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Study on the Receptivity of Breeds Silkworm, *Bombyx mori L.* to Artificial Diet low in Mulberry Powder

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A B S T R A C T

The use of silk moth, as an object of biological research, raises the need to create alternative sources of cheap food. The objective of this study was to determine the susceptibility of species *Bombyx mori* L. to artificial diets with reduced content of dried mulberry leaf. The study has been carried out at the educational and experimental facility of the Silkworm breeding section of the Faculty of Agriculture at Trakia University. The studied 45 breeds from the genetic bank of SAES - Vratsa were reared with artificial diet with 15% content of mulberry leaf powder, provided by SAES - Vratsa and prepared by methodology recommended by the manufacturer. The receptivity of specimens from the analyzed breeds was determined based on the survival rate of larvae in the Ist, IInd and IIIrd instar of the caterpillar stage. The results obtained showed that the vitality of the breeds included in this study varies widely: 53.76 - 100%; 26.49 - 98.75% and 0 - 95.67%, respectively for the first, second and third instar. Lower limit of survival in rearing with artificial food is over 70% vitality. In all three instars of the larval development, with over 70% vitality, and therefore high degree of receptivity to the artificial diet with low mulberry powder content was shown by the breeds "Almeria esp 1" (100.00% → 95.67%), "Nig 2" (97.94% → 80.05 %), "Vratsa 2003" (96.72% → 85.88%) and "Veslets 1" (95.91% → 72.99%). The high percentage of survival rate established in the majority (82.22%) of the analyzed 45 breeds during in the first instar and therefore, with high affinity to artificially prepared food low in dried mulberry leaf, is indicative of the biological potential of rearing them with artificial diet with low content mulberry powder (15%).

Introduction

Domesticated silk moth *Bombyx mori*L., has emerged as a very good object for conducting molecular genetic studies for solve a wide range of biological issues of major economic importance (Nagaraju, 2000). Favorable factors in this respect are the high fertility and short life cycle.

Limiting factors for intensification of cocoon production and for research purposes are the narrow food specialization and dependence on the terms of vegetation of the main food source –the mulberry tree.

The implementation of technologies for the use of artificial mixtures in the sericulture

practice,the testing and creation of highly productive hybrids suitable for cultivation with artificial diet, expands the area and opportunities for rearing and experimention with *Bombyx mori* L. in laboratory conditions (Ito and Kobayashi, 1978).

Artificial diet for young silkworms contains 50% mulberry meal for the output breeds and for hybrids 25% (Furusawa *et al.,* 1984; Murakami and Yamamoto, 1985).

Matsura and Matsuda (1993) developed three types of artificial diets suitable respectively for rearing silkworms in the first and second in star, third and fourth instar and fifth instar. The main differences of the diet for the fifth instar as compared with that of other instars, are the lower content of mulberry meal and agar-agar and the higher content of potato starch, sugar, citricacid, soybean meal and cellulose. Tsenov and Georgiev (2010) developed artificial diet suitable for feeding silk worms throughout the larval period. The authors point out that as the most optimal was established to be the composition of diet comprising 38% mulberry meal.

One of the main ideas in the development of artificial diet is to replace mulberry leaf in the nutrition of industrial hybrids (Ito, 1981). To increase the efficiency inrearing with artificial diet work is directed mainly to improvement of its composition, improved methods of cultivation and selectionof breeds of silkworms suitable for artificial feeding (Horie, 1981).

Seisho *et al.,*(1984)studied the possibilities of developing diet without mulberry meal for rearing parental forms in order to reduce the price of food, and hence the cost of the resulting produce. The authors use a diet in which the amount of mulberry meal contained is reduced stepwise (25, 20, 15,

10, 5 and 0%). In this regard Avramova and Grekov (2013) tested hybrids and as a result of the established tolerance of silkworms to rearing with artificial diet, they recommended developing recipes with different content of dried mulberry leaf. The results of the studies by Genova (1991) to feed silkworms with artificial food mixes with different percentage of mulberry meal in them show that better results are obtained with the hybrid forms compared to the basic breeds.

Panayotov (1995) found that in late autumn silkworm feeding rearing with artificial food has more favourable effect on the speed of development compared with rearing with mulberry leaf. The differences in the adaptation abilities of specimens from various crosses in the transition from natural to artificial diets have also been taken into account.

Hamano *et al.,* (1995) studied the effect of the basic types of energy sources included in the artificial diet on the silkworm respiration quotient. The results show that the RQ value can be changed depending on the components included in the artificial mix.

As a result of many years of research that leads to the need for selection of breeds and creating hybrids adapted to artificial diets (Azizov and Gulamova, 1982).

Kanda (1992) developed a quick and handy method for testing silkworm breeds regarding their eating behavior and found out that the use in practice of low cost artificial diet can be achieved by genetic change of the eating habits of the forms for industrial rearing.

As a result of the studies on the genetic control of the eating behavior in silkworms it has been found that the reaction of

silkworms to artificial diets is different depending on the breed and is probably controlled by numerous genes (Fujimori *et al.,* 1982; Yamamoto and Shimizu, 1982; Yamamoto, 1983; Tanaka and Midorikowa, 1984).

Mase *et al.,* (2007), based on the RFLPs analysis for the Sawa-J breed found out that the gene responsible for the food specialization of silkworms, called *"food preference"* belongs to a linked group of genes (RFLG9), comprising 7 more genes (m162, m227, m218, e42, m191, e73, ep16).

Nair *et al.,*(2011), through direct selection managed to enhance the receptivity of silkworms to artificial food more than85%. In generation nine the nutritive response of specimens from the studied breeds reaches over 78%. In hybrids from breeds selected in the course of 12 generations, adaptation to artificial diet is far greater (85%).

Important for the successful implementation of artificial diet low in dried mulberry leaf in rearing silkworms is the use of suitable *Bombyx mori* L. breeds and hybrids and the creation of forms adapted to rearing with such mix. That directed our efforts to the objective of this study.

Materials and Methods

The study was conducted at the Training Experimental Unit of Sericulture section at the department "Animal husbandry – non-ruminants and other animals" of the Faculty of Agriculture, Trakia University.

The object of the present study are the offspring of 45 breeds from the genetic bank of SAES – Vratsa that had demonstrated 70% survival rate when reared with artificial diet, comprising 25% mulberry powder in its composition. In the experiment for feeding silkworms we used artificial diet with 15%

content of dried mulberry leaf produced in SAES - Vratsa pulverized. The preparation of the diet to feed the silkworms was done by methods developed by the manufacturer, whereby 250g dry substance and 675ml 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 ~800W. The cooked food was stored in a refrigerator at temperature 2-5°C until using it for feeding.

In each of the breeds analyzed we formed 4 replicates of 50 pcs. of normal silkworm eggs previously disinfected with 2% formalin solution. The egg incubation, rearing and feeding of silkworms was done in sterile petri dishes in thermostats prepared in advance for this purpose. Silkworm rearing was carried out in the following temperature-humidity conditions. We laid the food every other day.

To establish the receptivity of specimens from the analyzed breeds, rearing to artificial diet, with 15% mulberry leaf powder content, we determined their survival rate in Ist, IInd, IIIrd instar. For this purpose, the number of living specimens was recorded after their mass awakening from first, second and third sleep. The survival rate of silkwormsat each instar was determined as percentage by the following formula:

$$V = \frac{N}{n} \, x \, 100 (\%), \qquad were$$

V– survival rate (%);

N– number of vital silkworms in the respective instar;
n – total number of pledged silkworms;

To process the data obtained we used the relevant modules from the STATISTICA software package of StatSoft and Microsoft Excel 2010.

Results and Discussion

When reporting the receptivity of silkworms to artificial food, one of the main signs is considered to be the number of normally developing specimens, calculated as percentage of vitality. The data analysis was carried out on the basis of the criteria specified in Table 2 defining the degree of receptivity of larvae to artificialdiet.

Table 3 presents data about the influence of the breed on the silkworms vitality, therefore receptivity to artificial diet with 15% content of mulberry meal during the first three instars of larval development.

The datain the table show that the breed has reliable influence on vitality (P≤0.001) during all three instars of the worm development.

Table 4 presentsdataabouttheaveragevitality percentage (LS-means) and LS-valuations for the influence of breedson the survival rate during the first three instars of agedevelopment.

The mean values given in the table show that the percentage of specimens that had survived during the age development from the studied breeds varies widely: 53.76-100%; 26.49- 98.75% and 0- 95.67%, respectivelyforfirst, second and third instar. There is different dynamics in the change of the analyzed trait in the direction from first to third instar.

With very high degree of receptivity (over 90% survival) in the first age are characterized 31.11% of the breeds, high (85-90%) - 37.78%, and with normal degree (70-85%) - 13.33% of the breeds included in the study. Low (60-70%) and very low (<60%) degree of receptivity have, respectively, 13.34% and 4.44% of the breeds.

The established% of survival of the breeds included in this study gives us reason to believe that the majority of them (82.22%) during the first ageshow high affinity to artificial diet low in mulberry meal, while only 17.78% have low and very low level of receptivity. In the second instar the proportionbetweenbreedswithhigh% of vitality to thosewithlow% is 60/40, respectively, and from all 45 surveyedbreeds, 8.9% haveveryhigh, 17.8% - highand 33.3% - normaldegree ofreceptivity. Those with low and very low degree are 8.9% and 31.1%, respectively. Thenumber of breedswithhighdegree of receptivity to artificial diet in the third instar is greatly reduced. Only 15.56% have over 70% vitality, of which 11.12% have normal, 2.22% - high and 2.22% - very high receptivity. Breeds with low (17.78) and very low (66.66%) degree of receptivity comprise a total of 84.44%. The specimens of 4.44% of the breeds do not survive the third instar.

The analysis of the data in Table 4 shows that despite the tendency of lowering the survival rate in the direction from first to third instar, some of the breeds retain high survival rate (over 70%) in all three instars of caterpillar stage. This gives us reason to believe that they have strong affinity to artificial diet with 15% content of dried mulberry leaf. These are the breeds "Almeria esp 1" (100.00% → 95.67%), "Nig 2" (97.94% → 80.05%), "Vratsa 2003" (96.72% → 85.88%) and "Veslets 1" (95.91% → 72.99%).

From presented LS-valuations in Table 4 it is evident that the degree and direction of diversion in the mean values of the survival rate of the breeds analyzed also vary widely compared to the average for the model: from 16.04 to -30.2 in the first; from 20.93 to -51.44 in the second and from 46.73 to -48.94 in the third instar.

Higher than average values for the model in the first instar are 68.89% of the breeds, the highest LS-valuations have: "Almeria esp. 1"(16.04), "Syria 1"(15.31),"Nig 2"(13.98), "Veslets 1"(12.79) and "Vratsa 2003" (12.76). Lower than the average survival rate for the model have 31.11% of the total number of breeds analyzed, the most pronounced this is in "Mayak 5" (-30.2) and "BN/ 4" (-29.14). In the second instar the number of breeds with positive LS-valuations is 8.89% less than in the first, while the number of those with negative values is 8.89% more. The highest positive LS-valuation shave "Nig 2" (20.93) and "Veslets 1" (20.69), and the negative ones are "BN/ 4" (-51.44), "Vratsa 38" (-44.04) and "Mir 5 "(-31.62).

The percentage of breeds with positive LS-valuations in the third instar is 53.33% and with negative - 46.67%. The most pronounced excess above the average survival rate for the model have the breeds: "Almeria esp 1" (46.73), "Vratsa 2003" (36.95) "Vratsa 2006" (35.70) "Vratsa 63" (31.67) and "Nig 2" (31.11). The lowest

values of survival compared to the average for the model have the breeds "BN/ 4" and "1g" with LS-valuations (-48.94), breed "20" with (-43.38) and "Vratsa 38" with (-42.69).

Lower limit of survival in rearing with artificial food is over 70% vitality.

Figure.1 graphically depicts the percentage of breeds above and below the value accepted as lower limit in the first three instars of silkworm age development.

The results of this study confirmed the high survival rate (over 70%) established by Guncheva *et al.,* (2015) in rearing breeds with artificial diet with 25% of mulberry leaf powder content.

The data in Figure 1 show that when reared with artificial diet with 15% content of dried mulberry leaf 82.22% of the breeds in first age also have > 70% vitality. Therefore, the majority of breeds retain high affinity to artificially prepared diet despite the lowered content of mulberry powder in it.

Table.1 Temperature-humidity Conditions for Rearing Silkworms with Artificial Diet

Instar	Temperature, °C	Humidity, %
I	29-30	85
II	29-30	85
III	27-28	80

Table.2 Criteria for Assessing the Degree of Receptivity of Silkworms to Artificial Food

Degree of receptivity	Vitality (%)
Very high	Over 90
High	85 - 90
Normal	70 - 85
Low	60 - 70
Very low	Under 60

Table.3 Influence of Breed on the Survival Rate during Age Development

| Sources of variation	df	F	P
First instar	44	6.42	0.00***
Second instar	44	4.72	0.00***
Third instar	44	3.80	0.00***

***P≤0.001

Table.4 LS-means and LS-valuations for the Impact of Breeds on the Survival Rate of
Silkworms during their Age Development

№	Breed	N	I instar		II instar		III instar	
			LS-mean±SE	LS-valuation	LS-mean±SE	LS-valuation	LS-mean±SE	LS-valuation
	Averagemodel	**180**	**83,96 ± 1,07**		**70,53 ± 1,80**		**48,94 ± 2,29**	
1	**Veslets1**	4	96,74 ± 2,36	12,79	91,21 ± 3,17	20,69	72,99 ± 8,2	24,06
2	**Ogosta 1**	4	87,07 ± 4,58	3,12	84,33 ± 5,23	13,81	69,01 ± 11,88	20,07
3	**Valve 111**	4	86,72 ± 3,22	2,76	82,77 ± 4,41	12,24	57,64 ± 2,25	8,70
4	**Vratsa 55**	4	88,31 ± 2,28	4,35	87,67 ± 1,68	17,14	64,13 ± 14,17	15,19
5	**Vratsa 63**	4	90,94 ± 4,23	6,98	88,37 ± 5,53	17,85	80,61 ± 2,96	31,67
6	**Hebur 1/18**	4	92,12 ± 2,97	8,17	86,88 ± 3,49	16,35	63,88 ± 8,27	14,95
7	**VBAS**	4	91,89 ± 3,69	7,94	79,14 ± 11,47	8,62	48,89 ± 23,99	-0,05
8	**SB- 1**	4	73,58 ± 4,69	-10,37	63,03 ± 1,33	-7,50	37,15 ± 5,89	-11,79
9	**VB- 1**	4	91,89 ± 4,39	7,94	50 ± 14,01	-20,53	39,74 ± 15,8	-9,19
10	**19**	4	91,57 ± 1,19	7,61	42,62 ± 17,13	-27,90	30,17 ± 18,69	-18,77
11	**1013**	4	87,58 ± 3,71	3,63	54,04 ± 21,34	-16,49	38,34 ± 22,15	-10,59
12	**Vratsa 38**	4	62,92 ± 5,48	-21,04	26,49 ± 8,13	-44,04	6,25 ± 6,25	-42,69
13	**157 К**	4	93,89 ± 1,4	9,94	81,13 ± 7,25	10,61	63,45 ± 11,13	14,51
14	**HB- 2**	4	77,05 ± 4,49	-6,91	64,21 ± 7,22	-6,32	51,87 ± 5,88	2,93
15	**HBShV**	4	85,57 ± 5,37	1,62	79,41 ± 6,93	8,89	46,75 ± 8,76	-2,19
16	**Ogosta 2/21**	4	79,15 ± 4,14	-4,8	58,72 ± 13,34	-11,81	43,97 ± 15,53	-4,97
17	**Vratsa 2003**	4	96,72 ± 2,57	12,76	89,4 ± 4,81	18,87	85,88 ± 3,71	36,95
18	**Vratsa2006**	4	87,83 ± 2,89	3,88	86,17 ± 2,25	15,64	84,64 ± 2,62	35,70
19	**Mayak 5**	4	53,76 ± 8,64	-30,2	48,76 ± 8,88	-21,77	24,32 ± 15,53	-24,61
20	**Mayak 6**	4	87,76 ± 3,48	3,8	59,2 ± 9,64	-11,32	38,8 ± 6,58	-10,14
21	**Vatan**	4	88,86 ± 6	4,9	84,26 ± 6,79	13,74	55,07 ± 7,08	6,13
22	**Sh – 3**	4	87,31 ± 6,46	3,36	84,82 ± 5,7	14,29	46,49 ± 16,4	-2,45
23	**Mir 5**	4	63,18 ± 12,77	-20,78	38,9 ± 14,1	-31,62	21,83 ± 10,1	-27,10
24	**E- 2**	4	78,28 ± 3,43	-5,68	71,76 ± 3,86	1,23	69,05 ± 4,39	20,12
25	**E- 9**	4	81,05 ± 4,02	-2,91	66,7 ± 13,45	-3,83	39,34 ± 19,43	-9,60
26	**E- 13**	4	85,6 ± 8,31	1,64	74,69 ± 12,48	4,17	66,92 ± 10,06	17,98
27	**E- 31**	4	87,17 ± 1,79	3,21	85,89 ± 2,2	15,36	68,26 ± 10,04	19,32

№	Breed	N	I instar		II instar		III instar	
			LS-mean±SE	LS-valuation	LS-mean±SE	LS-valuation	LS-mean±SE	LS-valuation
	Averagemodel	180	83,96 ± 1,07		70,53 ± 1,80		48,94 ± 2,29	
28	Mziuri1	4	65,23 ± 2,02	-18,73	57,92 ± 2,66	-12,60	34,21 ± 8,68	-14,72
29	Nig 2	4	97,94 ± 1,33	13,98	91,46 ± 3,92	20,93	80,05 ± 8,57	31,11
30	TV	4	62,67 ± 3,22	-21,29	56,75 ± 2,97	-13,78	49,51 ± 7,61	0,57
31	Banyasa Alba	4	89,68 ± 3,62	5,72	79,12 ± 4,15	8,59	33,02 ± 13,59	-15,91
32	Banyasa 1	4	89,16 ± 1,3	5,2	79,5 ± 5,41	8,98	71,4 ± 8,33	22,46
33	BN/ 4	4	54,82 ± 4,58	-29,14	19,08 ± 11,06	-51,44	0 ± 0	-48,94
34	VAR	4	83,85 ± 6,07	-0,1	61,23 ± 14,97	-9,30	29,34 ± 13,72	-19,60
35	Syria1	4	99,26 ± 0,74	15,31	88,69 ± 6,27	18,16	61,08 ± 18,7	12,14
36	Ukrainian 18	4	91,09 ± 2,9	7,14	86,82 ± 4,7	16,30	50,98 ± 9,64	2,04
37	Ukrainian20	4	86,07 ± 3,3	2,11	73,85 ± 6,41	3,32	59,29 ± 9,72	10,35
38	Merefa 6	4	94,44 ± 3,4	10,49	75,69 ± 5,43	5,17	54,78 ± 10,56	5,84
39	Merefa 7	4	94,23 ± 5,77	10,28	91,73 ± 5,07	21,21	56,39 ± 13,27	7,45
40	Line 22	4	89,75 ± 3,52	5,79	82,86 ± 3,02	12,34	51,5 ± 13,8	2,56
41	Almeria esp 1	4	100 ± 0	16,04	98,75 ± 1,25	28,22	95,67 ± 1,85	46,73
42	Almeria esp	4	87,57 ± 4,1	3,62	75,26 ± 8,9	4,73	37,91 ± 20,85	-11,03
43	1v	4	62,84 ± 5,22	-21,11	41,87 ± 12,2	-28,65	16,04 ± 10,1	-32,90
44	1w	4	67,99 ± 9,19	-15,96	47,56 ± 5,78	-22,96	0 ± 0	-48,94
45	20	4	86,9 ± 5,26	2,95	54,92 ± 11,91	-15,60	5,56 ± 5,56	-43,38

Figure.1 Percentage of Breeds with High (> 70%) and Low (<70%) Receptivity to Artificial Diet with Low Mulberry Powder Content
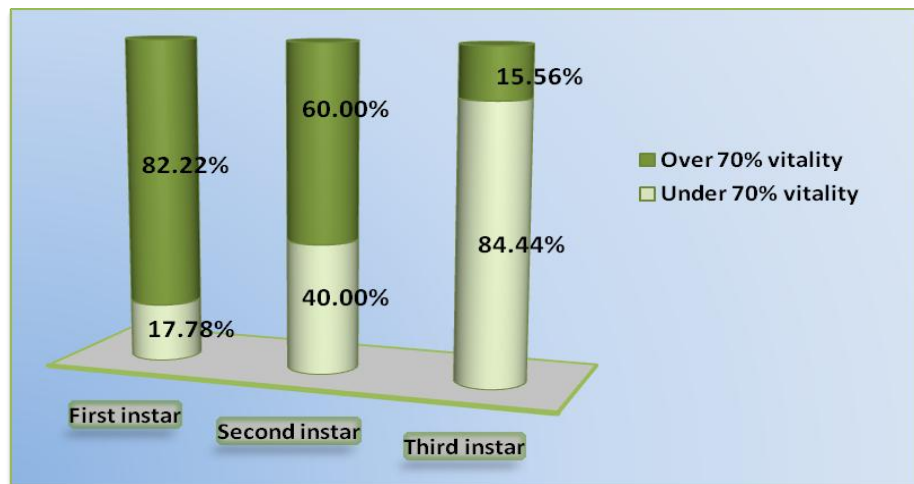
Despite the established trend for reducing the survival rate with advance in the age development, we believe that most of the breeds have high biological potential for rearing with artificial diet which can be used in the selection process. A reason for this are the results by Saviane *et al.,* (2013) and Nair *et al.,* (2011). Through direct selection the latter manage to increase significantly the receptivity of silkworms to artificial diet. In ninth generation the nutritional responseof specimens from the studied species reaches over 78%, while in hybrids bred in the course of 12 generations the adaptation to artificial diet is much greater (85%).

The 45 breeds from the genetic bank of SAES – Vratsa included in the study have the potential that allows their improvement interms of receptivity to artificial diet with reduced content of meal from dried mulberry leaf.

The breed has high reliable impactonthe survivalrate (P≤0.001) in all three instars of the agedevelopment.

The survival of the generations from the tested breeds (*Bombyx mori* L.) varies widely (53.76- 100%; 26.49- 98.75% and 0-95.67%, respectively for first, second and third instar).

Of the breeds under study survival rate over 70% have 82.22%, 60% and 15.56%, respectively for first, second and third instar. In addition to interbreed, there is also considerable intra breed variation of the survival rate during age development.

Strongaffinity to artificialdiet with 15% content of dried mulberry leaf in all three instars is shown by the breeds "Almeriaesp 1" (100.00%→ 95.67%), "Nig 2" (97.94%→ 80.05%), "Vratsa 2003"(96.72%→ 85.88%) and"Veslets 1"(95.91%→ 72.99%).

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