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[Review]

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Biotechnological Approaches in Sericultural Science and Technology of Uzbekistan

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Biotechnological researches in Central Asian Research Institute of Sericulture were developed since 1981 when laboratory of artificial diets for mulberry silkworm was created. The researches were directed not only to creation of accessible artificial diets (AD) for off-season mass rearing of economical beneficial insect and for industrialization of sericulture but also to obtaining of easy reproducible source of animal proteins, carbohydrates, lipids, enzymes, enzymes inhibitors and other metabolites - bioresource for manufacture of oriental medicine preparations. Space experiments with silkworms became possible with use of AD. These experiments used mulberry silkworm as high effective board test organism with outstanding developmental, reproductive and transgenic properties having important significance in future of cosmonautics. Introduction to practice of bioprotective and ecologically pure method of drying and sterilizing of green cocoons and by-products of silk manufacture allows to increase efficiency of cocoons reeling technology as well as to preserve nativity of biological active substances in mulberry silkworm pupae that is unachievable by traditional methods of raw material processing. New spheres of application of two important silk proteins - fibroin and sericin in medicine, cosmetics, for bio- and nanotechnology are opening with use of biotechnological approaches. At present the achievements and experience of R & D in these fields are used in creation of modern pests biocontrol agents. Schemes of efficient processing of cocoon raw material, by-products and wastes of sericulture and cocoon processing are developed for obtaining of traditional and new consumer products.

Key words: Silkworm, Nutrition, Physiology, Biochemistry, Artificial diets, Bioadditives, Space experiment, Fibroin, Sericin, Wastes, Bioconversion, Bio-and nanotechnology, Pests biocontrol

Introduction

The mulberry silkworm, one of two (including honey bee) economic beneficial insects, have been chosen by man as biotechnological object several thousand years ago because of its ability to produce a high-strength protein thread - fibroin, used for a long time for manufacture of a silk fabric. Centuries-old selection of a silkworm has strongly changed a wild variety, having transformed it in specialized biofactory of this unique biopolymer. The mulberry silkworm, conceding on development's velocity (increase of a biomass) only to bacteria, can become rather perspective biotechnological animal at the present not only in production of natural silk and biologically active substances but also as industrial transgenic insect. Use of environmentally appropriate and bioprotective methods of post harvest cocoon processing will allow both to improve its technological parameters and to make its processing wasteless, having expanded assortment of production. In a new millenium due to biotechnological approaches, proteins of cocoon shell (fibroin and sericin) will be used for obtaining of products with unique functional properties and with wide sphere of their practical use. Attempt to highlight (on the basis of own researches) results of application of biotechnological approaches in sericulture, sericultural science and technology, in silk material science and also in rational utilization of by-products and wastes of silk processing which can became useful for modern researchers are presented in this review.

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Biotechnological approaches in sericulture

Biotechnological developments have been purposefully started in Central Asian Research Institute of Sericulture since foundation of laboratory of artificial diet for silkworm by decree of State Committee of Science and Technology of the USSR in 1981. Creation of material and experimental bases for mass rearing of insects, development of AD contents with use of local raw materials and also new silkworm rearing biotechnology using developed AD formulas as well as screening silkworm races for chose of more adaptable to AD was the tasks of the laboratory that time. The first satisfactory by nutritiousness and assimilability of AD were already available in 1985 (Madyarov, 1985a, 1988a). Cocoons with average weight of 1.7 g have been obtained using these diets. This allowed to carry out their first trial. The problems of agrotechnical character which can not be solved within the framework of traditional sericulture have been revealed during this test (Madyarov, 1988b, 1989).

Large variety of potential ingredients of local production - substitutes of inaccessible protein, lipid, carbohydrate, mineral, vitamin, antibiotic, antioxidant and other biostimulating components were tested during development of optimized AD. Mulberry leaves powder, gossypolless cotton seed flour, deodorized soy flour, disintegrated chlorella and yeast, silkworm pupae defatted flour, sericin concentrates, acidic and enzymatic hydrolysates of non-traditional proteins and cotton cellulose, crude cotton oil and its fractions containing natural tocopherol have been obtained using of physical, chemical and biotechnological methods. They became accessible substitutes for protein and lipid components, starch, sugar, vitamins, minerals and other food products (Madyarov, 1985a, 1995; Madyarov *et al.,* 1986a; Tolibaev *et al.,* 1995a).

Numerous biologically active substances obtained in Institute of Sericulture and in number of Rresearch Institutes of Uzbekistan and Soviet Union - natural β-sitosterol, ecdysteroids and other insect hormones, polyphenolics, isoprenoids, alcoloids, crown ethers, polyunsaturated fatty and organic acids, phospholipids, thyroids and enzymes, deodorants-adsorbents, protein-vitamin-mineral complexes, phagostimulators, bacteriostatics, antibiotics and other biologically active substances (BAS) have been tested in content of AD in screening researches with use of silkworm larvae, (Madyarov, 1985a, 1989, 1990, 1996; Atabekova *et al.,* 1986; Khalmirzaev and Madyarov, 1986; Madyarov *et al.,* 1994). Precise and adequate physiological responses of larvae organism were marked in all these experiments finally reflected in behaviour and increase or decrease of biomass of insects, in their biological and biochemical characteristics, and also in technological parameters of cocoons, confirming practical use of silkworm as a high sensitive test-organism in screening of BAS including pesticides, toxic and poisonous compounds as well as microbial and viral pathogens.

Patents for the optimized AD containing the best from tested local nutrients and developed stimulating additives were the practical result of this work (Madyarov *et al.,* 1989a, b; Radjabov *et al.,* 1991) and also publications with the characteristics of the revealed resources of non-traditional food and fodder ingredients. Use of these ingredients is possible in fodder production not only for silkworm (Madyarov, 1990, 1995, 2005; Madyarov *et al.,* 1995a, 1997).

Development of young mulberry leaves meristem cultivation method (Ogurtsov *et al.,* 1986) was the certain achievement. It was planned to use it as an alternative diet and/or a component of AD and also as a producent of attractive and phagostimulating substances for mulberry silkworm.

Physiological and biochemical aspects of digestion at mulberry silkworm on level of digestive enzymes (proteases, carbohydrases and lipases) of intestinal juice, peritrophic membranes, intestine and also by criterion of digestibility of potential AD components (proteins, carbohydrates, lipids etc.) were also studied (Madyarov and Muminov, 1985; Madyarov *et al.,* 1986; Kocherovslaya *et al.,* 1987). Enzyme hydrolyzates of natural proteins, polysaccharides and lipids, a more assimilable and non allergic products have been obtained with the help of industrial enzymes and developed methods of immobilization for their rational use. Models of bioreactors have been created on the basis of principles of digestion in a simple mulberry silkworm digestive system (Madyarov, 1993). For the first time the phenomenon of synergism of water-soluble and immobilized enzymes is revealed (Madyarov and Mumiïnov, 1988). This phenomenon can be used in practice of pharmaceutical industry and in manufacture of biologically active additives (Madyarov and Islamova, 1989; Madyarov, 1990).

Processes of respiration and oxidative phosphorilation, taking place in mitochondrion of intestine tissue, and also respiration of eggs, silkworm germs, larvae, pupae and imago are studied in details in works (Almatov *et al.,* 1986; Madyarov *et al.,* 1993a). The respiratory and oxidative phosphorilation characteristics of mitochondrion, as it was shown in these works, is very close to similar parameters of gastrointestinal tract of homoiothermal organism, however they considerably differ in degree of a respiration/phosphorilation coupling and by number of other energetic reactions. Respiration processes of mitochondria are not equal on different parts and along the

length of intestines of mulberry silkworm, being gradually reduced by velocity from frontal to backward part and testifying about level of metabolic activity of intestinal tissue on a way of evacuation and assimilation of food nutrients (Almatov *et al.,* 1986). New hatched and young instars larvae had the highest velocity of oxygen consumption. About double decrease of energy consumption was observed at transition of larvae to the next instars.This tendency is kept up to a stage of larvae pupation, inclusive (Madyarov *et al.,* 1993a). Moreover, such periods of mulberry silkworm development as egg's incubation (embryo development) and also moulting of larvae ("sleep", sharply slowing of locomotion) are characterized by increased velocity of respiration (on 20 – 40%), testifying about high metabolic activity of insect and practically indicating on necessity of sufficient ventilation of incubators, silkworm rearing rooms and cocooneries especially in above mentioned stages of silkworm development. Furthermore, the results of such bioenergetic researches have allowed to find the effective bioregulators (bioadditives) which increase energetic potential of intestine and all organism.

Microflora of AD components and itself ADs of various methods of preparation, and also of eggs, healthy and sick larvae, air of rearing rooms and equipment were surveyed for dosed sterilization of their by autoclave and/or by antiseptics and antibiotics (Madyarov *et al.,* 1993b). It has been revealed, that before sterilization 10 fungus and 2 bacteria varieties were presented on AD components. From tested AD components growth only of *Aspergillus niger* fungus in corn starch has been found out after 30 minutes of sterilization at 0.5 atmosphere. *A. flavus* and *A. niger* is found to be pathogenic for larvae, *Mucorodae* varieties are slightly toxic and *Rhizopus nigricans* is not toxic, but weaving of new hatching larvae by gifs prevents them to move and eat normally and resulted in their death. Such fungi meet not only on components of artificial nutritious medium, but also on eggs, larvae, especially on sick and dead silkworms, in air and on equipment. This research became useful for development of pathogens control methods during rearing by artificial diets. The microflora of sick larvae and pupae is very various (more than 60 varieties of fungi) during feeding by mulberry leaves. They are saprophyte, semi-saprophyte and pathogenic varieties causing diseases both in silkworm and in mulberry (Madyarov *et al.,* 1993b).

The important questions of the process of preparation of paste like, porous and granulated consumer forms of AD for larvae of young and elder instars have been also solved (Madyarov, 1985a, 1989, 1990; Radjabov *et al.,* 1991).

Works on finding-out of the facts of unequal adaptation of various races of mulberry silkworm and the reasons of asynchronous development of their individuals on AD as well as on comparison reproductivity of races silkworm maintained on AD and mulberry leaves have been carried out (Djuraeva *et al.,* 1991, 1993; Madyarov, 1996).

For the first time space experiment with mulberry silkworm has been carried out with use of the improved AD (Madyarov, 1994; Madyarov *et al.,* 1995). Eggs and different instars larvae have been subjected to a unique complex of physical and environmental impacts of manless artificial satellite of Earth. In this experiment processes of hatching of larvae from eggs, their feeding by AD, larvae's development, and also silk production have been tested in conditions of g-load, weightlessness and pilotless flight on the Earth artificial satellite "Bion-10". Obtained experimental results have been compared with identical Earth control parameters and indexes, which had not noticeable differences excluding cocoon's weaving. Cocoon wasn't weaved although silk fiber was secreted. Despite of this mulberry silkworm can be used due to its outstanding biological characters as a board experimental animal and really biofactory for biotechnological (including gene engineering) researches and developments.

Principles of increasing of nutritiousness and assimilability of mulberry leaves and AD and also the concept of an effective feeding of insects at younger instars (Madyarov, 1996) when expenses for feeding and additives to it are not so high but the effect in end production was noticeable have been developed also. Bio-additives to natural or artificial diets (named by us as EVA, Energizer-Vitamizer-Appetizer) which stimulate fast growth and development of insects have been found for this purpose on the basis of results of physiological, biochemical and feeding experiments. Efficiency of such additives was shown in conditions of sharply continental climate of Central Asia and in tropical climate of Malaysia (Madyarov, 1990, 1996). Thus vitality is increased on 10 – 17%, weight of green cocoons - on 10 – 20%, green cocoon's shell percentage - on 2 – 3%, eggs laying ability - on 10 – 20%, cocoons reelability - on 8 – 15%, and length of thread - on 30 – 100 m. Feeding period was reduced for 1 – 3 days. Some unpublished results on bio-additive's EVA trials in conditions of tropical sericulture of Malaysia are presented in Tables 1 - 4. These trials were carried out with mulberry leaves and AD especially developed by us from local materials for tropical sericulture in 1995 - 1996 at Terengganu state's department of Malaysian Agriculture Research and Development Institute (MARDI, Kuala Terengganu) and at "Tersat" farm of "Suterasemay" silk Co. of State Economic Development Corporation, Terengganu (SEDC Terengganu, Malaysia). We tested races and hybrids of mulberry silkworm both for dry con-

Table 1. Comparison of the performance between artificial diet and mulberry leaves on different silkworm races and hybrids (MARDI, Terengganu: May, 1995)

Silkworm races*	Type of food**	Larval duration at each instar (hrs)						Larval wt. before cocooning (g)	Cocoon wt. (g)	Shell wt. (g)	RS (%)
		1	2	3	4	5	Total, days				
R_1	Artificial diet	72	62	62	76	168	22.3	3.82	2.03	0.30	14.4
	Mulberry leaves	72	48	62	68	182	22.9	4.27	1.84	0.27	15.4
R_2	Artificial diet	72	62	62	76	192	23.3	4.25	2.08	0.37	17.8
	Mulberry leaves	56	72	72	72	192	23.3	3.68	2.03	0.24	11.9
R_3	Artificial diet	72	62	62	100	144	22.3	4.19	1.93	0.25	13.5
	Mulberry leaves	56	68	62	68	168	21.8	3.87	1.97	0.31	16.0
H_1	Mulberry leaves	66	66	48	92	168	22.9	3.83	1.88	0.32	17.6
H_2	Mulberry leaves	68	58	76	82	168	23.2	4.02	1.91	0.42	18.9

*Races R_1 -Saniish 8, R_2 -Tashkent 8, R_3 -Bagdad 3; Hybrids H_1 -Tetrahybrid 3, H_2 -Ipakchi 1.
**Feeding by AD only during 1 - 3 instars, 4 - 5 instars, mulberry leaves feeding.

Table 2. Results of silkworm rearing experiment of Chul Thay variety with mulberry leaves (MARDI, Terengganu: July, 1995)

N	Method of rearing	Vitality (%)*	Average cocoon mass, (a), g	Average cocoon shell mass, (b), mg	Ratio (c) = (b)/(a) × 100%
1	Control,common method	73	2.16	447	20.68
2	Best method of MARDI	90	2.21	450	20.81
3	EVA treatment method	100	2.50	546	21.97

Effect of EVA treatment.
*Vitality of green cocoons estimated by exit of moth from cocoons.

Table 3. Results of silkworm rearing experiment of Chul Thay variety with mulberry leaves ('Tersat' farm, 'Suterasemai' Co., Terengganu: July, 1995)

N	Method of rearing	Average mass of dry cocoons, g	Reliability, %	Raw silk, %	Length of thread, m
1	Control, ML rearing	0.867	78.83	40.23	1405
2	EVA treated ML	1.003	88.44	43.15	1490

Effect of AVA treatment.

Table 4. Average weight of cocoons, cocoon's shells and shell/cocoon ratio (MARDI, Terengganu: April, 1996)

N	Variants methods of rearing	Average cocoon weight (a), g	Average cocoon shell wt.(b), g	Ratio (c) = (b)/(a) × 100%
1	Control (ML, Mulberry lives)	1.492	0.290	19.4
2	ML + EVA treatment	1.845	0.305	19.8
3*	AD of Japan	1.692	0.342	20.2
4*	AD of Malaysia + EVA	2.013	0.396	20.0
5*	AD of Malaysia	1.653	0.340	20.6

Effect of EVA preparation.
*Feeding by AD only during 1 - 3 instars, 4 - 5 instars, mulberry leaves feeding.

tinental and wet tropical climate in these works. It is shown that such breeding method with use bio-additives for local regional races of mulberry silkworm can be recommended for application at sericultural farms of south-east and other tropical countries.

Biotechnological approaches in rational cocoons processing

Fibroin and sericin are two main protein products which the silkworm synthesizes for creation of a cocoon besides

minor components - lipids cocoons reeling, pigments and minerals. Fibroin thread is easily separated from sericin in technological process keeping in a hot aqueous solution sericin and all other water soluble components. Enzymatic processing of this 'waste water' in a flow type reactor with immobilized enzymes allows to obtain hydrolysates (Kovalenko *et al.,* 1987; Madyarov, 1990) containing amino acids and peptides, and also free fatty acids, glycerin and the mineral substances possessing high nutritious properties. Study of comparative effects of different commercial proteases on fibroin and sericin can become a base for low temperature degumming of fibroin threads in reeling process (Madyarov, 1996; Madyarov *et al.,* 2001). Besides chemical and enzymatic hydrolysates of silk proteins can be obtained from waste cocoons and fibrous silk waste products of cocoons processing with the purpose of their use in dietary food and in cosmetics content (Madyarov, 1998; Madyarov *et al.,* 1999, 2001). Certainly more rational use of fibroin is achieved in its natural polymeric form rather than depolymerized. Fibroin possessing unique properties to exist in soluble, gel like and crystal like forms and to intertransform in the certain physical and chemical conditions can be possible to use for obtain huge variety of new products. So, for example, from fibroin thread it is possible to obtain microdispersed forms of silk adsorbent for immobilization of enzymes, functional proteins and various medicines on developed surface of fibroin particles. (Madyarov and Islamova, 1989; Madyarov, 1996, 2000b). On the other hand fibroin with its simplified protein structure which is remind of structure of synthetic blockpolymers, can be used as mild biopolymeric matrixes for obtaining of modern means of delivery of medicinal substances, proteins, nucleic acids, organells and even alive cells (Madyarov, 2001). The enzymes entrapped in such silk porous matrixes are capable to function in waterless organic media making easy stereospecific synthesis of medicines (Madyarov, 2001, 2002). Moreover fibroin possessing high plasticity, sanitary, biocompatible and biodegradable properties, can be used as irreplaceable biomaterial for creation and use of micro- or nanodevices mimicing of biocatalytic systems of intestinal mucous of animals or peritrophic membrane of insects, virus particles, inclusion body and also intracellular transport vesicles and many other smart functional parts of alive cells (Madyarov, 2002, 2004).

Bioprotective method of drying with use of functional ceramics will play the important role in utilization of silkworm larvae and pupae as rich sources of BAS (Madyarov, 2000a). AD, cocoons and pupae dried by this method completely kept all complex of BAS in their composition and could be used for feeding and for isolation from them high qualitative products: proteins, enzymes, enzymes inhibitors, chitin and other carbohydrates, lipids and phospholipids, etc. (Madyarov, 2005). As to chitin, mulberry silkworm (egg's shell, larvae and pupae cuticles, pupae and butterfly) can become additional source of chitin. Complex processing of silkworm pupae by wasteless, ecologically pure technology is developed.

In the conclusion it is necessary to note also, that the advanced biotechnological approaches and work experience achieved during creation of AD for artificial feeding of mulberry silkworm now are widely used in creation of baiting matrixes and baits for Turkestanic termites and other pests. Developed baits contain waste products of sericulture not only as nutrients, but also as multi functional additives. AD similarly to AD for mulberry silkworm, but more simple by nutrient's content for lesser mulberry pyralid *Glyphodes pyloalis* Wlk. (dangerous pest of mulberry plantations) as well as ADs for cotton ball worm *Helicoverpa armigera* Hbn. and some other noctuids have been developed. Various agents of the chemical or biological insects control can be include into these attractive diets (Madyarov *et al.,* 2002), that will allow to create modern means of integrated pest control for an agriculture. Recently isolated, neurotoxic components of venom gland of the parasite of many agricultural pests, *Bracon hebitor* Say not conceding on efficiency to the characterized neurotoxins of karakurts (Central Asian variety of black widow) can appear in the future as one of active agents for pest biocontrol (Madyarov *et al.,* 2003).

Thus twenty five year's experience of use of biotechnological approaches in various areas of sericulture and silk science and technology has shown the successful application of such approaches both in traditional silkworm nutrition and their cultivation on the base of AD, in rational utilization of raw silk, by-products and wastes as well as in the modern bio-and nanotechnological directions to obtain new consumer products.

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