4	5.3±0.4	5.0±0.2	2.7+0.4
July	5.3±0.4	3.3±0.4	4.7±0.4	4.0±0.8	4.7±0.4	4.3±0.4	2.7+0.2
August	4.7±0.4	3.0±0.3	4.0±0.8	2.7± 0.4	4.0±0.2	3.7±0.4	-
Mean value	5.4±0.6	3.2±0.6	5.5±0.5	5.0±0.7	5.2±0.4	5.1±0.5	2.4+0.3

Table 2: Quantitative composition of microorganisms in the contents of the intestines of young bees throughout the year

	Number of microorganisms, log CFU/g						
Month	Entero bacteria	Lactobacilli	Staphylococci	Enterococcus	Pseudomonas	Yeast	Mold fungi
September	3.0±0.8	-	2.7±0.4	-	2.3±0.4	3.0±0.2	-
October	3.7±0.9	-	2.7±0.4	-	2.7±0.4	3.0±0.2	-
November	4.0±0.8	-	4.0±0.8	-	3.0±0.8	3.7±0.4	-
December	4.3±0.4	-	4.7±0.4	2.7±0.4	3.7±0.4	4.0±0.6	-
January	5.3±0.4	-	5.3±0.4	4.3±0.4	4.7±0.4	5.0±0.2	-
February	5.7±0.8	-	6.3±0.2	6.7±0.4	6.0±0.8	6.0±0.2	0.7±0.1
March	6.7±0.4	2.3±0.4	6.3±0.8	7.0±0.6	6.0±0.8	6.0±0.8	1.3±0.2
April	6.0±0.2	3.0±0.8	6.0±0.8	7.0±0.6	6.0±0.6	5.7±0.4	1.0±0.4
May	5.7±0.4	3.3±0.2	6.0±0.8	6.3±0.4	5.0±0.6	4.7±0.4	0.7±0.4
June	5.7±0.2	1.0±0.4	5.7±0.2	6.3±0.4	5.0±0.2	4.0±0.2	1.0±0.4
July	4.7±0.4	0.7±0.2	4.7±0.4	3.7±0.4	4.0±0.6	4.0±0.2	-
August	3.7±0.3	0.7±0.2	3.7±0.4	2.3±0.4	3.0±0.2	3.0±0.6	-
Mean value	4.8±0.5	1.8±0.4	4.8±0.5	5.1±0.4	4.3±0.5	4.3±0.4	0.9±0.3

Results of the studies showed that microbial composition of the surfaces of various components of the hive during the whole year was rather meager (Table 3). The greatest number of microorganisms was detected on the aperture - 3.1+0.4 log CFU/cm^2 and the smallest at the bottom of the hive - 1.5+0.3 log CFU/cm^2. General tendency of the quantitative presence of microorganisms on the surface of the hive was their maximum quantity in June-August and minimum quantity in November-February.

It turned out that the species composition of microflora inhabiting the hive, as well as its quantitative number was poor. It was represented mainly by microscopic fungi of the genera *Aspergillus, Penicillium* and *Alternaria,* while the bacterial microbiota was represented by only *Enterobacter aerogenes* and *Escherichia coli.* These data were also confirmed by the other sources (Smirnov, 2004; 1987).

It turned out that aperture was the structure that was most rich in microorganisms. On its surface we found two species of bacteria and five species of fungi; microbiota of the walls was the poorest one, which in the majority of cases consisted of mold fungi of the genus *Penicillium*.

Thus, the conducted studies showed that the microbial landscape of various objects of the hive was represented by various organisms, but to a greater extent these were micromycetes, among which the prevailing position belonged to representatives of the genera *Aspergillus* and *Penicillium*. Meanwhile, there were particular differentiations even among these microorganisms. Aspergillus dominated among the microbial population inhabiting the aperture and the penicillium dominated on the remaining internal structure of the hive.

In the course of microbiological studies of washes from the inner walls of drinkers (three dots were examined in each drinker) it was established (Table 4) that microbiota of this beekeeping object was represented by *Enterobacter aerogenes*, *Escherichiacoli* and *Pseudomonas fluorescens*. Considering the fact that the bees were identical by the species composition of enterobacteria and pseudomonads, microbiota of the drinkers and intestinal tract, it could be assumed that the drinkers could be either a source of this microbiota or transmission factor.

Table 3: Total microbial contamination of different parts of hive during the year

	Number of microorganisms, log CFU/cm^2												
Object	September	October	November	December	January	February	March	April	May	June	July	August	Mean
Aperture	2.8±0.6	2.3±0.4	0	0	0	0	2.2±0.4	2.4±0.6	3.2±0.3	3.7±0.2	3.8±0.3	4.2±0.6	3.1±0.4
Frame	2.3±0.3	2.1±0.4	1.4±0.4	1.7±0.6	1.4±0.7	1.3±0.3	2.0±0.4	2.2±0.3	2.9±0.3	3.2±0.3	3.4±0.4	4.1±0.2	2.3±0.4
Bottom	1.8±0.2	1.3±0.3	1.0±0.3	0.7±0.3	1.0±0.1	1.1±0.1	1,2±0,2	1.6±0.3	1.8±0.5	2.1±0.4	2.0±0.6	2.0±0.4	1.5±0.3
Wall	3.0±0.3	1.7±0.1	1.3±0.4	1.3±0.4	2.0±0.2	2.3±0.3	3.0±0.2	3.7±0.4	3.7±0.5	4.3±0.3	4.0±0.4	3.7±0.3	2.8±0.3
Mean	2.5±0.3	1.9±0.3	1.2±0.4	1.2±0.4	1.5±0.3	1.6±0.2	2.1±0.3	2.5±0.4	2.9±0.4	3.3±0.3	3.3±0.5	3.5±0.4	2.4±0.3

Table 4: Species and quantitative composition of the microbiota of the inner walls of drinkers for bees

	Number of microorganisms, log CFU/cm^2	
Microorganism	Drinker number 1	Drinker number 2
Enterobacter aerogenes	6.3±0.9	6.1±0.7
Escherichia coli	5.5±0.6	5.3±0.9
Pseudomonas fluorescens	4.0±0.7	4.1±0.4

Thus, it should be noted that the hive objects being in the permanent contact with bees also have their own microbiocenosis, which is similar in many respects to the microbiocenosis of insects and therefore can participate as a mechanism and a factor of transmission of various microorganisms between insects. We should take it into account during the implementation of preventive and medical measures in case of infectious diseases of bees.

Interrelation Between Intestinal Microbiocenosis of Bees and their Physiological Activity

Symbiontic intestinal microbiota is important for life of bees, because it is established that due to bacterial enzymes, primarily glucosidase, carbohydrates are cleaved and nectar is converted into honey, protein components of the feed are digested and protection against pathogenic microorganisms is realized.

In this regard, we conducted comparative studies to identify the relationship between the state of intestinal microbiocenosis in bees at the end of wintering and their physiological activity. The study was carried out from February to September on the basis of the apiary of "IP Ovsyannikov A.A.". For the experiment, we selected families with different conditions of the intestinal tract which was determined by the method of Shagun (1983) and was indicated in points. As a result, we selected three families characterized by the state of intestinal tract as 2, 3 and 4 points. There were no families with intestinal condition equal to 1 point. Bacteriological evaluation was carried out in families selected for the study along with visual assessment of the intestinal state. The results are shown in Table 5.

As it turned out, the more enteric bacteria were in the intestinal tract, the lower was the point of their intestinal state (R = -0.83) and vice versa, the greater was the number of lactobacilli, the higher was the point of their intestinal state (R = 0.85). Certain correlative dependence was also established between the quantitative presence of enterococci, staphylococci, pseudomonads and mold fungi in the intestinal tract. This dependence in enterococci was direct (R = 0.75) and in staphylococci, pseudomonads and mold fungi it was inverse (-0.64, -0.51 and -0.56, respectively). Low degree of correlation was established between the quantitative presence of yeast and the anatomical state of the intestine (R = -0.44).

Consequently, the increased number of enterobacteria, staphylococci, pseudomonads and mold fungi in the intestines of bees leads to the development of dysfunction of the digestive tract of insects, up to significant anatomical defects. At the same time, quantitative prevalence of acid-producing microorganisms (lactobacilli and enterococci) in the intestine positively affects the structure and the state of the intestines of bees.

In families where the intestinal state was estimated at 4 points, the intensity of brood and honey production was 1.4-1.9 times higher than in families where intestinal state was estimated at 2-3 points.

This means that bees, which have a strong and elastic structure of the intestine, are more productive than bees, in which, after wintering, the intestinal tract is loose and filled with liquefied fecal masses, which is typical for dysbiosis.

Effect of Enrofloxacin on the Microbiocenosis of the Intestinal Tract of Bees

Enrofloxacin is widely used for the prevention and treatment of bacterial infections in beekeeping (Gordeev, 2008; Kaznowski, 2005).

In the apiaries of the Krasnodar Territory, enrofloxacin is used as a 10% solution. It is added to the sugar syrup at the dose of 10 mL per 1 L and fed to the bees by three courses with intervals of 7 days at the end of February - at the beginning of March. In order to study the effect of enrofloxacin on microbiota of the digestive tract of bees, we conducted appropriate studies where the preparation was given to bees of the experimental group according to the above-noted scheme, while the control bee families received feed supplementation without enrofloxacin.

Table 5: Quantitative presence of the main representatives of the intestinal microbiota in bees with different state of their intestines at the end of wintering (February)

	Number of microorganisms in different state of the intestinal tract of bees, log CFU/g			
Microorganism	2 Points	3 Points	4 Points	Correlation coefficient (R)
Enterobacteria	9.7±0.3	6.6±0.8	5.3±0.5	-0.83
Lactobacilli	3.7±0.4	5.3±0.4	6.3±0.9	0.85
Staphylococci	6.0±0.1	5.8±0.3	5.0±0.2	-0.64
Enterococcus	3.6±0.7	5.0±0.3	5.4±0.5	0.75
Pseudomonas	5.9±0.5	5.7±0.1	5.1±0.3	-0.51
Yeast	6.0±0.6	5.7±0.4	5.5±0.5	-0.44
Mold fungi	1.7±0.3	0.8±0.2	0.5±0.1	-0.56

Table 6: Qualitative and quantitative composition of the microbiota of the posterior part of intestines of bees that received sugar syrup with enrofloxacin (February-March), (n = 50)

	Number of microorganisms, log CFU/g			
	Control group		Study group	
Microorganism	Before the experiment	After the experiment	Before the experiment	After the experiment
Enterobacteria	5.9±0.6	6.9±0.4	5.8±0.6	0.6±0.1
Lactobacilli	4.3±0.5	5.0±0.5	4.1±0.4	1,2±0,2
Staphylococci	5.4±0.4	6.3±0.3	5.6±0.2	0.2±0.1
Enterococcus	6.0±0.3	6.6±0.6	5.9±0.3	4.7±0.3
Pseudomonas	5.9±0.5	6.0±0.5	5.7±0.5	0.3±0.1
Yeast	5.7±0.1	6.5±0.6	5.9±0.4	6.3±0.6
Mold fungi	1.1±0.1	1.9±0.2	1.3±0.2	2.0±0.3

Note: * - P<0.05 in relation to the control group and the background data

In 24 h after eating the last supplementation, qualitative and quantitative composition of the contents of the posterior part of the intestine was examined in the bees of the experimental and control groups. Results of the studies showed (Table 6) that the number of microorganisms in the intestinal tract of bees of the control group had increased after feeding with supplement: Enterobacteria - up to 6.9+0.4 log CFU/g, enterococci - up to 6.6+0.6 log CFU/G and yeast - up to 6.5+0.6 log CFU/g.

On the contrary, in the bees of the experimental group the number of representatives of the bacterial flora has decreased, while the amount of fungi has increased. The number of enterobacteria, staphylococci, pseudomonads and lactobacilli decreased to a greater extent (up to the complete disappearance from the intestinal tract). The amount of enterococci decreased insignificantly (from 5.9+0.3 to 4.7+0.3 log CFU/g). At the same time, unlike bacteria, the number of fungi, on the contrary, increased by 0.4-0.7 log CFU/g and was similar in the control group.

Consequently, feeding of sugar syrup to bees leads to stimulation of the reproduction of all microorganisms in the intestinal tract, but to a greater degree of enterobacteria, staphylococci, enterococci and fungi. Introduction of enrofloxacin to sugar syrup leads to significant suppression of the development of bacterial flora in the intestinal tract (except for enterococci), but this does not affect the development of fungal microflora. And this means that the use of sugar syrup without antibacterial drugs can contribute to the development of bees' diseases, the causative agents of which are various enterobacteria, staphylococci, enterococci and fungi. However, addition of enrofloxacin into the sugar syrup excludes the development of bacterial diseases, but at the same time it increases the risk of development of fungal diseases in bees. It leads to the development in the intestinal tract of insects of selective dysbacteriosis with the predominance of fungal (especially yeast) microbiota.

Effect of Ozone on Microbiocenosis of the Intestinal Tract of Bees

Treatment of hive with various bactericidal and fungicidal agents is an integral part of comprehensive antiepizootic measures aimed at the prevention and eradication of infectious diseases of bees (Ivashkevich, 2008; Plutakhin *et al*., 2016). Ozone, which is used for the treatment of bees, is the most promising and environmentally friendly agent (Ovsyannikov, 2006).

After the experiment, the number of enterobacteria, lactobacilli, staphylococci, enterococci and yeast increased by 0.2-0.5 log CFU/g and the number of pseudomonads and mold fungi decreased by 0.2-0.7 log CFU/g. More significant changes in the microbiocenosis of the intestinal tract of bees were found in the species from the experimental group: The amount of

staphylococci, enterococci and pseudomonas in them decreased by 1.8-3.2 log CFU/g; the amount of enterobacteria and lactobacilli decreased by 0.5-0.8 log CFU/g; and the amount of fungal microbiota increased by 0.4-0.5 log CFU/g.

Consequently, the use of ozone as a means of prevention and treating infectious diseases of bees can be justified. However, the number of fungi after ozonization in the intestinal tract of bees has been increased. This indicates the stability of these microorganisms to ozone. And therefore in order to suppress fungal microbiota, ozonization should be performed for a longer time, or higher ozone concentrations can be used.

Effect of Hydrohemol on the Microbiota of the Intestinal Tract of Bees

Beekeepers widely use feed supplementation with sugar syrup in order to stimulate the physiological activity of bees, especially in the absence of flowering honey-bees. It is also recommended to acidify sugar syrup with acetic acid and add protein-mineral additives into it.

We used hydrohemol in order to increase the biological value of sugar syrup. Hydrohemol is an acidic hydrolyzate of blood of animals with the addition of lactic, benzoic and succinic acids (Terekhov, 2001; Koshchaev *et al*., 2016).

Hydrohemol was added to 50% sugar syrup at the rate of 1:9. This feeding was given to bees during February-March 12 times with an interval of 2-3 days at the rate of 500 mL per hive. Families of the control group were fed with sugar syrup without additives. Prior to the experiment and 24 h after eating the last supplementation, we examined qualitative and quantitative composition of the intestinal microbiota in bees of both groups.

Results of the study showed (Table 8) that the amount of enterobacteria in bees of the control group in intestinal contents had increased by 1 log CFU/g; lactobacilli, staphylococci, enterococci and yeast had increased by 0.4-0.6 log CFU/g; and pseudomonads and mold fungi had increased by 0.1-0.3 log CFU/g.

Bees of the experimental group had slightly different picture. They demonstrated a decrease in enterobacteria, staphylococci, enterococci, pseudomonads, yeast and mold fungi by 0.4-3.4 log CFU/g. Only the amount of lactobacilli increased by 1.6 log CFU/g. Consequently, the use of a hydrohemol in the composition of 50% sugar feed supplementation allowed to affect the microbiota of the digestive tract of bees selectively, stimulating the reproduction of lactobacilli in them.

However, the use of the sugar syrup as feed supplementation in bees has a number of disadvantages. The main disadvantage is the fact that bees immediately take liquid feed supplementation and store it in honeycombs. When it is consumed, bees are excited, resulting in an increased ventilation of the nests and an increase in the hive temperature. In this regard, during the winter and early spring period it is recommended to feed bees with solid dough-like supplementation – candy. The latter is a mixture of powdered sugar (70-80%), honey (20-30%) and water (1-4%). For the experiment, during preparation of dough feed supplementation, we used hydrohemol instead of the water at the rate of 1.5 liters per 35 kg of sugar-honey mixture. This candy was fed to the bee families of the experimental group 2 times with an interval of 3 weeks at the rate of 1 g of candy for the hive. Bee-families of the control group were fed with an ordinary candy.

Studies established (Table 9) that in bees of the control group on completion of the feeding the number of escherichia, staphylococci, enterococci, pseudomonads and yeast was approximately the same and the number of lactobacilli was 1,000 times smaller. On the contrary, in the bees of the experimental group, in contrast to the control one, there was a 100-1,000-fold decrease in the amount of enterobacteria, staphylococci, pseudomonads, yeast fungi; and more than 1,000-fold increase in the number of lactobacilli.

Thus, the use of hydrohemol allowed to correct microecological processes in the digestive tract of honey bees in the period of emergence from wintering. Consequently, it can be used with both liquid and dough-like feed supplementation. However, one should give preference to the use of hydrohemol in the composition of candy in order to ensure a more stable result.

Table 7: Qualitative and quantitative composition of the intestinal microbiota of bees after treatment with ozone (March), (n = 50)

	Number of microorganisms, log CFU/g			
	Control group		Study group	
Microorganism	Before the experiment	After the experiment	Before the experiment	After the experiment
Enterobacteria	4.9±0.3	5.6±0.3	5.1±0.5	4.3±0.4*
Lactobacilli	5.0±0.1	5.2±0.5	4.9±0.6	3.4±0.4
Staphylococci	5.6±0.5	6.1±0.4	6.1±0.6	2.9±0.1*
Enterococcus	5.7±0.4	6.0±0.5	6.2±0.3	3.9±0.3*
Pseudomonas	6.0±0.7	5.8±0.6	5.7±0.2	3.9±0.3
Yeast	5.1±0.6	5.6±0.3	5.3±0.3	5.8±0.1
Mold fungi	2.3±0.3	2.8±0.2	2.0±0.4	2.4±0.2

Note: * - $P<0.05$ in relation to the control group and the background data

Table 8: Qualitative and quantitative composition of the microbiota of the posterior parts of the intestines of the bees that received 50% sugar syrup with hydrohemol (February-March), (n = 50)

	Number of microorganisms, log CFU/g			
	Control group		Study group	
Microorganism	Before the experiment	After the experiment	Before the experiment	After the experiment
Enterobacteria	4.8±0.4	5.9±0.2	5.0±0.3	3.6±0.1*
Lactobacilli	4.6±0.7	5.0±0.5	4.7±0.4	6.3±0.2*
Staphylococci	5.3±0.3	5.9±0.6	5.6±0.2	3.2±0.2*
Enterococcus	5.8±0.6	6.3±0.3	5.3±0.5	4.9±0.5
Pseudomonas	5.1±0.1	5.2±0.3	5.0±0.3	1.6±0.2*
Yeast	4.9±0.5	5.6±0.4	5.1±0.1	4.2±0.4
Mold fungi	2.0±0.1	2.3±0.3	1.9±0.2	0.3±0.1*

Note: * - P<0.05 in relation to the control group and the original data

Table 9: Qualitative and quantitative composition of microbiota of the posterior part of the intestine in bees receiving candy with hydrohemol (January-February), (n = 50)

	Number of microorganisms, log CFU/g			
	Control group		Study group	
Microorganisms	Before the experiment	After the experiment	Before the experiment	After the experiment
Enterobacteria	5.9±0.3	6.3±0.6	6.0±0.1	3.8±0.2*
Lactobacilli	2.8±0.6	3.2±0.3	2.9±0.4	6.4±0.2*
Staphylococci	6.0±0.4	6.5±0.8	6.1±0.3	3.3±0.1*
Enterococcus	5.3±0.5	5.9±0.2	5.0±0.3	4.5±0.4
Pseudomonas	5.5±0.6	5.9±0.3	5.4±0.5	2.0±0.1*
Yeast	5.6±0.2	5.8±0.1	5.3±0.3	3.8±0.3
Mold fungi	-	1.3±0.1	-	-

Conclusion

Microbiocenosis of the intestinal tract of adult bees in the conditions of the south of Russia is represented mainly by enterobacteria, lactobacilli, staphylococci, enterococci, pseudomonads, yeast and mold fungi. At the same time, enterobacteria (*Enterobacter aerogenes* and *Escherichia coli*) and staphylococci (*Staphylococcus warneri*) are the dominant members of the microbiocenosis. During the year, qualitative and quantitative composition of the intestinal microbiota undergoes significant changes characterized by a decline (up to the complete disappearance of enterococci and mold fungi) in August-September and peak in February-March.

Typical feature of microbiocenosis of the intestinal tract of young bees is a lower concentration of microorganisms (by 1.5 log CFU/g on average) than in adult bees and the absence of lactobacilli in the intestinal microbiota from September to February. Dominant species among the bacteria were *Enterobacter aerogenes*, *Staphylococcus warneri* and *Pseudomonas fluorescens* and *Penicillium glaucum* among the mold fungi, unlike adults.

Microbiocenosis of the surfaces of various objects of the hive (aperture, frames, walls, bottom) and drinkers is similar in their composition to the microbiocenosis of the intestinal tract of bees. The peak of the quantitative presence of microorganisms (3.3-3.5 log CFU/cm^2) in the hive occurs during the period from June to August (period of the main honey gathering). In this case, aperture and the frame are objects mostly populated by microorganisms (4.1-4.2 log CFU/cm^2).

There was a high correlation between the state of the intestinal tract of bees and the quantitative presence of enterobacteria, lactobacilli and enterococci in its contents. Anatomical state of the intestines of bees deteriorates, while their reproductive activity and honey production decrease along with an increase in the number of enterobacteria, staphylococci, pseudomonads, yeasts and mold fungi in the intestinal tract. If the content of lactobacilli in the intestinal tract is at least 6 log CFU/g, the state of their intestines is estimated at 4 points.

The use of the carbohydrate feed supplementation with enrofloxacin is accompanied by the development of selective dysbacteriosis in the intestinal tract. It is manifested by an almost complete disappearance of gram-positive and gram-negative bacteria and an increase in the quantitative presence of yeasts and mold fungi by 0.4-0.7 log CFU/g.

The use of ozone in the dose of 6 mg for the treatment of beehives in the period of 2 h for 7 consecutive days leads to the decrease in the intestinal tract of bees of the quantitative number of bacteria by

0.5-3.2 log CFU/g and, conversely, to an increase in a quantitative number of fungi by 0.4-0.5 log CFU/g. Inclusion of hydrohemol into the 50% sugar syrup or candy allows to regulate microecological processes in the digestive tract of bees, achieving a 100-1,000 fold decrease in the number of enterobacteria, staphylococci, pseudomonads, yeast and mold fungi and, conversely, leads to an increase in the same amount of lactobacilli.

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Author's Contributions

All authors equally contributed in this work.

Ethics

Authors declare no conflicts of interest.

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