Scientific Papers. Series D. Animal Science. Vol. LXV, No. 1, 2022
ISSN 2285-5750; ISSN CD-ROM 2285-5769; ISSN Online 2393-2260; ISSN-L 2285-5750

STUDIES CONCERNING THE EFFECT OF THE INBREEDING ON THE VIABILITY OF LARVAE AND LIVE PUPAE PERCENTAGE (*BOMBYX MORI* L.)

Georgeta DINIȚĂ[1], **Marius Gheorghe DOLIȘ**[2], **Minodora TUDORACHE**[1],
Ioan CUSTURĂ[1], **Ionela DOBRIN**[1]

[1]University of Agronomic Sciences and Veterinary Medicine of Bucharest,
59 Marasti Blvd, District 1, Bucharest, Romania
[2]University of Life Sciences “Ion Ionescu de la Brad“ Iasi, 3 Mihail Sadoveanu Alley, Iasi, Romania

Corresponding author email: georgetadinita@yahoo.com

Abstract

The silkworm amelioration program based on the use of the genetic resources and the inbreeding methods applicable in the case of Bombyx mori L. species targets mainly the creation of races and hybrids with superior traits. The improvement process by inbreeding is practiced in the creation of new populations and it requires the use in crossings of the inbred lines that meet the most valuable biological and productive characteristics, with a high combinative value. The possibility of rearing more silkworm generations per year, the physiological particularities and the variability of species characters constituted the base of the formation of silkworm races, lines and hybrids with high productive capacity.

Key words: *inbred lines, silkworm, viability of larvae, live pupae percentage.*

INTRODUCTION

The genetic amelioration of silkworms constitutes a complex driven by systems and methods of improvement of the hereditary base, of the races productive potential and of creation of new silkworm races and hybrids (Doreswamy & Gopal, 2012). In the process of silkworm amelioration is practiced the inbreeding which constitutes the mating system of related individuals belonging to the same race (Goldsmith et al., 2005). By inbreeding, phenotypic and especially genotypic changes take place, the main effect of inbreeding being the reducing of heterozygous genotypes frequency and the increasing of the homozygous genotypes frequency (Buhroo et al., 2016). The raising of the homozygosity degree has favorable effects materialized in fixing and amplifying some characters, as well as in increasing the hereditary transmission capacity of breeders. In case of silkworms the degree of kinship is very high because the mating system of the type brother x sister is most often practiced (Braslă & Matei, 1992; Haniffa & Thatheyus, 1992; Jamuna & Subramanya, 2012). In silkworm the obtaining of differentiated lines in terms of gene frequencies is easy to achieve due to the relatively small generation interval and the large number of offspring (Kumari & Tripathi, 2017). The species also allows the selection of lines according to the general and special combinative capacity, thus retaining those lines that present the highest values of the characters, but also those that are able to provide the best combinations with other lines, being known that not every inbred line in combination with another provides the same phenotypic effect (Kuzmanov & Petkov, 2000a). The selection of lines on the basis of their combinative capacity followed by the elaboration of various schemes of their crossing, in which each line is used as maternal and paternal form, results in the maximum capitalization of the heterosis phenomenon (Kuzmanov & Petkov, 2000b).

The amelioration works meant to lead to the creation of silkworm races and hybrids have in view the achievement of some forms superior to those existents, in terms of one or more biological, technological or economic characters (Bindroo & Moorthy, 2014). The researches on the practice of the amelioration process by inbreeding associated with the works of selection in silkworms, mention the intensification of some technological characters as the silk content of the cocoons and the filament length, but also the technological characters that refers to all the stages of the evolutionary cycle, these referring to embryos viability, materialized in the hatching energy and hatching percentage, viability of larvae, percentage of their transformation in pupae, moth viability and vigor (Diniță et al., 2019; Petkov et al., 1998; Ruiz & Almanza, 2018). As a result of the application of inbreeding a greater number of generations, the negative effects of this process appear, known as inbreeding depression, which in silkworms is manifested by a decrease in the hatching percentage, viability of larvae and prolificacy (Petkov et al., 1999; Nematollahian, 2010). However, the inbreeding depression doesn't affect the qualitative features such as length, finesse, elasticity and silk filament length (Nagaraju, 2002).

The purpose of the researches was on the one hand theoretical, contributing to the study of the effects on inbreeding on phenotypic and genotypic parameters, as well as a practical one, consisting in the use of inbred lines in the hybridization process, whose end result is to obtain commercial hybrids.

MATERIALS AND METHODS

In the experimental researches on inbreeding two line-founding races were used, both native, Alb Băneasa and Băneasa 75, respectively. The incubation process started with a number of 25 laying (lines) for each race. The silkworms rearing took place in the spring-summer season, using hibernating or non-hibernating eggs as appropriate.

In order to establish the inbred lines, families were structured within each race, practicing pairings of brother x sister type for six generations, the work stages being: lines extracting and their inbreeding a variable number of generations, until the fixation of the characters pursued in amelioration; the testing of general combinative capacity of the inbred lines by crossings with the tester race; the testing of specific combinative capacity of the inbred lines; the testing of general combinative capacity of the inbred lines by crossings with the tester race; the testing of specific combinative capacity of the inbred lines; the study of heterosis effect in hybrids obtained from inbred lines crossing.

In order to decrease the effects of the inbreeding depression was initially applied the inter-family (linear) selection based on the predefined performance criteria, continuing with the individual selection based with the performance of each family.

The inbreeding/generation coefficient was calculated according to Wright's formula:

$$F_x = (1/2)^{n_1 + n_2 + 1} \ (1 + F_A)$$

in which:

F_x – the inbreeding coefficient of the individual X;

n_1, n_2 – the changes number of generations between mother or father and the common ancestor;

F_A – the inbreeding coefficient of the common ancestor.

The viability of larvae has been calculated on 3 lots of 100 larvae for each line, at the beginning of the IVth stage, from the ratio between the number of cocoons and the number of larvae in the moment of the lot formation.

The percentage of live pupae was determined based on the ratio of the number of cocoons with live pupae to the total number of silk cocoons.

RESULTS AND DISCUSSIONS

The inbreeding effect on the larvae viability

The 15 inbred lines from the founding race Alb Băneasa presented in I_0 a viability percentage within the limits 88.82 - 82.88%, the lines average in this generation being 86.07% (Table 1).

In the first inbreeding generation, 10 of the 15 lines present a lower viability compared to the control, in two of them the differences in minus being significant, while three lines present a viability superior to the control, and in other two lines there are no differences from it.

Starting with I_2 the analyzed character was affected by the inbreeding depression, so that 9 of the 15 studied lines presented significantly lower values than the control, aspect that was also reposted in I_3.

In C_4 the lines average is with 4.89 percent lower than the control and in I_6 the difference to this records 8.56 percent. Comparing the lines' average by generations of inbreeding is found that each generation presents lower values than the previous generation, differences in minus statistically significant being found only in I_4 compared to I_3 and I_5 compared to I_4.

Following the effect of inbreeding within each line, it is found that the amplitude of variation of in minus differences between I_6 and I_0 is between 4.46 percent (AB-3/2) and 13.69 percent (AB-12/9).

It is also found that in some lines (AB-20/13 and AB-22/14) the inbreeding depression it has been manifested since I_1, but it has been generalized starting with I_3.

Also, in the case of the lines from Băneasa 75 race is found a progressive decrease of the viability percentage (Table 2), but differences in minus significant to the control appeared since I_3, generation in which 12 of the 15 analyzed lines are under the control (I_0). In I_4, I_5 and I_6 all the inbred lines present lower values than the control, the differences in minus being statistically significant.

Table 1. The inbreeding effect on the viability of larvae in the inbred lines – Alb Băneasa (%)

Line	Generation of inbreeding						
	I_0	I_1	I_2	I_3	I_4	I_5	I_6
	$\overline{X} \pm s_{\overline{X}}$	$\overline{X} \pm s_{\overline{X}}$	$\overline{X} \pm s_{\overline{X}}$	$\overline{X} \pm s_{\overline{X}}$	$\overline{X} \pm s_{\overline{X}}$	$\overline{X} \pm s_{\overline{X}}$	$\overline{X} \pm s_{\overline{X}}$
AB-1/1	88.20±0.86	87.80±0.66	87.20±0.16	86.22±0,08*	83.66±0.10**	79.16±0.12**	79.80±1.10**
AB-3/2	84.62±1.10	85.32±0.48	85.10±0.24	85.46±0.22	82.12±0.12**	80.32±0.14**	80.16±0.88**
AB-4/3	85.80±0.36	85.40±0.10	83.40±0.36**	84.16±0.16	80.40±0.42**	78.17±0.32**	79.13±0.82**
AB-5/4	87.77±0.18	86.88±0.12	85.60±1.10**	84.13±0.12**	81.26±0.48**	80.46±0.40**	78.15±0.16**
AB-7/5	83.40±0.36	84.62±0.63	85.10±0.68	83.80 ± 0.16	80.32±0.32**	79.90±0.44**	75.60±0.16**
AB-8/6	82.88±0.28	83.30±1.10	82.16±0.72	80.22±0.22**	79.88±0.16**	78.10±0.30**	75.30±0.10**
AB-9/7	87.73±0.46	86.82±0.88	86.40±0.63	85.40±0.18*	83.20±0.10**	80.42±0.18**	78.90±0.08**
AB-10/8	86.80±0.18	85.70±0.66	83.20±0.50**	82.60±0.60**	82.16±0.26**	80.14±0.12**	79.67±0.63**
AB-12/9	88.82±0.30	88.20±1.10	85.30±0.16**	82.20±0.54**	80.33±1.10**	78.16±0.16**	75.13±0.14**
AB-14/10	83.44±0.42	82.30±0.60	83.10±0.12	82.10±0.30	81.80±0.86	77.33±0.30**	76.22±0.10**
AB-15/11	85.72±0.60	84.10±0.48	80.88±0.28**	80.60±0.12**	80.22±0.72**	76.35±0.48**	77.10±0.12**
AB-18/12	87.47±0.48	86.12±0.36	82.66±0.32**	81.44±0.16**	79.66±0.17**	78.20±0.36**	76.16±0.14**
AB-20/13	86.30±0.10	83.13±0.76**	82.80±0.40**	82.20±0.42**	80.34±0.16**	77.16±1.10**	77.15±0.16**
AB-22/14	85.72±0.30	82.14±0.68**	83.66±0.12*	83.20±0.16**	81.22±0.36**	78.32±0.46**	77.80±032**
AB-25/15	86.40±0.42	85.76±0.14	84.40±0.40*	83.80±0.32**	81.10±0.18**	79.82±0.32**	76.40±0.40**
Average	86.07±0.47	85.17±0.49	84.06±0.44*	83.17±0.46**	81.18±0.31**	78.80±0.34**	77.51±0.44**

*P<0.05; **P<0.01; ***P<0.001.

Table 2. The inbreeding effect on the viability of larvae in the inbred lines – Băneasa 75 (%)

Line	Generation of inbreeding						
	I_0	I_1	I_2	I_3	I_4	I_5	I_6
	$\overline{X} \pm s_{\overline{X}}$	$\overline{X} \pm s_{\overline{X}}$	$\overline{X} \pm s_{\overline{X}}$	$\overline{X} \pm s_{\overline{X}}$	$\overline{X} \pm s_{\overline{X}}$	$\overline{X} \pm s_{\overline{X}}$	$\overline{X} \pm s_{\overline{X}}$
B75-2/1	89.30±0.20	88.76±0,46	87.27±0.52	86.40±0.36	76.66±0.18**	77.08±0.16**	72.20±0.23**
B75-4/2	86.40±0.36	87.32±0.82	86.32±0.42	80.80±0.72**	77.20±0.44**	71.88±0.22**	70.66±0.34**
B75-5/3	87.27±1.10	86.88±0.16	87.71±0.36	87.59±0.36	63.33±0.10**	68.20±0.46**	65.44±0.18**
B75-6/4	85.30±0.76	86.30±0.14	85.88±0.16	88.39±0.38	64.66±0.20**	77.50±0.38**	73.42±0.26**
B75-8/5	83.40±0.14	82.86±0.32	82.96±0.12	86.40±0.42	76.66±0.16**	76.50±0.14**	64.40±0.72**
B75-10/6	88.18±0.63	87.75±0.48	87.38±0.10	79.20±0.14**	74.32±0.46**	76.94±0.12**	76.60±1.01**
B75-11/7	87.36±0.22	87.20±0.56	86.86±0.22	84.40±0.16	77.33±0.18**	72.97±0.16**	73.90±2.36**
B75-13/8	88.20±0.86	85.30±1.06	87.40±0.46	83.20±0.10**	74.33±0.82**	70.93±0.28**	70.40±1.72**
B75-15/9	82.40±0.10	83.66±0.84	81.20±0.28	78.59±0.12*	72.46±0.60**	70.84±0.10**	69.60±1.10**
B75-16/10	88.60±0.80	85.40±0.36	84.80±0.16	82.00±0.62**	70.20±0.18**	70.11±0.26**	70.20±0.86**
B75-18/11	87.40±1.17	86.80±0.24	85.60±0.08	83.60±0.46*	74.85±0.22**	73.40±0.40**	72.20±0.72**
B75-19/12	88.20±0.22	88.10±0.28	87.98±0.18	80.32±0.32**	78.60±0.16**	75.00±0.26**	71.40±0.36**
B75-21/13	84.32±0.46	85.12±0.42	85.63±0.12	75.52±0.16**	76.14±0.12**	63.62±0.18**	66.60±0.42**
B75-23/14	82.20±1.10	81.20±0.72	80.88±0.11	78.44±0.46*	77.32±0.10**	73.79±0.60**	70.60±0.36**
B75-24/15	84.60±2.30	83.30±0.16	83.10±0.10	76.77±0.48**	75.44±0.40**	71.25±0.30**	69.96±0.18**
Average	86.21±0.60	85.73±0.56	85.40±0.60	82.11±1.04*	74.09±1.16**	72.67±0.98**	70.75±0.86**

*P<0.05; **P<0.01; ***P<0.001.

Comparing the inbreeding generations between them it is observed that the lines' average presents lower values in each of them compared with the previous generation, but they only become significant between I_4 - I_3 and I_5 - I_4. Inside the lines, in most cases, it is manifested the same decreasing tendency of the value of analyzed character from a generation to another, exceptions being noted in I_1 at the lines B75-4/2, B75-6/4, B75-15/9, B75-21/13, in I_2 at the lines B75-5/3 and B75-12/8 and in I_3 at the lines B75-6/4 and B75-8/5 when there is an increase in viability. In other cases, such as line B75-16/10 there is a stagnation of the viability percentage starting with I_4. As for the values reached by the I_6 inbred lines, they are between 64.40 - 76.60% and the differences in minus compared to the control (I_0) between -11.6 percent (B75-23/14) and -21.83 percent (B75-5/3).

Also, in the case of other works that aimed to achieve inbred lines in silkworms it was noticed the decrease of the percentage of larvae viability. So, after three generations of inbreeding is noticed a decreasing of the viability of larvae with 2 - 16 percent, the maximum inbreeding depression being recorded in I_6, the differences to the non-inbred control reaching the maximum value of -18,9% (Craiciu et al., 1971).

The inbreeding effect on the percentage of live pupae

The percentage of live pupae represents an important character of evaluation of silkworm races and hybrids and their promoting in production.

According to data presented by specialized publications, the value of this character represents 92.80 - 95.90% in silkworm races widespread in Korea, 95.60 - 97.50% in those reared in Japan and 88.60 - 90.80% in those in India.

Being a character with low heritability, the live pupae percentage is affected by the related crossing, aspect highlighted also by other authors who studied the inbreeding effect in silkworms (Craiciu et al., 1971).

In the case of inbred lines from Alb Băneasa race (Table 3) it is noticed that the percentage of live pupae is not visibly influenced in I_1, the lines' average in this generation being very close to I_0. Examining the percentage of live pupae for each line in this generation there are noticed slight differences more or less than I_0, but not being significant. In I_2 the inbreeding depression is manifesting in 10 lines that are below the values of the control with statistically significant differences.

The process of inbreeding depression extends in I_3 when the average of the lines is with 1.82 percent lower than the control and is generalized in I_5, generation in which the percentage of live pupae varies between 79.16 - 84.86% with a lines' average of 81.44 %. In I_6 the differences in minus to the control are between 1.80 - 6.60 percent, more affected being the lines AB-15/11 (78.16%), AB-25/15 (79.86%). As a whole, the lines present in I_6 a percent of transforming into pupae of 78.16-4.20%, being in average with 4.25 percent lower than in I_0.

Similar conclusions can be drawn also from the data on the percentage of live pupae in lines from Băneasa 75 group (Table 4). In I_1 no negative effect of inbreeding is reported, in this generation being observed an insignificant increase tendency of the percentage of live pupae in most lines. Starting with I_2, 6 of the 15 lines present significant in minus differences, their number increasing at 7 in I_3. In I_4 the inbreeding depression installs in most inbred lines and its effect is accentuated in the next two generations. In I_6 the average percentage of live pupae is of 79.25%, with 4.35 percent lower than in I_0. In the same generation the inbreeding depression is manifesting by a decrease of the value of the analyzed character with 1.82 - 6.34 percent, being more obvious in the case of 3 from the 15 lines.

CONCLUSIONS

The viability of larvae shows a tendency of progressive decrease with the increase of the coefficient of inbreeding depression, that become significant starting with I_3. The viability of larvae is in I_6 within the limits 75.13 - 80.16% in Alb Băneasa group of lines and 64.40 - 76.60% in Băneasa 75 group of lines. The differences in minus between I_6 and I_0 recorded by the average value of the lines represents 15.46 percent in Băneasa 75 lines and 8.56 percent in Alb Băneasa lines.

The percent of live pupae was influenced by the practice of re related crossings, aspect that manifested itself in the case of both groups of lines. The inbreeding depression has been installed in a small number of lines since I_1, expanding with the development of the inbreeding process and generalizing in I_5. The inbred lines in I_6 present a percentage of pupae between 78.16 - 84.20% in Alb Băneasa group and 76.10 - 80.86% in Băneasa 75 group, the differences in minus to I_0 being of 1.80 - 6.60 percent in the case of the first group of lines and of 1.82 - 6.34 percent in the case of the second group. The hatching percentage was affected by de inbreeding depression starting with I_3, in both groups of races. The differences in minus between I_6 and I_0 are between 6.10-15.66 percent in Alb Băneasa group of lines and 6.80-15.90 percent in Băneasa 75 group of lines.

The use of inbred lines in the process of silkworm hybridization led to the obtaining of commercial hybrids. This aspect made it possible to determine the magnitude of the heterosis effect for different characters as well as to elaborate some efficient schemes of hybridization, the work materializing by recommending some hybrid combinations for the sericultural production.

Table 3. The inbreeding effect on the live pupae percentage in the inbred lines – Alb Băneasa (%)

Line	Generation of inbreeding						
	I_0	I_1	I_2	I_3	I_4	I_5	I_6
	$\overline{X} \pm s_{\overline{X}}$	$\overline{X} \pm s_{\overline{X}}$	$\overline{X} \pm s_{\overline{X}}$	$\overline{X} \pm s_{\overline{X}}$	$\overline{X} \pm s_{\overline{X}}$	$\overline{X} \pm s_{\overline{X}}$	$\overline{X} \pm s_{\overline{X}}$
AB-1/1	87.26±0.12	86.22±0.40	85.10±0.32**	86.00±0,52	85.26±0.12**	84.86±0.18**	84.20±0.28**
AB-3/2	86.48±0.24	85.28±0.36	84.32±0.88**	84.12±0.44	85.10±0.40**	83.28±0.26**	82.26±0.32**
AB-4/3	87.10±0.14	87.27±0.14	86.16±0,72	85.26±0.66*	83.86±0.38**	84.10±0.40**	84.12±0.14**
AB-5/4	84.20±0.32	85.46±0.12	86.18±0.18**	85.30±0.18	83.36±0.18	82.66±0.52	82.10±0.42**
AB-7/5	84.31±0.40	83.32±0.26	84.66±0,16	82.26±0.24**	80.82±0.14**	81.82±0.38**	80.16±0.76**
AB-8/6	85.22±0.16	86.26±0.34	85.23±0.12	84.48±0.36	82.31±0.36**	81.45±0.18**	80.26±0.12**
AB-9/7	83.26±0.24	84.46±0.42	81.44±0,26*	83.25±0.40	80.27±0.22**	81.23±0.10**	81.46±0.14*
AB-10/8	84.44±0.30	83.88±0.11	82.60±0.34*	81.88±0.44**	79.96±0.54**	80.44±0.16**	80.55±0.26**
AB-12/9	86.21±0.36	85.22±0.17	84.32±0.16**	83.36±0.16*	81.25±0.17**	80.18±0.27**	81.32±0.30**
AB-14/10	87.42±0.24	87.36±0.24	85.26±0.42**	84.48±0.40**	84.10±0.46**	81.28±0.35**	81.10±0.44**
AB-15/11	83.46±0.12	84.42±0.30	85.22±0,46*	84.10±0.52	81.26±0.45**	79.86±0.14**	78.16±0.18**
AB-18/12	84.22±0.18	83.18±0.44	83.10±0.30	82.86±0.64	82.32±0.50**	80.32±0.44**	80.98±0.15**
AB-20/13	85.48±0.44	83.44±0.28**	82.86±0.22**	80.62±0.24**	81.82±0.44**	80.42±0.12**	80.26±0.27**
AB-22/14	85.26±0.14	84.16±0.36	83.22±0.40	82.44±0.38**	80.44±0.26**	79.16±0.34**	80.44±0.31**
AB-25/15	86.46±0.16	85.20±0.40	84.62±0.32*	83.18±0.22	81.25±0.30**	80.30±0.52**	79.86±0.42**
Average	85.39±0.36	85.01±0.35	84.29±0.35	83.57±0.37*	82.23±0.45**	81.44±0.42**	81.14±0.41**

*P<0.05; **P<0.01; ***P<0.001.

Table 4. The inbreeding effect on the live pupae percentage in the inbred lines – Băneasa 75 (%)

Line	Generation of inbreeding						
	I_0	I_1	I_2	I_3	I_4	I_5	I_6
	$\overline{X} \pm s_{\overline{X}}$	$\overline{X} \pm s_{\overline{X}}$	$\overline{X} \pm s_{\overline{X}}$	$\overline{X} \pm s_{\overline{X}}$	$\overline{X} \pm s_{\overline{X}}$	$\overline{X} \pm s_{\overline{X}}$	$\overline{X} \pm s_{\overline{X}}$
B75-2/1	86.40±0.46	85.80±0.30	84.42±0.18**	82.66±0.46**	81.82±0.18**	81.20±0.46**	80.86±0.22**
B75-4/2	83.20±0.32	84.22±0.22	85.26±0.38**	83.28±0.26	82.66±0,44	79.66±0.52**	80.20±0,18**
B75-5/3	85.60±0.18	81.66±0.16**	83.44±0.42**	81.62±0.18**	82.34±0.56**	81.32±0.38**	79.36±0.12**
B75-6/4	81.26±0.26	84.40±0.26**	82.86±0.22*	83.44±0.44**	81.62±0.62	80.86±0.40	79.44±0.46**
B75-8/5	82.40±0.54	83.26±0.48	83.20±0.28	82.62±0.55	80.36±0.74**	80.22±0.14**	80.10±0.32**
B75-10/6	86.42±0.52	85.20±0.36	85.44±0.18	83.46±0.72**	81.28±0.19**	81.10±0.21**	80.26±0.14**
B75-11/7	82.86±0.22	84.44±0.72*	83.26±0.36	83.22±0.14	82.46±0.26	81.40±0.88	79.68±0.28**
B75-13/8	83.22±0.46	84.62±0.86	81.48±0,56*	82.64±0.90	82.32±0.24	80.32±0.16**	79.46±0.22**
B75-15/9	86.44±0.30	86.22±0.92	82.66±0.62**	83.82±0.28**	81.66±0.12**	79.66±0.14**	80.10±0.36**
B75-16/10	80.22±0.10	82.30±0.40**	83.82±0.74**	81.20±0.32	82.46±0.14**	80.56±0.24	80.32±0.44
B75-18/11	81.45±0.72	82.46±0.36	82.60±0.22	80.46±0.40	79.98±0.28*	80.48±0.32	76.10±0.52**
B75-19/12	84.60±0.50	85.26±0.72	84.66±0.14	81.52±0.42	82.56±0.34**	80.88±0.28**	79.22±0.12**
B75-21/13	82.26±0.36	83.82±0,86	82.22±0.96	80.32±0.16**	80.20±0.44**	78.36±0.42**	77.16±0.15**
B75-23/14	83.52±0.28	82.66±0.16	81.86±0.80*	80.48±0.26**	78.36±0.22**	79.40±0.54**	78.30±0.21**
B75-24/15	84.10±0.10	83.46±0.42	82.24±0.76**	81.62±0.38**	80.98±0.14**	80.20±0.62**	78.24±0.30**
Average	83.60±0.51	83.99±0.35	83.29±0.31	82.16±0.31	81.40±0.32**	80.37±0.22**	79.25±0.34**

*P<0.05; **P<0.01; ***P<0.001.

REFERENCES

Braslă, A., & Matei, A. (1992). New silkworm hybrids prepared for spring breeding. *Review of Veterinary Medicine and Animal Breeding*, 11-12, 12-14.

Bindroo, B., & Moorthy, S. (2014). Genetic divergence, implication of diversity and conservation of silkworm *Bombyx mori*. *International Journal of Biodiversity*, 564850. doi:10.1155/2014/564850.

Buhroo, Z., Malik, M., Ganai, N., Kamili, A., & Bhat, B. (2016). Comparative performance and genetic polymorphism in some potential silkworm *Bombyx mori* L. genotypes. *Journal of Cell and Tissue Research* ,16(3), 5911-5920.

Craiciu, M., & Tițescu, E. (1971). The effect of inbreeding in silkworms (*Bombyx mori*). *Anale S.C.A.S.*, XI, 129-138.

Diniţă, G., Marin, M.P., Tudorache, M., Custura, I., & Șuler, A.D. (2019). Studies concerning the effect of the inbreeding on the prolificity and hatching percentage at silkworms (*Bombyx mori* L.). *Scientific Papers, Series D., Animal Science*, *LXII*(1), 198-202.

Doreswamy, J., & Gopal, S. (2012). Inbreeding effects on quantitative traits in random mating and selected populations of the mulberry silkworm, *Bombyx mori*. *J. Insect Sci*., 12, 140. doi: 10.1673/031.012.14001

Goldsmith, M.R., Shimada, T., & Abe, H. (2005). The genetics and genomics of the silkworm, *Bombyx mori. Annual Reviews Entomology*, 50, 71–100.

Haniffa, M.A., & Thatheyus, A.J. (1992). Effect of age-related mating schedule on reproduction in the silkworm, *Bombyx mori* L. *Sericologia*, 32(2), 217-223.

Jamuna, D., & Subramanya, G. (2012). Inbreedind effects on quantitative traits in random mating and selected populations on the mulberry silkworm, *Bombyx mori*. *J.Insect.Sci*., 12, 140-146.

Kumari, A., & Tripathi, N. (2017). Genetic diversity studies in six bivoltine races of *Bombyx mori* L., based on phenotypic characters. *International Journal Advanced Research*, *5*(1), 1048-1054.

Kuzmanov, K., & Petkov, N. (2000a). Selection asessment of silkworm lines (*Bombyx mori L.*) for hybridization. I. Chinese lines. *Animal Science, 37*(5-6), 56-59.

Kuzmanov, K., & Petkov, N. (2000b**).** Selection asessment of silkworm lines (*Bombyx mori L.*) for hybridization. I. Japanese lines. *Animal Science, 37*(5-6), 52-55.

Nagaraju, J. (2002). Application of genetic principles for improving silk production. *Current Science*, 83, 409–414.

Nematollahian, S.H., Ghanipoor, M., & Seidavi, A.R. (2010). Survey of inbreeding, coefficient on cocoon economical traits in ten commercial Iranian silkworm lines. *Journal of Animal Science*, 3, 27–34.

Petkov, N., Natcheva, Y., & Tzenov, P. (1998). Genetic studies of silkworm, *Bombyx mori* L. *Agricultural Science*, XXXIV, 17–22.

Petkov, N., Natcheva, Y., & Tzenov, P. (1999). About the problem of applying inbreeding to silkworm *Bombyx mori* L. *Agricultural Science*, 1, 37–39.

Ruiz, X., & Almanza, M. (2018). Implications of genetic diversity in the improvement of silkworm *Bombyx mori* L. *Chil. J. Agric. Res*., 78(4).