Available online at www.ijpab.com

DOI: http://dx.doi.org/10.18782/2582-2845.8867

ISSN: 2582 – 2845

Ind. J. Pure App. Biosci. (2021) *9*(4), 199-204

Indian Journal of Pure & Applied Biosciences

Peer-Reviewed, Refereed, Open Access Journal

Research Article

Studies on the Correlation between Haemolymph Glucose Level and Commercial Characters of *Bombyx mori* L.

P. H. Thejaswini[1] and H. B. Mahesha[2*]

[1]Department of Biotechnology, Maharani's Science College for Women, Mysuru-570 005

[2]Department of Sericulture, Yuvaraja's College, University of Mysore, Mysore-570 005, India

*Corresponding Author E-mail: hbmseri@gmail.com

Received: 9.06.2021 | Revised: 15.07.2021 | Accepted: 26.07.2021

ABSTRACT

Eight mulberry pure silkworm breeds namely Pure Mysore, Nistari, C. nichi & Hosa Mysore (multivoltines), CSR_2, NB_4D_2, NB_{18} & KA (bivoltines) and four hybrids (Pure Mysore x CSR_2, Nistari x NB_4D_2, C. nichi x KA and Hosa Mysore x NB_{18}) were selected for the present study. During fifth instar haemolymph glucose level was estimated with a regular interval of 24h and also average concentration was calculated. The commercial characters viz., fecundity, larval weight, cocoon weight, shell weight, shell ratio, filament length, denier and renditta were selected and rearing data was recorded. The average concentration of glucose was subjected to regression analysis against selected commercial characters. Of the 40 regression analysis eighteen results showed significant high positive correlation coefficient between haemolymph glucose level and commercial characters of silkworm Bombyx mori L.

Keywords: *Silkworm, Bombyx mori, Haemolymph, Glucose, Commercial Characters.*

INTRODUCTION

Ever since its inception, more than four thousand years ago, sericulture is playing an important role in the economic life of man (Krishnaswami et al., 1973). Due to its great economic value, more than 3000 different genotypes of silkworm were maintained in Europe and Asia. Recent advances in plant and animal breeding programmes have highlighted the prospects of using linked molecular markers for improvement of desirable traits. Therefore, identification of suitable markers, holds the key to successful implementation of marker assisted selection which is gaining ground fast in other fields of breeding Datta & Ashwath, 2000). Such supportive correlation studies in silkworm *Bombyx mori* were reported by several scientists *viz*., correlation between yield and biochemical parameters (Chatterjee, et al., 1993) between commercial characters with proteins (Kasmaei & Mahesha, 2012),

Cite this article: Thejaswini, P. H., & Mahesha, H. B. (2021). Studies on the Correlation between Haemolymph Glucose Level and Commercial Characters of *Bombyx mori* L., *Ind. J. Pure App. Biosci. 9*(4), 199-204. doi: http://dx.doi.org/10.18782/2582-2845.8867

commercial characters with amylase (Kasmaei & Mahesha, 2012), commercial characters with esterase (Kasmaei & Mahesha, 2012), commercial characters with alkaline phosphatase (Kasmaei et al., 2012), commercial characters with succinate dehydrogenase (Kasmaei & Mahesha, 2012) and commercial characters with fat body biomolecules (Mahesha & Fashid, 2013). Also, correlation studies between DNA, RNA & proteins of silkworm *Bombyx mori* (Mahesha et al., 2013) were reported. Haemolymph glucose level during cypovirus infection (Mahesha & Thejaswini, 2013) and in F_1 progeny raised from EMS treated silkworms (Mahesha & Thejaswini, 2013) were also reported. However, correlation studies between biomolecules like haemolymph glucose level and commercial characters of silkworm *Bombyx mori* are rather scarce. Hence, the present investigation was undertaken.

MATERIAL AND METHODS

Eight mulberry pure silkworm strains namely Pure Mysore, Nistari, C. nichi & Hosa Mysore (multivoltines), CSR_2, NB_4D_2, NB_{18} & KA (bivoltines) and four hybrids (Pure Mysore x CSR_2, Nistari x NB_4D_2, C. nichi x KA and Hosa Mysore x NB_{18}) were selected for the present study. Silkworm breeds obtained the Department of Studies in Sericultural Sciences, University of Mysore, Mysore and the silkworm rearing was conducted in the laboratory following the method described by Krishnaswami (Krishnaswami, 1978). All experimental batches were maintained in triplicate. In each replication 500 larvae were kept after third moult. The economic traits selected for present study included fecundity, weight of fifth instar larva, cocoon weight, shell weight, shell ratio, filament length, denier and renditta. After rearing the data on commercial characters were evaluated and recorded (Mahesha, 1997).

About 2-4 larvae were collected daily at regular interval of 24 hours from day one (after first two feedings) from each batch until the onset of spinning. The abdominal legs were punctured and the haemolymph was collected in pre-chilled eppondorf tubes containing 1mM thiourea to prevent oxidation (Mahesha, 1997).

Haemolymph glucose level was estimated by following the method of Folin-Wu as described by Oser (Oser, 1976). The blood glucose level was expressed as µg glucose per µl haemolymph.

The experimental data were statistically analyzed through SPSS by one way ANOVA (Fisher & Yates, 1953) and Scheffe's post hoc test (Scheffe, 1959) wherever they were applicable. The regression analysis was applied to determine the relationship between glucose and commercial characters relationship using the equation $Y = bx + a$.

RESULTS AND DISCUSSION

The summary of the studied commercial characters are presented in the table 1. From results, it is clear that the bivoltine breeds are superior for productivity aspects over multivoltines and the hybrid silkworms showed mid parental values. The results of one way ANOVA revealed that the variation in all commercial characters among the experimental batches are all significant at 0.1 % ($P<0.001$). The haemolymph glucose levels showed significant changes in their levels at every 24 hours till the end of fifth instar (Table 2). The average highest concentration of glucose among multivoltines breeds was observed in Hosa Mysore breed (3.378µg/µl) followed by Nistari (3.125 µg/µl), Pure Mysore (3.105 µg/µl) and C. nichi (2.669 µg/µl). The average highest concentration of glucose among bivoltines was observed in CSR_2 breed (2.714 µg/µl) followed by NB_4D_2 (2.64 µg/µl), KA (2.204 µg/µl) and NB_{18} (2.221 µg/µl). The average highest concentration among hybrids was observed in Hosa Mysore x NB_{18} (2.989 µg/µl) followed by Nistari x NB_4D_2 (2.928 µg/µl), Pure Mysore x CSR_2 (2.819 µg/µl) and C. nichi x KA (2.542 µg/µl). The results of statistical analysis revealed that the variation among the experimental batches are all found to be

significant at 0.1 % (P<0.001). The results of regression analysis between haemolymph glucose level and commercial characters are presented in Table 3. The results of regression analysis among multivoltines clearly showed that the haemolymph glucose level has highly positive correlation with cocoon weight (R^2=0.8971), shell weight (R^2=0.7094), shell ratio (R^2=0.5075), filament length (R^2=0.7385) and renditta (R^2=0.7648). Further, the haemolymph glucose level has moderately high positive correlation with larval weight (R^2=0.4408) and weak positive correlation with fecundity and denier exhibited weak negative correlation coefficient (R^2=0.01300). In case of bivoltines breeds, the results of regression analysis clearly indicated that haemolymph glucose level has highly positive correlation with cocoon weight (R^2=0.5053) and denier (R^2=0.5173) only. Further, the haemolymph glucose level exhibited moderately high positive correlation coefficient with shell weight (R^2=0.4671), shell ratio (R^2=0.4974), filament length (R^2=0.3987) and renditta (R^2=0.4720). In case of multi and bivoltines breeds with haemolymph glucose level exhibited moderately high positive correlation with renditta (R^2=0.1473) only. In case of hybrids, the results of regression analysis clearly indicated that haemolymph glucose level has highly positive correlation with larval weight (R^2=0.8861) and filament length (R^2=0.7501) only. The haemolymph glucose level has moderately high positive correlation with fecundity (R^2=0.1744), cocoon weight (R^2=0.4272), shell weight (R^2=0.4286), shell ratio (R^2=0.4575) and denier (R^2=0.05771). The results of regression analysis between haemolymph glucose level with commercial characters of all silkworm breeds showed negative correlation with all commercial characters of silkworm except renditta (+R^2=0.07603).

Analysis of correlation between silkworm haemolymph glucose level and commercial characters clearly indicated three types of correlations *i.e*., positive, negative or neutral correlations. Further, almost all significant positive correlations exhibited breed specificity that is limited to either multivoltines or bivoltines or hybrids only. And there is no significant positive or negative correlation coefficient between different voltine groups and/or their hybrids. Further, the haemolymph glucose level exhibited correlation with larval characters only and not with adult characters as it is indicated by both multi and bivoltines *i.e*., fecundity exhibited week positive and strong negative correlations in multivoltines and bivoltines respectively. Of the forty correlation studies, nine results exhibited highly strong positive correlation coefficient (*i.e.*, R^2 is more than 0.5) relationships. The studies on haemolymph glucose level is of paramount importance in the growth and development of organisms, as it is the carbon source of cells. Further, the haemolymph, the carrier of all nutrient substances distributes to each and every part of the body for cellular metabolism, wherein the micromolecules get converted into complex macromolecules like proteins and carbohydrates and also major reservoir of all nutrients received from midgut after digestion. The haemolymph blood glucose level showed variation among breeds and with growth. This difference in blood glucose level might be due to variation in ingestion, digestion and assimilation of food, as these are all directly depending up on various factors during silkworm rearing besides genetic material. Therefore, by studying haemolymph glucose level with commercial characters, it is possible to have a clear picture of the kind and degree of correlation coefficient between them. Such correlation studies between silkworm biomolecules and economically important characters are reported in our earlier work (Kasmaei & Mahesha, 2012), (Kasmaei & Mahesha, 2012), (Kasmaei & Mahesha, 2012), (Kasmaei et al., 2012), (Kasmaei & Mahesha, 2012), (Mahesha & Fashid, 2013), (Mahesha et al., 2013). An understanding of such correlations will help us to exploit the marker molecule during selection procedure for breeding of new breeds of silkworm *Bombyx mori* with improved commercial characters.

Table 1: Amount of Haemolymph Glucose during fifth instar (Values expressed as µg/µl)

Fifth Instar Development / Silkworm Breeds	1st Day	2nd Day	3rd Day	4th Day	5th Day	6th Day	7th Day	8th Day	Average concentration
Pure Mysore	3.921	3.890	3.660	2.865	2.174	2.440	2.985	2.910	3.105
Nistari	3.632	3.499	3.398	2.996	2.576	2.651	-	-	3.125
C. nichi	3.240	3.112	2.813	2.561	1.981	2.312	-	-	2.669
Hosa Mysore	3.962	3.912	3.781	2.985	2.254	-	-	-	3.378
CSR_2	1.923	1.842	2.83	2.912	2.962	3.981	-	-	2.741
NB_4D_2	1.882	1.671	2.573	2.617	2.877	3.980	-	-	2.64
KA	1.622	1.524	2.416	2.522	2.765	3.683	-	-	2.404
NB_{18}	1.520	1.489	2.125	2.132	2.621	3.442	-	-	2.221
Pure Mysore x CSR_2	3.412	3.371	3.430	2.751	1.941	2.192	2.642	-	2.819
Nistari x NB_4D_2	3.462	3.387	3.181	2.664	2.464	2.415	-	-	2.928
C. nichi x KA	3.121	2.921	2.814	2.314	1.871	2.213	-	-	2.542
Hosa Mysore x NB_{18}	3.221	3.323	3.481	2.481	2.241	-	-	-	2.989

The variation between experimental sets, between age groups and the interaction effect between experimental sets and age groups are all found to be statistically significant at 0.1% (P<0.001).

Table 2: Mean values ± SD of nine commercial characters in twelve breeds of silkworm, *Bombyx mori* L.

Commercial Traits / Silkworm Breeds	Fecundity	Larval Weight (g)	Larval Duration (h)	Cocoon Weight (g)	Shell Weight (g)	Shell Ratio (%)	Filament Length (m)	Denier	Renditta
Pure Mysore	467.22±10.96	2.01±0.06	660±10.39	1.02±0.07	0.12±0.01	12.57±0.49	426.44±19.83	1.77±0.09	11.77±0.82
Nistari	485.11±05.30	2.83±0.06	564.88±10	1.14±0.07	0.15±0.01	13.41±0.87	435.66±17.21	1.78±0.07	13.26±0.24
C. nichi	376.11± 18.06	2.14±0.05	537±28.16	0.890±0.0	0.114±0.02	12.8±0.947	390±18.22	1.88±0.08	14.12±0.23
Hosa Mysore	385.12±11.02	2.91±0.06	537±12.24	1.259±0.06	0.179±0.02	14.21±0.97	561±16.28	1.89±0.08	9.64±0.65
CSR_2	509.10±16.58	4.07±0.05	578.88±6.45	1.81±0.05	0.43±0.01	24.02±0.18	1011.99±12.3	2.93±0.22	5.78±0.23
NB_4D_2	520.55±16.65	4.16±0.05	576.67±11.1	1.76±0.03	0.35±0.01	20.27±0.15	1020±29.96	2.48±0.06	8.34±0.47
KA	518.41± 14.21	4.08±0.07	561±23.89	1.694±0.04	0.343±0.02	20.24±0.17	992±28.12	2.46±0.08	8.22±0.22
NB_{18}	528.23±16.66	4.11±0.06	576±12.1	1.74±0.04	0.35±0.02	20.11±0.16	1004±29.25	2.45±0.07	8.38±0.44
Pure Mysore x CSR_2	466.66±11.52	2.68±0.07	610±11.10	1.67±0.02	0.28±0.01	17.29±0.21	910±18.74	2.75±0.06	7.64±0.12
Nistari x NB_4D_2	490.77±06.81	3.46±0.04	557±10.21	1.47±0.02	0.23±0.01	16.06±0.85	805.99±12.4	1.83±0.02	9.22±0.85
C. nichi x KA	380.14±15.13	2.22±0.05	537±10.44	1.214±0.03	0.172±0.02	14.16±0.36	453.87±14.33	1.91±0.04	11.52±0.36
Hosa Mysore x NB_{18}	390.14±10,11	3.37±0.08	564±10.22	1.48±0.05	0.237±0.03	16.01±0.18	855±14.55	2.32±0.07	9.82±0.67

Values are the mean± SD of Pre monsoon, Monsoon and post monsoon observations. The variation between the breeds and hybrids is statistically significant at 0.1 % (P<0.001).

Table 3: Summary of the Regression Analysis between Glucose level and Commercial Characters

Glucose Level ↓	Fecundity	Larva weight	Cocoon weight	Shell weight	Shell ratio	Filament length	denier	Renditta
Among Multi voltines	Y = 46.61X + 285.3 R^2 = 0.06053	Y = 1.045X - 0.7343 R^2 = 0.4408	Y = 0.5098X - 0.4876 R^2 = 0.8971	Y = 0.08576X - 0.122 R^2 = 0.7094	Y = 1.774X + 7.802 R^2 = 0.5075	Y = 217.7X - 215.3 R^2 = 0.7385	Y = - 0.02470X + 1.91 R^2 = 0.01300	Y = - 5.829X + 30.09 R^2 = 0.7648
Among Bivoltines	Y = - 28.46X + 590.3 R^2 = 0.7175	Y = 0.001639X + 4.10 -	Y = 0.1458X + 1.386 R^2 = 0.5053	Y = 0.1205X + 0.0669 R^2 = 0.4671	Y = 5.742X + 6.796 R^2 = 0.4974	Y = 32.19X + 926.5 R^2 = 0.3987	Y = 0.7172X + 0.7860 R^2 = 0.5173	Y = - 3.719X + 16.98 R^2 = 0.4720
Between Multi and Bivoltines	Y = - 89.35X + 722.6 R^2 = 0.3288	Y = - 1.497X + 7.409 R^2 = 0.3914	Y = - 0.5691X + 2.999 R^2 = 0.3497	Y = - 0.2022X + 0.817 R^2 = 0.3926	Y = - 7.086X + 36.94 R^2 = 0.3898	Y = - 530.4X + 2207 R^2 = 0.4768	Y = - 0.6919X + 4.132 R^2 = 0.3935	Y = 2.807X + 2.120 R^2 = 0.1473
Among Hybrids	Y = 116.2X + 104.3 R^2 = 0.1744	Y = 2.802X - 4.967 R^2 = 0.3288	Y = 0.6181X + -0.2843 R^2 = 0.4272	Y = 0.1469X - 0.1843 R^2 = 0.4286	Y = 4.410X + 3.447 R^2 = 0.4574	Y = 902.9X - 1790 R^2 = 0.7501	Y = 0.5140X + 0.7534 R^2 = 0.05771	Y = - 4.559X + 22.40 R^2 = 0.3168
Among all Breeds	Y = 72.01X + 661.2 R^2 = 0.1554	Y = - 1.080X + 6.190 R^2 = 0.1886	Y = - 0.4469X + 2.68 R^2 = 0.2160	Y = - 0.1691X + 0.72 R^2 = 0.2862	Y = - 6.034X + 33.64 R^2 = 0.2939	Y = - 385.5X + 1817 R^2 = 0.2326	Y = - 0.5712X + 3.80 R^2 = 0.2112	Y = 2.043X + 4.096 R^2 = 0.07603

CONCLUSION

The present results clearly indicated that the haemolymph glucose level exhibited very high positive correlation with single cocoon weight (R2=0.8971), single shell weight (R2=0.7094), shell ratio (R2=0.5075) and filament length (R2=0.7385) in multivoltines breeds. In case of bivoltines breeds, single cocoon weight (R2=0.5053) and denier (R2=0.5173) showed highly positive correlation with glucose level. In hybrids, except larval weight (R2=0.8861) moderately high positive correlation with glucose level. The information gathered in this research work may be used during the breeding of new silkworm breeds with better economic characters.

Acknowledgments

Authors wish to thank University of Mysore for extending the facilities to carry out this work.

Funding

The author(s) received no financial support for the research, authorship, and/or publication of this article.

Conflict of Interest

The author declares no conflict of interest.

Author Contribution

Both author contributed equally to establishing the topic of the research and design experiment.

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