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Effect of cocoon fluorescence, silkworm hybrid and gender on sericin content of *Bombyx mori* L. silk thread

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Abstract. *The goal of the present study was to determine the influence of the ultraviolet fluorescence of cocoons, the hybrid, the sex and the interaction among them on the sericin content in silk threads. The study was performed with 3 di- and 2 tetra-cross silkmoth (Bombyx mori L.) hybrids, differentiated in three groups – with violet, intermediate and yellow fluorescence of the cocoons. The examined factors had a significant effect ($p \leqslant 0.001$) on the sericin content. The highest sericin content was detected in the silk threads of the violet-fluorescent and the lowest – in the yellow-fluorescent group. The analysed di-hybrids were distinguished by better characteristics in terms of sericin content, compared to the tetra-hybrids, most obvious for the yellow-fluorescent fraction.*

Keywords: *Bombyx mori* L. silk, fluorescence, hybrid, sex, sericin content

Introduction

Sericin is a type of scleroprotein similar to collagen, elastin, keratin, sponging etc. Along with fibroin it constitutes a primary building material of the silk thread. Its content, physical-mechanical and chemical properties are the main factors determining the technological qualities of the cocoons and the silk thread (Sadov et al., 1987). Sericin content is of great importance in the production of raw silk (Mano et al., 1988). Raw silk with lower sericin content is more resilient to friction than silk containing more sericin (Kuwahara et al., 1978). Sericin also affects the structure and thermal properties of the silk thread (Lee et al., 2005; Prasong et al., 2009). According to Narumi and Kobayashi (1997), Mondal et al. (2013), the amount of sericin is one of the factors determining the thickness of the silk thread from various breeds and hybrids. The rougher silk thread contains more sericin compared to the finer thread. Sericin content affects the process of silk dyeing (Kato and Hata, 1998). Along with quality, sericin content also has an influence on raw silk yield. For this reason, and due to the fact that sericin content is determined by inheritance, countries with developed sericulture make efforts to create breeds and hybrids with low sericin content (Gamo and Hirabayashi, 1984; Radhavendra Rao et al., 2004).

Sericin content is one of the criteria of the Standard of the National Bureau of Agricultural Commodity and Food Standards of the Ministry of Agriculture and Cooperative in Thailand. Sericin content below 30% determines a higher, and over 30% lower quality of the silk thread. Improving this parameter in the desired direction (towards reduction) can be achieved via selection or by creating and using hybrids with a maximum extent of negative heterosis expression of sericin content (Basavaraja et al., 2000; Raghavendra Rao et al., 2004; Gowda et al., 2013; Seetharamulu et al., 2013).

The scientific data accumulated thus far indicate that sericin makes up 20 – 30% of silk (Prasong et al., 2009; Seetharamulu et al., 2013; Chellamani et al., 2014). It is affected by factors with genetic (Gamo and Hirabayashi, 1984; Basavaraja et al., 2000; Radhavendra Rao et al., 2004 etc.), as well as non-genetic features (Santoshkumar et al., 2000; Vasarmidaki et al., 2000; Sanappa, 2002a,b; Amala Rani et al, 2011; Patil et al.,2013; Padma and Ramani., 2015). The significance of sericin to the efficiency of the spinning process has aroused the interest towards gathering new information about the factors that its amount in the cocoons and the raw silk depends upon. The insufficient information about its relation to the ultraviolet fluorescence of the cocoons directed us toward the goal of the present study.

The goal of the present study was to determine the influence of ultraviolet fluorescence of the cocoons, the hybrid and the sex on sericin content in the silk thread from *Bombyx mori* L cocoons.

Material and methods

The present study was conducted in the Experimental base of the Sericulture unit, Faculty of Agriculture at Trakia University, Stara Zagora. Silk threads from the cocoons of the following double- and tetra-cross *Bombyx mori* L. hybrids were used in the study:

- Double-cross hybrids: Super1 x Hesa2*, 19x20**, 1013 x 1014**
- Tetra-cross hybrids: $(CH_1 \times U_1) \times (M_2 \times H_2)$*, (19 x 1013) X (20 x 1014)**

Note:

* Hybrids were created and maintained in the Sericultural Experimental Station – Vratsa

** Hybrids were created and maintained in the Faculty of Agriculture, Trakia University, Stara Zagora.

Fluorescence of cocoons was evaluated by a quartz lamp with a filter transmitting ultraviolet rays within the range 334 – 400 nm. Depending on the effect of UV irradiation, samples were differentiated into three groups: with violet, intermediate and yellow fluorescence. Raw silk was obtained by unravelling of individual cocoons using a Fu apparatus (Hungary).

The sericin of silk threads was dissolved using the method of

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Komatsu (1975), through 180-minute boiling of silk skeins at 98 ^{0}C in M/5 borate buffer with pH 9, at a ratio of 1:750, modified by Bobov et al. (2006) by addition of 100 µl /ml 0.1 n NaOH. The content of sericin was evaluated on the basis of absorption measured on the 180th min from the beginning of boiling using the following relationship between sericin amount and absorption:

Sa (%) = 88.578 x A + 6.5576,

where Sa (%) is the percentage of sericin determined by spectroscopy, A is sericin solution absorption on the 180th min of boiling.

Spectroscopy was performed in the UV range at a wavelength of 280 nm.

The relationship between factors and variables was assessed through linear models with least-square means estimates (LSM) and ANOVA test.

Results and discussion

Table 1 presents the results from the dispersion analysis on the influence of cocoon fluorescence, hybrid and sex, as well as the interaction among them on sericin content in the raw silk of *Bombyx mori* L. The F-criterion values indicated that all analysed factors (fluorescence, hybrid, sex and interaction among them) had a significant ($p \leq 0.001$) effect upon the sericin content of the silk skeins.

The results presented in Table 2 show that sericin content decreased as cocoon fluorescence changed from violet towards yellow. Raw silk gathered from cocoons with intermediate and yellow fluorescence contained 0.81 and 1.52 units less sericin than the cocoons with violet fluorescence. The superior characteristics of the intermediate and yellow-fluorescent fractions, compared to the violet-fluorescent ones, were confirmed by the LS-means. There, the deviations from the model mean were in the desirable direction (decreasing), whereas in the violet-fluorescent ones the deviation from the mean was towards the undesired direction (increase) of the sericin content. These results indicated that the production in practice and the usage of yellow-fluorescent cocoons in the spinning process would contribute towards improving the sericin content parameter by 0.77%, while using violet-fluorescent cocoons would deteriorate it by 0.77%.

The creation of different silkworm forms (di, tri- and tetra-cross hybrids) is an object of constant interest to both science and practice. The results from testing various forms (Hirata, 1985; Petkov et al., 2000) are contradictory and do not allow for definitive evaluations of their advantages to be made. We believe that the study conducted by us and the data on sericin content in the silk thread of di- and tetra-cross hybrids would contribute to the available body of knowledge on the issue.

The results from the analysis of the LS means and the LS evaluations of the hybrid effect are presented in Table 3. The tetra-cross hybrids were distinguished by a higher sericin content (24.98 – 26.09%) compared to the di-cross hybrids (23.43 – 24.43%). The most apparent differences within the range from 1.74 to 2.66% were observed between the di- and tetra-hybrids (19x1013)X(20x1014). The LS evaluations produced during the present study, and the proven positive effect of low sericin content on the technological properties of the silk thread and the economic efficiency of the spinning process indicated a deviation in the desired direction from the model mean for the LS evaluations of the di-cross hybrids. This was more clearly exhibited in 1013 x 1014 and C_1 x $Xeca_2$. Their LS-evaluations indicated that using cocoons of the indicated hybrid would improve the analysed parameter by 1%. Using tetra-hybrid cocoons would lead to the parameter's (sericin content) deterioration by 0.52 – 1.63%. These results are a reason to consider that using cocoons of the di-hybrids analysed in the present study would be more economically beneficial in the production of raw silk, compared to the usage of tetra-hybrids. Analysing the productivity of the di-, tri- and tetra-cross hybrid forms, Gowda et al. (2013), determined a bidirectional influence of the hybrids on sericin content. Within the scope of the separate hybrid combinations, there were hybrids with high and low values of the parameter, but in general, sericin content increased from di- towards tetra-hybrids. The hybrid influence on sericin content established within the study corresponds to the results of Radhavendra Rao et al. (2004) from the study of 20 hybrids of *B. mori* L. We believe that the differences in the

Table 1. ANOVA results for influence of the fluorescence, hybrid, sex and their interaction on sericin content in raw silk filament

Source of variation	df	MS	F	P
Fluorescence	2	260.1	78.3	***
Hybrid	4	462.7	139.9	***
Sex	1	38.3	11.5	***
Interaction	8	20.9	6.3	***

***the factors have a statistically significant effect on the studied traits at P≤0.001

Table 2. Ls-means and LS-estimate for influence of the fluorescence on sericin content in raw silk filament

Source of variation	df	LS-mean ± SE	LS-estimate
Mean for the model	1969	24.46 ± 0.05	
Violet	677	25.23 ± 0.07	+0.77
Intermediate	779	24.42 ± 0.07	-0.04
Yellow	513	23.71 ± 0.09	-0.75

Table 3. Ls-means and LS-estimate for influence of the hybrid on sericin content in raw silk filament

Source of variation	df	LS-mean ± SE	LS-estimate
Mean for the model	1969	24.46 ± 0.05	
19 x 20	429	24.35 ± 0.10	-0.11
1013 x 1014	378	23.43 ± 0.09	-1.03
C_1 x X_2	425	23.44 ± 0.09	-1.02
(19x1013)x(20x1014)	393	26.09 ± 0.09	+1.63
$(CH_1xU_1)x(M_2xH_2)$	344	24.98 ± 0.14	+0.52

Table 4. Ls-means and LS-estimate for influence of the sex on sericin content in raw silk filament

Source of variation	df	LS-mean ± SE	LS-estimate
Mean for the model	1969	24.46 ± 0.05	
Female	931	24.30 ± 0.07	-0.16
Male	1038	24.62 ± 0.06	+0.16

phenotype expression were due to the participation of different genotypes in the creation of the analysed hybrids, as well as their different degree of combinative ability. Based on the statements of Gamo and Hirabayashi (1984) that the lower values of sericin content were determined by dominant and the higher values – by recessive genes, it could be assumed that the higher values in the examined tetra-hybrids were the results of the effect of recessive, and the lower values in the di-hybrids of dominant genes.

The analysis of the results from Table 4 indicates that the parameter variation in accordance with the factor of sex was within a smaller range (LS-means within 24.30 – 24.62%) compared to the hybrid as a factor. The differences between the LS-means and the model mean were with equal numerical value, yet differed in sign, and thus in the effect the two sexes would have on sericin content. Even though insignificant, female individuals would improve and males would deteriorate the characteristics of the silk thread, in terms of sericin content.

The mean values (LS-means) and the LS evaluations of the effect of the interactions among fluorescence, hybrid and sex on sericin content are presented in Tables 5 and 6. The results go along with the established trends in the individual influence of the three factors. In female silkworms (Table 5), the highest sericin content in the silk thread was observed in the group with violet fluorescence (24.00 – 26.93%), followed by the intermediate-fluorescent (23.29 – 26.10), with the lowest values being in the yellow-fluorescent group (21.44 – 24.76). In both females and males (Table 6), the highest amounts of sericin were measured in the violet-fluorescent silk threads (24.27 – 27.89%), followed by the intermediate-fluorescent (22.91 – 26.47%), and yellow-fluorescent (23.31 – 25.17%). The analysis of the LS means indicated that in both sexes, hybrids improved their characteristics in terms of sericin content, from violet towards yellow fluorescence. Di-cross hybrids had better characteristics than tetra-hybrids. Regardless of this, the latter also exhibited a considerable decrease in the mean values, compared to the model mean. For the females, it was from +2.47 and +0.36 in those with violet, and +0.3; -0.5% in those with yellow fluorescence, and in males: +3.43; +0.91 and +0.71; +0.17%.

Table 5. Ls-means and LS-estimate influence of the fluorescence and hybrid on sericin content in raw silk filament from female cocoons

Source of variation	df	LS-mean ± SE	LS-estimate
Mean for the model	1969	24.46 ± 0.05	
Violet			
19 x 20	81	24.90 ± 0.20	+0.44
1013 x 1014	54	24.17 ± 0.25	-0.29
C_1 x X_2	118	24.00 ± 0.17	-0.46
(19x1013)x(20x1014)	60	26.93 ± 0.24	+2.47
$(CH_1xU_1)x(M_2xH_2)$	69	24.82 ± 0.22	+0.36
Intermediate			
19 x 20	69	24.02 ± 0.21	-0.44
1013 x 1014	54	23.29 ± 0.25	-1.17
C_1 x X_2	72	24.01 ± 0.21	-0.45
(19x1013)x(20x1014)	60	26.10 ± 0.23	+1.64
$(CH_1xU_1)x(M_2xH_2)$	69	25.33 ± 0.22	+0.87
Yellow			
19 x 20	45	24.06 ± 0.27	-0.40
1013 x 1014	60	22.24 ± 0.23	-2.22
C_1 x X_2	40	21.44 ± 0.29	-3.02
(19x1013)x(20x1014)	72	24.76 ± 0.21	+0.30
$(CH_1xU_1)x(M_2xH_2)$	28	24.41 ± 0.64	-0.05

Table 6. Ls-means and LS-estimate influence of the fluorescence and hybrid on sericin content in raw silk filament from male cocoon

Source of variation	df	LS-mean ± SE	LS-estimate
Mean for the model	1969	24.46 ± 0.05	
Violet			
19 x 20	30	25.30 ± 0.33	+0.84
1013 x 1014	60	24.67 ± 0.23	+0.21
C_1 x X_2	63	24.27 ± 0.23	-0.19
(19x1013)x(20x1014)	52	27.89 ± 0.25	+3.43
$(CH_1xU_1)x(M_2xH_2)$	90	25.37 ± 0.19	+0.91
Intermediate			
19 x 20	129	24.16±0.16	-0.30
1013 x 1014	75	22.91±0.21	-1.55
C_1 x X_2	84	23.45±0.20	-1.01
(19x1013)x(20x1014)	80	26.47±0.20	+2.01
$(CH_1xU_1)x(M_2xH_2)$	87	24.53±0.20	+0.07
Yellow			
19 x 20	75	23.68 ± 0.21	-0.78
1013 x 1014	75	23.31 ± 0.21	-1.15
C_1 x X_2	48	23.48 ± 0.26	-0.98
(19x1013)x(20x1014)	60	25.17 ± 0.24	+0.71
$(CH_1xU_1)x(M_2xH_2)$	30	24.63 ± 0.33	+0.17

The effect of the breed (hybrid) was an object of study by other authors as well. According to Gamo and Hirabayashi (1984), and Radhavendra Rao et al. (2004), Sabina et al. (2012), the hybrid and the sex affected sericin content. The breed effect was established by Kerang et al., (1998), yet, according to them, there were no differences between the two sexes. The available literature offers studies on the influence of fluorescence on the behaviour of sericin while it is dissolved (Aoki et al., 1986; Chang and Nahm, 1988), but not about the sericin content. For this reason, we are unable to make a comparison of our results.

Conclusion

The results from the present study indicated the cocoon fluorescence, the hybrid, the sex, and their interaction had a highly significant ($p \leq 0.001$) effect on the sericin content of the silk thread, for the analysed hybrids of *Bombyx mori* L. Violet cocoon fluorescence deteriorated the sericin content factor in raw silk by 0.63%, and yellow improved it by 0.77%, compared to the model mean.

The analysed di-cross hybrids were distinguished by better characteristics in terms of sericin content, compared to tetra-cross hybrids. This trend being most strongly exhibited in the yellow-fluorescent fraction. The usage of cocoons from di-hybrid forms would lead to an improvement in the trait's values, mostly (by more

than 3%) in the yellow fluorescence group. Using cocoons from the analysed tetra-cross hybrids deteriorated the parameter, most pronounced (by up to 3.43%) in the violet fluorescence group.

The differences between the content of sericin in both sexes and the average for the model are the same in value but different in direction, more favourable in females.

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The manuscript should be structured as follows: Title, Names of authors and affiliation address, Abstract, List of keywords, Introduction, Material and methods,Results, Discussion, Conclusion, Acknowledgements (if any), References, Tables, Figures.

The title needs to be as concise and informative about the nature of research. It should be written with small letter /bold, 14/ without any abbreviations.

Names and affiliation of authors The names of the authors should be presented from the initials of first names followed by the family names. The complete address and name of the institution should be stated next. The affiliation of authors are designated by different signs. For the author who is going to be corresponding by the editorial board and readers, an E-mail address and telephone number should be presented as footnote on the first page. Corresponding author is indicated with *.

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Keywords: Up to maximum of 5 keywords should be selected not repeating the title but giving the essence of study.

The introduction must answer the following questions: What is known and what is new on the studied issue? What necessitated the research problem, described in the paper? What is your hypothesis and goal ?

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Figures should be sharp with good contrast and rendition. Graphic materials should be preferred. Photographs to be appropriate for printing. Illustrations are supplied in colour as an exception after special agreement with the editorial board and possible payment of extra costs. The figures are to be each in a single file and their location should be given within the text.

Discussion: The objective of this section is to indicate the scientific significance of the study. By comparing the results and conclusions of other scientists the contribution of the study for expanding or modifying existing knowledge is pointed out clearly and convincingly to the reader.

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Oldenbroek JK, 1999. Genebanks and the conservation of farm animal genetic resources, Second edition. DLO Institute for Animal Science and Health, Netherlands.

Book chapter or conference proceedings: Author(s) surname and initials, year. Title. In: Title of the book or of the proceedings followed by the editor(s), volume, pages. Name of publisher, place of publication. Example:

Mauff G, Pulverer G, Operkuch W, Hummel K and Hidden C, 1995. C3-variants and diverse phenotypes of unconverted and converted C3. In: Provides of the Biological Fluids (ed. H. Peters), vol. 22, 143-165, Pergamon Press. Oxford, UK.

Todorov N and Mitev J, 1995. Effect of level of feeding during dry period, and body condition score on reproductive performance in dairy cows,IXth International Conference on Production Diseases in Farm Animals, September 11–14, Berlin, Germany.

Thesis:

Hristova D, 2013. Investigation on genetic diversity in local sheep breeds using DNA markers. Thesis for PhD, Trakia University, Stara Zagora, Bulgaria, (Bg).

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