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Bioconversion of Nutrients and Biological Active Substances in Model Systems Chlorella-Insect-Livestock

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ABSTRACT

For decades, the most actual problem has been the deficiency of animal protein, and now this problem has become more urgent. Nowadays, animal products are not available to all levels of the population in many countries. New ways of receiving protein from nonconventional raw materials have been developed everywhere to solve this problem. At the moment, the selection of proteinaceous plants, biotechnology of microorganisms and bioengineering are on trend. Also, there are prerequisites for efficient development of animal breeding, which consist of searching for the alternate production technologies of the full-fledged enriched animal feeding stuffs received from the biomass of high-yield insects and products of their activity.

Keywords: Insecta*, Zophobas morio,Culex pipiens,Chlorella vulgaris,* chitin, protein, melanin, chlorelline, bioconversion.

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INTRODUCTION

The problem of the deficiency of the high-quality domestic feedstuffs ensuring a high level of the feed base has been known for many years, but it has not lost its importance. Nevertheless, there are prerequisites for the effective development of livestock. It is necessary to search for new biotechnological approaches for the production of feed and feed additives that have the desired properties; and new types of stuff and technologies for its processing should also be provided [18,23]. Thus, the task of modern agriculture is finding the solution which requires an integrated approach and original hypotheses.

One of the promising decisions of this task has been studying the mechanisms of interactions of the biologically active substances and products, the metabolism of the producers (Chlorella), and the first-order consumers with a high degree of feed conversion (highly productive insects in the larval stage of development) to create a renewable biotechnological cycle that would become the basis for the production of the functional feed additives [15,16].

The usage of chlorella as a producer in such systems that has been justified by the extremely high rate of biomass growth (up to 20 g / l per day), the high content of the high-grade protein – 50-55% in dry biomass (figure 1), and the presence of its own antibiotic – chlorellin, which has antibacterial and antimyotic effects, can influence the intestinal microflora [16,18]. The last property of chlorella has been especially valuable due to the fact that one of the main reasons that cause significant economic damage

to livestock is mass gastrointestinal diseases of young animals. These diseases are widespread, and cause a high mortality of newborns, which varies from 21% to 32% in different countries of the world. In addition, animals which have recovered from acute toxic diarrhea have been lagging behind in growth, and have been 10% to 12% below the level of the productivity in healthy animals. It has been assumed that the use of chlorellin will allow abandoning the synthetic potent antibiotics of a wide range of effects, often used in the fattening of young animals. Also, the high content of the natural antioxidants in chlorella: chlorophyll and β-carotene is noteworthy [20].

Figure 1. Dried biomass of Chlorella vulgaris

Nevertheless, the processing of chlorella has been associated with the certain difficulties that limit its use in an industrial scale. This is due to the fact that, despite the large amount of scientific data on the problems of cultivation and the use of chlorella, it has always been regarded as a separate feed additive. Traditionally, chlorella has been used in the form of a suspension or paste that has not been physically, chemically or otherwise processed. Problems with processing have been primarily associated with the thick cell membrane of chlorella (50-100 nm), which is poorly digested by most vertebrate species due to its complex multilayer structure [16]. However, the physiological properties of Chlorella suspension, tested previously on cows, showed a positive result. But, it was reliable only for cells in a state of division or death. In connection with this, the scientific interest has been in the search for the new methods of chlorella processing, which ensured the maximum assimilation of its contents. Earlier, in the project on "The development of innovative resource-saving technologies and equipment for intensification of raw materials processing in the production of meat products"(Russian State contract 254GS1/7927 dated 12.02.2015 by Fund for Assistance to Small Innovative Enterprises in the Scientific and Technical Sphere), the effects of the pulsed discharge treatment on the structure of the muscle fibers of livestock, and the hydration of biopolymers (figure 2) were investigated. The results of the research showed that the usage of the pulsed discharge treatment in the set established modes led to the destruction of myofibrils with a stiffness from 10 to 150 kPa, as well as the rupture of the cell membranes of the microorganisms with a tensile strength of 60-120 kPa [17,19]. The obtained results showed that the pulsed discharge treatment can be used for processing of chlorella biomass and obtaining an easily digestible suspension ready for use for feed purposes.

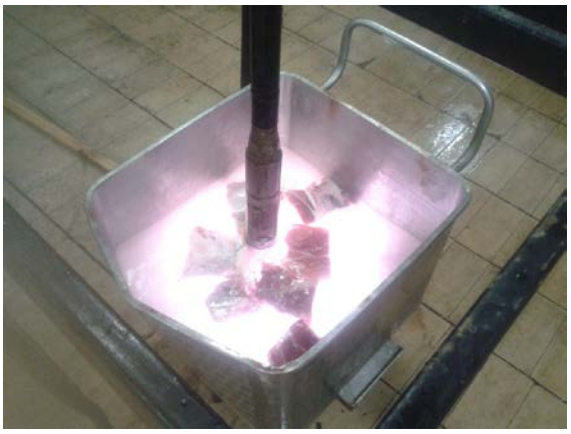

Figure 2. Pulsed discharge treatment of the meat stuff

Also chlorella can be processed in a biological cycle by bioconversion. As noted earlier, most vertebrates do not digest the cell membrane of chlorella. However, some insect species can digest it, while the conversion of chlorella biomass to insect biomass can reach 80% [1-3]. But, feeding insects with untreated chlorella, because of its slow digestion, in case of insects in the light, can cause destructive processes of fermentation. Thus chlorella complex processing will be optimized by pulsed discharge treatment and bioconversion. The urgency of the idea can

be justified by the fact that the management of the production process and the environment-improving potential of agroecosystems, and the use of useful insects with economically valuable characteristics are in line with the Prospective Plan for Fundamental Research on Priority Areas for the Development of Science, Technology and Engineering for the period up to 2025 by the Ministry of Education and Science of Russia that includes priority directions for agricultural science and scientific support of the agrarian and industrial complex of Russia. In addition, the Commission of the European food safety authority (EFSA, 08.11.2015) reported that about 800 insect species can be used in agriculture as sources of nutrients and biological active substances [5]. Such an approach carries with it the risks that have been described in this report. That's why it is important to study the effects of feeding the selected insects by chlorella after the pulsed discharges processing. This interest has been caused by the fact that theoretically, chitin of insects is able to form complexes with organic substances, including chlorellin, proteins, peptides, and also has a high potential for adsorption of other biologically active substances (chlorophyll, beta-carotene and melanin). So, it has been investigated by another State Contract 1701GS1/24288 dated 23.11.16 by Fund for Assistance to Small Innovative Enterprises in Science and Technology on the topic of "Development of a method for obtaining fodder and food products from insects with a high protein content, which have prophylactic and immunostimulating properties", and, a phenomenon of change of Zophobas moriolarvae color in case of feed bymelanin-containing stuff was discovered. The identified phenomenon has not been described in scientific literature and, apparently, has been associated with the complexing property of chitin, which has been manifested even in the process of vital activity.

Patent search showed that a row of inventors (Kuznetsov B of B, Aksenov of A B, 2013) has described the process of denigration of protein, most likely, the chemical process received from larvae of a fly, but the reason was also not revealed by them. In this study, it was assumed that change of color can be caused by a protein lysisby's own enzymes of larvae, or enzymes of microorganisms-symbionts. Also, there was a hypothesis, according to which during a molt secret of larvae select, phenol in the presence of oxygen of air can be oxidized by phenolase to the appropriate quinone. It has been known that quinones in water solutions and in the presence of air oxygen under the influence of enzymes will be polymerized, and will turn into melanin. The response to the raised questions will be received by studying the adsorption and the complex formation of the biologically active molecules in a chitinous cover of insects.

To study this process, this research was carried out on Chitinous cover of Zophobas morio by the atomic force microscopy and the computed tomography methods. The results of the research showed the presence of a complex cellular structure with located active centers. It has been well known that the biological activity of chitosan and melanin complex is higher than the biological activity of pure chitin or chitosan. It has been assumed that the compounds of chitin and chitosan with products of chlorella would have immunomodulatory, immunostimulating and immunocorrecting properties. For the biological processing of chlorella, the larvae of Culex pipiens and Zophobas morio were selected. The choice has been justified by the high rate of biomass growth, the high content of a high-grade balanced protein (20-25%), fat (16-20%) and the ability of these larvae to bio-converse to BAS (figure 3).

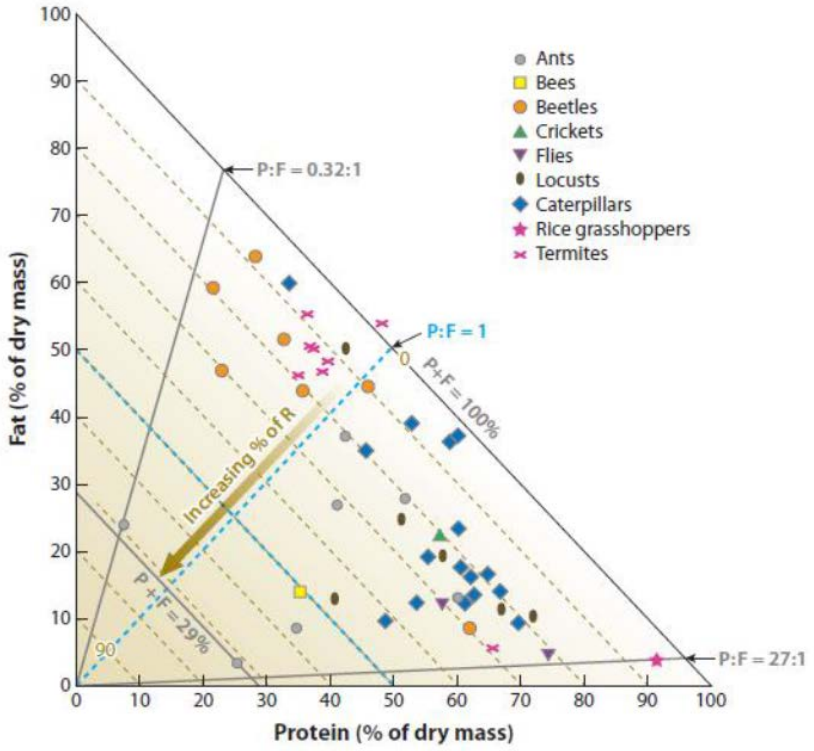

Figure 3. Fat and protein content in insect biomass (prof. Rui Costa edition)

Zophobas morio larvae are characterized by the ability to assimilate atmospheric nitrogen due to the symbiosis with nitrogen-fixing bacteria.

Thus, they can synthesize the entire spectrum of amino acid, and combine them with a protein by the bioconversion of cellulose. Proceeding from this, the use of chlorella, rich in both cellulose and protein, will contribute to the intensification of the described process. Culex pipiens larvae are interesting by the fact that they have an underdeveloped tracheal respiratory system, and therefore, there is hemoglobin in their hemolymph. In scientific literature, there are data that show they are able to synthesize it independently [6]. This issue requires a fundamental study, since the feed additives used in animal husbandry do not fully satisfy the need of agricultural animals in divalent iron.

It is worth noting that the creation of a renewable biotechnological cycle, including chlorella and selected insects, is not limited to the creation of functional complexes and the conversion of the plant biomass to an animal. For chlorella, as a producer with a high growth rate, a large amount of oxygen is produced. According to the monograph of Knut Schmidt-Nielsen "Why is animal size so important?" oxygen can be one of the main factors that limit the growth rate and the maximum size of insects [14]. In this case, the larvae of selected insects secrete CO_2 and NH_3 in volumes comparable to some agricultural animals (poultry). Previously, it was found that usage of a nutrient medium enriched with ammonia and carbon dioxide, allows a 60% increase in the rate of growth of chlorella. Thus, the maximum biomass yield of chlorella and insects will be achieved in the biotechnological cycle being created.

Scientific Challenge

For decades, the most actual problem was the deficiency of animal protein, and now this problem has become more urgent. According to the data of Institute of Nutrition RAMS, the annual deficiency of food protein in Russia exceeds 1 million tons. The protein lack is higher in the majority of the other countries. Moreover, animal products are not available to all levels of the population in many countries. New ways of receiving protein from nonconventional raw materials are developed everywhere in response to this problem. At this moment, the selection of proteinaceous plants, biotechnology of microorganisms and bioengineering are on

trend. There is a large number of organisms with genetically modified genes at the expense of which the foodstuff is enriched with protein due to the intensive development of genetic engineering of plants in the world market of agricultural production. However, harmlessness of GMO has still not been proved, as well the harm from using them is not reasonable [18].

It is expedient to stimulate the productivity growth in animal breeding because of agricultural ruminant animals' protein bioavailability. Unfortunately, the animal breeding has been in process of stagnation in Russia. The food supply is an important factor for low competitiveness of the Russian animal breeding. The production of qualitative fodder resources which has been reduced for many years, has disturbed the high efficiency of animals. Nevertheless, there have been prerequisites for the efficient development of animal breeding in Russia. The prerequisites consist of searching for the alternate production technologies of the full-fledged enriched animal feeding stuffs received from the biomass of high-yield insects and the products of their activity.

The importance of studying the mechanisms of the interactions between the biologically active agents and products, and the metabolism of the producers and consumers is of the first order with the high extent of feed converting (highly productive insects in a larval stage of development) for the creation of a system for a renewable bioproduction cycle which would become a base for the production of the functional feed additives.

Relevance

The majority of insect species have extremely high fertility, that exceeds a similar indicator for any objects of the modern animal breeding in hundred times. The question of their usage as a forage for farm animals was studied by the European Food Safety Authority (EFSA, 08.11.2015) [5,6]. The corresponding report on scratches and the prospects of insect consumption was published on the official site of the organization [http://www.efsa.europa.eu). In this report, it was concluded that the degree of possible risks from extensive cultivation of insects depended directly on the chemical agents and the fodder substrates applied at the cultivation. The scratches admitted the moderate application and not exceeding the scratches for the modern animal breeding. In this connection, it was concluded that the insects can become a rich source of complete proteins, fats, vitamins, food fibers, and other useful substances [1-4, 8-13, 25-30].

The main tendency of perfecting feeding technology and veterinary and preventive measures of the modern livestock production and poultry farming has been the development and deployment of the functional feed additives in the production process. On the assumption of the submitted report, it is possible to make a conclusion that the insect cultivation in agriculture and the development of technology for production of feed additives from insect biomass is the perspective and potentially realized direction.

Nowadays, there is a deficiency of the technologies allowing releasing the necessary range of new biologically active preparations based on cheap and available raw materials of vegetable and animal origin. In this connection, there has been a need for the development of new technologies for production of highly effective wide spectrum agents [21-25].

At the same time, the larger part of the deficiency has been occupied by the expensive foreign production and the lack of affordable domestic products.

Now, feed production based on chitin (the main substance of insect's cuticle) is promising to be applied in animals. The chitosan (deacetylated form of chitin) has the greatest biological activity among chitin derivatives. These natural polymers are highly adsorbed, immune-stimulating, antineoplastic, bacteriostatic, wound healing, and are bio-compatible to animal tissues. The antibacterial, antimicrobic, and anti-virus properties of chitosan have been used in biomedicine [7,10,22].

The solution of feeding problem in Russian Federation with ecological safety and usefulness, has been provided by the application of the modern production technology for the foodstuff of animal origin providing full feeding of animals with various bio-active materials. The application of such products can improve the eatability and digestibility of forages, regulate the metabolism, increase the resistance to

diseases, reduce chemotherapeutic medicine use, and finally allow reducing the material inputs by receiving quality production.

The objective interest in insects as the subject of the research studies, has been also proved by the phenomenon of bioconversion of various substances. For instance, some insect species, such as Zophobasmorio, are capable to convert cellulose into their biomass.

Current State of Research

The modern industrial production of the biosynthesis products represents a uniform biotechnological system which consists of consecutive stages and operations which numbers and features depend on the type of the made product.

The operated bioconversion - transformation of the components of the vegetables in the microbiological or enzymatic way in various useful substances and products can be carried out in adjustable conditions.

Now, for the bioconversion of the various microorganisms - bacteria, actinomycetes, yeast, micromycetes are generally used. Insects have been a perspective object for industrial cultivation, as they can process the most various substrata into products useful for people. However, today they are not broadly applied. Therefore, there is a need for the development of a unique technology for cultivation of each separate look. Similar technologies have been developed and realized only for several types, among which the greatest industrial value was received by bees and silkworms. The production volume made by these insects has had a considerable scale. So, annually, about 1,2 million tons of the honey produced by bees come to the market. At the same time, silkworms produce more than 90 000 tons of silk which is used in the industry, every year.

Seaweeds as well as insects are a perspective object for production. Unlike insects, for mono-celled seaweeds, there have been general regularities of cultivation which were well studied and realized in industrial scale. The most perspective view of mono-celled seaweeds for industrial production is Chlorella. However, its production has not been realized in large volumes, which has been connected with the lack of technology for processing the final biomass [18,20].

The products of bioconversion have a wide potential to be applied in food (bread, alcoholic beverages, fermented products, etc.), in feed, and also in the production of medicines, chemicals, fuel, etc. The operated bioconversion generally finds application in the food industry and agriculture. At the present stage of development in science, it is possible to allocate a number of perspective directions of applying bioconversion for fulfilling the needs of agriculture:

1. Receiving proteinaceous concentrates of food and fodder.
2. Proteinization of starch and cellulose-content raw materials.
3. Nonconventional ways of bioconversion of vegetable carbohydrates to ethanol.
4. Receiving biogas from the waste of farms and the vegetable remains.
5. Conservation of forages' products of fermentation (siloing).

CONCLUSION

Today, bioconversion has been generally carried out in one-component systems where only one species is used. Multicomponent systems with complex application of several species have been developed poorly. At the moment, the multicomponent systems have been generally developed for microorganisms. In the system of the bioconversion offered by this study for the development of the biological objects and insects, chlorella will appear. The similar combination is unique and has no analogs in the modern world. The use of similar system will allow resolving the issue of processing chlorella, and also increase the output of biomass in each of the cultivated objects. The pulsed discharge processing planned to be applied in the course of operation is this study's authoring method and has no analogs.

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Conflict of interest:
In the article, there has been no information capable of provoking conflicts of interest, with the exception of the information contained in previously published articles by Nagdalian[1], Andrey Ashotovich[1], Pushkin[1], Sergey Viktorovich[1], Lodygin Alexey Dmitrievich[1], Timchenko Lyudmila Dmitrievna[1], Rzhepakovsky[1], Igor Vladimirovich[1], Trushov Pavel Andreevich[1].

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