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Productivity of newly created F_1 tetrahybrids of the silkworm *Bombyx mori* L. reared with artificial diet low in mulberry powder

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Abstract

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Establishing the productivity of newly created F_1 tetrahybrids of the silkworm *Bombyx mori* L. when they are reared with artificial diet low in mulberry powder (15%) throughout the entire larval stage was the aim of this paper.

The study was conducted during 2020 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 × Line22/Nig2 × Merefa6" (1st experimental group) and "Nig2 × Merefa6/Vratsa63 × Line22" (2nd experimental group). Tetrahybrid "I1 x VB1/N2 × HB2" was used for the control group. Silkworms were reared with artificial diet containing 15% powder of dried mulberry leaf produced at Scientific Center on Sericulture – Vratsa and prepared by methods, developed by the manufacturer. From each of the analyzed hybrids, a dried average sample of cocoons was formed. The cocoons were assessed on the basic technological traits determining the productivity of the silkworms.

The results obtained showed that the studied tetrahybrids are relatively equivalent in quality of the obtained production such as the straight tetrahybrid form (1st experimental group), although insignificantly, superior the back cross tetrahybrid (2nd experimental group) in mass of cocoons, silk sheath and silk thread by 12.83 mg, 7.9 mg and 6.1 mg, respectively. The newly created F_1 hybrids do not deviate significantly in average values in all analyzed cases from the control, which gives reason to believe that they can be successfully reared throughout the larval development with artificial diet low content mulberry powder (15%). Therefore, they could be offered for testing by IASAS and approved as the first hybrids in Bulgarian sericulture, susceptible for rearing with artificial diet.

Keywords: Bombyx mori L.; hybrids; artificial diet; low mulberry content; cocoons; productivity

Introduction

The artificial diets for insects provides great opportunities for conducting precise research in a wide range of areas, incl. those of general biological significance and application. Research on the possibilities of rearing insects in controlled conditions and the use of artificial diets begun in the early 20th century and quickly gained popularity. Artificial diets can be used in the future in the field of biotechnology in the production of high quality proteins, lipids, carbohydrates, biologically active substances, as well as for the needs of alternative medicine (Madyarov & Khamraev, 2013). The rearing with artificial diets of economically important insect species, such as *Bombyx mori* L. and *Apis mellifera* L., is of great interest to science and practice in terms of increasing insect productivity (Dingle et al., 2005). Xu and Brochta (2016) call silkworm larvae "living bioreactors" that, after appropriate genetic modification, can produce valuable proteins and silk biomaterials. Scientists founds a high potential of the species as a suitable object for the study of various dosage forms, as well as therapeutic effects against drug addiction (Anantaworasakul et al., 2013).

Along with the growing range of non-agricultural application of silkworm products, research continues about the possibilities for the development of its main direction, namely the industrial yield of cocoons and raw silk, the efficiency of which is determined by basic quantitative and qualitative characteristics of the obtained products. Increasing the values of biological traits, along with those of technological traits remain the main goal of selection programs (Dong et al., 2017).

According to Etebari et al. (2004) most insect species have similar nutritional needs. However, the silkworm has many different nutritional requirements, which are almost entirely covered by the composition of mulberry leaves (Petkov, 1995; Vlaic et al., 2004). Dingle et al. (2005) claimed that most artificial diets for silkworms have a similar composition, containing about 25% dried mulberry leaf powder. According to Shinbo & Yanagaw (1994), this percentage of mulberry powder (25%) is high, increases the cost of artificial diet and makes it practically inapplicable in the industrial cultivation of silkworms. Therefore, an important condition to achieve a change in this direction is to reduce the prime cost of artificial diet, currently amounting to about 35–50% of the total costs. Tsenov (2012 a, b, c) conducted a number of studies on the partial or complete replacement of some ingredients in the silkworm diet in order to obtain cheaper artificial diet. On the other hand, it is necessary to create breeds and hybrids of silkworms with high efficiency of assimilation and conversion of mulberry proteins into silk proteins and therefore producing a large amount of quality silk (Kato et al., 2010; Tatemastu et al., 2012). According to Lamberti et al. (2019) the metabolism of silkworms, *Bombyx mori* L., rearing with artificial diet differs from those rearing with mulberry leaves, which can affect silk production. It was found that not all breeds of silkworms are receptive to artificially prepared foods, an an even smaller percentage of those are receptive to diets with reduced (15%) mulberry powder content (Guncheva et al., 2015, 2016). In confirmation of this, according to a number of authors, it is necessary to create suitable breeds and hybrids for rearing with such food (Horie, 1981; Nair & Kumar, 2004; Nair et al., 2011; Jula & Nirmal, 2011). Applying a purposeful strict selection of breeds, receptive to artificial diets and their use to create hybrids can lead to improve both susceptibility to artificial diets (Nair et al., 2013) and the productivity of silkworms (Singh et al. al., 2012; Saviane et al., 2014).

A review of the literature on the possibilities for the introduction of artificially prepared foods in the industrial rearing of silkworms raises a number of questions to science and practice, mainly related to reducing the prime cost of artificial diet and the creation of forms adapted to rearing with such a mixture. The search for decisions in this direction is the aim of the present study.

Material and Methods

The study was conducted during 2020 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 × Line22/ Nig2 × Merefa6” (1st experimental group) and “Nig2 × Merefa6/Vratsa63 × Line22” (2nd experimental group). The tetrahybrid “I1 × VB1/N2 × HB2” was used for the control group. Silkworms were reared with low cost artificial diet containing 15% powder of dried mulberry leaf produced at Scientific Center on Sericulture – Vratsa. The diet was 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 all repetitions of the respective hybrid, was formed an average sample in a volume of about 30 cocoons, which was 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:

$$I = l/ d,$$

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 (%) = MSh/ MC × 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 (%) = MS/ MSh × 100,

where MS is mass of the silk skein (mg) and MSh – mass of the silk sheath (mg).

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

R (%) = MS/ MC × 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 = G/ L × 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 quantitative and qualitative characteristics of the raw materials obtained from sericulture are leading indicators of the efficiency of silkworm rearing, which is also of paramount economic importance, especially when using diet with a lower prime cost, as is the case.

Technological characteristic of cocoons

Figure 1 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.

The assessment of the traits determining the linear (Figure 1a, b, c) and weight characteristics (Table 1) of the cocoons and silk sheaths of the individuals of the hybrids analyzed in the present study, shows that the size – length and width of the cocoon (mm), the shape (Index) and technological qualities of the cocoons, obtained from the experimental groups did not differ significantly, both from each other and from the control group. The control has only with 24.74 mg and 37.57 mg heavier cocoons and 2.74 mg and 10.64 mg higher average mass of silk thread, compared to cocoons of the 1st and 2nd experimental groups, respectively. The lower average values of the qualitative technological trait "Unwinding of the silk sheath", observed in the control, although less than 1% compared to the experimental groups, are followed by the lower average values of the signs silkiness and laboratory yield, which are also insignificant (Table 1).

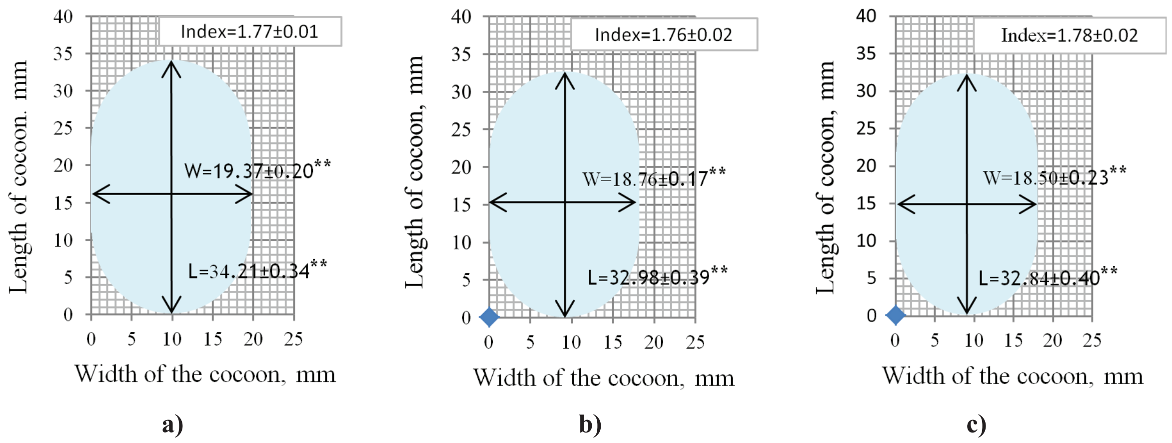

Fig. 1. Mean values of the traits, determined the size and shape of the cocoons:
a) Control group (I1 × VB1/ N2 × HB2); b) First experimental group (Vratsa63 × Line22/ Nig 2× Merefa6);
c) Second experimental group (Nig2 × Merefa6/ Vratsa63 × Line22)
**$p \leq 0.01$

Table 1. Mean values of the traits determined the technological qualities of the cocoons

Group	♀ individuals		♂ individuals		Total ♀ and ♂ individuals	
	n	x̄ ± SE	n	x̄ ± SE	n	x̄ ± SE
Mass of dry cocoon (mg)						
Control	15	655.53±25.36	15	531.67±27.65	30	593.60±21.73
1	15	612.07±18.88	14	522.57±15.54	29	568.86±14.76
2	13	599.00±32.03	17	523.06±23.74	30	556.03±20.23
Mass of silk sheath (mg)						
Control	15	278.13±12.76	15	239.20±12.10	30	258.67±9.37
1	15	270.47±8.92	14	240.36±7.91	29	255.93±6.53
2	13	260.31±14.58	17	238.65±9.52	30	248.03±8.39
Unwinding of silk sheath (%)						
Control	15	84.21±2.76	15	86.43±0.79	30	85.32±1.43
1	15	85.41±2.22	14	86.81±2.13	29	86.09±1.52
2	13	86.96±1.16	17	85.30±1.11	30	86.02±0.81
The silkiness of dry cocoon (%)						
Control	15	42.30±0.70	15	45.15±1.09	30	43.72±0.69
1	15	44.22±0.66	14	46.06±0.99	29	45.11±0.60
2	13	43.45±0.47	17	45.87±0.68	30	44.82±0.48
Laboratory yield of rew silk (%)						
Control	15	35.66±1.37	15	39.02±1.01	30	37.34±0.89
1	15	37.81±1.21	14	39.82±0.90	29	38.78±0.77
2	13	37.77±0.57	17	39.11±0.71	30	38.53±0.48

Table 2. Mean values of the traits determined the technological qualities of the silk thread

Group	♀ individuals		♂ individuals		Total ♀ and ♂ individuals	
	n	x̄ ± SE	n	x̄ ± SE	n	x̄ ± SE
Mass of the silk thread (mg)						
Control	15	234.00±13.23	15	207.13±11.30	30	220.57±8.90
1	15	231.07±10.04	14	208.28±8.21	29	220.07±6.77
2	13	227.23±13.98	17	203.82±8.97	30	213.97±8.05
Total length of the silk thread (m)						
Control	15	873.53±41.01	15	822.60±32.11	30	848.07±26.02
1	15	819.40±40.95	14	790.93±34.10	29	805.65±26.49
2	13	858.46±42.16	17	769.53±30.99	30	808.07±26.19
Continuous unwinding length of the silk thread (м)*						
Control	15	750.47±78.57	15	785.60±58.51	30	768.03±48.24
1	15	754.00±65.89	14	678.00±65.60	29	717.31±46.24
2	13	802.00±69.08	17	707.12±49.14	30	748.23±41.07
Silk thread thickness (Denier)[1]						
Control	15	2.40±0.07	15	2.26±0.08	30	2.33±0.05
1	15	2.57±0.07	14	2.39±0.08	29	2.48±0.06
2	13	2.38±0.08	17	2.41±0.10	30	2.39±0.06

* $p \leq 0.05$

[1] A higher value of the trait is equivalent to a thicker and therefore coarser thread

Technological characteristic of the silk thread

Table 2 presents the mean values of the traits determining the quantitative and qualitative characteristics of the silk thread.

The experimental groups (1st and 2nd) are characterized by not considerably lower average values of the total length (by 42.42 m and 40 m) and a continuous unwinding length of the silk thread (by 50.72 m and 19, 8 m), as well as with a slightly coarser silk thread (with 0.15 and 0.06 deniers) compared to the control (Table 2). However, with regard to the average duration of the continuous unwinding of the silk thread, they have values within the generally established limits in practice (from 600 to 1500 m), which is confirmed by the results obtained by Bai et al. (2001). The assessment of the traits, characterizing the technological qualities of the silk thread shows also that the backcross tetrahybrid "Nig2 × Merefa6 / Vratsa63 × Line22" (2nd experimental group) demonstrated by 6.1 mg and 6.6 mg the lower average silk thread mass compared to the 1st experimental group "Vraca63 × Line22 / Nig2 × Merefa6" and the control "I1 × VB1 / N2 × HB2", respectively (Table 2). The superiority of straight over the reciprocal tetrahybrid form, although insignificant, can be explained by the differences in the biological potential of maternal and paternal dihybrid forms in regard to the receptivity to artificial diet. In confirmed of this is the established by Guncheva et al. (2016) better receptivity to artificial diet containing 15% dried mulberry leaf powder of the dihybrid "Vratsa63 × Line22" which is mother form in the newly created straight crossbred, respectively, compared to that of the dihybrid "Nig2 × Merefa6".

The comparative characteristics of the two sexes confirm the generally established biological regularity between the sexes, according to which females have higher values than males. According to the method for determining the thickness of the silk thread (Denier), higher values of this trait in female individuals indicate that they have 0.14 deniers in the control and 0.18 deniers in the 1st experimental group thicker and a coarser thread compared to males, which is consistent with that found by Iizuka et al. (1996). An exception of that regularity between the sexes are the mean values of the continuous unwinding length of the silk thread in the control, where the males superior the females by 35.13 m and the thickness of the silk thread in individuals from the 2nd experimental group, which have almost equal values between the sexes but the male individuals, although only with 0.03 deniers, have a thicker silk thread than the females. That can be explained by the manifestation of individual features of the species.

Conclusions

The results obtained showed that the studied tetrahybrids are relatively equivalent in quality of the obtained production such as the straight tetrahybrid form, although insignificantly, superior the back cross in mass of cocoons, silk sheath and silk thread, by 12.83 mg, 7.9 mg and 6.1 mg respectively and do not deviate significantly in all analyzed cases from the control. That gives reason to believe that they can be successfully reared throughout the larval development with artificial diet (15%). Therefore, they could be offered for testing by IASAS and approved as the first hybrids in Bulgarian sericulture, susceptible for rearing with artificial diet.

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