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Characteristic of newly created F1 tetrahybrids of the silkworm *Bombyx mori* L. reared with artificial diet: Basic technological traits

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Abstract

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The aim of this paper was characteristic of newly created F_1 tetrahybrids of the silkworm *Bombyx mori* L. reared with artificial diet throughout the entire larval stage on basic technological traits, which are essential for determining the productivity of silkworms.

The study was conducted during 2018 – 2019 at the Training Experimental Station of the Sericulture section of the Faculty of Agriculture at Trakia University. Object of the study were the tetrahybrid forms of the silkworm (*Bombyx mori* L.) "Vraca63 x Line22 / Nig2 x Merefa6" (1st experimental group) and "Nig2 x Merefa6 / Vratsa63 x Line22" (2nd experimental group). The new, but already established in practice tetrahybrid "I1 x VB1 / N2 x HB2" was used for the control group. The necessary silkworm eggs for the present study were obtained and stored until the moment of their incubation by the method of Early wintering (Tsenov et al., 2009). Silkworms were reared with artificial diet containing 30% powder of dried mulberry leaf produced at Scientific Center on Sericulture – Vratsa and prepared by methods, developed by the manufacturer.

The comparative analysis of hybrids from the two experimental group show that with a greater total (837.17 m) and continuous unwinding (816.20 m) length, finesse (2.46 danier) and better unwinding (87.70%) of the thread, as well as with higher silkiness (44.98%) and yield (39.49%) of the cocoons is characterized the tetrahybrid form "Nig2 x Merefa6 / Vratsa63 x Line22". The analyzed hybrids are characterized by high mean values on the traits determining the quality of their production, which gives reason to believe that the newly created F1 hybrids have relatively high levels of technological traits when rearing throughout the larval development with artificial diet.

Keywords: silkworms; *Bombyx mori* L.; hybrids; artificial diet; cocoons; productivity; technological traits

Introduction

Sericulture is a sub-sector of agricultural production with over five thousand years of history. Till nowdays the silk produced from silkworm *Bomby mori* L. maintains to an unflagging interest of producers, processors and consumers of this products, thanks to its unique and unsurpassed hygienic and technological qualities. The technological characteristics, determined the productivity of the silkworms, are influenced by the maturity of the mulberry leaf (Homidi & Halmatov, 2005), as well as by the ingested and digested food by one silkworm (Bora et al., 1995). To a great degree, the productive characteristics of silkworms are also determined by the health of the larvae during age development. The most effective means of combating poisoning, pests and diseases is the cultivation of silkworms in sterile conditions with high-quality diet free of contaminants, including pathogenic microflora, such as artificial food. Using an artificial diet with a certain chemical composition, a lot of information has been accumulated about the needs of silkworms' growth, survival and productivity (Fukuda et al., 1960; Ito & Tanaka, 1960). The artificial diet, unlike the mulberry leaf, is not accompanied by seasonal changes in its quality (Scriber & Slansky, 1981) and is used in pathogen-free rearing systems (Sumida & Ueda, 2007).Considering that not all breeds of silkworms, as well as existing industrial hybrids are suitable for rearing with artificial foods, it is necessary to create artificial diet adapted to them by one side and by the other - suitable breeds and hybrids for rearing with such food (Horie, 1981; Nair et al., 2004; Jula & Nirmal, 2011; Nair et al., 2011). Lamberti et al. (2019) study the protein profile of silkworms (*Bombyx mori*) reared on an artificial diet compared to a mulberry diet.

They found differences in lipid transport and metabolism between experimental groups that may have a relevant effect on time and hormone secretion, which can also affect silk production. In order to improve the old and create new breeds and hybrids, it is necessary to deepen research about the factors that determine the productivity of silkworms.

The main method for increasing the yields and quality of cocoons and raw silk is hybridization, as a result of which the phenomenon heterosis, widely used in applied genetics of plants and animals, is observed. It is characterized by the superiority of the daughter generation (F1) in crossing between different species, varieties and inbred lines over the parental forms in regard to vitality, fertility, resistance to pathogens and productivity (Brigst et al., 1972), according to Ovesenska (1975). The benefits of hybridization in the field of silkworm breeding has been used earlier than in maize and has been the subject of research with over a hundred years of history (Nagaraju et al., 1996). It found practical application only in the twentieth century and for the first time in the breeding of *Bombyx mori* L. (Strunnikov & Strunnikova, 2003). According to Kanda (1992), the creation of new industrial breeds with a high receptivity to artificial diet is theoretically possible, as well as reducing the cost of artificial diets used for rearing, if the accumulated information is applied in practice in the selection process. In this regard, some authors are exploring the possibilities for mass implementation in practice of technologies for the application of artificial diet through selection methods (Kato et al., 2010; Tatemastu et al., 2012). The aim of the research is to create breeds and hybrids of silkworms with high efficiency of mulberry proteins transformation into textile fibers and therefore producing a large amount of silk, which makes growing with artificial food cheap and affordable enough. In order to increase the intensity of the industry, Shinbo & Yanagaw (1994) succeeded in implementing a system defined as the "One-week system for silkworms rearing", which allows to obtain 10 instead of 3-4 harvests per year, using cheap artificial diet, enabling their rearing until the end of the 3rd instar of larval development, without the additional investments in mulberry growing and equipment for silkworms rearing. At the same time, the biological characteristics of the species should be preserved, enabling effective mulberry leaf rearing, as well as its use as a biological model (Kaito & Sekimizu, 2007). An especially current topic in the research of a number of authors in recent years is the use of the silkworm, *Bombyx mori*, which represents a well -studied model among Lepidoptera, as a biological object in order to establish the mechanisms of autophagy during мetamorphosis processes (Romanelli et al., 2014, Romanelli et al., 2016) and for evaluating its role in postembryonic development using some morphological, biochemical, and molecular methods to monitor autophagy in silkworm organs (Montali et al., 2018).

Numerous studies have been conducted in Bulgaria to establish the effect of growing simple and complex hybrids (Yankov, 1973; Ovesenska et al., 1986; Petkov et al., 1998a; Tsenov et al., 2000; Ignatova et al., 2000a, 2000b and others.). Investigating the biological capabilities of silkworms, Petkov & Nacheva (1996) found that tetrahybrids are superior to the regionalized dihybrid. According to Petkov (1995), in 1995 the work of scientists towards the creation and use of F1 hybrid forms in *Bombyx mori* L. has reached such a scale that the entire industrial production of cocoons in countries with developed sericulture is on a hybrid basis.

Issues such as silkworms with weak vitality, low survival, low silk protein synthesis efficiency, and low silk yields in silkworms reared on artificial diets have not been resolved. Therefore, further improving the artificial diet formula and enhancing its metabolic utilization by silkworms are key issues that must be addressed (Dong et al., 2017). The aim of the present study was to establish the productive characteristics of newly created F1 hybrids of *Bombyx mori* L. reared with artificial diet throughout entire age development.

Material and Methods

The study was conducted during 2018 – 2019 at the Training Experimental Station of the Sericulture section of the Faculty of Agriculture at Trakia University. Object of the study were the tetrachybrid forms of the silkworm (*Bombyx mori* L.) "Vraca63 x Line22 / Nig2 x Merefa6" (1st experimental group) and "Nig2 x Merefa6 / Vratsa63 x Line22" (2nd experimental group). The new, but already established in practice tetrahybrid "I1 x VB1 / N2 x HB2" was used for the control group. The necessary silkworm eggs for the present study were obtained and stored until the moment of their incubation by the method of early wintering (Tsenov et al., 2009). Silkworms were reared with artificial diet containing 30% powder of dried mulberry leaf produced at Scientific Center on Sericulture – Vratsa and prepared by methods, developed by the manufacturer, whereby 250 g dry substance and 675 ml of distilled water are homogenized using a mixer. Placed in a box with a lid, the mixture is treated thermally in MW for 10' at ~800 W.

Of all breeds and hybrids object of this study, it was formed groups in 3 reps of 400 normal silkworm eggs previously disinfected with a 2% formalin solution. The rearing of the silkworms was done in specialized room prepared in advance for the purpose in established temperature -humidity conditions according to which in 1st, 2nd, and 3th instar air temperature was 28-30°C and air humidity – 80-85%, and in 4th and 5th instar – 24-26°C and 70-75%. From each repetition was formed an average sample in a volume of 30 cocoons which is dried in an electric dryer under standard conditions.

To establish the productivity of silkworms from the analyzed hybrids, the basic technological traits were controlled. The linear characteristic and shape of the cocoons were determined by individually measuring average cocoon samples with a caliper.

The cocoon index (I) was determined by the following formula:

$$I = \frac{l}{d},$$

where *l* is length of cocoon (mm) and *d* is the width of the cocoon (mm).

The weight characteristics of the cocoons, silk sheath and the obtained silk threads (skeins) – by weighing the components obtained by draining the cocoons and dried to a constant mass (frieze, skein, pellet and pupa) using a torsion balance (accuracy 0.001 g).

The silkiness (*S*) of dry cocoon was determined by the following formula:

$$S\,(\%) = \frac{MSh}{MC}\ x\ 100,$$

where *MSh* is mass of the silk sheath (mg) and *MC* - mass of the cocoon (mg).

The lengths (total and continuous unwinding) of the silk thread were determined by individually unwinding the cocoons from each sample using an apparatus "FU-30" - Hungary.

The unwinding of the silk sheath (*P*) was determined by the formula:

$$P\,(\%) = \frac{MS}{MSh}\ x\ 100,$$

where *MS* is mass of the silk skein and *MSh* - mass of the silk sheath.

The laboratory yield (R) of the raw silk was determined by the formula:

$$R\,(\%) = \frac{MS}{MC}\ x\ 100,$$

where *MS* is mass of the skein (mg) and *MC* - mass of the cocoon (mg).

To characterize the silk thread thickness, the *Denier* value is used, which was determined by the formula:

$$Denier = \frac{G}{L}\ x\ 9,$$

where *G* is the silk thread mass (mg) and *L* - length of the silk thread (m).

The obtained data were systemized and processed with the respective modules of STATISTICA software of StatSoft and Microsoft Excel 2010.

Results and Discussion

The technological characteristics of the produced cocoons and raw silk of different silkworm breeds and hybrids is of paramount importance for the efficiency of the silkworm rearing process. It can be used to judge both the quantitative and qualitative features of the obtained raw material.

Technological characteristic of cocoons

Tables 1 and 2 presents the mean values of the traits determining the linear and the weight characteristics of the cocoons and the silk sheaths from the individuals of the analyzed in the present study hybrids.

Table 1. Mean values of the traits determining the size and shape of the cocoons

Group	Hybrid	♀ individuals		♂ individuals		Total ♀ и ♂	
		n	x̄±SE	n	x̄±SE	n	x̄±SE
**Length of cocoon (mm) ** **							
Control	I1 x VB1 / N2 x HB2	16	35.88±0.44	14	34.13±0.59	30	35.06±0.39
1	Vratsa63 x Line22 / Nig 2x Merefa6	16	35.01±0.42	14	33.29±0.41	30	34.21±0.33
2	Nig2 x Merefa6 / Vratsa63 x Line22	13	34.10±0.40	17	32.63±0.34	30	33.27±0.29
Width of the cocoon (mm) **							
Control	I1 x VB1 / N2 x HB2	16	19.99±0.25	14	19.06±0.21	30	19.56±0.18
1	Vratsa63 x Line22 / Nig 2x Merefa6	16	19.01±0.21	14	18.73±0.27	30	18.88±0.17
2	Nig2 x Merefa6 / Vratsa63 x Line22	13	19.19±0.31	17	18.43±0.17	30	18.76±0.18
Index							
Control	I1 x VB1 / N2 x HB2	16	1.80±0.03	14	1.79±0.03	30	1.80±0.02
1	Vratsa63 x Line22 / Nig 2x Merefa6	16	1.84±0.03	14	1.78±0.03	30	1.81±0.02
2	Nig2 x Merefa6 / Vratsa63 x Line22	13	1.78±0.03	17	1.77±0.02	30	1.78±0.02

**p≤0.01

According to the traits determining the size of the cocoons, the tetrahybrids of the 1st and 2nd experimental groups for both sexes have lower mean values compared to those of the control, as the straight cross is on average by 0.94 mm in length and by 0.12 mm in width cocoons larger than those of the reciprocal. The information on the mean values of the cocoon index characteristic, characterizing its shape, shows that the cocoons of the straight tetrahybrid cross (1st experimental group) are more elongated on average by 0.01 and 0.03 indices compared to those of the control and 2nd experimental group. Generally females form bigger and more elongated cocoons in all three hybrids than male individuals, which is a confirmation of the generally established patterns between the sexes (Table 1).

Table 2. Mean values of the traits determining the technological qualities of the cocoons

Group	Hybrid	♀ individuals		♂ individuals		Total ♀ и ♂ individuals	
		n	x̄±SE	n	x̄±SE	n	x̄±SE
Mass of dry cocoon (mg)							
Contro 1	I1 x VB1 / N2 x HB2	16	682.94±21.83	14	546.78±25.63	30	619.40±20.69
1	Vratsa63 x Line22 / Nig 2x Merefa6	16	664.62±20.76	14	525.78±26.06	30	599.83±20.65
2	Nig2 x Merefa6 / Vratsa63 x Line22	13	652.00±22.43	17	522.76±17.02	30	578.77±17.95
Mass of silk sheath (mg)							
Contro 1	I1 x VB1 / N2 x HB2	16	289.31±9.72	14	238.50±8.81	30	265.60±8.03
1	Vratsa63 x Line22 / Nig 2x Merefa6	16	283.00±8.75	14	243.07±12.02	30	264.37±8.06
2	Nig2 x Merefa6 / Vratsa63 x Line22	13	288.38±8.46	17	236.47±7.22	30	258.97±7.21
Unwinding of silk sheath (%)							
Contro 1	I1 x VB1 / N2 x HB2	16	87.59±1.22	14	87.47±1.49	30	87.53±0.94
1	Vratsa63 x Line22 / Nig 2x Merefa6	16	87.82±1.22	14	85.93±1.46	30	86.94±0.94
2	Nig2 x Merefa6 / Vratsa63 x Line22	13	89.69±1.16	17	86.18±1.74	30	87.70±1.14
The silkiness of dry cocoon (%)							
Contro 1	I1 x VB1 / N2 x HB2	16	42.36±0.56	14	44.12±1.29	30	43.20±0.68
1	Vratsa63 x Line22 / Nig 2x Merefa6	16	42.64±0.60	14	46.32±0.93	30	44.35±0.63
2	Nig2 x Merefa6 / Vratsa63 x Line22	13	44.44±0.93	17	45.40±0.77	30	44.98±0.59
Laboratory yield of rew silk (%)							
Contro 1	I1 x VB1 / N2 x HB2	16	36.83±0.80	14	38.60±1.31	30	37.66±0.75
1	Vratsa63 x Line22 / Nig 2x Merefa6	16	37.49±0.88	14	39.84±1.15	30	38.59±0.73
2	Nig2 x Merefa6 / Vratsa63 x Line22	13	39.22±0.85	17	39.24±1.22	30	39.49±0.77

With regard to the traits forming the technological qualities of the cocoons presented on Table 2 the control group has the highest average weight in total for both sexes, both for the cocoon, whose average weight is 19.57 and 40.63 mg higher and for the silk sheath, which has a 1.23 and 6.63 mg higher average weight than those of the 1st and 2nd experimental groups, respectively. The comparative characteristics of the two experimental groups show that the highest average values of the silkiness, an important technological and selection feature, has the reciprocal tetrahybrid form (Nig2 x Merefa6 / Vratsa63 x Line22), in whose cocoons have 0.63% higher relative share of silk compared to the straight cross, and compared to the control – by 1.78%. Kumar et al. (2011) also reported higher values for some features in reciprocal variants in hybridization.The data in Table 2 also show that in regard to the trait, considered as one of the main technological features of cocoons, influencing the economic efficiency of the silk process, namely laboratory yield of raw silk, in total for both sexes with the highest average value (39.49%) is distinguished again tetrahybrid "Nig2 x Merefa6 / Vratsa63 x Line22", which is 0.9% and 1.83% higher laboratory yield of raw silk compared to the tetrahybrid form "Vratsa63 x Line22 / Nig2 x Merefa6" and the control "I1 x VB1 / H2 x HB2", respectively. The reciprocal cross (2nd experimental group) is also characterized by the highest average values (Table 2), although with slight differences (0.17 and 0.76%), and therefore with the best unwinding of the silk sheath (87.70%).

The comparative characteristic of the two sexes in all analyzed cases is in confirmation of the generally established biological regularity between the sexes, according to which females twist heavier cocoons with better unwinding ability and form silk sheaths with a larger average mass than males (Table 2).

While in terms of laboratory yield of raw silk and silkiness, males are characterized by more net production and form cocoons with a higher relative proportion of silk than females (Table 2).

Table 3. Mean values of the traits determining the technological qualities of the silk thread

Group	Hybrid	♀ individuals		♂ individuals		Total ♀ и ♂ individuals	
		n	x̄±SE	n	x̄±SE	n	x̄±SE
Mass of the silk thread (mg)							
Control	I1 x VB1 / N2 x HB2	16	252.44±10.89	14	209.21±9.57	30	232.27±8.24
1	Vratsa63 x Line22 / Nig 2x Merefa6	16	248.69±8.72	14	210.21±12.57	30	230.73±8.17
2	Nig2 x Merefa6 / Vratsa63 x Line22	13	259.30±9.60	17	203.94±7.94	30	227.93±7.89
Total length of the silk thread (m)							
Control	I1 x VB1 / N2 x HB2	16	889.31±35.17	14	816.93±30.94	30	855.53±24.22
1	Vratsa63 x Line22 / Nig 2x Merefa6	16	830.00±38.87	14	782.28±25.22	30	807.73±23.86
2	Nig2 x Merefa6 / Vratsa63 x Line22	13	875.61±36.05	17	807.76±30.03	30	837.17±23.53
Continuous unwinding length of the silk thread (м)*							
Control	I1 x VB1 / N2 x HB2	16	676.12±89.02	14	670.50±70.59	30	673.50±56.82
1	Vratsa63 x Line22 / Nig 2x Merefa6	16	821.56±40.64	14	725.86±54.38	30	776.90±33.96
2	Nig2 x Merefa6 / Vratsa63 x Line22	13	827.23±46.06	17	807.76±30.03	30	816.20±25.80
Silk thread thickness (Denier)[1]							
Control	I1 x VB1 / N2 x HB2	16	2.55±0.06	14	2.31±0.07	30	2.44±0.05
1	Vratsa63 x Line22 / Nig 2x Merefa6	16	2.76±0.14	14	2.41±0.12	30	2.61±0.10
2	Nig2 x Merefa6 / Vratsa63 x Line22	13	2.68±0.07	17	2.28±0.06	30	2.46±0.06

Technological characteristic of the silk thread

The assessment of the traits (Table 3) characterizing the technological qualities of the silk thread shows that the 1st experimental group for both sexes has 2.8 mg more average weight of the silk thread than that of the 2nd experimental group, while for in regard to the total length of the silk thread, the 2nd experimental group demonstrated superiority by 29.44 m compared to the 1st. On both traits, the experimental groups (1st and 2nd) are characterized by lower mean values than those of the control, respectively by 1.54 and 4.34 mg for the silk thread mass and by 47.8 and 18.36 m for the total length of the silk thread. The experimental groups (1st and 2nd) are characterized by lower mean values of the traits mass (by 1.54 mg and 4.34 mg) and total length (by 47.8 and 18.36 m) of the silk thread compared to the control. The tetrahybrid "Nig2 x Merefa6 / Vratsa63 x Line22" demonstrates the largest continuous unwinding length of the silk thread, significantly surpassing the control and the 1st experimental group with 142.7 and 39.30 m. The analyzed hybrids are characterized by mean values (673.50-816.20) within the limits indicated by Bai et al. (2001b), according to which the continuous unwinding length of the silk thread varies from 500 to 1000 .

The backcross tetrahybrid form has 0.15 denier thinner silk thread than a straight form which in turn has the highest average value (2.61 days) and respectively the coarsest thread. The higher average values of continuous unwinding length and thickness of the silk thread obtained from us (Table 3) in female individuals compared to males are one-way with the established by Iizuka et al. (1996a, 1996b).

Conclusions

The comparative analysis of hybrids from the two experimental group show that with a greater total (837.17 m) and continuous unwinding (816.20 m) length, finesse (2.46 danier) and better unwinding (87.70%) of the thread, as well as with higher silkiness (44.98%) and yield (39.49%) of the cocoons is characterized the tetrahybrid form "Nig2 x Merefa6 / Vratsa63 x Line22 ".

The analyzed hybrids are characterized by high mean values on the traits determining the quality of their production, which gives reason to believe that the newly created F1 hybrids have relatively high level of technological feature when rearing throughout the larval development with artificial diet.

References

Bai, L., Wang, J. & Zhou, Z. (2001б). On the division of distribution of non-broken filament length of cocoon, *J. Seric. Sci. Jpn., 70 (1),* 23-29.

Bora, B., Mahadevmurthy, T. S., Kumar, G. R. & Magdum, S. B. (1995). Studies on effect of feed quantum during fifth instar of the silkworm *Bombyx mori* L. on silk fiber. *Bull. Sericult. Res. (6),* 13-19.

Dong, H., Zhang, S. & Tao, H. (2017). Metabolomics differences between silkworms (*Bombyx mori*) reared on fresh mulberry (*Morus*) leaves or artificial diets. *Sci Rep 7,* 10972. https://doi.org/10.1038/s41598-017-11592-4

Fukuda, T, Suto, M. & Higuchi, Y. (1960). Silkworm raising on the artificial food. *J. Seric. Sci. Jpn., 29,* 1-3.

Homidi, H. & Halmatov, D. I. (2005). Application of Phisiological and biochemical criterions for forecasting of economically useful characters of mulberry and establishment optimum terms of realization of heeding of the silkworm larvae. *Int. Workshop on Revival and Promotion of sericultural Industries and small Enterprise Development in the Black, Caspian Seas and Central Asia Region Tashkent,* Uzbekistan, 11-15 April, 335-354.

Horie, Y. (1981). Development of artificial diet for the silkworm *Bombyx mori* and its practical application. *Plant protect, 35 (5),* 14-21.

Ignatova, L., Petkov, N. & Nacheva, J. (2000a). Influence of industrial hybridization methods and schemes on heterosis, depression and degrees of dominance in interbreed hybrids of the silkworm (*Bombyx mori* L.). II. Weight of cocoon and silk sheath. *Animal Sciences 1*, 48-58 (Bg).

Ignatova, L., Petkov, N. & Nacheva, J. (2000b). Influence of industrial hybridization methods and schemes on heterosis, depression and degrees of dominance in interbreed hybrids of the silkworm (*Bombyx mori* L.). IV. Number of normal eggs in one footnote. *Animal Sciences 2*, 74-79 (Bg).

Ito, T. & Tanaka, M. (1960). Rearing of the silkworm on an artificial diet and the segregation silkworm of pentamolters, *J. Seric. Sci. Jpn. 29*, 191-196.

Jula, S. N. & Nirmal, K. S. (2011). Development of bivoltine pure strains of silkworm, *Bombyx mori* L. to rear exclusively on artificial diet during young instar, *Abstracts of Golden Jubilee National Conference on Sericulture Innovations: Before and Beyond, CSRTI, Mysore,* 28-29th January 2011, pp. 69.

Kaito, C. & Sekimizu, K. (2007). A silkworm model of pathogenic bacterial infection. *Drug Discoveries & Therapeutics, 1 (2),* 89-93.

Kanda, T. (1992). Genetical study of feeding habits of the silkworm, *Bombyx mori*, to new lowcost diet designet by linear programming method and application to the breeding. *Bull. Natl. Seric. Entomol. Sci. 5,* 1-89.

Kato, T., Kajikawa, M., Maenaka, K. & Park, E. Y. (2010). Silkworm expression system as a platform technology in life science. *Applied Microbiology and Biotechnology, 85 (3),* 459-470.

Kumar, S. N., Murthy, D. P. & Moorthy, S. M. (2011). Analysis of heterosis over environments in silkworm (*Bombyx mori* L.). *Journal of Agricultural and Biological Science, Vol. 6 (3),* 39-47.

Lamberti, C., Gai, F., Cirrincione, S., Giribaldi, M., Purrotti, M., Manfredi, M., Marengo, E., Sicuro, B., Saviane, A., Cappellozza, S., Giuffrida, M.G. & Cavallarin, L. (2019). Investigation of the protein profile of silkworm (*Bombyx mori*) pupae reared on a well-calibrated artificial diet compared to mulberry leaf diet. *PeerJ 7,* 1-22. DOI 10.7717/ peerj.6723

Montali, A., Casartelli, M., Bruno, D., Grimaldi, A. & Tettamanti, G. (2018). Methods for monitoring autophagy in silkworm organs. In: Turksen K. (eds) Autophagy in Differentiation and Tissue Maintenance. *Methods in Molecular Biology, Vol 1854,* 159-174. https://

Nair, J. & Kumar, S. (2004). Artificial diet for silkworm, *Bombyx mori* L. - A retrospection throught the decades. *Indian Journal of Sericulture (43),* 1-17.

Nair, J. S., Nirmal, K. & Nair, K. (2011). Development of bivoltine pure strains of silkworm, *Bombyx mori* L. to rear exclusively on artificial diet during young instar. *Journal of Biological Sciences, 11 (6),* 423-427.

Ovesenska, L. G. (1975). Results of crossbreeding of populations of *Bombyx mori* L. in different degrees of homozygosity in a two-way team, Dis. DSN, Sofia, 219 (Ru).

Ovesenska, L. G. (1986). Study of the possibility of using laser and gamma radiation as stimulatory factors in the silkworm (*B. mori* L.), Habil. labor, St. Zagora*,* 305.

Petkov, N. (1995). Selection genetic investigation and results from the silkworm (*Bombyx mori* L.) races, linesand hybrid breedyng. Thesis degree Doctor of the Agricultural Science, *Agricultural Academy, Sofia,* 305 p. (Bg).

Petkov, N. & Nacheva, J. (1996). Studies on the economic characteristics of complex (tetra) hybrids of the silkworm (*Bombyx mori* L.). *Animal Sciences 4,* 63-64 (Bg).

Petkov, N., Nacheva, J. P. & Tsenov, P. I. (1998a). Characteristics of the tetrahybrid of the silkworm (*Bombyx mori* L.) Vratsa 35 for spring industrial feeds. *Animal Sciences, Prl.,* 110-112 (Bg).

Romanelli, D., Casati, B., Franzetti, E. & Tettamanti, G. (2014). A Molecular View of Autophagy in Lepidoptera. *BioMed Research International, vol. 2014,* 1-11, ID 902315. https://doi.org/10.1155/2014/902315

Romanelli, D., Casartelli, M., Cappellozza, S., De Eguileor, M. & Tettamanti, G. (2016). Roles and regulation of autophagy and apoptosis in the remodelling of the lepidopteran midgut epithelium during metamorphosis. *Scientific Reports, 6,* 1-15. DOI: 10.1038/srep32939

Scriber, J. M. & Slansky, F. J. (1981). The nutritional ecology of immature insects. *Ann. Rev. Entomol. 26,* 183-211.

Shinbo & Yanagaw (1994). Low-cost artificial diets for poliphagous silkworm. JARQ 28, 262-267.

Strunnikov, V. A. & Strunnikova, L. V. (2003). Heterosis can be fixed in the generation, *Nature 1*, 3-7.

Sumida, M. & Ueda, H. (2007). Dietary sucrose suppresses midgut sucrase activity in germfree, fifth instar larvae of the silkworm, *Bombyx mori*. *Journal of Insect Biotechnology and Sericology 37,* 31-37.

Tatemastu, K., Sezutsu, H. & Tamura, T. (2012). Utilization of transgenic silkworms for recombinant protein production. *Journal of Biotechnology & Biomaterials (S9:004)*, 1-8. http://dx.doi.org/10.4172/2155-952X.S9-004

Tsenov, P., Avramova, K. & Grekov, D. (2009). Study of possibilities for accelerated wintering of the eggs of the silkworms *Bombyx mori* L. and production of silkworm eggs suitable for hatching during the period from December to April. *Animal Sciences 6*, 36-39 (Bg).

Tsenov, P., Petkov, N. & Nacheva, J. (2000). Studies on heterosis and its components in hybrids of the silkworm (*Bombyx mori* L.) with the participation of the tropical breed Bonde 517. I. Traits characterizing the utilization of the mulberry leaf (ECS). *Zhiv. Science 2,* 62-73.

Yankov, A. (1973). Selection and methods of creating some highly productive white cocoon breeds and hybrids (polyhybrids) of the silkworm. *75 years OSB-Vratsa,* BAS, 55-66 (Bg).

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