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ORIGINAL ARTICLE

Features of histolism and hystogenesis in the vital temperature range in the organism of honey bee (*Apis mellifera* L.) in the postembrional period

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The productivity of bee families depends to a large extent on the influence of exogenous factors. The most important ones include: the presence of a honey-bearing base, climatic conditions, temperature of the environment, etc. It significantly affects the growth of families and the stability of the microclimate in the nest. The welcome temperature range in the postembryonic period fluctuates within 30-38 °C. Therefore, the purpose of the work was to find out the physiological and biochemical peculiarities of the adaptation of the body of honeybee bees under the influence of the lowered temperature in the nest. For research, two groups of honeybees were selected that differed in terms of maintenance. The control group was constantly in the nest of the bee family, and the experimental at the stage of the prepupa was placed in a thermostat where the temperature was maintained at 32 °C. Materials for researches were tissues of prepupa and pupa. The method of thin-layer chromatography identifies the classes of lipids, phospholipids and cholesterol. Based on the performed researches, it was found that the violation of the optimal parameters of the microclimate in the post-embryonal period affects the increase in the weight of the prepupa of the body by 19.4% (P < 0.001) and the decrease in the intensity of the processes of dehydration in tissues by 63.8% (P < 0.05), which is associated with the accumulation of bound water in cells. It is proved that the decrease of the optimum temperature of the incubation of the breeding breed at 2 °C leads to prolongation of the post-embryonic development period by 35–42 hours. Under such conditions, throughout the period of incubation in bees, high levels of total lipids are observed. At the stage of prepupa, the number of triacylglycerols in the lipids increases by 49.0% (P < 0.001). In ontogenesis in pupa tissues, a gradual decrease in the content of triacylglycerols, including the imaging stage, is evidently due to the effect of the stress factor. It was found that lowering the temperature on the second day of incubation of the breeding plant reduces the content of phospholipids in the lipid tissues by 11.9% (P < 0.05). At the same time, the decrease in the content of lysophosphatidylcholine was detected by 6.6% (P < 0.001). The violation of the optimum temperature during the development of bees leads to a steady increase in the content of esterified cholesterol by an average of 47.9% (P < 0.01). In the process of pupa formation, the ratio of classes of esterified cholesterol is changing. The conducted studies allow to correct the metabolic processes in the body of honey bees in order to adapt them to the lowered temperatures.

Key words: honeybee bees; keeping bees; optimal temperature; adaptation to cold; lipids, classes of phospholipids; classes of cholesterol

Introduction

Receiving the maximum amount of products from honey bees is impossible without the use of modern technologies of maintenance and care (Brovars'kij and Bagrij, 1995; Vishchur et al., 2016). This, in turn, is based on the knowledge of the biological features of insects (Taranov, 1961; Tyshhenko, 1986). These features include, for example, the stability of the incubation temperature of the breeding poultry. It is believed that the optimum temperature in the breeding zone varies within 34.0–35.0 degrees C. However, due to various factors, it can be reduced by 2–3 °C. Due to the influence of low temperature, the bees' club is sealed. Under these conditions there is a supercoiling of the breeder, which is located on the periphery of the cell. This is manifested in particular in the case of a sharp decrease in the temperature of the environment when there is a seedlings in the family, and in case of improper expansion of the nest with artificial wax frames. Adaptation of honey bees to the cold is accompanied by a change in the biochemical composition of tissues. Such changes allow to withstand sharp changes of ambient temperatures. Reducing the temperature by 3.5 °C leads to changes in development. Under these conditions, a decrease in the size of the exoskeleton was detected in the bees that came out of cells (Lobov, 1997; Yeskov, 2005). In some cases, a possible lethal consequence is possible. The temperature at which the cultivar grows affects the behavior of the bees and the transfer of information between the bees. In particular, if the seedlings were in an environment with a temperature of 32 °C, then the

Therefore, the purpose of the work was to study some of the adaptive mechanisms of the body of honey bees under the action of low temperature in the postembryonic period.

Material and methods

Work completed during 2012–2013. The research is carried out in the beekeeping department of the technology of production of small animals, as well as in the laboratory of ecological physiology of the Institute of Animal Biology of the Ukrainian Academy of Agricultural Sciences. For research, bee families, which were formed by the analog method, were selected. They had the same strength, the number of brood and feed. Honey in the nests was in the range of 6–8 kg, and pergs 0.7–0.9 kg. The bee family of the Carpathian breed was kept in a multi-hull 8-frame hive with a frame size of 435×300 mm. The material for biochemical studies was tissue, in front of prepupa, pupa and adult bees. The control served as a nursery, which was always in the hive (Yeskov, 2005). The experimental group was considered a nursery, which was placed in the thermostat at a temperature of 32 °C. After sealing the larvae in the experimental groups, after every 2 days, the dry mass was determined (Lebedev and Bilash, 1991), the content of total lipids (Folch et al., 1957), classes of lipids, phospholipids, esterified cholesterol (Tkachuk and Stapaj, 2011). The principle of the method for determining the number of common lipids is that the lipoprotein complexes are destroyed by a polar solvent (methanol), contributing to their extraction with a nonpolar solvent (chloroform). The method allows release of lipid extract from non-lipid substances by washing. Reagents: chloroform, methanol, methanol mixture with chloroform (2 : 1), 0.74% solution of KCl, washing mixture (chloroform: methanol: KCl – 8 : 4 : 3). In a cone with crumpled crust, 1 part of the crushed tissue was added and 20 parts of a mixture of chloroform-methanol were added in a ratio of 2 : 1. The resulting slurry was thoroughly shaken and left to stand for 12 hours at room temperature for extraction. The mixture was then filtered through a degreased filter, the precipitate was washed twice with an extraction mixture (5 ml), and the extracts were combined. To remove water-soluble non-lipid mixtures, 0.74 M KCl solution, equal to 1/5 volume of lipid extract, was added to the extract. The mixture was shaken again and left to stand for 12 hours. After a 12-hour advocacy, a two-phase system was formed. The upper aqueous methanol layer was sucked off with a water jet pump, and the bottom was concentrated on a rotary evaporator. The amount of lipids in the tissue was determined gravimetrically after concentration on the rotary evaporator. This method is most suitable for determining the total amount of lipids by weighing the dry residue.

The lipid extract obtained by the Folche method was dried by distillation of the evaporator, and then brought to a constant mass in a vacuum desiccator. For this test, they were placed in a desiccator filled with a moisture absorber (CaCl₂). After two hours, the samples were weighed to analytical grade and the number of lipids was determined by the formula :

 $(A - B) \times 100 / C = mg\%$

where: A – the weight of the buxa with lipid B – the mass of the bezx without lipids

C – mass of tissue, mg.

Results

Honey bees belong to insects with a full type of metamorphosis, which is called holometabolia. It is characterized by the transition of the larva to the imginal form through the intermediate stage – pupa. In the post-embryonic period, different tissues are formed, which will develop in the end organs of the bees at the stage of prepupa and pupa. Post-embryonic development is a long process characterized by relatively stable incubation rates. It can not be divided into certain phases, in which conditions of incubation can be changed. This is due to the peculiarity of placing the breeding nest in the nest, since there is a two-year nursery on one cell. Therefore, changing the temperature regime for some individuals can lead to irreversible processes in others. According to our studies, a steady decrease in the incubation temperature of the brood until 32 °C leads to an increase in the duration of development in the post-embryonal period. In the control group, its duration was 18 days. While in the experimental group he was bigger at 35–42 hours. The incidence of incubated bees was 8.0%. The seedling in a sealed state gradually uses spare substances in the tissues of bees before leaving the cell decreased by 48.6%, compared with the initial values. The amount of dry matter in the experimental group is high (P < 0.001) decreases by 39.2%. Figure 1 shows a diagram of the total number of lipids in the tissues of the prepupa and pupa.

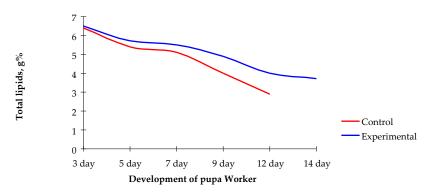


Fig. 1. The content of total lipids of prepupa and pupa, depending on the incubation temperature of the brood, g% (M \pm m, n = 20)

In the period of prepupa, the number of lipids is greatest and is 6.4 mg. For the entire period of prepupa and pupa, the total lipid content in the control group decreased by 42.18%. In the experimental group after the release of bees from the cells their weight was higher by 5.4%. During the whole period of incubation in the experimental group of bees, high levels of total lipids were recorded. The maximum deviation of 18% was found in the experimental group at day 15 of post-embryonic growth. Such a phenomenon can be interpreted as a deviation in the metabolism of the body of bees, when lipids are not used in full by the tissues for their construction. Loss of nutrients is associated with molting, secretion of the exudative fluid and significant transformations in body structure. During the histolytic and histogenesis process, there is a further loss of the total number of lipids. The loss of total lipids in the experimental group during the metamorphosis of pupa in adult bees is 38.09%. The next stage of our research has shown that due to non-compliance with the microclimate regime, changes in the ratio of lipid classes are taking place. Normally, with all the hygiene parameters observed, the number of phospholipids in the tissues of puppets of honey bees throughout the development period is practically stable (Fig. 2).

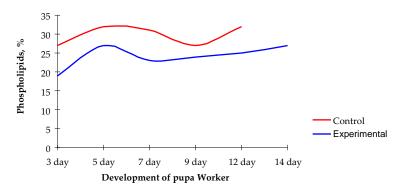


Fig. 2. Dynamics of phospholipids in lipids of tissues of prepupa and pupa depending on incubation temperature of brood,%

Their content ranges from 28.0 to 31.1% of the total number of lipids. In the structure of lipids, it was found that a decrease in the temperature of the incubation of the bream at 2 °C leads to changes in the ratio of, in particular, the content of phospholipids. In the tissue pupa of the experimental group, their number does not exceed 26.4%. Their lowest content in the experimental group was recorded during the fourth day of the development of the pupa. In this period there are intense histolytic processes. The number of phospholipids in the experimental group was 20.23% lower than the control group (P < 0.001). Such a decrease in the number of protoplasmic lipids is negatively reflected in the processes of histogenesis. This is due to the fact that phospholipids form the overwhelming part of the nucleus lipids, mitochondria, ribosomes and hyaloplasmic cells. In the preimaginal period in the pupa there are the most pronounced changes associated with the formation of tissues. Therefore, during this period, the content of phospholipids is particularly necessary. Inconsistency of the incubation parameters of the ram leaf results in prolongation of the development period. In the last day, the control group has the highest content of phospholipids – 31.1% of all lipids. On the ninth day in the research of the experimental group, it was found that the content of phospholipids was only 23.0%. In the preimaginal period, their content increases to 27.4%. Obviously, because of the significant need for phospholipids in pupa of the experimental group and there is a fluctuation in their content.

Phospholipids of prepupa and pupa tissues are represented by the following classes: lysophosphatidylcholine (lysolecithin), sphingomyelin, phosphatidyl ethanolamine (kefalin), phosphatidylcholine (lecithin) (Table 1).

Table 1. Correlation of classes of phospholipids of lipids of tissues of a prepupa, depending on incubation temperature ofbrood,% (M±m, n=20)

Indicator	Family Group	
	control	experimental
Lysophosphatidylcholine	19.6 ± 0.26	18.6 ± 0.78
Sphingomielin	17.9 ± 0.32	17.1 ± 0.30
Phosphatidylethanolamine	29.9 ± 0.17	31.4 ± 0.45
Phosphatidylcholine	32.4 ± 0.59	32.7 ± 0.69

The data in Table 1 indicates that the stage of prepupa in the tissues amount the largest number of phosphatidylcholine was detected. Moreover, in the experimental groups, its contents are practically the same. Fewer phosphatic di-ketanolamine is used. Its amount in the control group is 4.90% lower than the experimental one. In the structure of phospholipids the fifth part is occupied by lysophosphatidylcholine. The decrease in the temperature of the incubation of the brood leads to the fact that in the tissues of experimental puppies, the content of this class is 5.0% smaller. Such a slight difference may indicate that the temperature regime during this period does not significantly affect the intensity of the histolylastic process at the stage of the prepupa.

The smallest part in the tissues of the prepupa is sphingomyelin. In particular, in the experimental groups the content of sphingomyelin ranges from 17.18 to 17.92% Inconsistency of comfort parameters for breeding honey bees leads to further

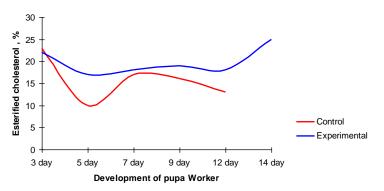
In the control group of all classes, the content of phosphatidylcholine remains the largest. In the structure of the phospholipids of the control group, he occupies a third of all classes. In ontogenesis, there is a decrease in phosphatidylcholine in the experimental groups. In particular, the control group found a decrease in its number by 9.86%, and in the research – by 3.20%. The temperature factor also affects the amount of phosphatidylcholine. Thus, in the experimental group, its content is higher by 6.55% (P < 0.05). The predecessor of phosphatidylcholine is phosphatidyl ethanolamine. Phosphatidylethanolamines - esters of phosphatidylcholines, they form the bulk of phospholipids. In the ontogenesis, the nine-hour pupa metamorphosis is accompanied by a decrease in the phosphatidylethanolamine content in the control group by 9.71%, and in the experimental group by 6.02%. In case of violation of the microclimate parameters in the phospholipids of the experimental group, a high probable increase of phosphatidylethanolamine was found at 8,15% (P < 0.01). Preparation for the imgant form is manifested by an increase in the structure of phospholipids sphingomyelin. This class in the structure of phospholipids remains at the lowest level. In particular, during this period, its amount in control increases by 29.51%. The violation of the temperature regime affects the probable decrease in sphingomyelin by 16.73% (P < 0.01).

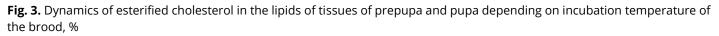
Regarding the amount of lysophosphatidylcholine, an increase in its amount in the control group was 12.03% in the control group and 12.03% in the experimental group. In the experimental group due to the violation of the temperature of the incubation, a probable decrease in the content of lysophosphatidylcholine was found at 5.95%.

The bees of the control group for the 21st day in this period have already left the cells and were selected to study histochemical changes. A seedling of the experimental group was still in the thermostat. In lipids of tissues of honey bees at the beginning of the immaginal phase most phosphatidylcholine (33.26%) and phosphatidylethanolamine (28.28%) were found. Other fractions of phospholipids are found in lesser quantities.

Due to the violation of the microclimate of the conditions for keeping bees, changes in the content were found not only in the ratio of phospholipid classes but also in the ratio of other classes of common lipids. In particular, such changes are characteristic of esterified cholesterol (Fig. 3).

According to the data of Chart 3, the second day of the pupal stage in bees of the control group revealed low content of esterified cholesterol. In bees of the experimental group during this period its quantity was higher in 1.7 times (P < 0.01). The dynamics of esterified cholesterol in the tissues of the pupa of the control group has the form of an increasing parabola. The peak of esterified cholesterol was detected on the fourth day of the pupal stage. In particular, in this period, in comparison with the second day, pupa of the control group its content increases by 1.7 times. In the experimental group, the amount of esterified cholesterol remains unchanged. The decrease in the temperature of the incubation of the brood in the bees of experimental groups results in a steady increase in the content of esterified cholesterol. Thus, in the preamaginal stage of development in bees of the experimental group, its content, in comparison with the second day, increases by 1.5 times. Etheric cholesterol predominates in hemolymph and is deposited in small cells. In this case, cells use it for the synthesis of other substances. The dynamics of esterified cholesterol looks like hyperbole. In bees of the control group in the preamaginal stage its content is 11.7%.





As a result of the decrease in the temperature of the incubation of the brood during the intensive metabolic processes in the bees of the experimental group, the growth of this fraction is 1.5 times. The esterified cholesterol in the tissues of puppets of honey bees is represented by the following classes: esters of saturated acids, esters of monounsaturated acids, dieths, triene esters, tetraeines, other polyene esters. Reducing the incubation temperature of the sprout at 2 °C leads to changes in the ratio of classes of esterified cholesterol. At the stage of prepupa in the classes of esterified cholesterol in the experimental group, the smallest number of detected tetraene esters. However, among the experimental groups, their content is practically the same and varies within 11.14–12.08%. In the control group, the esters of saturated, monounsaturated acids, as well as diene and teraenic esters occupy practically the same amount. Additionally, in the control group, a significant amount of 22.05% is carried by trine ethers, which is 28.02% higher than the experimental group (P < 0.01) (Table 2).

Table 2. The correlation of the classes of esterified cholesterol of lipids in the tissues of honey bees at the stage of a prepupa of the second day, depending on the incubation temperature of the brood, % (M ± m, n = 20)

Indicator	Family Group	
	control	experimental
Ethers of saturated acids	13.31 ± 0.30	15.30 ± 0.68
Ethers of monounsaturated acids	14.11 ± 0.43	17.43 ± 0.32**
Diethanes	12.32 ± 0.62	13.36 ± 0.23
Trine ethers	22.05 ± 0.51	15.87 ± 0.63**
Tetraene esters	12.08 ± 0.35	11.14 ± 0.06
Other polyene ethers	26.01 ± 0.27	26.88 ± 0.06*

Note: The probable difference between the control and the experimental group * - P < 0.05; ** - P < 0.01; *** - P < 0.001.

In the experimental group among these classes, the increase in the number of esters of mono-unsaturated acids was found to be 23.53% (P < 0.01). Of all classes of esterified cholesterol, the highest number of polysaccharides was found. The differences found in the pupal stage are no significant changes and make up 26.0% in control versus 26.8% in the experiment (P < 0.05). In addition to the structural function of the cholesterol esters responsible for the binding and transport of polyunsaturated fatty acids between the organs and tissues of the lipoproteins. As can be seen from these studies, most of cholesterol in the body of bees is esterified with fatty acids. It is with fatty acids that one of the functions of lipids is associated with energy. Due to the oxidation of fatty acids, body tissues receive energy.

In the preimaginal period in the lipids of tissues of honey bees there are intense changes. In particular, this is clearly seen from the data in Table 3, which shows the dynamics of the classes of esterified cholesterol in the tissues of honey bees at the stage of the metamorphosis of the pupa of the ninth day.

Table 3. The correlation of the classes of esterified cholesterol of lipids in the tissues of a pupa of the ninth day, depending on the incubation temperature of the brood, % (M ± m, n = 20)

Indicator	Family Group	
Indicator	control	experimental
Ethers of saturated acids	9.82 ± 0.40	13.63 ± 0.77*
Ethers of mono-unsaturated acids	13.51 ± 1.20	21.20 ± 0.52**
Diethyl esters	17.53 ± 0.75	18.15 ± 0.71
Trene ethers	11.17 ± 0.56	13.58 ± 0.70
Tetraene esters	15.78 ± 0.84	10.19 ± 0.72**
Other polyene ethers	32.15 ± 0.78	23.19 ± 0.64***

Note: The probable difference between the control and the experimental group * – P < 0.05; ** – P < 0.01; *** – P < 0.001.

Under the influence of the temperature factor of the classes of esterified cholesterol, changes were found regarding the content of saturated esters. In particular, with the age in the control group on the eighteenth day of post-embryonic development their number increases.

In the experimental group, their number is higher by 38.80% (P < 0.05). In the structure of esterified cholesterol, the largest share is occupied by other polyester esters. In the experimental group there is a decrease in their number by 27.87% (P < 0.001). Due to the influence of the temperature factor, the most dynamic changes were found in the analysis of the content of monosodium monoxic acid esters. Thus, in the experimental group, their number is higher by 56.9% (P < 0.01). The content of diene esters, both in the control and in the experimental groups, remains practically the same. An increase in the amount of trichine esters by 21.57% occurs on the background of a decrease in the content of tetraene esters by 35.42% (P < 0.01). During this period, the amount of tetraene esters in the control is 15.78%. In the ontogenesis of all classes of esterified cholesterol in control, the most dynamic changes were detected with respect to the content of triene esters. In particular, in the control group at the eighteenth day of post-embryonic development their number is reduced to 11.17%. In the experimental group, the triesters drop to a mark of 13.58%. In the research group, the content of other polyene esters decreases, while in control it grows. Ethers of saturated acids in the control are characterized by stable indicators, but in the experimental group there are significant changes in their content. In particular, the growth and development of pupa is accompanied by an increase in the content of this fraction by 1.4 times. More dynamics of growth are characterized by esters of monounsaturated acids. In the experimental group, their content is 21.2% of the mass fraction of esterified cholesterol, and in the control group – 13.51%. The content of diene esters in the experimental groups varies within 15–19%. In the experimental group in nine-hour pupa, 1.2 times, their content was found to be higher than in the six-day period. The content of tetra-ethers in the control at the final stage of development becomes higher. In the control group their content is reduced by 1.6 times. As for the experimental group, then, on the contrary, their number is less and is 10.19%.

Discussion

In general, lipids enter the body of a honey bee larva when consuming perg. (Brodschneider and Crailsheim, 2010). Virtually all insects receive sterols only when they consume food. The body is unable to synthesise it independently (Somerville, 2005), sterols are precursors of hormones that regulate growth and molting (Harano, 2013; Collison et al., 2015). In this case, lipids play an important role in the formation of the protective coating - cuticles (Fröhlich et al., 2000; Arsene et al., 2002). In the body, with the optimal composition of the diet, the lipid components are pheromones, pharyngeal and wax glands (Blomquist and Bagners, 2010; Nelson et al., 1997).

The stage of the prepupa of a working bee lasts for three days. During this period she does not eat, but cocoon spins. External signs of the exterior of the prepupa of honey bees are practically no different from the larvae. The ratio of lipid classes depends on the influence of some factors (Svoboda et al., 1980). Our research revealed biochemical differences in the tissues of bees, depending on the mode of incubation of the brood. In particular, they relate to changes in the ratio of lipid classes: phospholipids, mono- and diglycerides, free cholesterol, unesterified fat acid, triacylglycerol, esterified cholesterol. In lipids of tissues, prepupa of all classes are dominated by phospholipids (Singh and Singh, 1996). In the structure of lipids, they occupy 25–27%. In turn, the classes of phospholipids are represented by the following classes: lysophosphatidylcholine (lysolecithin), sphingomyelin, phosphatidyl ethanolamine (kefalin), phosphatidylcholine (lecithin). Of these, the largest percentage is occupied by phosphatidylethanolamine and phosphatidylcholine. Their total number is more than 60%. The cell membrane is a liquid dynamic system with a mosaic arrangement of proteins and lipids. The basis of the membrane is the molecules of phospholipids, the polar (ionic) heads of which are directed to the aqueous medium (hydrophilic zone), and the nonpolar parts - "tails" - inside the membrane (hydrophobic zone) (Lenindzher, 1985). In addition to phospholipids, a significant share in lipids is esterified cholesterol. Its amount in control is 24.09%. Evidence of a metabolic abnormality of esterified cholesterol is the index of its content in the experimental group, where its amount is 22.7%. In the structure of esterified cholesterol, 6 classes have been identified, namely, esters of saturated acids, ethers of monounsaturated acids, dieths, triene ethers, tetraeines, and other polyene esters. In the prepupa of the specimens, the largest number is occupied by other polyene ethers – 26.0%. In an ontogeny in tissues of a four-day pupa of the experimental group, an increase in the number of free cholesterol was detected in comparison with the control.

Honey bees store energy in the form of fats (Young and Tappel, 1978). Fats are stored in the form of drops in adipocytes (Winston, 1987). Triacylglycerols are neutral fats if all three hydroxyl groups of glycerol are esterified with fatty acids. Most of the fatty acids that are part of the molecule of triacylglycerols, differ from each other. Neutral fats are found in the body or in the form of protoplasmic fat, which is a structural component of cells, or in the form of spare, reserve fat. The role of these forms is different: protoplasmic fat has a stable chemical composition and is contained in tissues in a certain amount, which does not change even in pathological obesity, while the amount of reserve fat varies considerably. The main function of fats - the source of energy in the cell. In this case, fats perform a protective function, in the composition of adipose tissue protect the body and individual organs from mechanical damage, temperature fluctuations, and others. Fats are an important source of endogenous water in the body (Boyechko and Boyechko, 1993, Gubs'kij, 2000). Analyzing the indicators of triacylglycerols, we assume that the energy costs are more detected in the prepupa of the control group. As a result of the welcome temperature range, it is likely that the content of triacylglycerols in the prepupa of the experimental group increases by 48.96%. In the ontogeny in the tissues of puppets of the experimental group, a gradual decrease in the content of triacylglycerols was observed. This may be the cause of the stress factor due to hypothermia, which negatively affects lipid metabolism during this period.

Conclusions

Violation of the microclimate parameters of the bees' nest, in particular the lowering of the temperature of the incubation of the bream, affects the change in body weight in all stages of ontogenesis. In this case, the extension of the post-embryonic period of development was revealed. During the whole period of incubation in the experimental group of bees, high levels of total lipids were recorded. In the ontogenesis of the pupa of the experimental group in tissues, a gradual decrease in the content of triacylglycerols was observed, including the imgasal stage, which was caused by the influence of the stress factor. Reducing the incubation temperature of the sprout at 2 ° C leads to a change in the ratio of lipid classes and esterified cholesterol.

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