Studies of the silkworm enzyme activity and their correlations with economic variables

Mohd Younus Wani[1,*], Nisar Ahmad Ganie[1], Mushtaq Rasool Mir[2], Shakeel Ahmad Mir[3], Munazah Yaqoob[4], Sergiy Yeremenko[5,*], Imtiyaz Murtaza[6], Tanveer Ahmad Wani[7], Mohd Farooq Baqual[8], Oleksii Sukennikov[9]

[1]Division of Cocoon Crop Production, COTS, Mirgund, SKUAST-K, Shalimar, Srinagar, India
[2]Division of BSH, COTS, Mirgund, SKUAST-K, Shalimar, Srinagar, India
[3]Division of Agristatistics, SKUAST-K, Shalimar, Srinagar, India
[4]Division of Entomology, FOA, Wadura SKUAST-K, Shalimar, Srinagar, India
[5]Institute of Public Administration in the Sphere of Civil Protection, Kiev, Ukraine
[6]Division of BSH, SKUAST-Kashmir, Shalimar, Srinagar, India
[7]Division of Plant Pathology, FOA, Wadura SKUAST-K, Shalimar, Srinagar, India
[8]Division of Host Plant Production, College of Temperate Sericulture, Mirgund, SKUAST-K, Shalimar, Srinagar, India
[9]Open International University of Human Development "Ukraine"

*Corresponding author's e-mail: Mohd Younus Wani, younus.wani6922@gmail.com; Sergiy Yeremenko, esamns71@gmail.com

Received: 30 November 2022 / Accepted: 7 February 2023

Abstract. The experiment was conducted to analyse the larval performance and economic traits of bivoltine silkworm breeds of silkworm (SK-1, SK-6, SK-22, SK-28, SK-33, CSR4, CSR2, NB4D2, DUN6 and APS4) during spring season. The haemolymph total protein, succinate and glutamate dehydrogenase activities were estimated and their correlation with economic traits were also worked out. The results of the study confirmed that among ten bivoltine silkworm breeds, highest haemolymph SDH activity of 3.47 µmoles/ml/mgprotein/min was recorded in the silkworm breed SK1 and least SDH activity of 1.58 µmoles/ml/mg protein/min was recorded in the breed APS4. The highest peak of succinate dehydrogenase activity of 2.65 µmoles/ml/mg protein/min was observed on 7th day of the 5th instar and lowest peak of succinate dehydrogenase activity of 2.39 µmoles/ml/mg protein/min was observed on 4th day of the 5th instar. GDH activity of 0.46 µmoles/ml/mg protein/min was recorded highest in the silkworm breed SK1 and lowest of 0.15 µmoles/ml/mg protein/min was recorded in silkworm breed APS4. The highest peak of haemolymph GDH of 0.36 µmoles/ml/mg protein/min was recorded on 7th day of 5 th instar and lowest peak of 0.26 µmoles/ml/mg protein/min was recorded on 4th day of 5th instar. The correlational studies revealed that haemolymph total protein, SDH and GDH were found to be positively corelated with yield by weight and number (cocoon), weight of mature larvae, shell weight, cocoon weight, shell ratio percent, silk productivity, rate of pupation, fecundity, raw silk percentage and length of filament. Thus, the study revealed that silkworm breeds like SK1, SK6, SK22 and SK28 as productive breeds and hence may be used for future breeding programmes for evolution of new robust silkworm breeds.

Key words: Fifth instar, Haemolymph, Silkworm, Textiles, activities of succinate and glutamate dehydrogenases, Peak value.

Abbreviations:

SK-1, SK-6, SK-22, SK-28, SK-33, CSR4, CSR2, NB4D2, DUN6 and APS4 - different breeds of silkworm

DCCI – Division of cocoon crop improvement

CoTS – College of temperate sericulture, Mirgund

BSH – Basic science and humanities, SKUAST-Kashmir

FOH - Faculty of horticulture, SKUAST-Kashmir

SDH - succinate dehydrogenase

GDH - gultamate dehydrogenase

CRD- Completely randomised design

CPCS – Statistical software

PTU - phenyl thiourea

PBS - phosphate buffer saline

BSA – bovine serum albumin

INT - p-Iodonitrotetrazolium Violet

1. Introduction

The quantity and quality of silk is affected by a variety of elements such as mulberry leaf quality, ambient circumstances, and breed nature. The union territory of Jammu and Kashmir is situated at the same height as the world's leading bivoltine countries and enjoys a favourable climate to produce high-quality bivoltine silk. It's critical to find potential genotypes that are well-suited to the valley agroclimatic conditions to boost raw silk production. The second largest silk producing country is India after China, with an annual raw silk production to about 36152 MT during 2019-20, besides providing employment to 9.25 million persons in 2019-20 compared to 9.17 million persons in 2018-2019 (Anonymous, 2020). The union territory of Jammu and Kashmir has 2800 villages and about 30300 families are engaged in silkworm rearing and raw silk production (Anonymous, 2019). The availability of energy for various activities is directly related to the spinning of quality cocoons so the breeds having high succinate dehydrogenase and glutamate dehydrogenase activities spin quality cocoons. Furthermore, the nitrogen metabolism in insects leads to formation of ammonia, which is highly toxic, glutamate dehydrogenase pathway plays a key role to synthesis of amino acids from ammonia, hence improves the quality of cocoons. Larval haemolymph contains most abundant storage proteins or hexamerins (Sumino et al., 1980). As enzymes are proteins due to which they participate in balancing the sub cellular functions. Proteins make antibodies, hormones, and contractile elements of the cells. In insects' proteins are present abundantly in fat bodies and in the haemolymph. The fibre of silk is completely dependent on the protein content of the silkworm. The fifth instar haemolymph protein contributes towards silk protein biosynthesis in the silk gland and the final products are

fibroin and sericin which represents the main components of silk fibre (Shivkumar & Subramanya, 2015). The growth and development of silk gland depends on the health of silkworm and its nutritional status (Kumar & Gangwar, 2010). Dehydrogenases are of vital importance for analysing the metabolic activities and for carbohydrate metabolism (Horie, 1968). The relative activities of dehydrogenases may be related to the function and energy yielding demands of the tissues in insects (Dickinson & Sullivan, 1975). As no sufficient information is available regarding the enzyme activities in different bivoltine breeds of silkworm and their association with economic parameters under temperate conditions of Kashmir, hence the study was conducted.

2. Materials and Methods

In the spring 2018 and 2019, four disease-free layings of each of the ten silkworm breeds - SK-1, SK-6, SK-22, SK-28, SK-33, CSR4, CSR2, NB4D2, DUN6, and APS4 were purchased from the Division of Sericulture Crop Improvement at the College of Temperate Sericulture in Mirgund. These moths had previously been tested for pebrine disease by mother moth examination. The eggs were incubated for roughly 10–13 days until they hatched at a temperature of 25°C and a relative humidity of 80–85%. The farm of CoTS in Mirgund provided the two mulberry kinds Ichinose and Goshoerami, and selected leaves were sliced into appropriate sizes based on the stage of the silkworm larvae. After the second moult, the silkworm bed was cleaned once, and daily after that. The silkworms were kept in good condition till spinning. The cocoons were collected on the sixth day of spinning, and the following day, a selection of cocoons was made and they were stored in plastic trays. The moths that emerged on days 10 through 12 were given about 4 hours to pair up. The despaired females were kept in the oviposition cellules on craft brown paper. The egg sheets were cleaned by soaking them in a 2% formalin solution, and they were then allowed to dry at room temperature. Following oviposition, mother moth inspection was used to discover pebrine in the female moths. Within 24 hours following oviposition, all races' layings underwent acid treatment to stop the formation of diapause and to continue embryonic development (Raja, 2000; Anonymous, 2003). Three replications of each treatment were used in the CRD of the experiment. Each replication includes 200 silkworms that have survived the third moult and are the same age and size. In the springs of 2018 and 2019, the biochemical experiments were carried out in the biochemistry lab at DBSM, FOH, SKUAST-Kashmir. CPCS software was used to analyse the two years of data.

Figure 1. Different breeds of silkworm.

2.1. Haemolymph collection

On the fourth, fifth, sixth, and seventh days of the fifth instar larva, the thoracic legs were cut using a sterilised needle for haemolymph extraction, and the blood was promptly collected into Eppendorf tubes. The larvae were carefully squeezed from the anterior and posterior ends at the same time until no more haemolymph seeped out of the wound in order to ensure thorough extraction of the fluid. In pre-cooled tubes with a few crystals of PTU @ 1 pinch per tube, the haemolymph was collected. The haemolymph samples were mixed with PBS in a 1:5 ratio, succeed by centrifugation at 12000 rpm for five minutes to remove the haemoglobin and tissue debris. Phenyl thiourea was used to prevent the activity of prophenol oxidase followed by the melanization (Etebari et al., 2005) (Fig. 2). Haemolymph samples were kept in storage at -20°C until further research.

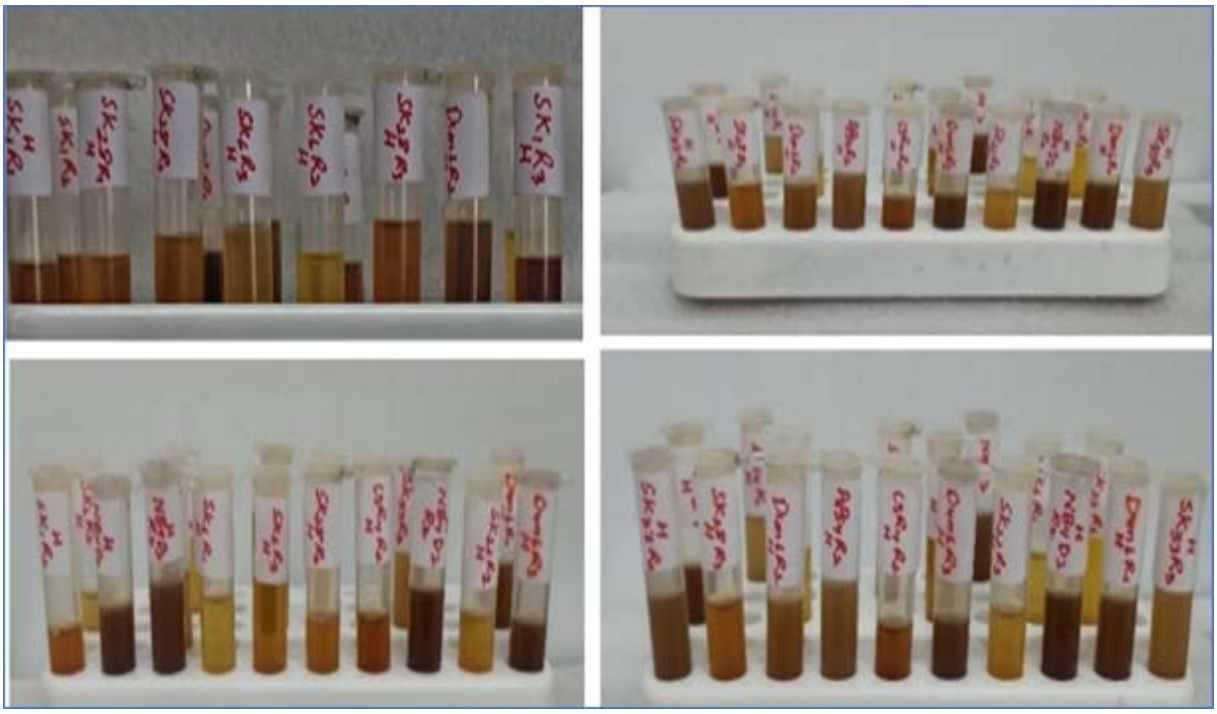

Figure 2. Haemolymph samples of 4th, 5th, 6th and 7th days of 5th instar

2.2. Quantitation of total protein

The quantitative estimation of total protein in haemolymph was calculated by (Lowry et al., 1951) using BSA as standard and expressed as mg of protein per ml of haemolymph.

2.3. Equalization of haemolymph protein in different races

The volume of haemolymph was made equal in terms protein so that the estimation of the enzyme activities was calculated more precisely (Table 1 and Figs. 3 and 4)

Table 1. Volume of haemolymph (microlitres) having one milligram protein in different breeds of silkworm (*Bombyx mori* L.) during spring season (Pooled of 2018 and 2019).

Season	**Spring 2018**					**Spring 2019**					**Pooled**				
Days / **Genotypes**	4th Day	5th Day	6th Day	7th Day	Mean	4th Day	5th Day	6th Day	7th Day	Mean	4th Day	5th Day	6th Day	7th Day	Mean
G_1:SK1	20.38	19.97	19.21	19.2	19.69	20.73	19.99	18.86	18.44	19.51	20.56	19.98	19.03	18.82	19.60^{i}
G_2:SK6	20.4	20.7	19.51	19.48	20.02	20.39	20.32	19.5	19.46	19.92	20.4	20.51	19.51	19.47	19.97^{h}
G_3:SK22	20.8	20.8	19.95	19.58	20.28	21.17	20.77	19.93	19.74	20.4	20.99	20.79	19.94	19.66	20.34^{g}
G_4:SK28	21.17	22.81	20.19	19.03	20.8	21.15	20.74	20.23	18.98	20.28	21.16	21.77	20.21	19	20.54^{f}
G_5:SK33	22.16	20.23	20.82	19.1	20.58	22.13	20.35	20.73	20.1	20.83	22.15	20.29	20.78	19.6	20.70^{f}
G_6:NB4D2	22.62	22.08	20.54	19.51	21.19	22.6	21.93	20.88	19.5	21.23	22.61	22.01	20.71	19.5	21.21^{e}
G_7:CSR4	28.36	27.12	26.42	22.45	26.09	28.35	28.02	26.49	22.46	26.33	28.36	27.57	26.46	22.46	26.21^{d}

G_8:CSR2	29.17	28.21	27.37	25.35	27.52	29.13	28.2	27.38	24.93	27.41	29.15	28.2	27.37	25.14	27.47^{c}
G_9:Dun6	30.08	29.23	27.46	25.95	28.18	30.08	29.23	27.46	25.96	28.18	30.08	29.23	27.46	25.95	28.18^{b}
G_{10}:APS4	49.46	45.17	41.12	38.1	43.46	49.43	45.21	41.14	38.2	43.49	49.44	45.19	41.13	38.15	43.48^{a}
Mean	26.46	25.63	24.26	22.77		26.52	25.48	24.26	22.78		26.49^{a}	25.55^{b}	24.26^{c}	22.78^{d}	
CD ($p \leq 0.05$)		Day: 1.32					Day: 0.33						Day: 0.29		
		Genotype: 0.35					Genotype: 0.41						Genotype: 0.32		
		Day x Genotype: 1.87					Day x Genotype: 1.85						Day x Genotype: 1.82		

Note: Each value represents the mean of three replications; Figures super scripted with same letter are statistically nonsignificant.

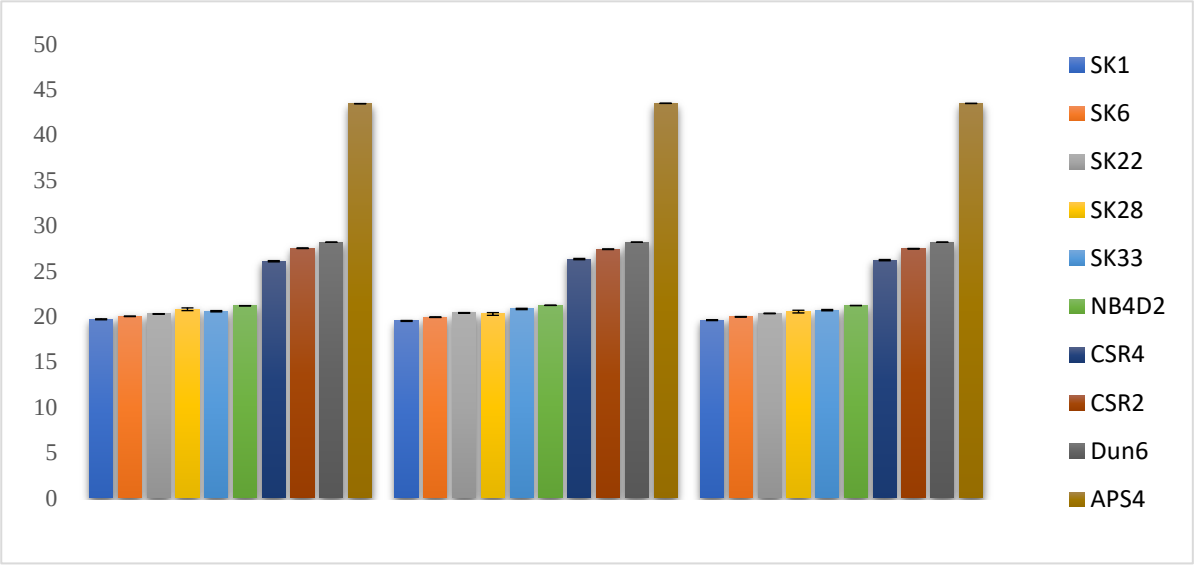

Figure 3. Protein equalisation of haemolymph in different races of silkworm.

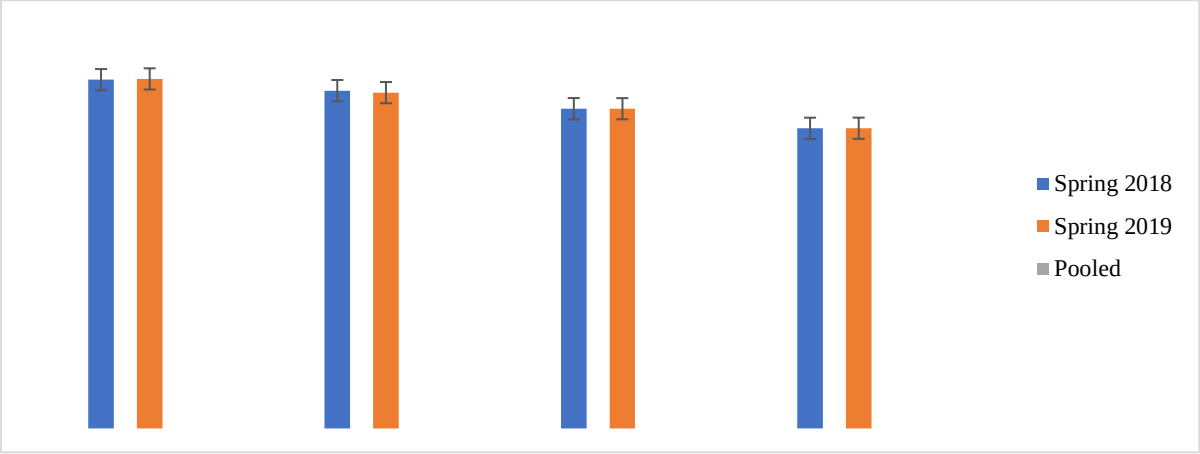

Figure 4. Protein equalisation of haemolymph on different days of 5th instar.

2.4. Quantification of succinate dehydrogenase (SDH) activity

Using the Nachlas et al. (1960) approach, the succinate dehydrogenase activity in the silkworm's haemolymph was calculated. Phosphate buffer, 0.1 mM pH 7.4: 100 ml of double-distilled water was used to dissolve 20 grammes of sodium monobasic phosphate, $Na_2HPO_4 \cdot 7H_2O$. 100 ml of double-distilled water were used to dissolve 4 grammes of sodium dibasic phosphate, $NaH_2PO_4 \cdot H_2O$. The two solutions are combined in an 87:13 ratio, and an acid or base is used to adjust the pH of the mixture to 7.4, Mono sodium succinate at 0.2M: 100 ml of double-distilled water are mixed with 0.2M sodium succinate to make 2.8 grammes. A freezing solution of sucrose: 10 M INT was used to dissolve 68.5 grammes of sucrose in 100 ml of double-distilled water. In 100 ml of water, glacial acetic acid, formazone, and toluene, 0.5 mg of INT was dissolved. As shown in table 1, different standardised amounts of haemolymph from various races were combined with 1 ml of chilled sucrose solution (0.25M) and centrifuged for 15 minutes at 3000 rpm. The supernatant was utilised as an enzyme source. Three test tubes were used for each race on each day of the fifth instar, and haemolymph was introduced in the amounts listed in Table 1 along with one ml of substrate buffer, 0.2 ml of phosphate buffer pH 7.4, 0.2 ml of sodium succinate, and 1 ml of 0.01 mM INT. The mixture was incubated at 37°C for 30 minutes, and then 6.0 ml of glacial acetic acid was added to stop the reaction. The formazan produced was extracted into 6 ml of toluene and left overnight at 0°C. The optical density of the colour produced was then measured in a spectrophotometer using a 495 nm wavelength. taking 0.5 ml of distilled water from a blank. As moles of formazan/ml of hemolymph/mg protein/min, the enzyme activity was measured.

2.5. Quantification of glutamate dehydrogenase (GDH) activity

Using the Lee and Lardy (1965) method, glutamate dehydrogenase activity (GDH) was quantitatively measured in the haemolymph. Different standardised quantities of haemolymph from various races were combined with 1 ml of cold (0.25 M) sucrose solution, centrifuged at 3000 rpm for 15 minutes at 2°C, and the clear cell free extract was then dialyzed against 0.25 M sucrose at 2°C to 4°C for 24 hours.

0.5 ice-cold sucrose solution: 8.6 grammes of sucrose were combined with 100 ml of double-distilled water, 100 mM sodium monohydrogen phosphate heptahydrate ($Na_2 HPO_4 \cdot 7H_2O$), 2.7 milligrams of monobasic sodium phosphate, and 100 mM sodium dihydrogen phosphate monohydrate ($NaH_2PO_4 \cdot H_2O$), 1.4 milligrams of dibasic sodium phosphate, respectively 200 ml of distilled water, 400 M mono sodium glutamate monohydrate (0.8 milligrams of mono sodium glutamate are dissolved in 100 ml of double distilled water), 6.6 micrograms of nicotinamide adenosine dinucleotide, and 4 M INT were used to dilute the

mixture of 8.5 ml of A and 91.5 ml of B. Toluene, glacial acetic acid, and 100 ml of water were used to dissolve 0.2 milligrams of INT.

Three test tubes were used for each race on each day of the fifth instar. Amount of haemolymph was added according to the table1, along with 0.2 ml of phosphate buffer pH 7.4, 0.2 ml of sodium glutamate, 0.2 ml of nicotinamide adenosine dinucleotide, and 1 ml of INT. By introducing several standardised quantities of haemolymph from various races, the reaction was started. A thermostatic water bath was used to incubate the mixture at 37°C for 30 minutes before adding 5 ml of glacial acetic acid to stop the process. The formazan was extracted into 5.0 ml of toluene and stored at 5°C overnight. At a 495 nm wavelength, the optical density values were measured. taking 0.5 ml of distilled water from a blank. In addition, INT standards were created for comparability. In terms of moles of formozon released/ml of hemolymph/mg protein/min, the enzyme activity was expressed.

On each day of the fifth instar, three replications of each treatment were collected to determine the average value.

2.6. Correlation

The nature and magnitude of correlation between economic parameters, total protein, succinate dehydrogenase and glutamate dehydrogenase were figured out.

3. Results and Discussion

3.1. Haemolymph total protein

Pooled analysis revealed hat highest haemolymph total protein (51.10 mg/ml) was recorded in the silkworm breed SK1, which was statistically higher than rest of the genotypes. The total protein content recorded in other breeds include: SK6 (50.11 mg/ml), SK22 (49.19 mg/ml), SK28 (48.85 mg/ml), SK33 (48.41 mg/ml), NB4D2 (47.31 mg/ml), CSR4 (38.47 mg/ml), CSR2 (36.52 mg/ml), and Dun6 (35.60 mg/ml). The least value of haemolymph total protein (23.22 mg/ml) was recorded in the silkworm breed APS4 (Table 2). During the study it was also reported that highest peak of haemolymph total protein (45.94 mg/ml) was achieved on seventh day of fifth instar which was statistically higher than rest of the days. The total protein content recorded on other days of fifth instar include: (43.49 mg/ml) on sixth day and (41.58 mg/ml) on fifth day. The least value haemolymph total protein (40.50 mg/ml) was recorded on fourth day of the fifth instar (Figs 5 and 6). Standardization of haemolymph volume (in terms protein equalization) for estimation of enzyme activities.

Table 2. Total protein content (mg/ml) in different breeds of silkworm (*Bombyx mori* L.) during spring season (Pooled of 2018 and 2019)

Season	Spring 2018					Spring 2019					Pooled				
Days / Genotypes	4th Day	5th Day	6th Day	7th Day	Mean	4th Day	5th Day	6th Day	7th Day	Mean	4th Day	5th Day	6th Day	7th Day	Mean
G_1:SK1	49.06	50.07	52.06	52.08	50.82	48.23	50.02	53.03	54.23	51.38	48.65	50.05	52.55	53.16	51.10[a]
G_2:SK6	49.02	48.32	51.25	51.33	49.98	49.04	49.21	51.28	51.39	50.23	49.03	48.77	51.27	51.36	50.11[b]
G_3:SK22	48.08	48.07	50.13	51.07	49.34	47.23	48.14	50.18	50.65	49.05	47.66	48.11	50.16	50.86	49.19[c]
G_4:SK28	47.23	43.85	49.53	52.55	48.29	47.28	48.22	49.42	52.69	49.4	47.26	46.04	49.48	52.62	48.85[d]
G_5:SK33	45.13	49.43	48.03	52.36	48.74	45.18	49.15	48.23	49.75	48.08	45.16	49.29	48.13	51.06	48.41[e]
G_6:NB4D2	44.21	45.29	48.69	51.26	47.36	44.25	45.59	47.89	51.29	47.26	44.23	45.44	48.29	51.28	47.31[f]
G_7:CSR4	35.26	36.87	37.85	44.54	38.63	35.27	35.69	37.75	44.52	38.31	35.27	36.28	37.8	44.53	38.47[g]
G_8:CSR2	34.28	35.45	36.54	39.45	36.43	34.33	35.46	36.52	40.11	36.61	34.31	35.46	36.53	39.78	36.52[h]
G_9:Dun6	33.25	34.21	36.41	38.54	35.6	33.24	34.21	36.42	38.52	35.6	33.25	34.21	36.42	38.53	35.60[i]
G_{10}:APS4	20.22	22.14	24.32	26.25	23.23	20.23	22.12	24.31	26.18	23.21	20.23	22.13	24.32	26.22	23.22[j]
Mean	40.57	41.37	43.48	45.94		40.43	41.78	43.5	45.93		40.50[d]	41.58[c]	43.49[b]	45.94[a]	
CD (p≤0.05)		Day: 0.064 Genotype: 0.100 Day x Genotype: 0.201					Day: 0.052 Genotype: 0.082 Day x Genotype: 0.163						Day: 0.039 Genotype: 0.062 Day x Genotype: 0.124		

Note: Each value represents the mean of three replications; Figures super scripted with same letter are statistically nonsignificant.

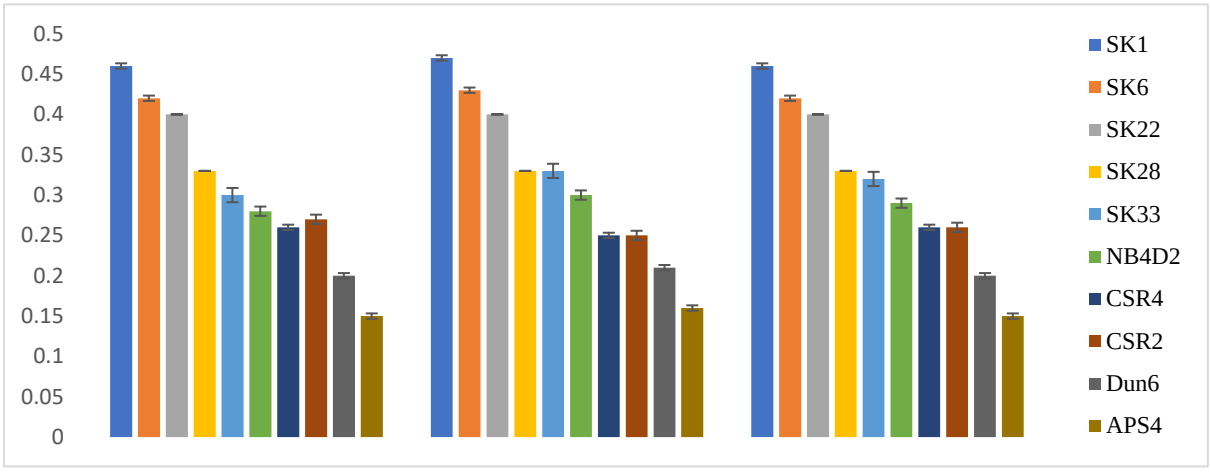

Figure 5. Total protein in different races of silkworm.

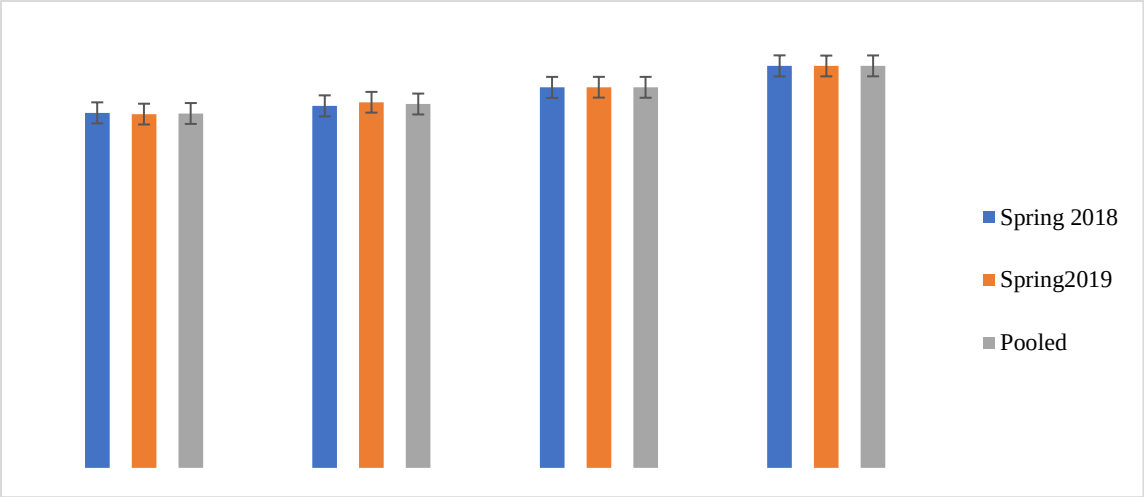

Figure 6. Total protein on different days of 5th instar.

During the study, haemolymph volume having one milligram protein was estimated in various silkworm breeds, during spring season. Pooled analysis of the two-year data (spring 2018 and spring 2019) revealed that highest volume of haemolymph having one milligram protein (43.48 µl), was observed in the breed APS4, which was statistically higher than rest of the genotypes. The volume of haemolymph having one milligram protein in other breeds include: Dun6 (28.18 µl), CSR2 (27.47 µl), CSR4 (26.21 µl), NB4D2 (21.21 µl), SK28 (20.54 µl) which was statistically at par with SK33 (20.70 µl), SK22 (20.34 µl) and SK6 (19.97 µl). The least volume of haemolymph having one milligram protein was recorded in the silkworm breed SK1 (19.60 µl). Highest value of the volume of haemolymph having one milligram protein was recorded on fourth day (26.49 µl) of fifth instar having statistical significance and different from other days. The haemolymph volume on other days include: (25.55 µl) on 5^{th} day and (24.26 µl) on 6^{th} day of 5^{th} instar. The least volume of haemolymph having one milligram protein (22.77 µl) was recorded on 7^{th} day of 5^{th} instar.

3.2. Haemolymph glutamate dehydrogenase activity

Hemolymph glutamate dehydrogenase activity was estimated in different breeds of silkworm. Pooled analysis of the two-year data (spring 2018 and spring 2019) revealed that highest haemolymph glutamate dehydrogenase activity of 0.46 µmoles was recorded in the silkworm breed SK1, which was statistically higher than rest of the genotypes under study. The glutamate dehydrogenase activities in other breeds include: SK6 (0.42), SK22 (0.40), SK28 (0.33 µmoles), SK33 (0.32 µmoles), NB4D2 (0.29 µmoles), CSR4 (0.26 µmoles), CSR2 (0.26 µmoles) and Dun6 (0.20 µmoles). The least value of haemolymph glutamate dehydrogenase activity (0.15 µmoles) was recorded in the silkworm breed APS4 (Table 3). During the current study, it was

also revealed that highest peak of haemolymph glutamate dehydrogenase activity of 0.36 µmoles was recorded on 7th day of 5th instar which was statistically higher than rest of the days. The lowest peak of haemolymph glutamate dehydrogenase activity of 0.26 µmoles was recorded on 4th day of 5th instar (Figs 7 and 8).

Table 3. Haemolymph glutamate dehydrogenase activity (µmoles of formazon released /ml /mg of protein/min.) in different breeds of silkworm (*Bombyx mori* L.) during spring season (Pooled of 2018 and 2019).

Season	**Spring 2018**					**Spring 2019**					**Pooled**				
Days / Genotypes	4th Day	5th Day	6th Day	7th Day	Mean	4th Day	5th Day	6th Day	7th Day	Mean	4th Day	5th Day	6th Day	7th Day	Mean
G_1:SK1	0.41	0.44	0.48	0.51	0.46	0.43	0.45	0.48	0.51	0.47	0.42	0.45	0.48	0.51	0.46[a]
G_2:SK6	0.38	0.41	0.44	0.45	0.42	0.37	0.42	0.45	0.47	0.43	0.38	0.42	0.45	0.46	0.42[b]
G_3:SK22	0.33	0.38	0.42	0.45	0.40	0.34	0.37	0.42	0.46	0.40	0.34	0.38	0.42	0.46	0.40[c]
G_4:SK28	0.29	0.31	0.34	0.37	0.33	0.28	0.31	0.33	0.39	0.33	0.29	0.31	0.34	0.38	0.33[d]
G_5:SK33	0.27	0.29	0.31	0.34	0.30	0.28	0.31	0.35	0.38	0.33	0.28	0.30	0.33	0.36	0.32[e]
G_6:NB4D2	0.24	0.27	0.29	0.33	0.28	0.25	0.28	0.31	0.34	0.30	0.25	0.28	0.30	0.34	0.29[f]
G_7:CSR4	0.18	0.25	0.28	0.34	0.26	0.17	0.22	0.27	0.33	0.25	0.18	0.24	0.28	0.34	0.26[g]
G_8:CSR2	0.23	0.25	0.28	0.33	0.27	0.22	0.24	0.26	0.29	0.25	0.23	0.25	0.27	0.31	0.26[g]
G_9:Dun6	0.15	0.18	0.21	0.24	0.20	0.16	0.19	0.22	0.25	0.21	0.16	0.19	0.22	0.25	0.20[h]
G_{10}:APS4	0.12	0.14	0.16	0.18	0.15	0.13	0.15	0.17	0.18	0.16	0.13	0.15	0.17	0.18	0.15[i]
Mean	0.26	0.29	0.32	0.35		0.26	0.29	0.33	0.36		0.26[d]	0.29[c]	0.32[b]	0.36[a]	
CD (p≤0.05)		Day: 0.007 Genotype: 0.011 Day x Genotype: 0.023					Day: 0.007 Genotype:0.011 Day x Genotype: 0.022					Day: 0.005 Genotype: 0.008 Day x Genotype: 0.016			

Note: Each value represents the mean of three replications; Figures super scripted with same letter are statistically nonsignificant.

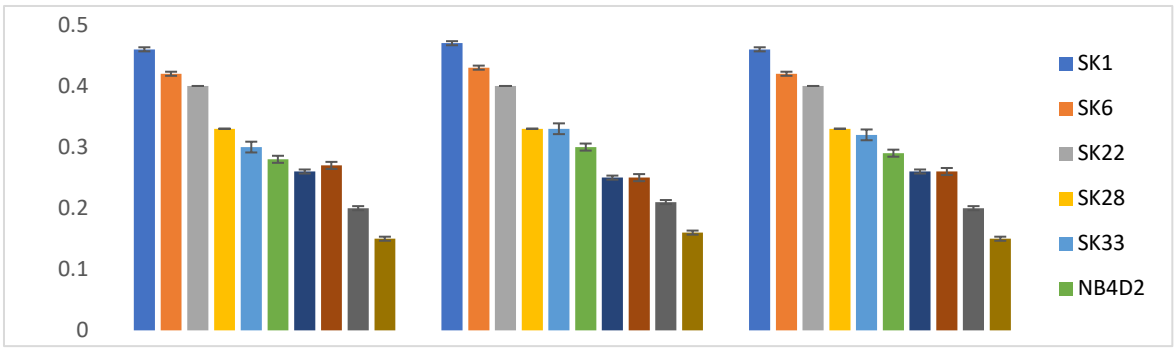

Figure 7. Haemolymph glutamate dehydrogenase activity in different races of silkworm.

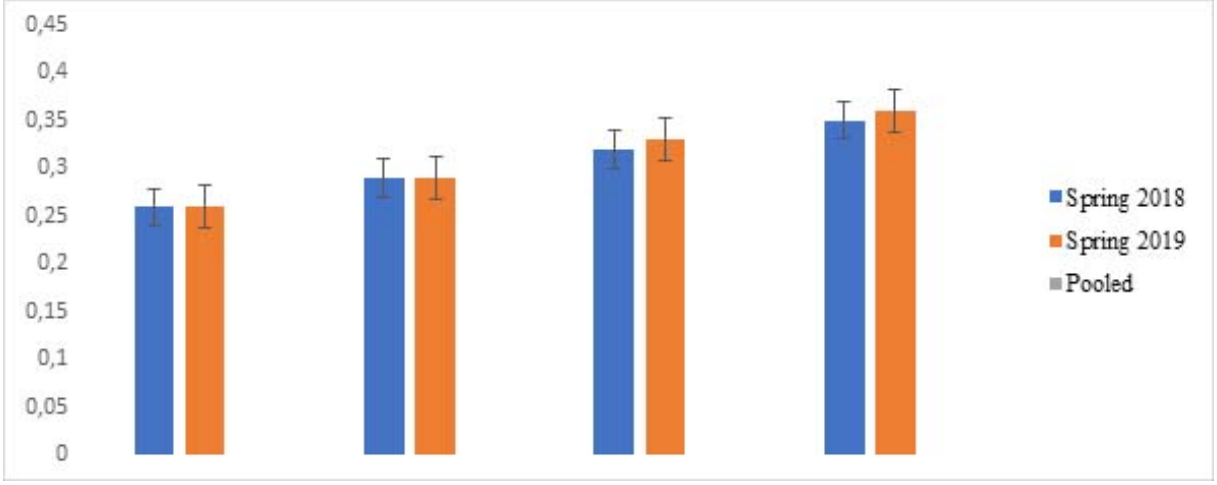

Figure 8. Haemolymph glutamate dehydrogenase activity on different days of 5th instar.

3.3. Haemolymph succinate dehydrogenase activity

Succinate dehydrogenase activity was estimated in various breeds of silkworm. Pooled analysis of the two-year data (spring 2018 and spring 2019) revealed that highest haemolymph succinate dehydrogenase activity (3.47µmoles) was recorded in the silkworm breed SK1, which was statistically higher than rest of the genotypes. The haemolymph succinate dehydrogenase activity in other breeds include: SK6 (2.92 µmoles), SK28 (2.84 µmoles), SK22 (2.63 µmoles), CSR2 (2.58 µmoles), SK33 (2.57 µmoles), CSR4 (2.51 µmoles), NB4D2 (2.35µmoles) and Dun6 (1.68 µmoles). The least value of haemolymph succinate dehydrogenase activity (1.58 µmoles) was recorded in the silkworm breed APS4 (Table 4). During the current study it was also revealed that highest haemolymph succinate dehydrogenase activity (2.65 µmoles) was recorded on seventh day of fifth instar which was statistically higher than rest of the days. The succinate dehydrogenase activity on other days include: (2.55 µmoles) on sixth day, (2.46 µmoles) on 5th day, which was statistically at par with 4th day (2.39 µmoles) of the 5th instar (Figs 9 and 10).

Table 4. Succinate Dehydrogenase (µmoles of formazon released /ml /mg of protein/min.) in different breeds of silkworm (*Bombyx mori* L.) during spring season (Pooled of 2018 and 2019).

Season	**Spring 2018**					**Spring 2019**					**Pooled**				
Days	4thDay	5thDay	6thDay	7thDay	Mean	4thDay	5thDay	6thDay	7thDay	Mean	4thDay	5thDay	6thDay	7thDay	Mean
Genotypes															
G_1:SK1	3.31	3.37	3.56	3.77	3.50	3.33	3.41	3.45	3.55	3.44	3.32	3.39	3.51	3.66	3.47[a]
G_2:SK6	2.81	2.86	2.96	3.11	2.94	2.85	2.88	2.92	2.98	2.91	2.83	2.87	2.94	3.05	2.92[b]
G_3:SK22	2.53	2.59	2.68	2.76	2.64	2.55	2.57	2.65	2.69	2.62	2.54	2.58	2.67	2.73	2.63[c]

G_4:SK28	2.78	2.86	2.88	2.91	2.86	2.75	2.79	2.86	2.92	2.83	2.77	2.83	2.87	2.92	2.84^{b}
G_5:SK33	2.48	2.51	2.52	2.69	2.55	2.51	2.55	2.61	2.65	2.58	2.50	2.53	2.57	2.67	2.57^{c}
G_6:NB4D2	1.93	2.23	2.50	2.67	2.33	1.88	2.23	2.53	2.83	2.37	1.91	2.23	2.52	2.75	2.35^{e}
G_7:CSR4	2.43	2.47	2.47	2.56	2.48	2.39	2.41	2.63	2.68	2.53	2.41	2.44	2.55	2.62	2.51^{d}
G_8:CSR2	2.53	2.59	2.69	2.78	2.65	2.43	2.48	2.53	2.59	2.51	2.48	2.54	2.61	2.69	2.58^{c}
G_9:Dun6	1.62	1.66	1.69	1.71	1.67	1.65	1.68	1.71	1.75	1.70	1.64	1.67	1.70	1.73	1.68^{f}
G_{10}:APS4	1.51	1.57	1.62	1.69	1.60	1.52	1.55	1.59	1.62	1.57	1.52	1.56	1.61	1.66	1.58^{g}
Mean	2.39	2.47	2.56	2.67		2.39	2.46	2.55	2.63		2.39^{c}	2.46^{c}	2.55^{b}	2.65^{a}	
CD ($p \leq 0.05$)		Day: 0.081					Day: 0.049						Day: 0.05		
		Genotype: 0.128					Genotype: 0.079						Genotype: 0.08		
		Day x Genotype: 0.257					Day x Genotype: 0.156						Day x Genotype: 0.15		

Note: Each value represents the mean of three replications; Figures super scripted with same letter are statistically nonsignificant.

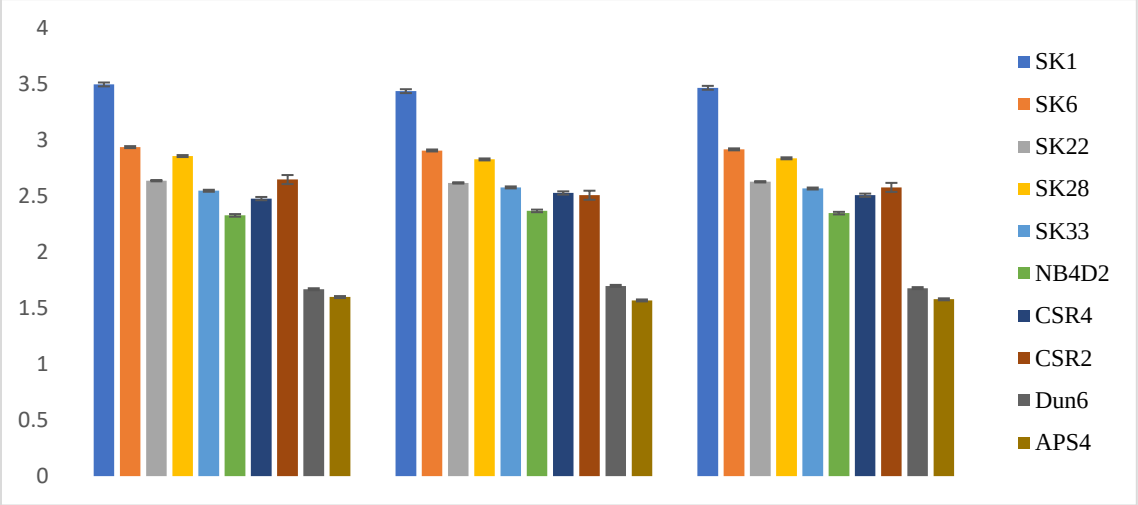

Figure 9. Haemolymph succinate dehydrogenase activity in different races of silkworm.

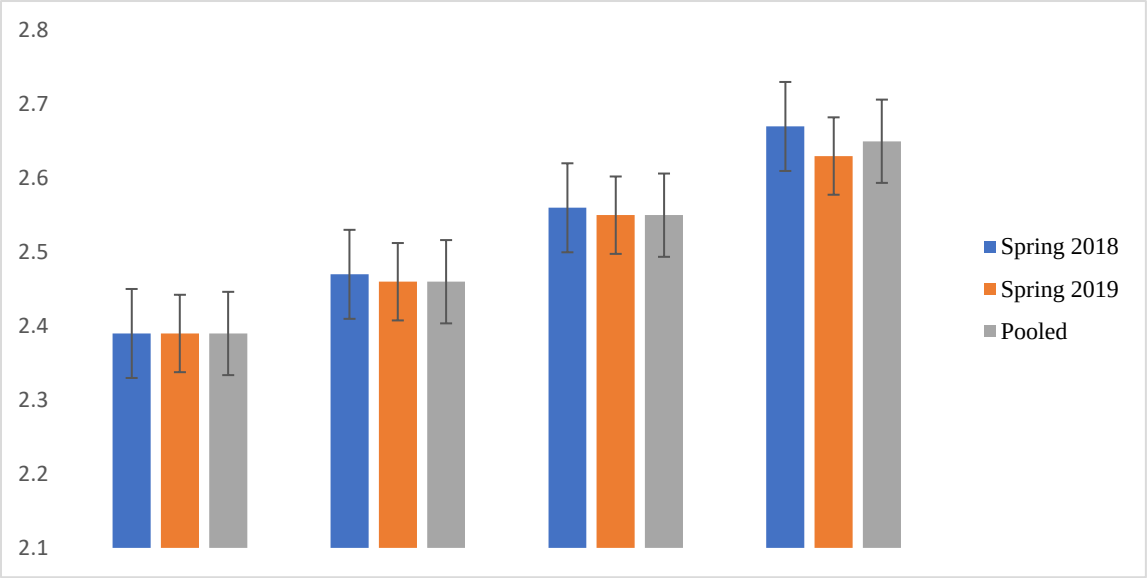

Figure 10. Haemolymph succinate dehydrogenase activity on different days of 5th instar.

3.4. Correlation of haemolymph total protein with economic parameters during spring

During the study, it was found that moderately significant ($p \leq 0.05$) positive correlations were obtained between haemolymph total protein and weight of 10 mature larvae on fifth day (r=+0.621), haemolymph total protein and single cocoon weight (r=+0.704), haemolymph total protein and single shell weight (r=+0.680), haemolymph total protein and shell ratio (r=+0.745), haemolymph total protein and cocoon yield per 1000 larvae by number (r=+0.711), haemolymph total protein and cocoon yield per 1000 larvae by weight (r=+0.738), haemolymph total protein and fecundity (r=+0.820), haemolymph total protein and filament length (r=+0.704) and haemolymph total protein and raw silk percentage (r=+0.651).The moderately significant ($p \leq 0.05$) negative correlations were obtained between haemolymph total protein and fifth instar larval duration in days (r=-0.632) and haemolymph total protein and total larval duration in days (r=-0.639) (Table 5).

Table 5. Correlation matrix of economic parameters with total protein, Succinate dehydrogenase activity and Glutamate dehydrogenase activity.

Economic Parameters	Total protein	Succinate dehydrogenase activity	Glutamate dehydrogenase activity
Fifth instar larval duration in days	-0.632*	0.172	-0.212
Total larval duration in days	-0.639*	0.148	-0.325
Weight of ten mature larvae on fifth day of fifth instar	0.621*	0.672*	0.628*
Single shell weight	0.680*	0.721*	0.634*

Single cocoon weight	0.704*	0.637*	0.755*
Shell ratio	0.745*	0.629*	0.665*
Silk productivity	0.321	0.712*	0.633*
Cocoon yield per 10000 larvae by number	0.711*	0.719*	0.646*
Cocoon yield per 10000 larvae by weight	0.738*	0.742*	0.857*
Pupation rate	0.284	0.812*	0.710*
Fecundity	0.820*	0.618*	0.625*
Average filament length	0.704*	0.713*	0.766*
Filament size	0.598	0.322	-0.158
Raw silk percentage	0.651*	0.741*	0.452

*Indicates Significant at 0.05 and ** indicates significant at 0.01

3.5. Correlation of haemolymph succinate dehydrogenase activity with economic parameters during spring

During the study, it was found that positive correlations ($p \leq 0.05$) were obtained between haemolymph succinate dehydrogenase activity and weight of ten mature larvae on fifth day (r=+0.672), haemolymph succinate dehydrogenase activity and single shell weight (r=+0.721), haemolymph succinate dehydrogenase activity and single cocoon weight (r=+0.637), haemolymph succinate dehydrogenase activity and shell ratio (r=+0.629), haemolymph succinate dehydrogenase activity and silk productivity (r=+0.712), haemolymph succinate dehydrogenase activity and cocoon yield per 10000 larvae by number (r=+0.719), haemolymph succinate dehydrogenase activity and cocoon yield per 10000 larvae by weight (r=+0.742), haemolymph succinate dehydrogenase activity/pupation rate (r=+0.812), haemolymph succinate dehydrogenase activity and fecundity (r=+0.618), haemolymph succinate dehydrogenase activity and filament length (r=+0.713) and haemolymph succinate dehydrogenase activity and raw silk percentage (r=+0.741) (Table 5).

3.6. Correlation of haemolymph glutamate dehydrogenase activity with economic parameters during spring

During the study, it was found that moderately significant ($p \leq 0.05$) positive correlations were obtained between haemolymph glutamate dehydrogenase activity and weight of ten mature larvae on fifth day (r=+0.628), haemolymph glutamate dehydrogenase activity and single shell weight (r=+0.634), haemolymph glutamate dehydrogenase activity and single cocoon weight (r=+0.755), haemolymph glutamate dehydrogenase activity and shell ratio (r=+0.665), haemolymph glutamate dehydrogenase activity and silk productivity (r=+0.633) haemolymph glutamate dehydrogenase activity and cocoon yield per 10000 larvae by number (r=+0.646),

haemolymph glutamate dehydrogenase activity and cocoon yield per 10000 larvae by weight (r=+0.857), haemolymph glutamate dehydrogenase activity and pupation rate (r=+0.710), haemolymph glutamate dehydrogenase activity and fecundity (r=+0.625) and haemolymph glutamate dehydrogenase activity and filament length (r=+0.766) (Table 5).

4. Discussion

Crop productivity depends on the dimensions of genetic variability in different breeds. Thus, the evaluation of genetic resources is the most important aspect of germplasm management for evaluating the promising genotypes. Estimation of genetic variability in the breeding material and magnitude of genetic effects controlling the yield and other economic traits are highly useful for successful breeding programmes.

Proteins are biomolecules made of different amino acids. Proteins play very important role in growth, development and silk protein synthesis. During present studies, it was found that SK1 silkworm breed excelled in terms of total protein content and it was recorded to the extent of 51.10 mg/ml in haemolymph during spring season. Rajannan et al. (1994) while working on the biochemical aspects of various silkworm breeds has reported that total protein content differed among silkworm breeds and there is a profound influence of breed and feed on the expression of proteins. Therefore, such results are in consonance with observations of the present research Programme. During the current study it was also found that highest haemolymph total protein content (45.94 mg/ml) was recorded on seventh day of fifth instar which was higher than rest of the days. The least value haemolymph total protein content (40.50 mg/ml) was recorded on fourth day of the fifth instar. The results are supported by the findings of Yogananda Murthy (2015), who reported 30.40 mg/ml, 36.71 mg/ml, 39.71 mg/ml and 43.30 mg/ml protein contents on fourth, fifth, sixth and seventh days of fifth instar in the haemolymph of bivoltine silkworm. The results are also supported by Wani et al. (2021). Variation in protein concentration in haemolymph maybe due to differential rate of metabolism and synthesis of more proteins on seventh day of fifth instar. Krishnaswami (1978) found that increase in the protein concentration in the silkworm during the fourth moult is due to the regular feeding of mulberry leaves.

Succinate dehydrogenase (SDH) is an enzyme found in all eukaryotic organisms and participates both in citric acid cycle and electron transport chain (Alberts et al., 2007). The enzymes catalyze the oxidation of succinate into fumarate in the Krebs cycle (McCammon et al., 2003). During present study, it was found that highest haemolymph succinate

dehydrogenase activity vary among bivoltine breeds with highest value (3.47 µmoles) recorded in SK1 silkworm breed and lowest 1.58 µmoles) in silkworm breed APS4.

During the current study it was also found that highest haemolymph succinate dehydrogenase activity (2.65 µmoles) was recorded on seventh day and lowest (2.39 µmoles) on fourth day of fifth instar. The possible reason for highest succinate dehydrogenase activity on seventh day of fifth instar might be due to more energy requirement of the silkworm larva for its muscular activity during spinning of cocoon. The results are governed by Hemavathi et al. (2002), which presents the increase in level of succinate dehydrogenase activity in haemolymph was in synchrony with glycolytic pathway which indicates that Tri Carboxylic Acid cycle was also elevated in terms of pyruvate production due to increased energy demands of the insect. Rajasekhar (1993) also reported that increased succinate dehydrogenase activity during final instar was accompanied by accelerating Krebs cycle and glycolysis.

GDH is localized mainly in the mitochondrial part of the cell (Harper et al., 1993). *Glutamate dehydrogenase* (GDH) catalyzes the reversible inter-conversion of glutamate to α-ketoglutarate and ammonia. During the current study the results showed that highest haemolymph glutamate dehydrogenase activity (0.46 µmoles) was recorded in SK1 silkworm breed having statistical importance and higher to other genotypes. The least value of haemolymph glutamate dehydrogenase activity (0.15 µmoles) was recorded in silkworm breed APS4. During the present study it was also found that highest value haemolymph glutamate dehydrogenase activity (0.36 µmoles) was recorded on seventh day of fifth instar which has statistical significance and higher to remaining days and the lowest haemolymph glutamate dehydrogenase activity (0.26 µmoles) was recorded on fourth day of fifth instar. The results are supported by Hemavathi et al. (2002) and Dasamahapatra et al. (1990), who presented the amplified activity of glutamate dehydrogenase, had positive metabolic modulation leading to synthesis of more energy required to produce improved quality cocoons. The possible reason for the progressive increase in haemolymph glutamate dehydrogenase activity during fifth instar might be due to increasing energy demands for muscular activity for the formation of quality cocoon and for formation and mobility of sperms. Osanai et al. (1987), who reported the α-ketoglutarate generated by this enzyme is probably used-up in ensuring sperm mobility in silkworm. Lehninger (1993) and (Herrero-Yraola et al. (2001) who reported the dehydrogenases also helps in the transfer of amino group of most amino acids into α-ketoglutarate through transamination reaction by forming glutarate with the release of ammonia.

Significant ($p \leq 0.05$) positive correlations were obtained between haemolymph total protein and weight of 10 mature larvae on fifth day (r=+0.621), haemolymph total protein and single cocoon weight (r=+0.704), haemolymph total protein and single shell weight (r=+0.680), haemolymph total protein and shell ratio (r=+0.745), haemolymph total protein and cocoon yield by number (r=+0.711), haemolymph total protein and cocoon yield by weight (r=+0.738), haemolymph total protein and fecundity (r=+0.820), haemolymph total protein and filament length (r=+0.704) and haemolymph total protein and raw silk percentage (r=+0.651).The moderately significant ($p \leq 0.05$) negative correlations were obtained between haemolymph total protein and fifth instar larval duration in days (r=-0.632) and haemolymph total protein and total larval duration in days (r=-0.639). The results are supported by Kasmaei and Mahesha (2012a), who reported that haemolymph protein had positive correlation with larval weight, shell ratio, single shell weight, single cocoon weight, denier, raw silk percentage, yield and fecundity and had significant negative correlation to fifth instar larval duration and total larval duration in mulberry silkworm.

During the study, it was found that moderately significant ($p \leq 0.05$) positive correlations were obtained between haemolymph succinate dehydrogenase activity and weight of ten mature larvae on fifth day (r=+0.672), haemolymph succinate dehydrogenase activity and single shell weight (r=+0.721), haemolymph succinate dehydrogenase activity and single cocoon weight (r=+0.637), haemolymph succinate dehydrogenase activity and shell ratio (r=+0.629), haemolymph succinate dehydrogenase activity and silk productivity (r=+0.712), haemolymph succinate dehydrogenase activity and cocoon yield per 10000 larvae by number (r=+0.719), haemolymph succinate dehydrogenase activity and cocoon yield per 10000 larvae by weight (r=+0.742), haemolymph succinate dehydrogenase activity and pupation rate (r=+0.812), haemolymph succinate dehydrogenase activity and fecundity (r=+0.618), haemolymph succinate dehydrogenase activity and filament length (r=+0.713) and haemolymph succinate dehydrogenase activity and raw silk percentage (r=+0.741).The results are in conformity with Maqbool (2010), who reported that positive correlation of succinate dehydrogenase activity with cocoon yield per 10000 larvae by weight (r=+0.701), single cocoon weight (r=+0.660), single shell weight (r=+0.548).The results are also supported by Kasmaei and Mahesha (2012b), who reported that the activity of succinate dehydrogenase in haemolymph had positive correlation to filament length (r=+0.415, cocoon weight (r=+0.319), shell weight (r=+0.246), shell ratio (r=+0.214), larval weight (r=+0.591), fecundity (r=+0.003), pupation rate, silk productivity and raw silk percentage.

Moderately significant ($p \leq 0.05$) positive correlations were obtained between haemolymph glutamate dehydrogenase activity and weight of ten mature larvae on fifth day (r=+0.628), haemolymph glutamate dehydrogenase activity and single shell weight (r=+0.634), haemolymph glutamate dehydrogenase activity and single cocoon weight (r=+0.755), haemolymph glutamate dehydrogenase activity and shell ratio (r=+0.665), haemolymph glutamate dehydrogenase activity and silk productivity (r=+0.633) haemolymph glutamate dehydrogenase activity and cocoon yield per 10000 larvae by number (r=+0.646), haemolymph glutamate dehydrogenase activity and cocoon yield per 10000 larvae by weight (r=+0.857), haemolymph glutamate dehydrogenase activity and pupation rate (r=+0.710), haemolymph glutamate dehydrogenase activity and fecundity (r=+0.625) and haemolymph glutamate dehydrogenase activity and filament length (r=+0.766). The results are supported by Bannikov et al. (1982), who reported that glutamate dehydrogenase activity had positive correlation with weight of silkworm larva. The results are supported by Pant and Jaiswal (1981), who reported that glutamate dehydrogenase, had positive correlation with growth and survival. The results are supported by Naga Jyothi et al. (2009), who reported under the influence of ultrasound protein metabolism and glutamate dehydrogenase activity are stimulated to achieve greater turnover of silk proteins, greater spinning activity and cocoon yield by weight and number, filament length and silk productivity. The results are supported by Yungen et al. (2002), who reported effects of prostaglandin F2α on cocoon variables and biosynthesis of silk proteins in the multivoltine silkworm, *Bombyx mori* L. Increases in biochemical constituents, including total proteins, free amino acids, RNA content, glutamate dehydrogenase activity and fibroin concentration in the posterior silk gland after treatment with prostaglandin F2α corresponded to increases in whole cocoon weight, shell weight, shell ratio, fecundity and silk quality.

5. Summary

Among different breeds of silkworm SK1, SK6, SK22, SK28 excelled in total protein content of haemolymph during spring. The haemolymph total protein contents in these breeds include SK1 (51.10 mg/ml), SK6 (50.11 mg/ml), SK22 (49.19 mg/ml), SK28 (48.85 mg/ml) whereas lowest haemolymph total protein content (23.22 mg/ml) was found in silkworm breed APS4.Among different days of fifth instar highest haemolymph total protein content (45.94 mg/ml) was found on sixth day whereas lowest haemolymph total protein content (40.50 mg/ml) was found on fourth day of the fifth instar. The breeds excelled in haemolymph

succinate dehydrogenase activity during spring season. The haemolymph succinate dehydrogenase activity in these breeds SK1 (3.47µmoles), SK6 (2.92 µmoles), SK28 (2.84 µmoles), SK22 (2.63 µmoles) and lowest haemolymph succinate dehydrogenase activity (1.58 µmoles) was recorded in silkworm breed APS4. Among different days of fifth instar highest haemolymph succinate dehydrogenase activity (2.65 µmoles) was recorded on seventh day of fifth instar and lowest haemolymph succinate dehydrogenase activity (2.39 µmoles) was recorded on fourth day of fifth instar. The breeds also excelled in haemolymph glutamate dehydrogenase activity during spring season. The haemolymph glutamate dehydrogenase activity in these breeds SK1 (0.46 µmoles), SK6 (0.42), SK22 (0.40), SK28 (0.33 µmoles) and lowest haemolymph glutamate dehydrogenase activity (0.15 µmoles) was recorded in silkworm breed APS4. Among different days of fifth instar highest haemolymph glutamate dehydrogenase activity (0.36 µmoles) was recorded on seventh day and lowest haemolymph glutamate dehydrogenase activity (0.26 µmoles) was recorded on fourth day. Haemolymph total protein, succinate and glutamate dehydrogenase activities were having positive correlation with cocoon yield per 10000 larvae by weight and number, weight of ten mature larvae on fifth day, single cocoon weight, single shell weight, shell ratio, silk productivity, pupation rate, fecundity, filament length and raw silk percentage

6. Conclusions

The bivoltine breeds SK1, SK6, SK22, SK28, and SK3 exhibit strong SDH and GDH activities, according to the study. Most economic metrics have a positive link with the breeds/enzymes. As a result, they have been selected as potential breeds. The breeds have the potential to be used as markers for future breeding programmes aimed at developing a prolific silkworm breed suitable for the Kashmir valley.

Acknowledgements

Authors thank Head, DCCI, CoTS, Mirgund for giving different silkworm breeds. The authors also extend a deep sense of gratitude to the Head, Division of BSH, SKUAST-Kashmir for providing the laboratory facilities to conduct this work. The author wishes to extend his heartfelt thanks to Associate Dean and Major advisor, CoTS, Mirgund, SKUAST-Kashmir.

Funding

CoTS, Mirgund, SKUAST-Kashmir for financial support.

Conflict of interest: The authors have no conflict of interest and Nothing to disclose.

References

Alberts B., Johnson A., Lewis J., Raff M., Roberts K. & Walter P., 2007, Molecular biology of the cell, 5th edition. Chapter 14: Energy Conversion: Mitochondria and Chloroplasts, p. 813-878. Garland Science, New York.

Anonymous, 2003, Package of practices for silkworm rearing and mulberry cultivation in Kashmir. Technical Document, Directorate of Extension Education, Sher Kashmir University of Agricultural Sciences and Technology of Kashmir, 5 pp.

Anonymous, 2019, Annual report. Vision document and road map for cocoon and silk production in Jammu & Kashmir, p. 2-3. Sericulture Development Department.

Anonymous, 2020, Functioning of central silk board and performance of Indian silk industry, p.19-21.

Bannikov V.M., Bachikova A.P., Ushkova G.I. & Fillippovich Y.B., 1982, Study on sub cellular localization of some enzymes in silkworm, *Bombyx mori* L. eggs. Biokhimiya 47: 1386-1391.

Dasamahapatra A.K., Ray A.K. & Medda A.K., 1990, In vitro demonstration of putative nuclear 3, 5, 3-triiodo thyronine receptor in isolated liver nuclei of singi fish, Heteropneustes fossilis (Bloch). Hormone and Metabolic Research 22: 221-224.

Dickinson W.J. & Sullivan D.T., 1975, Gene enzyme system in Drosophila. Springer, Berlin 11: 12-18.

Etebari K., Mirhodeini S.Z. & Matindoost L., 2005, A study on intraspecific biodiversity of eight groups of silkworms (*Bombyx mori*) by biochemical markers. Insect Science 12(2): 87-94.

Harper H.A., Rodwell V.W. & Mayes P.A., 1993, Review of physiological chemistry (21st Ed.). Lange Medical Publication. Los Altos, California.

Hemavathi B., Thygaraju K. & Bharathi D., 2002, Effect of thyroxine on the activities of dehydrogenases in silkworm, *Bombyx mori* L. Indian Journal of Comparative Animal Physiology 20: 59-65.

Herrero-Yraola A., Bakhit S.M.A., Franke P., Weise C., Scheiger M., Jorcke D. & Ziegler M., 2001, Regulation of glutamate dehydrogenase by reversible ADP-ribosylation in mitochondria. European Molecular Biology Organization Journal 20(10): 2404-2412.

Horie Y., 1968, The oxidation of NADPH by the soluble fraction of the fat body of the silkworm, *Bombyx mori* L. Journal of Insect Physiology 14: 417-424.

Kasmaei F.G. & Mahesha H.B., 2012a, Studies on succinate dehydrogenase and its relationship with economic characters of silkworm, *Bombyx mori* L. Annals of Biological Research 3(7): 3638-3651.

Kasmaei F.G. & Mahesha H.B., 2012b, Correlation studies on haemolymph and midgut tissue proteins with commercial characters of silkworm, *Bombyx mori* L. Asian Journal of Experimental Biological Sciences 3(3): 642-653.

Krishnaswami S., 1978, New technology of silkworm rearing. Bulletin No. 2 of Central Sericultural Research & Training Institute (CSRTI), Mysore, India, p. 1-24.

Kumar R. & Gangwar S.K., 2010, Impact of varietal feeding on *Samia ricini* Donovan in spring and autumn season of Uttar Pradesh. ARPN Journal of Agricultural and Biological Science 5(3): 46-51.

Lee Y.L. & Lardy H.A., 1965, Influence of thyroid hormones on L-glycerophosphate dehydrogenase and other dehydrogenases in various organs of the rat. Journal of Biological Chemistry 240:1427-1436.

Lehninger A.L., 1993, Biochemistry (2nd Ed.). Kalyani Publishers, Ludhiana, New Delhi, India.

Lowry O.H., Rosebrough N.J., Farr A.L. & Randall R.S., 1951, Protein measurement with the Folin Phenol reagent. Journal of Biological Chemistry 93: 265-275.

Maqbool A., 2010 Genetic divergence in some bivoltine silkworm, *Bombyx mori* L. breeds. PhD Thesis Submitted to Sher-e-Kashmir University of Agricultural Sciences and Technology of Kashmir, p. 1-76.

McCammon M.T., Epstein C.B., Przybyla-Zawislak B., McAlister-Henn L. & Butow R.A., 2003, Global transcription analysis of Krebs tricarboxylic acid cycle mutants reveal an alternating pattern of gene expression and effects on hypoxic and oxidative genes. Molecular Biology of the Cell 14: 958-972.

Nachlas M.M., Morgulis S.P. & Seligman A.M. 1960, A calorimetric method for the determination of Succinate dehydrogenase activity. Journal of Biological Chemistry 235: 499-505.

Naga Jyothi P., Nagalakshmamma K., Suneetha Y. & Sarma P.A., 2009, Enhancement of protein metabolism and enzyme activities in haemolymph of silkworm, *Bombyx mori* L. by ultrasound treatment during fifth instar development. World Journal of Zoology 4(3): 225-231.

Osanai M., Aigaki T. & Kosuga H., 1987, Arginine degradation cascade as an energy yielding system for sperm maturation in the spermatophore of silkworm, *Bombyx mori*, [in:] P.P. Mohr (ed.) New Horizons in Sperm Cell Research, p. 185-195.

Pant R. & Jaiswal G., 1981, Photoperiodic effect on transaminase activity, protein and total free amino acid content in the fat body of diapausing pupae of the tasar silkworm, *Antheraea mylitta*. Indian Journal of Experimental Biology 19: 998-1000.

Raja R. 2000, Appropriate Silkworm Rearing Technology, [in:] H.O. Agrawal, M.K. Seth (eds.) Sericulture in India, p. 289-302. Bishen Singh Mahendra Pal Singh Press, Dehradun, India.

Rajannan K.L., Pattarju H.P., Manjula A.C.A. & Yadav P.R., 1994, Biochemical differences among some selected lines of two different voltine races of *Bombyx mori* L. Journal of the Entomological Research Society 18(1): 53-60.

Rajasekhar R., 1993, Studies on the temperature induced modulations on the growth and reproductive physiology of silkworm *Bombyx mori* L., p. 45-48. Ph.D. Thesis, Sri Venkateswara University, Tirupati, India.

Shivkumar G. & Subramanya G., 2015, Quantitative estimation of haemolymph protein during different days of fifth instar larvae in bivoltines, multivoltines and mutants of the silkworm *Bombyx mori*. Global Journal of Bioscience and Biotechnology 4(3): 239-241.

Sumino T., Masao N. & Masahiko K., 1980, Storage proteins in the silkworm *Bombyx mori* L. Insect Biochemistry 10: 289-303.

Wani M.Y., Ganie N.A., Mir M.R., Mir S.A., Wani T.A., Yaqoob M. & Murtaza I., 2021, Total protein and total free amino acid estimation in peculiar bivoltine breed of mulberry silkworm (*Bombyx mori* L.). J. Exp. Zool. India 24(2): 901-908.

Yogananda Murthy V.N., 2015, Estimation of protein concentration in different tissues of popular silkworm (*Bombyx mori* L.) races. International Journal of Advanced Research 3(1): 254-261.

Yungen M., Lijun J. & Bharathi D., 2002, Impact of Prostaglandin F2 on Silk Quality and Biosynthesis of Silk Proteins of the Silkworm, *Bombyx mori* L. J. Agric. Urban Entomol. 19(2): 65-71.